Development of Biodegradable Films Using Polysaccharides Extracted from *Ulva intestinalis*: A Comparative Study of Plasticizers

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Abstract:

This study explores the preparation of biodegradable films from the green algae *Ulva intestinalis*, a rich source of sulfated polysaccharides. Extraction procedures employed comprised both microwave-assisted extraction (MAE) and aqueous acidic extraction with citric acid and hydrochloric acid. On safety grounds, the MAE procedure was abandoned in favor of mild thermal extraction under acidic conditions. The solution was subsequently screened with various plasticizers—glycerol and polyethylene glycol (PEG)—in concentrations between 30% and 70%, with and without citric acid as a crosslinker. The film-forming behavior, physical state, and stability were screened under controlled drying conditions. The results indicated that the formulations at 70% Ulva content with citric acid crosslinking, particularly in combination with 30% glycerol, showed the highest potential for producing elastic, semi-solid films. This thesis offers new knowledge on sustainable biofilm formation and establishes important parameters for the optimization of polysaccharide-based biodegradable package materials.

Keywords:

Seaweeds, polysaccharides, biofilms, biopolymers, algae

Contents

1 Introduction

The global environmental crisis has intensified the search for biodegradable and renewable alternatives. Plastic packaging is one of the most widespread sources of pollution in both terrestrial and marine environments, contributing significantly to the degradation of ecosystems, accumulating microplastics, and contributing long-term threats to biodiversity and human health. With increasing consumer awareness, stricter environmental regulations, and urgent climate change mitigation, we are currently looking toward natural, bio-based polymers as sustainable replacements. Bioplastics derived from renewable biomass have emerged as an environmentally responsible solution. Among the various sources of natural polymers, marine algae have received growing attention for their abundance, low cultivation cost, and promising properties. Unlike traditional terrestrial crops used in bioplastic production, seaweed farming does not require arable land, fertilizers, or additional freshwater resources, making it an economically viable option that does not compete with food crops for land or water. For example, farmed seaweed has demonstrated a harvested mass of 13.1 kg/m² over seven months, significantly outperforming conventional land plants, which yield between 0.5 and 4.4 kg/m² over 12 months, and can be cultivated in marine environments with minimal ecological impact (Moore & Colbert, 2024). Particularly, green macroalgae such as *U. intestinalis* are rich in polysaccharides that constitute the primary component of their cell walls, accounting for nearly 18% of the dry weight. These polysaccharides exhibit strong film-forming abilities, making them promising biopolymers for applications such as biodegradable films. Despite their complex chemical composition, ulva polysaccharides hold significant potential for development into sustainable materials due to their favorable physicochemical properties and versatility in processing (Ning et al., 2022).

While polysaccharide-based films derived from *U. intestinalis* offer a sustainable and biodegradable alternative to petroleum plastics, they also present several technical limitations. These films tend to suffer from high water sensitivity, low tensile strength, and poor flexibility when compared to conventional packaging films (Davoodi et al., 2021). As such, their commercial viability depends heavily on optimizing extraction techniques and formulation strategies to enhance film properties. The general purpose of this project is to investigate the development of biodegradable films using sulfated polysaccharides extracted from the *U. intestinalis*. This involves a detailed exploration of extraction methods, such as

water-based extraction with mild acidification, and the use of functional additives like plasticizers and crosslinkers to improve the physical characteristics of the final film. Specifically, the study examines the performance of glycerol and polyethylene glycol (PEG) as plasticizers, and the contribution of citric acid as a natural crosslinker that could improve the film's structural integrity. The effectiveness of different extract-to-plasticizer ratios is tested to assess their impact on film texture and stability, with the ultimate goal of identifying the optimal formulation for biodegradable packaging applications.

This research is timely and relevant within the broader field of sustainable materials science. It addresses the challenges of reducing environmental pollution while creating high-performance alternatives to conventional plastics. By characterizing different film formulations and establishing clear relationships between ingredients and resulting material properties, this study provides foundational knowledge that can support future efforts in boosting seaweed-based bioplastics. The findings will contribute to practical development in industries aiming to transition toward circular, bio-based packaging solutions. The thesis begins with an overview of the environmental and scientific context motivating the research, followed by a comprehensive review of relevant literature covering seaweed biopolymers, extraction techniques, and film-forming chemistry. Subsequent chapters outline the experimental methods, present the findings in structured form, and analyze the implications of those findings. The document concludes with a critical reflection on limitations and recommendations for future development and application of ulva-based biodegradable films.

This work aims to lay groundwork for unlocking the potential of marine algae-derived polysaccharides as sustainable alternatives to fossil-based plastics. By exploring this, we strive to bridge the gap between natural bioresources and high-performance materials.

The first chapter of this thesis introduces the topic of research, explaining the justification for the study and outlining its main aims. Chapter 2 provides an extensive review of relevant literature, covering environmental concerns related to plastics, the development of bioplastics, and key information on seaweed and ulvan extraction methods. This provides the background essential for understanding the context of the research.

Chapter 3 details the materials and methods employed throughout the experimental protocols, outlining ulvan extraction and film fabrication and analysis procedures. Chapter 4 presents

the experimental findings, setting out the principal results without presenting any interpretative discussion. Chapter 5 discusses the findings, taking into account their relevance and placing them within the framework of current research.

Lastly, Chapter 6 summarizes the findings, acknowledges the study's limitations, and offers recommendations for future research. Generally, this structure guides the reader in a logical flow through the research process from background to findings and implications.

2 Literature Review

2.1 Bioplastics and Sustainable Polymers

The increasing worldwide recognition of the environmental damages wrought by petroleum-based plastics has spurred the pursuit of sustainable, renewable alternatives. Bioplastics—polymeric products based on biological feedstocks—have become an important class with potential for lower carbon emissions and less environmental impact than traditional plastics. These materials are made to be biodegradable, compostable, or both, thereby confronting major concerns regarding plastic wastes and pollution. However, challenges remain in finding a balance between mechanical performance, processability, and environmental sustainability (Sinha, 2024).

In the wide variety of available feedstocks for bioplastics, marine biomass, and more specifically seaweeds, have garnered significant interest. Seaweed-derived biopolymers have the advantages of renewability, abundance, and reduced competition with food crops, which set them apart from terrestrial biomass sources such as corn or sugarcane (Kumar et al., 2021). The marine environment offers an untapped reservoir of polysaccharides with unique chemical structures and functional properties, making seaweed a compelling choice for the production of next-generation bioplastics.

2.2 Seaweed as a Sustainable Biomass Source

Seaweeds grow rapidly in the oceanic habitat without requiring arable land, freshwater irrigation, or fertilizers as opposed to terrestrial crops (Khan, Sudhakar, & Mamat, 2024).

Such low-input cultivation dramatically reduces environmental stresses, including water scarcity and land degradation. Moreover, seaweed aquaculture can also provide ecosystem services such as carbon sequestration, nutrient remediation in eutrophic waters, and habitat for marine biota, and support blue economy and coastal community livelihoods (Lim et al., 2021). Seaweed use as a bioplastic material thus not only offers a circular material solution but is also congruent with broader ecological and socioeconomic sustainability goals.

2.3 *Ulva intestinalis* and Polysaccharides

Among the great diversity of seaweed species, the green alga *U. intestinalis* stands out for having high levels of sulfated polysaccharides called ulvans. The structural complexity and unusual characteristics of ulvans are defined by their high sulfate content and unusual sugar monomers, including rhamnose and glucuronic acid, that dictate distinctive physicochemical and biological functionalities (Figueira et al., 2020). Those functionalities encompass an outstanding film-forming capacity, alongside gelling, stabilizing, and thickening properties that are particularly valuable for bioplastic applications.

2.3.1 Properties of Ulvan Solutions

Understanding the rheological behavior of ulvan solutions to be able to optimize their utilization for film formation. Ulvan exhibits non-Newtonian flow behavior with shear-thinning characteristics, i.e., its viscosity drops upon shear stress—a very good characteristic for processing (Amin, 2020). Molecular weight, concentration, ionic strength, and temperature significantly affect viscosity and gelation behavior.

The rheological characteristics also significantly influence the capacity of the solution to create even, continuous films and influence interactions with plasticizers and crosslinkers, subsequently determining mechanical strength, flexibility, and water resistance (Wang et al., 2014).

