DEVELOPMENT AND CHARACTERIZATION OF SEAWEED-BASED FILM FROM *Kappaphycus alvarezii* WITH ALOE VERA GEL PLASTICIZER

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CHAPTER 1

THE PROBLEM

Introduction

Traditional plastic films commonly used in packaging, agriculture, and various other applications present significant environmental issues due to their non-biodegradability and persistence in ecosystems. According to Geyer et al. (2017), more than 8.3 billion metric tons of plastic have been produced since the 1950s, with a mere 9% being recycled, 12% incinerated, and the remaining 79% either stored in landfills or dispersed throughout the environment. The Philippines, in particular, ranks as one of the leading sources of ocean plastic pollution, producing approximately 2.7 million tons each year (Jambeck et al., 2015). Moreover, in 2019, the country was recognized as the primary source of annual plastic emissions into the ocean (Meijer et al., 2015). The rampant plastic waste severely affecting the ecosystems highlights the urgent need for sustainable alternatives to synthetic plastics.

Developing biodegradable films using biopolymers has emerged as a viable solution to the environmental challenges posed by traditional plastic packaging. These environmentally friendly materials break down into simpler molecules through biological processes, thus presenting options for different medical, packaging, and agriculture applications due to their biocompatibility and biodegradability (Mapossa, 2023; Chandra & Rustgi, 1998). Among the various natural sources of polymers, *Kappaphycus alvarezii*, a red seaweed extensively cultivated due to its economic value and readily managed production methods (Bindu & Levine, 2010; Rupert et al., 2022), emerges as a promising source for biodegradable packaging materials. This potential is primarily due to the film-forming characteristics of its carrageenan content (Tye et al., 2018), which is beneficial given the increasing demand for sustainable alternatives to traditional plastics. Moreover, its ranking as a leading aquaculture commodity (Irawati & Affandi, 2024) indicates cost-effectiveness and availability, positioning *K. alvarezii* as an appealing choice for large-scale bioplastic production.

In addition to carrageenan's economic benefits, it also has functional properties that aid in bioplastic production. It provides a strong and flexible base for film formation (Rhein-Knudsen et al., 2015) and possesses antimicrobial properties that can extend the shelf life of packed products (Rizwan et al., 2018). Carrageenan demonstrates high versatility with applications ranging from pharmaceuticals to food, cosmetics, printing, textiles, and packaging (Rupert et al., 2022). Given its natural abundance and ease of production, *K. alvarezii* appears to be a sustainable alternative to conventional plastics.

Similarly, plasticizers are essential in developing biofilms, as they improve flexibility and process them quickly. Plasticizers commonly used in plastic production contain phthalates, a toxic substance that negatively affects human health and the environment (Hauser & Calafat, 2005). This concern leads to a demand for healthy and eco-friendly substitutes. Aloe vera gel, known for its medicinal properties, emerges as a suitable material in biopolymer research. Its abundant components of polysaccharides, vitamins, enzymes, and amino acids indicate that it may have some inherent plasticizing effects that enhance the flexibility and mechanical strength of the films (Hamman, 2008). Several studies assessed the use of aloe vera gel as a plasticizer and crosslinker in biodegradable films. Results show its ability to enhance the biodegradable films' moisture content, mechanical strength, and gas barrier properties (Hadi et al., 2022; Tambe, 2024). Aside from plasticization, it also contains film-forming properties, antimicrobial, and biodegradability, making it a good natural preservative coating for food products (Berihu & Zegeye, 2022; Misir et al., 2014). These biochemical properties make aloe vera gel an interesting material for developing a seaweed-based film with enhanced qualities.

While both *K. alvarezii* and aloe vera gel show promise as viable materials, further research is necessary to explore the potential of combining these materials in film formulation. This approach could use the beneficial features of each component to complement the other to create films with enhanced properties. Such research could help develop sustainable packaging materials, which offer a solution to the increasing need for eco-friendly alternatives in different industries.

Objectives of the Study

This research aims to investigate the potential of kappa-carrageenan combined with aloe vera gel as a plasticizer in developing seaweed-based films. Specifically, the study aims to:

1. Formulate seaweed-based films from *K. alvarezii* and aloe vera gel from various concentrations and evaluate the properties of the developed film, such as:
    a. physical (water solubility)
    b. mechanical (tensile strength and elongation at break)
    c. biodegradability
2. Determine the microbial load through the aerobic plate count of the best-formulated film.
3. Conduct a simple cost analysis.

Significance of the Study

This study is very significant and is expected to provide benefits to the following individuals/ groups, private and governmental entities, and institutions:

General Public. The development of biodegradable films from *K. alvarezii* with aloe vera gel as a plasticizer would offer benefits by reducing plastic waste and environmental pollution, thereby promoting cleaner and healthier communities. These films provide a safer alternative to conventional plastics, minimizing exposure to harmful chemicals associated with traditional plastic production and use.

Fisheries Industry. This research investigates the utilization of locally sourced *K. alvarezii* for producing biodegradable films, thereby offering a multifaceted contribution to the fishing industry. By demonstrating the potential for sustainable economic development through the diversification of marine-based industries and the promotion of eco-friendly practices, the study aligns with the mandates of the Bureau of Fisheries and Aquatic Resources (BFAR). Furthermore, it advances the Department of Science and Technology's (DOST) objectives by developing sustainable materials from indigenous resources, fostering innovation in product and technology development, and serving as a model for collaborative research that exemplifies the application of scientific principles for sustainable development within the sector.

Plastic Industry. This study can lead to the production of eco-friendly packaging materials that meet industry standards while reducing the ecological footprint. Adopting these biodegradable films can help the plastic industry transition towards more sustainable practices, enhance corporate social responsibility, and meet increasing consumer demand for environmentally friendly products.

Students and Future Researchers. This study would offer valuable insights and methodologies that can be adapted for other biopolymer combinations, encouraging further innovation in the field of sustainable materials.

Science and Body of Knowledge. This study would contribute to the scientific understanding of the interactions between kappa-carrageenan and aloe vera gel, thereby furthering the scientific understanding of natural plasticizers within biodegradable films.

Scope and Delimitation

This research centered on the development of seaweed-based films utilizing kappa-carrageenan sourced from *K. alvarezii* and aloe vera gel as a plasticizer. The investigation encompassed the formulation of these films and a comprehensive evaluation of their physical (water solubility), mechanical properties (tensile strength and elongation at break), and biodegradability. Furthermore, the determination of the microbial load of the most promising film formulation was assessed. A simple cost analysis was also performed. This study was confined to using *K. alvarezii* as the sole source of carrageenan and aloe vera gel as the only plasticizer, excluding other potential seaweed varieties or plasticizer options. While the research explored the potential of these films for packaging applications, actual testing in products was not conducted. The research timeframe was limited to six months, restricting the long-term assessment of film properties, stability, and performance under extended storage or use conditions. Moreover, the study was conducted on a laboratory scale, focusing on small-batch film production.

Definition of Terms

Alkali-treated Cottonii Chips (ATCC). A treated seaweed with an alkali solution, predominantly potassium hydroxide (KOH), to optimize the extraction of carrageenan by breaking down cell wall structures and facilitating the release of soluble polysaccharides (Masud et al., 2019; Nurmiah et al., 2013). In this study, it is the primary ingredient for developing the seaweed-based film.

Aloe Vera Gel (AVG). Derived from the succulent leaves of the Aloe vera plant (Aloe barbadensis Miller), a viscous substance characterized by its high-water content, comprising approximately 99% water and 1% solid components, including various minerals and bioactive compounds (Gentscheva et al., 2022). In this study, AVG is used as a natural plasticizer in the development of seaweed-based films.

Biodegradable Film. A type of film made with materials that can be broken down into smaller substances by microorganisms under natural environmental conditions (Shah et al., 2008). In this study, it is the seaweed-based film produced using kappa-carrageenan from *K. alvarezii* with aloe vera gel plasticizer.

Carrageenan. A family of linear sulfated polysaccharides extracted from red seaweeds, known for their gelling, thickening, and stabilizing properties in various applications (Necas & Bartosikova, 2013). In this study, it is the semi-refined kappa-carrageenan extracted from *K. alvarezii*, used as the primary film-forming material.

Elongation at Break. The maximum percentage increase in length a material can withstand before rupturing under tensile stress (ASTM D882-12, 2012). In this study, it is the percentage change in length of the film sample at the point of breakage during tensile testing using a Universal Testing Machine (UTM).

Kappaphycus alvarezii. A species of red seaweed widely cultivated for its high carrageenan content, valued for its commercial applications in food, pharmaceutical, and cosmetic industries (Bixler & Porse, 2011). In this study, it is the specific red seaweed species used as the source of kappa-carrageenan for seaweed-based film production.

Mechanical Properties. The characteristics of a material that describe its behavior under applied forces, including its strength, stiffness, and deformation response (Callister & Rethwisch, 2010). In this study, it encompasses the tensile strength (resistance to breaking under tension) and elongation at break (maximum stretchability) of the film, measured using a UTM.

Plasticizer. A substance added to a material to improve its flexibility, workability, and reduce brittleness by decreasing intermolecular forces between polymer chains (Rahman & Brazel, 2004). In this study, it is aloe vera gel, incorporated into the carrageenan film formulation to enhance its flexibility and processability.

Seaweed-based films. Films made from seaweed-derived polysaccharides, such as carrageenan, alginate, and agar, combined with additional components to enhance strength and functionality (Abdul Khalil et al., 2017). In this study, they are evaluated in terms of solubility, mechanical properties, biodegradability, and microbial load to determine their potential as an alternative to traditional plastics.

Semi-refined carrageenan (SRC). A polysaccharide derived from red seaweeds, primarily *K. alvarezii*, where the seaweed is treated with an alkaline solution, typically potassium hydroxide (KOH) or sodium hydroxide (NaOH), followed by the extraction of carrageenan using hot water to remove a certain amount of fibrous materials and impurities (Yahaya et al., 2023). In this study, it is the process of dissolving the ATCC to further purify the carrageenan.

Tensile Strength. The maximum stress a material can withstand before fracturing when subjected to tensile loading (ASTM D882-00, 2000). In this study, it is the maximum force per unit area that the film can resist before breaking during tensile testing using a UTM.

CHAPTER 2

REVIEW OF RELATED LITERATURE AND STUDY

This chapter examines the existing literature and studies related to the proposed research. It summarizes the current state of knowledge in the field and establishes the conceptual and theoretical framework guiding this study.

Related Literature

The Use of Synthetic Plastic and Its Associated Issues. Synthetic plastics made from petrochemicals or biomass raise environmental concerns because of non-biodegradability, non-recyclability, and high carbon emissions (Favian, 2023; Gbadeyan et al., 2022; Maniglia et al., 2022). Long degradation periods of plastics can take decades or even centuries to break down, posing a sustainability issue (Li et al., 2021). The excessive use of synthetic polymers has led to land and water contamination, stressing the critical need for more efficient and sustainable use of these materials (Pathak & Bithel, 2017; Shanthi, 2024). Plastic waste from discarded food packaging is a known cause of environmental pollution (Ncube et al., 2020). The buildup of this waste, including microplastics, harms the ecosystem's health and biodiversity (Kibria et al., 2023; Pandey et al., 2023). Even the tiny particles in many personal care products are threatening because they can easily pass through our wastewater treatment systems (McMackin, 2024). The environmental effect of synthetic plastics shows more than the visible pollution; it also stresses major concerns, including resistance to decomposition, contribution to carbon emissions, and the risks they present to natural ecosystems.

