Valorisation of side streams from alginate extraction process using Deep Eutectic Solvents

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Valorisation of side streams from alginate extraction process using Deep Eutectic Solvents

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Abstract

Seaweed-based products and production chains offer well-documented advantages over fossil and plant-based ones. Today, widespread adoption has yet to materialise due to insufficient product yields regarding total biomass. Conventional brown seaweed processing valorises mainly alginate, where the solid residues are now sold as feed or fertiliser. Therefore, valorising this sidestream effectively could improve production chain sustainability through greater adoption of seaweed.

Mapping the lab-scale alginate extraction process's component balances revealed the process streams' chemical value. Combining these and reported results, possible isolation methods, and the component chemical properties, a selection of sustainable extraction methods was tested, revealing parameters used for a proposed purification model.

The lab-scale conventional *Saccharina latissima* alginate extraction process had an alginate yield of 59%. Within the solid residues, 65 w% of the seaweed lipids and 66 w% of fucoxanthin are allocated. Menthol-levulinic acid (M-L) was the most effective DES in fucoxanthin and free fatty acid (FFA) extraction, with FFA yields of 76% higher than the standard hexane. Fucoxanthin yields within the M-L extracts were only 28% lower than those of MeOH standards. Further isolation with the anti-solvent Choline Chloride-Levulinic acid (CC-L) has been shown to be effective with yields for both FFA and fucoxanthin that are lower than 10%.

Using the mass balances and parameters derived by this research and others, a biphasic separation and double anti-solvent isolation process has been modelled. This process indicates that a minimum DES recirculation percentage of 80% is required to remain viable with substantial solvent purification capacity. These results combined indicate that M-L is an effective solvent extraction platform. With additional proof of the isolation method, they could promote the creation of more viable seaweed multi-product biorefineries.

Keywords: *Saccharina latissima*, hydrophobic deep eutectic solvents, multi-product refinery, fucoxanthin, lipids, extraction, isolation

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1 Introduction

Seaweed, also known as macroalgae, is a rising star in the search for sustainable growth models able to replace fossil-based or other energy-intensive production chains. Seaweed's advantages are many and well-documented. Some of these are: higher production rates and relatively elevated carbon fixating properties compared to land-based plants, their ability to deacidify bodies of water, the decrease of land pressure by replacing land-based cultivars, removal of excess runoff nutrients, and the minimal need for fertilisers and freshwater (Ciravegna et al., 2023; Duarte et al., 2022; Jagtap & Meena, 2022; Kraan, 2010).

Additionally, its unique chemical composition can directly offer many products, often as an alternative to fossil-intensive or based ones, e.g., emulsifiers, gels, pharmaceuticals, protein isolates, and nutraceuticals (Li et al., 2021). Additional processing can replace more carbon-intensive products such as fuels, plastics, oils, and cosmetics depending on the starting biomass (Tullberg et al., 2022). In short, further adoption of seaweed has a general positive effect on current global dynamics by making production chains more sustainable.

1.1 Brown macroalgae

Besides being the seaweed group most cultivated (Figure 1) in terms of weight use and mass brown macroalgae has a unique chemical profile. Classified based on their pigmentation, brown macroalgae (Phaeophyceae) have the average highest total polysaccharides dry weight composition (50-60%) compared to their red (Rhodophyta) and green (Chlorophyta) macroalgae counterparts. Within this composition, brown macroalgae offer valuable compounds like fucoxanthin and alginate, which almost exclusively reside in this group (Handå et al., 2013; Kraan, 2010; Pocha et al., 2022).