Physical- and enzyme-extraction techniques, such as ultrasound- and enzyme-assisted techniques, are employed to extract ulvan fractions with stability and high molecular weight that are required for the fabrication of stable and functional films (Alves et al., 2010). The extraction techniques enable the maintenance of the native structure and bioactivity of ulvan, and this further supports the viability of ulvan in the production of bioplastic materials.

2.4 Extraction Techniques for Ulvan Polysaccharides

Precious polysaccharides' extraction from seaweeds is a critical operation within the bioplastic production process. Traditional extraction operations typically involve prolonged heat treatment under acidic or basic conditions, leading to the degradation of bioactive molecules. Microwave-assisted extraction (MAE), on the other hand, has gained popularity as a low-energy, fast, and solvent-minimizing process. MAE uses microwave radiation to warm polar solvents in plants or algal biomass, leading to cell wall breakdown and the release of target compounds. Torres, Kraan, and Dominguez, in their article on sustainable seaweed technologies, write that MAE significantly minimizes processing time as well as solvent consumption and makes it ecologically friendlier than conventional techniques, which often require extensive filtration (Figure 1 shows the vacuum filtration setup used during water extraction).

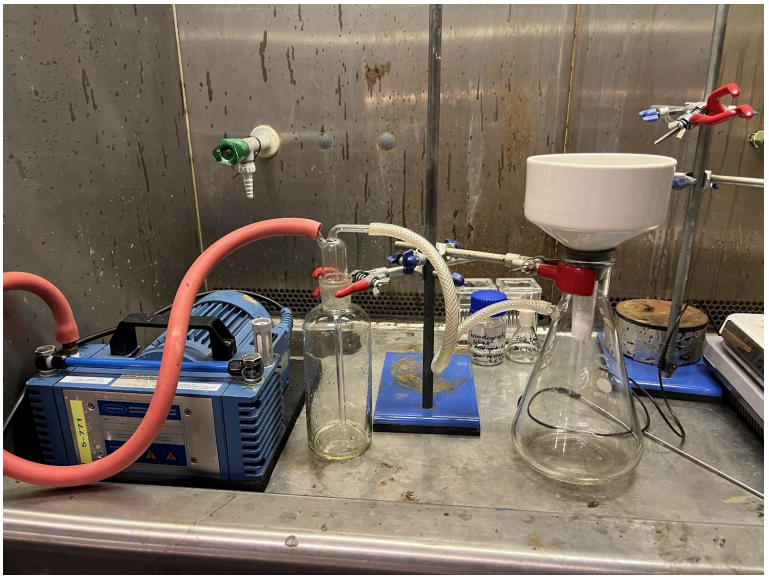

Figure 1: Vaccuum filtration during water extraction trials

While useful, MAE does come with issues. Processing involves advanced equipment that can handle the internal pressure generated upon extraction safely, posing potential safety hazards and limiting its accessibility.

To mitigate the issues, hybrid techniques combining MAE with other extraction techniques such as ultrasound-assisted extraction have been explored to ensure the highest bioactive

compound yield possible while potentially addressing equipment limitations (Garcia-Vaquero et al., 2020).

2.5 Plasticizers and Film Formation

Once extracted, the seaweed polysaccharides are blended with plasticizers to create flexible films. Plasticizers are significant additives that reduce intermolecular interactions between polymer chains, making the films more flexible and reducing brittleness. Some of the most commonly used plasticizers in seaweed films are glycerol and polyethylene glycol (PEG), which both add varying characteristics to the final product (Davoodi et al., 2021). Glycerol is a hydrophilic low-molecular-weight molecule that increases the elasticity and flexibility of biopolymer films. However, it also has the ability to absorb moisture from the surroundings, which can compromise the water resistance and durability of the film over time (Jost & Stramm, 2016).

PEG is less hygroscopic, however, and forms a denser, more cohesive film matrix. Davoodi investigated the mechanical properties of Ulva films and concluded that PEG-plasticized films showed improved tensile strength and reduced oxygen permeability than their glycerol-based counterparts. Though these advantages were obtained at the cost of reduced flexibility. The mechanical properties of films are highly susceptible to the concentration of plasticizers. The plasticizer content would usually cause decreased tensile strength and increased elongation at break, as well as increased water solubility and water content (Davoodi et al., 2021).

2.6 Crosslinking for Enhanced Properties

Crosslinking is another method of improving the mechanical and barrier property of seaweed films. Citric acid, a naturally occurring tricarboxylic acid, has also received attention as a bio-based crosslinker for polysaccharide films due to its biocompatibility and ability to form ester linkages between hydroxyl functionalities in the polymer matrix (Dudeja, Mankoo, Singh, & Kaur, 2023). The addition of citric acid into the film composition not only enhances tensile strength and thermal stability but also reduces the hydrophilicity of the film to enhance it for water-resistant packaging applications.

A case in point is provided by Raghav Poudel, who prepared citric acid-modified yam starch films with significantly improved mechanical strength and water resistance. The films also contained anthocyanin, a pH-sensitive dye, so the films could act as intelligent indicators of fish spoilage. Such multi stimulation highlights the ability of bioplastics not only to substitute conventional packaging but also to complement it through functionality of added value (Poudel, Dutta, & Karak, 2023).

Whereas Poudel's work focused on starch, the citric acid crosslinking mechanism is applicable to polysaccharides from ulva and other seaweeds (Poudel, Dutta, & Karak, 2023). Incorporating citric acid into Ulva-derived films in this study resulted in improved gel structure and elastic films when used in combination with glycerol. Most interesting of these are recipes in which a 7:3 mixture of Ulva solution to glycerol and citric acid produces semi-solid elastic films suitable for use in soft packaging or for biomedical purposes. These results show that the appropriate balance of plasticizers and cross linkers can significantly influence the functionality of seaweed-derived films, and optimization may result in materials whose properties can be tuned for any intended use.

2.7 Sustainability of Seaweed Bioplastics

The potential of such films is beyond the conventional packaging to the field of smart and functional materials. Emerging trends in the research on bioplastics are centered around creating materials that encompass antimicrobial activity, pH or even electrical conductivity sensitivity in order to broaden the application areas (Abang, Wong, Sarbatly, Sariau, Baini, & Besar, 2023). For instance, plant-based dyes or metal oxide nanoparticles-based dyes are being designed to be utilized in sensing food spoilage, biosensing, or antimicrobial packaging (Jayakumar et al., 2019; Shalabi et al., 2022). Although the present research did not entail smart sensing agents, the elasticity and semi-solid texture events occurring in certain of the formulations indicate shape-conforming and bio-interfacing compatibility.

Besides, the use of *U. intestinalis* as a base material supports larger environmental goals. Because seaweed culture is a low-input, high-yield biomass production system with low ecological disturbance. Seaweed aquaculture can also be used to sequester carbon, remediate nutrient in eutrophic water and drive coastal community economic development (Lim, Yusoff, Ng, Lim, & Ching, 2021). The use of seaweed in bioplastics thus not only represents

a technological innovation, but also a strategic approach towards reaching environmental and socioeconomic sustainability.

2.8 Life Cycle Considerations

It should be noted that the post-use life cycle of such bioplastics, biodegradability within natural environments, compostability under industrially-managed conditions, and the generation of microplastic-similar fragments upon degradation are all considerations that must be critically examined. The studies have shown that while polysaccharide films degrade at a quicker rate than conventional plastics, the rate and degree of degradation are subject to environmental conditions such as humidity, microbial action, and temperature (Folino, Karageorgiou, Calabrò, & Komilis, 2020). Life cycle assessment (LCA) can match total ecological footprint of bioplastics from cradle to grave. Such comparisons are incorporated to guarantee that products presented as sustainable will function better during their entire environmental impact.

Economically, seaweed-based bioplastic production upscaling depends on the creation of low-cost cultivation and processing infrastructure. Integrated Multi-Trophic Aquaculture (IMTA) is a promising framework via the co-cultivation of seaweed with fish or shellfish to create synergistic systems optimizing resource use with reduced environmental effects (Knowler et al., 2020). Public-private partnerships, as well as government incentives, would also drive adoption with market creation, research, and investment in infrastructure. Standardized testing and certification of bioplastic properties and environmental profiles would similarly be the main driver in consumer and industry adoption.

