Bioactives in Ulva

Aveiro, Portugal

June 24-25. 2024

Ulva
The Future Wheat of the Sea

ORGANIZERS

Prof. Dr. Rosário Domingues, Departament of Chemistry, University of Aveiro, Portugal
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Muki Shpigel (University of Haifa, IL),
Rosário Domingues (University of Aveiro, PT),
Thomas Wichard (University of Jena, DE)

The COST Action SeaWheat, the University of Aveiro, the Friedrich Schiller University Jena, and the Company AlgaPlus joined forces to organize a 2-day Workshop on "Bioactives in Ulva" (Working Group 4), a topic of significant importance in the research field. This collaborative effort, held from 24.06 – 25.06. 2024 with the Department of Chemistry of the University of Aveiro graciously hosting the workshop, demonstrated a shared dedication to advancing the understanding of macroalgal bioactives.

RATIONAL AND OBJECTIVES

The green seaweed *Ulva* (Chlorophyta) is a valuable source of diverse bioactive compounds with significant potential in health, nutrition, cosmetics, and pharmaceuticals. Its rich content of polysaccharides, proteins, phenolics, vitamins, minerals, fatty acids, and pigments offers a wide range of potential biological activities that can be harnessed for promoting health and well-being. The workshop aimed to bring scientists together to uncover the full potential of these compounds, as *Ulva* is assumed to play an increasingly important role in various industries. Established and young scientists and representatives from Small and Mid-sized Enterprises came together to discuss the status quo of the current research on macroalgal bioactives.

ORGANIZATION

The workshop was part of Working Group 4's (WG4) planned list of events for the current grant period. The local organizing committee, led by Prof. Dr. Rosário Domingues, Dr. Ana Moreira, and Dr. Cláudia Nunes, played a pivotal role in ensuring the workshop's smooth operation. They were supported by Dr. Thomas Wichard (University Jena, DE). The workshop program was discussed and decided upon by experts who constituted the scientific committee. These were (listed alphabetically): Rosário Domingues (PT), Thomas Wichard (DE), Céline Rebours (NO), and Muki Shpigel (IL). Ana Moreira and Rosário Domingues edited the abstract book (Annex of the report). News and information about the workshop were posted on the SeaWheat COST Action social media channels (such as LinkedIn) and the Action websites.
The local host of the workshop, the University of Aveiro (Universidade de Aveiro), located in Aveiro, Portugal, is a prominent institution known for its academic excellence, research contributions, and innovative approach to education. Established in 1973, the university has become one of Portugal's leading educational and research institutions. The University of Aveiro hosts several research centers and institutes focusing on areas such as, e.g. marine sciences, environmental studies, materials science, and education and was a perfect location for the COST Action Workshop on "Bioactives in Ulva".

PARTICIPANTS

The COST Action workshop was well received, with 60 registered participants from 14 countries (6 ITC countries). Combining it with the subsequent Training School in Aveiro at the same location proved excellent. In addition to the COST Action registered participants, further scientists attended the presentations throughout the workshop.

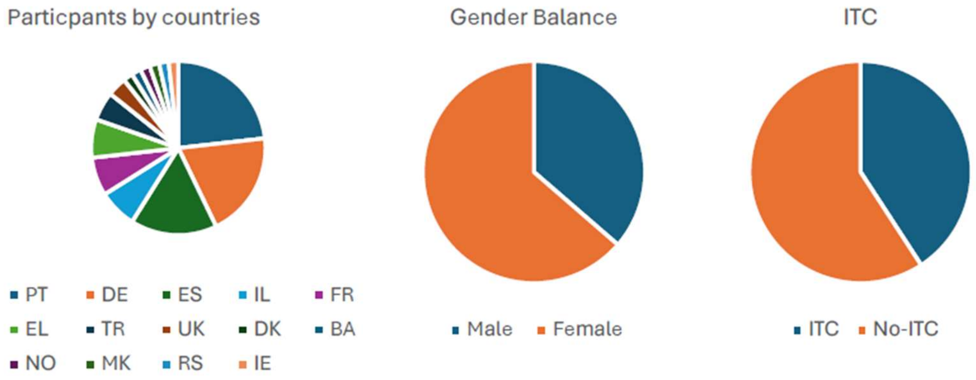

Figure 37 Distribution of the Workshop participants by countries, gender, and members of ITC

WORKSHOP DESIGN AND FUNCTIONING

Amongst the participants, current research topics on bioactives in macroalgae and their applications were presented in four sessions chaired by experts on the topic (total of 20 talks, each 30 min) : (i) "Extraction Approaches," (ii) "Omics Analysis," (iii) "Bioactive Compounds," and (iv) "Targeted Analysis of Bioactive Phytochemicals". Each session comprised 4-6 presentations by experts in the field as an overview, including discussions after each presentation.

Within these sessions, the following topics were addressed by the speakers (examples are given):

- **Extraction Techniques:** Methods for extracting bioactive compounds from Ulva
  (e.g., Hugo Pereira, Greencolab, Portugal).
- **Bioactive Properties:** Health benefits and potential applications of bioactives.
  (e.g., Dieter Lütjohann, University, Hospital Bonn, Germany)
- **Industrial Applications:** Use of *Ulva* bioactives in pharmaceuticals, cosmetics, and nutraceuticals.
  (e.g., Pi Nyvall Collén, Olmix, France)
- **Research Advances:** Latest research findings and future directions in the study of *Ulva* bioactives.
  (e.g., Vassilios Roussis, University of Athens, Greece)

As can be seen from the list of speakers, proximity to the application of bioactives was sought through reports from industry (AlgaPlus (PT), Omix (Fr), GreenCoLaB (PT). The breaks between the sessions were used for further discussions and exchanges on other activities of the COST Action participants. Networking was used to establish new projects and existing co-operations were deepened. The status quo of various COST Action activities of the WG4 were discussed (e.g., the ongoing research exchange via STSMs, the review initiative about bioactives in Ulva).

Notably, on the afternoon of the event's second day, the young scientists (Master's students, PhD students, and Postdoctoral students) who participated in the subsequent training school gave short presentations. These 15 5-minute presentations (pitch sessions) again demonstrated the growing interest in bioactives in macroalgae and provided an exciting overview of the ongoing European research.

Figure 38 Group photo of 65 participants of the Seawheat workshop outside the venue, Department

CONCLUSIONS

This workshop focused on the bioactive compounds found in Ulva, a genus of green algae commonly known as sea lettuce. The event brought together researchers, industry professionals, and students interested in extracting and applying bioactives from Ulva species. An overview of *Ulva* bioactives, extraction techniques, and initial research findings was given. The organizing team resumed that the workshop promoted research and the cohesion of research, often scattered throughout Europe.

Bioactives in Ulva

Aveiro, Portugal

PROCEEDINGS

Ulva
The Future Wheat of the Sea

HYDROTHERMAL EXTRACTION AND DEPOLYMERIZATION OF ULVANS

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Ulvan composition and structural features, closely related to the biological properties, are influenced by the extraction and purification stages. Water extraction at temperatures higher than 100 °C allowed shortened operation time [1]. Acidic conditions aid in the de-stabilization of aggregates enhancing the yields [2] and bioactivity due to partial reduction of molecular weight [3], although coextraction of other undesirable components may occur [4]. Hydrothermal extraction with water under subcritical conditions offers advantages derived from the change in physicochemical properties of the solvent, such as the lowered relative dielectric constant, which enhances the extraction of nonpolar substances. However, the ionic product increases converting water is an interesting reaction medium acting as an acidic or basic catalyst. The release of sulfate and carboxylic acid groups in the aqueous phase generates an acidic medium promoting autohydrolysis and depolymerization of polysaccharides to oligomers and to monomers without externally added chemicals. Operation times could be additionally shortened during operation with microwave assisted heating [5].

In this study the potential of hot pressurized water or autohydrolysis was studied with conventional or with microwave heating for solubilising the ulvan fraction from *Ulva sp.* The initial defatting stage was avoided due to the low lipid and extractives content and to conform a simpler process. Different recovery strategies were tried, including the conventional cold precipitation with ethanol, with bioionic liquid and the fractionation with ultrafiltration membranes. Further characterization of the chemical, structural, rheological and cytotoxic properties were assessed.

The operation temperature strongly influenced the process performance and up to 60% overall extraction yield was attained. The maximum ulvans yield recovered by ethanol precipitation was 23% from the extract obtained during heating up to 160 °C, which required 23 min during conventional heating up to the maximum temperature, and was 32% after 6 min of isothermal operation at the same temperature with microwave heating and precipitation with chloline chloride. The lowered pH induced by hydrothermal operation, due to the presence of organic acids in the liquid phase, influences the extraction efficiency of ulvan [2], but the decrease was less pronounced than with a strong acid leading to pH below the pKa of glucuronic acids and facilitating its selective extraction over glucuronan, xyloglucan, and soluble protein [4].

The crude ulvan recovered after ethanol precipitation exhibited carbohydrate and uronic acids contents in the range 66-77%, with 7-11% sulfate and a protein content under 1%. The apparent viscosity decreased with increasing shear rate, exhibiting a clear pseudoplastic behaviour. The highest values of apparent viscosity were observed for the ulvan extracted at 160 °C, followed by those extracted at 140 and 120 °C, and were comparable to those of commercial products. The viscoelastic features of representative ulvan-based films exhibited mechanical profiles corresponding to a typical gel behaviour. The product obtained at 160 °C presented two peaks, corresponding to 150 kDa and to 24 kDa, comparable to those attained with acidic extraction, but the enzymatically obtained extract are mainly formed by high molecular weight fractions [3, 6]. Cell viability assays with the mouse fibroblasts NCTC clone 929 was not affected by ulvan samples at concentrations up to 1.0 mg/mL in the growth medium.

The ulvan free extract showed a low protein and phenolic content, this latter correlating with the reducing and antiradical properties. The hydrothermal treatment caused changes in the microstructure of the residual solid phases, from the smooth, compact and regular morphology of untreated seaweed to the shrinkage of the cell wall in extracted biomass, particularly at treatments beyond 180 °C. These residual solid fractions are enriched in protein, suggesting potential applications, i.e. for food or feed. The ash content in the residual solids was progressively reduced with increasing temperature, and also the sulfate content. The lipid content slightly followed this trend. However, a lowered carbohydrate content, and the reduction in the glucose content confirms that only 20% of the initial glucose remained in the solid suggesting that this fraction corresponded to the cellulose.

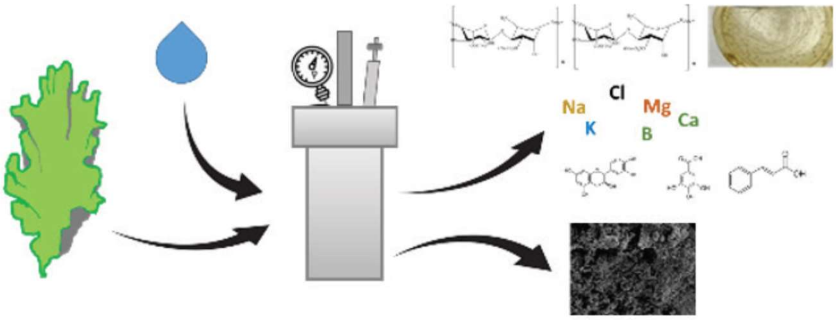

Figure 39 Hydrothermal extraction and depolymerization of ulvans

In summary, hydrothermal processing of *Ulva* sp is an efficient, rapid and tuneable technology valid to fractionate the wet algal biomass in less than 20 min with conventional heating and in 6 min with microwave assistance. No previous drying, solvent extraction and chemical addition were needed. The ulvan yields could be enhanced and the mechanical properties of the recovered ulvan were comparable to those of commercial counterparts.

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Acknowledgments

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SCALABLE BIOREFINERY PIPELINES FOR THE PRODUCTION OF ALGAE-BASED BIOACTIVE INGREDIENTS

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Algae feedstocks offer remarkable potential for diverse biotechnological applications. However, the current production costs of algae biomass in industrial-scale facilities often limit its use in high-value markets like food, nutraceuticals, and cosmetics. Although significant advances are currently being undertaken to improve the cost-effectiveness of algae production at the industrial scale, one of the most promising approaches to fully exploit the potential of algae biomass is the development of cost-effective biorefineries. Multiproduct biorefineries are an innovative approach that promotes the exploitation of the different biochemical components present in algal biomass through their sequential extraction, fractionation, and processing into high-value ingredients. In this context, GreenCoLab and associated partners have developed and optimized several biorefinery pipelines to process different algal biomasses (micro and macroalgae) into multiple ingredients at laboratory scale in the last few years. The established pipelines followed a logical sequential extraction and separation from high-value to lower-value compounds, using an array of different techniques for the valorization of the different fractions, including biomass pre-treatment, membrane filtration, enzymatic hydrolysis, and green chemistry extractions. More recently, a pilot-scale biorefinery platform - ALGAEREFINE prototyping center - was implemented at GreenCoLab to validate the scalability of the developed biorefinery pipelines and generate relevant quantities of ingredients to be tested in multiple applications. Results demonstrate the feasibility of different biorefinery processes in establishing different ingredients and upgrading the value of algae biomass. In addition, the potential of these ingredients and their valorization for different biotechnological applications (e.g., aquafeeds, cosmetics, agriculture) was also assessed, highlighting that the biological activities can be enhanced in the ingredients. The environmental and economic performance of the most promising biorefinery routes are currently being evaluated using life cycle assessment (LCA) and techno-economic analysis (TEA). Overall, the implementation of scalable biorefineries is key to maximizing the market value of the different biochemical components contained in algae biomass and ensuring their valorization in new value chains.