In response to these issues, there is a growing interest in biodegradable alternatives to synthetic plastics. Biodegradable polymers, such as polyhydroxyalkanoates and chitosan-based materials, are being explored for various applications, including food packaging, to address the environmental concerns associated with traditional plastics (Kumar et al., 2020; Paulraj et al., 2019; Watcharakul et al., 2012). Among various synthetic films, biodegradable polymeric choices offer a possible solution to lessen environmental pollution (Islam et al., 2020). Developing composite films for food packaging using resources like cellulose nanofibers and carrageenan has gained recognition because of their good barrier qualities and environmental benefits (Ramadas, 2024; Shanmugam, 2023). The shift to renewable and sustainable materials for plastic production shows a hopeful path for food packaging in many industries.

Biodegradable Films as An Alternative to Synthetic Plastic. Biodegradable polymers, as defined by Saha et al. (2003), undergo complete degradation through the action of biological organisms. These polymers can be synthetic or natural organic compounds that break down with the active involvement of microorganisms (Sander, 2019). Commonly used materials in biodegradable polymers include thermoplastic polyesters like poly(L-lactide-co-glycolide) (PLGA) for drug delivery systems and medical implants, and poly(4-hydroxybutyrate) (P4HB) for disposable products (Bettinger & Bao, 2010). Among these, bacterially-synthesized poly(3-hydroxybutyrate) (PHB) is extensively studied and has been recognized as a prominent biodegradable polymer (Lee & Yang, 2016). Therefore, biopolymers, whether synthetic or natural, undergo complete degradation through biological organisms and are utilized in various applications such as drug delivery systems, medical implants, and disposable products.

Developing biodegradable plastics often involves blending traditional plastic formulations like polyethylene and polystyrene with natural polymers such as cellulose or starch, known for their biodegradability (Rivard et al., 1992). The biodegradability of these polymers can be influenced by the presence of hydrolyzable groups in comonomers, which enhance biodegradability, while aromatic structures or rigid groups in comonomers may reduce biodegradability (Kijchavengkul & Auras, 2008). Furthermore, microorganisms can break down biodegradable plastics into smaller molecules like water, carbon dioxide, and methane (Kim et al., 2023). Using composites and blends is common, where natural polymers like starch, cellulose, and proteins are readily biodegradable through enzymatic hydrolysis and oxidation (Schimmel et al., n.d.). Additionally, incorporating environmentally friendly parameters to assess biodegradability emphasizes the importance of these materials breaking down into harmless components like carbon dioxide and water in natural environments (Guo et al., 2012). This aligns with the broader goal of creating biodegradable polymers that contribute to a free microplastic environment (Agarwal, 2020). In conclusion, this development of bioplastics with the blending of traditional natural polymers enhances environmental sustainability by ensuring these materials break down into harmless components like carbon dioxide and water.

Research has extensively explored the use of various biopolymers, including protein, cellulose, chitosan, starch, and lipids, for producing biodegradable films, highlighting the versatility of materials in sustainable packaging solutions (Go & Song, 2019; Wang et al., 2017). Starch, in particular, has gained attention as a promising candidate, with both native and chemically modified forms being studied to enhance film properties (Fonseca et al., 2016; Onyeaka et al., 2022). Similarly, proteins from sources such as gelatin, soy protein isolates, fish myofibrillar protein, whey protein concentrate, and even unconventional sources like black soldier fly prepupae proteins have been investigated for their potential in biodegradable film production, focusing on their mechanical, physical, chemical, thermal, and barrier properties (Kaewprachu et al., 2015; Setti et al., 2020). Additionally, the role of lipids in biofilm formation and resistance has been emphasized, with studies showcasing lipid-based materials as effective in combating biofouling, such as the lipid-hydrogel-nanostructure hybrid introduced by Park et al. (2018) and the surfactant glycerol monolaurate identified by Hess et al. (2015). The significance of lipids in biofilm studies, including those involving *Candida albicans* and *Legionella* species, further underscores their role in cell attachment and biofilm formation (Alim et al., 2018; Kusić et al., 2015). By leveraging natural and synthetic materials that can degrade through biological processes, these biopolymers offer a sustainable solution to reduce plastic waste accumulation.

The Seaweed Kappaphycus. As one of the top products in the country, seaweed is significant for the aquaculture industry in the Philippines. According to the Bureau of Fisheries and Aquatic Resources (BFAR) in 2020, seaweeds accounted for the majority of aquaculture production, representing 64.15% of the total output (Andriesse & Lee, 2021). Production value for seaweed in the Philippines was estimated at Php10.9 billion in the year 2018, which was equivalent to US$215 million, a significant input in the aquaculture industry (Andriesse, 2022). It ranks fourth for seaweed production, following China, Indonesia, and the Republic of Korea worldwide (Tahiluddin & Terzi , 2021). It takes a leading position in Philippine aquaculture and is a significant economic contributor.

The Philippines' seaweed aquaculture industry employs more than 116,000 families across 58,000 hectares of cultivation areas (Mahalik & Kim, 2014). Cultivating seaweed, particularly *K. alvarezii*, has been a long-standing practice in the Philippines since the 1960s (Alibon et al., 2019). Seaweed farming not only contributes to the economy but also plays a role in climate change mitigation by absorbing CO2 through photosynthesis (Erlania & Radiarta, 2014). Additionally, seaweed aquaculture provides ecosystem services by dampening wave energy, protecting shorelines, and improving water quality by elevating pH levels and supplying oxygen (Duarte et al., 2017; Kim et al., 2017). The growth of seaweed aquaculture is seen as a promising avenue for economic development, with high economic value and market opportunities both domestically and for export (Pratama et al., 2021).

Furthermore, the industry has the potential to support the blue economy, representing a sustainable and economically viable sector (Mendes, 2024). Seaweed cultivation also offers environmental benefits, such as carbon sequestration through carbon absorption from the environment (Radiarta, 2015). As the aquaculture sector continues to expand globally, seaweed aquaculture stands out as one of the fastest-growing segments, with an annual growth rate of 8% (Froehlich et al., 2019). Its cultivation supports livelihoods and offers environmental benefits and opportunities for sustainable development in the aquaculture sector.

The emphasis on sustainable materials has progressively included marine resources, and one of them focused on *K. alvarezii*, a red macroalgal species native to Southeast Asia (Hayashi et al., 2017; Mantri et al., 2024). *K. alvarezii*, commonly known as *Cottonii*, has many applications and properties that make it important (Jalal et al., 2023). Botanically, *Eucheuma denticulatum* or *Kappaphycus alvarezii* is a red seaweed extracting κ-carrageenan (Yahaya, 2023). This seaweed species has been widely studied for various uses, including its potential in sustainable packaging applications. Research has shown that *K. alvarezii* is suitable for developing biodegradable films and packaging materials

(Sathiamurthy, 2024). Its diverse application as a sustainable material has increasingly encompassed marine resources.

They are primarily cultivated through aquaculture practices, often involving a long line system in coastal areas, such as Gerupuk Bay in West Nusa Tenggara, Indonesia (Erlania & Radiarta, 2014). The cultivation period of *K. alvarezii* requires suitable environmental conditions, including appropriate water temperature, salinity levels, and nutrient availability (Hayashi et al., 2007). The species has been successfully cultivated in subtropical waters, such as regions like Indonesia, Philippines, Vietnam, and Malaysia – which is valued for its rich chemical composition, particularly its polysaccharides and other nutrients (Kumar et al., 2020; Rupert et al., 2022). Harvesting of *K. alvarezii* involves manually collecting mature seaweed from cultivation sites. The species is typically harvested after a specific growth period to ensure optimal carrageenan content and quality. Studies have focused on the growth characteristics and biochemical composition of different strains of *K. alvarezii* to determine the most suitable harvesting time for maximum yield (Narvarte et al., 2022). The harvested seaweed is then processed to extract carrageenan, a valuable phycocolloid used in various industries. These make the *K. alvarezii* known for its high growth rate and carrageenan content, making it a valuable resource for multiple applications.

Carrageenan is a hydrophilic polysaccharide exists in different forms like κ-carrageenan, ι-carrageenan, and λ-carrageenan, categorized based on the number of sulfate groups in the disaccharide repeating unit of the galactose/anhydrogalactose chain (Wang et al., 2016). Studies have highlighted the diverse biological properties of carrageenan, such as its antioxidant activity, antimicrobial properties, and potential as a drug delivery system (Pacheco-Quito et al., 2020). Carrageenan has also been utilized in various applications, including food packaging, wound dressing films, and as a stabilizer in dairy-based products (Ajithkumar, 2017; SRIPHOCHAI, 2024).

It has been utilized in the development of biodegradable films that show promising characteristics as packaging material, where its polysaccharides serve as a base matrix for applications in plasticulture and as an alternative to synthetic compounds (Khalil et al., 2018; Khalil et al., 2019; Kocira et al., 2021). One of the key properties relevant to film formation is its mechanical properties. Studies have focused on enhancing the mechanical strength of biopolymeric films derived from *K. alvarezii* by incorporating fillers such as calcium carbonate (CaCO3) to improve their properties for plasticulture applications (Khalil et al., 2018). Additionally, it has been investigated for its potential to produce biodegradable films with desirable physico-mechanical functions, highlighting its versatility in film development (Hasan et al., 2019). Research has shown that seaweed-based bioplastic sheets derived from *K. alvarezii* have high biodegradability, making them environmentally friendly options for food packaging applications (Sari, 2024). Also, it contains antioxidant properties and has the ability to inhibit bacterial growth. Research has demonstrated that carrageenan extracted from *K. alvarezii* exhibits antioxidant properties essential for sustainable packaging applications (Yahaya, 2023). Furthermore, seaweed has been found to possess antibacterial properties, showing bacteriostatic effects against various bacteria such as *Bacillus cereus*, *Streptococcus mutans*, *Staphylococcus aureus*, and *Salmonella typhimurium* (Hlila et al., 2017; Deepa et al., 2023). These antimicrobial properties make *K. alvarezii* a valuable resource for developing packaging materials with enhanced functionality.

Marine Algae Utilized in Biodegradable Film Production. The development of biodegradable packaging materials from marine biomaterials underscores the potential of algae in promoting sustainability (Sarkar, 2023). Comparative analysis among marine algae-based films highlights their superior qualities to those derived from *K. alvarezii*. For instance, a study by Agarwal et al. (2022) has compared the mechanical properties and biodegradability of films derived from *K. alvarezii* with those from *U. lactuca*, a green alga. The findings revealed that while both types of films exhibited excellent biodegradability, *K. alvarezii* films showed superior tensile strength and thermal stability.