Finally, regulatory frameworks and market forces will determine future trends for seaweed bioplastics. Policy support to bioplastics currently is highly variant between regions, with some governments subsidizing or mandating in favor of bio-based packaging, while others are even focused on recycling-based solutions (Rosenboom, Langer, & Traverso, 2022). Education campaigns at the public level, labeling and education for proper disposal also needs to prevent bioplastics from ending up in inappropriate waste streams such as being discarded at landfill or placed in among conventional plastics.

2.9 Conclusion

Seaweed-derived bioplastics, like those of *U. intestinalis*, represent an outstanding opportunity for green material design, uniting environmental gains and versatility of performance. Recent advances in the formulation of sustainable extraction procedures, the optimization of plasticizers, and the improvement of crosslinking chemistry have enabled the production of biodegradable films with tunable mechanical and barrier properties. Nevertheless, there are still important research gaps, especially a comprehensive understanding of ulvan's rheological behavior in multicomponent formulations, optimization of extraction procedures for the highest possible bioactive compound retention, and the addition of novel functional additives to enhance application efficiency.

The study seeks to address the mentioned limitations through a detailed examination of ulvan solution properties, the evaluation of novel plasticizer and crosslinker combinations, and the evaluation of film performance under industrial application. The work seeks to push the development of industrial-scale, high-performance seaweed bioplastics that can meet a range of industry requirements while being environmentally friendly.

3 Materials and Methods

3.1 Materials

Green seaweed (*U. intestinalis*) was used as the primary polysaccharide source. The powder was sieved to below 300 μm particle size and stored at room temperature. All extractions used distilled water. Plasticizers included glycerol and polyethylene glycol (PEG 600), both of analytical grade. Citric acid and hydrochloric acid (5%) were used to adjust the pH of solutions. Equipment included a household microwave, a magnetic stirrer with a temperature probe, a laboratory-scale oven, a centrifuge, vacuum filtration units with mesh and paper filters, a mechanical pipette, and a micro weighing scale.

3.2 Microwave-Assisted Extraction Trials

Microwave-assisted extraction (MAE) was explored as a promising approach to achieve fast and efficient extraction of polysaccharides from *U. intestinalis*, exploiting the convenience and availability of household microwave ovens. Preliminary experiments were carried out in standard laboratory containers in a household microwave oven to test for technical feasibility and also for the response of seaweed biomass to microwave exposure.

MAE is achieved through the application of non-ionizing electromagnetic waves, normally in the range of 300 MHz to 300 GHz, on a sample matrix. Ionic conduction and dipole rotation are mainly responsible for energy transfer in MAE (Torres, Kraan, & Dominguez, 2020). During ionic conduction, the ions in the solvent move upon exposure to the electromagnetic field, leading to even warming because the solvent opposes their movement. At the same time, dipole rotation is taking place as the polar molecules reorient themselves with the changing electric field, resulting in thermal agitation. Once the field is removed, the molecules return to their disordered state and release energy in the form of heat. The synergistic effects produce cellular disruption, which increases the release of intracellular material like ulvan, as illustrated by the mass and heat transfer gradients in Figure 2.

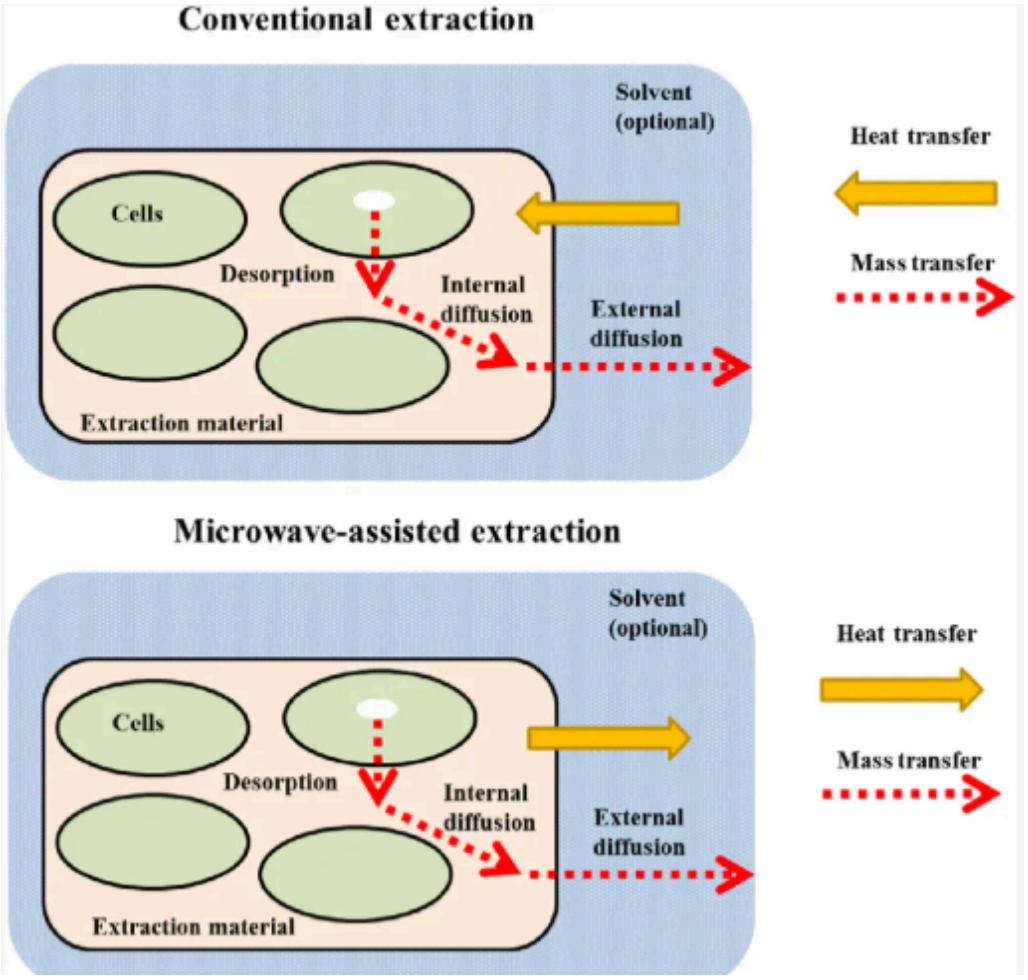

Figure 2: Mass and heat transfer gradients in conventional and microwave-assisted extraction (MAE). Reprinted from Torres, M. D., Kraan, S., & Dominguez, H. (2020). Sustainable seaweed technologies: Cultivation, biorefinery, and applications (p. 210). Elsevier. © 2020 Elsevier. Used under fair use.

The primary aim of these trials was to evaluate whether commonly available containers could withstand the internal pressure generated during the process. MAE is known to reduce extraction time and potentially increase yield by rapid heating. However, it became evident that using a domestic microwave is not designed to safely manage the pressure build-up associated with heating aqueous seaweed mixtures. This led to safety concerns, including cap failure and the risk of equipment damage.

Due to these limitations, MAE was deemed unsuitable for further use in this study, and alternative, safer extraction methods were pursued.

3.2.1 Microwave Tests with Seawater and Ulva

Microwave-assisted extraction (MAE) was explored first as a potentially rapid and efficient method of extracting polysaccharides from *U. intestinalis*, exploiting the general availability and convenience of household microwave ovens. Initial trials were performed in conventional laboratory vessels in a household microwave oven to examine both the technical viability and the response to microwave irradiation of seaweed biomass.

Three pilot microwave-assisted extraction (MAE) experiments were conducted to ascertain the efficacy and safety of *U. intestinalis* polysaccharide microwave extraction in a conventional domestic microwave oven setting. For Trial 1, 500 mL water and 10 g Ulva were extracted in a loose-fitting lid at 600 W for 40 minutes. This setup caused too much foaming, condensation, and spilling of the water, and almost all of the water had evaporated by the time the cycle finished. In Trial 2, the amount of water was reduced to a minimum and the container was sealed with a tight-fitting lid, then microwaved at 600 W for 20 minutes. This trial produced a burning smell after 10 minutes, which meant overheating.

Lastly, Trial 3 was run with a closed cap at the same 600 W power for 40 minutes. This setup led to cap failure within a period of around 7 minutes owing to pressure inside (Figure 3). All these trials were beset by dangerous safety issues, such as equipment instability and possible container rupture, and hence microwave-based extraction had to be abandoned in favor of safer techniques.