WATER SOLUBLE EXTRACTS OF *ULVA* USE AS PLANT BIOSTIMULANT

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Seaweed-derived extracts are promising sources of novel biostimulants [1,2] that could increase crop yields sustainably. The objective of this work was to determine whether ulvan crude extract could be used as a biostimulant for terrestrial plants. Herein we showed that crude ulvan extract, derived from cultivated local to Israel *Ulva sp.*, promotes the growth of Arabidopsis thaliana in its early germination stage. This study addressed the challenges of identifying an appropriate extraction method for ulvan, examining its bioactive concentration and timing of supplementation, and testing its effect on A. thaliana seedlings and mature plants. We identified and addressed the following knowledge gaps: (i) specification of a reliable and repeatable method for ulvan extraction from a local *Ulva* species based on a nondestructive procedure, (ii) determination of the bioactive ulvan concentration and its effect on inducing the highest stimulation to A. thaliana sprouts, (iii) examination of the effect of the timing of ulvan supplementation during the early-stage germination of A. thaliana sprouts, and (iv) determination of the impact of ulvan supplementation at the early stage of plant development on the final seed yield of mature A. thaliana. Our attempt to fill these gaps began with examining the chemical composition of our extracted ulvan and comparing it to compounds identified in the literature. We then observed the specific bioactivity effects of the biostimulant on A. thaliana seedlings. We measured morphological characteristics after three weeks under two light conditions. We divided early germination into two stages (at sowing and one week after sowing) to examine the effect of adding ulvan in those two steps separately and together. We also determined several photosynthetic parameters. Finally, we ascertained whether ulvan supplementation at the early stage of plant development affected the final seed yield of mature A. thaliana. This research identified the ulvan extraction method and the bioactivity concentration required to enhance A. thaliana seedling development, We identifying 0.2 mg/ml as the optimal ulvan concentration for stimulating A. thaliana germination. We showed that the timing of ulvan supplementation during early germination of A. thaliana is essential. We revealed that the positive effects of our ulvan extract on A. thaliana germination (enhanced root length and shoot area, recorded at the end of the three-week germination period) occurred if we added the ulvan at the beginning of this first week (ulvan present at sowing). The roots of the treated sprouts were significantly longer (+13.6%, mean 5.55 cm) than the control (mean ~4.88 cm). The treated sprouts also had a higher shoot area (+45.2%, 58.06 mm2) than the control (33.98 mm2). Adding ulvan at the beginning of the second week (after sowing) had no significant effect on these parameters. Finally, we found that the stimulation in the early-stage germination positively affected the final seed yield of mature plants. We show that the seed yield was ~43% higher for the ulvan-treated group than the control, untreated group. These results demonstrate that ulvan biostimulants could potentially reduce the use of harmful agrochemicals, thus mitigating their negative environmental impacts while increasing crop yield and contributing to global food security.

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INNOVATIVE APPROACHES FOR THE SELECTIVE RECOVERY OF NUTRIENTS AND BIOACTIVE COMPOUNDS FROM ULVA: BENEFITS AND LIMITATIONS

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Over the last years, a growing interest has shown in the use of *Ulva* from different points of view such as food, cosmetics, pharmaceuticals, etc. In this sense, one of the possible uses is through its use as such, for example, incorporating it into food, but another possible use is through the recovery of high-added-value compounds, and in some cases their subsequent purification. Some of the main advantages of this strategy are the concentration of the active ingredients, as well as an improvement in acceptance by consumers, who can reject the consumption of *Ulva* as such, but not in the form of its derivatives (i.e, additives). In this sense, the use of solid-liquid extraction techniques for the recovery of *Ulva* compounds is one of the most followed strategies, but it has different drawbacks such as the use of solvents, which in many cases are toxic, consumption of a large amount of solvents, high temperatures and extraction times, totally in disagreement with current standards regarding green extraction and sustainability [1]. Thus, different green and eco-friendly strategies have been proposed, such as pulsed electric fields, supercritical fluids and pressurized liquid extraction, among others, as possible alternatives to conventional processes. However, although the use of these technologies is of great interest and has many advantages, it also has some important disadvantages, mainly related to the scalability of the processes and other limiting factors of the process. The following presentation will evaluate some of the main advantages and disadvantages derived from the use of innovative extraction processes such as pulsed electric fields, supercritical fluids and pressurized liquid extraction.

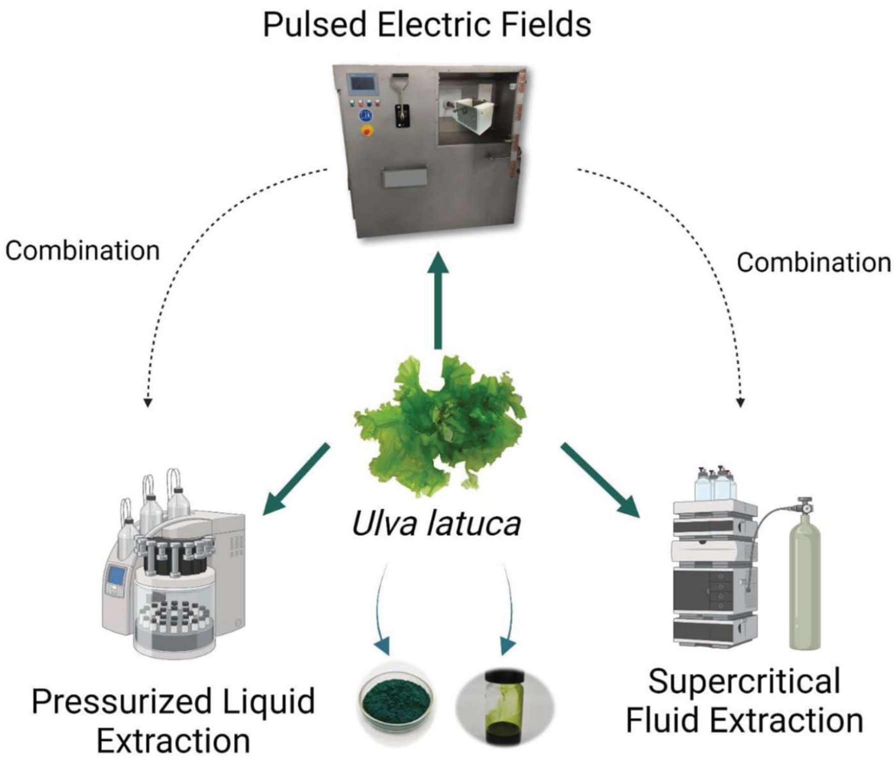

Figure 40 Innovative approaches for the selective recovery of nutrients and bioactive compounds from ulva: benefits and limitations

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EXTRACTION STRATEGIES OF BIOACTIVE COMPOUNDS AND ENVIRONMENTAL CONTAMINANTS FROM ULVA

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Seaweed constitutes an important group of marine organisms with a vast potential as sources of bioactive compounds, which have been gaining attention from the scientific community due to the broad range of biological effects they display, including antimicrobial, anti-inflammatory, antiviral, and antifungal activities. However, marine species have lower concentrations of bioactive compounds than terrestrial organisms, sometimes only trace amounts [1]. In another hand, the possible use of seaweed as bioindicator of environmental contaminants, has also the constraint of dealing with residual concentrations [2,3]. Thus, it is paramount that the extraction techniques available reflect the nature of the organism from which the extract will be obtained, as well as the characteristics of the compound to be isolated. The efficiency of the extraction methods is influenced by several factors, such as seaweed species, solvent selection, extraction temperature, pressure, and time, with optimization of these parameters crucial to maximizing the yield and quality of the bioactive compounds extracted. While traditional methods such as solvent extraction, maceration, and Soxhlet extraction have been widely employed, the concern with more clean and environmentally safe methods has led to the development of more efficient techniques. Innovative extraction methods like supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and enzyme-assisted extraction have emerged as promising alternatives, offering higher extraction yields, shorter extraction times, and better preservation of bioactivity.

Figure 41 Extraction strategies of bioactive compounds and environmental contaminants from ulva

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AN INORGANIC ANTIOXIDANT IN A LIVING SYSTEM IMPACTING ATMOSPHERIC AND MARINE CHEMISTRY: IODIDE IN SEAWEEDS (KELP)

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Brown algae of the Laminariales (kelps) are the strongest accumulators of iodine among living organisms. They represent a major pump in the global biogeochemical cycle of iodine and in particular, the major source of iodocarbons in the coastal atmosphere. Nevertheless, the chemical state and biological significance of accumulated iodine have remained unknown. Elucidation of these questions was the objective of this study. Using an interdisciplinary array of techniques, chiefly relying on synchrotron X-ray absorption spectroscopy, we show that the accumulated form is iodide, which readily scavenges a variety of reactive oxygen species (ROS). We have shown that its biological role is that of an inorganic antioxidant, the first ever to be described in a living system [1]. Upon oxidative stress, iodide is effluxed. On the thallus surface and in the apoplast, iodide detoxifies both aqueous oxidants and ozone, the latter resulting in the release of high levels of molecular iodine and consequent formation of hygroscopic iodine oxides leading to particles, which are precursors to cloud condensation nuclei. When kelp thalli are submerged, this process impacts iodine speciation in seawater. In several aspects, iodide is unique as a biological antioxidant. Among the halides, it has by far the best antioxidant properties; yet, bromide complements it for the detoxification of superoxide. This paper will also report on the latest tomography- and micro-XANES-based findings concerning the subcellular localization of iodine in cells, with implications for its mechanism of uptake, storage and efflux.

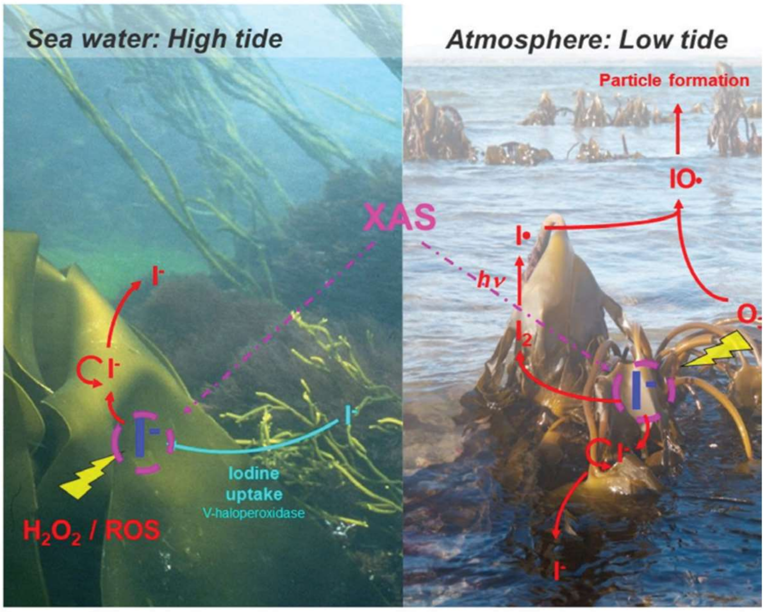

Figure 42 An inorganic antioxidant in a living system impacting atmospheric and marine chemistry: iodide in seaweeds (kelp)

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ADVANCES AND PERSPECTIVES ON SEAWEED METABOLOMICS

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Seaweed metabolomics is an emerging field with immense promise for understanding seaweed biochemical processes, metabolic pathways, and bioactive compounds [1-4]. Metabolomics, which involves the comprehensive analysis of small molecules present in a biological sample, will enable researchers to delve deeper into the metabolic profile of seaweeds and unlock their potential for various applications in industries such as food, pharmaceuticals, agriculture, and bioenergy. For example, perspectives on seaweed metabolomics can be identified in elucidating pathways, identifying bioactive compounds, or the environmental stress response of seaweeds such as the green macroalga *Ulva* [4]. Many recent studies have identified metabolic changes in the thallus of *Ulva* (endo-metabolomics) because of diurnal growth, gametogenesis, proliferation, and environmental stresses [e.g., 3,5,6]. Identifying metabolites affecting Ulva's development in the culture medium (exo-metabolomics) [1] is an additional challenge, considering the low concentration of bioactives. The metabolomics experiment design will be a specific focus of the talk. It requires careful consideration of multiple factors to ensure reliable and interpretable results. For example, it includes sample preparation, which involves extracting metabolites from biological samples while minimizing degradation and contamination. After all, finding unknown compounds is common in metabolomics, although "known unknowns" can be utilized to make descriptive comparisons between treatments like stress stimuli. Under laboratory conditions, metabolomics can detect any changes (e.g., caused by reintroduced bacteria or abiotic stresses) in *Ulva* [7] and benefits from a combined approach with transcriptome data. Finally, multivariate methods must exploit the high covariation between omics measurements. Hereby, the metabolome is of special interest since it integrates all molecular and environmental effects, which will be illustrated in case studies.

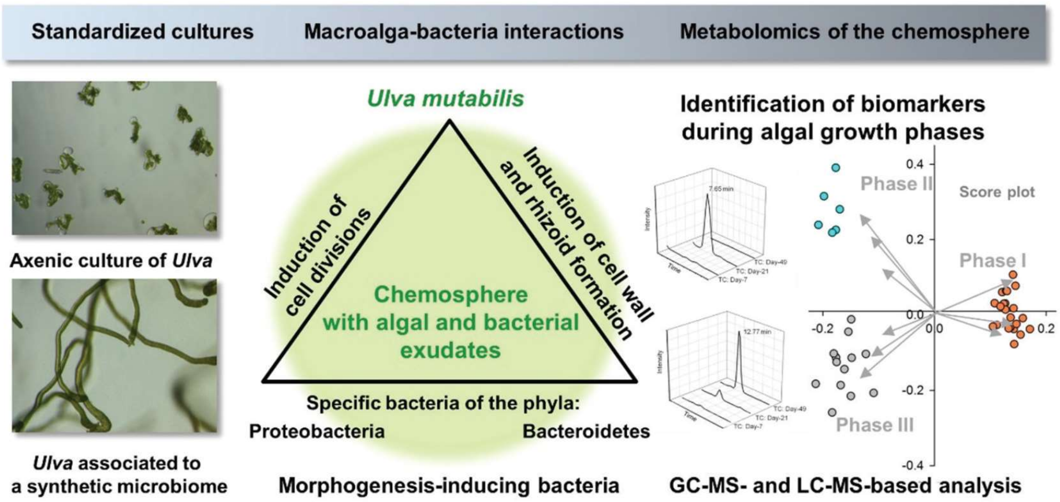

Figure 43 Advances and perspectives on seaweed metabolomics

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LIPIDOMICS OF ULVA: UNVEILING NOVEL INSIGTHS INTO ADDED VALUE AND BIOACTIVE PROPERTIES

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Macroalgae are rich sources of valuable compounds, including polysaccharides, proteins, lipids, phenolic compounds, and pigments. Among these, lipids stand out as essential nutrients, some with notable bioactive properties, making algae a valuable resource for enhanced health [1]. Lipidomics has significantly contributed to comprehending the diverse lipid profiles of *Ulva* species, unraveling their potential as sources of omega-3 fatty acids and membrane polar lipids with promising bioactive properties, as anti-oxidant and anti-inflammatory potential [2]. Also the added value and the lipidomic profile of *Ulva* species may be modulated under varying environmental conditions and seasonal changes. Recently, it was shown that *Ulva sp.*exhibited clear seasonal variations in its lipid profile, with highest abundance of unsaturated fatty acids, including omega-3 polyunsaturated fatty acids (PUFAs), during winter and lowest during summer [3]. Moreover, regardless of seasonal fluctuations, *Ulva sp.* consistently maintained a low n-6/n-3 ratio, associated with health benefits. Analysis of polar lipids revealed variations in glycolipids and phospholipids, with glycolipids, often associated with anti-inflammatory activities, showing higher content during spring and winter, when compared to summer. The phospholipids are increasing notably during autumn and spring. Understanding the modulation of lipid composition and bioactive properties of *Ulva* lipids is crucial for selecting optimal biomass suited for diverse applications, ranging from functional foods to pharmaceuticals.

