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Polyphenol extraction from *Alaria esculenta* and *Ascophyllum nodosum* with different process technologies

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Abstract

This study explores the effectiveness of different processing technologies; pulsed electric field processing, ultrasound assisted extraction, and blanching in extracting polyphenols from two types of brown algae; *Ascophyllum nodosum* (rockweed) and *Alaria esculenta* (winged kelp). Algae, known for their high antioxidant, lipid and carbohydrate content, hold significant potential for addressing energy security, climate change and food scarcity. These marine organisms are not only a promising source for biofuel production but are also rich in proteins, vitamins and minerals. Making them valuable for nutritional supplements and various bioproducts.

The research focuses on enhancing the extraction of polyphenols, which are bioactive compounds recognized for their antioxidant properties. To achieve this, the study compares polyphenol extraction from untreated, freeze-dried and processed samples of the algae. The pretreatment methods used in the study include pulsed electric field processing, which creates small pores in cell membranes to facilitate the release of intracellular contents; ultrasound assisted extraction, which employs high-frequency sound waves to disrupt cell structures; and blanching, which briefly heats the samples to inactivate enzymes that might degrade sensitive compounds.

Through comprehensive analyses, including color analysis, dry matter and ash content, weight analysis and polyphenol content, this study evaluates the best parameters and the efficiency of these processing techniques. The results highlight the varying effectiveness of each technique on polyphenol extraction and preserving the nutritional and functional properties of the algae. Among the methods tested, pulsed electric field showed the most promise in enhancing polyphenol extraction while preserving the nutritional and functional properties of the algae

Preface/Acknowledgments

This bachelor thesis is submitted to the Department of Chemistry, Bioscience and Environmental Engineering with the University of Stavanger. The work was carried out from January to June 2024, and the experiments were performed in Nofima AS research rooms and laboratories at Måltidets Hus, Stavanger.

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Contents

List of Abbreviations

ACS	=	Artic Seaweed AS
CIE	=	Commission International de l'Eckaurage
CO_2	=	Carbon Dioxide
NMLK	=	Nordic-Baltic committee on food analysis
PEF	=	Pulsed electric field
PGE	=	Propyl gallate equivalents
SD	=	Standard deviation
TPC	=	Total phenolic content
TS	=	Total solid
UN	=	United Nation
UAE	=	Ultrasound assisted extraction
WA	=	Weight after
WB	=	Weight before
WD	=	Weigh difference
ww	=	Wet weight

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1. Introduction

1.1 Background

Our planet is experiencing a decline in environmental health and resilience, characterized by widespread ecological degradation (destruction of large natural environments), loss of biodiversity and escalating threats to an ecosystem vital for sustaining life. The global average temperature has risen with 1 °C since pre-industrial times (United Nations Association of Norway, 2023c).

Algae represents a versatile and sustainable resource with the potential to address some of the worlds pressing challenges. This includes energy security, climate change and food scarcity. Algae are promising for biofuel production, because of their high lipid and carbohydrate content, which can be converted into biodiesel without competing with food crops (Hannon et al., 2010; Skjermo, 2016). Additionally, algae are rich in proteins, vitamins and minerals, which can be used for nutritional supplements. It has been found that brown algae (*Saccharina latissisma*) can produce 170 tons of biomass per hectare, in comparison to wheat which produce 3-5 tons per hectare. The algae biomass can be used as food for humans, but also as animal and fish feed (Skjermo, 2016). Algae play a crucial role in carbon sequestration, absorbing carbon dioxide (CO_2) during photosynthesis and can be used in wastewater treatment to remove contaminants and nutrients (Hagen, 2008). It can also be processed into bioplastics and various bioproducts. This offers eco-friendly alternatives to conventional plastics and contributes to sustainable agriculture through biofertilizers. Algae contain polyphenols and minerals, like iodine (Skjermo, 2016). The potential for algae to create new economic opportunities and renewable energy sources further underscores their significance.

At the same time as our planet is experiencing a climate crisis, our population is increasing. The world population surpassed 8 billion people in late 2022 and is expected to increase every year and it is estimated to surpass 9.7 billion by 2050 (United Nations Association of Norway, 2023b). United Nations (UN) sustainability development goal number 2 is to eradicate hunger. One of the main reasons for hunger is the increasing difficulties agriculture is facing. The heightened temperature has led to extreme weather, changes in climate and rainfall (United Nations Association of Norway, 2023a). According to the UN, one of the main changes a person

can do to help the climate crisis is to eat more sustainable (United Nations Association of Norway, 2023c).

Food from the sea is often promoted as part of the solution to climate change. It is estimated that we harvest 2 % of our food from the ocean (Skjermo, 2016). Increased utilization of the ocean's resources can contribute to a solution to ensure enough food to meet the global demand. At the same time food from the ocean is often of nutritionally good quality, being a source of essential vitamins, omega-3 fatty acids and proteins (Lock et al., 2022). The ocean can also be a source of completely new food products (Skjermo, 2016). To increase our food production from the sea, we have to source food from further down in the food chain than we do today (Lock et al., 2022). Algae is a resource that can be cultivated as food products for human consumption, as well as feed for farmed salmon and livestock. In addition, the use of algae as food for humans and animals has a low environmental impact when comparing it to other sources (Albrektsen et al., 2022; Lock et al., 2022). Better utilization of oceans' recourses will help lower food shortages and contribute to reduce CO_2 emissions. At the same time, it's only possible if we utilize the resources in the ocean sustainably and respect our planets tolerance limits (Lock et al., 2022).

Despite their potential, algae remain underutilized due to high production costs, technical challenges and regulatory hurdles. The cultivation, harvesting and processing of algae require investment and energy consumption, making them less economically competitive with traditional alternatives. Technical challenges include optimizing algae strains and developing efficient cultivation and harvesting systems (Skjermo, 2016). Regulatory frameworks and market acceptance also pose barriers. Strict approvals are needed for food and pharmaceutical products (European Commission, 2024a, 2024b). Furthermore, limited consumer awareness and price can hinder demand (Hannon et al., 2010). The infrastructure for large-scale algae production is not well developed and can lead to conflicts with other ocean-based uses and facilities. More research and development are needed to improve cost-effectiveness and discover new applications (Skjermo, 2016). Addressing these economic, technical, regulatory and market challenges is essential to fully harness the potential of algae.

1.2 Aim of study

This bachelor study aims to evaluate the effectiveness of different process technologies, pulsed electric field (PEF), ultrasound assisted extraction (UAE) and blanching in extracting polyphenols from two types of brown algae; *Ascophyllum nodosum* (rockweed) and *Alaria esculenta* (winged kelp). The study compares polyphenol extraction from algae (including both the solid and liquid fractions), which are untreated, dried, freeze-dried and processed with PEF, UAE and blanching. The study also focuses on comparing the dry matter and ash content of the algae samples under these different treatment methods to determine which method is most effective in improving the extraction of bioactive compounds. It aims to provide insight into the most effective processing techniques for improving the nutritional and functional properties of brown algae and with that contributing to sustainable food production and better utilization of the oceans resources.

1.3 Algae

The term algae refers to a diverse array of organisms that, despite their taxonomic differences, share common characteristics such as the ability to photosynthesize as primary producers in aquatic ecosystems. This group encompasses cyanobacteria, eukaryotic microalgae and macroalgae. Macroalgae are categorized into three groups; brown, red and green algae.
Each group contains a variety of bioactive compounds with diverse properties that can be utilized for various biotechnological applications (Lomartire et al., 2021).

Algae is an important part of our ecosystem and innovative solutions have been proposed to utilize algae for human endeavors that needs oxygen. Algae can be employed to treat oxygenated wastewater by breaking down pollutants (Hagen, 2008). An example of this is found in Senja, Norway. The company Finnfjord AS, which makes ferrosilicon, collaborates with the Artic University of Norway to cut the company's CO_2 emissions. They use the tanks at the smelter to cultivate diatoms and produces fish feed (Aaraas, 2021; SINTEF, 2022). The diatoms, a kind of microalgae, trap CO_2 from the production (United States Environmental Protection Agency, 2024). This concertation of CO_2 to oxygen can also be used for astronauts in space and treat patients in hospital with severe respiratory conditions or during surgeries that needs external oxygen (Hagen, 2008).

With a rich tradition of incorporating algae, also known as seaweed, into their diets as a staple plant, countries in Asia have long valued seaweeds for their nutritional benefits and medicinal properties (Indergaard, 2010; Wiborg, 1980). There is a growing interest in seaweed for human consumption in the western world, however, it has not reached the same level as in the east. Seaweeds can be utilized as a vegetable or processed ingredient. However, excessive consumption of seaweed may pose challenges for human digestion and should therefore be consumed in moderation (Indergaard, 2010).

1.3.1 Brown algae

Brown algae belong to the class *Phaeophyceae* and are a diverse group of marine multicellular algae. They are predominantly found in cold-water coastal environments. The main pigments responsible for the brown and green color in brown algae are fucoxanthin, chlorophyll a and chlorophyll c (K. Kumar et al., 2008). They range in sizes, all from very small filaments to the largest seaweeds in the ocean (Rueness, 1998). Brown algae can reproduce both sexually and asexually. They produce reproductive organs called sporophytes, which release spores that grow to new individuals (Costa et al., 2024).

Additionally, they contribute to the health of marine life by providing habitats (Norwegian Environment Agency, 2024). Some lager brown algae make up huge kelp forests around the shallow coastal zones (Garcia, 2023) and like forests on land they serve as the environment for a wide array of marine organisms. Smaller red algae grow on the seabed and on the stem of the kelp. These small algae attract crustaceans and mollusks, which in turn attracts fish. The fish attracts sea birds and marine mammals, like otters and sea lions (Fothergill et al., 2020; Norwegian Environment Agency, 2024).

Humans use brown algae in a variety of ways. Brown algae contain alginic acid which can make up to 40 % of its dry weight. Alginic acid is an anionic polymer, called alginates, which can be used as a thickening agent. It is extensively utilized in the food industry, textiles, cosmetics and pharmaceuticals because of its gelling properties, viscosity and stability. Additionally, it has a

strong affinity for heavy metals, making it an effective alternative treatment for aqueous effluents (Bertagnolli et al., 2014).

Norway has perhaps the best conditions in Europe for the development of the algae industry, with the second longest coastline in the world and its rough environmental conditions. This makes it a prime area for the establishment of brown algae production and breeding (Norways Ministry of Climate and Envrioment, 2015; Skjermo, 2016). Seaweed bind CO_2 from the sea water, absorbs nutrients and salts, and use sunlight as an energy source. This can produce a large amount of biomass, helping the environment and contributing to Norway's economy. Seaweed can be used as an ingredient in food, pesticides, fertilizer, medicine and cosmetics (Skjermo, 2016).

1.3.1.1 Rockweed (*Ascophyllum nodosum*)

Ascophyllum nodosum, also known as rockweed, is a brown algae found along the European coast, from the west of Portugal to the north of Norway. Growing on large rocks in the tidal zone, it forms a vegetation belt in the more wind protected areas (Norwegian Institute of Marine Research, 2022).

Figure 1.1 Drawing of rockweed (Halmø et al., 1981).

The thallus (body) of rockweed is brown, giving it the characteristic brown algae color. The shade of brown can vary and may appear olive-green or dark brown. The morphology of rockweed is characterized by a thick body and no central rib. The thallus is covered in air bladders which help the algae float in the water and these bladders have a knotted appearance. A drawing of rockweed is shown in Figure 1.1. The size of rockweed varies, but it typically grows to be between 0.5-2 meters and lives for 40-60 years. The age of the algae can be estimated by counting the number of bladders and adding the age when the first bladder forms, which is typically between 2-3 years old (Norwegian Institute of Marine Research, 2022).

In late spring and early summer rockweed has light brown reproductive organs attached to the sides of its entire body. When they mature the rockweed disconnect them and they spring out new specimens. (Rueness, 1998). Figure 2.1 shows a picture taken in late spring (May), showing the reproductive organs.

In 1970 there was estimated to be around 1.8 million tons of rockweed along the coast of Norway (Baardseth, 1970). Each year, 20 000 tons of rockweed are harvested. Since 1937 the company Algea have harvested rockweed in Norway to use as ingredients in agriculture and in animal feed because of its nutritional content (Algea, n.d.). This is obtained by extraction of hydrocolloid by dehydration. The leftover biomass (70%) is then discarded (Hrólfsdóttir et al., 2022). In Norway there are no regulations regarding the harvesting of rockweed. This is because it grows in places shallower than two meters deep and falls under private law. This means that the harvester only needs permission from the property owner (Norwegian Institute of Marine Research, 2022).

Rockweed is ecologically important as it provides habitat and food for various marine organisms close to shore. A reduction in these habitats will impact the ecological balance. Rockweed is under threat from several sources such as harvesting (Pocklington et al., 2018) and point source pollution (Bellgrove et al., 1997). Studies show that large brown algae like rockweed is expected to be increasingly affected in the future due to local impacts and climate change (Hawkins et al., 2009). To combat the impact of harvesting rockweed, it is recommended to cut less than half of the thallus. A study published by Cambridge University revealed that when less than 50% of the thallus remains, the algae's ability to regulate temperature and light is reduced (Pocklington et al., 2018).

1.3.1.2 Winged kelp (*Alaria esculenta*)

Alaria esculenta, commonly known as winged kelp, is a species of brown algae found along the coasts of the North Atlantic Ocean, particularly in colder regions. It is native to the northern Atlantic, ranging from the eastern coast of North America to the western coast of Europe. This includes the shores of Iceland, Greenland and Scandinavia (Barsanti & Gualtieri, 2014; Nordbakke, 2002). The regional distribution of winged kelp is controlled by temperature, which means that it is not found in areas where summer temperatures exceed 16 °C. During summer the winged kelp can disappear, but it will grow deeper in the sea where the temperature is lower (Barsanti & Gualtieri, 2014; Lüning, 1990). They attach themselves to rocks or other substrates by a holdfast. Winged kelp typically grows in the intertidal and subtidal zones (Nordbakke, 2002).

Figure 1.2 Drawing of winged kelp (Halmø et al., 1981).

The morphology of winged kelp is characterized by long, ribbon-like fronds that can grow up to 4 m in length and 25 cm wide (Barsanti & Gualtieri, 2014; Greville, 1830). The fronds are usually brown or olive-green in color and have a leather-like texture. They are attached to a flexible central rib that helps them to attach the algae to the surface inhibiting it being washed away by strong currents and waves. It is common to see parts of the winged kelp missing, as shown in Figure 1.2 (Greville, 1830). The morphology of the leaves and stems can vary due to different wave exposure and the appearance of the species will vary somewhat depending on the place of growth (Kraan et al., 2000).

One of the distinguishing features of winged kelp are its reproductive structures, which consist of small, dark sporophylls arranged in distinct patterns along the fronds. These structures contain reproductive cells that enable the seaweed to reproduce and spread (Greville, 1830).

In terms of culinary and cultural significance, winged kelp have traditionally been harvested for food in regions where they are abundant. They are rich in nutrients such as vitamins,

minerals and dietary fiber, often consumed in various dishes such as soups, salads and stews. Winged kelp is counted as the best source of protein among the kelp species and can be eaten both raw and cooked (Indergaard & Jensen, 1991). In addition to its culinary uses, winged kelp have also been used in traditional medicine for its potential health benefits (Anis et al., 2017).

However, like many marine species, winged kelp faces threats from habitat destruction, pollution and overharvesting. One of the biggest threats is grazing by sea urchins. The natural predators of sea urchins, like the Atlantic wolffish (*Anarhichas lupus*) have been overfished for a long time (Bluemel et al., 2021), increasing the threat to winged kelp and other seaweed species (Fothergill et al., 2020; Garcia, 2023). Sustainable harvesting practices and conservation efforts are important for ensuring the long-term viability of winged kelp populations and the ecosystems they inhabit (Fothergill et al., 2020).

1.3.2 Conservation of brown algae

Conserving algae with the help of pretreatment methods gives minimal loss of quality and nutritional content while it helps maintain its economic and environmental value. Conserving algae typically involves drying, freezing, blanching, fermentation and acid preservation (Akomea-Frempong et al., 2021), but research and industry developments have given space for new pre-treatment methods like PEF and UAE processing (Both et al., 2014; Janahar et al., 2022).

1.4 Pretreatments methods

The first steps in processing is pretreatment of raw material (Both et al., 2014). As mentioned above, brown algae is a good source of different phenolic compounds. To find out what method extracts the most polyphenols, a series of methods were tested. The methods includes; 1. PEF, which creates small pores in the cell membrane. The increased permeability enables a more efficient extraction of polyphenols (Zimmermann, 1986, as cited in Demirci & Ngadi, 2012); 2. UAE, which uses high-frequency sound waves to break down cell structures (Demirci & Ngadi, 2012), and also enhance extraction of polyphenols (Both et al., 2014); 3. Blanching,

which heats the sample briefly to inactivate enzymes that could degrade sensitive compounds (Fellows, 2009).

1.4.1 Pulsed electric field processing

PEF-processing is a non-thermal process, allowing for production of food that is of nutritional good quality (Demirci & Ngadi, 2012; Y. Kumar et al., 2015). PEF is used in the food industry for food pasteurization, which eliminate pathogens and extend shelf life. In literature, PEF processing is discussed as an energy-efficient and greener way of improving the extraction yield of intercellular components (Eing et al., 2013). An example of this is the increase of lipid yield from microalgae after PEF-processing (Zbinden et al., 2013). PEF-processing is also shown to be more effective than more traditional pre-treatments like freezing to decrease the content of potentially toxic elements in brown algae. Iodine and mercury were significantly reduced (-40 %) after PEF-processing for *Saccharina latissima* (Blikra et al., 2022).

In PEF treatment the product is placed in a chamber between two electrodes and exposed to pulsating electric beams (Y. Kumar et al., 2015), which create small pores in the cell membranes leading to structural changes in the product. These small pores increase membrane permeability to ions and macromolecules. This breakdown of the cell membrane is also referred to as electroporation, a process that can be both reversible and irreversible with complete destruction of the membrane, depending on the strength of the electrical pulse (Zimmermann, 1986, as cited in Demirci & Ngadi, 2012). One of the purposes of PEF is to change the properties of the product (Demirci & Ngadi, 2012) by inactivating microorganisms by the high-voltage pulses rupturing their cell membranes (when used for microbial inactivation). This will lead to a leak of intracellular content and results in a loss of cellar metabolic activity (Janahar et al., 2022) as shown in Figure 1.3.

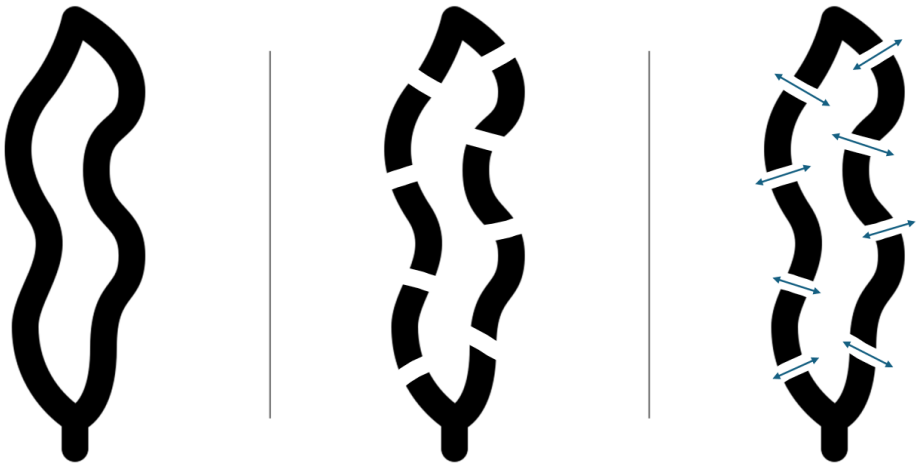

Figure 1.3: Illustration of how pulsed electric field processing create pores in the cell membrane and gives a flow of intercellular content.

PEF alters the structure of the product and can minimize the force needed to change the texture by for example cutting, as shown with red onion (Tantamacharik et al., 2019). In addition, PEF causes electroporation of the cell membrane, which could reduce water content in the product as the holes in the cell membrane led to the release of more liquid from the product. This can give a higher juice yield, for example in sugar beets (Knorr et al., 2001) and a higher extraction yield in microalgae (Carullo et al., 2022).

In PEF-processing a lower energy consumption than traditional pre-treatments (like blanching) is achieved due to reduced temperatures used, resulting in a decreased environmental impact (Demirci & Ngadi, 2012). PEF is often employed as a pre-treatment in industry before drying, as it is showed to improve the drying process (Barba et al., 2015).

Deashing is the process of removing ash, or mineral content, from biological materials (Merriam-Webster, n.d.). In the context of algae processing, deashing is used for producing high-purity extracts. Particularly for food, pharmaceuticals, and cosmetics. Studies show that

PEF technology is an effective method for deashing. The permeabilization of the membrane release intracellular contents. This includes minerals, while leaving other valuable compounds like polyphenols intact (Carullo et al., 2020; Robin et al., 2018).

1.4.2 Ultrasound assisted extraction

UAE is a nonthermal technique that uses ultrasonic waves to improve the extraction of bioactive compounds from bioactive materials, like algae (Adam et al., 2012). Ultrasound waves generate mechanical vibrations that spread through a liquid medium, like Figure 1.4, leading to the formation of microscopic bubbles. These bubbles grow and collapse in a phenomenon known as cavitation as shown in Figure 1.5. The implosion of these bubbles produces intense local pressure and temperature changes and this creates shockwaves that disrupt cell walls and enhance the release of intracellular content (Sanjaya et al., 2022).

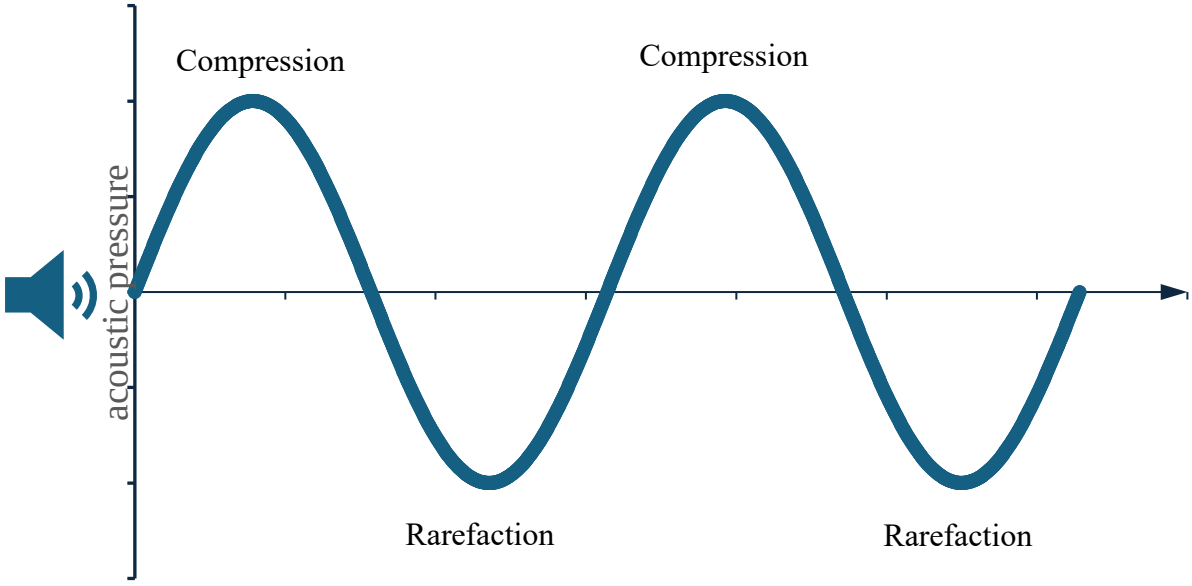

Figure 1.4: A bubble oscillates in phase with the applied sound wave, contracting during compression and expanding during rarefactions, modified from (Leong et al., 2011).

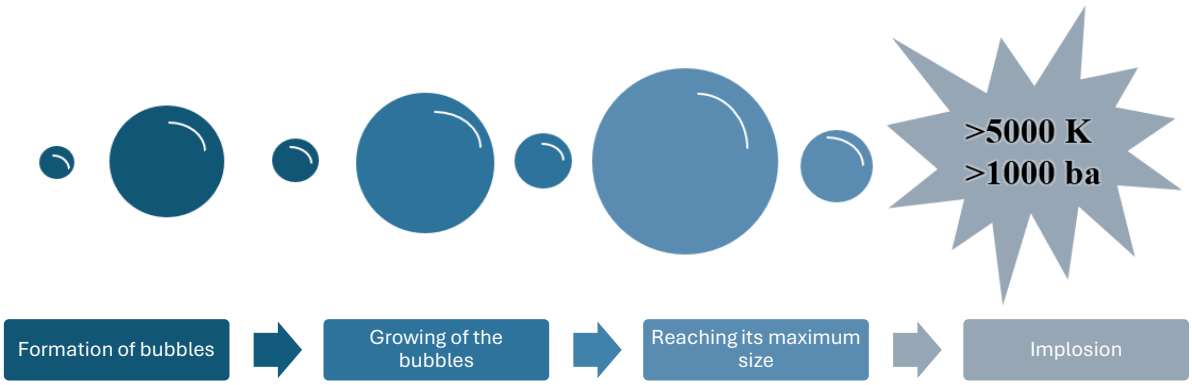

Figure 1.5: The growth and collapse of bubbles in acoustic cavitation process, modified from (Leong et al., 2011).

The development of ultrasonic processing has accelerated in recent years, driven by its potential benefits across various fields such as biology, pharmaceuticals and the food industry (Bui et al., 2020). UAE offers numerous advantages over traditional extraction methods. It provides higher yields in shorter times and reduces the need for large volumes of organic solvents, making the process more environmentally friendly (Sanjaya et al., 2022). UAE is widely used for extraction of essential oils, polyphenols and other phytochemicals from plant materials (Bui et al., 2020).

In algae it is also used for the extraction of lipids, protein and pigments. The unique structure of algal cells, which often have tough cell walls, makes traditional extraction methods less efficient (Bleakley & Hayes, 2017). UAE can increase lipid yield and reduce extraction time compared to conventional methods. For instance, research on microalgae such as *Nannochloropsis osculata* has demonstrated higher lipid extraction efficiencies using UAE, which then can be converted into biofuel (Adam et al., 2012). Beyond biofuels, UAE is also valuable in the extraction of protein and ammino acids. Alkaline extraction is a common process for extracting algal protein. However, alkaline extraction is often not effective, because it is hindered by the tough cell walls (Görgüç et al., 2020). Algae has been reported to have a higher protein content than most plants (Fleurence et al., 2018). A study shows that UAE have a high potential for extracting these proteins effectively (Braspaiboon et al., 2022). Moreover, in wastewater treatment, UAE aids in the breakdown and removal of algal biomass by blocking

the algae's access to sunlight, improving the overall efficiency of the process (Huang et al., 2021).

The cavitation process in UAE disrupts plant cell walls, enhancing the release and dissolution of polyphenols into the solvent. For example, UAE has been shown to significantly increase the extraction efficiency of polyphenols from green tea leaves. The technique does not only enhances the yield but also preserves the antioxidant activity of the extracted polyphenols, making them more effective for use in dietary supplements and food (Luo et al., 2020). The method is also adaptable, allowing for the extraction of polyphenols from a wide range of plant materials under relatively mild conditions, which helps in maintaining bioactivity of sensitive compounds (Bin Mokaizh et al., 2024).

1.4.3 Blanching

Water blanching is a thermal processing technique involving briefly submerging a product (in this case seaweed) in water or steam before being cooled down. Blanching is widely used in the food industry today. It is a common pre-treatment for processes such as drying, freezing and canning (Heldman & Moraru, 2010; Xiao et al., 2017). The effectiveness of blanching depends on the temperature and duration used. Blanching can enhance drying efficiency, increase yield, reduce pesticides residues, improve product quality and texture (Fellows, 2009; Heldman & Moraru, 2010; Xiao et al., 2017). The process works by denaturing enzymes that are responsible for deterioration, such as polyphenol oxidase and peroxidase. By deactivating these enzymes, hot water blanching helps to preserve the quality and extend the shelf life of food (Fellows, 2009).