Figure 3: Results of microwave-assisted extraction trials using different Ulva–water configurations resulting in bottle cap Failure.

Despite modifications, all three setups failed to yield extract safely. Most notable were cap failures and severe overheating.

3.2.2 Bottle Pressure Resistance Tests

A series of pressure resistance tests were conducted on various containers under microwave-assisted extraction (MAE) conditions to evaluate their suitability and safety. In one trial, a 120 mL polyethylene bottle was exposed to 180 W of microwave power for 10 minutes; however, the container exploded after 8 minutes of exposure. A separate trial using a 75 mL glass beaker with a lid under identical conditions resulted in boiling but no structural failure.

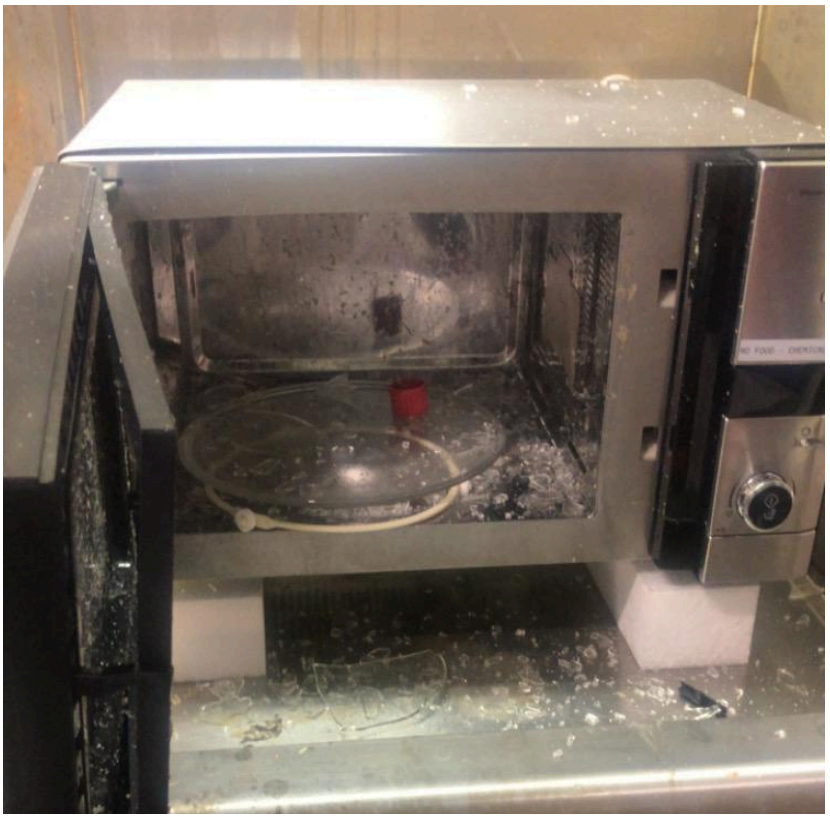

Figure 4: Destruction of microwave unit and container during pressure testing. This led to discontinuation of MAE

In a more intensive test, a glass container filled with 75 mL of water and 26.24 g of wet Ulva was subjected to 180 W for 20 minutes. This trial caused catastrophic failure, leading to the destruction of the microwave unit itself (as seen in Figure 4).

These results highlighted significant safety concerns and limitations in using household microwave equipment for MAE, particularly regarding pressure build-up in water seaweed mixtures. Consequently, MAE was discontinued in favor of safer and more controlled extraction methods to ensure safety.

3.3 Water-Based Extraction

Given the safety risks associated with microwave-assisted extraction (MAE), a water-based extraction method was adopted. This approach involved mild acidification and low-temperature stirring, based on protocols reported in the literature (Davoodi et al., 2021).

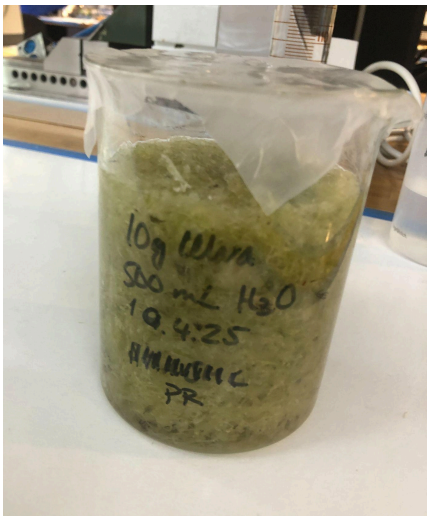

Figure 5: Preparation of Ulva extract using 10 g of U. intestinalis in 500 mL of distilled water

In this method, 10 g of Ulva powder was mixed with 200 mL of distilled water and acidified to pH 4.4 using 5% hydrochloric acid (HCl) as depicted in Figure 5. The mixture was subjected to magnetic stirring and gently heated to 70 °C. Stirring was maintained for 6 hours to facilitate extraction. Subsequently, the solution was refrigerated overnight at 2 °C to promote phase separation.

3.3.1 Separation and Filtration

Purification of the extract was essential to remove particulates and impurities that could compromise film quality. Initial separation was attempted via centrifugation at 6,500 RPM for 10 minutes. However, due to the break of a glass centrifuge tube, this method was discontinued for safety reasons.

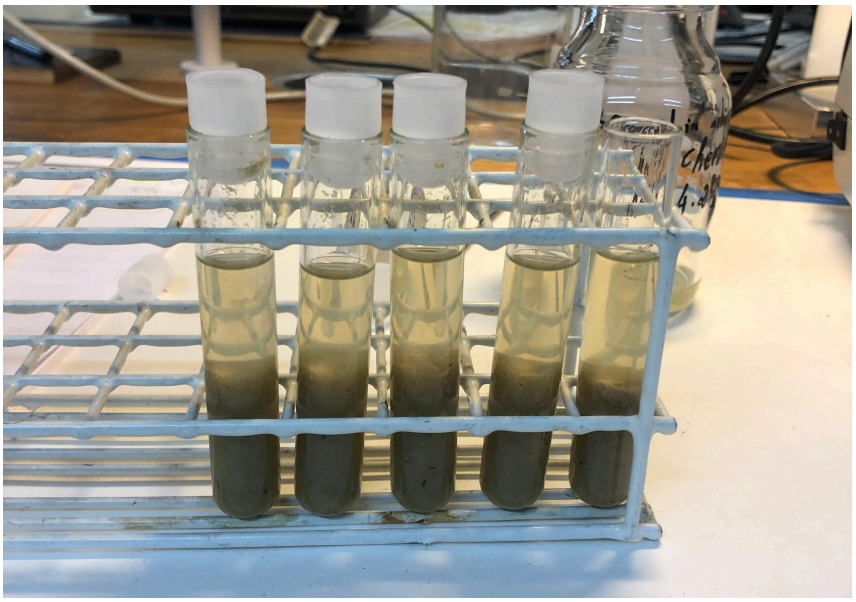

Figure 6: Ulva extract showing upper and lower phases

As an alternative, static refrigeration was employed to promote gradual phase separation. After six days at 2 °C, the samples exhibited clear separation, allowing for continued processing, as seen in Figure 6, this refrigeration process led to the formation of distinct upper and lower liquid phases.. To further purify the extract, filters with decreasing pore sizes were used. Samples were first passed through a 25 µm mesh, followed by filtration with 5–13 µm paper filters.

Although centrifugation was initially preferred for its speed and efficiency, the combination of refrigeration and stepwise filtration provided a practical and effective means of obtaining a clarified extract suitable for film casting.

3.4 Plasticizer Film Trials

Using 50 mL of extracted solution, six samples were prepared with varying Ulva-to-plasticizer ratios, as summarized in Table 1. The ratios were reversed compared to those used in Davoodi et al. (2021) to explore new mechanical properties.

Table 1: Film Formation Results with Various Ratios of Ulva Extract to Plasticizer (35°C, 8 Days)

Sample	Ulva Extract (mL)	Plasticizer (mL)	Plasticizer Type	Result (35°C, 8 days)
A	5	5	PEG	Gelatinous
B	4	6	PEG	Gelatinous
C	3	7	PEG	Gelatinous
D	5	5	Glycerol	Unstable, liquid
E	4	6	Glycerol	Unstable, liquid
F	3	7	Glycerol	Unstable, liquid

All films were dried at 35°C for approximately 8 days.