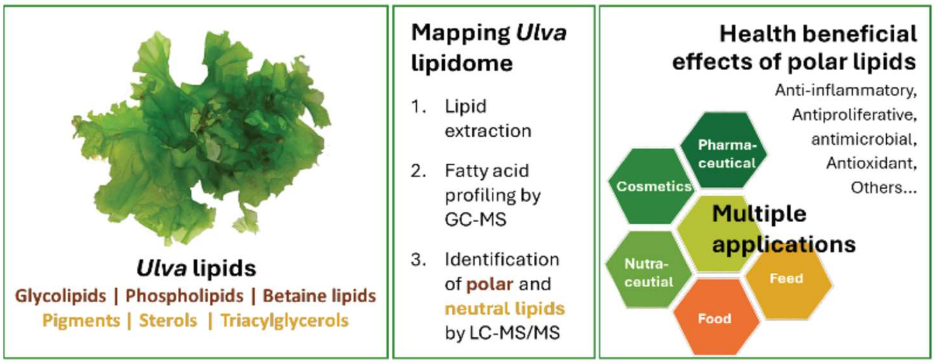

Figure 44 Lipidomics of Ulva: unveiling novel insigths into added value and bioactive properties

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Acknowledgments

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WHEN TOTAL SYNTHESIS MEET LIPIDOMICS...

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A vast array of oxygenated metabolites derived from polyunsaturated fatty acids (PUFAs) has been uncovered and named oxylipins. Radical cascades play a pivotal role in the biosynthesis of these metabolites, with radical initiation occurring either within the active site of an enzyme[1,2] in the extracellular environment or within membrane[3,4] on phospholipids/glycolipids. The advancement of convergent and adaptable chemical methodologies by organic chemists, coupled with the refinement of sophisticated mass spectrometry techniques, has significantly expanded our understanding of PUFA metabolites. Therefore, these oxygenated fatty acid metabolites have emerged as crucial indicators of oxidative stress in marine environments. This presentation will provide a brief overview of the biosynthetic pathways leading to oxylipins, chemical strategies for obtaining pure compounds, and examples of LC/MS-MS quantification of oxylipins in marine matrices such as algae and corals.

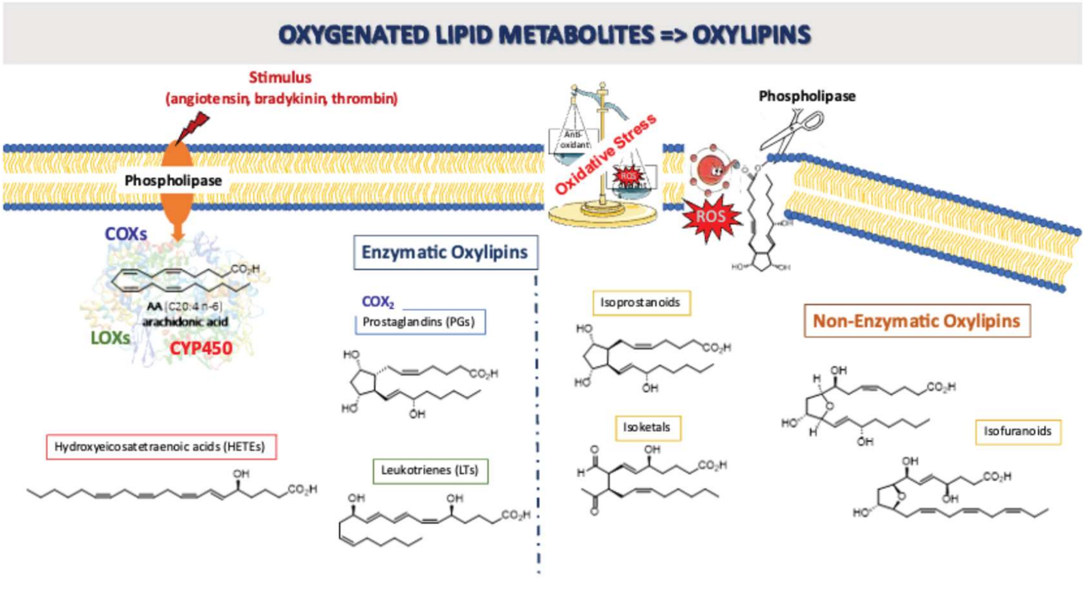

Figure 45 When total synthesis meet lipidomics...

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ALGAPLUS: A PERSPECTIVE FROM A LAND-BASED SEAWEED PRODUCER

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ALGAplus is a private Portuguese company that initiated its activity in 2012, in a property located at Ria de Aveiro with 14 ha. ALGAplus produces customised seaweed and seaweed-based products mainly for the food and well-being markets, reaching the B2B (companies & restaurants) and B2C (retail) sectors with different brands. ALGAplus seaweed are organic certified, assuring warranties of sustainability, quality standards through time and traceability to the customers. At ALGAplus, the production is done under the Integrated Multi-Trophic Aquaculture (IMTA) concept, with a strong focus in R&D and continuous support to the customers. All seaweed production phases are carried out in-house: biomass production in a land-based modular IMTA system, processing (washing, drying, salting, cutting, milling), and packaging. Besides, ALGAplus works with their customers on tuning the seaweed biomass to the desired characteristics or on the domestication of new species of interest, giving them the product differentiation needed in highly competitive markets. The main species in production at this Portuguese company is *Ulva sp.*, however, other species native from the Atlantic ocean are also farmed and commercialized year-round, such as Porphyra dioica, Porphyra umbilicalis, Gracilaria gracilis, Codium tomentosum, among others.

Since 2011, many of the achievements of the company have been reached in the frame of internal and collaborative innovation projects. In 2016, the company reached a milestone with 600 m2 of surface of production, however, this meant still a small production in comparison with the market demand and the upscaling production/processing was mandatory. Since 2020, ALGAplus is actively working on the processes optimization and upscaling, that goes from the nursery to outdoors tanks, including the update of protocols of cultivation. In 2022, ALGAplus reached another milestone with the implemtation of 15 raceways for seaweed production under the same IMTA concept in the frame of the European project GENIALG. Currently, ALGAplus is optimising the system in terms of energy and labour costs, manipulation of production factors according to downstream and upscaling needs. Today, these keystone tasks are being carried out in the frame SeaMark (Horizon Europe) and Pacto da Bioeconomia Azul (02/C05-i01/2022); and also together with strategic partners with expertise in the field of macro- and microalgae, such as GreenCoLab.

Wordwide there has been a significant interest of the academic institutions and companies not only on the upstream processes associated to the cultivation/harvesting of seaweed (and algae in general) but also related to downstream and application of these resources. In this regard, the Algae Vertical (part of the project Pacto da Bioeconomia Azul) was created intending to develop i) methodologies to obtain more sustainable biomass, and also ii) innovative algae-based applications for the nutraceutical and cosmeceutical markets, ready-made meals and novel foods for human consumption, new functional foods for aquaculture and the development of new agricultural solutions (i.e. biofertilizers, biopesticides and microbiota stimulants).

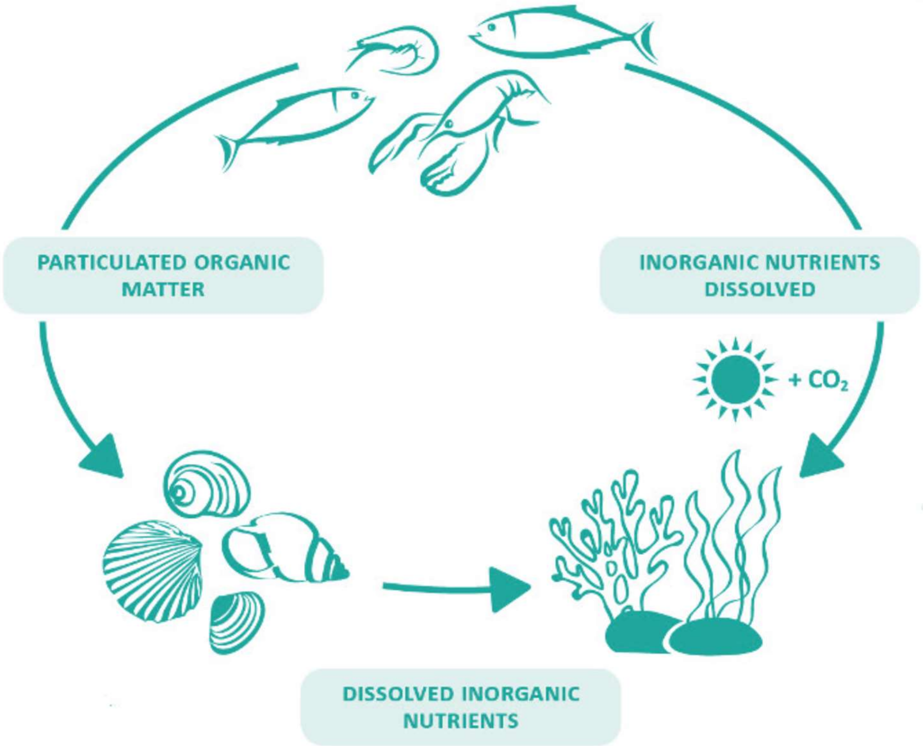

Figure 46 Algaplus: a perspective from a land-based seaweed producer

Acknowledgments

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BRITTANY ULVAN: A MALEDICTION OR A GOLDEN RESOURCE?

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Ulva species present a dual nature: Responsible for numerous strandings, green tides, or blooms worldwide, *Ulva* species are perceived negatively by coastal populations. Algal blooms and strandings have gained significance worldwide due to climate change and anthropogenic activities. They are considered a nuisance due to decomposition and the production of toxic vapors for the tourism industry in coastal areas and marine ecosystems (inhibition of seaweed zygote germination, decrease in algal species growth rates). They result in financial losses for resort operators, who must also cover the costs of removing and disposing of the thousands of beached algae.

Conversely, they have been utilized as vegetables for decades in certain Asian countries and also find numerous applications in the food, feed, or biofuel sectors. Indeed, *Ulva sp.* contains commercially valuable components such as bioactive value-added products (Ulvan, nutrients, starch, PHA). The aim of this presentation is to highlight the potential benefits of harnessing the biomass of *Ulva* species. Various strategies for valorization have been investigated in the domains of human health and material science in our laboratory. Ulvan and oligo-ulvan fractions obtained after Extraction Assisted by Enzyme have been shown to stimulate human dermal fibroblasts proliferation. An increase in the synthesis of extracellular matrix components such as type I and III collagens, and also of glycosaminoglycans has been demonstrated. Fractions also stimulate the expression, synthesis and MMP-1 enzymatic activity. At skin microbiota level, the fractions do not alter the growth of commensal bacteria S. epidermidis, S. aureus and C. acnes, but may alter the biofilm formation. Ulvan and oligo-ulvan fractions also reduce the inflammatory potential of HaCaT keratinocytes induced by C. acnes. In a distinct domain, we have investigated the utilization of *Ulva* and ulvan in materials science, specifically focusing on their impact on the fresh and hardened properties of raw earth materials

24(R,S)-SARINGOSTEROL – FROM ARTEFACT TO A BIOLOGICAL MEDICAL AGENT

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Enhancing the cholesterol turnover in the brain via activation of liver x receptors (LXR) can restore memory in a mouse model for Alzheimer's disease (AD) [1]. The edible Asian brown alga Sargassum fusiforme (Hijiki) contains high amounts of oxysterols such as (3β, 24ξ)-stigmasta-5, 28-dien-3, 24-diol (24[R, S]-saringosterol) that are a potent liver x receptor (LXR) agonists. We aimed to find native European seaweed species with contents of 24(R, S)-saringosterol that are comparable to those found in S. sargassum fusiforme. Additionally, we hypothesize that seasonal variations modify the amount of 24(R, S)-saringosterol in seaweeds. Sterols and oxysterols were extracted with chloroform/methanol from various seaweed species harvested in the Eastern Scheldt in different seasons between October 2016 and September 2017. Identification and quantification of the lipids was performed by gas chromatography-mass spectrometry and gas chromatography - flame ionization detection. We confirmed that brown algae Undaria pinnatifida harvested in February and S. muticum harvested in October contained the highest amounts of 24(R, S)-saringosterol (32.4 ± 15.25 µg/g, mean ± S.D. and 32.95 ± 2.91 µg/g, respectively) and its precursor fucosterol (1.48 ± 0.11 mg/g), higher than S. fusiforme (20.94 ± 3.00 µg/g, mean ±S.D.), while Ascophyllum nodosum and Fucus vesiculosus and F. serratus contained amounts of 24(R, S)-saringosterol (22.09 ± 3.45 µg/g, 18.04 ± 0.52 µg/g and 19.47 ± 9.01 µg/g, mean ± S.D., respectively) comparable to S. fusiforme. In other algae only minor amounts of these sterols were observed. The green algae *Ulva lactuca* contained only 0.29 mg/g fucosterol and 10.3 µg/g 24 (R, S)-saringosterol, while all investigated red algae did not contain any 24(R, S)-saringosterol or fucosterol. In the Eastern Scheldt algae harvested in September/October delivered the highest yield for 24(R, S)-saringosterol, with the exception of U. pinnatifida that showed the highest levels in February. We showed that exposure of lipid extracts of *Ulva lactuca* to sunlight at room temperature or in the presence of oxygen to UV-C light lead to the quantitative conversion of fucosterol into 24(R, S)-saringosterol. Exposing pure fucosterol to UV-light did not convert any fucosterol into 24(R, S)-saringosterol underscoring the requirement of seaweed constituents in the conversion of fucosterol into 24(R, S)-saringosterol. In conclusion, we showed that brown seaweeds harvested from the Eastern Scheldt contain amounts of 24(R, S)-saringosterol comparable to S. fusiforme, varying per season and showing the highest amounts in spring. In accordance with these observations the amount of 24(R, S)-saringosterol in the brown seaweeds can be modulated by light.