Brown algae contain a high concentration of iodine, which can be dangerous for humans in high amounts. Research shows that blanching of brown algae lowers the concentration of iodine, without reducing other important compounds like polyphenols (Nielsen et al., 2020).

Despite its benefits, blanching also has drawbacks. Water blanching often leads to significant nutrient loss, as the water-soluble components leach into the blanching water (Xiao et al., 2017).

Moreover, blanching is an energy-intensive process that uses a lot of water. Therefore, there is a need to explore alternative technologies to replace or minimize the usage of blanching in the food industry and to reduce the overall production energy consumption (Heldman & Moraru, 2010).

1.5 Bioactive compounds

Bioactive components are interesting to extract because they offer numerous health benefits, including antioxidant, anti-inflammatory, antimicrobial and anticancer properties (Samtiya et al., 2021). Marine algae are rich in bioactive compounds. Extracting these components from natural sources like algae is environmentally friendly and economically valuable (Tan et al., 2020)

Studies have shown a positive relationship between the phenolic content and the antioxidant activity in algae. Polyphenols are therefore considered one of the primary contributors to antioxidant capacity seen in brown algae (Athukorala et al., 2006).

1.5.1 Phenolic compounds

Polyphenols are a collective term for molecules with complex phenolic structures. The fundamental unit in polyphenols consists of a phenolic ring. Phenolic rings consist of one or more benzene rings attached to one or more hydroxyl groups (-OH) (Al Mamari, 2022) as shown in Figure 1.6 a. They can be categorized into phenolic acids and phenolic alcohols (Abbas et al., 2017). Phenolic compounds are categorized into three classes; simple phenols, consisting of a single phenol unit; flavonoids, which consist of two phenol units; and tannins, consisting of three or more phenol units. The last two classes are known as polyphenols, and each major group is further divided into subgroups (Al Mamari, 2022). Polyphenols can be classified depending on the potency of the phenolic ring. The primary classes of polyphenols include phenolic acids, flavonoids, stilbenes, phenolic alcohols and lignans (Abbas et al., 2017). An example of a phenolic acid is shown in Figure 1.6 b. Phenolic compounds and antioxidants are major contributors to the antioxidant activity in plants (Ratnavathi, 2019). Polyphenols are often found in free form (water-soluble) in water, but can also be bound to the cell wall (insoluble) (Abbas et al., 2017).

(a) (b)

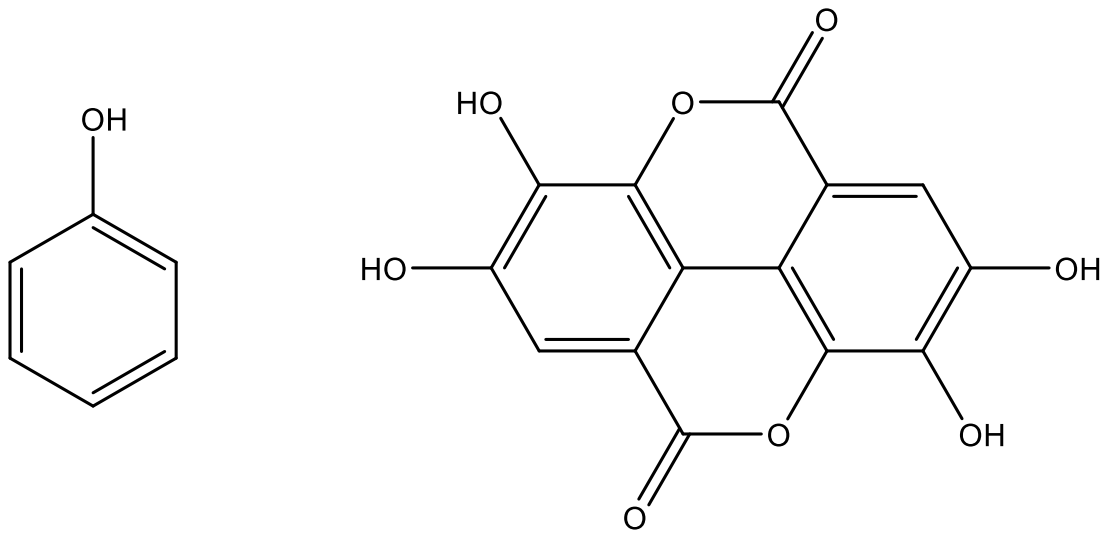

Figure 1.6: Chemical structure of a phenol group (a) and ellagic acid (b), an example of a polyphenol found in algae (Sharifi-Rad et al., 2022).

Polyphenols are used as a protective layer for plants, as they absorb light from different wavelengths and the plant uses them as protection against UV-B rays, thus preventing cell death (Dixon, 2021, as cited in Cheynier et al., 2012). They are also used as color pigments (anthocyanins) to give a strong color to flowers to attract animals that disperse seeds or pollinate. Some polyphenols repel predators by giving a bitter taste to the plant (Oancea, 2021, as cited in Straßmann, 2021). Furthermore, polyphenols can be used as a bio stimulant in agriculture, as they enter the soil they will have a positive effect on decomposing microorganisms (Horner et al, 1988, as cited in Hättenschwiler & Vitousek, 2000). Elevated polyphenol levels leads to greater conversion of plant material, enhancing the nutrient density within the soil (Northrup, 1995, as cited in Hättenschwiler & Vitousek, 2000).

The quantity of polyphenols present in algae varies depending on the season and species. It has been documented that brown algae contain more polyphenols than green and red algae, shown in Table 1.1 (Castejón et al., 2021).

Table 1.1: Total phenolic content of seaweeds extracts (*A. esculenta, Palmaria palmata* and *Ulva lactuca*) produced by the investigated extraction methods; hit water extraction and pulsed electric field (Castejón et al., 2021).

	Total Phenolic Content (mg GAE/g dry weight)		
Seaweed species	HW	PEF	HW+PEF
A. esculenta (brown)	8.94 ± 0.79	9.37 ±0. 41	8.30 ± 0.59
P. palmata (RED)	1.85 ± 0.12	1.81 ± 0.10	1.76 ± 0.10
U. lactuca (GREEN)	1.95 ± 1.10	1.59 ± 0.10	1.71 ± 0.05

HW = hot water extraction, PEF = pulsed electric fields-assisted extraction; PEF+HW = combination of botch techniques. (*n =3*). All numbers were converted from µg/g to mg/g.

Polyphenols are bioactive compounds known for their antioxidant properties. The antioxidant effect of polyphenols is primarily related to the phenol rings, which act as electron traps by foraging free radicals such as peroxyl, superoxide anions and hydroxyl radicals. The more interconnected the phenol rings are, the more potent the compound becomes in neutralizing free radicals (Wang et al., 2009). Additionally, polysaccharides also possess high antioxidant potential. Sulfated polysaccharides, such as fucoidan and laminarin, can be found in brown algae and exhibit antioxidant properties (Moroney et al., 2015), highlighting its rich antioxidant profile (Rupérez et al., 2002). Extracting them from algae presents an opportunity for multiple applications due to their health benefits and potential industrial uses. These strong antioxidants profiles and anti-inflammatory properties make them valuable in the food-and pharmaceutical industries (Pastore et al., 2009; Rupérez et al., 2002), where they can be used to develop supplements and medications (Pandey & Rizvi, 2009). Additionally, the natural origin of these compounds appeals to the growing consumer preference for plant-based and sustainable products (Cardello et al., 2022).

1.6 Analysis

Analysis involves the systematic examination and interpretation of data in order to understand and determine essential features (Merriam-Webster, 2024). Analysis of algae involves identifying its chemical components and their behavior to understand its nutritional value, ecological role and potential industrial applications (Anis et al., 2017).

1.6.1 Color analysis

The food industry places an significance on color and visual preferences (Lee et al., 2013). Color is linked to the chemical composition of food and commonly assessed using variables from the CIELAB color space coordinate system. CIELAB was established as an internal standard by the Commission International de l'Eckaurage (CIE) in 1976. It employs a three-dimensional model with vertical (L*) and horizontal (a* and b*) axes. The L* value ranges from 0, representing black, to 100, represent white, describing the brightness. The a* axis indicate positive values for reddish hues and negative values for greenish hues, while the b* axis indicates positive values for yellowish hues and negative values for blueish hues (Rodríguez-Pulido et al., 2012). This is represented in Figure 1.7. The advantage of this system is that each variable can be assessed independently from the others.

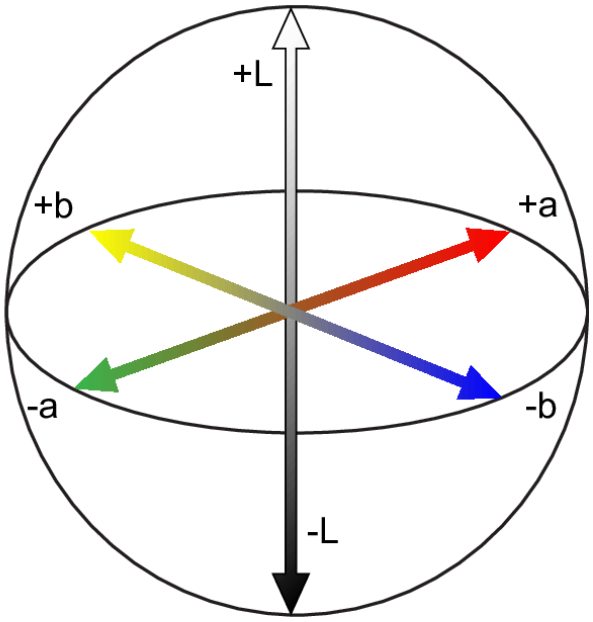

Figure 1.7: Model of CIELAB Color Space: 3-D representation of perceptible color change (Bisulca et al., 2012).

DigiEye is a color measurement system and software used in a range of industries. It consists of a digital camera, placed on top of an illustration box. The pictures are taken and processed by the DigiEye software in a computer. The system uses digital images and measure color in millions of pixels (MacDougall, 2002). This enables the measurement for larger areas, even when color distribution is not homogeneous (Fernández-Vázquez et al., 2011). The illustration

box can be enclosed to eliminate ambient light, allowing for contactless color measurement of objects. The light source, CIE standard illuminant D65, simulates natural daylight with a color temperature of 6400 K. The object is illuminated by two lamps positioned at 45° angles on each side (MacDougall, 2002). The system is shown in Figure 1.8.

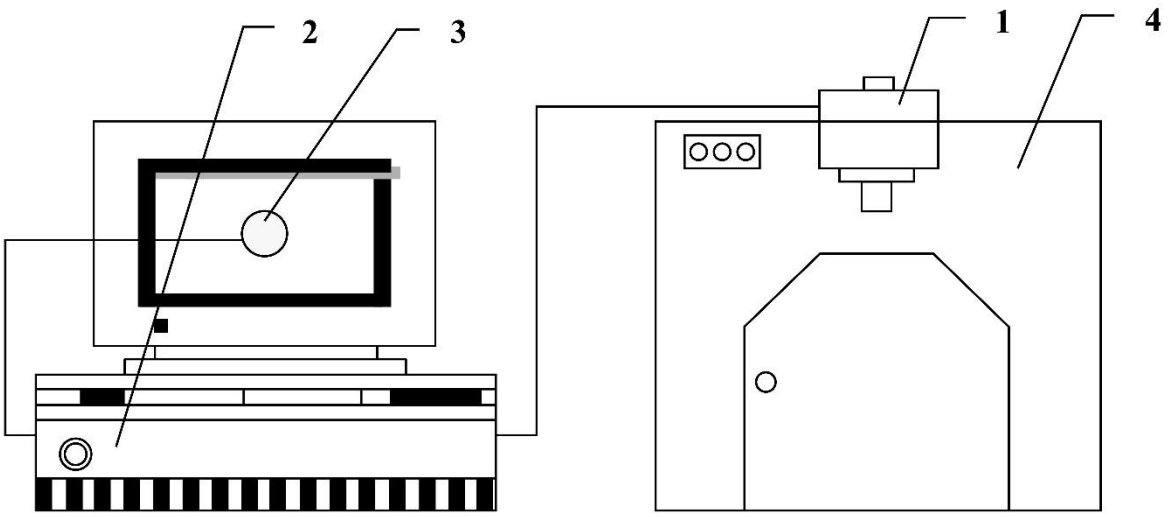

Figure 1.8: The DigiEye imaging system consists of a digital camera (1), computer (2), color sensor (3) and illustration box (4) (MacDougall, 2002)

Color analysis can serve various purposes across different fields. Researchers at Tjärnö Marine laboratory, University of Gothenburg determined nitrogen content in algae using color image analysis and made a color guide accessible for seaweed farmers- and researchers (Stedt et al., 2022). Nitrogen is a major component of the chlorophyll molecule (Fathi, 2022). To calculate protein, the Kjeldahl methods is a widely used technique. It is a method used for determining nitrogen content in organic compounds and calculate the amount of protein based on the results (González López et al., 2010). Based on the study from Tjärnö Marine laboratory, the protein content can be determined by color analysis in the same way as with the Kjeldahl method. Showing how color can estimate multiple nutritional components of algae (Stedt et al., 2022).

1.6.2 Dry matter and ash content

Dry matter content is a parameter in the analysis of biological materials, including plants and algae. It represents the portion of the material that remains after all the water has been removed. This is typically done through drying at a specified temperature until a constant weight is achieved. This measure is essential for understanding the true concentration of nutrients and bioactive compounds in the sample, as water content can significantly dilute these components (Zhu & Lee, 1997).

Dry matter content in algae is significant for several reasons, particularly in the context of biofuel production, nutritional supplements and industrial applications. In biofuel production, the dry matter content of algae is directly related to the efficiency and yield of biofuel conversion processes (Gallagher et al., 2017). Algae with a high dry matter content are preferable because they contain higher concentrations of lipids, carbohydrates and proteins. These are essential for producing biodiesel, bioethanol, and other biofuels (Trivedi et al., 2015). The dry matter content of algae is also important for understanding their value as dietary supplements, considering that algae are rich in essential nutrients, including proteins, vitamins and minerals. By analyzing the dry matter content, researchers and manufacturers can determine the actual concentration of these nutrients in the algae biomass (Wu et al., 2023). In industrial applications, the dry matter content of algae will affect the processing and handling of the biomass. High dry matter content is beneficial because it reduces the volume and weight of the biomass which makes it easier and more economical to transport and store (Gallagher et al., 2017).

Ash content refers to the inorganic residue that remains after a biological sample is completely burned at high temperatures, typically around 500-600 °C (Liu, 2019). This residue consists of minerals such as calcium, magnesium, potassium, and trace elements that are essential for various physiological functions in plants and animals (Vassilev et al., 2017). The determination of ash content is a standard procedure in food, feed, and agricultural industries to assess the mineral composition and overall quality of the material (Liu, 2019).

It has been shown that algae have high levels and variations in ash content. It has also been shown that algae that grow in certain locations contain ash levels as high as 70 % (Liu, 2017). High ash content in food and feed could indicate a rich mineral profile, which is beneficial for nutritional purposes (Liu, 2019). However, excessively high ash content could also imply contamination with soil or other inorganic materials, which again could affect the digestibility and safety of the product. In algae, ash content analysis is important for understanding the mineral composition and potential applications in nutraceuticals, fertilizers and biofuel production (Liu, 2017).

1.6.3 Freeze-drying

Both freeze-drying commonly used for drying seaweed because fresh seaweed biomass contains large amounts of water (up to 70-90 %), which increase the volume and weight of the algae (Amorim et al., 2020). Removing water not only makes storage easier, but also slows down growth of microorganisms. Enzymatic and non-enzymatic processes may occur during drying, potentially affecting the chemical composition of phytochemicals and antioxidant properties (Capecka et al., 2005). However, drying also prevents decomposition, increases shelf life and aids in the extraction of some chemical compounds (Ito & Hori, 1989).

Freeze-drying is a process that begins with freezing a product, turning the moisture content to ice. The next step, primary drying, involves creating a vacuum that make the ice sublimate. This means that it goes from solid ice to vapor without going into a liquid phase in between. This requires carefully applied heat to help the sublimation process. After the primary drying, secondary drying removes any residue moisture by increasing the temperature gradually under vacuum. Freeze-drying preserves nutritional value, flavor and texture of products, as the temperature is low during the process. It is also shown that products that are freeze-dried have an extended shelf life and rehydrate quickly without liquid being added. However, the process is expensive and slow, often taking hours to days. It also requires specialized equipment and careful control of processing parameters (Prosapio & Lopez-Quiroga, 2021).

Despite the high production costs and high energy consumption associated with freeze-drying, it is considered the best method for producing high-quality dried products (Prosapio & Lopez-

Quiroga, 2021). Research indicates that freeze-drying better maintains the nutritional composition of certain seaweeds compared to other drying methods like oven, which can be more suitable for protein extraction (Wong & Cheung, 2001). This highlights that the choice between freeze-drying and oven-drying can also depend on the specific properties and intended use of the dried product. In summary, both freeze-drying and oven-drying have specific applications.

2. Methods

2.1 Raw materials

2.1.1 Rockweed

Fresh rockweed was harvested before undergoing the experiments. The rockweed was harvested from Emmaus, Stavanger. The brown algae were found during low tide, around 2 meters from the shore. During harvest, wrack siphon weed (*Vertebrata lanosa*, a red algae) had grown on the rockweed as shown in Figure 1.2 but were removed during cleaning.

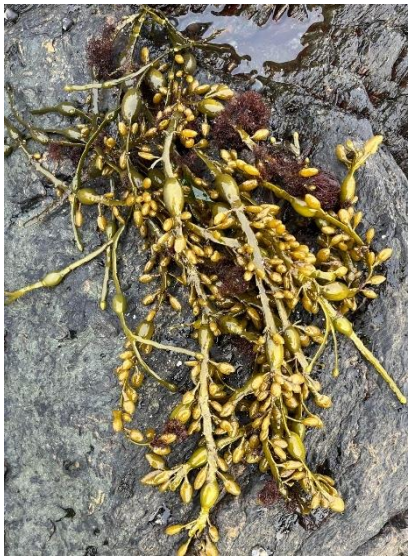

Figure 2.1: Rockweed from early spring, red algae shown growing on it.

To obtain sustainability and ensure protection of the environments and the organisms in the area, the harvesting was distributed over a larger area so as not to clean the seabed of this specific species. A picture of some of the area is shown in Figure 2.2 a.

During harvest, the rockweed was cut 2/3 up the stem. This is to ensure the attachment organ remained, and the rockweed could grow back. After harvest, the rockweed was placed in a cooler bag with a cover with no sea water and transported to Nofima Stavanger. Around half an hour after harvesting it was placed into a refrigerator at 4 °C in a closed environment, without water as shown in Figure 2.2 b. For each day of experiments around 8-10 kg of rockweed was harvested.

Figure 2.2 (a) Area where rockweed was harvested and (b) fresh rockweed stored with a lid and no water in a refrigerator.

2.1.2 Winged kelp

The harvesting of farmed winged kelp was done by Artic Seaweed AS (ACS), Flekkefjord. Winged kelp where grown on ropes in ACS farm in Askøy and was reached by boat. The ropes with algae were then hoisted into the boat, simultaneously cutting the algae and the rope with scissors.

Figure 2.3: Winged kelp and sea water in insulated buckets.

Right after harvest the winged kelp was placed in insulated buckets with sea water covering the algae as shown in Figure 2.3. After harvest the buckets was kept in a car awaiting travel from Askøy to Stavanger. The winged kelp arrived at Nofima laboratories, Stavanger at 12:30 the next day.

Figure 2.4: Winged kelp and sea water drained in mesh strainers at Nofima laboratories.

At Nofima laboratories the kelp was taken out of the seawater. Approximately 2 kg was then drained for 10 minutes, moving it around halfway through. It was then kept in a dark refrigerator at 4°C.

2.1.3 Harvest coordinates

A detailed map of where rockweed was harvested is shown in Figure 2.5, with location being spread in a general area of Emmaus, Stavanger. A detailed map of where the winged kelp was harvested is shown in Figure 2.6, in one area of Trætteosen, Askøy. A Table with information of the common names, location in map, coordinates and date harvested is shown in Table 2.1.

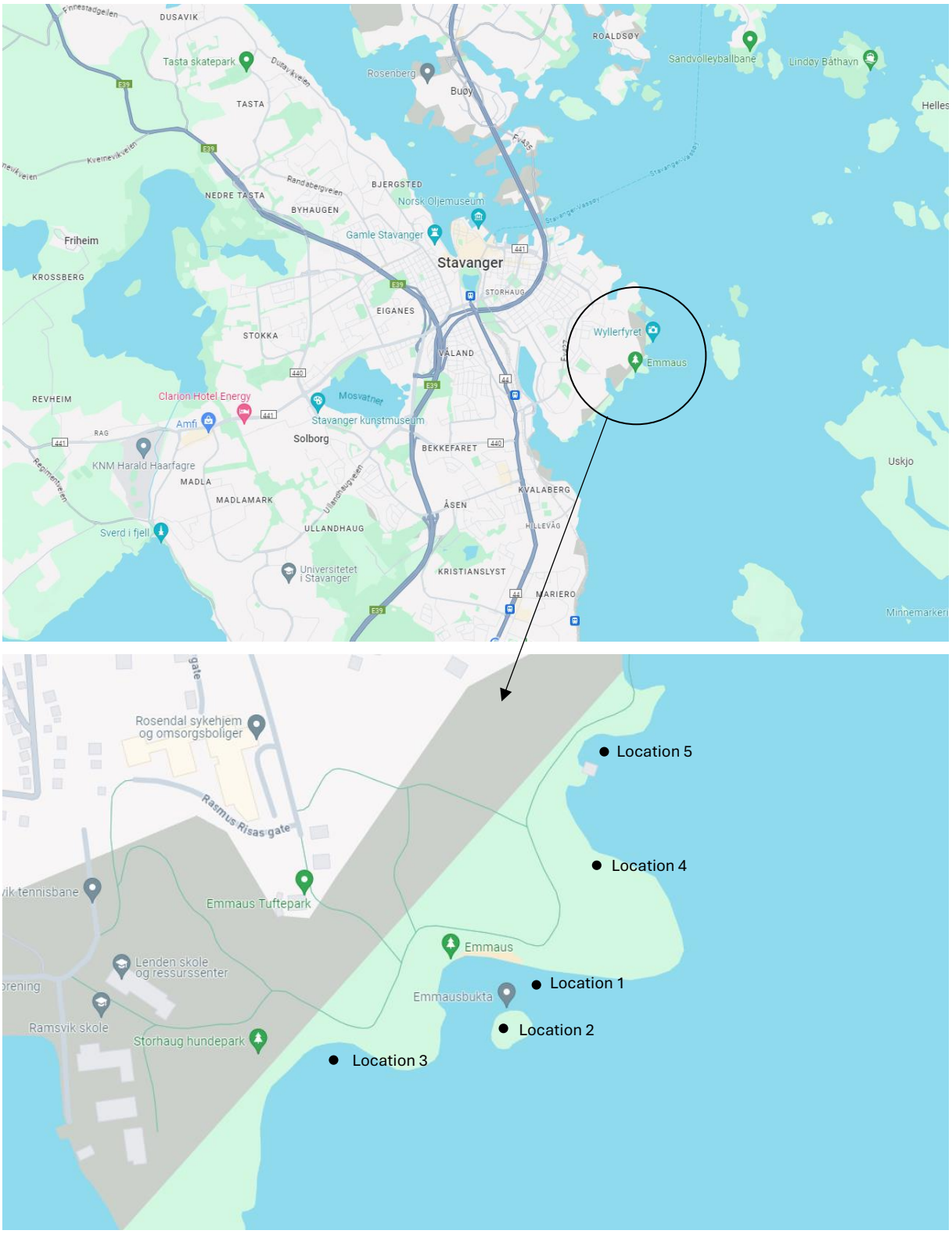

Figure 2.5: Map of the collection points for harvest of rockweed in Emmaus, Stavanger (Google LLC, n.d.-b).

Figure 2.6: Map of the collection points for harvest of winged kelp from Trætteosen, Askøy (Google LLC, n.d.-a).

Table 2.1: Overview of collection locations and associated coordinates for collection samples.

Place	Species	Location in map	Coordinates	Harvest date
Emmaus, Stavanger	Rockweed	1	58.958747, 5.766880	08.01.24
		2	58.958482, 5.766393	18.03.24 21.03.24
		3	58.958305, 5.764399	20.03.24
		4	58.959510, 5.767422	11.03.24 14.03.24
		5	58.960231, 5.767557	12.03.24
Trætteosen, Askøy	Winged kelp	6	60.497613, 5.014545	23.04.24

2.2 Preparation

2.2.1 Storing

All brown algae samples, both before and after extraction, were stored in the same dark refrigerator with a temperature of 4 °C, only being taken out when used. The algae- and water samples were constantly covered with aluminum foil to minimize light exposure as much as possible, to avoid break down the polyphenols and was only uncovered when in use.

2.2.2 Sampling

It was conducted four experiments, named A, B, C and D. Where experiment A was a pre-experiment to decide the best parameters to use for rockweed under each process to extract the most polyphenols. Experiment B and C were the main experiments for rockweed with chosen parameters, an added experiment for freeze-drying, and analysis like color, dry matter, ash content and weight. Experiment D was the main experiment for winged kelp, with the same

parameters as rockweed and color- and polyphenol analysis. Figure 2.7 shows a flowchart of the similarities between experiments A, B, C. Experiment D (winged kelp) looks the same but was only ground once (before process).

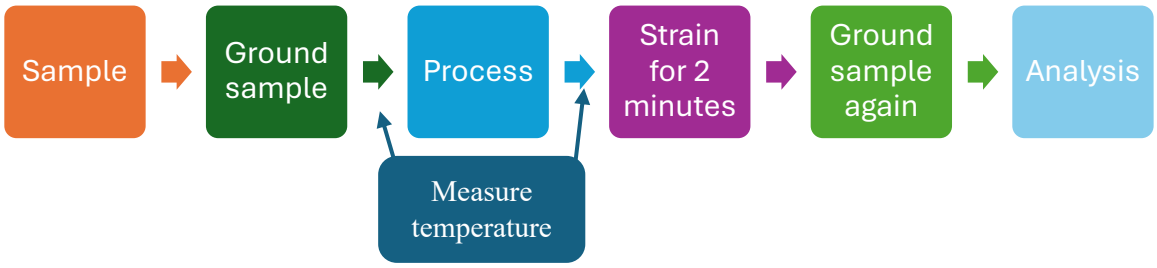

Figure 2.7: Shows treatment similarities between experiments A, B and C.

For experiment A, the algae were harvested, processed and analyzed in the same week. For experiment B and C, the algae were harvested less than 16 hours before each experiment. The process-experiments and the color- and polyphenol analysis were done the same day. This was done to combat any polyphenol-loss. For experiment D, the process-experiments and analysis were done the same and the next day as harvest. A detailed Table of experiments, algae species and dates of processes and analyses is found in Table 2.2. The dry matter analysis started the same day as each polyphenol analysis for experiment C. The ash content analyses began the day after the dry matter analysis.

Table 2.2: Shows information about which experiment with what algae species, date the algae was harvested and date and what process was done, date and what analysis was done.