3.5 Citric Acid Crosslinking Trials

Citric acid (CA) was employed as a bio-based crosslinking agent to improve the structural stability of ulvan-based films by facilitating covalent bonding within the polysaccharide matrix. Its introduction was based on established evidence of its efficacy in enhancing the mechanical integrity of carbohydrate-based films. In this phase of the experiment, nine samples were formulated using 4 mL of seaweed extract, with varying concentrations of citric acid and glycerol. Adjusting these concentrations allowed for the optimization of film properties, specifically targeting a balance between firmness and elasticity.

Cross-linking refers to the formation of covalent (and occasionally ionic) bonds that connect polymer segments from different chains, creating a three-dimensional network structure. This mechanism is visualized in Figure 7, which demonstrates how these bonds can form either during polymer synthesis or through post-synthetic reactions involving crosslinking agents, which enable long macromolecules to be joined into spatially organized, durable frameworks.

Notably, only small quantities of crosslinker are required to induce significant changes in polymer behavior due to the extended nature of the polymer chains.

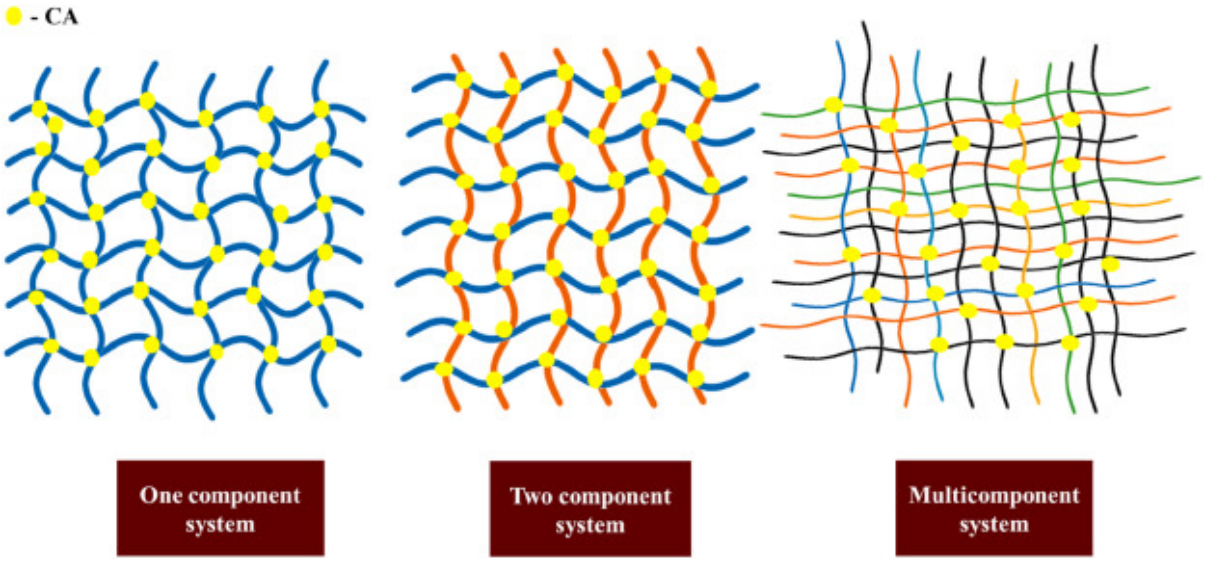

Figure 7: Polymer networks formed by CA cross-linking of biopolymers Adapted from Dudeja, Mankoo, Singh, & Kaur (2023). © Elsevier, 2023. Used under fair use.

In this study, citric acid was selected for its three carboxylic acid (-COOH) groups, which are capable of reacting with the hydroxyl (-OH) groups found along the ulvan backbone. This facilitates intermolecular esterification, resulting in enhanced film cohesion and mechanical performance.

Table 2 summarizes the film consistency outcomes at 50 °C with varying levels of citric acid and glycerol:

Table 2: Film Consistency Outcomes from Varying Citric Acid and Glycerol Concentrations (50°C)

Citric Acid (%)	Glycerol (%)	Citric Acid (g)	Glycerol (mL)	Observation (50°C)
0.5	5	0.02	0.2	Gelatinous
1.0	10	0.04	0.4	Gelatinous
1.5	15	0.06	0.6	Gelatinous

All samples were dried at 50 °C to allow proper film formation under controlled thermal conditions.

3.6 Final Formulation Using Ulva, Citric Acid, and Plasticizer

A final concentrated extract was prepared under optimized conditions to maximize polysaccharide content for effective film formation with citric acid crosslinking and plasticizer. The extraction process was repeated using citric acid to adjust the solution to pH 4.4. The mixture was then heated to 86 °C and maintained for 90 minutes. Following extraction, the solution was reduced by evaporation to a final volume of 50 mL to increase polymer concentration.

Concentration by evaporation ensured that the extract contained a sufficient amount of polysaccharides to support film formation. A series of films were then produced by varying the ratios of plasticizer and citric acid crosslinker. This approach demonstrated the tailorability of the system.

These final combinations and their physical outcomes after extended drying are presented in Table 3:

Table 3: Physical Properties of Final Ulva-Based Films with Citric Acid and Plasticizer After Extended Drying (50°C, Few Weeks)

Sample	Ulva Solution + Citric Alcid (mL)	Plasticizer (mL)	Plasticizer Type	Result (50°C, few weeks)
A	5	5	PEG	Soft, gelatinous
B	6	4	PEG	Slightly firm
C	7	3	PEG	Crumbly, brittle
D	5	5	Glycerol	Soft, unstable
E	6	4	Glycerol	Elastic
F	7	3	Glycerol	Very elastic, semi-solid

4 Results

This study explored different extraction methods and film formulations using *U. intestinalis* as a polysaccharide source for biodegradable films. Microwave extraction proved unsafe

without proper equipment. Water-based extraction under mild acidic conditions showed promising yields. Citric acid significantly improved the film's physical characteristics by promoting cross-linking.

An overview of films cast under various conditions is presented in Figure 8, which compares trays with PEG and glycerol alone (A), glycerol with citric acid (B), and a combination of PEG, glycerol, and citric acid (C).

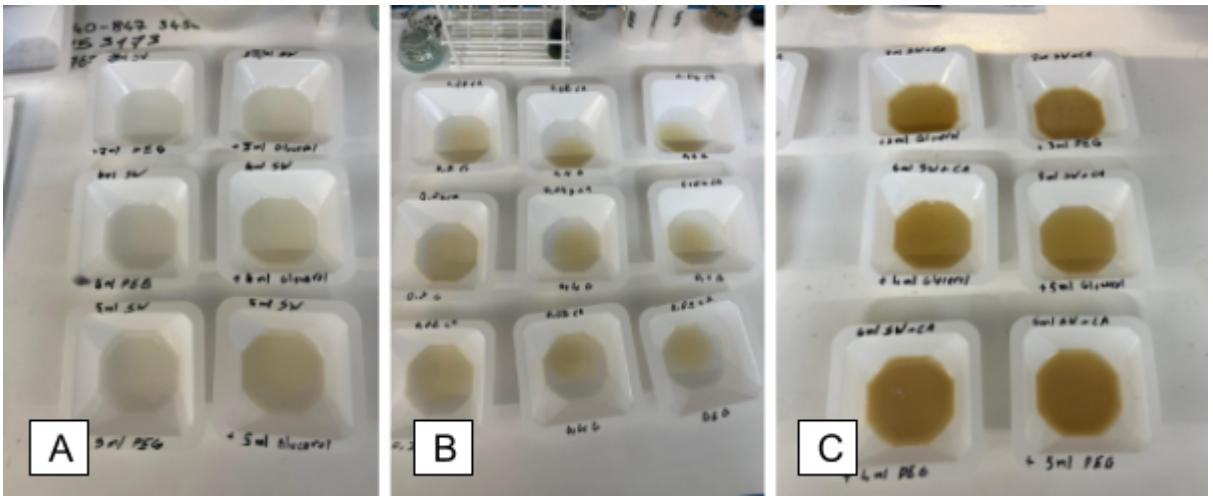

Figure 8: Ulva-based film trays under different conditions (A) Films with PEG and glycerol after 8 days at 35°C (B) Films with glycerol and citric acid after 5 days at 50°C (C) Films with citric acid, PEG, and glycerol after 5 days at 50°C.