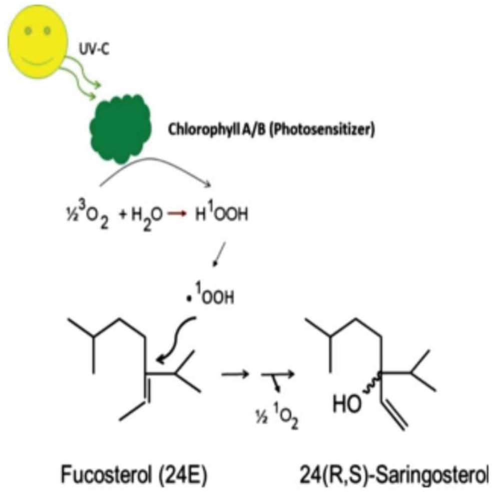

Figure 47 24(R,S)-Saringosterol – from artefact to a biological medical agent

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IMMUNE MODULATING PRODUCTS FROM *ULVA* FOR ANIMAL CARE

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The valorization of opportunistic seaweed biomass arriving in thousands of tons on the coasts of Brittany have allowed OLMIX to develop natural solutions for Agriculture and Animal husbandry based on seaweed. Today the green seaweed *Ulva sp.* containing MSP (Marine Sulfated Polysaccharides) are used in a range of animal-Care products designed to manage mycotoxin risk, digestive health and efficiency as well as immunity.

Scientific proof of efficacy, knowledge of the mode of action and dose effects are all important for regulatory compliance, to define product positioning and provide sales arguments. For these and many other reasons Olmix invests extensively into research on it's actives.

The immune system is continuously challenged in livestock animals and is a key factor of animal performance. This is why this was one of the first issues Olmix addressed with its seaweed extracts and products. Today, demonstration have been made, of the capacity of an ulvan extract from *Ulva sp.* to modulate the immune system in pig, poultry as well as aqua species, both in vitro [1 ,2, 3] and in vivo [2, 4, 5]. Using both reporter cell lines and specific inhibitors, TLR2 and TLR4 have been identified as the main target receptors in different cell types (2, 6). The in vitro activation of heterophils and monocytes by the ulvan extract leads to a temporary release of pro-inflammatory cytokines, including interleukin1-β, interferon α and interferon γ, through partly identified pathways. When ulvan is given per os to animals in vivo the activation of heterophils and monocytes is induced in a similar manner. The same protocol was used to evaluate the impact of a formulated version of this extract, called Searup®. A single oral administration at the dose recommended for use in the farm, results in heterophils and monocytes activation in vivo similarly to the purified extract. The products are also evaluated in field conditions to confirm positive effects on animal performance.

Together our results confirm the capacity of the ulvan extract to modulate immunity in several species that

the formulated solution, Searup®, similarly to the purified extract allow to activate monocytes and heterophils and may provide protection against a larger variety of pathogens including virus. This may explain the recently identified capacity of the ulvan extract, and Searup®, to limit dissemination of Marek's disease virus in vitro and to promote viral reactivation in lymphoid cells.

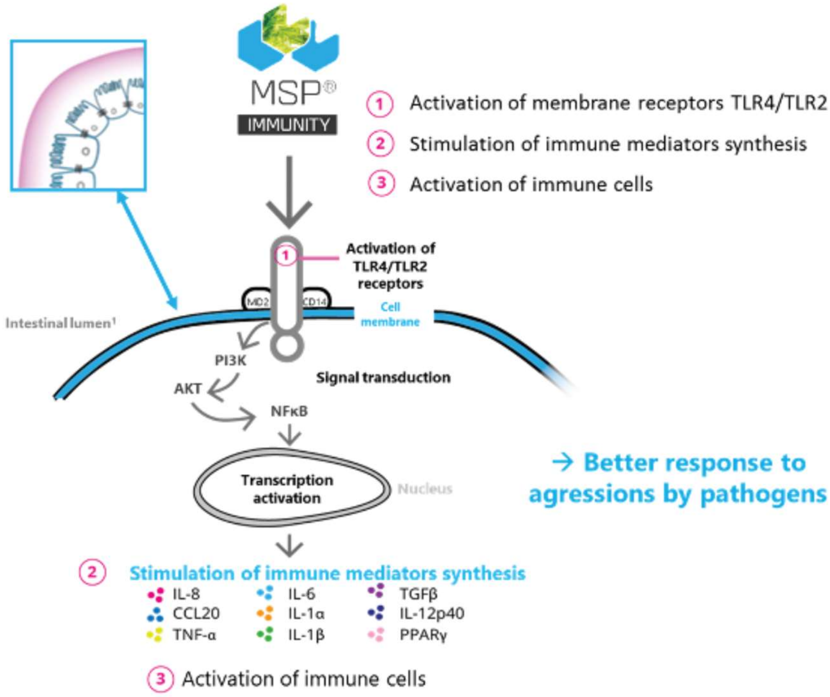

Figure 48 Immune modulating products from Ulva for animal care

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EXAMPLES OF ELISA AND REPORTER ASSAY TO ASSESS HEALTH RISKS AND BENEFITS OF DIETARY MACROALGAE

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There is an increasing interest in using macroalgae as food ingredients and the consumption of macroalgae is considered healthy due to their content in numerous bioactive compounds [1]. However, the safety of seaweed products and the health effects of its consumption are less understood. For example, correct labelling of allergens is an important aspect of food safety to provide consumers with safe food products [2]. Of the established food allergens, which must be labelled according to food regulations, cohabitant fish, mollusc and crustaceans are the most likely source of possibly contaminating allergens that could be found in seaweed products. ELISA (Enzyme-linked Immunosorbent Assay) is the most common and internationally accepted detection method for established allergenic proteins in food by direct antibody recognition of the protein and easy to perform in any laboratory. The principle of the method will be explained, followed by examples of detection of crustacean, mollusc and fish allergens in samples from Norwegian farmed seaweed [3]. Complementary methods for allergen detection will also be discussed. Further, cell cultures are interesting models to simulate conditions of health and disease. ELISA is one of the methods to assess the level of target proteins, mostly in case of secretion from the cells, such as for inflammatory signalling molecules. Here we will discuss the effects of supplementation with extracts from *Ulva* fenestrata and Saccharina latissima on the human monocyte cell line THP-1 with focus on the used methods such as ELISA and others [4].

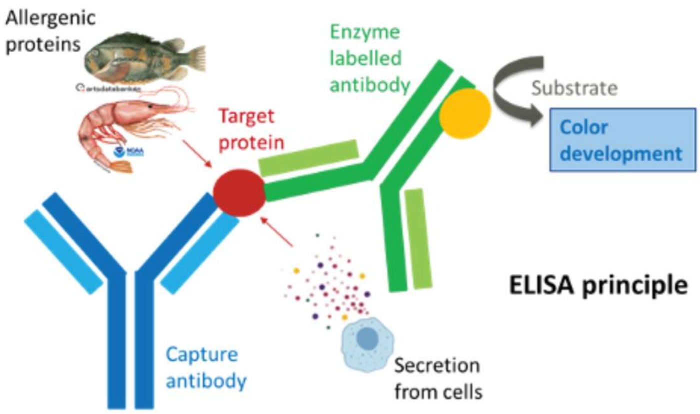

Figure 49 Examples of elisa and reporter assay to assess health risks and benefits of dietary macroalgae

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Acknowledgments

Bettina Wickman Kvamme (Akvahub AS) and the operating personnel from Osland Havbruk AS, Sulefisk AS and Engesund Fiskeoppdrett AS are gratefully acknowledged for collecting, washing and packing the seaweed at the IMTA sites and for funding the allergen project. The work has been carried out under the funding of the Research Council of Norway (326803, 319577, 312947, 326803), Møre og Romsdal country council and Møreforsking AS.

ULVANS AS VERSATILE BIOACTIVE BUILDING BLOCKS FOR THE DEVELOPMENT OF HYBRID BIOMATERIALS FOR BIOMEDICAL APPLICATIONS

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Polysaccharides are highly appreciated as valuable ingredients of biomaterials and are widely exploited in the biomedical field due to their biocompatibility and biodegradability. Marine polysaccharides, incorporating various functionalities and exhibiting interesting physicochemical properties and significant biological activities, are attractive materials for the development of novel systems for bioapplications, such as drug delivery and tissue engineering.

Algal polysaccharides, in contrast to those of animal origin, are regarded as safer and non-immunogenic. Among them, the sulfated polysaccharides ulvans located in the cell walls of green algae of the order Ulvales (Chlorophyta), due to their physicochemical properties and the wide range of biological activities they exhibit, are increasingly studied for applications in the pharmaceutical field [1].

The utilization of ulvans as a biopolymer has not yet been comprehensively investigated. In the recent years, our group has successfully prepared and characterized a number of ulvan-based hybrid scaffolds of various structures, such as membranes, particles, hydrogels, 3D porous structures or nanofibers, and has utilized them for drug delivery, tissue engineering and wound healing, among other applications [2-13].

Our current results highlight the potential uses of ulvans as bioactive building blocks towards the design of hybrid biomaterials for biomedical applications.

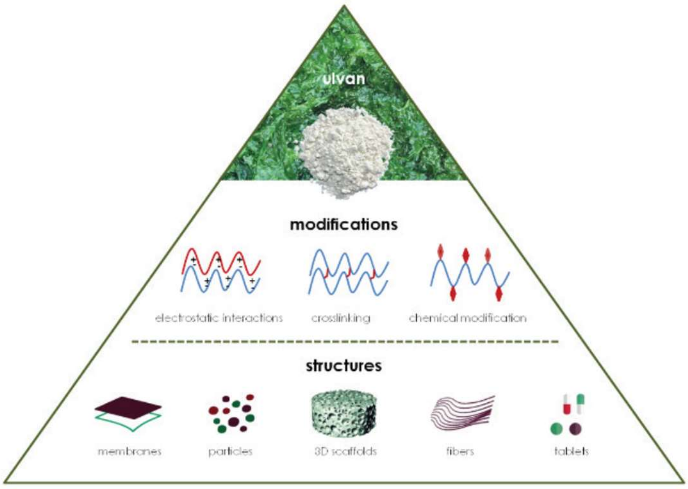

Figure 50 Ulvans as versatile bioactive building blocks for the development of hybrid biomaterials for biomedical applications

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MICROBIAL CONTRIBUTION TO FOOD DIGESTION IN THE GUT OF ALGIVOROUS SEA URCHIN: A NOVEL RESOURCE OF ALGAL POLYSACCHARIDES DEPOLYMERIZING BACTERIA

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An ecological insight into the spatial arrangement in the gut bacterial community of the algivorous sea urchin *Tripneustes gratilla elatensis* will improve our knowledge of host-microbe relations concerning the involved taxa, their metabolic repertoire, and the niches of activity. We hypothesized that alterations in the bacterial compositional structure under different diets and along the gut are associated with the potential contribution to food digestion. Toward this goal, we investigated the bacterial communities in the esophagus, stomach, and intestine of the sea urchin when fed a mono-specific diet of either *Ulva* fasciata or Gracillaria conferta, or an algal-free aquafeed. The study combined 16S rRNA amplicon sequencing, followed by bioinformatics analyses of community structure, interactions, and the prediction of their functional genes.
Sea urchins fed with *U. fasciata* grew faster and their gut microbiome network was rich in bacterial associations (edges) and networking clusters [1]. Bacteroidetes was the keystone phylum in the gut with few microbes of this phylum being central hub nodes that maintained community connectivity, while others were driver microbes that led the rewiring of the assembly network based on diet type [1]. Spatial distribution was evidenced by communities with distinct features in the esophagus and intestine. Bacteria that can contribute to *Ulva* digestion are common in the stomach and intestine and consist of genes for carbohydrate decomposition, fermentation, synthesis of short-chain fatty acids, and various ways of N and S metabolism [2]. Bacterial enrichment was performed in a selective medium with *Ulva* polysaccharides extract or ulvan. Among the isolated bacteria, a novel strain of *Alkalihalobacillus sp.* was found capable of in vitro degradation of several algal polysaccharides [3]. Carbohydrate-active enzymes of this and other bacteria are being used for the development of a bacterial based process for depolymerization of seaweed polysaccharides in a designated bioreactor.