Ex	Algae species	Date harvested	Date ex	Process	Date of analysis	Analysis
A	Rockweed	08.01.24	13.01.24	PEF	19.01.24	Polyphenol (water sample)
					25.01.24	Polyphenol (Algae sample*)
		30.01.24	31.01.24	UAE	01.02.24	Polyphenol
			01.02.24	PEF	03.02.24	
			05.02.24	Blanching	06.02.24	
B		11.03.24	12.03.24	Blanching	12.03.24	Polyphenol and color
		12.03.24	13.03.24	PEF	13.03.24	
		14.03.24	15.03.24	UAE	15.03.24	
C		18.03.24	22.03.24	Freeze-drying	22.03.24	Polyphenol and color
		20.03.24	21.03.24	Blanching	21.03.24	
		21.03.24	22.03.24	PEF UAE	22.03.24	
D	Winged kelp	23.04.24	23.04.24	UAE	23.04.24	Polyphenol and color
			24.04.24	PEF Blanching	24.04.24	

Ex = experiment
* Frozen

2.2.3 Preparation for analysis

Right before experiment A, B and C, the rockweed was put in a bowl chopper (Robot-Coupe R5, 2 V) for 10 seconds and a picture of the chopped rockweed is found in Figure 2.8 a. After each experiment and before the analysis the rockweed was placed in an immersion blender (Braun Vitro, 300 W) on full speed for 20 seconds and a picture of the blended rockweed is found in Figure 2.8 b. Figure 2.9 shows a simplified workflow of this process.

Figure 2.8: Rockweed before and after 10 seconds in a bowl shopper (a) and Blanched rockweed before and after 30 seconds in immersion blender (b).

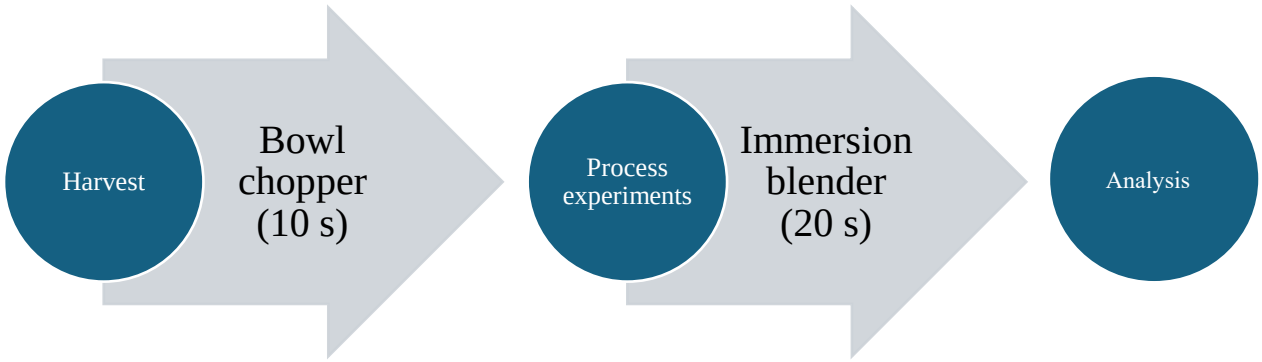

Figure 2.9: Preparation or analysis for rockweed.

For the winged kelp, it was first drained in pools of 2 kg for 10 minutes. The algae were shaken after 5 minutes. Before each experiment the algae were put in a commercial meat grinder (Figure 2.10 a), and result is shown in Figure 2.10 b. Nothing was done with the winged kelp between the experiment and the analysis, The winged kelp was not ground between experiments and analysis because it did not change texture when ground the second time.

Figure 2.10: Picture of (a) grounding process and (b) showing the difference between whole and grounded winged kelp.

2.3 Experiment A

To optimize PEF, UAE and the blanching techniques for rockweed polyphenol analysis, a series of pre-experiments (Experiment A) were carried out.

2.3.1 Pulsed electric field

Samples of brown algae underwent PEF-processing by PEF Pilot Dual (Elea Technology, Germany). The brown algae were placed in tap water at room temperature right before going

into the PEF-machine and strained for 2 minutes straight after. The temperature was measured both before and after the PEF-treatment. The PEF treatment was performed with the following machine settings; 24 kV, 50 Hz (frequency) and 6 μs (pulse width). The energy used was determined with formula 6.6 (Blikra et al., 2022) (Appendix I) and shown in kJ/kg.

For the rockweed harvested 08.01.24 (A1) an experiment was conducted to determine the best ratio to use for the machine. Two samples with a pulse count of 1000 were conducted with a ratio of 1:5 and 1:3 of seaweed and water. A sample with a pulse count of 4000 and a ratio of 1:5 was also conducted. These samples were named A, B and C, and with one biological parallel each. A control sample was made by adding a ratio of 1:5 rockweed in tap water at the same time as one of the samples and strained at the same time as said sample for 2 minutes. The control sample was in tap water for around 2.5 minutes.

After the first pre-experiment it was decided to keep using a ratio of 1+5. The rockweed harvested 29.01.24 (A2) underwent PEF treatment two days after harvest. The experiment as described earlier in this section, with the same amount of straining time and with the same machine settings. The pulse counts were changed to find the best suitable pulse count (Experiment C and D). The pulses tested where 1000, 1500, 2000, 2600, 3000 and 3400 and was given names according to the pulse count (PEF1000, PEF1500... to PEF3400). These samples had two biological parallels each.

2.3.2 Ultrasound assisted extraction

All algae samples underwent UAE-processing with an Ultrasonic cleaner (Ultrasonic Power Corporation, USA), with 3 generators (Model 5300, Ultrasonic Power Corporation, USA) with different voltages. A picture of the machine is shown Figure 2.11 (a). Using four different voltages (40, 68, 170, 170+68 kV), three biological parallels foe each voltage was treated for 30 minutes. The samples were named UAE40, UAE68, UAE170 and UAE170+68 (A-C). The machine was filled with approximately 16 liters of tap water, before being turned on for 5 minutes. Approximately 100 g rockweed and 500 g tap water (in a ratio of 1+5) is used for each parallel. The brown algae and tap water were placed in glass jars with lids, with the same temperature as the water in the machine (approximately 16 °C). Each glass jar was placed with

similar distance between them and the walls of the machine. A weight was placed on top of each jar to keep them under water during the treatment process. A picture of the glass jars placed in the machine and with weight is shown in Figure 2.11 b. After the UAE-processing each parallel was strained for 2 minutes. A control sample was made and kept in a closed glass jar for 30 minutes before straining for 2 minutes.

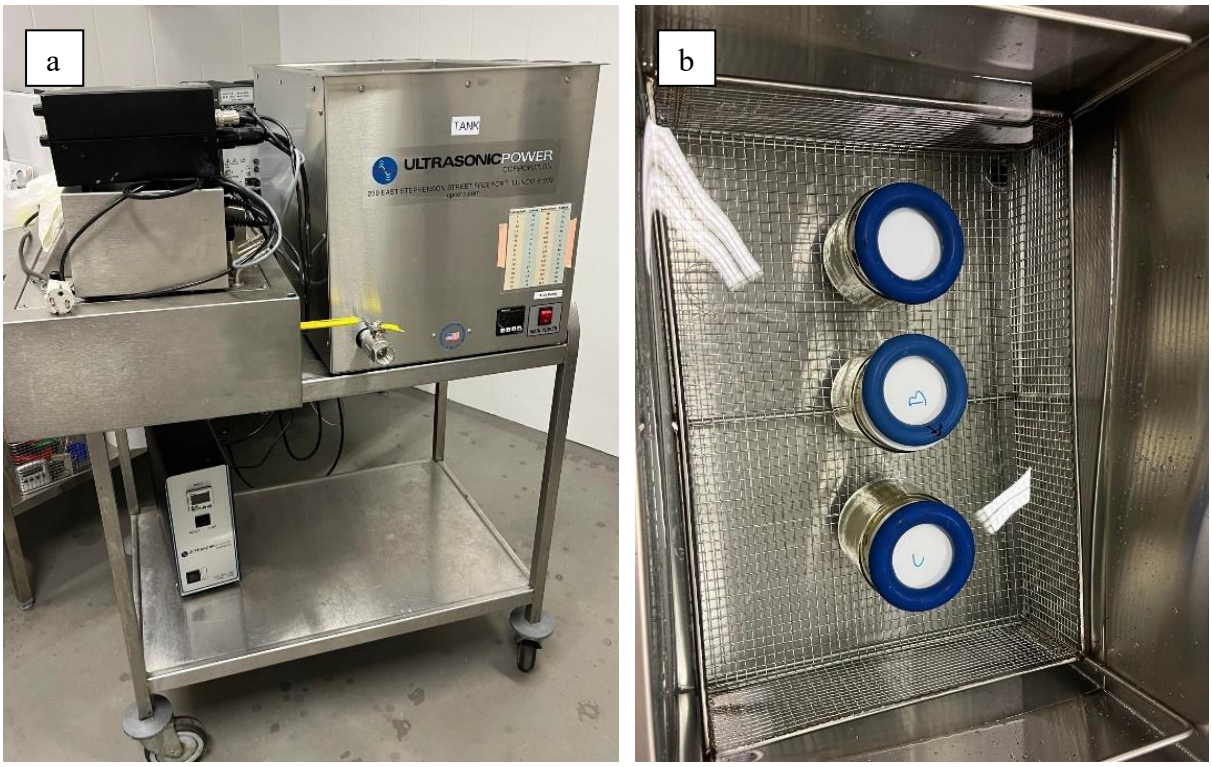

Figure 2.11: (a) Ultrasonic Power Corporation Ultrasonic cleaner with one generator on the bottom shelf and (b) samples of rockweed and tap water in glass jars, placed in a mesh basket, and held under water with weights.

2.3.3 Blanching

The rockweed was blanched at temperatures of 40, 50, 60 and 100 °C with a TM6 (Thermomix, USA) as pictured in Figure 2.12. These samples were named BL40, BL50, BL60 and BL100 with three biological parallels at each temperature (A-C). In a ratio of 1+5, 100 g rockweed and 500 g tap water were used for each parallel. The brown algae were put in the machine when the tap water reached the desired temperature of 40, 60, 80 and 100 °C and stirred for one minute. A picture of blanched rockweed at 40 and 100 °C is found in Figure 2.12 a and b, respectively. The brown algae and water were then strained for two minutes. A control sample was made by stirring the same ratio by hand at room temperature for 1 minute, before being strained for 2 minutes.

Figure 2.13: Thermomix TM6 (USA) used for blanching.

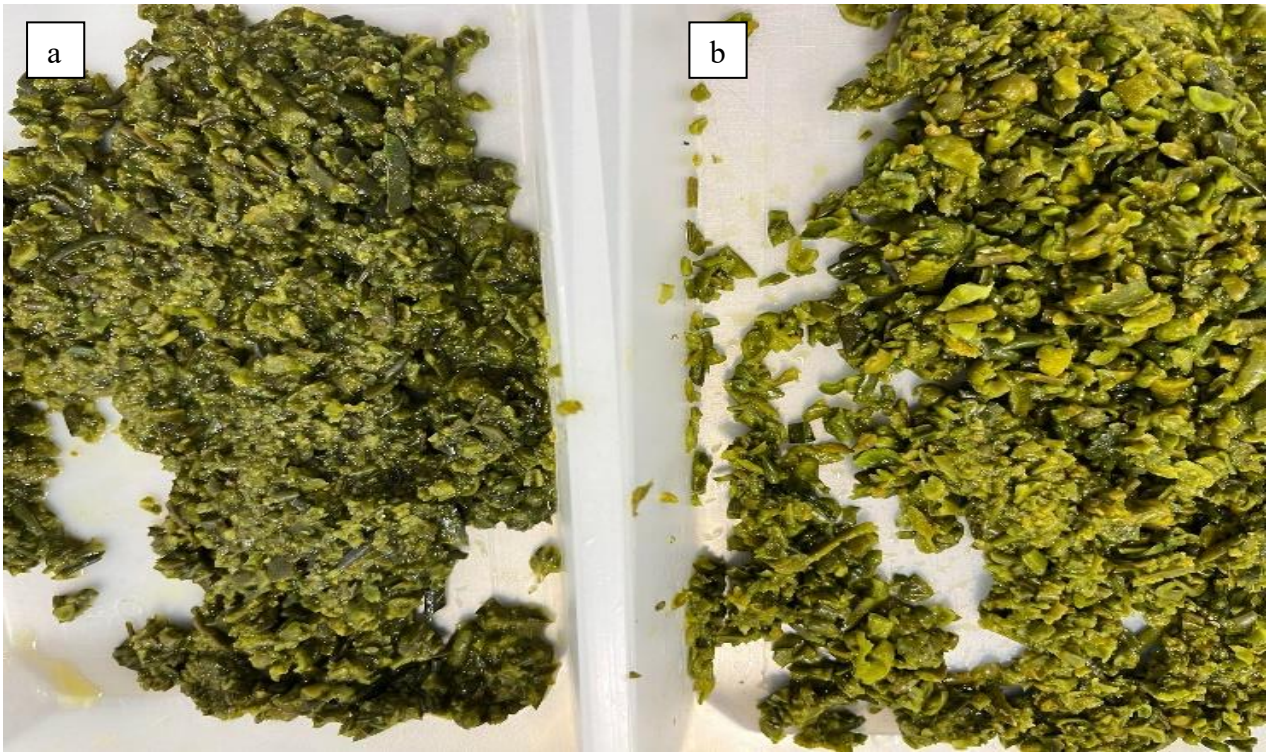

Figure 2.12: Picture of rockweed blanched at (a) 40 and (b) 100 °C.

2.4 Experiment B, C and D

2.4.1 Changes in between experiments

The preliminary experiments were done to find the best parameters for each process. The main experiment for rockweed was done twice (B and C), since additional changes to the experimental conditions were needed after experiment B. These are explained in Figure 2.14. Just one main experiment (D) was done for winged kelp, with the exact same methods and parameters as used for rockweed in experiment C.

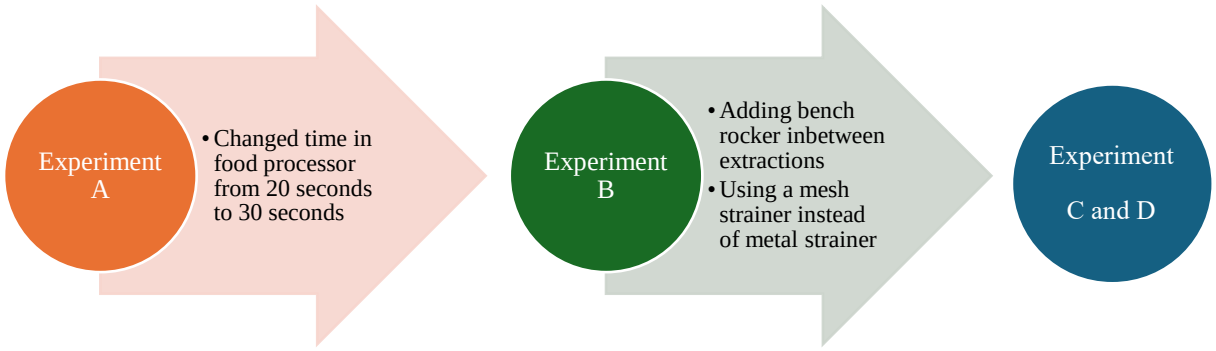

Figure 2.14: Changes between experiments.

The grounding time before the analysis was changed from 20 seconds to 30 seconds, to try to make the rockweed samples smaller and easier to extract. After 20 seconds the rockweed samples where at the size of salt flakes (approx. 2-3 mm), after 30 seconds it became more of a porridge consistency (approx. 1-2 mm). After experiment B a bench rocker was added to constantly move the algae flakes in the test tubes under the phenol extraction. In experiment C and D, a mesh strainer replaced the metal strainer used under experiment A and B, a detailed explanation is under 2.4.2.

For TPC analysis done up to this point (Experiment A), 250 µl was used in the 96-well plate under the reading of absorbance. Because of a misunderstanding in the laboratory, all samples in further experiments are using 300 µl in the 96-well plate.

2.4.2 Mesh strainer

Experiment A and B used a strainer of metal, with bigger and fewer holes to try to combat rockweed loss in the groves of a mesh strainer when tipped into a bowl. This led to less water getting strained in the two minutes of straining. An extra experiment was conducted in between experiment B and C to find the best way to strain brown algae using fresh rockweed chopped in a food processor for 30 seconds. Using a mesh strainer, tipping it fast and hard against a metal bowl yields minimal loss of product and was used as a solution for experiment C and D. Figure 2.15 shows the mesh strainer with the minimal loss of product.

Figure 2.15: Mesh strainer used with minimal loss of product.

2.4.3 Pulsed electric field

After polyphenol analysis for experiment A, it was decided to keep using the same machine settings; 50 Hz (frequency) and 6 μs (pulse width), as well as the same ratio of 1+5 with a constant pulse count of 1800. The voltage changed from 24 to 8 kV, because the PEF-chamber was changed from a big one (24 cm diameter) to a small one (8 cm diameter). Experiments B, C and D were performed as described in section 2.3.1, with changes from 2.3.1. The samples were given name PEF1800, with three biological parallels each given the names A-C. Three control samples were made during each experiment as described in 2.3.1

2.4.4 Ultrasound

After polyphenol analysis for experiment A, it was decided to use 40 kV for the main experiments. Experiments B, C and D were performed as described in section 2.3.2, with changes from 3.2.1. The samples were given name UAE40, with three biological parallels each (A-C). Control samples were made for each experiment as described in 2.3.2.

2.4.5 Blanching

After polyphenol analysis after experiment A, it was decided to use 40 °C and 100 °C in the main experiments (Experiment B, C and D). All main experiments were performed as described

in Section 2.3.3. The samples were given the names BL40 and BL100 with three biological parallels (A-C). One to three control samples were made each day in the same way as described in 2.3.3.

2.5 Analysis performed on samples from experiments

2.5.1 Color analysis

The color measurements were performed using the DigiEye instrument (DigiGrade system, VeriVide, United Kingdom). The instruments light fixtures (D65) to achieve standardized daylight (6500K). The instrument was calibrated each day before the analysis. The sample plates were put inside the DigiEye cube and photographed with a digital camera (D80, 35 mm lens, Nikon) mounted on top of the cube and pointed down on the samples. Processed algae and liquid phase were placed on a transparent white plate, shown in Figure 2.16. It was measured out approximately 100 g algae and 30 g water. The grey plate was used as a neutral background. The processed seaweed was placed on the plates with a thickness of 0.5 cm, while the dried seaweed was placed as thinly as possible. DigiPix software (Verivide Ltd) was used to analyze the images. The colors were quantified in the L*a*b system by measuring three different places on each sample.

Figure 2.16: Transparent white plates in the DigiEye instrument.

2.5.2 Dry matter and ash content

Dry matter analysis was conducted by drying both algae and liquid samples at 105 °C in a Termaks T1056 (Nordic Labtech, Sweden) for 18 hours as shown in Figure 2.17 (a), this leading to a constant weight, based on earlier research from Nofima (Sund et al., 2024). After drying the samples were placed into a Star-Vitrum desiccator (Sicco, Denmark) for 30 minutes as show in Figure 2.17 (b). The samples were weighed before and after analysis, and dry matter content was calculated using formula 6.3 (Nordisk metodikkomité for levnedsmidler, 2005) (Appendix I) and shown in % of wet weight (ww).

Figure 2.17: (a) Termaks T1056 (Nordic Labtech, Sweden) and (b) Star-Vitrum desiccator (Sicco, Denmark).

Using Nordic-Baltic committee on food analysis (NMKL) ash content method, the ash content was determined from the dried algae samples by combustion in a muffle furnace (More than Heat 30-3000 °C, with B170 control panel, Nabertherm, Germany) as shown in Figure 2.18 (a). The muffle used 3 hours to reach 550°C, and then left for 18 hours (Nordisk metodikkomité for levnedsmidler, 2005). The oven was turned off and the door was kept closed for 3 hours to cool down. The samples were weighed before and after analysis, and ash content was calculated with formula 6.4 (Nordisk metodikkomité for levnedsmidler, 2005) (Appendix I) and shown in % of total solid (TS).

Figure 2.18: More than Heat 30-3000 °C, with B170 control panel (Nabertherm, Germany).

2.5.3 Polyphenol

Using a method conducted as a part of a previous Nofima project PROMAC, the phenol extraction started with adding 0.5 g brown algae to 10 ml 80 % acetone (Sigma-Aldrich, Germany) before incubating for 1 hour and rocked with a ProBlot bench Rocker 25 (Labnet, USA). The samples were decanted, and the supernatant was kept. This was repeated once more on the same raw material and the supernatants were pooled and filtered through a 0.45 µm filter (Stévant et al., 2018).

All samples (algae extraction and water) from experiment A, B, C and D underwent polyphenol analysis using the *Folin-Ciocalteu assay* (Singleton et al., 1999). A propyl gallate stock solution was prepared by dissolving 0.53 g of propyl gallate powder (Sigma-Aldrich, Switzerland) in 250 ml of 80 % methanol (Sigma-Aldrich, France). Standard samples were made by dilutions of the propyl gallate stock solution in 0.5, 1, 1.5, and 2 mM. Then 5 ml of deionized water, 0.5 ml of Folin-Ciocalteu phenol reagent (Sigma-Aldrich, Switzerland), and 0.5 ml of sample/standard/blank (80 % methanol) were combined by vortexing. After precisely 3 minutes, 1 ml of 20 % Na_2CO_3 (Sigma-Aldrich, USA) was added, followed by an additional 3 ml of deionized water to bring the total volume to 10 ml. The solutions were vortexed again, and the tubes were sealed with airtight caps before being stored at room temperature for 1 hour.

During the analysis the samples were covered by aluminum foil as often as possible to avoid light exposure.

For all samples, the absorbance was measured at 725 nm using the BioTek synergy H1 microplate reader (Agilent Technologies, USA). For experiment A it was used 250 µl in each section of the microplate, while for all main experiments (B, C and D) it was used 300 µl. Using the standard curve 6.1 (Appendix I) for experiment A, 6.2 (Appendix I) for experiment B and C, and 6.3 (Appendix I) for experiment D. Total phenolic content (TPC) was calculated with formula 6.2 (Kupina, 2019) (Appendix I) and is shown in mg PGE (propyl gallate equivalents)/g ww algae.

2.5.4 Freeze-drying

Before freeze-drying the sample was placed in square plastic containers and frozen at -80 °C. The sample was dried using a Gamma 2-16 LSCplus laboratory freeze dryer (Martin Christ, Germany).

Table 2.3 shows the set- and final values of the main- and final freeze-drying process of rockweed. During the main drying, the set values where 10 °C shelf temperature and 0.1 mbar vacuum. The vacuum pump created a low-pressure environment (0.0821 mbar) and the shelf temperature increased over a span of 28 hours and 19 minutes (main drying) to 17.9 °C. This was to make sure the ice goes from solid to vapor and won't turn to liquid in between. During the final drying, the shelf temperature was set to 30°C and the vacuum at 0.01 mbar. After 18 hours and 53 minutes the shelf temperature was at 30 °C and the vacuum was at 0.0601 mbar. The temperature was increased slowly to remove bound moisture and the vacuum was lowered to remove any remaining water molecules. After the sample was freeze-dried, the gas was slowly let out before removing the samples.

Table 2.3: Set setting and final settings of main- and final drying of rockweed.

	Main drying	**Final drying**
Set vacuum (mbar)	0.1	0.01
Set shelf temperature (°C)	10.0	30.0
Time used (h:m)	28:19	18:53
Final vacuum (mbar)	0.0821	0.0601
Final shelf temperature (°C)	17.9	30.0
Final ice conductor temperature (°C)	-89.4	91.0

3. Results

The results section is divided into multiple parts, each describing the findings from different experiments. In pre-treatments (Experiment A) the parameters used for PEF, UAE and blanching for rockweed is studied by trying different parameters to get the highest yield possible of TPC. In bioactive contents (Experiment B, C and D) the chosen parameters are further studied by polyphenol analysis, dry matter, ash content, color analysis and weight analysis. Experiment D is conducted on winged kelp.

3.1 Pre-treatments (Experiment A)

3.1.1 Algae amount

An experiment was conducted to find the amount of algae to use in polyphenol assay. Frozen rockweed samples of 0.5, 1, 2.5 and 10 g were analyzed. The result is shown in Table 3.1. It was decided that 0.5 g rockweed would be used for the rest of the polyphenol analysis based on the standard curve (Figure 6.1, Appendix I) as the amount of polyphenols withing the range of the standard curve, without having to be further diluted.

Table 3.1: Absorbance at 725 nm and corresponding weights of frozen rockweed samples.

	Absorbance at 725 nm	**Weight (g)**
RW0.5	1.052	0.5
RW1	2.010	1
RW2	3.195	2
RW5	-	5
RW10	-	10

RW0.5, RW1, RW2.5 to RW10 corresponds with weight used respectively.

- No data was obtained from BioTek synergy H1 microplate reader (Agilent Technologies, USA).

3.1.2 Pulsed electric field processing

A series of pre-experiments (experiment A) were conducted to find the best parameters for TPC which used a low amount of energy. The first experiment (A1), with algae harvested 08.01.24, was done to determine the ratio of algae and tap water best suited for brown algae using PEF-processing. Table 3.2 shows the parameters used, data obtained and energy calculated by

equation 6.5. All samples were separated into a algae (solid) and a water (liquid) sample after each pretreatment process.

Table 3.2: Showing the ratio between algae and water, voltage, pulse count, frequency, energy and energy per kg sample (n = 1).

Parallel	Ratio	Voltage (kV)	Pulse count	Frequency /Hz)	* Energy (J)	Weight (g)	Energy pr kg (kJ/kg)
A	1:5	24	1000	50	23151.4	2399.7	9.65
B	1:3	24	1000	50	23144.8	1602.5	14.89
C	1:5	24	4000	50	143635.1	2400.3	14.96

Weight (g) = total weight of algae and tap water in PEF-chamber

* Numbers received from PEF Pilot Dual (Elea Technology, Germany)

A and B showed that a ratio of 1:5 has a lower energy pr kg than a ratio of 1:3. A higher pulse count (4000) was tried with 1:5 ratio to determine if the machine was able to read energy with the same amount of algae and tap water used at a higher pulse count. Since the high pulse count measure were possible, a smaller PEF-chamber (8 cm instead of 24 cm diameter) was used for experiment B, C and D to minimize the amount of algae required for the experiment.

In the second experiment (A2), with algae harvested 29.01.24, the parameters were used to determine the best suited pulse count, based on energy used during the PEF-processing calculated with formula 6.5 (Appendix I). This is shown in Table 3.3. Varying the number of pulses resulted in a distinct energy consumption pattern, and a correlation between the numbers of pulses and energy required during PEF-processing was found and shown in Figure 3.1.

Table 3.3: Showing the ratio between algae and water, voltage, pulse count, frequency, energy and energy per kg sample (n = 2).