The study of Zhang et al. (2020) evaluated the water vapor barrier characteristics of films fabricated from *K. alvarezii, S. muticum,* and *G. dura*. The findings reveal that *K. alvarezii* films have the best moisture barrier properties because they provide sufficient protection for packaging needs that require moisture protection. The research conducted by Kim et al. (2021) compared the environmental impact and biodegradability of film materials derived from *K. alvarezii* and *L. digitata.* The base films of *K. alvarezii* decompose faster in composting environments, suggesting they can reduce plastic waste better than other film types. Many researchers use marine algae to create eco-friendly, sustainable methods for packaging and material manufacturing. *K. alvarezii*-derived biodegradable films present outstanding performance capabilities because they demonstrate better mechanical strength, thermal stability, and moisture resistance than other marine algae-based films.

However, a study by Chia et al. (2020) claims that biodegradable films derived from *K. alvarezii* needed plasticizer agent(s) to improve their physicochemical properties due to their brittleness. Studies have shown that incorporating materials like alginate, polyethylene glycol (PEG), chitosan, cellulose nanocrystals, and polyvinyl alcohol into κ-carrageenan films can enhance their mechanical strength, water vapor barrier properties, and biodegradability (Ili Balqis *et al.,* 2017; Martiny *et al.*, 2020; Panatarani *et al.,* 2020;

Prasetyaningrum *et al.,* 2020). When combined with different plasticizers and additives, these films exhibit various physical, mechanical, and barrier properties that make them suitable for food packaging and other commercial uses. Also, adding plasticizers like PEG and chitosan has been found to improve the flexibility and water solubility of κ-carrageenan-based films (Panatarani *et al.,* 2020; Zarina & Ahmad, 2014). Incorporating plasticizers into biofilms enhances many of its properties, which are crucial in achieving a good quality of biodegradable films.

The Use of Plasticizers in Film Development. In the production of biodegradable films, the addition of plasticizers such as glycerol, sorbitol, and polyethylene glycol has been shown to reduce molecular chain interactions, lower melting temperatures, and improve the film-forming process (Lin & Tung, 2010). Plasticizers are essential additives that help overcome issues such as brittleness and improve handling, packaging, and patient compliance (Krull et al., 2016). It modifies the three-dimensional networks within the films, affecting properties like hydrophobicity, permeability, and mechanical strength (Díaz-Montes, 2022). The addition of plasticizers to film-forming materials like whey protein and xylan has been shown to favorably impact the mechanical properties of the resulting films, making them more flexible and easier to work with (Carvalho et al., 2020; McHugh & Krochta, 1994). Furthermore, the choice of plasticizer is critical as it directly influences the physical properties and stability of the films (Luangtana-anan et al., 2010).

Studies have evaluated the impact of various natural-based plasticizers, including dicarboxylic acid-based esters, acetyl tributyl citrate, and polyethylene glycol, on the biodegradation of films made from materials like polylactic acid (PLA) (Brdlik et al., 2022). Research has demonstrated that plasticizers like glycerol and sorbitol are commonly used in various film formulations to enhance properties such as tensile strength, elongation, and flexibility (Fulzele et al., 2002; Mikkonen et al., 2009; Zhang & Whistler, 2004). Furthermore, the incorporation of plasticizers like glycerol into biopolymer matrices, such as semi-refined carrageenan, has been shown to enhance the mechanical and barrier properties of the resulting edible and biodegradable packaging films (Hamid et al., 2019). Additionally, the type and concentration of plasticizer are key factors affecting the time required to achieve stable films with desired characteristics (Wurster et al., 2007). Plasticizers with specific characteristics, such as small size, high polarity, and more polar groups per molecule, have been shown to have a greater plasticizing effect on polymeric systems (Cheng et al., 2006). Moreover, incorporating antimicrobial bio-based carriers onto film surfaces demonstrates the diverse applications of plasticizer modifications in enhancing antimicrobial activity and reducing contamination risks (Huang et al., 2021). This demonstrates plasticizers' significant role in improving biodegradable films' processability and enhancing their functional properties for various applications, including food packaging.

The Aloe vera gel and its Properties. Aloe vera gel, a natural substance extracted from the leaves of *Aloe barbadensis Miller* or the aloe vera plant, is rich in bioactive 18 compounds such as anthraquinones, vitamins (including Vitamin C, B1, B2, B6, and niacin), minerals, enzymes, amino acids, and polysaccharides (Edwards et al., 2015; Elgegren et al., 2020; Janurianti et al., 2021; Rahman et al., 2017). It has been investigated as a potential additive for developing biodegradable films, offering a natural and sustainable alternative to traditional plasticizers (Maan et al., 2021). It is known for its diverse chemical composition, which contributes to its various medicinal properties. The primary chemical constituents of aloe vera include amino acids, anthraquinones, enzymes, minerals, vitamins, lignins, monosaccharides, polysaccharides, salicylic acid, saponins, and phytosterols (Choudhary et al., 2011). It contains over 110 potentially active substances falling into categories such as flavonoids, phenylpropanoids, coumarins, chromones, and their glycoside derivatives, anthraquinones and their glycoside derivatives, phenylephrine and its phenol derivatives, phytosterols, and others (Satruhan & Patel, 2022). The plant is reported to contain as many as 75 nutrients and nearly 200 distinct physiologically active components (Shah, 2024). It has been found to contain various carbohydrate polymers, notably glucomannans, along with a range of other organic and inorganic components (Kojo & Qian, 2004). The diverse chemical composition of aloe vera, including amino acids, enzymes, and polysaccharides, can contribute significantly to its numerous medicinal properties.

The gel of aloe vera, commonly used in various applications, contains many components with varying chemical composition and biological activity (Langmead et al., 2004). The phytochemical analysis of aloe vera gel has revealed compounds such as polysaccharides, steroids, organic acids, antibiotic agents, amino acids, and minerals, contributing to its skin-soothing and cell-protecting effects (Akinola et al., 2021). The bioactive components of aloe vera, such as anthraquinones and saponins, have been studied for their effects on tissue engineering applications, showing antibacterial, anti-inflammatory, antioxidant, and immune-modulatory effects that promote tissue regeneration and growth (Rahman et al., 2017). Aloe vera products have been found to contain multiple constituents with potential biological activities, although the active components are still not fully defined (Boudreau & Beland, 2006). Its chemical composition is rich and diverse, encompassing a wide array of compounds that contribute to its various medicinal properties and potential health benefits.

Aloe vera gel has been extensively studied for its antimicrobial properties, showcasing effectiveness against a variety of pathogens. Quaye et al. (2023) demonstrated that aloe vera gel exhibits antimicrobial properties against pathogenic bacteria like *Staphylococcus aureus* and *Escherichia coli*. This finding is further supported by (Sajadi & Bahramian, 2015), who highlighted that aloe vera gel contains a combination of carbohydrates, glycoproteins, vitamins, and minerals that contribute to its antimicrobial, antifungal, and antioxidant properties. Moreover, Mosaad (2021) suggested that aloe vera's antifungal, antibacterial, and antimicrobial properties make it a viable alternative to synthetic antimicrobial agents. These studies collectively emphasize the broad spectrum of antimicrobial activity possessed by Aloe vera gel. It makes a versatile natural ingredient and can inhibit the growth of pathogens and prevent biofilm formation which underscores its importance as a natural antimicrobial agent.

Driven by the surge in demand for healthy food products, the potential of various herbal compounds as base materials for edible films and coatings is being actively studied (Maan et al., 2021). Due to its non-toxicity and eco-friendly characteristics at levels used for food protection, Aloe vera is considered a promising, sustainable alternative to synthetic chemical preservatives, antioxidants, and antimicrobial agents (Kumar et al., 20 2022). Its potential use as an additive for developing biodegradable films offers a natural and sustainable alternative to traditional plasticizers (Maan et al., 2021). Its physicochemical properties are essential for its application in film formation. Research has demonstrated that aloe vera gel can act as a plasticizer in biodegradable films, improving their thermal stability and antibacterial activity (Sherani, 2022; Tambe, 2024). These aloe vera gel-infused edible films demonstrate high transparency with minimal reduction in transmittance, structural integrity, antimicrobial properties, and suitability for printing, indicating their potential for food packaging applications (Birtane & Çiğil 2022). Due to its film-forming abilities, antimicrobial actions, and biodegradability, aloe vera gel is suitable for producing edible coatings that can enhance the quality and shelf-life of various food products (Berihu & Zegeye, 2022; Sharmin et al., 2015). The qualities and attributes of aloe vera show potential in the packaging industry, which offers biodegradability, antibacterial properties, and the ability to enhance the shelf life of food products.

Related Studies

Some studies, like the one from Young & Byon (2022), demonstrated the exceptional biodegradability of films produced from *G. lichenoides* and *S. horneri*, outperforming other biodegradable options. Their research highlighted the excellent biodegradability of the developed seaweed-based film, surpassing other biodegradable films in terms of biodegradation rates. Farghali et al. (2022) expanded on this potential by investigating the creation of bioplastics from seaweed-derived alginate. They found that films produced using alginate powder from seaweeds and sorbitol treated with calcium chloride exhibited favorable bioplastic film characteristics.

Khalil et al. (2019 and 2017) further explored the versatility of seaweed-derived polymers like alginate, carrageenan, and agar in film production. Their work highlighted the potential of *K. alvarezii* for producing films with substantial mechanical strength and functional properties suitable for various industrial applications. Additionally, they demonstrated the enhancement of film properties by incorporating nanofillers like oil palm shell particles. To address the challenge of microbial contamination, Kumar et al. (2020) integrated neem leaf extract into seaweed-based biopolymer films, improving their hydrophilic nature and antimicrobial properties. By incorporating neem leaf extract into the seaweed matrix, the researchers observed improvements in the hydrophilic nature of the films, leading to enhanced contact angles. Collectively, these studies underscore the potential of seaweed as a sustainable resource for developing biodegradable and functional packaging materials. Future research can focus on optimizing film properties, exploring a wider range of seaweed species, and developing large-scale production processes to facilitate commercialization.

Exploring sustainable packaging solutions extends beyond seaweed, with various plant-based sources also showing promise in developing biodegradable films. Taro starch, for instance, has been investigated for its potential in creating biodegradable films and its application in specific food products, highlighting the versatility of plant-based resources and the possibility of tailoring films to specific packaging needs (Callada & Velilla, 2019). Similarly, the feasibility of using arrowroot starch for producing edible films and their sensory and physicochemical properties has been studied, emphasizing the importance of characterizing film properties and the potential of starch-based materials for edible packaging (Bobis et. al., 2023). Furthermore, the use of sweet potato starch in creating edible films and their suitability for packaging selected foods has been explored, demonstrating the potential for expanding the range of food products that can benefit from biodegradable packaging (Armando & Bonagua, 2022). Barbadillo and Paderon (2022) extracted starch from banana peels and incorporated it into a film, which was then evaluated for its characteristics and potential application in wrapping various food products. The positive findings in terms of consumer acceptability and economic feasibility further highlight the promising role of agricultural byproducts in creating sustainable packaging solutions.