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BASICS, DOS AND DON'TS IN CAROTENOID ANALYSIS

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Carotenoids are widespread and versatile isoprenoid compounds that intervene in many processes in Nature. They are essential in photosynthesis, acting as secondary light harvesters but also in the assembly of the pigment-protein complexes and in photoprotection. They are also important in the communication between species through colour, which is relevant for plant pollination, seed dispersal and for the mating of some animals, such as flamingoes. Some carotenoids are precursors of vitamin A, which is an essential nutrient. Besides, a large body of evidence indicates that these compounds can intervene in biological actions and help reduce the risk of diverse diseases. Importantly, carotenoids can be cleaved into derivatives (collectively termed as apocarotenoids) including aromas, important hormones or many signals that have been shown to play important roles in plants. Taken together, carotenoids and their derivatives are very important for food security and health promotion through the diet [1,2].
The analysis of carotenoids is challenging for different reasons. On one hand, with very few exceptions, they are lipophilic compounds that are located in lipidic environments (membranes, lipid globules, plastids) that make their extraction more difficult. Besides, their structure, featuring a long system of electron-rich conjugated double bonds, make them very susceptible to oxidation and geometrical isomerization. Some carotenoids, especially those with 5,6-epoxide groups, can be easily transformed upon contact with even traces of alkali [3,4].
Rather than dealing with state-of-the-art methodologies for the analysis of carotenoids, this work deals with basics and advice about their handling and choice of methodological aspects. In this sense, advice about sample preparation and storage, extraction, saponification or choice of stationary phases, among others, is given. As a general rule, carotenoid analysis should be done as soon as possible, although this is not always possible. Then, samples need to be stored. Ideally, they should be freeze-dried and kept as intact as possible, as the damage of the cells increase the risk of degradation. The approach for extraction depends largely on the purpose of the analysis. If this is to identify as many carotenoids as possible from a source, large amounts of samples and solvents will be needed. If the goal is to analyze the major carotenoids of a source, microextractions can be performed, with the consequent gain in throughput and savings in solvents. Saponification is usually done to reduce the lipidic content, get rid of interferent compounds (for instance chlorophylls) and/or to simplify the chromatograms when carotenoid esters are present. However, this step can lead to important losses of carotenoids, so it is important to think carefully if it is really a necessary step in our methodology. For many years, C18 reverse-phase columns were the stationary phases of choice for carotenoid separation in HPLC. With the advent of C30 reverse-phase columns, these gained much popularity. The choice of either type of stationary phase is another aspect that can help us optimize our analyses and workflow [3–6].
Last, but not least, it is important to start replacing traditional methodologies for carotenoid analysis with more environmentally-friendly alternatives. In this sense, studies on the replacement of typical solvents for carotenoid analysis (some of which are petroleum-derived and are toxic) for more greener alternatives (2-methyl oxolane, natural deep eutectic solvents, etc.) appear as a timely topic. The recently presented AGREEprep tool, which helps assess the greenness of sample preparation can be very helpful for this purpose [7].

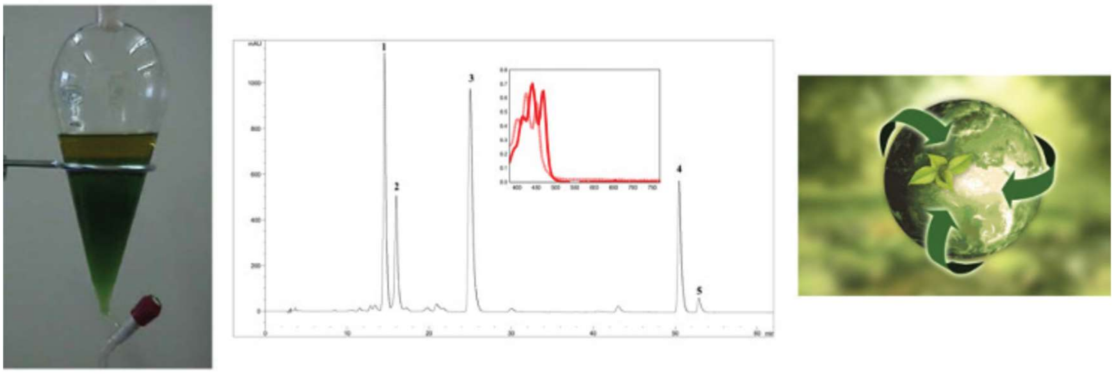

Figure 51 Basics, dos and don'ts in carotenoid analysis

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SEASONAL DYNAMICS OF NUTRITIONAL COMPOSITION AND NUTRIENT DIGESTIBILITY OF *ULVA LACTUCA*: A PERSPECTIVE OF A MEDITERRANEAN LAGOON IN MOROCCO

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Continuously growing world population has increased the demand for plant and animal origin foods thus enhancing the competition for land use and crops between food and feed. This requires the addition of new resources without shifting the balance between the competing forces. Algae growing due to the water pollution in response to the increased human activity present such resources. *Ulva lactuca*, a green algae belonging to Ulvaceae family, has been the specie of great interest for food and feed since its abundant availability in the oceans around the world. Wild growing *U. lactuca* in the oceans is not suitable for use in food because of the bioaccumulation of impurities and toxic materials in *U. lactuca*. Therefore, wild growing *U. lactuca* can be used in animal diets provided a continuous supply of uniform biomass is maintained. Marchica lagoon of Nador, Morocco receives green tides that brings a considerable amount of *U. lactuca* every year that can be valorized in animal diets. However, great discrepancies in the nutritional content of *U. lactuca* have been reported most likely arising from varying abiotic factors associated with seasonal and geographical differences. No earlier study has evaluated the seasonal dynamics of nutritional composition and nutrient digestibility of *U. lactuca* growing in the Marchica lagoon of Nador, Morocco. The resent study was aimed to investigate the seasonality of nutrient composition and nutrient digestibility of *U. lactuca* from Marchica lagoon.

U. lactuca was collected from Marchica lagoon during the year 2022-2023. Collection of samples started in summer 2022 through autumn 2022, winter 2022-2023 to spring 2023. A total of four samples were collected in each season, dried in shade, and finally dried in greenhouse facility in Nador, Morocco. Then, samples were transported to the animal nutrition laboratory in Turkey. The samples were analyzed for nutritional composition according to methods described previously by the Association of Official Analytical Chemists. Fiber fractions were measured using the methods described by Van Soest et al. in an automatic fiber analyzer (ANKOMA2000 Fiber Analyzer, ANKOM Technology, NY, US). In vitro digestibility of nutrients was measured using ANKOM DaisyII incubator (ANKOM Technology, NY, US) after 48 hours of incubation. In situ digestibility of nutrients was measured in in heifers fitted with rumen cannula. Data were subjected to statistical evaluations using appropriate tests in a computer based statistical software package. The study revealed that the nutritional composition and nutrient digestibiilities differed significantly as a function of season ($P < 0.05$).

This study suggested that the chemical composition, in vitro and in situ rumen digestibility of nutrients is dependent on the harvesting season. Accordingly, adjustments are required while including *U. lactuca* in animal diets.

Acknowledgments

The study was partially funded under the Short Term Scientific Mission (STSM) framework of EU COST Action CA20106 - Tomorrow's 'Wheat of the Sea' : *Ulva* a Model for an Innovative Mariculture. The authors gratefully acknowledge Amine Kharoub, Sylvia Strauss, and Stefan Kraan at The Seaweed Company B.V. (The Netherlands) for hosting and assisting in the collection, drying, and dispatch of samples.

IDENTIFICATION AND FUNCTION OF ALGAL GROWTH PROMOTING FACTORS IN *ULVA* (CHLOROPHYTA): ADVANCES IN OUR UNDERSTANDING OF CHEMICALLY MEDIATED BACTERIA-INTERACTIONS

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The marine macroalgae *Ulva sp.*, widespread in aquatic habitats, bears considerable ecological relevance and enormous economic potential across numerous industries such as food, feed, pharmaceuticals, cosmetics, and sustainable biofuel generation [1]. For optimal cultivation, *Ulva* requires specific symbiotic bacteria that release algal growth- and morphogenesis-promoting factors (AGMPF's) [2, 3]. The symbionts interact through chemical signalling molecules. The Ulva-associated Roseovarius sp. cells are chemotactically attracted to dimethyl sulfoniopropionate (DMSP) released by the alga [4], and *Ulva* provides glycerol as a carbon source for Roseovarius sp. [4]. In turn, the bacterium supports Ulva's cell division and growth by secreting unknown morphogens. This is followed by the interaction with the second Ulva-associated symbiont Maribacter sp., which releases thallusin and induces Ulva's rhizoid and cell wall formation [5].

This study embarks on a quest to identify novel AGMPF's and unravel their mode of action. Leveraging highresolution mass spectrometry, it opens doors to structure elucidation, subsequent organic synthesis, and the establishment of structure-activity relationships. These findings could potentially improve our understanding of chemically mediated bacteria-algae interactions in *Ulva* (Chlorophyta).

Thallusin, the first identified morphogen in Ulva's chemosphere [5], exhibits remarkably high activity in the picomolar range [6]. The stereochemistry and the three carboxylic acid groups play a pivotal role in the activity of the steroid-like hormone thallusin. The two carboxy groups in the dipicolinic acid facilitate the iron complex unit, ensuring the acquisition of the Fe-thallusin complex through a siderophore-like uptake system [6, 7]. To monitor thallusin in aquaculture and to determine ecologically relevant concentrations in nature, a highly sensitive method for quantifying thallusin has been developed, capable of detecting concentrations close to its half-maximum effective concentration (EC50) [8]. Thallusin has been quantified in various natural systems, often at concentrations multiple times higher than its EC90 value, demonstrating the precision and sensitivity of our method.

In addition to thallusin, the still unknown Roseovarius-AGMPF would complete a fully axenic cultivation of Ulva. With a sterile-filtered supernatant of Roseovarius sp. supplemented with thallusin, it is already feasible to grow bacteria-free *Ulva* cultures. Initial experiments using solid phase extraction and bioassay-guided fractionation indicate that Roseovarius releases multiple potentially synergistically acting AGMPF's. Moreover, recent observations support a long-standing hypothesis [2] that Roseovarius supports the growth of Maribacter. The partially purified and identified AGMPFs, pave the way for a deeper ecological understanding of the symbiotic network of the tripartite community Ulva-Roseovarius-Maribacter. Understanding the mechanism of algal growth-promoting substances not only improves our understanding of *Ulva* ecology and microbial interactions, but it also opens up new biotechnological possibilities for marine bioactives, both in aquaculture and on land.

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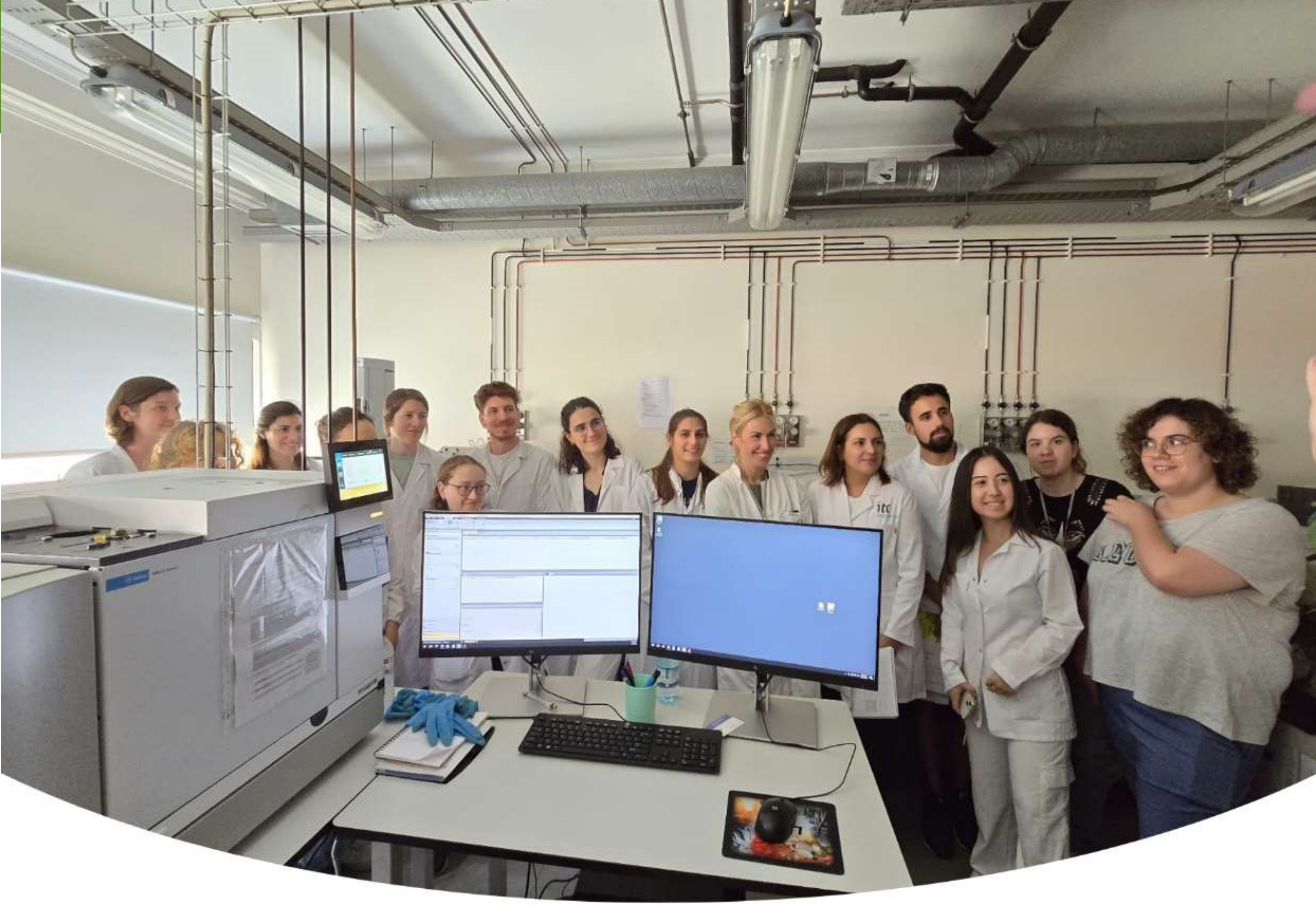

Bioactives in Ulva

Aveiro, Portugal

ABSTRACTS OF TRAINEES

Ulva
The Future Wheat of the Sea

STRAIN IMPROVEMENT: A KEY-ISSUE IN LAND-BASED CULTIVATION OF *ULVA SP.*

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The European seaweed industry relies mostly on wild stock harvesting, which adds pressure on natural populations and displays seasonal variability reflected in biochemical composition shifts and presented phenotypes. Sustainable cultivation is essential to fulfil the demands of locally sourced seaweed of consistent quality, high-grade, traceable and with predictable biomass yield. Biobanking of genetically diverse strains promotes food security, biosecurity and safeguarding biodiversity conservation under the threat of climate change and anthropogenic pressures.