Parallel	Ratio	Voltage (kV)	Pulse count	Frequency /Hz)	*Energy (J)	Weight (g)	Energy pr kg (kJ/kg)
PEF1000	1+5	8	1000	50	6500	609	10.73
PEF1500	1+5	8	1500	50	11 000	618	18.84
PEF2000	1+5	8	2000	50	17 000	613	28.03
PEF2600	1+5	8	2600	50	25 000	609	40.95
PEF3000	1+5	8	3000	50	30 000	611	48.97
PEF3400	1+5	8	3400	50	40 000	613	65.84

* Numbers received from PEF Pilot Dual (Elea Technology, Germany)

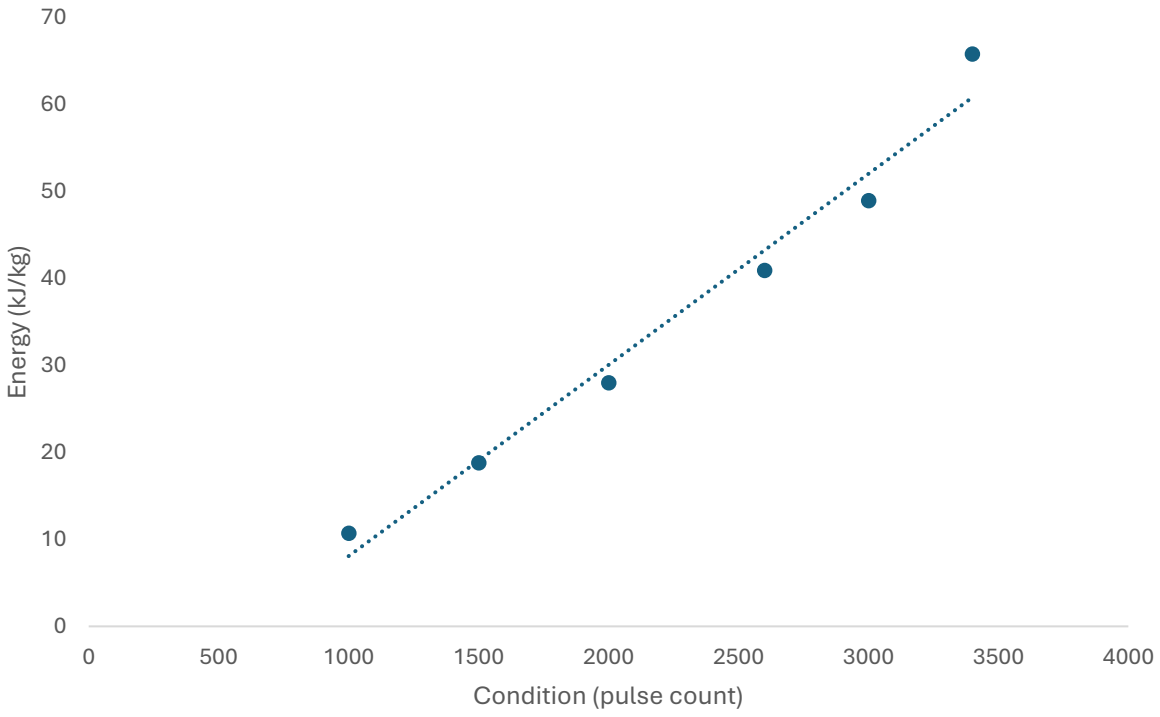

Figure 3.1: Point diagram with trend line of different pulse counts versus energy used (kJ/kg) using pulsed electric field processing (n = 2).

The TPC of rockweed from experiment A2 following PEF-processing are summarized in Figure 3.2. The TPC increased with the number of pulses, reaching a peak at 2600 pulses (67.70±0.04 mg PGE/g ww algae), followed by a slight decrease for both solid (67.70±0.04 mg PGE/g ww algae) and liquid samples (34.05±0.07 mg PGE/g ww algae). Both control samples, without

PEF-treatment, had a significantly lower TPC of 27.87±0.04 mg PGE/g ww algae and 17.92±0.01 mg PGE/g ww algae, for solid and liquid respectively. These results demonstrate that PEF processing significantly enhances the extraction of phenolic compounds from rockweed into the water, whit the maximal phenolic content being obtained with a pulse count of 2600.

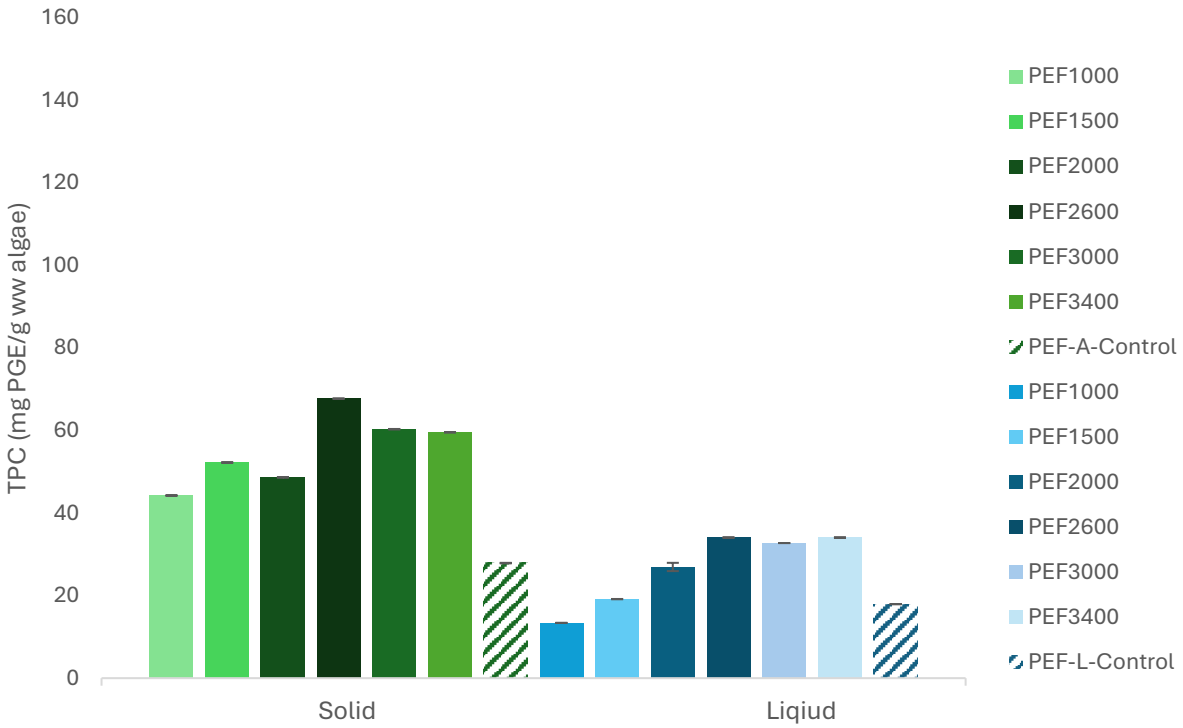

Figure 3.2: Total phenolic content of pulsed electric field processed algae (solid, green) and algae-water (liquid, blue) with settings; 8 kV, 50 Hz (frequency) and 6 μs (pulse width) with different pulse counts; 1000, 1500, 2000, 2600, 3000 and 3400 (n = 6 and error bars are standard deviation).

For the solid samples, TPC varied significantly across different energy inputs, with values ranging from 44.24±0.01 to 67.70±0.01 mg PGE/g ww algae for a pulse count of 1000 to 3400 respectively as shown in Figure 4.2. The corresponding energy used per kilogram of solid sample also fluctuated, reaching up to 65.84 kJ/kg for 3600 pulses. The sample with a pulse count of 2600 had the highest amount of TPC (67.70±0.01 mg PGE/g ww algae) and had an energy consumption of 40.95 kJ/kg. In comparison, the liquid samples had a lower TPC which ranged from 13.39±0.00 to 34.05±0.00 mg PGE/g ww algae while the energy consumption was

the same as with the solid samples. The samples with a pulse count of 2600- and 3000 and the highest amount of TPC (67.70±0.01 and 60.30±0.00 mg PGE/g ww algae) had an energy consumption of 40.95±0.01 and 48.97±0.00 kJ/kg respectively. The parameters of energy used and TPC is plotted against each other in Figure 3.3.

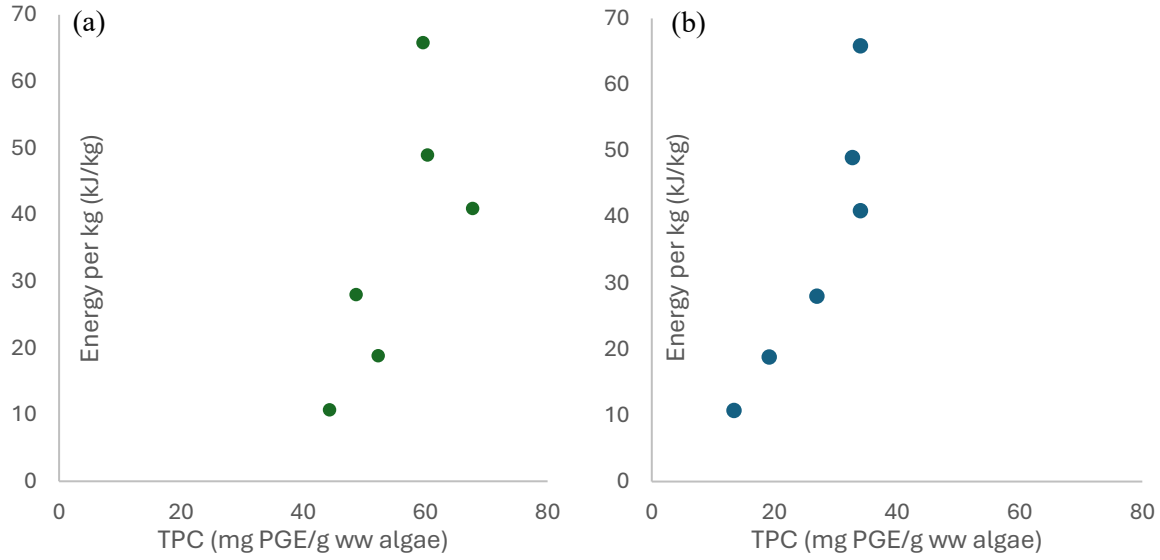

Figure 3.3: Comparison of total phenolic content in solid (a) and liquid (b) rockweed samples and energy used per kg (kJ/kg) during pulsed electric field processing (n = 2).

PEF treatment showed that there was a difference in the phenolic content based on the form of rockweed processed, with the solid form generally retaining higher phenolic content at comparable energy levels as compared to the liquid water form. Based on these findings, it was determined to try once more with 3000, 2000 and a new pulse count of 1800 to keep the energy level low and still get a high yield of TPC.

Experiment B (with rockweed harvested 12.03.24) was conducted to determine the final pulse count for the rest of the experiments. The two pulse counts (3000 and 2000) that yielded the highest TPC was tried again (A and B), with an addition of a pulse count of 1800 (C) to try to lower the energy usage without sacrificing the TPC. The parameters are shown in Table 3.4. It was determined to use a ratio of 1+5 for the rest of the experiments, to try to keep the resistance

in the PEF-machine low. The resistance in the machine was too low when using the pulse counts of 2000 and 3000, and therefore no data of energy usage was received. A pulse count of 1800 gave an energy per kg of 45.53 kJ/kg.

Table 3.4: Showing ratio between rockweed and water, voltage, pulse count, frequency, energy, weight of algae and water, and energy per kg sample (n = 2).

Parallel	Ratio	Voltage (kV)	Pulse count	Frequency (Hz)	Energy (J)	Weight (g)	Energy per kg (kJ/kg)
***A**	1+5	8	3000	50	-	598.6	-
***B**	1+5	8	2000	50	-	602.2	-
C	1+5	8	1800	50	27336.10	600.4	45.53

*Resistance was too low to receive numbers from PEF Pilot Dual (Elea Technology, Germany)

Since 3000 and 2000 pulses gave no value of energy, 1800 pulses were used for the rest of the experiments. The energy used at 1800 pulses for rockweed had a mean of 44.64±0.62 kJ/kg and 38.03±1.37 kJ/kg for winged kelp (n = 6 and n = 3, respectively).

3.1.3 Ultrasound assisted extraction

The results of the UAE-processing illustrated in Figure 3.4, show the variations in TPC between solid and liquid samples subjected to different voltage levels. Solid samples processed at 40 kV (UAE40A) exhibited the highest phenolic content at 47.07±0.04 mg PGE/g ww algae, while those treated at 68 kV (UAE68A), 170 kV (UAE170A), and a combination of 68 and 170 kV (UAE68-170A) had lower phenolic contents of 35.29±0.00, 29.95±0.01 and 32.04±0.02 mg PGE/g ww algae, respectively. The control sample for solids (UAE-A-Control) had the lowest phenolic content at 27.83±0.01 mg PGE/g ww algae. In contrast, liquid samples showed a different trend, with the control having the highest phenolic content at 36.66±0.00 mg PGE/g ww algae, while UAE40L, UAE68L, UAE170L, and UAE68-171L displayed lower phenolic contents of 19.68±0.00, 16.06±0.00, 18.94±0.00, and 18.39±0.00 mg PGE/g ww algae, respectively.

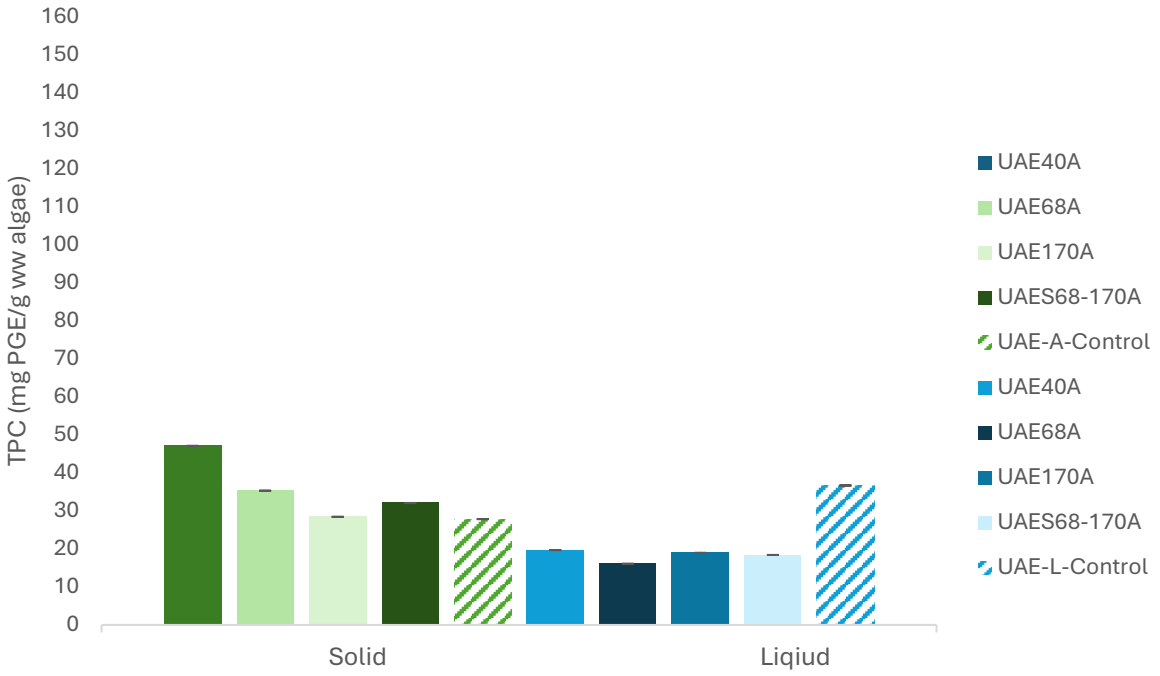

Figure 3.4: Total phenolic content of ultrasound processing with 40 kV, 68 kV, 170 kV and 68-170 kV, shown in algae (solid, green) and algae-water (liquid, blue).

This data indicates that UAE-processing at different voltages impacts the phenolic content differently in solid and liquid forms, with solids generally retaining higher phenolic content compared to liquids under the same conditions. It was decided to use 40 kV in the main experiments (B, C and D), because of the higher yield in solid algae, while there was no significant difference between treatments in the liquid samples.

3.1.4 Blanching

For solid samples, phenolic content increased with temperature, starting at 65.39±0.00 mg PGE/g ww algae for BL40, then slightly decreasing to 55.27±0.00 mg PGE/g ww algae for BL50 and peaking at 75.24±0.00 mg PGE/g ww algae for BL60 and 82.41±0.00 mg PGE/g ww algae for BL100 as depicted in Figure 3.5. The control solid sample (BL-A-Control) had a significantly lower phenolic content of 29.90±0.01 mg PGE/g ww algae. Liquid samples showed a dramatic increase in phenolic content with temperature. BL40 liquid samples started at 31.69±0.00 mg PGE/g ww algae, rising sharply to 69.18±0.00 mg PGE/g ww algae for BL50,

110.03±0.00 mg PGE/g ww algae for BL60 and reaching a maximum of 149.55±0.01 mg PGE/g ww algae for BL100. The control liquid sample (BL-L-Control) had the lowest phenolic content at 14.45±0.00 mg PGE/g ww algae.

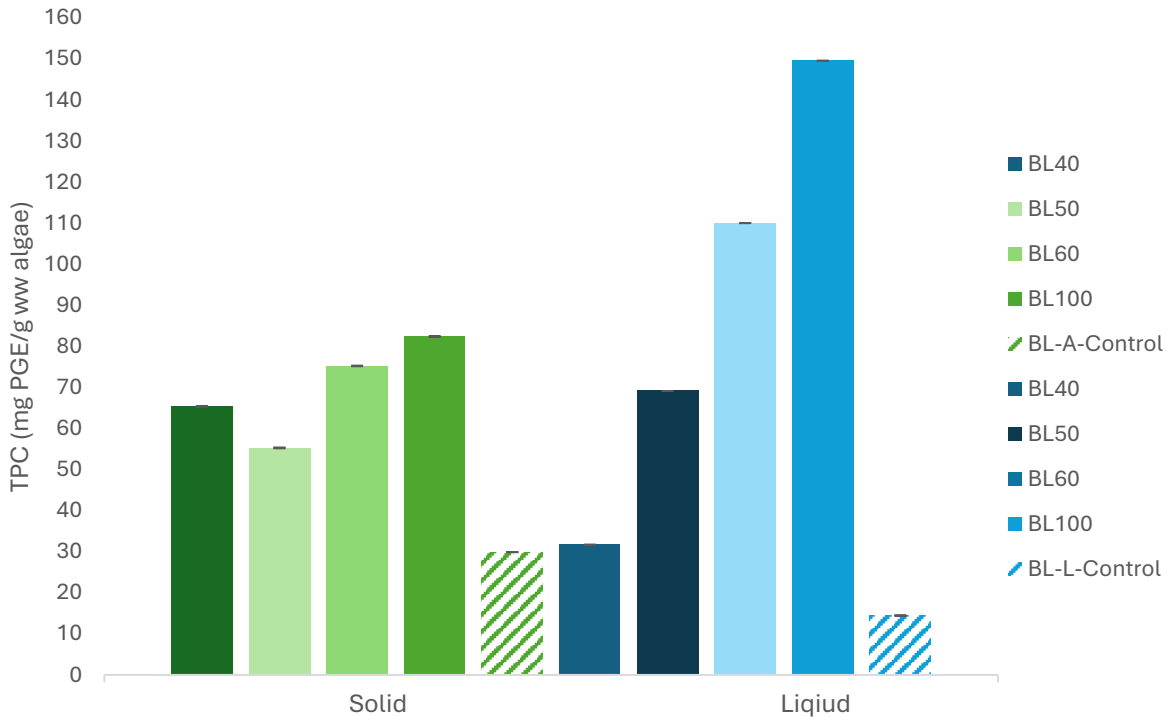

Figure 3.5 Total phenolic content of blanching with 40, 50, 60 and 100 °C, shown in algae (solid, green) and algae-water (liquid, blue).

The results of the blanching found the TPC in both solid and liquid samples varied at the different blanching temperatures (40 °C, 50 °C, 60 °C and 100 °C). These results indicate that higher blanching temperatures significantly enhance the extraction of phenolic compounds into the liquid samples, suggesting that temperature is a crucial factor in optimizing phenolic extraction during the blanching process. It was decided to use 40 and 100 °C in experiment B, C and D, because 40°C had more TPC than 50°C, but 100°C had the highest TPC.

3.2 Bioactive contents (Experiment B, C and D)

3.2.1 Total phenolic content

For the rockweed samples harvested in early spring (Experiment B and C), the solid samples showed significant variability in TPC depending on the processing method shown in Figure 3.6. The highest TPC was observed in the freeze-dried samples, with a value of 434.82±0.05 mg PGE/g ww algae, indicating that this method obtained the extraction of TPC compared to the other treatments. The lowest TPC was recorded in the BL40 with a value of 123.58±0.09 mg PGE/g ww algae. With UAE40 being close with 128.781±0.24 mg PGE/g ww algae TPC. For the other treatments, BL100 resulted in TPC and 258.84±0.40 mg PGE/g ww algae, indicating higher phenolic content at increased blanching temperatures.

All the control samples had similar TPC values, where UAE-C had the highest amount of 91.08±0.01 mg PGE/g ww algae and BL-C with the lowest TPC at 44.94±0.09 mg PGE/g ww algae. Indicating lower phenolic content compared to their processed counterparts.

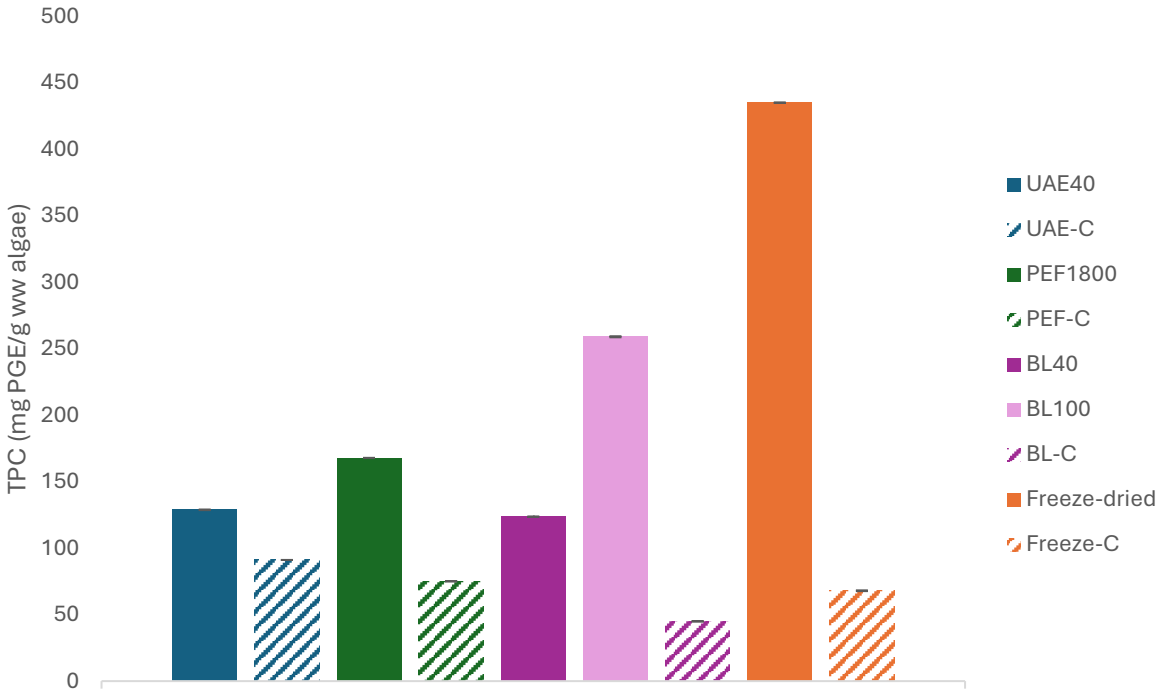

Figure 3.6: Total phenolic content of ultrasound processed, pulsed electric field processed, blanched and freeze-dried solid samples of rockweed with control samples. (n = 3 for freeze-dried and freeze-c and n = 12 for the rest of the samples)

For the liquid algae samples shown in Figure 3.7, the samples blanched at 100°C (BL100) had the highest TPC at 315.94±0.91 mg PGE/g ww algae, being a lot higher in TPC compared to other treatments. In contrast, the lowest TPC was observed using the UAE40 samples, with a value of 120.67±0.18 mg PGE/g ww algae. BL40 resulted in a TPC of 155.12±0.41 mg PGE/g ww algae, showing a substantial increase in phenolic content that went even higher when blanched at a higher temperature.

The UAE-control had a TPC of 111.28±0.20 mg PGE/g ww algae, the highest among the control samples. The other controls, BL-C and PEF-C which was 56.01±0.06 mg PGE/g ww algae and 54.123±0.01 mg PGE/g ww algae, respectively. Both the solid and liquid blanched and PEF samples were close in TPC.

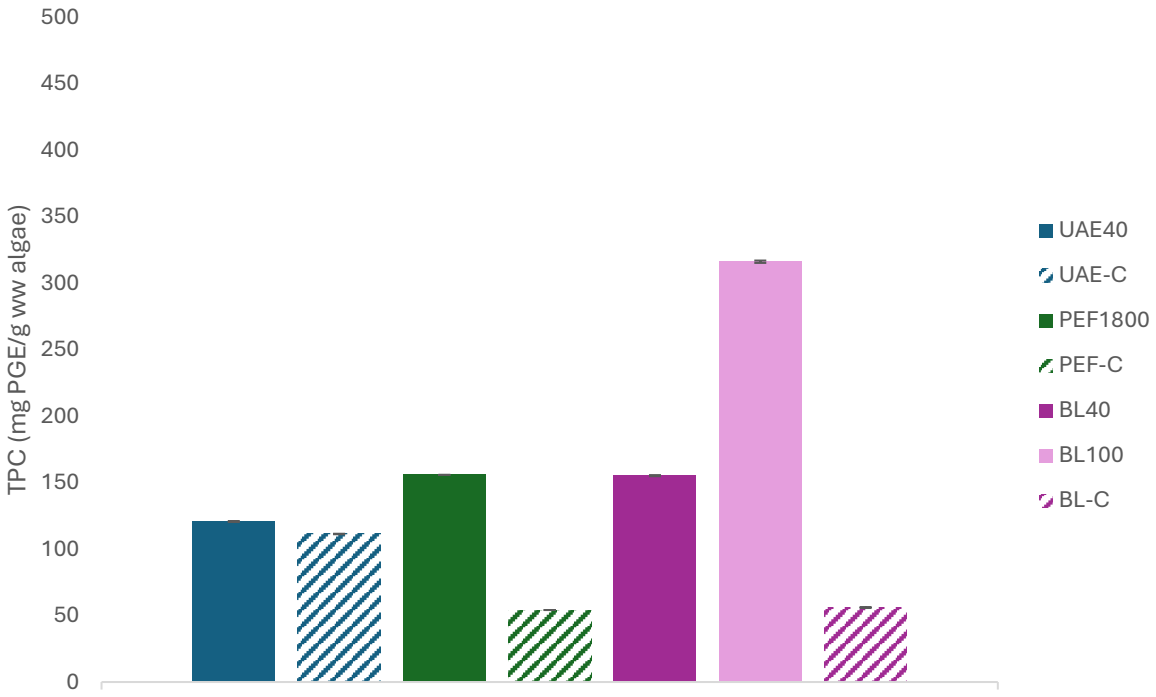

Figure 3.7: Total phenolic content of ultrasound processed, pulsed electric field processed and blanched liquid samples rockweed with control samples (n = 12).

These results demonstrate that different processing methods significantly affect the TPC in rockweed samples, with freeze-drying leading to the highest TPC in solid samples. Blanching at higher temperatures also resulted in increased TPC, while PEF processing was effective but not as effective as freeze-drying. It is also interesting to note that even though blanching at higher temperatures does not result in a significant difference for the solid sample, the liquid samples acquired a substantial higher yield using blanching as a pre-treatment method. Control samples generally had lower TPC values across all processing methods expect for liquid UAE samples.

For the solid samples of winged kelp, shown in Figure 3.8, BL40 resulted in the highest TPC at 18.56±2.34 mg PGE/g ww algae. The other processed samples were not far behind. UAE40 had a TPC of 10.30±1.45 mg PGE/g ww algae, while PEF-processing at 1800 pulse count (PEF1800) showed a TPC of 15.05±1.98 mg PGE/g ww algae. Blanching at 100°C (BL100) resulted in a TPC of 14.48±1.67 mg PGE/g ww algae.

In comparison, the control samples exhibited a similar pattern for TPC. The control for PEF had a the highest TPC of 14.12±1.80 mg PGE/g ww algae. UAE-C had a TPC of 10.71±1.29 mg PGE/g ww algae, and BL-C showed a TPC of 12.51±1.56 mg PGE/g ww algae.