These studies, encompassing both seaweed and plant-based materials, collectively contribute to the growing body of research on biodegradable film development and highlight the vast potential of natural resources for sustainable packaging solutions. Future research can focus on optimizing film properties, exploring a wider range of both seaweed and plant species, and developing large-scale production processes to facilitate commercialization.

Some studies, like those by Tambe (2024) and Berihu & Zegeye (2022), have demonstrated the effectiveness of aloe vera gel in reinforcing biodegradable starch-based blends for sustainable packaging applications. These findings highlight the role of aloe vera gel in improving the mechanical strength and quality of biodegradable films, showcasing its potential as a valuable ingredient in eco-friendly packaging solutions. Moreover, research by Hadi et al. (2022) has explored the development of sustainable food packaging films using alginate and aloe vera, emphasizing the biodegradability and eco-friendliness of films incorporating aloe vera gel. This study underscores the importance of utilizing natural ingredients to create environmentally friendly packaging materials that align with sustainable practices. Additionally, the work by Salman (2023) found that aloe vera gel can preserve food and extend shelf life due to its polysaccharide content, acting as a natural barrier against moisture and oxygen, which could translate to similar benefits when used as a plasticizer in various materials. In conclusion, aloe vera gel's properties make it a versatile and sustainable alternative to traditional plasticizers, with potential applications in food packaging, medicine, and materials science. It offers biodegradability, antimicrobial properties, film-forming abilities, and mechanical reinforcement to enhance product performance and sustainability.

Various methods and techniques are employed in synthesizing and characterizing biodegradable films. For film synthesis, standard techniques include casting, extrusion, and molding (Vonnie et al., 2022). These methods are crucial in creating films from biodegradable materials such as aloe vera, alginate, tapioca starch, and thermoplastic starch. Aloe vera gel is known for its film-forming properties and has been utilized in developing biodegradable films due to its antimicrobial and biochemical characteristics (Hadi et al., 2022). Similarly, alginate-based films reinforced with carboxymethyl cellulose and hydroxypropyl methylcellulose have been explored for their sustainability in food packaging applications (Othman et al., 2019). Therefore, the synthesis and characterization of biodegradable films involve various techniques and property assessments to ensure the development of sustainable packaging materials.

Characterization of these biodegradable films involves assessing various properties such as mechanical strength, barrier properties, and biodegradability. Mechanical properties like tensile strength and elongation are essential for determining the film's strength and flexibility (Todhanakasem et al., 2022). Biodegradability tests are also conducted to assess the film's environmental impact and degradation over time (Karim et al., 2022). Optimizing the properties of biodegradable films often involves studying the impact of different components. Studies have shown that incorporating additives like glycerol can improve the properties of edible films made from starch, enhancing their elongation and flexibility (Sim & Raj, 2019). Utilizing natural biopolymers and additives aims to optimize these films' mechanical, barrier, and biodegradable properties for a wide range of applications.

Gap Bridged by the Study

The review of related literature emphasizes the methods used in this study to identify the need for innovative processing techniques and interdisciplinary approaches that integrate insights from material science, environmental science, and economics. Biodegradable films have garnered significant attention as sustainable alternatives to traditional plastics (Rhim et al., 2006; Santos et al., 2021; Sudhakar et al., 2021). There is an abundance of studies that have demonstrated that *K. alvarezii* can be explored to create biodegradable films with notable biocompatibility and biodegradability (Chia et al., 2020; Preetha, 2021; Moey et al., 2015). However, a study by Kumar et al. (2020) examined the physiological responses of *K. alvarezii* to elevated temperatures, suggesting potential implications for its mechanical strength and barrier properties. Therefore, developing a plasticizer for biodegradable films is essential to address the current limitations in low mechanical and barrier properties of *K. alvarezii* and improve the overall sustainability and performance of these materials.

Research has explored various plasticizers such as glycerol, sorbitol, and acetyl tributyl citrate (ATBC Citroflex A4) (Brdlik et al., 2022; Díaz-Montes, 2022; Lin & Tung, 2010). However, there is a need to study natural plasticizers because current plastic pollution demands cost-effective, high-performance packaging solutions. Misir et al. (2014) investigated the incorporation of aloe vera gel in biodegradable films and found enhanced moisture retention and microbial management capabilities. Aloe vera gel coatings on fruits have also been studied for their antimicrobial properties in food preservation applications (Kahramanoğlu et al., 2019). Yet, there is limited existing research regarding the potential use of aloe vera gel as a plasticizer for biodegradable films produced from *K. alvarezii*. Combining Aloe vera gel with conventional biopolymers and lipids has demonstrated potential improvements in film and coating characteristics, including clarity, surface texture, strength, flexibility, moisture barrier effectiveness, and biological activity, with further research needed to overcome technological challenges for easily tunable properties (Maan et al., 2021). Research indicates that the combination of these materials holds promise since the aloe vera gel and traditional biopolymer emulsion show potential. Still, the researchers need to study the optimal blending ratios and processing methods (Maan et al., 2021). This research aims to evaluate the impact of adding aloe vera gel to *K. alvarezii*-based films to improve the mechanical and barrier properties of new eco-friendly packaging materials and address the knowledge gap in the literature.

Synthesis of the State–of–the–Art

Recent advancements in biodegradable film technology have led to a surge in research focusing on creating environmentally friendly packaging materials. The transition towards biodegradable films from conventional non-biodegradable plastics is primarily motivated by environmental concerns (Ramírez-Brewer et al., 2021). Researchers are actively investigating the use of agro-industrial wastes and renewable agricultural materials 26 to develop biodegradable films, aligning with consumer preferences for eco-friendly packaging solutions (Choudhary et al., 2022). Seaweed-based films have emerged as a promising alternative, demonstrating superior biodegradability compared to traditional options like polybutylene succinate (Young & Byon, 2022). Incorporating seaweed, such as *K. alvarezii*, along with additives like aloe vera gel as a plasticizer, offers an opportunity to enhance the properties of biodegradable films.

The global plastic pollution crisis necessitates sustainable alternatives, with biodegradable films emerging as a promising solution, particularly those derived from natural materials like *K. alvarezii* and aloe vera gel (Imre & Pukánszky, 2013; Tharanathan, 2003). Understanding the use of natural-based plasticizers in many biodegradable films is crucial to achieve a cost-effective alternative in improving their physicochemical properties. Current research indicates that aloe vera gel has been utilized to enhance film flexibility and bio-functionality, though achieving the desired mechanical strength and uniformity (Maan et al., 2021). Also, *K. alvarezii*-based films exhibit good biocompatibility and biodegradability but often lack sufficient mechanical strength and barrier properties (Rhein-Knudsen et al., 2015). For these reasons, experts have extensively worked on modifying and enhancing these biopolymers by blending or grafting them with other polymers or incorporating fillers to improve their properties and make them more competitive with conventional polymers (Khalil et al., 2017; Khalil et al., 2017).

Evaluating key properties such as mechanical strength, barrier properties, and biodegradability is essential for development (Liu et al., 2017). Moreover, cost-effective and scalable production techniques are necessary for commercial viability (Rhim & Ng, 2007). Incorporating advancements in composite film structures (Subramanian*, 2019) and nanocomposite technology (Wang & Gunasekaran, 2009) can significantly enhance film properties. Understanding the role of fillers (Hasan et al., 2019) and the film's rheological behavior (Ramírez-Brewer et al., 2021) is crucial for tailoring the material to specific packaging requirements.

To extend the shelf life of packaged food, the film must possess antimicrobial and antioxidant properties. Integrating substances like neem leaf extract (Kumar et al., 2020) or green tea and palm oil extracts (Perazzo et al., 2014) into the film matrix can provide these benefits. Additionally, studying the biodegradation kinetics (Hidayati et al., 2021) of the developed film is essential for assessing its environmental impact and optimizing its performance for specific packaging applications.

By leveraging insights from recent research on biodegradable films and incorporating innovative elements into the study design, there is potential to create a biodegradable film with enhanced properties suitable for diverse packaging applications. This approach aligns with current trends and innovations in biodegradable film development, ensuring the study is at the forefront of this evolving field.

Theoretical Framework

Developing biodegradable films from natural sources like *K. alvarezii* and aloe vera gel addresses the critical issue of plastic pollution. These films offer a sustainable alternative to conventional plastics, contributing to environmental conservation (Freile-Pelegrín & Madera-Santana, 2017). Utilizing abundant natural resources like seaweed and aloe vera for bioplastic production can boost local economies, particularly in regions where these resources are readily available. This can lead to new market opportunities and economic growth (Freile-Pelegrín & Madera-Santana et al., 2015). The framework integrates polymer science principles with material science and environmental sustainability to evaluate the effects of different carrageenan and aloe vera gel plasticization concentrations on *K. alvarezii* biodegradable film properties.

Polymer Science Theory. Carrageenan is a natural polysaccharide derived from red seaweed, particularly *K. alvarezii*, known for its gelling, thickening, and stabilizing properties. In polymer science, the film-forming ability of polysaccharides like carrageenan is attributed to their molecular structure, which allows for the formation of hydrogen bonds and cross-linking, creating a cohesive film matrix (Campo et al., 2009). The theoretical understanding of carrageenan's role as a film-forming agent underpins its selection as a base material for biodegradable films.

The addition of plasticizers is a common practice to enhance the flexibility and processability of polymer films. The plasticization mechanism involves the plasticizer molecules intercalating between polymer chains, reducing intermolecular forces and increasing chain mobility. This results in improved mechanical properties such as increased flexibility and reduced brittleness (Lim & Hoag, 2013). Aloe vera gel, with its high polysaccharide content, acts as a natural plasticizer, and its effect on carrageenan films can be understood through this theoretical lens.

Material Science Theory. The mechanical properties of biodegradable films, such as tensile strength, elongation at break, and Young's modulus, are critical for their practical applications. According to material science theories, the mechanical properties of polymer films can be significantly influenced by the type and concentration of plasticizers used. The introduction of aloe vera gel is expected to enhance these properties by providing additional flexibility and improving the overall mechanical performance of the films (Nur Hanani et al., 2014).

Environmental Sustainability Theory. The environmental sustainability theory emphasizes the importance of developing materials that are not only effective but also environmentally friendly. Biodegradable films made from natural polymers like carrageenan are expected to degrade into non-toxic by-products, reducing their environmental footprint (Siracusa et al., 2008). The integration of aloe vera gel, a natural and renewable resource, aligns with the principles of environmental sustainability, further enhancing the biodegradability and eco-friendliness of the films.