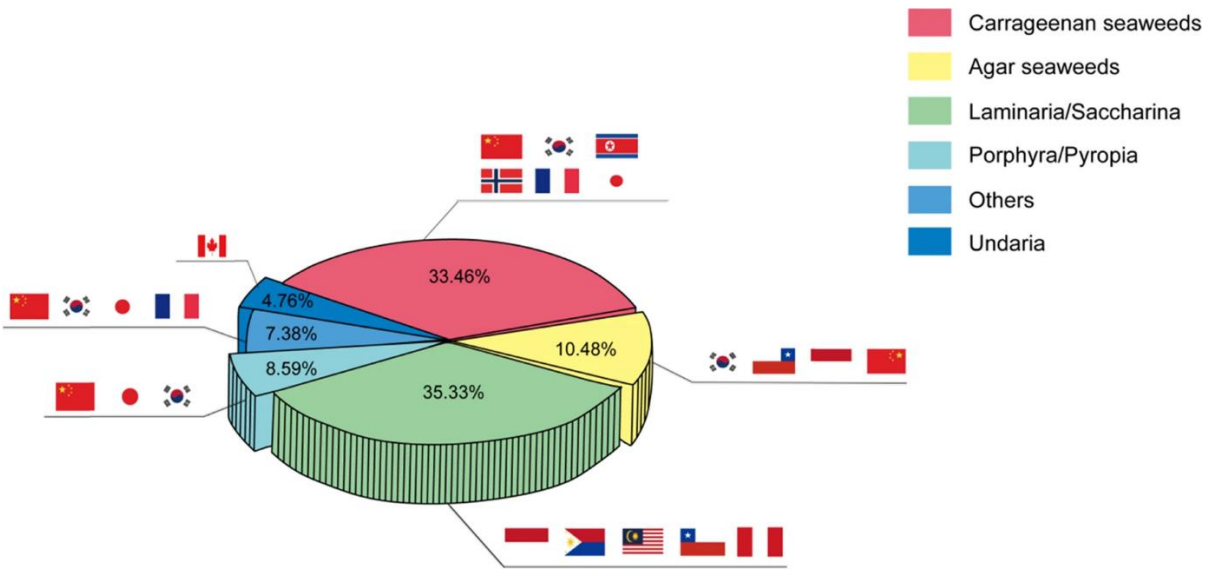

Figure 1: Seaweed aquaculture production per species and countries – 2014. Brown seaweed species in green and shades of blue. Source: (FAO, 2021).

Although preferring temperate waters, brown seaweed species are found in every ocean, among which *Laminariales* species dominate the European coast. Of all the macroalgae, *Saccharina latissima* is the fastest-growing, widespread in European waters, and one of the species used for human consumption (J. Naylor, 1976; Kraan, 2010). *S. latissima* was the brown seaweed species used in the research.

1.2 Chemical composition

Brown macroalgae are known to have many components with wide-spread use and proven applications, e.g., fucoxanthin, fucoidan, alginate, lipids, proteins, mannitol, laminarin, and phlorotannin ordered in descending market value (Table 1). The relative abundance of each of these components varies by species, time of harvest, location and other growing conditions such as current strength and water nutrient levels (Marinho et al., 2015; Monteiro et al., 2020, 2021; Samarasinghe et al., 2021). Only the components with widespread use, an existing market and a significant DW% within seaweed are mentioned. Other peculiarities and compounds reported in brown seaweed by various studies, such as halogenated metabolites, cellulose, etc., are beyond the scope of this research and will not be further discussed (Cabrita et al., 2010; He et al., 2018; Figure 2).

Table 1: Composition of Saccharina latissima in DW% and their corresponding current market values.

Compound	Average content DW% (ranges)	Market value ($/kg)	Reference for content	Reference for market value
Alginate	16.5 (6-27)	23	Handå et al., 2013	(Alginic Acid, Thermo Scientific Chemicals, 2024)
Laminarin	6 (3-9)	51.7	Handå et al., 2013	(*Laminarin Ebiochem*, 2024)
Fucoidan	3.9 (2.3-6.2)	150	Bruhn et al., 2017	(*Fucoidan Price, MOLBASE*, n.d.)
Mannitol	10 (2-18)	7.3	Handå et al., 2013	Saha & Racine, 2011
Xylose	0.2	5.2	Samarasinghe et al., 2021	(*Xylose Ebiochem*, n.d.)
Cellulose	6 (2-10)	-	Samarasinghe et al., 2021	-
Total carbohydrates	42.4 (30-50)	-	Handå et al., 2013	-
Proteins	12 (3-21)	5	Fleurence 1999, Holdt and Kraan, 2011	Kraan, 2013
Phenols (phlorotannins)	0.73 (0.2-20)*	20	Sardari et al., 2020	(*Phlorotannin Kelp Extract*, n.d.)
Lipids	2.5 (1.9-3.6)	77	Rey et al., 2019	(*DHA Micro Algae Oil*, n.d.)
Fucoxanthin	$2.0x10^{-3}$ (1.3-6.0) $x10^{-3}$	60,000	Afonso et al., 2022.; H.Zhang et al., 2015	Pocha et al., 2022
Ash	31.8 (25.3-41)	-	Sharma et al., 2018	-

*Genaral brown seaweed range.