4.1 Extraction Performance

4.1.1 Microwave-Assisted Extraction (MAE)

Initial experiments aimed to assess the feasibility of microwave-assisted extraction (MAE) as a rapid method for isolating ulvan. However, all attempted configurations failed due to unsafe pressure buildup and thermal instability of standard laboratory containers. In one trial, a plastic bottle exploded after 8 minutes at 180 W. In another, a sealed glass container ruptured, resulting in the destruction of the microwave unit. Across all setups, severe foaming,

overheating, and equipment failure were consistently observed. Due to these safety concerns and equipment limitations, MAE was deemed impractical and subsequently abandoned in favor of a safer water-based extraction approach.

4.1.2 Water-Based Extraction

The selected extraction procedure employed mildly acidified water (pH 4.4 adjusted) and gentle heating at 70 °C, in addition to magnetic stirring at all times to permit polysaccharide release from *U. intestinalis* biomass. Following six hours of stirring, chilling of the extract in the fridge was employed to promote phase separation. Mechanical separation was halted owing to damage of one of the centrifuge tubes in early trials. The extract was left to stand with static refrigeration for six days instead, which effectively achieved visual separation of the liquids and solids. The supernatant was then purified by successive filtration using a 25 µm mesh and then 5–13 µm paper filters. The resultant ulvan-rich solution was clear and viscous, indicating successful extraction and purification, and was determined to be adequate for film formation experiments.

4.2 Film Formation Trials

4.2.1 Plasticizer Trials (No Citric Acid)

Films were cast using varying ratios of ulvan extract to either PEG or glycerol. After drying at 35°C for 8 days, the films showed different levels of gelation and stability.

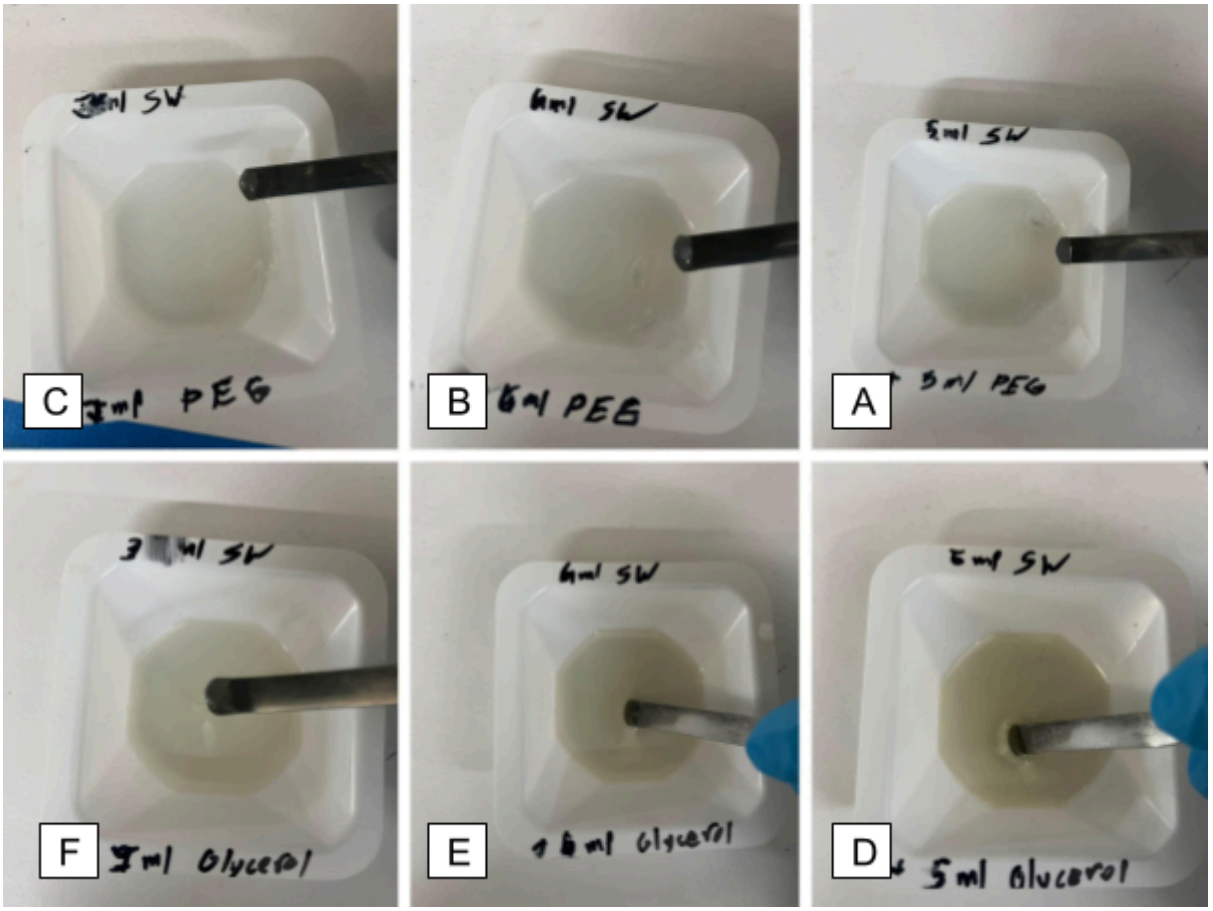

Figure 9 Ulva-based films prepared with varying PEG and glycerol ratios, after 8 days at 35 °C. A metal lab spatula is used to visually assess film consistency.

Table 4. Effect of PEG and Glycerol Plasticizers on Ulva-Based Film Formation (No Citric Acid)

Sample	Ulva Extract	Plasticizer	Result
A	5 mL	5 mL PEG	Gelatinous
B	4 mL	6 mL PEG	Soft gel
C	3 mL	7 mL PEG	Gel-like

D	5 mL	5 mL Glycerol	Liquid
E	4 mL	6 mL Glycerol	Liquid
F	3 mL	7 mL Glycerol	Liquid

The specific ratios and the corresponding visual and textural results are outlined in Table 4. PEG-containing films exhibited partial gelation and retained their shape upon drying, though they lacked mechanical strength or elasticity. Figure 9 shows these outcomes visually, comparing the consistency of PEG- and glycerol-based films after drying, as the proportion of PEG increased and ulvan decreased (Samples B and C), the films became softer and more loosely structured, indicating that PEG may facilitate partial gelation but not full network formation in the absence of a cross-linking agent.

In contrast, glycerol-based samples failed to form any solid structure, remaining entirely liquid or semi-fluid even after extended drying. This suggests that glycerol, while typically effective as a plasticizer in other polysaccharide-based films, may not interact strongly enough with ulvan alone to support gelation or film cohesion under these conditions.

Overall, these trials demonstrated that while PEG is more favorable than glycerol for initial ulvan film formation, neither plasticizer alone was sufficient to produce robust or elastic films. These results prompted further investigation into the role of citric acid as a cross-linking agent to enhance film structure.

4.2.2 Citric Acid Crosslinking Trials

To investigate the cross-linking potential of citric acid on ulvan-based films, nine small-scale samples were prepared using a fixed volume of *U. intestinalis* extract (4 mL), while varying citric acid and glycerol concentrations.