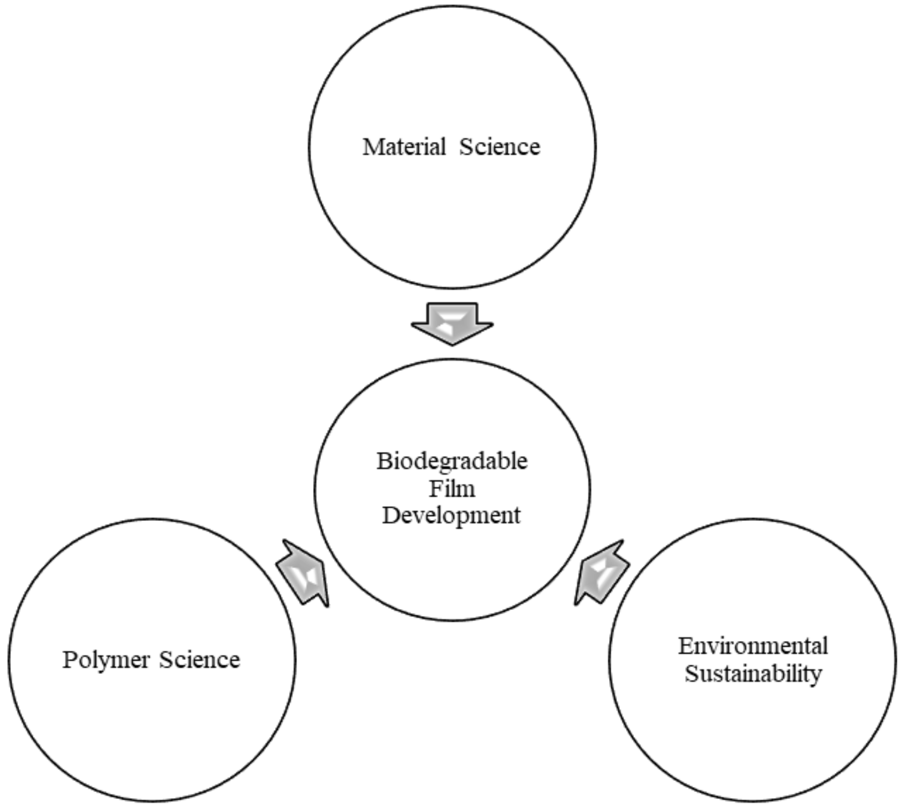

Figure 1. Theoretical framework on developing Biodegradable films using *K. alvarezii* with Aloe vera gel

Conceptual Framework

The development of films through *K. alvarezii* and aloe vera gel follows an input-process-output framework. The model demonstrates how different variables and development stages connect, thus creating an organized path from starting inputs to final outputs.

The independent variables of this study consist of natural polymer sources, which include *K. alvarezii* and aloe vera gel. The film-forming properties of *K. alvarezii* work effectively because it possesses superior gelling and thickening abilities and stabilizing characteristics. The aloe vera gel functions as a natural plasticizer, enhancing mechanical properties.

The film production process requires mixing carrageenan with aloe vera gel, followed by adjusting their concentrations to reach optimal film characteristics. The research process includes testing and analysis of the film's physical properties (solubility), mechanical properties (tensile strength and elongation at break), biodegradability and antimicrobial properties.

The study's dependent variables or outputs consist of film properties that achieve improved mechanical properties through optimized concentrations of carrageenan and aloe vera gel. The films are expected to demonstrate improved antimicrobial characteristics, which will make them appropriate for packaging applications while providing a natural, non-toxic substitute for synthetic packaging materials. The films are also expected to have positive environmental characteristics by breaking down into harmless byproducts that support sustainability.

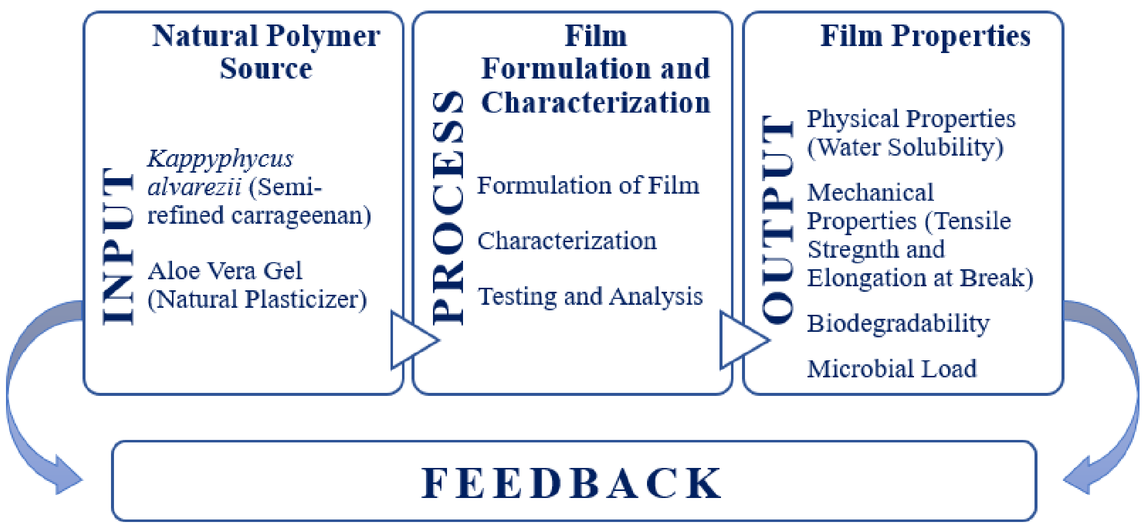

Figure 2. Conceptual framework on developing biodegradable films using *K. alvarezii* with aloe vera gel

CHAPTER 3

RESEARCH DESIGN AND METHODOLOGY

This chapter details the methodologies and approaches necessary to develop and test the properties of the seaweed-based film. Such approaches include the preparation, testing, and analysis of the different properties of the developed films.

Research Design

This study employs an experimental research design to evaluate the effects of varying concentrations of aloe vera gel (AVG) plasticizer on the physical properties (water solubility), mechanical properties (tensile strength and elongation at break), biodegradability, and microbial load of *K. alvarezii*-based films. Glycerol was utilized as a control plasticizer for comparative analysis.

Preparation of seaweed-based film

a. Materials

The Alkali-treated Cotonii Chips (ATCC), a form of semi-refined carrageenan (SRC), were sourced from the Bureau of Fisheries and Aquatic Resources—National Seaweeds Technology Development Center (BFAR-NSTDC) in Cabid-an, Sorsogon. Fresh aloe vera leaves were purchased from Tabaco City. Glycerol and analytical-grade reagents were purchased from reputable sources.

b. Preparation of aloe vera gel (AVG)

The extraction of AVG followed a procedure from Maan et al. (2021). Aloe vera leaves were selected as mature and undamaged, with uniform sizes measuring 55-80 cm in length. They were washed with tap water and then rinsed with distilled water or a mild chlorine solution as a disinfectant. One inch was trimmed from the base of each leaf, and they were immersed vertically in distilled water for 1 hour to facilitate aloin removal. This is essential as it is a compound with laxative properties and a strong, bitter taste that may not be suitable for all applications, particularly in food and cosmetics (Wang et. al 2017). However, this step may be optional depending on the intended use of the film. After which, the leaves were prepared by cutting off the tapering tip (top 2-4 inches) and removing sharp spines along the margins. One side of the outer leaf layer was carefully peeled away to extract the gel matrix using a stainless-steel spoon. The extracted gel was homogenized in a blender for approximately 3 minutes, then filtered through cheesecloth to remove impurities and obtain pure aloe vera gel. Finally, the filtered gel was transferred to a glass jar and refrigerated for preservation (Figure 3).

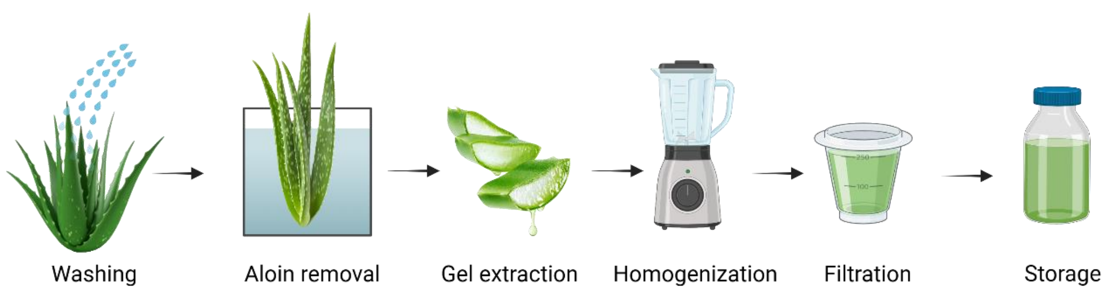

Figure 3. Schematic diagram of aloe vera gel extraction

c. Preparation of film treatments

The solution was prepared following a slightly modified method based on Farhan and Hani (2017). The ATCC was dissolved in distilled water (2% w/w), heated to 90°C, and continuously stirred using a hot plate magnetic stirrer for 30 minutes to ensure adequate dissolution. Any volume loss due to evaporation was adjusted with distilled water to return the final volume to 200 mL. The resulting solution was then filtered through cheesecloth to remove undissolved impurities. AVG was subsequently added to the filtered solution at varying concentrations (10%, 20%, and 30%, w/w based on the dry weight of ATCC) (detailed composition in Table 1). The solution was stirred for an additional 2 minutes to ensure homogeneity. 150 mL of the resulting solution was poured onto a casting plate and dried in an air oven (Biobase Forced Air Drying Oven BOV-V30F) at 60°C for 4 hours. After drying, the films were carefully peeled from the casting plates. For comparison, the control κ-carrageenan films were prepared similarly but with glycerol as a controlled plasticizer. All experiments were carried out in triplicates.

Table 1. Composition of seaweed-based film treatments using Alkali-treated Cotonii Chips (ATCC) with varying plasticizers.