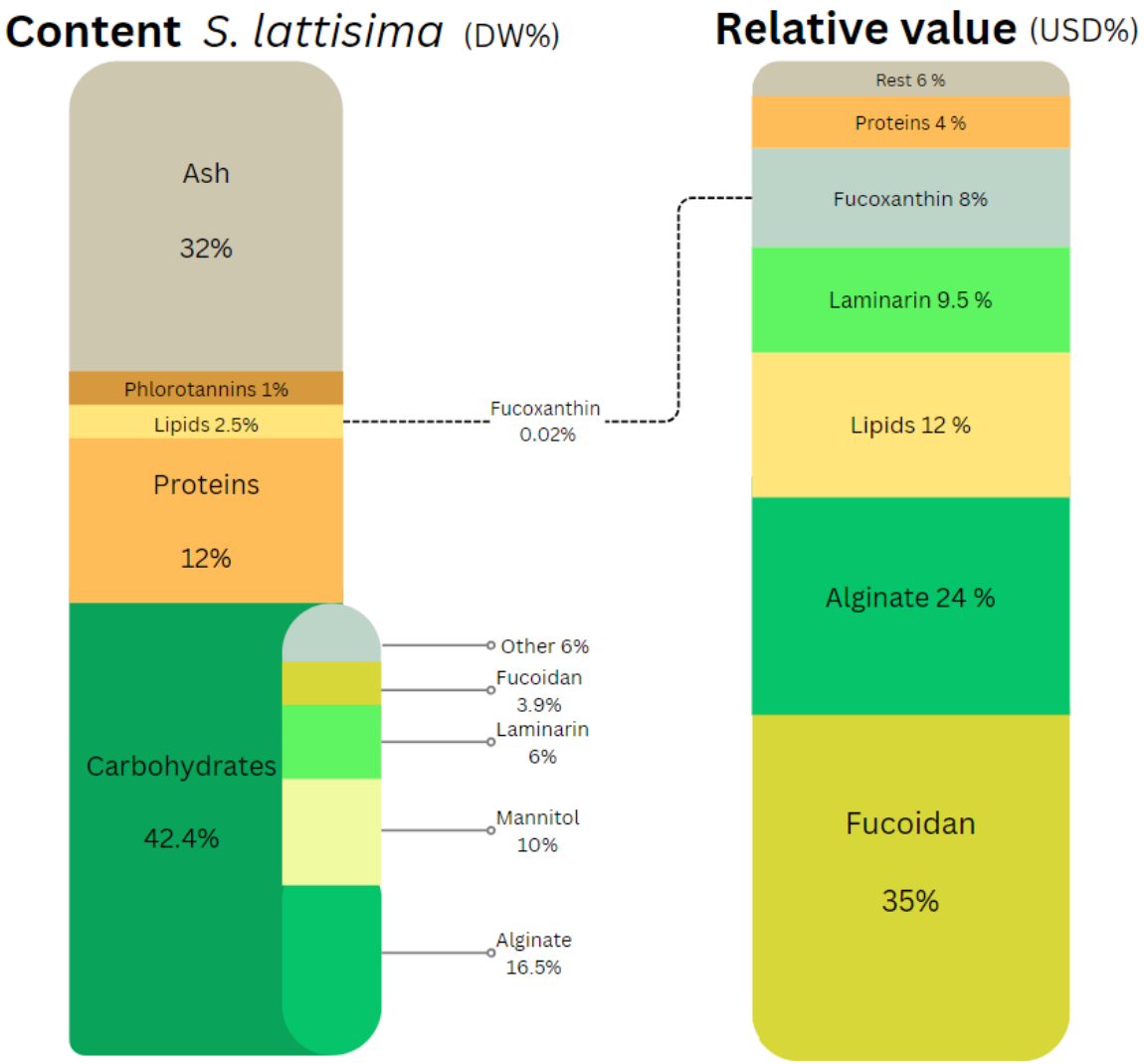

Figure 2: Averaged dry weight content in S. latissima and the relative value. Average values are estimated using the data displayed in Table 1.