Since 2012, ALGAplus has been cultivating Atlantic seaweed species in an integrated multitrophic system (IMTA). *Ulva* is the species with the most significant production at the company, leading to a special interest in strain selection for this species. In January 2023, the ALGAplus biobank collection counted with 20 strains of *Ulva sp.*

In the framework of the project Seamark (Horizon Europe), ALGAplus carried out multiple sampling campaigns, increasing the number of *Ulva* strains to 104 in biobank. Samples were collected from the natural populations growing in the surrounding earthen ponds, comprehended in the 14ha property, but also arbitrary individuals from our high performing batch of mixed industrial strains, in cultivation since 2022. Novel strains are being screened, in partnership with NUIGalway, based on genotyping and expressed phenotypes, to detect the highest yielding, value added, resilient strains, to factors such as herbivory and environmental variability. The best performing strains will be validated at pilot scale at the company. In this work, the biobanking and preliminary results of screening will be presented.

Figure 52 Strain improvement: a key-issue in land-based cultivation of Ulva sp.

Acknowledgments

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EXPLORING THE BIOACTIVE POTENTIAL OF ADRIATIC SEAWEED (*ULVA SP.*): GREEN EXTRACTION AND MICROENCAPSULATION TECHNIQUES FOR POLYPHENOLICS AND NATURAL PIGMENTS

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Seaweed *Ulva sp.* as a potential innovative product from the sea till now is not examined and sufficiently exploited [1-4]. The main focus of this research was to establish new ways of utilizing *Ulva* and to show the possibility of applying more advanced, green, and safe technologies in the industry as well as to develop new technologies that will preserve valuable bioactive compounds from *Ulva sp.* The sampling of *Ulva sp.* In Boka Kotorska Bay (Adriatic Sea) was performed from June to July 2023. The dried samples were ground into powders in a laboratory mill and prepared for the extraction process. The goal of the extraction process was to be economically justified and environment friendly, based on the principles of "green extraction". The effects of several extraction parameters (solvent type, time, solid-solvent ratio, extraction method) on the extraction efficiency of solid/liquid extraction (maceration and ultrasound) were examined. To determine the influence of total polyphenols and flavonoid extraction, three levels of green solvent (ethanol-water) (30, 50, and 70%) and two levels of the solid–solvent ratio (1:10 and 1:20) were examined. Based on the results, an ethanol-water mixture (50%, solid solvent ratio 1:20) was chosen as optimal extract conditions. With the optimal extraction conditions, maceration was performed for 1h, 2h, and 18h. The next step was a comparison of maceration-MAC (2h) vs. ultrasound-assisted extraction method-UAE (0.5h), with optimal conditions. The maceration method of 2h was a superior (more powerful) extraction method and was used for the production of a higher amount of extract which was subjected to a microencapsulation process. To preserve bioactive compounds, the optimal extract was microencapsulated. The *Ulva sp.* extract, possessed a high content of total polyphenols, chlorophyll A and B, and carotenoids (3.55 mg GAE/g, 84.53 mg/100g Chl A, 68.41 mg/100g Chl B, 5026.82 mg/100 g), respectively. The novel microencapsulation technologies were used for the preservation of *Ulva sp.* Extracted bio-compounds. *Ulva* liquid extract was spray-dried (Labtex ESDTi spray dryer) and freeze-dried (Beta 1-8 Freeze Dryer), with and without carriers' addition. Microencapsulates were prepared using maltodextrin as a conventional carrier and an innovative one, polydextrose. The obtained powders improved Ulva's stability, limiting the inactivation of the sensitive polyphenolic compounds, achieving their high encapsulation efficiency associated with good bioactive protection. The examined microencapsulates demonstrated good powder properties, exhibited high powder yield, parameters important for further food or pharmaceutical applications. Based on the results, it can be concluded that the microencapsulation techniques and carrier addition had an impact on the protection of *Ulva sp.* bioactive compounds. The *Ulva* microencapsulates demonstrated high antioxidant activities determined by DPPH, RP, and ABTS assays. The thermal analysis proved *Ulva* microencapsulates have good thermal stability. FTIR method indicated that microencapsulation developed effective preservation by physical incorporation, not chemical transformation. All fragments from this valuable sea resource may be used as uncostly bio-products rich in functional compounds, which could have commercial utilization and benefit. To overcome the limitations of the *Ulva* application, through the proposed research we intend to make efforts to demonstrate characteristics of *Ulva* species present along the Adriatic Sea. Outputs from this research will provide a source of valuable research data in the promotion and development of Ulvabased blue-biotech industries.

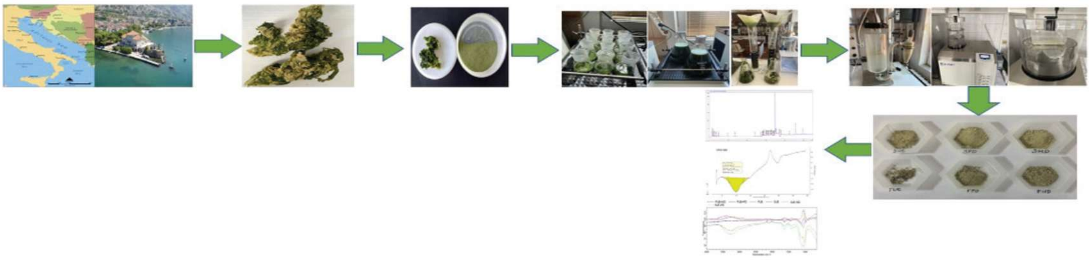

Figure 53 Exploring the bioactive potential of adriatic seaweed (Ulva sp.): green extraction and microencapsulation techniques for polyphenolics and natural pigments

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ASSESSMENT OF *ULVA SP.* RESPONSE TO DIFFERENT ABIOTIC FACTORS UNDER LABORATORY CONDITIONS

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Macroalgae represent a source of high value substances for food, pharmaceuticals, and cosmetic industries. Algae are thus a valuable resource for a greener economy, providing a natural source of a variety of chemicals; they improve the sustainability of industrial processes, thereby contributing to the European Circular Bioeconomy. A variety of abiotic parameters can influence the physiology of macroalgae, such as light, temperature, salinity, nutrient availability, and wave action. Consequently, the composition of algal biomass is affected by these factors, and therefore its commercial use. Within the framework of the MSCA SeaChem Doctoral Network, this PhD project investigates responses of *Ulva* intestinalis under different temperature and salinity regimes. The methods consist of eco-physiological analyses to evaluate changes in photosynthetic activity (oxygen evolution and chlorophyll fluorescence), and biochemical analyses to assess biomass composition (pigments, lipids, and proteins) using HPLC and GC. Further experiments analysing the effects of light and nutrient starvation were conducted in spring 2024, applying the similar analytical approach on *Ulva* compressa, in collaboration with PureAlgae, a startup company based in Denmark. The overall project aim is to determine the optimal growth conditions for the targeted algal species, to obtain the desirable biomass composition. Through identification of the main factors influencing the metabolism of the seaweed, ultimately the accumulation of high value compounds can be enhanced.

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MICROBIAL INTERACTIONS IN *ULVA* HOLOBIONT WITH IMPLICATIONS IN AQUACULTURE

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The continuous growth of Aquaculture and its expected intensification could lead to environmental concerns, such as high nutrient effluent discharges, resulting into eutrophication events, or an abusive usage of disinfectants or antibiotics in culture infection treatments, which could contribute to the spread of antimicrobial resistances. An alternative reduce environmental impact of intensive aquaculture is Integrated Multi-Trophic Aquaculture (IMTA), that portrays the co-culture of species belonging to different trophic levels so that the waste from one is the nourishment for the other [1]. IMTA systems using sea lettuce (*Ulva sp.*) associated to fish cultures have been developed, due to the remarkable biofiltration capacity of *Ulva* in the removal of nitrate and phosphate from the environment, high growth rate, vegetative reproduction and high ecophysiological plasticity [2]. Furthermore, *Ulva* may provide a niche for bacteria that can help prevent diseases in the aquaculture systems. Bacteria of the Rhodobacteraceae family are often abundant on the surface of *Ulva* [3], and, among them, genera such as Phaeobacter have been highlighted as potential probiotics for use in aquaculture [4]. Phaeobacter is described as a good colonizer of abiotic surfaces and also to integrate microbiomes of living organisms, including algae species [5]. These bacteria have the ability to produce Tropodithietic acid (TDA), which is an antibiotic capable of preventing the appearance of pathogenic bacteria in aquaculture facilities such as those belonging to the Vibrio genus [6]. In recent years, an approach combining IMTA recirculation system (IMTA-RAS) with the usage of host-associated beneficial bacteria was proposed [7]. This IMTA-RAS comprised the usage of a co-culture of sole with *Ulva* ohnoi inoculated with Phaeobacter sp. 4UAC3, previously isolated from a wild *Ulva* microbiome. *Ulva* colonized with Phaeobacter reduced significantly the mortality of turbot larvae. However, the disappearance of the bacteria from the *Ulva* microbiome was observed in the scaling up of the cultures, and the effect was attributed to light. Del Olmo et al. [8] confirmed that the inoculated Phaeobacter eventually disappeared in *Ulva* cultures with high light intensities, which was not the case in dark conditions. It was also shown that this disappearance was not directly caused by the light, since on non-living surfaces, such as glass or autoclaved Ulva, the inoculated Phaeobacter did not disappear when high light intensity was applied, hypothesizing that the disappearance of Phaeobacter could be caused by changes in the physiology or chemical microenvironment of the *Ulva* holobiont in response to light. To test this hypothesis, an experiment was carried out in 500 mL flasks, where *Ulva* ohnoi was cultured under the following treatments: a) non-inoculated Ulva-Control in light (UCL), b) Ulva-Phaeobacter in light (UPL), c) non-inoculated Ulva-Control in darkness (UCD), and, d) Ulva-Phaeobacter in darkness (UPD). In UPL and UPD, Phaeobacter was inoculated at a concentration of ~106 CFU·mL-1, whereas UCL and UCD were left uninoculated. Light intensity, in UPD and UCL, was adjusted to 120 µmol photon·m-2·s-1 under a regular photoperiod (12h:12h). The experiments lasted 14 days and for each sampling (0, 2, 6 and 14 days), 4 independent replicates were collected of each condition to analyse Ulva's microbiome (CFUs and 16S rRNA gene sequencing) and metabolome (Liquid and gas chromatography mass and spectrometry). After day 2, Phaeobacter disappears completely from the microbiome of *Ulva* cultivated in light conditions but maintained in darkness. Time and the light regime were the main factors affecting both microbial communities of U. ohnoi biofilm and the chemical compounds of the U. ohnoi tissues. We found that under total darkness compared to regular light conditions, there were marked differences in Ulva's metabolome composition, regardless of the presence of Phaeobacter bacteria. This could be due to compounds produced by *Ulva* in light that could be unfavourable to Phaeobacter. Additionally, different carbon-rich compounds were found in *Ulva* under different light conditions, suggesting *Ulva* promotes different microbial communities in each environment. Targeted analysis showed that TDA was not identified in the controls nor in the UPL treatment at any time, but it was found in the UPD treatment through the four samplings. This knowledge will help to better understand the interactions between *Ulva* and Phaeobacter in order to optimize their co-culture in aquaculture applications.

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ULVA INCLUSION DIET WITH PROBIOTICS SUSTAINABLE FEED FOR THE AQUACULTURE

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Aquaculture is currently the fastest-growing industry in the animal protein production landscape. While being pushed to its limit, high productivity should not compromise the well-being of cultured organisms [1]. In aquaculture, probiotics confer several benefits and play important roles in fish growth and health [2]. In addition, the practical application of probiotics in aquaculture diets could minimize the adverse impacts associated with the use of antibiotics. Recently, we have isolated a novel strain of bacterium Alkalihalobacillus from the gut of an algivorous sea urchin (Tripneustes gratilla elatensis) that was fed on a mono-specific diet of *Ulva sp.* [3]. In vitro studies have confirmed Alkalihalobacillus to be a probiotic bacterium [4]. In a preliminary study, we assessed the Alkalihalobacillus as a probiotic for seabream (Sparus aurata) to feed a commercial diet with different inclusion levels of probiotics to enhance growth, biochemical composition, immune status, and digestibility. Moreover, macroalgae *Ulva* offers an added-value dietary ingredient in formulated diets for fish. Aside from theirbasic nutritional value, they contain antioxidants, pigments, polysaccharides, and bioactive compounds that could benefit farmed fish [5]. However, specific polysaccharides in *Ulva sp.* may act as prebiotics, promoting the growth of beneficial gut bacteria in aquaculture species [6,7]. Future studies will focus on the effects of *Ulva* inclusion in fish diets with probiotic bacteria, which will be a novel approach toward sustainable aquafeed for seabream.

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DIGESTIBILITY, NUTRITIONAL QUALITY AND FUNCTIONAL PROPERTIES OF ALTERNATIVE PROTEIN-RICH EXTRACTS FROM GREEN SEAWEED *ULVA SP.*

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Seaweeds have a significant potential as a sustainable source of alternative proteins of non-animal origin [1-7]. Besides their high protein and dietary fiber contents, seaweeds are garnering attention for their numerous advantages over land-based biomass, including their high mineral and vitamin content, cost-effectiveness and wide acceptance. Despite these benefits, seaweeds are still largely unexplored as food ingredients and their techno-functional and nutritional properties are yet to be investigated. The aim of this work was to characterize the protein digestibility, nutritional quality and functional properties of hybrid protein-polysaccharide extracts obtained from the green seaweed *Ulva sp.* through different extraction protocols and with varying degrees of protein purification. The extraction method was based on a pH-shifting protocol and the application of an ultrasound pre-treatment to disrupt the seaweed cell walls was also evaluated. The gross composition of the extracts was determined, as well as a more exhaustive characterization of the protein and polysaccharide fractions. Subsequently, the digestibility of the whole seaweed and the different extracts was determined by means of in vitro gastrointestinal digestions following the standardized INFOGEST Protocol. Additionally, the extracts' digestibility was compared with the digestibility of traditional plant-based protein sources. Although all the obtained extracts were mainly composed of proteins and polysaccharides, the different steps of the extraction protocol had a significant impact on their composition and functional properties, such as solubility and surface charge. In general, all the extracts showed improved digestibility with respect to the native seaweed, whose digestibility was quite low due to the tough cell walls. However, the presence of polysaccharides in some of the extracts, mainly ulvans, was also determinant on the digestibility, being lower in the extracts with higher amount of polysaccharides. Our results provide a basis for the rational design of strategies to produce nutritious protein-rich ingredients from *Ulva* and evidence the potential of this seaweed as an alternative protein source.