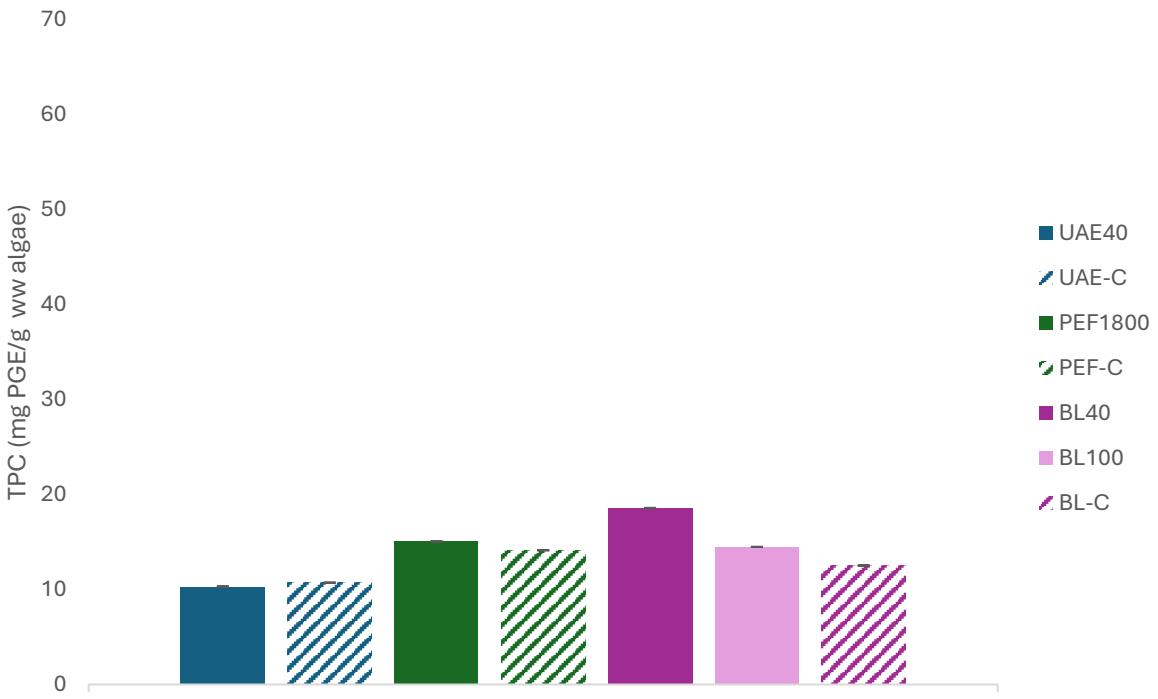

Figure 3.8: Total phenolic content of ultrasound processed, pulsed electric field processed and blanched solid samples of winged kelp with control samples (n = 12).

For the liquid samples of winged kelp, as shown in Figure 3.9, BL40 again yielded the highest TPC at 65.30±3.45 mg PGE/g ww algae. While UAE40 resulted in the lowest TPC of 36.35±2.12 mg PGE/g ww algae, yielding significantly higher than the solid samples. In comparison, the control samples for the liquid winged kelp showed different TPC values. The UAE-C sample had the highest TPC of 44.38±2.43 mg PGE/g ww algae. PEF-C had the lowest TPC of 19.44±1.56 mg PGE/g ww algae, while and the-C sample showed a TPC of 25.37±1.89 mg PGE/g ww algae.

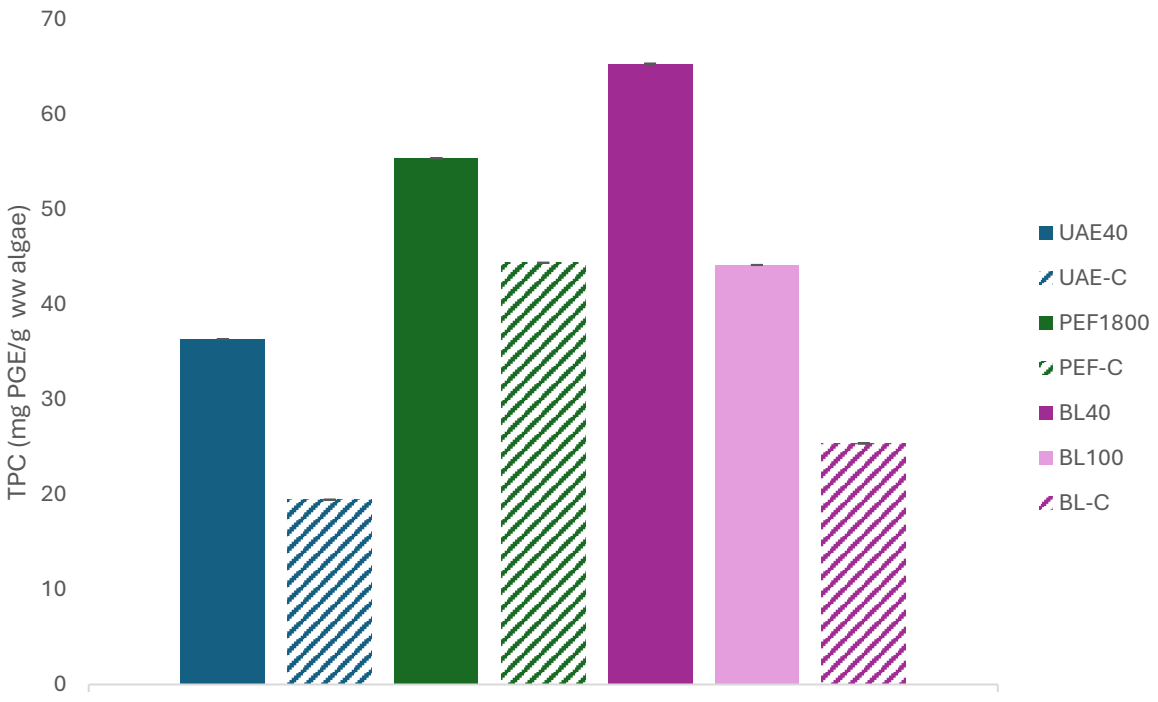

Figure 3.9: Total phenolic content of ultrasound processed, pulsed electric field processed and blanched liquid samples of winged kelp with control samples (n = 12).

Blanching at 40°C resulted in the highest TPC for both solid and liquid samples of winged kelp, with standard deviations (SD) indicating the consistency of these measurements. UAE40 showed a significant increase in TPC compared to the control for both solid and liquid samples, with the SD values showing relatively consistent measurements. PEF1800 had a higher TPC compared to the control in both solid and liquid samples, though this increase was less pronounced in liquid samples. The control samples generally had lower TPC values and their corresponding standard deviations compared to their processed samples, indicating that the various processing methods effectively increased the TPC of the winged kelp

Figure 3.10 shows comparisons of solid rockweed and winged kelp. Significant differences are observed. For solid rockweed samples, the highest TPC is achieved with blanching at 100°C yielding 258.85±0.40 mg PGE/g ww algae, which is substantially higher than any other method. The lowest TPC for solid rockweed is found in the blanched control at 44.94±0.09 mg PGE/g ww algae. For solid winged kelp, the highest TPC is recorded in the BL40 treatment at 18.56±2.34 mg PGE/g ww algae, while the lowest TPC is the UAE-processing at 10.30±1.45

mg PGE/g ww algae. These results highlight that solid rockweed samples generally possess higher TPC compared to winged kelp across all processing methods.

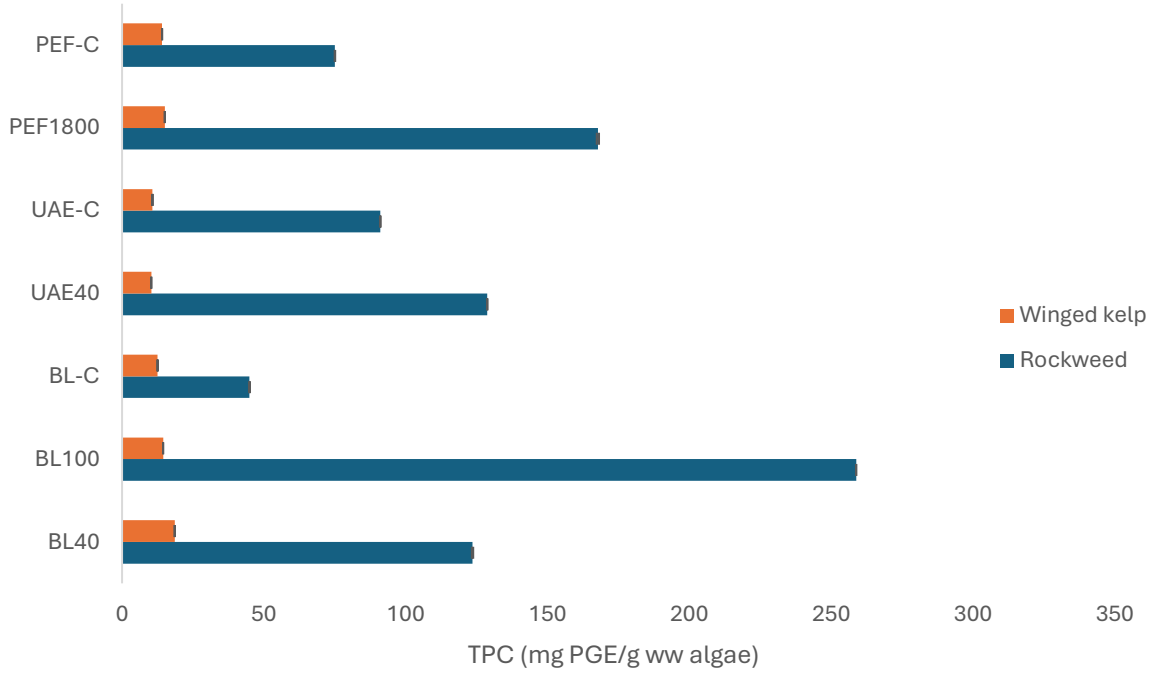

Figure 3.10: Diagram comparing TPC of solid winged kelp and rockweed samples after PEF-processing, UAE-processing, blanching at 40 and 100 °C and their control samples (n = 12 and standard deviation).

The TPC in liquid samples of rockweed and winged kelp also displays notable variations across different processing methods as shown in Figure 3.11. Liquid rockweed samples exhibit the highest TPC, again, with blanching at 100°C reaching 315.94±0.91 mg PGE/g ww algae. The lowest TPC for liquid rockweed is found in PEF-C with 54.12±0.01 mg PGE/g ww algae. For liquid winged kelp samples, the highest TPC is observed in the UAE-processing control treatment at 65.30±3.45 mg PGE/g ww algae, while the lowest TPC is recorded in UAE-processing at 19.44±1.56 mg PGE/g ww algae.

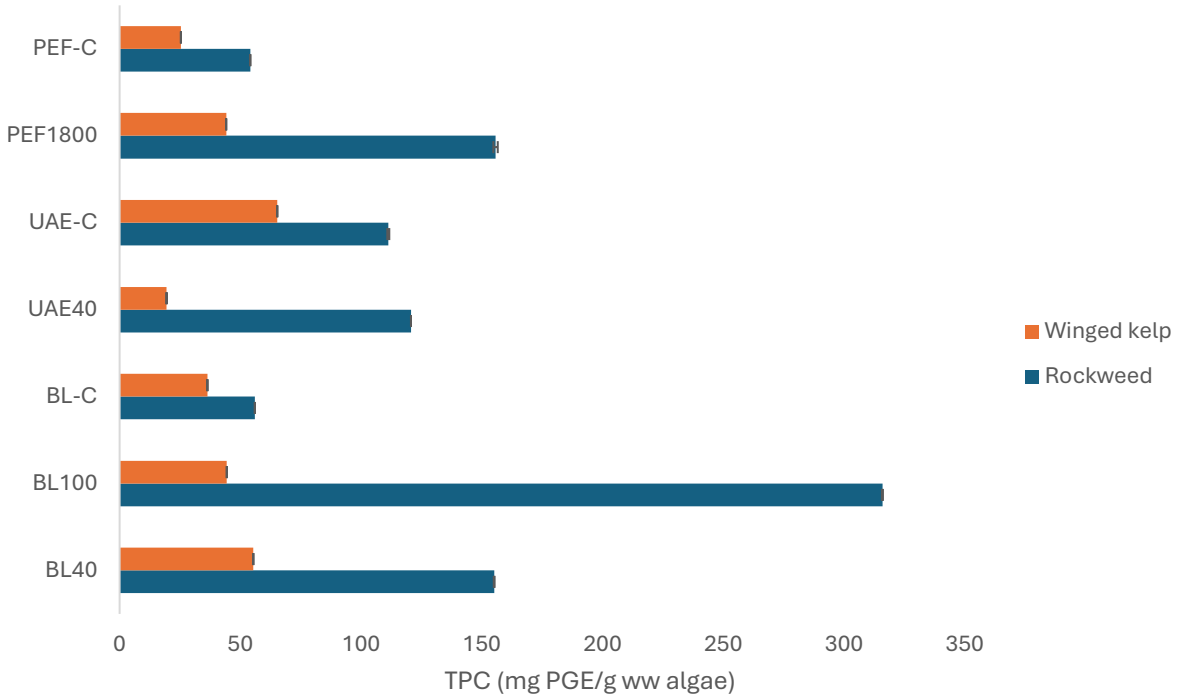

Figure 3.11: Diagram comparing TPC of liquid rockweed and winged kelp samples after PEF-processing, UAE-processing, blanching at 40 and 100 °C and their control samples (n = 12 and standard deviation).

3.2.2 Dry matter and ash content

The dry matter of the algae samples from experiment C and D was measured as a percentage of ww (Table 3.5). The untreated sample had the highest dry matter for both algae (22.51±0.01 and 8.87±0.28 %, respectively), while the lowest dry matter content was found in the BL-C control samples (15.68±0.01 and 4.00±0.58 %, respectively). There was also no distinct difference between the processed samples and their controls.

Table 3.5: Dry matter for rockweed and winged kelp, measured in % of wet weight (n = 6).

	Dry weight (% ww)	
	Rockweed	Winged kelp
Untreated	22.51±0.01	8.87±0.28
PEF1800	18.21±0.01	5.67±0.71
PEF-C	18.73±0.01	4.33±0.26
UAE40	16.30±0.02	4.23±0.61
UAE-C	16.73±0.02	4.17±1.59
BL40	18.64±0.08	6.50±0.34
BL100	18.35±0.01	6.67±0.24
BL-C	15.68±0.01	4.00±0.58

Table 3.6 details the dry matter content of liquid samples from processed rockweed and winged kelp, measured as a percentage of ww. The highest dry matter content of water for rockweed was observed in the PEF1800-treated samples at 0.43±0.00 %, while the lowest was recorded for UAE-C at 0.23±0.00 %. For winged kelp, the highest value was seen in the BL100 and BL40 samples, at 1.00±0.02 and 0.01±0.2 % respectively, and the lowest in UAE40 at 0.01±0.03 %. There is more of a substantial difference between the processed liquid samples and their controls, than with the algae samples.

Table 3.6: Dry matter for processed rockweed and winged kelp with standard deviation. (n = 6).

	Dry weight (% ww)	
	Rockweed	Winged kelp
PEF1800	0.43±0.00	0.36±0.07
PEF-C	0.28±0.00	0.32±0.02
UAE40	0.40±0.00	0.01±0.03
UAE-C	0.23±0.00	0.50±0.15
BL40	0.30±0.00	1.00±0.06
BL100	0.93±0.00	1.00±0.02
BL-C	0.67±0.01	0.17±0.01

Table 3.7 presents the ash content, measured as a percentage of TS for untreated and processed rockweed and winged kelp, including their respective control samples. For rockweed, the untreated samples had the highest ash content at 22.61±0.31 %, while the PEF-sample had the lowest at 6.03±0.83 %. In the case of winged kelp, the highest ash content was also found in the untreated samples at 39.39±7.34 %, whereas the lowest was observed in the BL100 samples at 18.78±2.75 %.

Table 3.7: Ash content for untreated and processed rockweed and winged kelp and their corresponding control samples. (Measured in % of dried weight, n = 6, SD is given).

	Ash content (% TS)	
	Rockweed	Winged kelp
Untreated	22.61±0.31	39.39±7.34
PEF1800	6.03±0.83	19.25±0.43
PEF-C	15.68±0.89	24.06±0.54
UAE40	18.67±0.55	36.79±14.65
UAE-C	19.55±0.56	19.46±5.07
BL40	19.56±0.67	33.52±12.11
BL100	18.51±1.00	18.78±2.75
BL-C	22.24±0.33	28.91±2.00

3.2.3 Color analysis

The color analysis of the algae samples harvested in spring (experiment B, C and D) was subjected to different processing methods. For rockweed, it was also conducted a freeze-dry experiment. All color analysis is compared to an untreated sample. For this color analysis, it is used L* (Where the higher the number, the lighter the sample), a* (Where + numbers are more red, and – numbers are more green), and b* (Where + numbers are more yellow and – numbers are more blue) color parameters.

In the solid samples shown in Figure 3.12, PEF1800 resulted in L* = 27.12±0.46, making it one of the darker treatments compared to UAE40, which had a high L* value of 70.46±0.42, indicating a much lighter sample. Blanching treatments showed an average lightness with L* values of 32.10±0.45 for BL40 and 34.66±0.58 for BL100. The a* values indicated the most significant shift towards a green hue in BL40 (-2.89±0.21), while UAE40 had the least green shift (-1.52±07). The b* values were highest in BL100 (36.43±1.06), suggesting a significant yellow hue, while PEF1800 had a lower b* value (17.67±0.29) showing a bluer hue.

In solid control samples, the PEF-C samples had the lowest L* value (25.92±21), indicating it was the darkest among the controls. UAE-C had the highest L* value (73.01±0.59), making it the lightest sample. For the a* value, BL-C recorded the lowest (-2.12±0.07), indicating a shift towards green, while UAE-C had the highest (-1.52±0.05), indicating a less green hue. The b* value was highest in PEF-C (18.93±24), suggesting more yellow, and lowest in UAE-C (10.00±0.19).

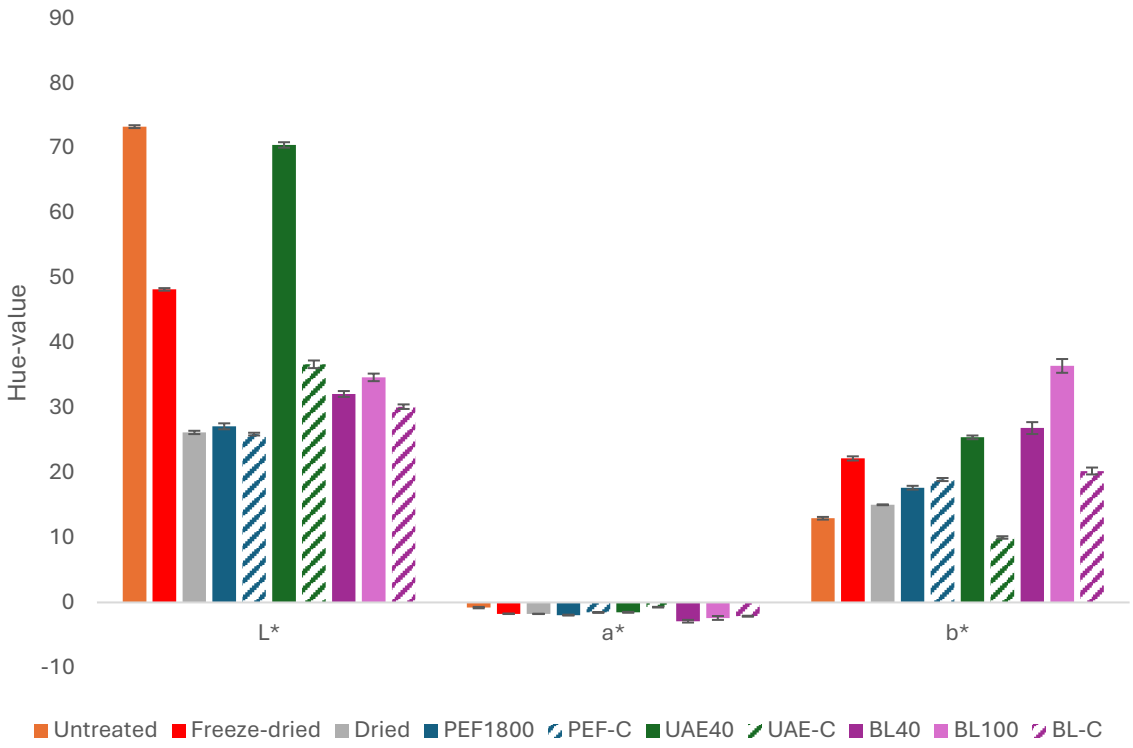

Figure 3.12: Mean of color analysis L*, a* and b* for solid rockweed that's untreated, freeze-dried, dried, pulsed electric field processed, ultrasound processed and blanched at 40 and 100 °C (n = 9 and error bars are standard deviation).

In the liquid samples shown in Figure 3.13, PEF1800 had a high L* value (73.54±0.44), indicating lightness similar to the control PEF-C. UAE40 showed a much lower L* value (24.87±1.12), making it one of the darkest treatments. Blanching at 40°C and 100°C (BL40 and BL100) resulted in relatively high L* values (65.83±0.35 and 67.09±0.43, respectively), showing these samples remained light. The a* values were highest in BL100 (5.57±0.27), indicating a shift towards red, and lowest in UAE40 (-1.24±0.42), indicating a green shift. The b* values were highest in BL100 (41.16±0.96), suggesting a strong yellow hue, while UAE40 had the lowest b* value (10.17±0.58).

For liquid samples, the PEF-C group had the highest L* value (75.63±0.24), making it the lightest among the controls. Both UAE-C and UAE40 showed the same L* value (24.87±1.36), making them the darkest. The lowest a* value was found in PEF-C (-1.13±0.24), showing a

greener hue, while the highest was in BL-C (-0.79±0.21). The b* value was highest in BL100 (41.16±0.21), indicating a strong yellow hue, and lowest in UAE-C (9.93±1.29).

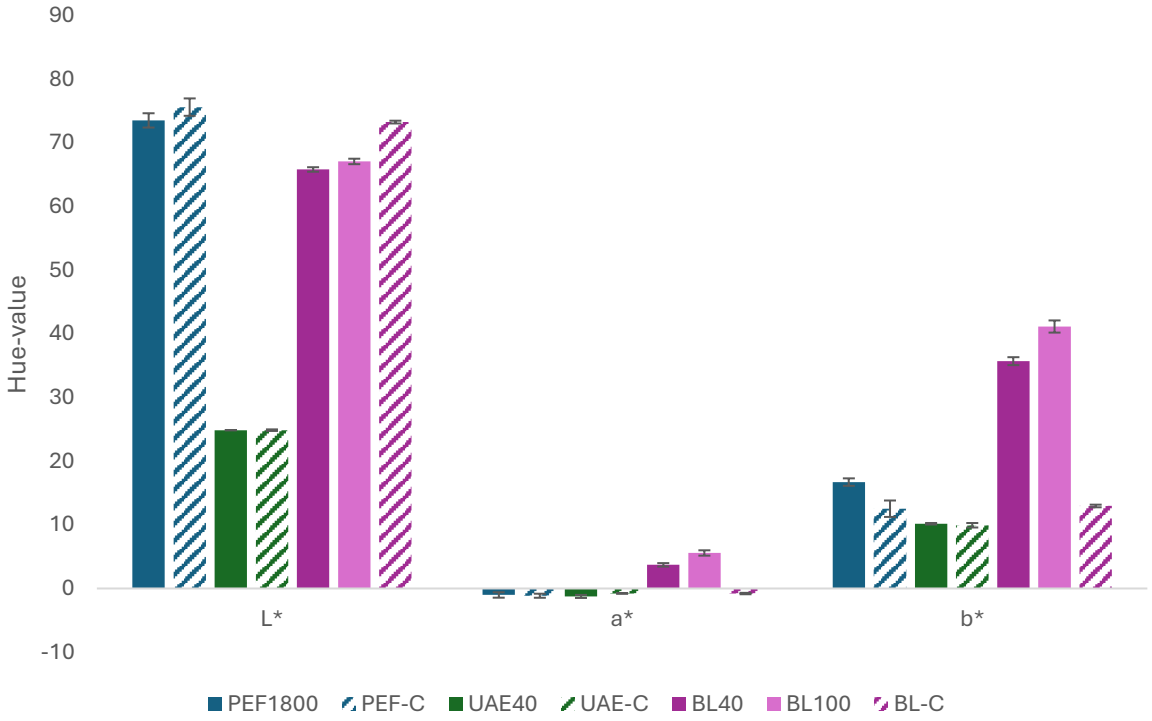

Figure 3.13: Mean of color analysis L*, a* and b* for liquid rockweed samples that's pulsed electric field processed, ultrasound processed and blanched at 40 and 100 °C (n = 2).

These results demonstrate that different processing methods significantly impact the color parameters of rockweed samples. In both solid and liquid samples, UAE treatments result in lighter colors with a lower tendency towards green hues. Blanching, particularly at higher temperatures, significantly increases the yellow hue of the solid samples. PEF-treatments tend to produce darker and more yellow samples in solid form but maintain lightness in liquid form. For solid samples, the control samples stayed similar in all hues, while in the liquid forms, the PEF-C and the BL-C did become much lighter than the UAE-C.

In the solid winged kelp samples, as shown in Figure 3.14, the samples were similar in lightness. BL100 resulted in an L* value of 18.39±0.10, making it the lightest treatments compared to the rest, which had a lower L* value of 12.18±0.32, 11.31±0.22 and 10.39±0.11 for UAE40, BL40

and PEF1800, respectively. The a* values indicated the most significant shift towards a green hue in UAE40 (-18.84±0.51), while BL100 had the least green shift (-11.57±0.36). The b* values were highest in BL100 (17.35±0.48), suggesting a significant yellow hue, while PEF1800 had a lower b* value (7.90±0.27), indicating a less yellow and more neutral hue.

In the solid control samples, the PEF-C group had an L* value of 10.39±0.11, indicating it was similar in darkness to the untreated and UAE-C samples. For a*, BL-C had a value of -4.77±0.21, similar to the untreated sample, indicating a moderate shift towards green. The b* value was highest in the untreated and BL-C samples (6.56±0.19), suggesting a mild yellow hue, while PEF-C had a slightly lower b* value (6.04±0.18). All in all, the control samples were similar in all hues.

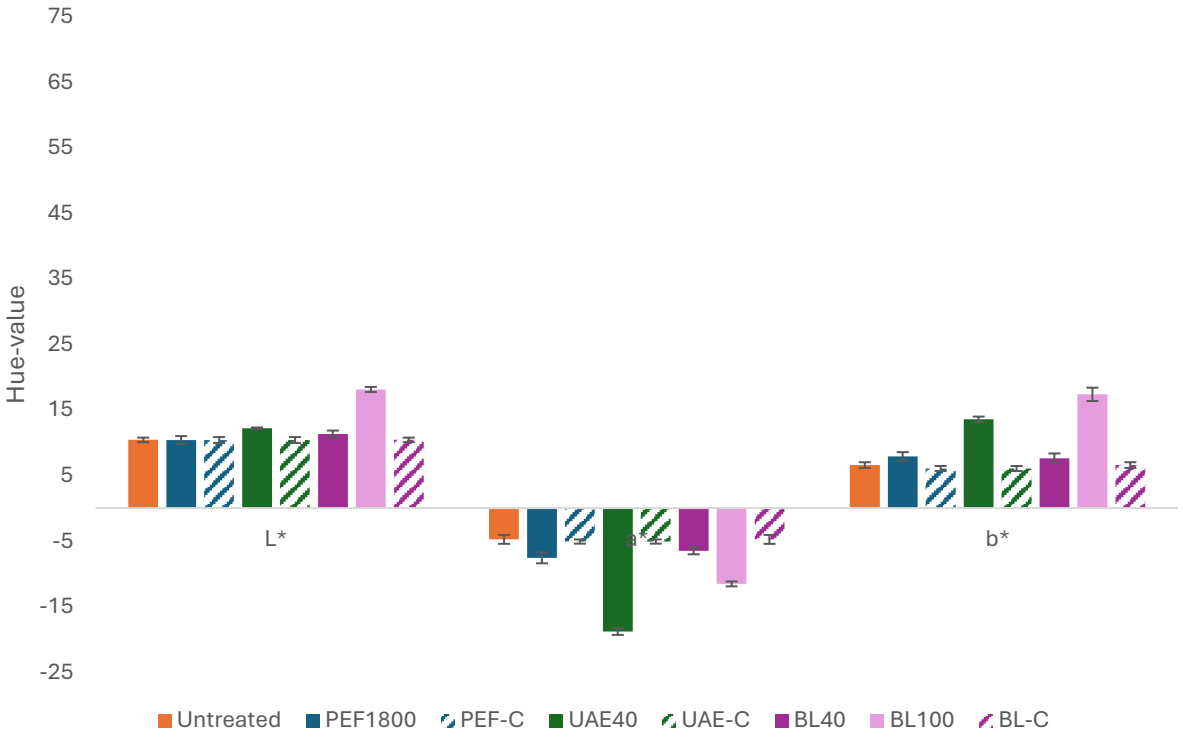

Figure 3.14: Mean of color analysis L*, a* and b* for solid winged kelp samples that are untreated, pulsed electric field processed, ultrasound processed and blanched at 40 and 100 °C (n = 3 and error bars are standard deviation).