1.2.1 Alginate

Starting with the first significant carbohydrate, alginate is a hydrocolloid associated with the cell wall structure and intracellular spaces. Alginate is the major structural polysaccharide of most brown macroalgae (Sellimi et al., 2015). Due to its emulsifying, edible and complex but flexible structure, alginate has numerous applications such as tissue engineering, thickening and gelling agents, films and coatings (Fawzy et al., 2017; Sellimi et al., 2015). Within a natural environment, alginate exists as calcium, magnesium and sodium salts of alginic acid, providing both strength and flexibility to macroalgae (Kraan, 2010; Sellimi et al., 2015; Zdanowicz et al., 2018).

The polysaccharide consists mainly of 2 monomers, mannuronic (M) and guluronic (G) acid, in roughly 1:1 ratio. This ratio varies a lot between species and growing conditions and

determines the properties of the alginate (Draget, 1998). Alginates are typically described by their M/G ratio and average molecular weight since these parameters are closely related to the functionality of the alginates. Alginates rich in G residues have higher water solubility than those rich in M residues, exhibiting more substantial stiffness and gelling properties when metal ions such as Ca^{2+} are present (Li et al., 2021).

The mannuronic acid forms β (1–4) linkages. The guluronic acid has risen to α (1–4) linkages, which gives the chain a steric hindrance around the carboxyl groups (Figure 3). This hindrance gives the polymer water- and metal-ion-absorbing capability and the associated flexibility and emulsifying effects.

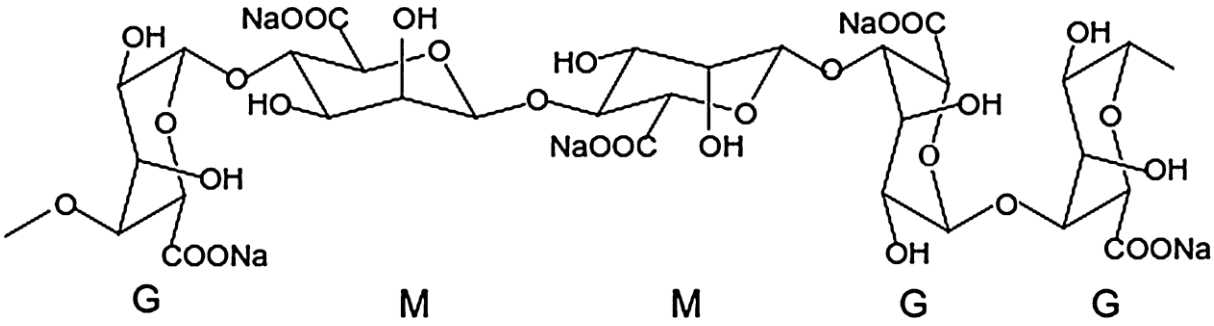

Figure 3: Molecular structure of Alginate. Source: Sellimi, et al., 2015.

In the presence of divalent cations such as calcium, a strong interaction with the COO− groups of guluronic acid from different chains is formed. Giving rise to a water-insoluble and thermo-irreversible three-dimensional network (gel), whose conformation is also called the “egg box” (Sellimi et al., 2015). This makes the polymer versatile and relatively easy to isolate.

1.2.2 Laminarin & Mannitol

Laminarin and mannitol are the main major storage carbohydrates of brown macroalgae. Laminarin is commonly found in the fronds of *Laminaria* and *Saccharina* macroalgae, although it is also found in *Ascophyllum*, *Fucus* and *Undaria* (Holdt & Kraan, 2011). Laminarin is a β-glucan, mainly composed of glucose residues. Laminarin linked to d-mannitol at the reducing end of the chain is called an M chain, while laminarin without mannitol at the reducing end is a G chain (Figure 4). Mannitol exists in 2% of laminarin in M-chains (Li et al., 2021)