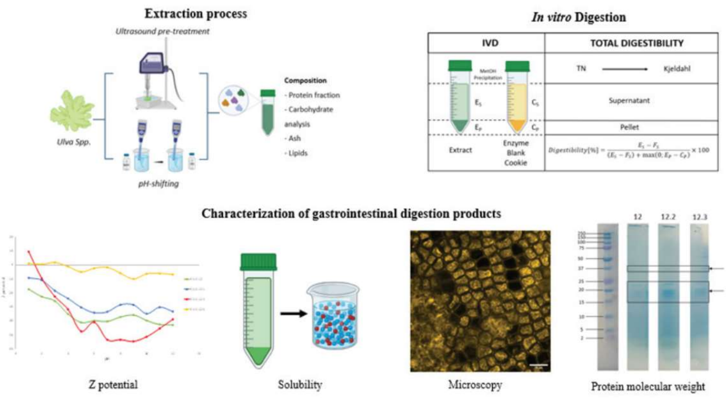

Figure 54 Digestibility, nutritional quality and functional properties of alternative protein-rich extracts from green seaweed Ulva sp.

Acknowledgments

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IDENTIFICATION AND COMPARISON OF PHOTOPIGMENTS BETWEEN *ULVA* LACINULATA AND CLADOPHORA SP. USING HPLC ANALYSIS

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Seaweeds contain different photopigment compositions, which reflect their adaptation to various light environments and ecological niches [1-5]. In this study, we identified and compared the photopigments (chlorophylls and carotenoids) in two green macroalgal strains, *Ulva* lacinulata, and Cladophora sp., cultivated unialgally under identical laboratory conditions, using High-Performance Liquid Chromatography (HPLC). We testified a lot of analytic methods and we concluded to a specific one, which we are going to suggest in this research. Both species shared similar photopigment profiles, with major differences though in the amount of chlorophylls a and b, and carotenoids. The most exciting thing from our results was the identification of fucoxanthin in both species, a photopigment that is not related to the green algae.

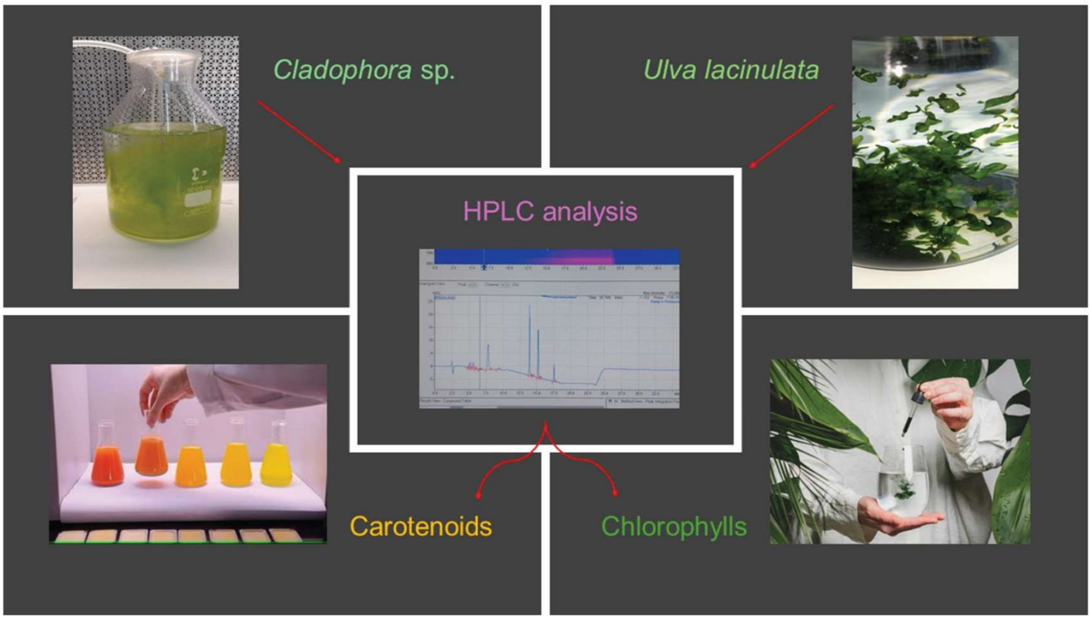

Figure 55 Identification and comparison of photopigments between Ulva lacinulata and cladophora sp. using hplc analysis

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Acknowledgments

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EFFECT OF MACROALGAE EXTRACTS ON SEED GERMINATION AND IN VITRO MICROPROPAGATION OF AQUATIC PLANTS

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Macroalgae, also called seaweed, are multicellular (eukaryotic) organisms [1-5]. Turkey is a country surrounded by seas on three sides. Therefore, there are many algae species in the Mediterranean, Aegean, Black and Marmara Seas. *Ulva* species are especially common in the Aegean Sea. Algae, which has important uses in food, agriculture, pharmacy, medicine and energy fields due to its important contents, has started to be used in in vitro nutrient media and seed germination studies in recent years [1-4]. Therefore, in our study, the effects of *U. lactuca* and Jania rubens extracts on germination of tomato and pepper seeds and on in vitro micropropagation of important aquatic/ aquariums plants Anubias barteri and Micranthemum tweediei (Monte Carlo) were investigated. *Ulva lactuca* and J. rubens species were collected from Izmir Bostancı and Urla seas from June to September. The collected algae were then washed 4 times with tap water and then rinsed with distilled water. After washing, the algae were completely dried in the shade for a week. The dried algae were then roughly ground. 500 g of ground algae was added to 1 liter of sterile distilled water and autoclaved at 121 °C for 30 minutes. The autoclaved samples were filtered through muslin cloth and allowed to cool at room temperature. The filtered solution was stored at +4 °C and used as stock solution. To pre-treat the seeds of tomato and pepper with algae extracts, the seeds were kept in 50 ml of solution containing different amounts of extracts (1%, 2%, 4%, 8% and 16%) at 25 °C in the dark for 16 hours. Seeds used as control were treated with distilled water. Then, two pieces of blotting paper placed in a 90X10 mm petri dish were moistened with 4.75 ml of water and 50 seeds were placed on them. Seeds were germinated in the dark at 20 °C. Seed germination counts were made every other day for 14 days. At the end of the experiment, germination rates and speeds of the seeds were determined. The use of algae extracts especially increased the seed germination speed. MS containing 20 g/l sucrose and 3 mg/l agar was used as the basic in vitro nutrient medium. 10, 20, 40 and 80 ml/l algae extract was added to each of the full, 1/2 and 1/4 strength MS nutrient media [5] and the nutrient media without MS. MS media without algae were used as control. Shoot clumps taken from plants A. barteri and M. tweediei species growing under in vitro conditions were cultured in 100X10 mm glass petri dishes containing MS media with algae extracts. Four clumps of shoots were placed in each petri dish. Shoot cluster diameter, number of shoots, number of roots and root length were counted 4 weeks after the culture initiation in Monte Carlo and 8 weeks after the start of culture in Anubias. Chlorophyll measurements were also carried out in these cultures. Addition of A. barteri and M. tweediei algae extracts, especially to nutrient media containing low amounts of MS, had a positive effect on shoot regeneration and rooting (Figure). Moreover, algae extracts in the nutrient media also increased the chlorophyll content of in vitro growing plantlets. These results we obtained showed that addition of algae extracts to nutrient media at certain concentrations will increase in vitro plant micropropagation.

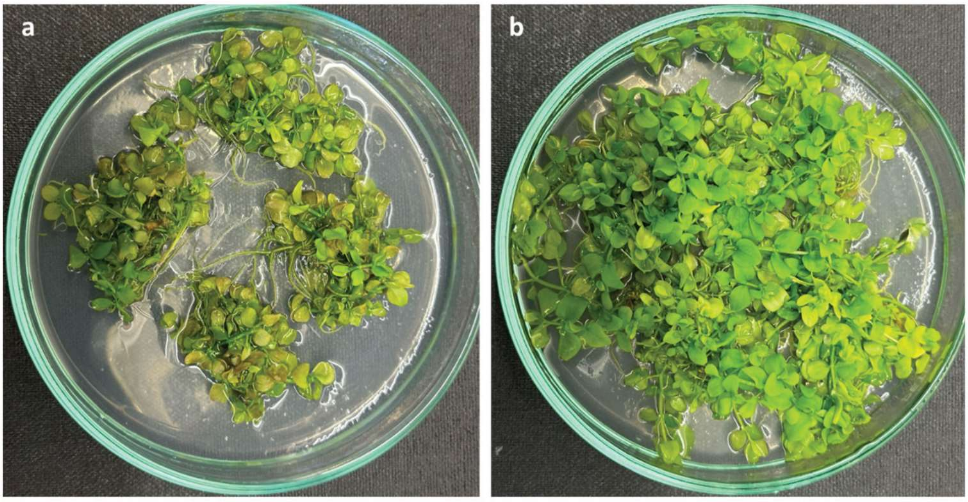

Figure 56 Effect of Jania rubens extract added to in vitro nutrient medium on in vitro micropropagation of Micranthemum tweediei plant. a) Growth in ¼ MS nutrient medium without extract, b) Growth in ¼ MS nutrient medium with 20 ml/l extract.

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ENZYMATIC TREATMENT OF A PHENOLIC-RICH EXTRACT FROM GREEN MACROALGAE *ULVA SP.* FOR THE ENHANCEMENT OF ITS BIOACTIVITY

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Biomass from green marine macroalgae *Ulva sp.* is recognized as a plentiful reservoir of bioactive substances. Specifically, *Ulva* genus species have been demonstrated to metabolize biomolecules with pharmacological potential, including polysaccharides, lipids, proteins and phenols. Alongside, enzymatic biocatalysis is extensively employed for the purposeful biotransformation of natural products to enhance their properties. In this context, a recent work from the Laboratory of Biotechnology at University of Ioannina in Greece demonstrated an increase in the bioactivity of a phenolic-rich extract from green marine macroalgae *Ulva* intestinalis following treatment with two fungal laccases. A comparative investigation was conducted into the antioxidant, enzymeinhibitory, antimicrobial, and cytotoxic activities as well as into the phenolic composition of the enzymatically modified and non-modified extracts. Depending on the laccase utilized, the enzyme-modified extracts displayed improved bioactivities compared to untreated extracts. Specifically, the enzymatically modified extracts exhibited enhanced antimicrobial and enzyme-inhibitory activities along with mild cytotoxicity suggesting that the proposed biocatalytic approach could be employed to produce green macroalgal natural extracts with enhanced biological activities and potential applications in the food, pharmaceutical, and cosmeceutical sectors [1].

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ULVA BIOMASS PRODUCTION IN AN INNOVATIVE BRINE-BASED CULTIVATION SYSTEM: ENHANCING THE NUTRITIONAL QUALITY OF SEAWEED.

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With the exponential growth of the human population and the decline of arable land, food security is a major challenge of this century. In addition to adaptations of traditional agricultural techniques, alternative food sources, such as edible macroalgae, are urgently required to diversify the human food spectrum. In this context, the “food4future” project (https://www.food4future.de/en/home), funded by the German Federal Ministry of Education and Research (BMBF) under the “Agricultural Systems of the Future” program, aims to develop innovative cultivation approaches capable of providing fresh and healthy biomass in urban areas. One creative solution being studied is using marine macroalgae cultivated in brine-based systems. Initial results have shown that regional brine from Brandenburg, Germany, can be utilized to cultivate *Ulva* compressa, even enhancing the nutritional value of this sea vegetable [1]. This approach is promising as it eliminates the need for natural seawater and freshwater usage (required to dilute commercial salt solutions). To enhance the production capacity of innovative systems, the current research focuses on investigating and evaluating the quantity and nutritional value of the produced algal biomass. Apart from different abiotic factors (e.g., light, temperature, or substrates), direct interactions with the associated microbiome influence the development and chemistry of edible macroalgae. For example, the phytohormone-like compound thallusin, produced by the bacterium Maribacter sp., plays a crucial role in the morphology of its hosting macroalga. Thus, it has been identified to affect cell walls and rhizoid formation in *Ulva* compressa [2]. Understanding microbiome data can offer approaches to enhance algae-based nutrient production by engineering its microbiome, particularly in standardized land-based aquaculture systems. In this context, the presented study investigates the cultivability of two different strains of U. compressa: i) a descendant of the isolate of B. Føyn, serving as a model organism for macroalgae, and ii) a more recently isolated strain (by Fricke in 2019). Both strains will be exposed in parallel to the food4future developed brine-based system for at least 3 months. Microbiological composition will be determined at the experimental start and during cultivation. Different cultivation parameters will be monitored (e.g., pH, salinity, minerals), and biomass quantity (e.g., growth rates) and quality (e.g., protein, fat, carbohydrates content, and carotenoid and chlorophyll content) will be determined during the cultivation process. The study will help identify the core microbiome in this innovative system and provide a better understanding of the dynamics in algal biomass production, which is crucial for adapting a suitable harvest strategy.

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GREEN BEAUTY: UTILISATION OF ULVA'S BIOACTIVES FOR ECO-FRIENDLY COSMETIC SOLUTIONS THROUGH ADVANCED ENCAPSULATION AND EXTRACTION METHODS

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In recent years, concerns about the reliability of synthetic substances and their effects on human health have been increasing [1-2]. Alongside the growing public awareness of environmental issues -such as global warming and ecological problems, consumers have started to prefer green, healthy, and chemical-free products [3]. This trend has been reflected in the global market, leading producers to focus on sustainable, natural, and clean ingredients. Concerns about the reliability of synthetic substances used in cosmetics and their effects on human health are increasingly growing. This situation has directed producers towards sustainable, natural, and clean ingredients [4]. Cosmetic raw materials can be obtained from various natural sources such as plants and animals. The use of components obtained from natural sources in cosmetics attracts consumers' interest [5]. Components isolated from marine organisms and algae are also present in the content of many cosmetic products. Nowadays, macroalgae emerge as potential raw material sources due to their novelty and sustainability. It has been found that bioactive ingredients extracted from macroalgae, known as seaweeds, and a range of secondary metabolites have beneficial properties for the skin, such as photoprotective, moisturizing, antioxidant, and anti-inflammatory effects. *Ulva* species belonging to the Ulvaceae family, is a green macroalgae. *Ulva sp.*, a green algae, is a macroalgae family frequently encountered along the Aegean coasts of our country, causing harm to the marine ecosystem, creating odor and visual pollution, and classified as an invasive species when the season comes. Sustainability has become one of the most important issues both globally and in our country. Extraction of bioactive compounds from *Ulva sp.* and encapsulating them by applying a gradual extraction method of the same species will make it possible to develop natural, sustainable, and multifunctional new generation cosmetic raw materials by increasing their stability.