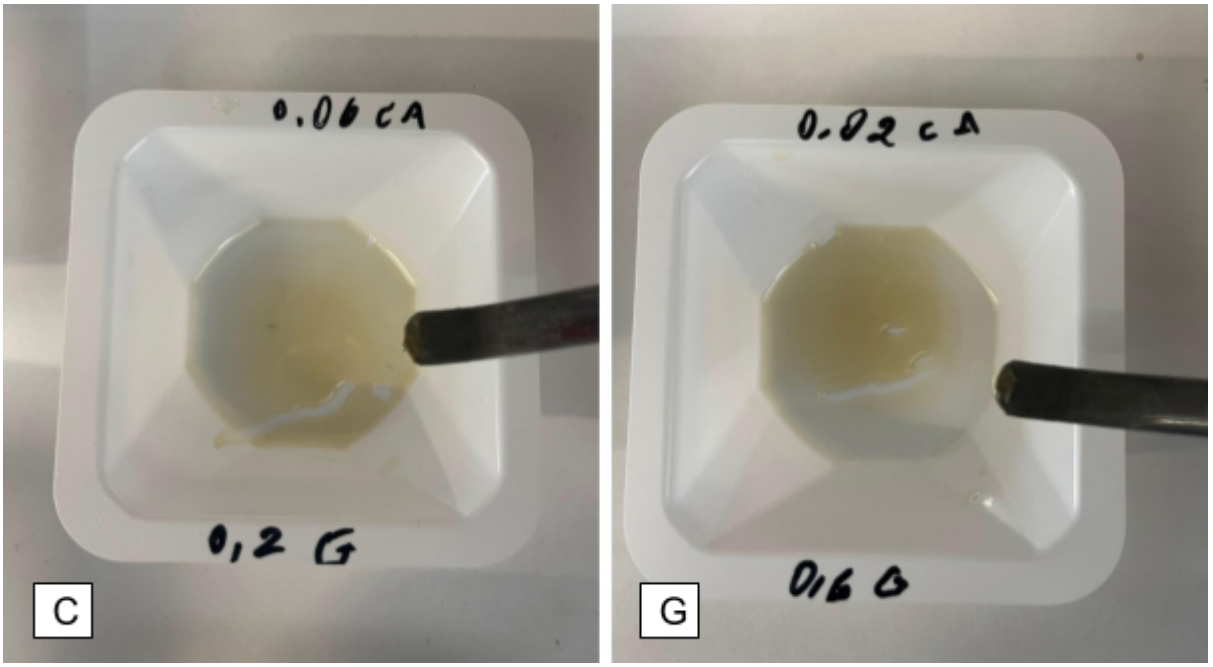

Figure 10 Trays with Ulva-based films prepared with varying glycerol and citric acid ratios, after 5 days at 50°C. A metal lab spatula is used to visually assess film consistency.

Table 5: Effect of Citric Acid and Glycerol Concentration on Ulva-Based Films (Fixed Ulva Volume: 4 mL)

Sample	Citric Acid (%)	Glycerol (%)	Citric Acid (g)	Glycerol (mL)	Result
A	0.5	5	0.02	0.2	Gelatinous
B	1.0	5	0.04	0.2	Gelatinous
C	1.5	5	0.06	0.2	Gelatinous
D	0.5	10	0.02	0.4	Gelatinous

E	1.0	10	0.04	0.4	Gelatinous
F	1.5	10	0.06	0.4	Gelatinous
G	0.5	15	0.02	0.6	Gelatinous
H	1.0	15	0.04	0.6	Gelatinous
I	1.5	15	0.06	0.6	Gelatinous

All sample compositions and their gelatinous outcomes are listed in Table 5, the resulting films maintained a gelatinous texture, with no transition to solid or fully cohesive film structures. Despite varying both citric acid (0.5–1.5%) and glycerol (5–15%) concentrations, no significant enhancement in structural integrity was observed. The mixtures thickened slightly at higher concentrations of citric acid and plasticizer, suggesting some interaction between the ulvan chains and citric acid; however, this was insufficient to promote full cross-linking or form stable, peelable films.

Visually, the samples appeared uniform and translucent, with increasing viscosity correlating to higher additive content. However, all samples lacked the firmness, elasticity, or dryness necessary for practical film applications. Figure 10 illustrates how these results confirm their gelatinous nature, and suggest that citric acid alone was not an effective crosslinker for ulvan films at this scale and concentration range. Additional factors such as drying conditions, higher acid concentrations, or complementary crosslinkers may be required to achieve functional films.

4.2.3 Final Batch Trials

A refined extraction was performed (86°C, 90 minutes) and concentrated to ~50 mL. Six formulations were cast at 50°C using various extract-to-plasticizer ratios.

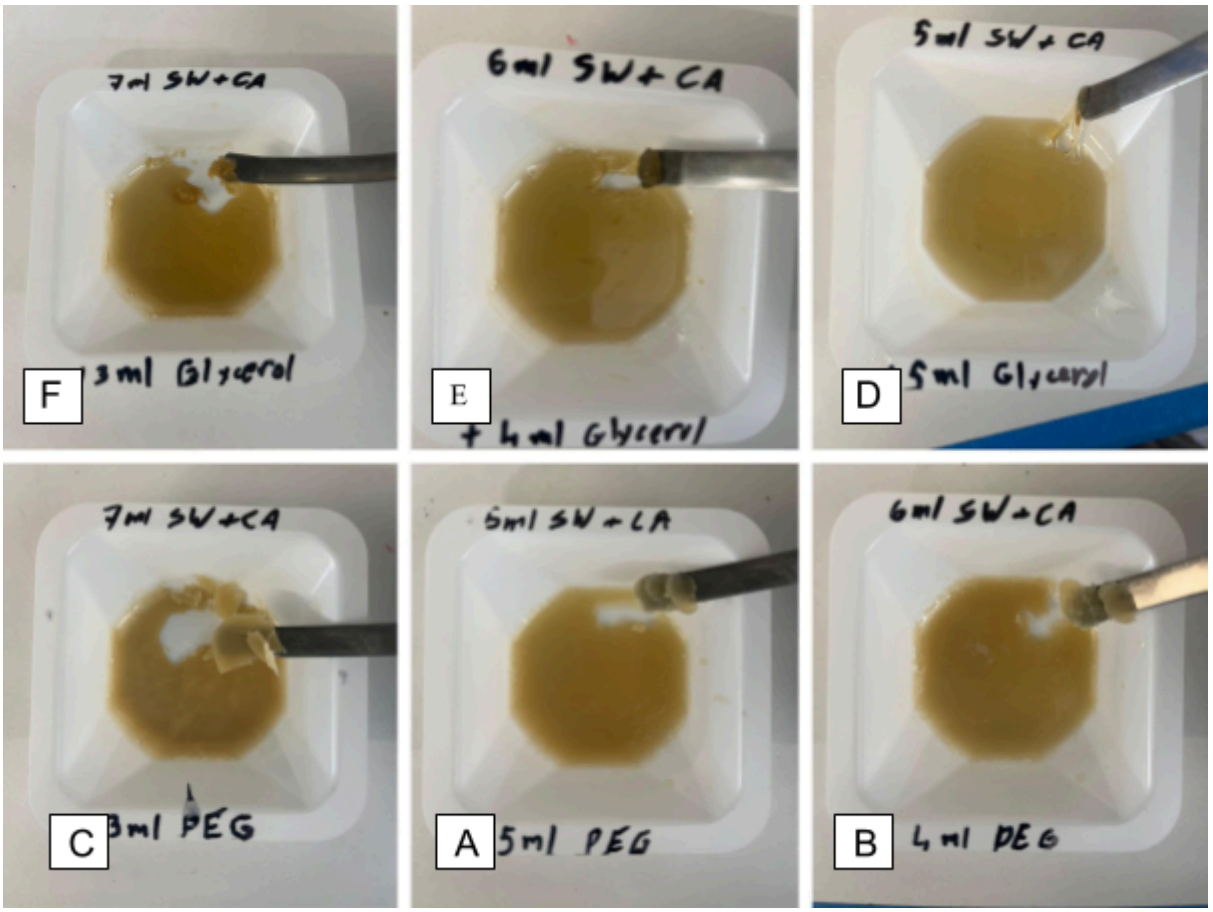

Figure 11 Trays with Ulva-based films prepared with Citric Acid with varying PEG and glycerol ratios added, after 5 days at 50°C. A metal lab spatula is used to visually assess film consistency.

Table 6: Final Film Formulations Using Extract, Citric Acid, and Varying Plasticizers

Sample	Ulva + Citric Acid (mL)	Plasticizer (mL)	Plasticizer Type	Result
A	5	5	PEG	Soft, gelatinous

B	6	4	PEG	Slightly firm
C	7	3	PEG	Crumbly, brittle
D	5	5	Glycerol	Soft, unstable
E	6	4	Glycerol	Elastic
F	7	3	Glycerol	Very elastic, semi-solid

Films containing both citric acid and a high concentration of Ulva extract demonstrated the greatest degree of structural integrity among all formulations. These samples retained a gelatinous but cohesive texture, indicating that citric acid promoted some degree of cross-linking within the ulvan matrix. While increasing the citric acid concentration appeared to slightly improve film consistency, it did not lead to complete solidification or the formation of fully dry, peelable films under the tested drying conditions.