In the liquid winged kelp samples, shown in Figure 3.15, PEF1800 had a high L* value (72.68±0.36), indicating lightness similar to the control PEF-C. UAE40 showed a lower L* value (67.41±0.43), making it one of the darker treatments. BL40 and BL100 resulted in relatively high L* values (66.73±0.39 and 63.81±0.41, respectively), indicating these samples remained light. The a* values were highest in BL40 (7.00±0.24), indicating a shift towards red, and lowest in UAE-C (-7.16±0.29), indicating a green shift. The b* values were highest in BL100 (32.74±0.45), suggesting a strong yellow hue, while UAE40 had a lower b* value (27.70±0.41).

For liquid samples, the PEF-C group had a high L* value (72.49±0.35), making it the lightest among the controls. UAE-C showed a lower L* value (49.09±0.38), making it the darkest. The lowest a* value was found in UAE-C (-7.16±0.29), indicating a strong green hue, while BL40 had the highest a* value (7.00±0.24), indicating a shift towards red. The b* value was highest in BL100 (32.74±0.45), indicating a strong yellow hue, and lowest in PEF-C (18.70±0.33).

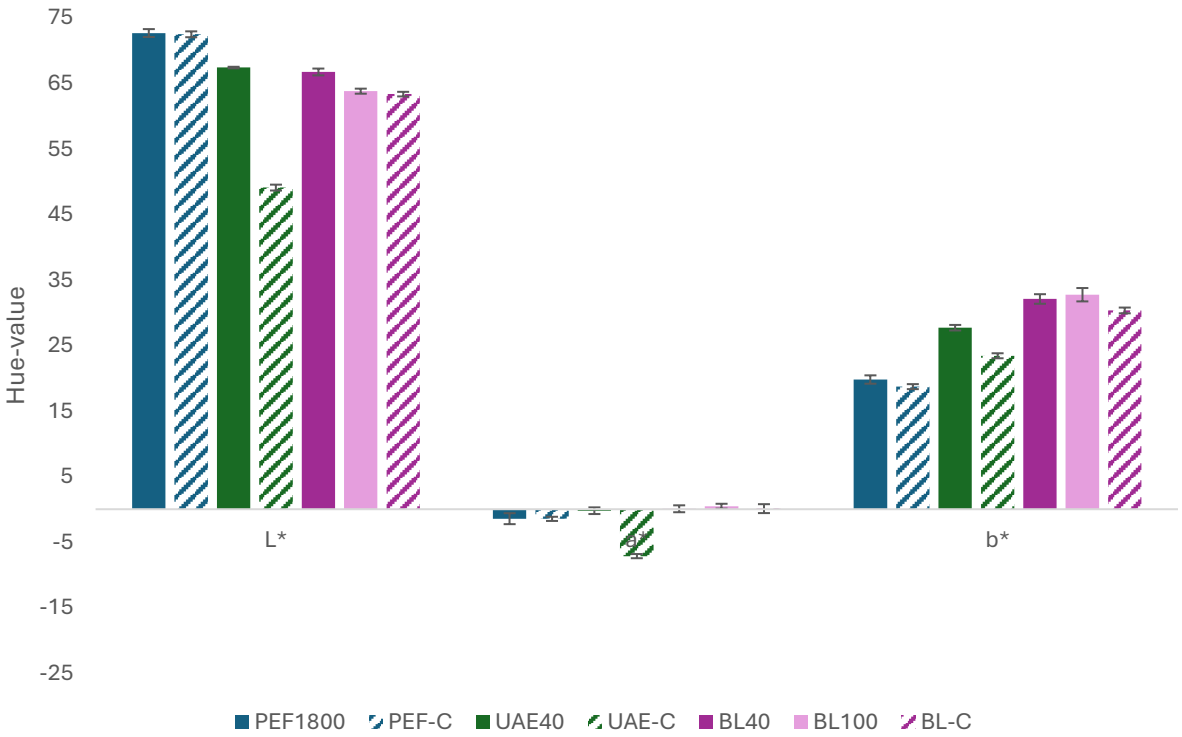

Figure 3.15: Mean of color analysis L*, a* and b* for liquid winged kelp samples that are pulsed electric field processed, ultrasound processed and blanched at 40 and 100 °C (n = 3 and error bars are standard deviation).

In both solid and liquid samples, UAE-treatments result in lighter colors with a higher tendency towards green hues. Blanching, particularly at higher temperatures, significantly increases the yellow hue in both solid and liquid samples. PEF treatments tend to produce darker samples in solid form but maintain lightness in liquid form. Control samples in solid form stayed similar in all hues, while in liquid form, the PEF and blanching controls were much lighter than the UAE control, indicating that the water becomes darker the longer the algae is mixed in it, while the algae itself does not change color.

The comparison between the color of solid rockweed and winged kelp samples after processing reveals distinct differences in their color values as shown in Figure 3.16. The results shows that rockweed consistently exhibited higher L* values, indicating a lighter color, and higher b* values, indicating a stronger yellow hue. In contrast, winged kelp displayed lower L* values and stronger green tints with higher negative a* values. These differences suggest rockweed is lighter and yellower, while winged kelp is darker and greener.

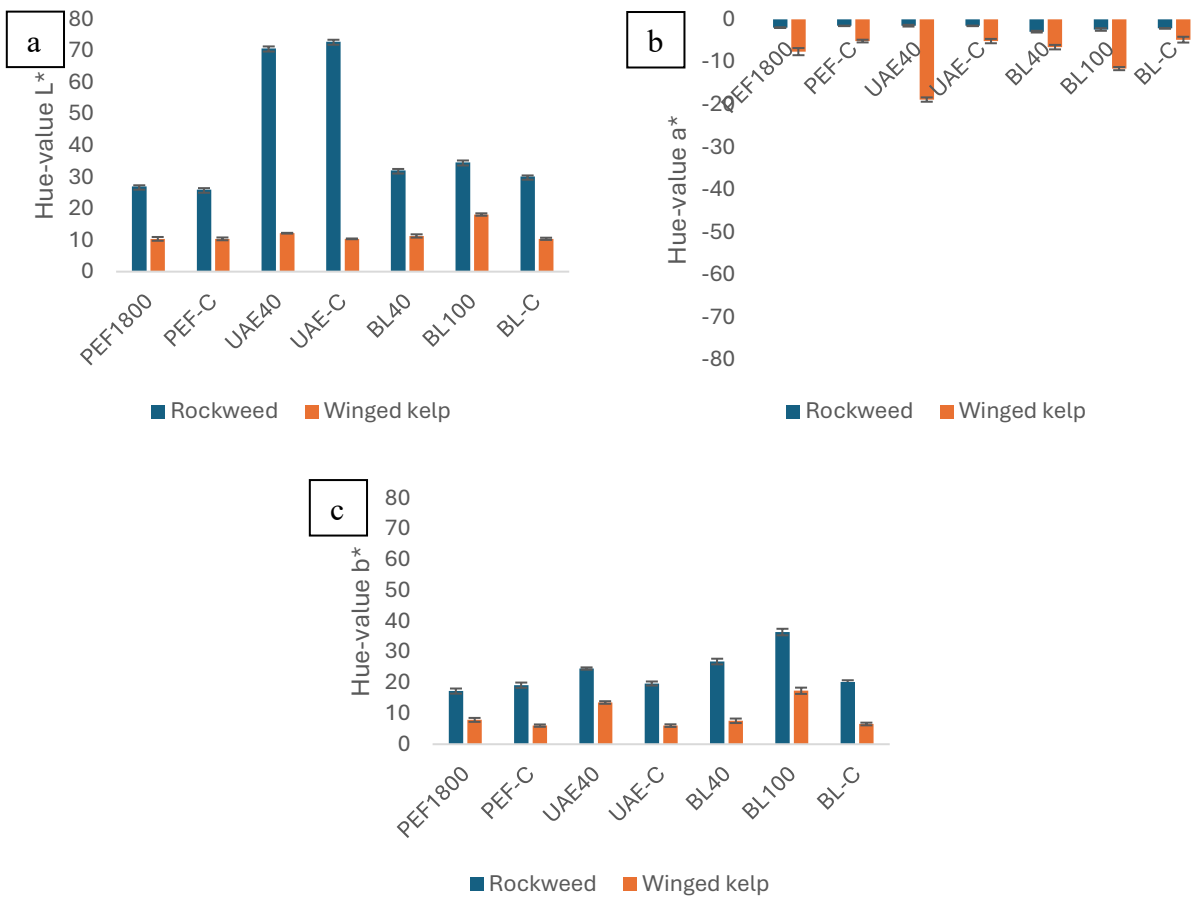

Figure 3.16: Diagram comparing L* values (a), a* values (b) and b* values (c) of color analysis of solid rockweed and solid winged kelp samples after PEF-processing, UAE-processing, blanching-processing, and their control samples (n = 12 and standard deviation).

3.2.4 Weight analysis

The results in Table 3.8 indicate that UAE40 and its control resulted in the highest increase in algae weight (increase of 21.13 and 21.44 %), suggesting the most significant hydration effect among the treatments. This is the sample that was together the longest (30 minutes). The PEF1800 showed a moderate increase in algae weight, indicating a moderate hydration effect. BL40 and its control (BL-C) showed a decrease in algae weight, indicating weight loss. BL100 showed the least change in algae weight, suggesting minimal impact on hydration.

Table 3.8: Shows the weight of rockweed and tap water before and after pulsed electric field processing, ultrasound processing and blanched at 40 °C and 100 °C, with their control samples (n = 6).

	Algae before (g)	Algae after (g)	Algae difference (%)	Tap water before (g)	Tap water after (g)	Tap water difference (%)
PEF1800	103.01±2.27	130.91±5.52	11.93±4.96	503.92±8.68	474.13±7.56	-3.05±4.88
PEF-C	103.10±6.30	155.3±14.00	20.2±7.70	512.47±0.33	457.35±1.05	-5.68±0.72
UAE40	103.33±1.74	158.7±4.65	21.13±5.29	502.05±6.85	435.18±6.06	-7.13±10.04
UAE-C	102.05±0.75	157.75±1.05	21.44±1.80	502.2±0.90	436.45±6.75	-7.00±5.85
BL40	104.24±3.18	146.25±16.57	16.77±14.66	501.65±4.57	448.62±20.98	-5.58±21.83
BL100	104.75±4.33	110.77±3.73	2.79±2.10	504.88±3.91	450.67±4.4	-5.67±2.99
BL-C	102.75±7.35	144.4±4.2	16.85±3.15	509.9±11.10	459.6±10.3	-5.19±0.80

The results in Table 3.9 indicate that PEF1800 and its control (PEF-C) resulted in significant increases in algae weight (14.97% and 51.64%, respectively), suggesting substantial hydration effects among the treatments. UAE40 and its control (UAE-C) also showed notable increases in algae weight (35.79% and 25.43%, respectively), also indicating hydration. On the contrary, BL40 and BL-C showed a slight decrease (-0.30%) and an increase (36.85%) in algae weight, indicating minimal weight loss and hydration, respectively, but BL100 resulted in a decrease in algae weight (-6.81%), suggesting a weight loss effect, the opposite of the results for rockweed.

Table 3.9: Shows the weight of winged kelp and tap water before and after pulsed electric field processing, ultrasound processing and blanched at 40 °C and 100 °C, with their control samples (n = 3).

Sample	Algae before (g)	Algae after (g)	Algae difference (%)	Tap water before (g)	Tap water after (g)	Tap water difference (%)
PEF1800	500.23±0.45	588.30±22.56	14.97±22.30	5008.37±9.64	4864.80±33.31	-2.87±25.64
PEF-C	50.77±0.82	104.97±1.11	51.64±1.77	507.93±1.41	450.77±4.48	-11.25±3.18
UAE40	101.17±1.72	157.57±7.11	35.79±7.11	503.23±2.57	438.83±6.39	-12.80±4.14
UAE-C	102.90±1.63	138.00±11.32	25.43±11.32	502.47±2.71	449.93±13.02	-10.46±10.71
BL40	100.67±0-09	100.37±1.51	-0.30±1.51	500.80±3.56	488.30±1.51	-2.50±9.77
BL100	100.37±0-29	93.97±2.05	-6.81±2.05	501.40±2.53	454.70±20.12	-9.31±22.39
BL-C	100.10±0.29	158.50±3.40	36.85±.3.23	503.67±0.45	484.23±12.28	-3.86±12.71

4. Discussion

4.1 Polyphenols

Extracting polyphenols from algae is valuable due to their potent antioxidant properties and wide range of health benefits, including anti-inflammatory and anti-cancer effects. Algae are also a sustainable source, making them ideal for applications in the food, pharmaceutical and cosmetic industries. This study investigated the optimal parameters for extracting the maximum TPC for each process. The processed samples were compared to show which pre-treatment would give the highest yield.

The pretreatment method which yielded the highest TPC for rockweed solid samples, was freeze-drying with TPC levels of 424.81±0.05 mg PGE/g ww algae. Due to the time constraints of this project freeze-drying was not tested for the winged kelp. In rockweed liquid samples, blanching at 100°C resulted in the highest TPC at 315.94±0.91 mg PGE/g ww algae, indicating substantial extraction of phenolic compounds into the water. PEF, UAE and blanched at 40 °C showed lower TPCs in liquid samples, where PEF and BL40 were similar (155.71±0.04 and 151.13±0.14 mg PGE/g ww algae, respectively). While for the winged kelp liquid samples, UAE-processing resulted in the highest TPC at 55.38±0.00 mg PGE/g ww algae. PEF-treated liquid samples had the lowest TPC of 36.36±0.00 mg PGE/g ww algae of processed samples. Control samples had generally lower TPCs across all processing methods, confirming that active treatments significantly enhance phenolic extraction.

Figure 3.10 and 3.11 shows a comparison of rockweed and winged kelp, solid and liquid samples, respectively. These results show a significant difference in TPC between the different brown algae. UAE-processing shows variable results, being less effective than PEF and blanching in solid samples for both algae, but more effective in liquid samples for winged kelp. For winged kelp liquid samples, blanching was an effective method for the preservation of phenolic compound. Rockweed is a part of the algae family Fucaceae, known for being rich in bioactive polyphenols. Rockweed is often used for polyphenol extraction because of its abundance in the Atlantic Ocean (Catarino et al., 2017). It can then be argued that the use of winged kelp for polyphenol extraction is not as lucrative as rockweed. However, it can also be argued that if the algae can be used for food after extraction, there could be potential in using

winged kelp as well, as rockweed is not typically used for human consumption (Hrólfsdóttir et al., 2022).

Freeze-drying is the most effective method for preserving phenolic content in solid rockweed samples, which corresponds with literature showing how freeze-drying maintains the nutritional composition of phenolic compounds in brown algae (Prosapio & Lopez-Quiroga, 2021; Wong & Cheung, 2001). It is a slow and expensive process that changes the texture of the algae considerably (Prosapio & Lopez-Quiroga, 2021). For liquid rockweed samples, PEF and BL40 had similar TPC. This showing how PEF extract as much as blanching at 40 °C extract similar amount of polyphenols. PEF is considered a more environmentally friendly and cheaper process than traditional pre-treatments like blanching (Demirci & Ngadi, 2012). It can therefore be argued that PEF is the best suited process, of the ones researched in this study, for TPC for a commercial and industrial level usage.

PEF proved to be an effective method for the extraction of polyphenol, for both the rockweed and winged kelp species. Rockweed showed significant increase in TPC, with a peak at 167.77±0.09 mg PGE/g ww algae, which is a significant increase in the TPC compared to the controls (75.06±0.04 mg PGE/g ww algae). Thus, demonstrating the effectiveness of PEF in enhancing phenolic extraction. Similar results were seen for the winged kelp treated with PEF as they also had higher TPC compared to the untreated sample. PEF treatment resulted in a TPC of 15.06±0.00 mg PGE/g ww algae, showing effectiveness but less than blanching. This is a higher yield than literature (9.37 ±0. 40 mg PGE/g ww algae) (Castejón et al., 2021), but it should be kept in mind that the samples from Castejón et al (2021) were freeze-dried before PEF-processing. It is shown in this study that freeze-drying is effective as a pre-treatment, and that may be a reason for the literature-numbers being higher. The effectiveness of PEF treatment can be due to how PEF processing creates small pores in the cell membrane (Y. Kumar et al., 2015), increasing permeability and therefore enhancing the extraction of polyphenols.

This study found that blanching significantly enhances TPC in both rockweed and winged kelp liquid samples, but it should be considered how blanching takes more energy than the other processes. The high yield of TPC in liquid samples can be explained by literature, as blanching

briefly heats the samples to inactivate enzymes that degrade sensitive compounds, such as polyphenols (Fellows, 2009). This means that blanching with water can lead to nutrient extraction as the water soluble components leak into the blanched water (Sterling, 2006; Xiao et al., 2017). The antioxidant activity was not researched in this study, so it can not be concluded if extracted polyphenols was degraded or not.

As mentioned in 2.4.1, the TPC results from experiment B, C and D are higher than in experiment A because of the change from 250 to 300 µl in 96-well plates used for absorbance reading. It was calculated as an 18.20±2.46 % higher yield from 250 to 300 µl for n =13 rockweed, shown in Figure I.1 (Appendix I). This is considered a methodical source of error in this study. Other sources of error can be variable of weight or other measurements (±1.0 g for scale used). The time between harvest should be mentioned here, because the seasons changed in between experiment A (January) and experiment B and C (March). The rockweed harvested later in the year did have more reproductive organs, which may have impacted the results of the PEF-energy level and TPC of all processed algae. The temperature in the ocean at the different times of harvest could also impact the results. Lastly, all experiments were only conducted 1-2 times, with at most 3 parallels. This is indicated in the SD-calculations, which are smaller than usual in a study like this.

4.2 Dry matter and ash content

Analyzing dry matter and ash content in algae assesses its nutritional value and quality. Dry matter analysis helps determine the concentration of essential nutrients and optimize biomass production for better yield and efficiency (Wu et al., 2023). Meanwhile, ash content analysis provides insights into the mineral content of algae, indicating the presence of essential minerals or potential contaminants (Liu, 2019). In this study these analyses were done to compare the different processes and the two algae.

The dry matter content for untreated winged kelp was calculated to be 9.0±0.0 % ww. Literature reported a dry matter content of 14.5±2.5 % ww for winged kelp, based on average values from algae harvested regularly throughout the year. It has also been indicated that dry matter can vary with seasonal difference during harvesting (Schiener et al., 2015). The literature dry matter

values (14.5±2.5 % ww) are higher than the values found in this study. This discrepancy can be attributed to differences in harvesting location and times, since the winged kelp in this study was harvested once in late spring (March). The literature study also mentions that winged kelp did not grow well in the locations where they were harvested, while the winged kelp in this study was farmed for research. It should also be noted that the algae in literature was frozen before analysis, while this study used fresh algae. The dry matter content in the untreated samples is the highest in this study, being a good baseline for comparison between the samples.

The results show that PEF processing significantly reduces the dry matter content in rockweed and winged kelp, both in the solid and liquid samples. Indicating effective cellular disruption and extraction of the solids into the liquid phase. This agrees with the theory that PEF-processing causes electroporation, leading to s holes in the cell membrane and the release of more dry matter (Janahar et al., 2022). UAE processing also reduce dry matter content (36.79±16.05 % ww), with high variability in winged kelp suggesting inconsistent effects of UAE. The blanched samples for both algae at both temperatures resulted in a reduction in dry matter content from the untreated sample, indicating substantial leak in the cell membrane, which consist with literature (Xiao et al., 2017). One of the uses for blanching in the food industry is as a pre-treatment before drying, because it enhances drying efficiency (Fellows, 2009; Heldman & Moraru, 2010). The comparison between untreated samples and blanched samples supports this theory, since the blanched samples do have a higher dry matter content.

The ash content has been reported to be 28.7 % TS for untreated rockweed. The ash content for rockweed in this study is a little lower, at 22.51±0.01 % TS. The ash content for the untreated winged kelp was 34.39±3.32 % of dry weight. Schiener et al. (2015) reported a lower ash content value of 25.3±5.8% TS. This study shows that PEF processing is effective in reducing ash content in both rockweed and winged kelp, where there is a significant reduction in the ash content of the rockweed. For winged kelp the UAE treated sample had a higher ash content than the untreated sample. The standard deviation for these samples was also high, which can explain this. Blanching at higher temperatures (100 °C) is more effective than at lower temperatures (40 °C) for both types of algae, but still less effective than PEF with the smallest percentage TS. This showing an effect of the processing methods. The control samples

(especially PEF-C) also showed some lower percentage and suggest that the ash content can be reduced even without aggressive treatment.

Schiener et al. (2015) reported that throughout the year, ash levels varied from 20-25% dry weight for winged kelp. High ash contents can be due to the accumulation of potassium and sodium ions, which peak during the winter months and decrease in autumn (Schiener et al., 2015). In this study, the two brown algae were harvested in March, which is early spring and can explain why the ash content in this study is higher for winged kelp than those reported in the literature. It should also be noted that the results for the ash content had a high variability in general, which suggests the need for further optimization of the method used to determine ash content. Location and ecological conditions can affect ash and nutrient contents due to environmental factors such as light, nutrients, water temperature and currents. Some examples of this is light exposure; where in shallow sunlit areas, algae can accumulate nutrients leading to a higher concentration of ash, and water currents; where coastal areas with strong currents can lead to an increased sediment mixing and redistribute ash and nutrients (Marinho-Soriano et al., 2006) Overall, the present study found lower dry weight and higher ash content for winged kelp compared to the literature values. The untreated samples maintained a high ash content, indicating a high natural mineral level in the algae which is supported by literature (Anis et al., 2017).

It should be noted that the samples in this study were placed in the muffle oven at different times over a two-week period, with 16 samples processed at a time. This may lead to inconsistency in drying time, where there was a maximum difference of 40 minutes in the muffle oven between the samples. It could also be environmental factors during the two weeks, as the room where the muffle oven is, had temperature fluctuates because of a broken air condition.

4.3 Color analysis

There are multiple reasons to do color analysis on algae, like species identification or water quality assessment (Lee et al., 2013). In this study color analysis was done to see if there were any big differences between the different processes, that can influence the consumers opinion on if the algae are suitable for eating. It is also interesting to know that the color of algae can inform about the biomass productivity. Denser and healthier algae tend to have a richer color, which is an indicator of higher biomass (Singh & Singh, 2015).

The untreated sample serves as a baseline for comparison. In Figure 3.10, the untreated rockweed sample has the highest L* value, meaning it is the lightest sample. The hue value dips significantly for all processes, except UAE40. Showing how UAE40 keeps the light-hue intact. For a*, the untreated sample has the highest hue value, but there is no significant difference between any of the samples. Meaning that the processes may not make a change in how green the sample is. This should be researched further and with a statical analysis. For b*, the untreaded sample has the second lowest hue, where UAE-C is lower. This shows that the processed samples, and control samples there are in tap water for a short amount of time does get affected and become more yellow. For winged kelp, the hues do not change significantly from the untreated sample, but there is a small difference in the UAE40 and the BL100 sample. They both become lighter, more green and more yellow.

Figure 3.16 shows that even though rockweed has higher amounts of yellow and blue pigments and are lighter than winged kelp, the different pretreatment methods affect both types of algae similarly. On the other hand, rockweed spiked at L* with untreated and UAE samples, while winged kelp spiked with BL100. Then both algae have a small spike at L* with their BL100 samples. Overall, rockweed shows higher lightness (L*) and yellow hues (b*) compared to winged kelp, which showed stronger green tints (a*). The processing methods did influence the color parameters, with UAE-processing and blanching at 100 °C leading to the highest lightness values in both rockweed and winged kelp. Control samples showed that rockweed had consistently higher lightness and yellow hues, while winged kelp had stronger green tints. This indicates distinct color characteristics between the two types of algae, with rockweed generally being lighter and more yellow, and winged kelp being darker and greener. It can then be argued that the color analysis does show that PEF and blanching processed rockweed have a higher

biomass of the processed samples, but the untreated sample have the highest, which is also backed up by the dry matter and ash content results.

Literature shows a pattern of brown algae turning lighter and greener at higher temperatures. It also demonstrates that the timing and location of the photographs of the algae, as well as whether the samples are fresh or frozen, are important factors (Blikra et al., 2019). This is something that could be researched further, and how it connects to polyphenol-contents. It is a red line between how the color of brown algae reacts to light and heat, just as polyphenols react from literature.

Algae has high content of the pigment fucoxanthin which is easily degradable. It has been shown that brown algae have an instability to light, because light promotes trans-cis isomerization (Hii et al., 2010) (the transformation of a molecule to a different isomer (Herceg & Murr, 2011)). Isomerization increases the rate of degradation of pigments and results in a color change (Hii et al., 2010).

When heat was applied to the brown algae the color changed. The higher the temperature, the lighter the color became. When brown algae are dipped in hot water the color will change from brown to green, due to the degradation of fucoxanthin (Bast, 2014) making the chlorophyll-pigments more visible. This degradation can be related to the pigment-protein complex. Most of the fucoxanthin content in brown algae is found in the fucoxanthin-chlorophyll a/c protein complex in the thylakoid membrane and is synthesized as needed. This complex is more loosely bound to the membrane, in comparison to chlorophyll-protein complexes (Wolstenholme & FitzSimons, 1979). That's why it can be argued that when the algae were blanched at a high temperature, went into a hot ultrasound chamber or in a drying rack with heat some of the fucoxanthin-complex detached and the fucoxanthin was degraded in response to the heat. This making the results from Figure 3.12, where a* UAE40 drop, reasonable. In contrast, the chlorophyll-protein complexes are more tightly bound to the membrane (Wolstenholme & FitzSimons, 1979) providing greater stability against external factors. How much of the fucoxanthin was degraded is not known since it was not measured, but the color analysis showed a lighter coloring and a more greenish hue when heat was applied, which indicates that

the chlorophyll-pigments are more stable than the fucoxanthin (that detached) to external influences. The same argument can be used for the algae reaction to light and how much the wait time between processing and color analysis had to say for the accuracy of these results.

All the sample algae in this study were covered by aluminum foil when stored, to prevent degradation of polyphenols. The color was not analyzed 2-3 hours after processing, which can lead to a change in the pigments. The degree to which these factors impacted the color analysis is impossible to tell, but it can be argued that it did. The algae for each process were harvested on different days, and at different locations close to each other (<0.5 km). This could affect the color, depending on the weather, and ties back to the argument regarding the algae's reaction to light. All the color analysis of the different processed algae was conducted at different days. The machine was calibrated each day, which could lead to calibration errors. The samples used for color analysis was prepared by different researchers, and this could lead to variability in sample thickness and surface uniformity. This could lead to inconsistent color reading.