Treatments	Composition
Control	4g ATCC + 20% glycerol
Treatment 1	4g ATCC + 10% AVG
Treatment 2	4g ATCC + 20% AVG
Treatment 3	4g ATCC + 30% AVG

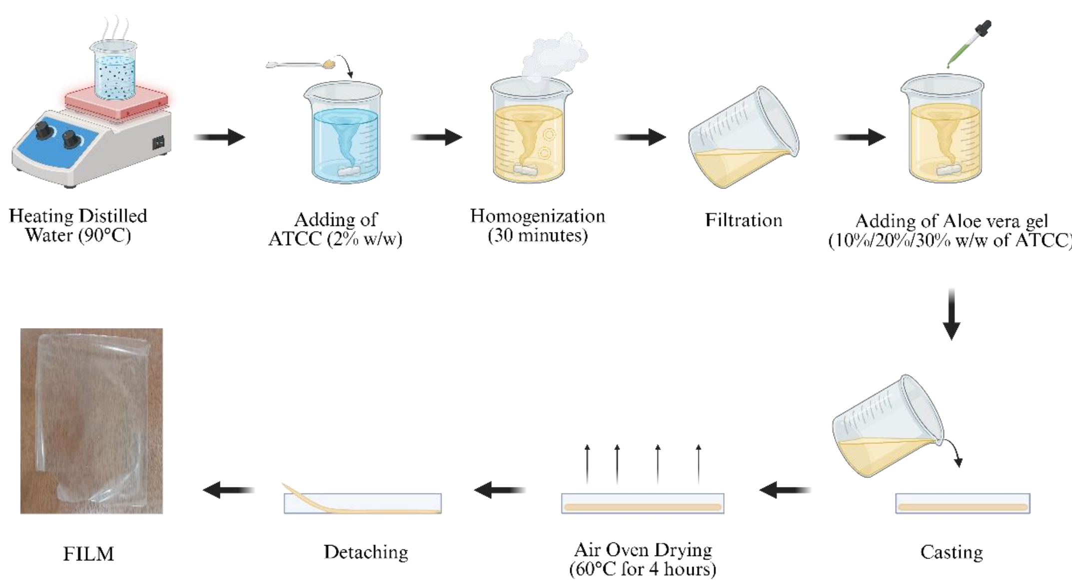

Figure 4. Schematic diagram of *K. alvarezii* and aloe vera gel incorporation into the seaweed-based film

Determination of film properties

a. Solubility in Water

Water solubility was determined using the method described by Romero-Bastida et al. (2005), with few modifications. The film was cut into 2 cm^2 samples, dehydrated in an oven at 60°C for 1 hour to establish the initial dry weight (s_i). Subsequently, each dehydrated sample was immersed in 50 ml distilled water at room temperature for 4 hours with periodic agitation. After immersion, the samples were filtered using a mesh to separate the immersed film. The pieces of film were then taken out and dried again in an air oven at 60°C for 3 hours to determine the final dry weight (S_f). Water solubility, expressed as the percentage weight loss of the film after immersion, was then calculated using the formula:

$$\text{Solubility (\%)} = [(S_i - S_f) / S_i] \times 100$$

b. Tensile Strength and Elongation at Break

Tensile strength and elongation at break were determined using a Universal Testing Machine (UTM) extensometer operated in accordance with the standard method ASTM D882. The prepared film samples were sent to the Materials R&D Consulting Facility at the University of the Philippines Diliman for testing.

c. Biodegradability

For the biodegradability test, soil and burial tests were conducted based on the methods of Sari et al. (2024) with a few modifications. The films were cut into 2 x 3 cm strips and were buried in 10 cm diameter plastic pots. Each pot contains 300 g of damp soil and sand. The samples were retrieved every 4 days, cleaned with tissue to remove excess debris, dried in an oven at 60°C for 1 hour, and then weighed. The weight loss percentage (WL%) was calculated using the formula:

$$WL\% = ((W_i - W_f) / Wi) \times 100$$

where W_i is the film's initial weight, W_f is its weight after degradation.

d. Microbial Load

The microbial load was analyzed through aerobic plate count using the Compact Dry TC (total count) – AOAC 010404 method from the film with the best mechanical properties to indicate bacterial growth. The film samples were sent to the Bicol University Regional Center for Food Safety and Quality Assurance (BURCFSQA) for testing.

Following the standard method, the film samples were cut into thin strips and diluted at a ratio of 1:10 with distilled water. After dilution, the samples were tested using the 0.5 McFarland turbidity test to standardize the suspension of microorganisms, ensuring that the bacterial concentration fell within a specific range. One ml of the diluent was plated on the Compact Dry TC and incubated within 24 hours at 35 ± 1°C. After the incubation period, the plates were examined for bacterial growth. The number of colonies formed on the plates was counted to quantify the bacterial presence.

Data Analysis

Statistical analysis was performed using the analysis of variance (ANOVA) to evaluate the significant differences among the experimental groups. The assumptions of normality and homogeneity of variances were checked using appropriate tests. The Shapiro-Wilk test was utilized to assess the normality of the distribution, whereas Levene's test was applied to examine the homogeneity of variances. In cases of significant ANOVA results, Tukey's multiple-range test was utilized to determine which experimental group had a significant difference from the others. All statistical analyses were performed using a 5% alpha level. The statistical analyses were conducted using Jamovi, Version 2.3.28

(Jamovi Project, Sydney, Australia). Preliminary data calculations and exploration were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA). Figures and visual representations of data were created using BioRender (BioRender, Toronto, Canada) and Microsoft Excel.

Cost Analysis

The production cost analysis was thoroughly determined, encompassing raw materials, labor, electricity, and equipment. The raw materials were calculated by multiplying the cost per unit of each material by the quantity used in each formulation. These costs were then summed with the calculated labor costs and electricity consumed during the process. To determine the unit cost, the total production cost was divided by the number of yields, providing a clear estimate per unit cost.

CHAPTER 4

DEVELOPMENT AND CHARACTERIZATION OF SEAWEED-BASED FILM FROM *Kappaphycus Alvarezii* WITH ALOE VERA GEL PLASTICIZER

This chapter presents the comprehensive research findings, followed by an in-depth discussion of film solubility in water, mechanical properties (tensile strength and elongation at break), biodegradability, microbial load, and cost analysis.

1. Formulation of Seaweed-Based Films and Evaluation of Film Properties

1.1 Film Solubility in Water

Significant variations in water solubility were observed among treatment groups (Figure 5). Specifically, Treatments 2 and 3 have significantly low water solubility as compared to the control and Treatment 1 ($p<0.05$). On the contrary, Treatment 1 shows a similar solubility to the control ($p>0.05$). This result indicates that incorporating a relatively higher amount of AVG (>20%) will result in lower solubility in water.

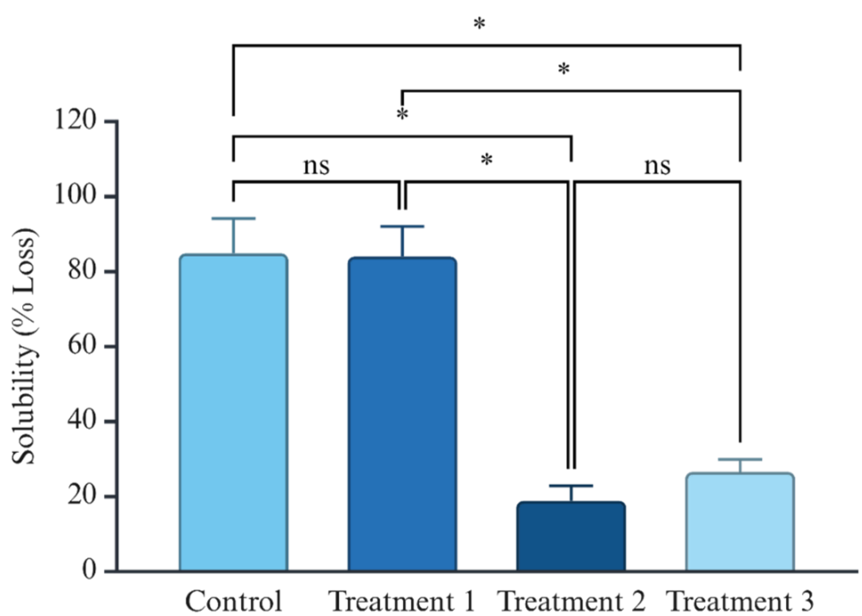

Figure 5. Comparison of the solubility properties of the different film treatments and the control. The * indicates a significant difference ($p<0.05$), while "ns" means no significant difference between groups ($p>0.05$).

The high solubility of Treatment 1 (84.02%) is comparable with the control film (84.87%), indicating that the addition of 10% AVG contributes to the film's water absorption and breakdown. This occurrence can be attributed to the inherent hydrophilic nature of AVG. For instance, adding AVG to sodium alginate has resulted in a composite film with increased hydrophilic properties, thereby enhancing the solubility of the overall structure (Hadi et al., 2021). The plasticizer effect provided by AVG further aids this process by increasing the space between the polymer chains, allowing easier migration of water molecules into the film matrix (Hadi et al., 2021). The comparable solubility of Treatment 1 suggests that a low concentration (10%) of AVG does not significantly alter this property, indicating that at this level, AVG behaves similarly to glycerol in terms of water solubility.

However, a significant decrease in solubility was observed in Treatments 2 (18.87%) and 3 (26.57%), with 20% and 30% AVG, respectively. This observation contrasts with the findings reported by Hadi *et al*. (2022), showing a gradual increase in film solubility, rising from 45.45% in the control to 59.12% with a 50% AVG concentration. Potential explanations for this discrepancy include variations in the base film composition, differing interactions between aloe vera gel and other film components, and possible differences in the properties of the aloe vera gel itself.

The varying solubilities of the different treatments suggest a range of possible applications for the films. For instance, the high solubility of the film with 10% AVG makes it suitable for applications requiring rapid dissolution. These potential applications are beneficial in the food and detergent industries. For example, polyvinyl alcohol (PVOH)-based films have been identified as excellent candidates for use in such applications, their complete water solubility, even at lower temperatures, aligns with the requirements of detergent capsules, facilitating immediate release upon contact with water (Byrne et al., 2021). Furthermore, edible films with higher solubility can be used to encapsulate dry food items that require heating before consumption, such as instant soups, enhancing the product's eating experience (Andrade et al., 2016). Therefore, the inherent characteristic of rapid dissolution in these films unlocks a spectrum of practical applications, improving consumer convenience and product efficacy and fostering the development of sustainable and environmental solutions across diverse industries.

Conversely, the reduced solubility of the films with 20%-30% AVG is more appropriate for applications where water resistance is prioritized. For example, biodegradable films developed from materials like starch are utilized in food packaging for perishable goods that serve as barriers against moisture (Chavan et al., 2022). The solubility of the films falls within the range of values reported for various starch-based films (5.5%-19.12%) in Chavan et al. (2022), highlighting the potential of these films for similar applications. Additionally, films with reduced solubility are beneficial in agricultural applications for resilient coatings against wash-off during rainfall or irrigation, ensuring the sustained efficacy of the chemicals they encapsulate (Alahmed & Şimşek, 2024). Thus, the balance between solubility and water resistance plays a crucial role in determining the suitable application for aloe vera gel-based films. High-solubility films are advantageous when rapid breakdown is required, whereas low-solubility films are essential for applications demanding extended durability. This versatility enables a broad range of practical uses within food processing and agricultural domains.

1.2 Mechanical Properties

Tensile Strength and Elongation at Break

All treatments with AVG incorporation show no significant variations when compared to the control, with an average tensile strength of 42.64 ± 6.32 N/mm² (≈ 4.35 ± 0.64 kgf/mm²) ($p>0.05$). On the contrary, Treatment 1 (61.63 ± 10.54 N/mm², ≈ 6.28 ± 1.07 kgf/mm²) has significantly higher tensile strength compared to Treatment 3 (29.90 ± 1.42 N/mm², ≈ 3.05 ± 0.14 kgf/mm²). In addition, Treatment 1 also shows the highest tensile strength among the treatments and the control, indicating that 10% aloe vera gel is the most effective for enhancing the film's tensile strength ($p<0.05$). Further increasing the concentrations of aloe vera gel leads to a reduction in the tensile strength (Figure 6).