When comparing plasticizers, PEG-based formulations generally formed gelatinous films but were prone to brittleness and cracking once dried. In contrast, glycerol-plasticized films exhibited greater flexibility and elasticity, particularly in samples with higher Ulva content (Figure 11). The structural improvements seen with glycerol suggest that it may be more compatible with ulvan for applications where flexibility is essential.

The component ratios and film characteristics from the final batch are presented in Table 6. The most promising outcome was observed in the sample containing 7 mL of Ulva extract and 3 mL of glycerol (Sample F), which produced an elastic, semi-solid film with the best balance of structure and pliability. This formulation showed potential for future development and may be a suitable candidate for mechanical testing to quantify its performance in terms of tensile strength, elongation, and other relevant properties.

5 Analysis

The results of this study clarify many of the formulation-based problems and preparation-related differences involved in the preparation of ulvan films. The main aim of utilizing microwave-assisted extraction (MAE) was not achievable with the constraints of safety. Even though MAE is one of the best techniques with regards to the efficiency of polysaccharide extraction, in the current laboratory setup, it was considered to be risky. The increase in pressure-related problems coupled with indications of equipment instability made the use of MAE no more feasible, except in the case of pressurized apparatus. A mild acidic aqueous extraction process was thus used accordingly.

The plasticizers were also found to have a critical bearing on the physical properties of the resultant films. Films that used the plasticizer polyethylene glycol (PEG) were found to have a tendency for higher densities and were more structurally stable during and after the process of drying.

The addition of citric acid to the formulations greatly impacted the structural characteristics of the films. The addition caused significant enhancement of the formerly poorly solidified samples, specifically those which used glycerol as a plasticizer. The new formulations produced films that were more uniform in nature and thus output more cohesive films with increased apparent resiliency. The results indicate that citric acid promotes intermolecular interactions in the polymer matrix, thus strengthening the coherence and pointing to its possible use as a stabilizer.

The proportion of ulvan to plasticizer is of paramount importance. The addition of ulvan to the composite enabled better film formation, which was attested to by the better behavior of samples with higher extract content. Out of the formulations tested, the 7:3 ratio of ulvan to glycerol was found to possess the best characteristics, such as viscoelastic consistency. Compared to other samples, this particular ratio of the components displayed lower deformation and higher resistance to rupture. From the findings, higher levels of ulvan with glycerol and citric acid result in films with higher stability but for this to be definitively proven would require quantitative analysis.

Although mechanical testing, including barrier properties, elongation, and tensile strength, was not within the current study scope, the visual observations were valuable in terms of revealing properties of film behavior as well as component interactions. Perhaps most significantly, however, some of the blends—namely those including citric acid and

glycerol—consistently yielded more homogeneous and more cohesive results than formulations where one or more of the additives were omitted. These observations emphasize the indispensable importance of careful optimization of plasticizer types as well as the levels of additives in seaweed film formulations.

6 Conclusion

This study has demonstrated the feasibility of developing biodegradable films based on *U. intestinalis*, a green marine alga, using green, available extraction methods. The work was directed at synthesizing ulvan films by the utilization of green chemistry principles aimed at sustainability and scalability of polymer extraction and film synthesis. Microwave-assisted extraction (MAE) has been hoped to be a quick and efficient extraction technique for polysaccharides from seaweed and other biomasses; however, its application has been hindered by safety issues as well as equipment constraints in our lab. The intense internal pressure developed during MAE and the lack of pressure-withstanding equipment made it impossible to apply this technique under the given circumstances. This called for a change to a gentle, water-based extraction under mildly acidic conditions, which was a safer and more controllable option that still resulted in polysaccharide-rich extracts that were amenable to film formation. This illustrates a key factor to consider when taking up green extraction processes: a balance between efficiency and safety and ease of operation during the initial development stages.

The ulvan extracted was used to form films with various plasticizers and additives and showed that the choice of plasticizer has a pronounced effect on the physical and mechanical properties of the resulting films. PEG-based formulations produced compact, highly structured films with greater initial firmness. However, those films were comparatively lacking in elasticity and flexibility, properties that are critical for many packages needing to resist some level of stretching or deformation without cracking. Films with glycerol alone were semi-fluid or unstable after extended drying, indicating that the polymer network was not established or that insufficient intermolecular bonding was present to provide structural stability.

The incorporation of citric acid in the formulations significantly contributed to the improvement of film properties. As an organic acid, citric acid is recognized as having the ability of a natural cross-linking agent, which facilitates reactions and bond formation between polymer chains. This impact was greatest with the more concentrated ulvan films with glycerol and citric acid, where the resultant materials were more elastic and had a degree of solidification than glycerol films alone. The 7:3 ratio of ulvan to glycerol with the addition of citric acid had the most potential.

In spite of these promising findings, the research had some limitations that inhibit its immediate applicability. Notably, there were no quantitative mechanical tests. Tensile strength, elongation at break, Young's modulus, and tear strength are essential in establishing bio-based films as an alternative to traditional plastics or other biodegradable films. In the absence of standardized measurements, it is hard to ascertain whether the films are suitable to compare them with other plastics. Further, measurement of barrier properties of the films - such as water vapor transmission rate, oxygen permeability, and solubility - was not conducted. These are essential to ascertain the ability of the films to prevent packaged contents, especially foodstuffs, from moisture evaporation, oxidation, or microbial contamination. The visual observations performed offered useful initial findings, but they do not possess the thoroughness required for regulatory approval processes.

Looking ahead, a number of key research directions must be prioritized in order to fully unlock the potential of ulvan-based films. First and foremost, thorough mechanical characterization is needed. Tensile testing to measure strength, elasticity, and stiffness will provide performance metrics that can be directly compared to synthetic plastics as well as other biodegradable polymers such as polylactic acid (PLA) or starch-based films. These numbers are instrumental in the development of tailored compositions that address the requirements of individual packaging applications, including flexible wraps, rigid packages, or secondary packaging layers.

Second, barrier property testing is required. Water vapor permeability, oxygen transmission rate, and solubility in relevant solvents will divulge films' ability to prevent moisture and oxidation - two of the main agents affecting food quality and shelf life. Such tests also direct modifications for performance improvement, i.e., multilayer films or coatings.

Thirdly, there should be ecological effects measurements. Long-term biodegradation experiments performed under controlled conditions mimicking composting, terrestrial, freshwater, and marine environments will yield critical information on the environmental fate of ulvan films. Further, ascertaining the stability of shelf-life during use and storage is of paramount concern, since premature degradation or excessive brittleness may restrict their usefulness.

Expansion of the palette of plasticizers and cross-linkers is another research opportunity. While glycerol and citric acid were effective, other biodegradable compounds like xylitol, sorbitol, or natural calcium salts can potentially offer superior mechanical or barrier functionality.

Re-evaluation of MAE and other advanced extraction techniques under well-controlled, pressure-resistant conditions is suggested. These methods can possibly enhance the yield, purity, and reproducibility of ulvan, thus making economically viable, large-scale production for industrial use possible. Further, incorporation of functional additives - i.e., natural antimicrobials, antioxidants, or active nanoparticles - may provide bioactive characteristics, making it possible to develop intelligent or active packaging materials. These products can extend the shelf life of food, inhibit the growth of microbes, or provide environmental sensing capabilities, meeting emerging trends in eco-friendly packaging technology.

Overall, this study offers a strong foundation for the elaboration of biodegradable films manufactured with ulvan from *U. intestinalis*. The exploitation of green extraction processes and natural additives aligns with global sustainability objectives and the growing demand for oil-based plastic alternatives. Despite remaining challenges, particularly regarding mechanical properties, barrier properties, and industrial viability, the prospects of controlling film properties by adjusting the plasticizers and cross-linkers employed are high. Ongoing interdisciplinary studies combining polymer chemistry, materials science, environmental studies, and process engineering are necessary to translate these initial findings into commercially relevant and green packaging solutions. The sea-derived ulvan films discussed in this article thus constitute a significant step in the growing repertoire of sustainable biopolymers that simultaneously resolve pressing environmental and economic challenges on a global scale.

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