4.4 Weight analysis

A weigh analysis was done to see if the brown algae absorbed the tap water more effectively in any of the processes and if this is connected to the polyphenol extraction.

The results from Tables 3.7 and 3.8 demonstrate that UAE-processing and its control resulted in the highest increases in algae weight for both rockweed and winged kelp, indicating significant hydration effects (21.13 % and 21.44 % for rockweed; 35.79 % and 25.43 % for winged kelp), likely due to the mechanical effects of ultrasound waves enhancing water uptake. PEF showed moderate hydration for both algae species (11.93% for rockweed and 14.97 % for winged kelp), with its control sample exhibiting even higher hydration (20.20 % for rockweed and 51.64% for winged kelp), suggesting that winged kelp have a greater water absorption, even in a short amount of time (2.5 minutes). BL40 resulted in a lower biomass weight for rockweed (-16.77 %) but minimal weight loss for winged kelp (-0.30 %), whereas blanching at 100°C (BL100) caused weight loss in both alga types (-2.79 % for rockweed and -6.81 % for winged kelp).

Overall, the changes in tap water weight reflected the inverse relationship, with decreases corresponding to the increases in algae weight, supporting the hydration trend observed. It can be argued that the lower weight for solid winged kelp blanching samples is not necessarily water loss, whereas the algae can lose other mineral and substances like ash. The standard deviation being higher in the after samples indicates that both algae absorb water, but it may be different outside factors that decide how much. In this study, this may be human error. The duration before straining the algae and water mixture may have varied. It could also be because of inaccuracies in the measurement equipment (which was ±1.0 g for scale used in this study). UAE40 had the lowest TPC of all processes, and freeze-dried had the highest for rockweed (Table 2.6). This is interesting to note, because UAE30 was the longest in tap water (30 minutes) and freeze-dried algae was never in water. This is also reflected in in the polyphenol analysis of the liquid samples, where UAE40 and its control had a similar amount of TPC to PEF1800, while PEF1800 for the solid samples had a much higher TPC than UAE40. These results suggest that longer water exposure affects TPC.

5. Conclusion

This study evaluated various processing technologies on their effectiveness in improving the extraction of polyphenol from two different species of brown algae; rockweed (*Ascophyllum nodosum*) and winged kelp (*Alaria esculenta*). The processing methods investigated included PEF, UAE, and blanching. The results demonstrate that the choice of processing method significantly impacts the color, dry matter, ash content and TPC in both algae species.

The color analysis revealed that different processing treatments affect the visual quality of the solid samples, but not the liquid samples. PEF and UAE treatments generally preserve the natural color of the algae better than blanching, which often leads to color degradation. The preservation of color indicates that certain pigments and phenolic compounds have been maintained, these are important for both the nutritional quality and marketability of algae products.

The dry matter values were lower than those reported in the literature, which could possibly be attributed to seasonal variations and differences in harvesting times. The ash content was found to be higher than in literature. This suggests an accumulation of minerals, likely affected by the harvesting period being at the transition between winter and summer. These results highlight the importance of considering seasonal and environmental factors when evaluating the nutritional content of algae.

The study demonstrates that freeze-drying, PEF processing, UAE processing and blanching are effective methods for extracting polyphenols from brown algae. Even though freeze-drying did yield more TPC, PEF processing showed the most significant improvement in TPC with optimized pulse counts and energy inputs. UAE processing is the least effective in improving the TPC in comparison to their control, while blanching at lower temperatures preserves higher phenolic content in both solid and liquid samples.

Overall, the findings underscore the importance of choosing and optimizing processing methods to maximize the extraction and preservation of bioactive compounds in algae. This

information contributes to improving the nutritional and functional properties of algae-derived products, supporting sustainable food production and the utilization of ocean resources.

6. Future research

Future research should focus on further optimizing processing parameters for PEF, UAE, and blanching to maximize the extraction and preservation of bioactive compounds in brown algae. More specifically, studies could explore the effects of varying the duration of each process on the TPC, but also how these processes would affect other nutritional components. The seasonal and environmental factors affecting the TPC, dry matter and ash content should be further investigated to understand how different harvesting times and locations influence the nutritional quality of algae.

In addition, future research should include the investigation of combined pretreatment methods. For instance, combining PEF with UAE or blanching could potentially improve the extraction efficiency of polyphenols and other valuable compounds. It would also be interesting to determine how different pre-treatment methods can influence polyphenol extraction, like freeze-drying before PEF. Various combinations of different processes could be investigated to increase both the yield and quality of algae extracts.

Furthermore, the impact of these processing methods on the bioavailability and bioactivity of the extracted compounds needs to be evaluated. This would include studying how the treatments affect the antioxidant activity, antimicrobial properties and the potential health benefits of algae extracts. Further research should also be conducted on potential uses for the leftover biomass of the algae. Research like this could provide valuable insight into the functional properties of algae-derived products and their applications in food, pharmaceutical and cosmetic industries.

This study showed and discussed which pre-treatment enchanted the most TPC in general for both solid and liquid samples. Future research should focus on bringing as much of the TPC into one of the phases, to simplify the extraction process and potentially increase the yield of phenolic compounds.

Finally, upscaling of the optimized processes for industrial applications would be a critical step in future research. This research should address the technical and economic possibility of large-

scale implementation. This includes energy consumption, cost-effectiveness and the environmental impact. This would help in transitioning the findings from laboratory research to practical applications, thus promoting the sustainable and efficient use of algae resources.

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Appendix I Calculations

Standard deviation

Standard deviation for all measurements were determined by Excel build in function STDAV.S, with formula:

$$\sigma = \sqrt{\frac{\sum(x_i - \bar{x})^2}{N - 1}} \tag{6.1}$$

Calculation of total phenolic content

The total phenolic content [mg PGE/g ww algae] was determined with formula:

$$TPC = \frac{cV}{m} \tag{6.2}$$

Were,

c [mg/ml] = concentration obtained by the standard curve, further converted from [mM] to [mg/ml] with the molar mass of propyl gallate (212.2 g/mol)

V [ml] = volume of solvent used in extraction

m [g] = mass of algae used

Calculation of ash content

Ash content was determined by formula:

$$\frac{ash}{100}(g) = \frac{(a-c)}{(b-c)} \times 100 \quad (6.3)$$

Were,

a [g] = final weight of crucible and ash

b [g] = weight of crucible and original sample

c [g] = weight of empty incinerated crucible

Calculation of dry matter

Dry matter content was determined by formula:

$$Dry\ matter\ content\ (\%) = \frac{(a-c)}{(b-c)} \times 100 \quad (6.4)$$

Were,

a [g] = final weight of crucible and dry matter

b [g] = weight of crucible and original sample

c [g] = weight of empty incinerated crucible

Calculation of energy usage of pulsed electric field processing

$$Energy\ \left(\frac{kJ}{kg}\right) = \frac{Energy\ per\ pulse}{w_{algae} + w_{water}} \quad (6.5)$$

Were

Energy per pulse = Obtained from PEF Pilot Dual (Elea Technology, Germany) machine

W_{algae} = weight of algae used

W_{water} = weight of tap water used

Table I.1: Calculated difference between different amounts of sample in 96-well plate (n = 2).

		250 µl	SD	300 µl	SD	% difference
Solid	PEF1800	0.58	0.00	0.69	0.01	19.57
	PEF-C	0.29	0.00	0.34	0.01	15.27
	UAE40	0.29	0.00	0.35	0.00	22.13
	UAE40-C	0.30	0.00	0.35	0.00	17.53
	BL40	0.48	0.00	0.56	0.02	15.40
	BL100	0.49	0.00	0.58	0.01	16.49
Liquid	PEF1800	0.51	0.00	0.61	0.01	20.37
	PEF-C	0.20	0.00	0.24	0.00	17.41
	UAE40	0.55	0.00	0.64	0.00	17.22
	UAE40-C	0.56	0.00	0.64	0.00	14.89
	BL40	0.84	0.01	1.01	0.01	20.06
	BL100	1.71	0.02	2.08	0.01	22.08
Mean						**18.20**
SD						**2.46**

Appendix II Energy consumption of pulsed electric field

Experiment A

Table II.1: Parameters used and data received from PEF Pilot Dual (Elea Technology. Germany) and corresponding energy usage for rockweed.

Parallel	Ratio	Voltage (kV)	Pulse count	Frequency (Hz)	Pulse peak voltage max (kV)	Pulse peak current max (A)	Energy (J)	Energy per kg (kJ/kg)
A	1:5	24	1000	50	24.43	167.00	23151.4	9.65
B	1:3	24	1000	50	23.78	167.00	23144.8	14.89
C	1:5	24	4000	50	23.82	261.00	143635.1	59,.4

Table II.2: Parameters used and data received from PEF Pilot Dual (Elea Technology. Germany) and corresponding energy usage for rockweed.

Parallel	Ratio	Voltage (kV)	Pulse count	Frequency (Hz)	Pulse peak voltage max (kV)	Pulse peak current max (A)	Energy (J)	Energy per kg (kJ/kg)
10-1	1+5	8	1000	50	7.63	143	6301.10	10.20
10-2	1+5	8	1000	50	7.61	155	6770.40	11.28
20-1	1+5	8	2000	50	7.61	193	16743.20	27.39
20-2	1+5	8	1500	50	7.60	172	11260.50	18.49
30-2	1+5	8	2000	50	7.58	205	17615.70	28.66
40-1	1+5	8	3000	50	7.55	232	29729.60	48.90
50-1	1+5	8	1500	50	7.57	184	12002.00	19.17
50-2	1+5	8	3000	50	7.56	236	30073.40	49.03
60-1	1+5	8	2600	50	7.58	225	24954.50	40.95
60-2	1+5	8	3400	50	7.49	286	40385.00	65.84

Experiment B

Table II.3: Parameters used and data received from PEF Pilot Dual (Elea Technology. Germany) and corresponding energy usage for rockweed.

Parallel	Ratio	Voltage (kV)	Pulse count	Freq (Hz)	Pulse peak voltage max (kV)	Pulse peak current max (A)	Energy (J)	Energy per kg (kJ/kg)
3000a	1+5	8	3000	50	7.35	387	-	-
2000a	1+5	8	2000	50	7.38	386	-	-
1800a	1+5	8	1800	50	7.43	376	27130.41	45.32
1800b	1+5	8	1800	50	7.41	384	27541.81	45.74

- Resistance too low in machine to collect data

Experiment C

Table II.4 Parameters used and data received from PEF Pilot Dual (Elea Technology. Germany) and corresponding energy usage for rockweed.

Parallel	Ratio	Voltage (kV)	Pulse count	Freq (Hz)	Pulse peak voltage max (kV)	Pulse peak current max (A)	Energy (J)	Energy per kg (kJ/kg)
1800a	1+5	8	1800	50	7.32	385	-	-
1800b	1+5	8	1800	50	7.42	370	26830.45	44.30
1800c	1+5	8	1800	50	7.36	378	26998.71	44.98

- Resistance too low in machine to collect data

Experiment D

Table II.5: Parameters used and data received from PEF Pilot Dual (Elea Technology, Germany) and corresponding energy usage for winged kelp.

Parallel	Ratio	Volt (kV)	Pulse count	Freq (Hz)	Pulse peak voltage max (kV)	Pulse peak current max (A)	Energy (J)	Energy per kg (kJ/kg)
1800a	01:10	24	1800	50	22,72	882	200394.80	36.42
1800b	01:10	24	1800	50	22,37	925	208395.60	37.88
1800c	01:10	24	1800	50	22,02	998	219652.00	39.77

Appendix III TPC standard curves and absorbance

Standard curves for TPC by Folin-Ciocalteu assay

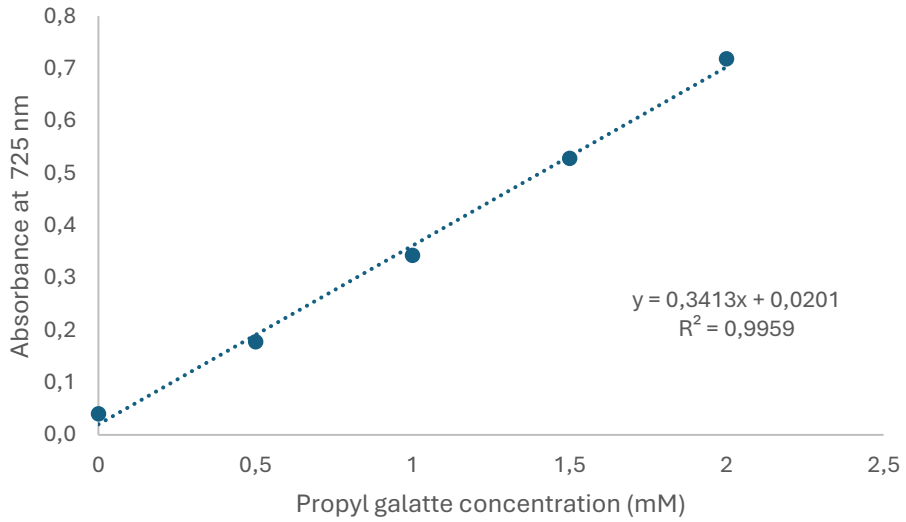

Figure III.1: Standard curve for dilution series of propyl gallate for calculations of TPC for rockweed samples for 250 µl used in 96-well plate.

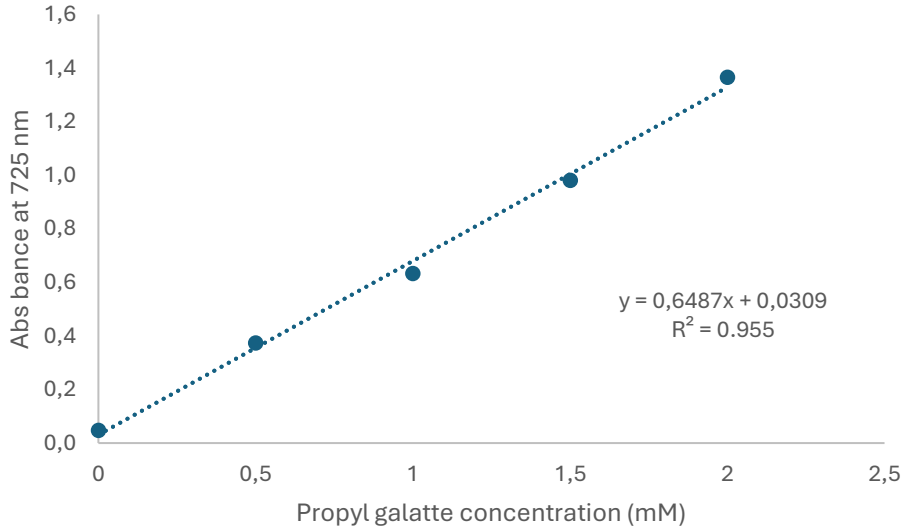

Figure III.2: Standard curve for dilution series of propyl gallate for calculations of TPC for rockweed samples for 300 µl in 96-well plate.

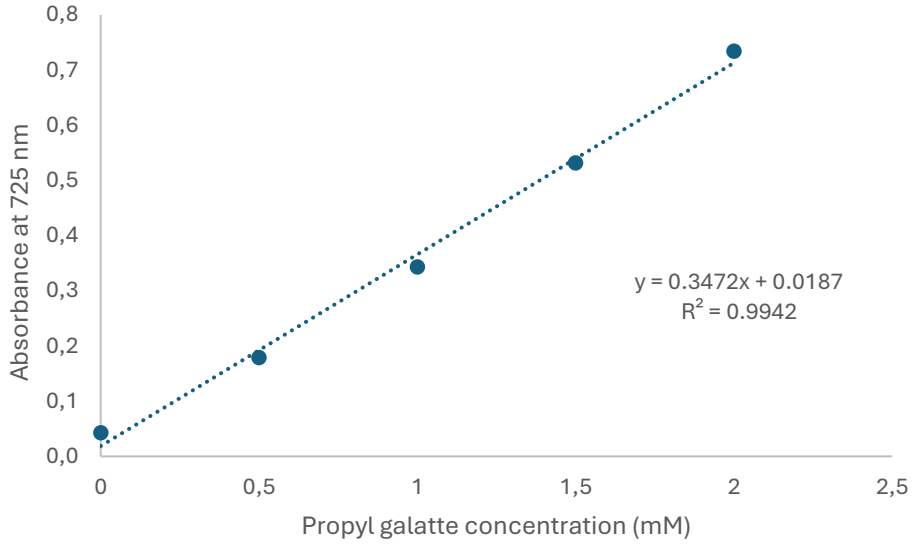

Figure III.3: Standard curve for dilution series of propyl gallate for calculations of TPC for experiment samples for fresh winged kelp.

Absorbance measurements at TPC the Folin-Ciacalteu assay, 725 nm

Experiment A

Table III.1 Absorbance of pulsed electric field processed solid rockweed.

Parallel	1	2	3	Weight (g)	SD
10-1	0.326	0.323	0.321	0.56	0.002
10-2	0.236	0.234	0.272	0.46	0.017
20-1	0.293	0.293	0.303	0.53	0.005
20-2	0.365	0.36	0.362	0.48	0.002
30-2	0.275	0.364	0.366	0.53	0.042
40-1	0.288	0.287	0.29	0.55	0.001
50-1	0.396	0.37	0.363	0.49	0.014
50-2	0.502	0.504	0.506	0.47	0.002
60-1	0.453	0.432	0.457	0.56	0.011
60-2	0.387	0.378	0.408	0.57	0.013
Control	0.167	0.175	0.175	0.50	0.004

Table III.2 Absorbance of ultrasound processed solid rockweed.

Parallel	1	2	3	Weight (g)	SD
UAE40a	-	-	-	0.51	-
UAE40b	0.274	0.254	0.262	0.51	0.008
UAE40c	0.345	0.345	0.349	0.52	0.002
UAE68a	-	-	-	0.53	-
UAE68b	0.222	0.219	0.221	0.50	0.001
UAE68c	0.225	0.229	0.225	0.51	0.002
UAE170a	0.188	0.189	0.19	0.49	0.001
UAE170b	0.165	0.163	0.168	0.49	0.002
UAE170c	0.173	0.172	0.177	0.50	0.002
UAE17068a	0.178	0.179	0.176	0.51	0.001
UAE17068b	0.23	0.229	0.232	0.52	0.001
UAE17068c	0.194	0.194	0.198	0.48	0.002
Control	0.381	0.374	0.368	0.49	0.006

- no data obtained from absorbance analysis

Table III.3 Absorbance of blanched solid rockweed.

Parallel	1	2	3	Weight (g)	SD
40a	0.536	0.541	0.527	0.56	0.006
40b	0.325	0.328	0.331	0.55	0.002
50a	0.398	0.396	0.391	0.56	0.003
50b	0.603	0.604	0.604	0.53	0.000
60a	0.492	0.489	0.501	0.49	0.005
60b	0.656	0.656	0.56	0.48	0.045
100a	0.534	0.525	0.523	0.57	0.005
100b	0.493	0.525	0.523	0.50	0.015
Control	0.178	0.191	0.190	0.50	0.006

Liquid

Table III.4 Absorbance of pulsed electric field processed rockweed.

Parallel	1	2	3	SD
10a	0.101	0.101	0.099	0.040
10b	0.046	0.043	0.044	0.033
20a	0.148	0.123	0.145	0.033
20b	0.047	0.047	0.048	0.045
30a	0.193	0.192	0.194	0.065
40a	0.21	0.211	0.214	0.031
50a	0.177	0.178	0.175	0.065
50b	0.199	0.201	0.2	0.028
60a	0.214	0.215	0.216	0.048
60b	0.217	0.278	0.228	0.046
Control	0.104	0.103	0.104	0.073

Table III.5 Absorbance of ultrasound processed liquid rockweed.

Parallel	1	2	3	SD
UAE40a	0.117	0.118	0.116	0.001
UAE40b	0.116	0.116	0.114	0.001
UAE40c	0.116	0.115	0.114	0.001
UAE68a	-	-	-	-
UAE68b	0.095	0.095	0.093	0.001
UAE68c	0.087	0.088	0.087	0.000
UAE170a	0.119	0.119	0.12	0.000
UAE170b	0.108	0.108	0.109	0.000
UAE170c	0.105	0.104	0.104	0.000
UAE17068a	0.114	0.111	0.114	0.001
UAE17068b	0.114	0.111	0.113	0.001
UAE17068c	0.095	0.095	0.095	0.000
Control	0.243	0.241	0.239	0.000

Table III.6 Absorbance of blanched liquid rockweed.

Parallel	1	2	3	SD
40a	0.230	0.227	0.227	0.001
40b	0.169	0.171	0.168	0.001
50a	0.405	0.406	0.414	0.004
50b	0.509	0.505	0.506	0.002
60a	0.733	0.720	0.722	0.006
60b	0.760	0.752	0.750	0.004
100a	1.029	1.010	1.012	0.009
100b	1.004	1.003	1.016	0.006
Control	0.079	0.080	0.079	0.000

Experiment B

Algae

Table III.7 Absorbance of pulsed electric field processed solid rockweed.

Parallel	1	2	3	Weight (g)	SD
1800a	1.016	0.989	0.97	0.51	0.019
1800b	0.685	0.666	0.668	0.54	0.009
1800c	0.523	0.592	0.524	0.58	0.032
1800d	0.537	0.533	0.543	0.51	0.004
Control	0.268	0.267	0.267	0.42	0.000

Table III.8 Absorbance of ultrasound processed solid rockweed.

Parallel	1	2	3	Weight (g)	SD
40a	0.991	0.975	0.981	0.53	0.008
40b	0.585	0.587	0.581	0.48	0.002
40c	0.366	0.361	0.368	0.54	0.003
Control	0.382	0.374	0.368	0.47	0.006

Table III.9 Absorbance of blanched solid rockweed.

Parallel	1	2	3	Weight (g)	SD
40a	0.344	0.352	0.345	0.47	0.004
40b	0.467	0.486	0.479	0.48	0.008
40c	0.386	0.384	0.378	0.52	0.003
100a	1.346	1.347	1.324	0.54	0.011
100b	1.354	1.354	1.542	0.52	0.089
100c	1.338	1.337	1.348	0.52	0.004
Control	0.105	0.103	0.102	0.51	0.001

Liquid

Table III.10 Absorbance of pulsed electric field processed rockweed.

Parallel	1	2	3	SD
1800a	0.651	0.645	0.654	0.004
1800b	0.598	0.600	0.585	0.007
1800c	0.608	0.572	0.605	0.016
1800d	0.550	0.549	0.542	0.004
Control	0.218	0.219	0.220	0.001

Table III.11 Absorbance of ultrasound processed liquid rockweed.

Parallel	1	2	3	SD
40a	0.299	0.299	0.299	0.000
40b	0.268	0.267	0.265	0.001
40c	0.333	0.313	0.315	0.009
Control	0.233	0.230	0.233	0.001

Table III.12 Absorbance of blanched liquid rockweed.

Parallel	1	2	3	SD
40a	0.215	0.219	0.212	0.003
40b	0.202	0.204	0.209	0.003
40c	0.129	0.128	0.13	0.001
100a	0.295	0.294	0.294	0.000
100b	0.264	0.273	0.264	0.004
100c	0.261	0.26	0.255	0.003
Control	0.173	0.172	0.191	0.009

Experiment C

Algae

Table III.13 Absorbance of pulsed electric field processed solid rockweed.

Parallel	1	2	3	Weight (g)	SD
1800a	0.682	0.69	0.697	0.682	0.006
1800b	0.618	0.607	0.626	0.618	0.008
1800c	0.783	0.773	0.775	0.783	0.004
Control	0.325	0.351	0.343	0.325	0.011

Table III.14 Absorbance of ultrasound processed solid rockweed.

Parallel	1	2	3	Weight (g)	SD
40a	0.349	0.348	0.355	0.51	0.004
40b	0.345	0.345	0.344	0.51	0.000
40c	0.355	0.376	0.362	0.52	0.009
Control	0.353	0.343	0.35	0.53	0.004

Table III.15 Absorbance of blanched solid rockweed.

Parallel	1	2	3	Weight (g)	SD
40a	0.582	0.531	0.584	0.52	0.025
40b	0.528	0.555	0.548	0.52	0.011
40c	0.575	0.552	0.558	0.51	0.010
100a	0.557	0.557	0.553	0.53	0.002
100b	0.573	0.571	0.553	0.5	0.009
100c	0.604	0.602	0.614	0.5	0.005
Control	0.287	0.284	0.284	0.47	0.001

Liquid

Table III.16 Absorbance of pulsed electric field processed rockweed.

Parallel	1	2	3	SD
1800a	0.679	0.682	0.686	0.003
1800b	0.572	0.625	0.559	0.029
1800c	0.576	0.575	0.571	0.002
Control	0.234	0.234	0.24	0.003

Table III.17 Absorbance of ultrasound processed liquid rockweed.

Parallel	1	2	3	SD
40a	0.657	0.654	0.642	0.006
40b	0.652	0.643	0.647	0.004
40c	0.631	0.629	0.631	0.001
Control	0.633	0.642	0.639	0.004

Table III.18 Absorbance of blanched liquid rockweed.

Parallel	1	2	3	SD
40a	0.973	0.966	0.970	0.003
40b	1.023	1.070	1.070	0.022
40c	0.988	0.988	1.0000	0.006
100a	2.110	2.099	2.051	0.026
100b	2.048	2.066	2.062	0.008
100c	2.116	2.091	2.102	0.010
Control	0.289	0.292	0.289	0.001

Experiment D

Algae

Table III.19 Absorbance of pulsed electric field processed solid winged kelp.

Parallel	1	2	3	Weight (g)	SD
1800a	0.099	0.102	0.101	0.51	0.001
1800b	0.139	0.138	0.139	0.49	0.000
1800c	0.122	0.125	0.123	0.50	0.001
Control1	0.109	0.115	0.115	0.51	0.003
Control2	0.110	0.110	0.116	-	0.003
Control3	0.112	0.125	0.119	-	0.005

Table III.20 Absorbance of ultrasound processed solid winged kelp.

Parallel	1	2	3	Weight (g)	SD
40a	0.082	0.082	0.082	0.51	0.000
40b	0.091	0.091	0.090	0.51	0.000
40c	0.092	0.095	0.093	0.51	0.001
Control1	0.093	0.093	0.093	0.49	0.000
Control2	0.090	0.091	0.094	0.50	0.002
Control3	0.086	0.09	0.093	0.49	0.003

Table III.21 Absorbance of blanched solid winged kelp.

Parallel	1	2	3	Weight (g)	SD
40a	0.102	0.101	0.101	0.5	0.000
40b	0.179	0.178	0.162	0.51	0.008
40c	0.16	0.16	0.159	0.51	0.000
100a	0.1	0.101	0.103	0.52	0.001
100b	0.124	0.125	0.124	0.51	0.000
100c	0.125	0.125	0.126	0.50	0.000
Control1	0.097	0.099	0.109	0.49	0.005
Control2	0.101	0.107	0.108	0.50	0.003
Control3	0.102	0.102	0.108	0.49	0.003

Liquid

Table III.22 Absorbance of pulsed electric field processed liquid winged kelp.

Parallel	1	2	3	SD
1800a	0.293	0.29	0.285	0.003
1800b	0.254	0.255	0.257	0.001
1800c	0.25	0.253	0.252	0.001
Control1	0.148	0.147	0.148	0.000
Control2	0.155	0.152	0.161	0.004
Control3	0.149	0.147	0.149	0.001

Table III.23 Absorbance of ultrasound processed liquid winged kelp.

Parallel	1	2	3	SD
40a	0.392	0.391	0.393	0.000
40b	0.394	0.395	0.398	0.000
40c	0.396	0.395	0.397	0.001
Control1	0.315	0.313	0.314	0.000
Control2	0.334	0.329	0.333	0.002
Control3	0.319	0.313	0.309	0.003

Table III.24 Absorbance of blanched liquid winged kelp.