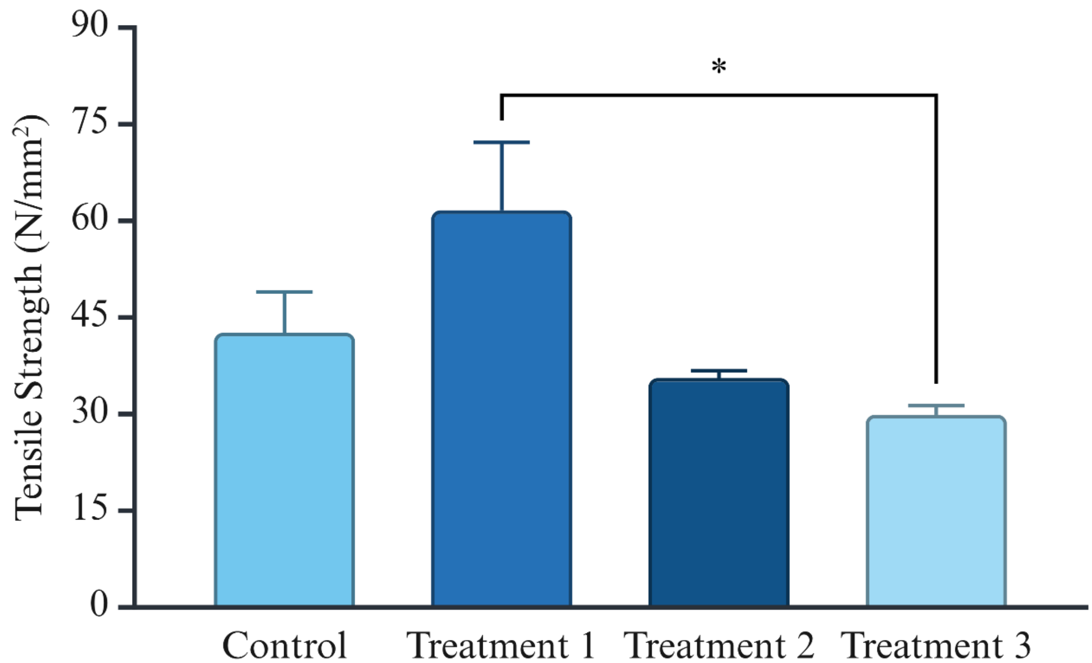

Figure 6. Comparison of the tensile strengths of the different film treatments and the control. The presence of asterisks indicates significant differences between groups ($p<0.05$)

Additionally, incorporating AVG into the *K. alvarezii*-based films significantly affected elongation at break (Figure 7). Analysis showed statistically significant reductions in elongation across all aloe vera gel treatments compared to the glycerol control (17.81 ± 3.34%, mean±SE) ($p<0.05$). Subsequently, mean values (± SE) decreased from 3.07 ± 0.73% (10% AVG) to 1.49 ± 0.06% (20% AVG) and 1.37 ± 0.06% (30% AVG), indicating a clear inverse relationship between AVG concentration and film flexibility.

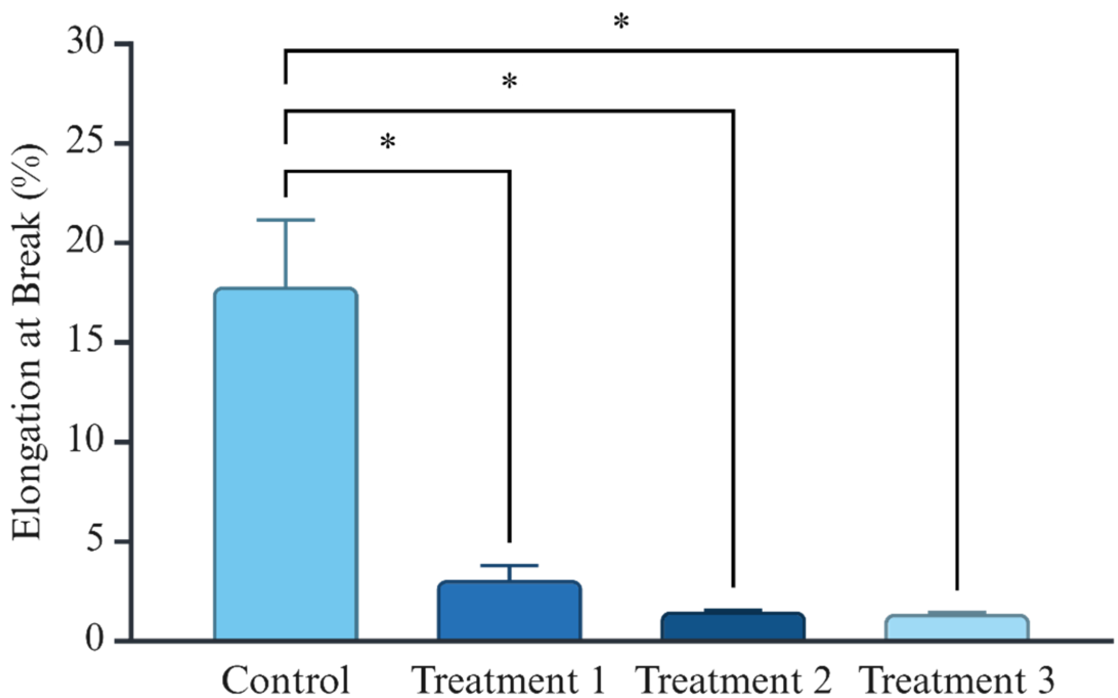

Figure 7. Comparison of the different elongation at break (%) of the different film treatments and the control. The presence of asterisks indicates significant differences between groups ($p<0.05$)

The preceding results highlight a complex relationship between tensile strength and elongation at break in the *K. alvarezii*-based biodegradable films. As observed, the control films exhibited a moderate tensile strength and a significantly high elongation at break, suggesting a balanced material that effectively combines strength and flexibility.

The introduction of 10% aloe vera gel (Treatment 1) significantly increased tensile strength, along with a decreased elongation at break. The observed data in Figure 8 demonstrate a well-known material science relationship between strength enhancement and ductility reduction (Tambe et al., 2024; Hadi et al., 2022). The increase in tensile strength results from stronger intermolecular bonds that form within the film matrix through hydrogen bonding or other cohesive forces, supported by studies that show aloe vera gel creates a crosslinked structure that improves mechanical properties (Tambe et al., 2024; Rahmiatiningrum et al., 2019). However, these interactions may restrict the mobility of polymer chains, leading to increased brittleness and reduced elongation, a trend observed in various polymer blends where increased hydrogen bonding correlates with decreased flexibility (Chen et al., 2019). This suggests that the addition of low aloe vera gel concentration leads to improved tensile strength but reduced film flexibility.

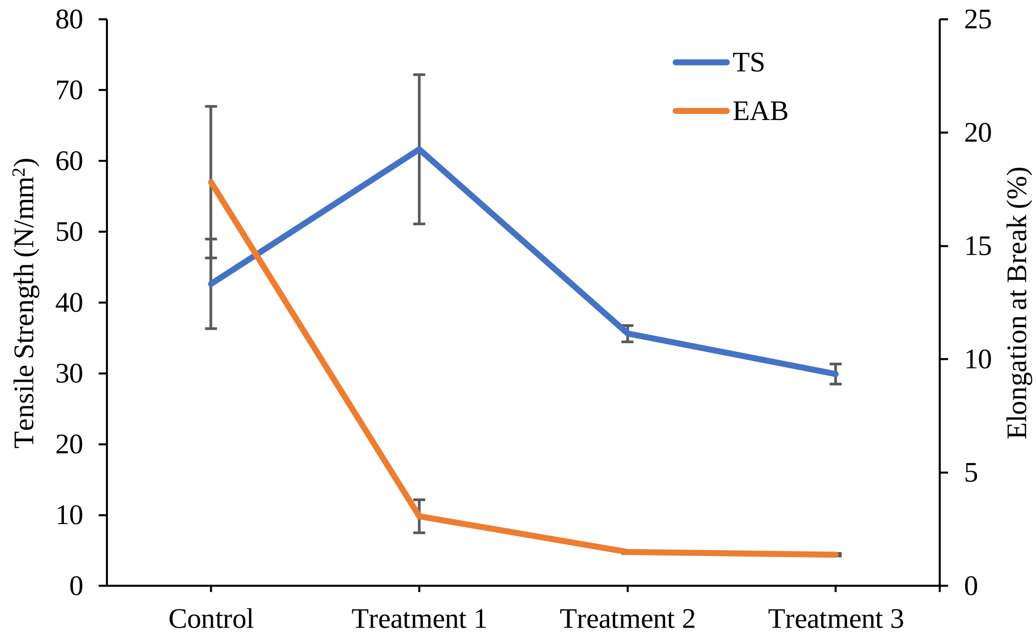

Figure 8. Relationship of tensile strength (TS) and elongation at break (EAB) of the different film treatments and the control. The error bars represent the standard error (SE) of the mean.

The *K. alvarezii*-based film treatments exhibit variations in properties when compared to traditional plastics. For instance, Pradhan et al., (2020) has shown that the High-Density Polyethylene (HDPE) has a tensile strength of 20-40 N/mm² with high elongation at break (100-1000%), while Low-Density Polyethylene (LDPE) shows 7-17 N/mm² tensile strength with elongation at break of 200-900%. In addition, the same study suggests that Polypropylene has 28-40 N/mm² tensile strength with moderate elongation at break of 20-75%. In contrast, the 10% AVG film achieved in the present study has higher tensile strength (61.63 N/mm²) but significantly reduced elongation (3.07%).

On the other hand, the tensile strength decreased when the AVG concentration exceeded 10%, while the elongation at break stayed consistently low. This pattern suggests that increasing AVG concentration negatively impacts the structural integrity of the films. It may disrupt the formation of the polymer network or cause phase separation within the film matrix (Lagos et al., 2015; Ramos et al., 2013). The low elongation at break values in Treatments 1, 2, and 3 demonstrate the difficulty of obtaining flexibility when AVG functions as a plasticizer in these biodegradable films.

The results indicate that adding aloe vera gel to biodegradable films enhances tensile strength but only at low concentrations. The tensile strength and elongation at break decrease substantially when the concentration exceeds 10%. The results demonstrate the requirement to find optimal plasticizer concentrations that will produce suitable mechanical strength and flexibility in biodegradable film formulations.

Comparatively, studies on other biodegradable films have reported similar trends. For instance, Sari et al. (2023) found that the tensile strength of biodegradable plastics increased with the addition of glycerol, reaching a peak at an optimal concentration, after which the strength began to decline. Similarly, Hadi et al. (2021) noted that structural discontinuities occurred at higher AVG concentrations, leading to decreased mechanical strength. This is consistent with the findings from the current study, which highlight the importance of optimizing plasticizer concentrations to maintain a balance between mechanical strength and flexibility in biodegradable film formulations.