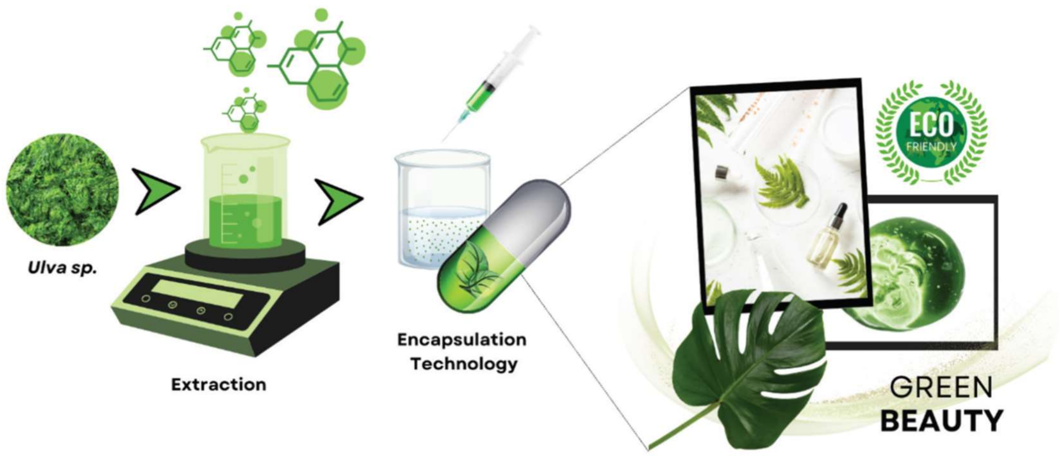

Figure 57 Green beauty: utilisation of ulva's bioactives for eco-friendly cosmetic solutions through advanced encapsulation and extraction methods

By changing the parameters affecting extraction, the extraction method can be optimized by detailed analysis of the bioactivities identified using statistical methods. Turning into particles through encapsulation methods is a highly advantageous feature. Encapsulation is the process of enclosing natural compounds in a protective shell or coating. Encapsulation provides long-term stability to natural compounds sensitive to light and heat. It also enables controlled release of natural compounds, making them useful for drug delivery systems and nutraceutical applications [6]. This way encapsulated bioactive compounds from derived *Ulva sp.* will be valuable raw materials with high added value on their own and can be used for cosmetic purposes.

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TRANSFORMING MACROALGAE INTO VALUABLE PRODUCTS

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Macrocarbon was born out of the Carbon-to-Value Challenge of the German Innovation Agency SPRIN-D in 2023. It is a Canary Islands based start-up developing an integrated supply chain for growing and converting seaweed into sustainable feedstocks for the European chemical industry. Joaquin Ortiz-Cortes will present the solutions that Macrocarbon is developing to achieve large scale, high yield seaweed farming on one side and smaller scale, high value compound farming on the other. The former takes a modular, scalable approach to floating seaweed farming aimed at reliably producing industrial quantities of biomass. The latter focuses on integrated multitrophic aquaculture with local partners, with more controlled conditions allowing for targeted manipulation of environmental conditions.

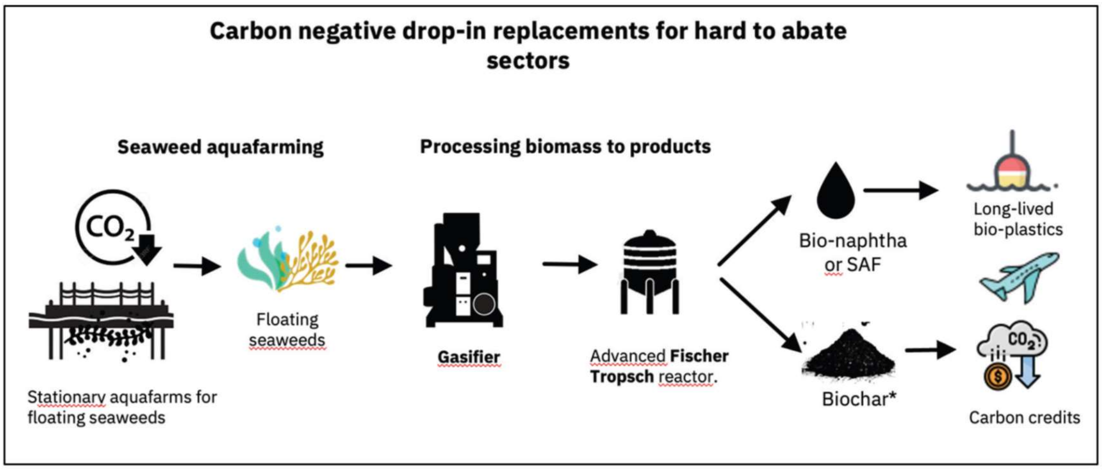

Figure 58 Carbon negative drop-in replacements for hard to abate sectors

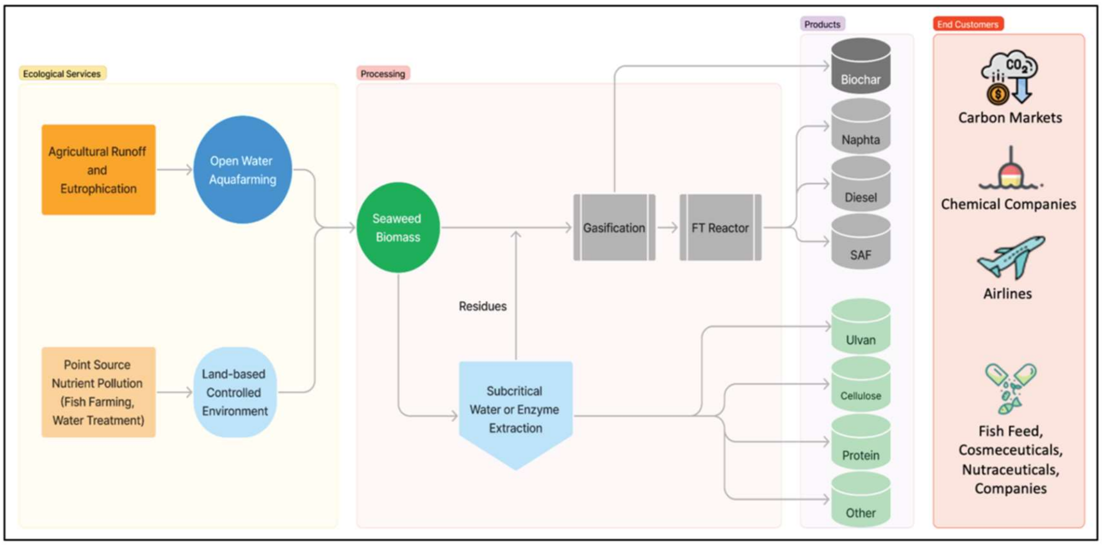

Figure 59 Transforming macroalgae into valuable products

BIOREMEDIATION IN MARINE RAS: TESTING ULVA'S CAPACITY FOR LOWERING TOXIC OZONATION BY-PRODUCTS

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The maintenance of good water quality is crucial for production success in recirculating aquaculture systems (RAS). Ozone (O3) has seen an increase in interest among aquafarmers worldwide as a water treatment tool with a wide array of beneficial effects. Besides its germicidal properties, O3 can enhance solid removal, oxidise toxic nitrogen compounds and degrade a broad spectrum of biogenic and man-made molecules. While its application in freshwater is mostly unproblematic, ozonation of seawater creates by-products termed 'ozone-produced oxidants' (OPO). These can accumulate in a system when process water is recirculated and are detrimental to animal health if species-specific thresholds are exceeded. In this experiment, we tested the seaweed *Ulva sp.* For its OPO-bioremediation capacity in an outdoor IMTA (Integrated Multi-Trophic Aquaculture)-RAS-setup with Gilthead seabream (*Sparus aurata*). Effluent seawater was ozonated and subsequently led through an *Ulva* cultivation unit. OPO-concentrations in the seawater were measured before and after the seaweed unit and the decline was compared to controls without *Ulva*. Additionally, the impacts of OPO on growth, metabolic composition and photosynthetic efficiency of *Ulva* were investigated by comparing OPO-exposed seaweed to controls cultivated without ozonation. 16S rRNA gene sequence analyses were performed to monitor the *Ulva* associated microbiomes in response to ozonation. While seaweed-containing systems exhibited a significantly higher reduction in OPO, ozonation also diminished *Ulva* growth (32%) and caused a darker, fringier, and more rigid morphology. Moreover, ozonation elevated chlorophyll -, total phenolic – and flavonoid contents, and shifted the amino acid composition in *Ulva* towards more glutamine, glutamic acid and serine. With this study we showed that *Ulva* can reduce OPO-concentrations in marine RAS, but with impacts on growth, chemical composition, and morphological properties of algal biomass.

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OPTIMIZATION OF HIGH VALUE COMPOUNDS EXTRACTION FROM ALGAE BIOMASS: SFE AND AUXILIARY EQUIPMENT IN A SEMI-INDUSTRIAL SCALE SCENARIO

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Supercritical fluid extraction (SFE) plays an important role in traditional organic solvent extraction replacement due to its several advantages: higher selectivity and purity, reduced product contamination; minimal organic solvent consumption and lower environmental footprint. In addition, the lack of light and air during the extraction process decreases the highly oxidizable compound degradation. Due to these reasons and among the myriad of methods employed to extract valuable compounds from algae, supercritical fluid extraction (SFE) has emerged as a powerful and efficient technique. SFE has shown significant benefits in high value compound extraction from natural biomass due to its gentle treatment for heat-sensitive materials. Moreover, SFE technique can be developed at different scales: from laboratory scale to pilot plant scale and up to large industrial scale. However, scientific literature has not provided yet an effective approach for this process. Studies based on scale-up are inconclusive [1, 2] because of the interaction among several parameters. The purpose of this work was to highlight the advantages of this technique, design a supercritical extraction system (taking into account factors such as materials, wall thickness, valves, Joule-Thompson phenomena, number and types of separators, among many others) and scale-up the process based on the proposed design. This study provides an overview of the supercritical fluid technique applications on algae biomass, equipment design, technique scale-up and whole process design for the extraction of bioactive compounds. Due to the diversity of algae biomass a process flow diagram was designed, encompassing both processing and post-processing stages. Moreover, various factors such as sample moisture, particle size, matrix effects, etc., were taken into consideration to achieve the extraction objective. The various processing techniques available at the ITC facilities were studied to accomplish an optimal overall process/ an optimization of the whole process.

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SCREENING SEAWEEDS LIPIDS AS SUSTAINABLE SOURCES OF BIOACTIVE AND PREMIUM INGREDIENTS FOR NUTRICOSMETICS

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Lipids and their derivatives play an essential role in maintaining healthy skin since provide a protective barrier that prevents water loss, keeps the skin hydrated, and blocks the entry of external elements [1]. Imbalance of skin lipids have been associated with inflammatory skin diseases (ISD), either to change in metabolism, or due to with oxidative stress, that can induce lipid peroxidation that promote the overproduction of proinflammatory cytokines [2,3]. Polar lipids from seaweeds bearing omega-3 polyunsaturated fatty acids (PUFA) have antioxidant, anti-microbial, and anti-inflammatory properties [4], suggesting that they could be used as new therapeutical agents to prevent or treat ISD [5]. Despite being a highly attractive topic, it remains scarcely explored. Therefore, the research work proposed with the scope of my PhD thesis aims to bioprospecting of polar lipids bearing omega-3 PUFA from different edible seaweeds as a source of bioactive and premium ingredients for cosmetics and cosmeceutical applications as well as for nutricosmetic products. By using high throughput mass spectrometry-based lipidomic approaches as well as in chemico and in vitro models using cells lines, it is expected to highlight the bioactive lipid from green, and *Ulva rigida* and *Codium tomentosum*, and red seaweeds, *Gelidium corneum, Palmaria palmata, Porphyra dioica* for application in cosmetic/cosmeceutics to takle the burden of ISD. Up to this time, we have characterized the lipidome of Gelidium corneum, which is commonly used for agar production and extraction. These processes generate a subsequent biomass residue, which is poorly valorized and this by-product can be a potential source of high-value compounds. The gas chromatography-mass spectrometry analysis performed after transmethylation of the lipid extract revealed a total of 9 fatty acids in both biomass and residue. The arachidonic acid (omega-6 C20:4) and eicosapentaenoic acid (omega-3 C20:5) were more abundant in the biomass, while linoleic acid (omega-6 C18:2) was more abundant in the residue. The lipidome profiling obtained using reverse-phase liquid chromatography-mass spectrometry showed that both biomass and residue contain several classes of phospholipids, glycolipids, and sphingolipids, with a significant decrease of glycolipids was observed in *G. corneum* residue. Interestingly, the lipidome of this seaweed in rich in several classes of ceramides, a predominant lipid fractions present in skin cells, highlighting its potential as a source of ingredients for cosmetics and cosmeceuticals. Both lipid from *G. corneum* biomass and residue demonstrated antidiabetic activity by inhibiting the activity of α-glucosidase, with a slightly higher effect promoted by the lipids from G. corneum residue, and antioxidant activity by scavenging the 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS●+) and the 2,2-diphenyl-β-picrylhydrazyl radical (DPPH●), with a slightly higher effect promoted by the lipids from G. corneum biomass. It is expected that the results gathered under this PhD thesis will contribute to the valorization of seaweeds as sustainable sources of high-value premium ingredients, including bioactive lipids for usage in a variety of industries, such as cosmetics, cosmeceuticals, and nutricosmetics.

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