Parallel	1	2	3	SD
40a	0.41	0.404	0.403	0.003
40b	0.456	0.465	0.451	0.006
40c	0.527	0.513	0.528	0.007
100a	0.331	0.323	0.324	0.004
100b	0.306	0.299	0.321	0.009
100c	0.321	0.318	0.322	0.002
Control1	0.182	0.182	0.183	0.000
Control2	0.199	0.201	0.198	0.001
Control3	0.192	0.192	0.189	0.001

Appendix IV Color measurements with DigiEye

Experiment B

Table IV.1: Mean of three parallels of color analysis of pulsed electric field processed solid rockweed and their corresponding standard deviation (n = 3).

Algae	L*	a*	b*	C*	h
1800a	25.57	-1.86	18.75	18.84	95.67
1800b	28.70	-2.00	17.27	17.38	96.58
1800c	27.37	-1.96	18.20	18.31	96.14
1800d	26.60	-1.93	16.32	16.43	96.78
Control	26.01	-1.53	19.17	19.24	94.58
SD					
1800a	0.70	0.10	0.50	0.49	0.44
1800b	0.37	0.18	0.35	0.37	0.48
1800c	0.48	0.13	0.48	0.48	0.41
1800d	0.31	0.03	1.18	1.17	0.54
Control	0.47	0.06	0.85	0.85	0.37

Table IV.2: Mean of three parallels of color analysis of ultrasound processed solid rockweed (n = 3).

Algae	L*	a*	b*	C*	h
40a	67.51	-1.37	29.59	29.62	92.65
40b	69.58	-1.69	28.37	28.42	93.40
40c	73.15	-1.37	20.65	20.70	93.80
Control	72.94	-1.53	19.72	19.78	94.45
SD					
40a	0.24	0.19	0.50	0.49	0.42
40b	1.03	0.22	0.16	0.15	0.46
40c	0.60	0.20	0.45	0.44	0.60
Control	0.59	0.07	0.66	0.65	0.35

Table IV.3: Mean of three parallels of color analysis of pulsed electric field processed solid winged kelp and their corresponding standard deviation (n = 3).

Algae	L*	a*	b*	C*	h
40a	11.77	-6.05	8.44	10.43	125.74
40b	11.72	-6.18	7.36	9.63	130.21
40c	10.45	-7.36	7.03	10.21	136.34
100a	18.05	-11.79	17.61	21.19	123.87
100b	18.83	-10.96	16.66	19.95	123.41
100c	17.44	-11.96	17.81	21.46	123.89
Control1	10.84	-4.83	5.96	7.78	128.50
Control2	9.89	-5.07	7.11	8.77	125.62
Control3	10.49	-4.43	6.63	7.97	123.74
SD					
40a	0.60	0.71	0.78	0.20	5.70
40b	0.41	0.27	0.77	0.50	3.80
40c	0.55	0.60	0.64	0.31	4.57
100a	0.21	0.33	1.10	1.09	0.97
100b	0.91	0.58	1.35	1.38	1.50
100c	0.04	0.20	0.61	0.61	0.51
Control1	0.42	1.28	0.53	0.47	9.52
Control2	0.30	0.62	0.69	0.21	5.91
Control3	0.33	0.13	0.10	0.12	0.84

Liquid

Table IV.4: Color analysis of pulsed electric field processed liquid rockweed and their corresponding standard deviation (n = 3).

Liquid	L*	a*	b*	C*	h
1800a	72.72	-1.48	19.44	19.50	94.38
1800b	71.67	-1.28	17.72	17.77	94.15
1800c	73.99	-0.66	16.64	16.65	92.28
1800d	74.00	-1.13	15.55	15.59	94.17
Control	75.63	-1.14	12.53	12.59	95.26
SD					
1800a	0.45	0.20	0.71	0.70	0.75
1800b	0.79	0.04	0.59	0.58	0.27
1800c	0.28	0.06	0.64	0.63	0.23
1800d	0.22	0.02	0.24	0.24	0.08
Control	0.25	0.20	0.70	0.68	1.14

Table IV.5: Color analysis of ultrasound processed liquid rockweed and their corresponding standard deviation (n = 3).

Liquid	L*	a*	b*	C*	h
40a	25.66	-0.67	10.01	10.04	93.74
40b	24.38	-1.83	10.63	10.78	99.72
40c	24.57	-1.24	9.88	9.99	97.37
Control	24.88	-0.77	9.93	9.97	94.47
SD					
40a	1.03	0.37	0.73	0.75	1.90
40b	0.61	0.22	0.42	0.45	0.75
40c	1.72	0.69	0.60	0.52	4.36
Control	1.37	0.32	1.29	1.29	1.99

Table IV.6: Color analysis of blanched liquid rockweed and their corresponding standard deviation (n = 3).

Algae	L*	a*	b*	C*	h
40a	63.34	4.57	37.04	37.32	82.96
40b	65.15	3.33	34.57	34.73	84.51
40c	69.01	3.25	35.54	35.69	84.78
100a	66.27	4.59	38.15	38.43	83.15
100b	64.79	5.34	40.55	40.90	82.50
100c	70.24	6.81	44.78	45.30	81.37
Control1	73.28	-0.80	12.96	12.99	93.52
Control2	63.34	4.57	37.04	37.32	82.96
Control3	65.15	3.33	34.57	34.73	84.51
SD					
40a	0.17	0.30	0.66	0.69	0.33
40b	0.22	0.31	0.67	0.70	0.42
40c	0.67	0.20	0.54	0.56	0.25
100a	0.49	0.38	0.91	0.94	0.41
100b	0.16	0.33	0.59	0.62	0.35
100c	0.63	0.56	1.37	1.43	0.46
Control1	0.22	0.11	0.21	0.20	0.54
Control2	0.17	0.30	0.66	0.69	0.33
Control3	0.22	0.31	0.67	0.70	0.42

Experiment C

Table IV.7: Mean of three parallels of color analysis of pulsed electric field processed solid rockweed and their corresponding standard deviation (n = 3).

Algae	L*	a*	b*	C*	h
1800a	25.53	-1.84	18.81	18.83	95.63
1800b	28.78	-2.08	17.30	17.39	96.51
1800c	27.49	-1.96	18.14	18.42	96.16
Control	25.85	-1.55	18.69	18.87	95.01
SD					
1800a	0.58	0.10	0.33	0.48	0.37
1800b	0.38	0.07	0.29	0.36	0.34
1800c	0.43	0.03	0.25	0.32	0.43
Control	0.21	0.05	0.24	0.26	0.13

Table IV.8: Mean of three parallels of color analysis of ultrasound processed solid rockweed (n = 3).

Algae	L*	a*	b*	C*	h
40a	67.84	-1.32	29.54	29.66	92.66
40b	69.44	-1.89	28.51	28.50	93.38
40c	73.13	-1.45	20.78	20.66	94.05
Control	73.01	-1.52	20.29	20.18	94.79
SD					
40a	0.00	0.03	0.46	0.48	0.51
40b	0.85	0.05	0.04	0.02	0.45
40c	0.41	0.13	0.36	0.40	0.33
Control	0.59	0.05	0.19	0.24	0.36

Table IV.9: Mean of three parallels of color analysis of blanched processed solid rockweed and their corresponding standard deviation (n = 3).

Algae	L*	a*	b*	C*	h
40a	29.43	-3.10	20.32	20.56	98.66
40b	35.68	-2.19	37.47	37.53	93.33
40c	31.21	-3.39	22.78	23.03	98.50
100a	33.75	-2.41	36.99	37.07	93.72
100b	35.68	-2.19	37.47	37.53	93.33
100c	34.57	-2.57	34.83	34.93	94.20
Control1	30.15	-2.12	20.25	20.36	95.97
SD					
40a	0.04	0.11	0.86	0.87	0.06
40b	0.79	0.28	0.94	0.95	0.39
40c	0.52	0.23	0.94	0.89	0.92
100a	0.38	0.27	0.16	0.17	0.40
100b	0.79	0.28	0.94	0.95	0.39
100c	0.58	0.35	2.08	2.10	0.40
Control1	0.35	0.07	0.53	0.53	0.05

Table IV.10: Color analysis of dried rockweed and their corresponding standard deviation (n = 3).

Solid	L*	a*	b*	C*	h
A	48.32	-1.72	22.55	22.61	94.36
B	48.65	-1.73	22.45	22.64	92.38
C	47.99	-1.71	22.51	22.89	94.98
SD	0.27	0.01	0.04	0.13	1.11

Table IV.11: Color analysis of freeze-dried rockweed and their corresponding standard deviation (n = 3).

Solid	L*	a*	b*	C*	h
A	26.35	-1.75	15.04	15.14	96.64
B	25.99	-1.75	15.15	15.14	96.58
C	26.12	-1.71	14.95	15.04	96.60
SD	0.15	0.02	0.08	0.05	0.02

Liquid

Table IV.12: Color analysis of pulsed electric field processed liquid rockweed and their corresponding standard deviation (n = 3).

Liquid	L*	a*	b*	C*	h
1800a	73.23	-1.185	19.56	19.495	93.97
1800b	71.57	-1.33	17.73	17.61	94.17
1800c	73.81	-0.72	17.53	16.63	92.29
Control	75.54	-1.18	12.60	12.50	95.23
SD					
1800a	0.45	0.11	0.80	0.52	0.29
1800b	0.80	0.04	0.26	0.34	0.77
1800c	0.66	0.04	0.42	0.50	0.63
Control	0.12	0.11	0.29	0.44	1.01

Table IV.13: Color analysis of ultrasound processed liquid rockweed and their corresponding standard deviation (n = 3).

Liquid	L*	a*	b*	C*	h
40a	25.61	-0.42	10.22	9.98	93.07
40b	25.34	-2.00	10.75	10.63	99.47
40c	24.68	-1.44	10.24	10.33	98.87
Control	26.09	-0.85	9.98	9.46	95.09
SD					
40a	0.93	0.04	0.45	0.28	0.21
40b	0.39	0.11	0.17	0.34	0.51
40c	1.72	0.08	0.18	0.08	0.62
Control	0.38	0.29	0.18	0.59	2.93

Table IV.14: Color analysis of blanched liquid rockweed and their corresponding standard deviation (n = 3).

Liquid	L*	a*	b*	C*	h
40a	63.34	4.57	37.04	37.32	82.96
40b	65.15	3.33	34.57	34.73	84.51
40c	69.01	3.25	35.54	35.69	84.78
100a	66.27	4.59	38.15	38.43	83.15
100b	64.79	5.34	40.55	40.90	82.50
100c	70.24	6.81	44.78	45.30	81.37
Control1	73.28	-0.80	12.96	12.99	93.52
Control2	63.34	4.57	37.04	37.32	82.96
Control3	65.15	3.33	34.57	34.73	84.51
SD					
40a	0.17	0.30	0.66	0.69	0.33
40b	0.22	0.31	0.67	0.70	0.42
40c	0.67	0.20	0.54	0.56	0.25
100a	0.49	0.38	0.91	0.94	0.41
100b	0.16	0.33	0.59	0.62	0.35
100c	0.63	0.56	1.37	1.43	0.46
Control1	0.22	0.11	0.21	0.20	0.54
Control2	0.17	0.30	0.66	0.69	0.33
Control3	0.22	0.31	0.67	0.70	0.42

Experiment D

Solid

Table IV.15: Mean of three parallels of color analysis of pulsed electric field processed solid winged kelp and their corresponding standard deviation from experiment D (n = 3).

Algae	L*	a*	b*	C*	h
1800a	10.13	-7.61	7.90	11.01	133.83
1800b	10.76	-7.19	8.33	11.06	130.83
1800c	10.27	-7.97	7.48	10.98	136.70
Control1	9.89	-5.69	6.15	8.41	132.74
Control2	10.62	-4.97	5.64	7.52	131.40
Control3	10.67	-4.65	6.34	7.86	126.26
SD					
1800a	0.43	0.75	0.55	0.18	4.72
1800b	0.61	0.83	0.86	0.10	6.19
1800c	0.77	0.92	0.52	0.32	5.31
Control1	0.71	0.68	0.58	0.53	4.98
Control2	0.35	0.23	0.24	0.13	2.35
Control3	0.28	0.04	0.33	0.27	1.44

Table IV.16: Mean of three parallels of color analysis of ultrasound processed solid winged kelp and their corresponding standard deviation from experiment D (n = 3).

Algae	L*	a*	b*	C*	h
40a	12.29	-16.58	11.90	20.41	144.33
40b	11.86	-20.78	14.49	25.34	145.12
40c	12.39	-19.18	14.25	23.90	143.38
Control1	9.89	-5.69	6.15	8.41	132.74
Control2	10.62	-4.97	5.64	7.52	131.40
Control3	10.67	-4.65	6.34	7.86	126.26
SD					
40a	0.14	0.41	0.42	0.58	0.33
40b	0.04	0.24	0.34	0.37	0.43
40c	0.18	0.88	0.51	1.01	0.29
Control1	0.71	0.68	0.58	0.53	4.98
Control2	0.35	0.23	0.24	0.13	2.35
Control3	0.28	0.04	0.33	0.27	1.44

Table IV.17: Mean of three parallels of color analysis of blanched solid winged kelp and their corresponding standard deviation (n = 3).

Algae	L*	a*	b*	C*	h
40a	11.77	-6.05	8.44	10.43	125.74
40b	11.72	-6.18	7.36	9.63	130.21
40c	10.45	-7.36	7.03	10.21	136.34
100a	18.05	-11.79	17.61	21.19	123.87
100b	18.83	-10.96	16.66	19.95	123.41
100c	17.44	-11.96	17.81	21.46	123.89
Control1	10.84	-4.83	5.96	7.78	128.50
Control2	9.89	-5.07	7.11	8.77	125.62
Control3	10.49	-4.43	6.63	7.97	123.74
SD					
40a	0.60	0.71	0.78	0.20	5.70
40b	0.41	0.27	0.77	0.50	3.80
40c	0.55	0.60	0.64	0.31	4.57
100a	0.21	0.33	1.10	1.09	0.97
100b	0.91	0.58	1.35	1.38	1.50
100c	0.04	0.20	0.61	0.61	0.51
Control1	0.42	1.28	0.53	0.47	9.52
Control2	0.30	0.62	0.69	0.21	5.91
Control3	0.33	0.13	0.10	0.12	0.84

Table IV.18: Mean of three parallels of color analysis of dried solid winged kelp and their corresponding standard deviation (n = 3).

Liquid	L*	a*	b*	C*	h
Dried-a	12.29	-16.58	11.90	20.41	144.33
Dried-b	11.86	-20.78	14.49	25.34	145.12
Control1	9.89	-5.69	6.15	8.41	132.74
Control2	10.62	-4.97	5.64	7.52	131.40
SD					
Dried-a	0.14	0.41	0.42	0.58	0.33
Dried-b	0.04	0.24	0.34	0.37	0.43
Control1	0.71	0.68	0.58	0.53	4.98
Control2	0.35	0.23	0.24	0.13	2.35

Liquid

Table IV.19: Color analysis of pulsed electric field processed liquid winged kelp (n = 3).

Liquid	L*	a*	b*	C*	h
1800a	73.53	-1.30	20.08	20.12	93.70
1800b	72.75	-1.52	18.90	18.96	94.61
1800c	71.76	-1.51	20.40	20.45	94.22
Control1	72.97	-1.38	16.83	16.89	94.70
Control2	72.56	-1.62	17.22	17.29	95.37
Control3	72.09	-1.57	16.37	16.44	95.48

* No SD

Table IV.20: Color analysis of ultrasound processed liquid winged kelp (n = 3).

Liquid	L*	a*	b*	C*	h
40a	66.42	0.03	26.99	26.99	89.95
40b	68.98	-0.75	27.80	27.81	91.55
40c	66.84	0.04	28.33	28.33	89.92
Control1	11.47	-20.77	14.14	25.12	145.76
Control2	12.12	-17.94	13.67	22.88	147.20
Control3	12.65	-15.35	11.41	19.13	143.44

* No SD

Table IV.21: Color analysis of blanched liquid winged kelp (n = 3).

Liquid	L*	a*	b*	C*	h
40a	66.42	0.03	26.99	26.99	89.95
40b	66.74	0.07	32.09	32.09	89.88
40c	63.81	0.46	32.74	32.75	89.19
100a	63.36	0.09	30.34	30.34	89.82
100b	64.30	-1.34	32.66	32.69	92.36
100c	66.03	-1.74	31.11	31.16	93.20
Control1	64.65	-1.47	31.32	31.35	92.69
Control2	71.20	-0.80	23.37	23.39	91.97
Control3	68.64	-0.65	24.85	24.85	91.49

* No SD

Appendix V Fluid loss and dry matter

Experiment C

Solid

Table V.1: Mean of two parallels of fluid loss (g and %) and dry weight of untreated ultrasound. pulsed electric field and blanched processed solid rockweed their corresponding standard deviation (n = 6).

	Fluid loss (g)	Fluid loss (%)	Dry matter (% of ww)	SD (Dry matter)
PEF1800	4.117	81.790	18.210	0.010
PEF-Control	4.092	81.392	18.733	0.008
UAE40	4.194	83.701	16.299	0.021
UAE-Control	4.272	83.275	16.725	0.022
BL40	4.026	81.365	18.635	0.077
BL100	4.128	81.654	18.346	0.010
BL-Control	4.275	84.317	15.683	0.006
Untreated	3.885	77.487	22.513	0.010

Liquid

Table V.2: Mean of two parallels of fluid loss (g and %) and dry weight of untreated ultrasound. pulsed electric field and blanched processed liqiud rockweed their corresponding standard deviation of dry matter (n = 6).

	Fluid loss (g)	Fluid loss (%)	Dry matter (% of ww)	SD (dry matter)
PEF1800	4.707	99.573	0.427	4.707
PEF-Control	4.699	99.725	0.275	4.699
UAE40	4.692	99.599	0.401	4.692
UAE-Control	4.710	99.770	0.230	4.710
BL40	4.890	99.698	0.302	4.890
BL100	4.837	99.075	0.925	4.837
BL-Control	4.814	99.335	0.665	4.814
Untreated	4.707	99.573	0.427	4.707

Experiment D

Solid

Table V.3: Mean of two parallels of fluid loss (g and %) and dry weight of untreated ultrasound. pulsed electric field and blanched processed solid winged kelp their corresponding standard deviation of dry matter (n = 6).

	Fluid loss (g)	Fluid loss (%)	Dry matter (% of ww)	SD (Dry matter)
UAE	5.064	0.957	4.315	0.606
UAE-Control	5.034	0.958	4.233	1.585
PEF	4.781	0.944	5.628	0.708
PEF-Control	4.807	0.957	4.329	0.254
BL40	4.718	0.933	6.723	0.336
BL100	4.684	0.934	6.572	0.242
BL-Control	4.807	0.957	4.329	0.577
Untreated	4.657	0.911	8.867	0.283

Liquid

Table V.4: Mean of two parallels of fluid loss (g and %) and dry weight of untreated ultrasound. pulsed electric field and blanched processed liquid winged kelp their corresponding standard deviation of dry matter (n = 6).

	Fluid loss (g)	Fluid loss (%)	Dry matter (% of ww)	SD (dry matter)
UAE	4.967	0.994	0.590	0.023
UAE-Control	4.873	0.995	0.506	0.145
PEF	4.444	0.996	0.355	0.067
PEF-Control	4.424	0.997	0.349	0.016
BL40	4.398	0.993	0.688	0.063
BL100	4.381	0.992	0.815	0.016
BL-Control	4.394	0.996	0.442	0.012

Untreated	4.967	0.994	0.590	0.023

Appendix VI Water percentage and ash content

Experiment C

Table VI.1: Mean of two parallels of water (%) and ash content (% of TS) of untreated ultrasound. pulsed electric field and blanched processed solid winged kelp the corresponding standard deviation of ash content (n = 6).

	Water (%)	Ash content (% TS)	SD (ash conent)
PEF1800	0.182	81.835	6.034
PEF-Control	0.192	80.754	15.682
UAE40	0.163	83.701	18.674
UAE-Control	0.147	85.322	19.552
BL40	0.186	81.365	19.558
BL100	0.183	81.654	17.508
BL-Control	0.166	83.435	22.241
Untreated	0.225	77.471	22.616

Experiment D

Table VI.2: Mean of two parallels of water (%) and ash content (% of TS) of untreated ultrasound. pulsed electric field and blanched processed solid winged kelp the corresponding standard deviation of ash content (n = 6).

	Water (%)	Ash content (% of TS)	SD (ash content)
UAE	91.13	39.39	14.65
UAE-Control	95.68	36.79	5.07
PEF	95.77	19.46	0.43
PEF-Control	94.37	19.25	0.54
BL40	95.67	24.06	12.11
BL100	93.28	33.52	2.75
BL-Control	93.43	18.78	7.34
Untreated	95.11	28.91	2.00

Appendix VII Weight measurements

Experiment A

Table VII.1: Weight difference of pulsed electric field processed rockweed and tap water for experiment A1.

	WB algae (g)	WA algae (g)	WD algae (g)	WB liquid (g)	WA liquid (g)	WD liquid (g)
10-1	101.23	122.40	21.17	516.50	470.46	-46.04
10-2	99.28	126.18	26.90	500.81	472.63	-28.18
20-1	104.38	128.47	24.09	506.92	476.61	-30.31
20-2	101.80	126.44	24.64	507.16	482.36	-24.80
30-1	100.14	124.50	24.36	514.50	481.96	-32.54
30-2	102.01	131.77	29.76	505.99	475.26	-30.73
50-1	110.06	141.90	31.84	515.99	478.94	-37.05
50-2	109.56	130.22	20.66	503.76	464.98	-38.78
60-1	104.80	128.75	23.95	504.54	490.45	-14.09
60-2	99.87	124.50	24.63	513.47	490.54	-22.93
Control	100.40			489.9		

WB = weight before. WA = weight after. WD = weigh difference

Experiment B

Table VII.2: Weight difference of pulsed electric field processed rockweed and tap water.

	WB algae (g)	WA algae (g)	WD algae (g)	WB liquid (g)	WA liquid (g)	WD liquid (g)
1800a	104.4	134.2	29.8	501.6	464.5	-37.1
1800b	99.6	132.1	32.5	501.6	461	-40.6
1800c	100.8	134.1	33.3	497.8	451.9	-45.9
1800d	106.5	136.1	29.6	495.7	549.4	53.7
Control	109.4	169.3	59.9	512.1	456.3	-55.8

WB = weight before. WA = weight after. WD = weigh difference

Table VII.3: Weight difference of ultrasound processed rockweed and tap water.

	WB algae (g)	WA algae (g)	WD algae (g)	WB liquid (g)	WA liquid (g)	WD liquid (g)
40a	100.9	157.1	56.2	493.3	426.8	-66.5
40b	102.6	165.6	63	497.1	438.4	-58.7
40c	102.8	159.5	56.7	513.1	429.5	-83.6
Control	101.3	158.8	57.5	501.3	429.7	-71.6

WB = weight before. WA = weight after. WD = weigh difference

Table VII.4: Weight difference of blanched rockweed and tap water.

	WB algae (g)	WA algae (g)	WD algae (g)	WB liquid (g)	WA liquid (g)	WD liquid (g)
40a	99.5	136.1	-36.6	510.1	445.8	-64.3
40b	105.45	175.1	-69.7	498.9	411.9	-87
40c	109.6	159.9	-50.3	503.4	433.5	-69.9
100a	103.8	107.2	-3.4	504.5	452.2	-52.3
100b	97.5	105.0	-7.5	507.3	447.4	-59.9
100c	110.2	116.2	-06	511.5	459.1	-52.4
Control	95.4	140.2	-44.8	521	469.9	-51.1

WB = weight before, WA = weight after, WD = weigh difference

Experiment C

Table VII.5: Weight difference of pulsed electric field processed rockweed and tap water.

	WB algae (g)	WA algae (g)	WD algae (g)	WB liquid (g)	WA liquid (g)	WD liquid (g)
1800a	106.2	137	30.8	523.5	476.5	-47.0
1800b	102.4	123.6	21.2	503.3	457.9	-45.4
1800c	101.0	122.5	21.5	499.2	465.3	-33.9
Control	96.8	141.3	44.5	512.8	458.4	-54.4

WB = weight before, WA = weight after, WD = weigh difference

Table VII.6: Weight difference of ultrasound processed rockweed and tap water.

	WB algae (g)	WA algae (g)	WD algae (g)	WB liquid (g)	WA liquid (g)	WD liquid (g)
40a	106.4	159.9	53.5	498.3	445.3	-53.0
40b	102.7	160.1	57.4	508.7	434	-74.7
40c	104.6	150	45.4	501.8	437.1	-64.7
Control	102.8	156.7	53.9	503.1	443.2	-59.9

WB = weight before, WA = weight after, WD = weigh difference

Table VII.7: Weight difference of blanched rockweed and tap water.

	WB algae (g)	WA algae (g)	WD algae (g)	WB liquid (g)	WA liquid (g)	WD liquid (g)
40a	105.2	143.3	-38.1	502.6	472.4	-30.2
40b	101.6	125.0	-23.4	495.6	461.3	-34.3
40c	104.1	138.1	-34	499.3	466.8	-32.5
100a	109.2	113.4	-4.2	504.9	448.9	-56.0
100b	105.9	111.2	-5.3	499.1	445.3	-53.8
100c	101.9	111.6	-9.7	502	451.1	-50.9
Control	110.1	148.6	-38.5	498.8	449.3	-49.5

WB = weight before, WA = weight after, WD = weigh difference

Experiment D

Table VII.8: Weight difference of pulsed electric field processed winged kelp and tap water.

	WB algae (g)	**WA algae (g)**	**WD algae (g)**	**WB liquid (g)**	**WA liquid (g)**	**WD liquid (g)**
1800a	500.6	620.2	119.6	5001.9	4823.9	-178
1800b	499.6	571.7	72.1	5001.2	4865	-136.2
1800c	500.5	573	72.5	5022	4905.5	-116.5
Control 1	51.8	103.5	51.7	509.8	457.1	-52.7
Control 2	49.8	105.2	55.4	506.4	447.4	-59
Control 3	50.7	106.2	55.5	507.6	447.8	-59.8

WB = weight before. WA = weight after. WD = weigh difference

Table VII.9: Weight difference of ultrasound processed winged kelp and tap water.

	WB algae (g)	**WA algae (g)**	**WD algae (g)**	**WB liquid (g)**	**WA liquid (g)**	**WD liquid (g)**
40a	103.3	160.1	56.8	504.9	439.5	-65.4
40b	101.1	148.6	47.5	505.2	446.3	-58.9
40c	99.1	164	64.9	499.6	430.7	-68.9
Control 1	103.1	140.6	37.5	505.5	462.6	-42.9
Control 2	100.4	138.1	37.7	503.1	446.7	-56.4
Control 3	105.2	135.3	30.1	498.8	440.5	-58.3

WB = weight before. WA = weight after. WD = weigh difference

Table VII.10: Weight difference of blanched winged kelp and tap water.

	WB algae (g)	WA algae (g)	WD algae (g)	WB liquid (g)	WA liquid (g)	WD liquid (g)
40a	100.6	98.5	2.1	505	493.2	-11.8
40b	100.6	100.2	0.4	496.3	495.4	-0.9
40c	100.8	102.4	-1.6	501.1	476.3	-24.8
100a	100.1	96.7	3.4	502.1	446.5	-55.6
100b	100.5	94.3	6.2	501.2	464.9	-36.3
100c	100.5	90.9	9.6	500.9	452.7	-48.2
Control1	98.9	165.6	-66.7	500.2	448	-52.2
Control2	102	159.5	-57.5	500.2	550.3	50.1
Control3	99.4	150.4	-51	510.6	454.4	-56.2

WB = weight before. WA = weight after. WD = weigh difference