1.3 Biodegradability

The results demonstrate that AVG significantly enhances the biodegradability of the films compared to the glycerol-plasticized control (Figure 9). Notably, all treatments incorporating AVG resulted in over 50% film degradation after 12 days. Specifically, the biodegradability of Treatment 2 (53.55%) is significantly higher than the control (37.63%) ($p<0.05$). In addition, Treatments 1 (50.87%) and 3 (52.31%) show higher biodegradability as compared to the control, although not statistically significant ($p>0.05$). These findings confirm the positive impact of aloe vera on film degradation and prompt further examination of the optimal concentration range for maximum biodegradability.

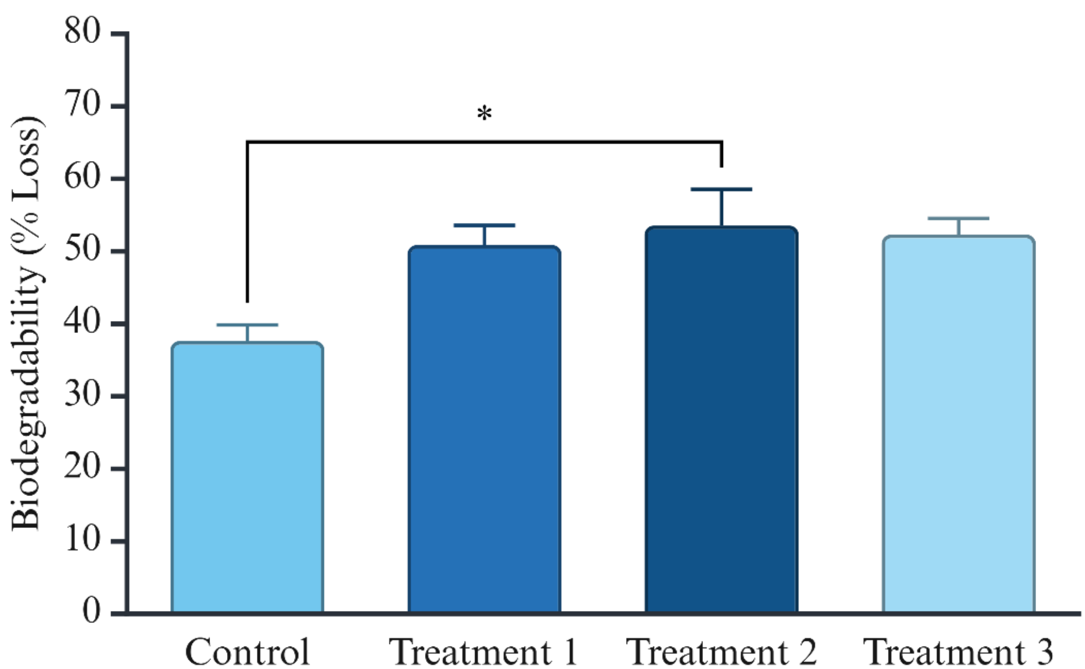

Figure 9. Comparison of biodegradability of the different treatments and the control. The error bars represent the standard error (SE) of the mean.

This rapid degradation, achieving over 50% within 12 days, suggests that AVG may contribute to a faster biodegradation process compared to some glycerol-plasticized carrageenan films, as reported by Sari et al. (2024), where several treatments took 40 days to achieve >50% degradation. However, it's important to note that the final biodegradability levels after a more extended period (e.g., 40 days) might be comparable between AVG and glycerol-plasticized films. The study by Sari et al. (2024) also highlights the importance of optimizing the plasticizer concentration, as they observed increased biodegradability with higher glycerol content.

The concentration-dependent relationship observed in other research aligns with the biodegradability results of the films, where increasing aloe vera gel concentrations positively impacted biodegradability until an optimal point was reached, after which further increases in concentration could hinder degradation due to structural changes in the film matrix or the formation of degradation byproducts that disrupt microbial action (Hadi et al., 2022). The slight decrease in biodegradation observed in Treatment 3 compared to Treatment 2 could be attributed to several factors, including changes in the film's microstructure, the formation of degradation byproducts, or a shift in degradation pathways. High concentrations of aloe vera gel may alter the film's microstructure, reducing its accessibility to microorganisms.

The consistently higher degradation rates of the aloe vera gel-plasticized films highlight the potential of AVG as an effective plasticizer for enhancing the environmental biodegradability of polymer matrices. These findings are of significant relevance to developing sustainable plastic alternatives, as they demonstrate that incorporating AVG can significantly improve the biodegradation of seaweed-based films. Consequently, this improvement makes these films highly suitable for applications prioritizing ecological compatibility.

2. Determination of Microbial Load

The addition of 10% AVG to the film decreased aerobic bacterial counts, demonstrating its antimicrobial potential. The minimal bacterial count (1.0 cfu/g) in Treatment 1 indicates the effective control of bacterial accumulation. The control film demonstrated bacterial contamination with a high level too numerous to count (TNTC), indicating the lack of antimicrobial properties. These differences show AVG's antimicrobial ability as an essential additive for creating biodegradable plastics.

The observed antimicrobial effects are consistent with previously published research. Ghafoor et al. (2016) observed that biodegradable polymer films containing aloe vera exhibited significant bacterial growth reduction, supporting the current study's results. Similarly, Yoshida et al. (2021) discovered that chitosan biofilms containing aloe vera gel had improved antimicrobial properties. Research evidence shows that specific compounds in aloe vera, including anthraquinones, saponins, tannins, flavonoids, ascorbic acid, and pyrocatechols, demonstrate antibacterial effects against a wide range of bacteria. Anthraquinones in aloe vera exhibit antimicrobial properties because they block bacterial protein synthesis, according to Fani & Kohanteb (2012) and Zahra et al. (2022). The compounds interact with microbial membranes to cause bacterial cell lysis, resulting in decreased bacterial viability, especially against Gram-positive *Staphylococcus aureus* strains (Chelu et al., 2023; Parnomo & Pohan, 2021). The current research and previous studies show how aloe vera gel improves biodegradable film antimicrobial properties, which leads to the development of safer and more sustainable alternatives.

3. Cost Analysis

The total cost of two pieces of 30 cm x 20 cm film per production is Php 135.80, making their per-unit cost Php 67.90. The cost of labor and electricity comprises most of the total expenses. An hour and thirty minutes of labor are worth Php 68.44, while using 3.2 kW of electricity costs Php 41.60 for four hours. Material costs are manageable, with distilled water being most costly at Php 20.00 per 500 mL, while semi-refined carrageenan (Php 3.60) and aloe vera gel costs (Php 2.16) contribute minimally.

Table 2. Cost analysis of the developed film.

Materials	Amount Used	Price per Unit	Total
Semi-refined Carrageenan (ATCC)	8 g	Php 450/1000 g	Php 3.60
Aloe Vera Gel	0.8 g	Php270/100g	Php 2.16
Distilled Water	500 mL	Php 10/250 mL	Php 20.00
Labor	1 hour 30 mins	Php 365/8 hours	Php 68.44
Electricity	3.2 KW (4 hours)	Php 13/KW	Php 41.60
		Total Production Cost:	Php 135.80
		No. of Yields (30cm by 20cm Film):	2 pcs
		Unit Cost (Total Production Cost/No. of Yields):	**Php 67.90**

CHAPTER 5

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

This chapter summarizes the key findings and conclusions from the researchers' experimental investigation. It highlights the study's outcomes, including the film's solubility in water, mechanical properties (tensile strength and elongation at break), biodegradability, antimicrobial properties, and cost analysis.

SUMMARY

The study sought to investigate how aloe vera gel affects the characteristics of a seaweed-based film against conventional plasticizers such as glycerol. The main focus was to assess how aloe vera gel affects the solubility, mechanical properties, biodegradability, and the microbial load of the film, as a reference for its possibility as a sustainable replacement for conventional plastic products.

The amount of aloe vera gel used as a plasticizer influences the biodegradable film's solubility. Films using glycerol as a plasticizer and films with lower aloe vera gel concentrations displayed excellent water solubility. Meanwhile, films with high concentrations are less soluble, meaning they have better water resistance. Possible application in food packaging, agriculture, pharmaceuticals, and personal care products depends on the film's solubility, which is affected by the variation in aloe vera gel concentrations as plasticizers.

For its mechanical properties, the films showed different responses depending on the concentration of aloe vera gel. Lower concentration improved tensile strength, making these films stronger than those plasticized with glycerol. However, increased concentrations lessened both tensile strength and flexibility, producing more brittle and less stretchable films.

Furthermore, the biodegradability assessments revealed that films plasticized with aloe vera gel decomposed faster than those plasticized with glycerol. The films with aloe vera gel showed higher degradation rates throughout the testing period, highlighting the role of aloe vera in enhancing biodegradability. This improvement makes these films suitable for applications where quick environmental degradation is essential.

Additionally, adding aloe vera gel significantly affects the seaweed-based films' microbial load. Films containing aloe vera gel effectively inhibited bacterial growth, while control films without aloe vera gel showed substantial bacterial contamination. This indicates the potential use of aloe vera gel in developing packaging materials requiring antimicrobial efficacy.

Lastly, a detailed cost analysis showed that developing seaweed-based films is financially attainable. The costs of raw materials, labor, and other expenses were acceptable, so developing biodegradable films was doable for commercial applications.

CONCLUSION

In conclusion, this study demonstrates the promising potential of aloe vera gel as a sustainable plasticizer in seaweed-based biodegradable films. Varying the concentration of aloe vera gel produced films with properties suitable for a wide array of applications. Notably, aloe vera gel enhanced biodegradability and provided significant antimicrobial activity, surpassing the performance of traditional glycerol-plasticized films. While lower concentrations improved tensile strength, higher concentrations led to reduced solubility and increased brittleness, highlighting the importance of precise formulation. Furthermore, the economic feasibility of producing these films, as demonstrated by the cost analysis, underscores their potential for commercial viability. Overall, the findings suggest that aloe vera gel offer a promising alternative to conventional plasticizers, contributing to the development of enhanced, environmentally friendly packaging materials. Future research into the optimization of aloe vera gel concentration and its applications within specific packaging applications would be useful in exploring the full extent of its potential.

RECOMMENDATIONS

In light of the study's results, the researchers propose the following recommendations:

1. Conduct an optimization study of the film formulations to enhance compatibility and performance.
2. Explore the application of aloe vera gel-plasticized films in specific packaging sectors, such as food packaging, medical packaging, and agricultural packaging.
3. Conduct additional evaluations of the effectiveness of aloe vera gel-based films against various relevant foodborne pathogens (e.g., *E. coli*, *Salmonella*, *Listeria*, *Staphylococcus aureus*) to assess their suitability for food packaging applications.
4. Conduct additional testing to characterize other film properties, such as water vapor permeability, oxygen permeability, oil permeability, and film morphology.
5. Conduct long-term studies to assess the complete biodegradation process of the films in various environmental conditions (soil, water, compost).
6. Conduct a comprehensive life cycle assessment to evaluate the environmental impact of the films from production to disposal.

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