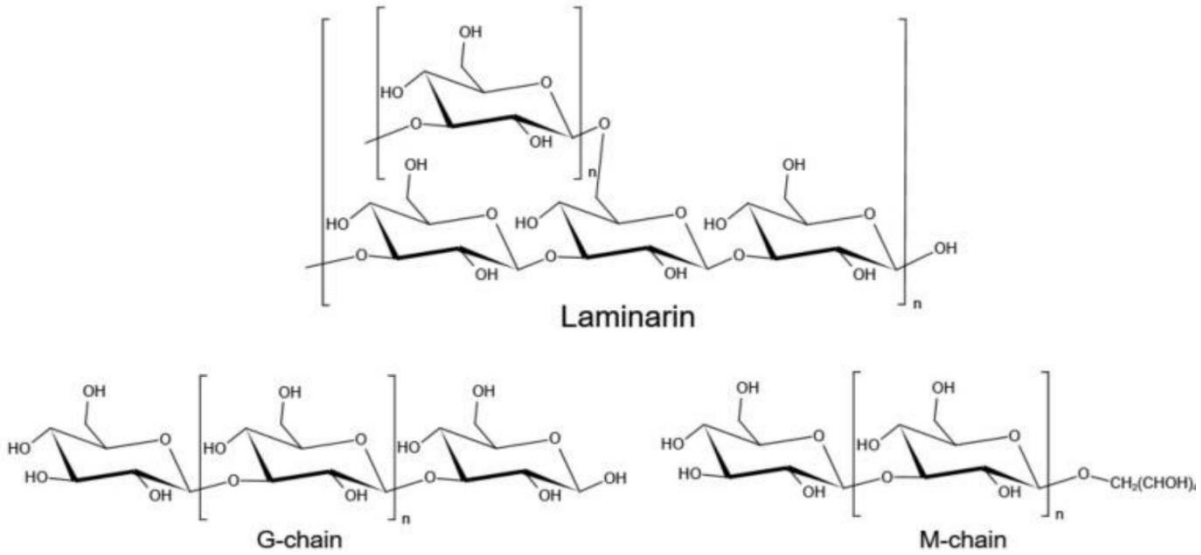

Figure 4: Laminarin chemical structure. Source: *(Li et al., 2021)*

Besides being part of the larger polysaccharides such as laminarin, mannitol is, for the most part, unbonded intracellularly in brown macroalgae. Mannitol is intimately involved in osmotic adjustment, and its levels are regulated by macroalgae in response to changes in salinity (Reed et al., 1985). Mannitol is classified as a sugar alcohol, derived from the six-carbon sugar D-mannose and appears to be the primary product of photosynthesis (Li et al., 2021). Due to their function and localisation with the larger macroalgae, both mannitol and laminarin vary mostly within season and growing conditions. This makes it difficult to extract these carbohydrates in consistent levels predictably (Li et al., 2021).

1.2.3 Fucoidan

Fucoidan is a long-sulphated polysaccharide in numerous marine species, such as echinoderms and algae, which are predominantly brown algae. These polymers mainly consist of sulfonated monomers such as fucose, α-l-fucopyranose residues and glucuronic acid (Bilan et al., 2002). Previous reports also suggest the presence of an extensive range of other carbohydrate monomers such as g; glucose, galactose, xylose, mannitol and glucuronic acid. This heterogeneity is especially observed in macro-algae species, giving it variability and a certain unpredictability in composition and functionality (Z. Zhang et al., 2015).

Regarding functionality, multiple bio-medical effects have been reported, such as anti-inflammatory, anti-hyperglycaemic, anti-coagulant and even anti-cancer effects. These bio-medical properties have given the molecule its current valuation (Bruhn et al., 2017, Table 1). Previous research suggests that these effects, although different for each, are primarily due to fucoidans enzyme-inhibiting properties. The length, sulphate content and fucose presence have

all been suggested to be vital for its medical effectiveness, although more research is needed to quantify the importance of the fucose. Long-chain fucoidan has been proven to have adequate medical properties (Pozharitskaya et al., 2020). However, more elaborate and comparative studies show that small-chain fucoidan of around 130-160 kDA with high fucose percentages are more biochemically active (Zhang et al.,2015). Hence, these parameters are essential for assessing fucoidan quality.

1.2.4 Proteins

The global protein demand is steadily increasing and is expected to do so in the coming decades, especially for proteins as added ingredients (Marinho et al., 2015). This is even more the case for plant-based protein sources with good amino acid profiles and essential amino acids (EEA) ratios due to their nutritional and sustainable nature.

Brown seaweed protein b(3-21% DW) generally contains most amino acids, with glycine, alanine, arginine, proline, and glutamic and aspartic acids mostly represented.(Holdt & Kraan, 2011; Marinho et al., 2015) In some macroalgae species, essential amino acids (EAAs) can account for almost half of the total amino acids (Černá, 2011; Holdt & Kraan, 2011.). EAAs in human nutrition include leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine, and histidine (Friedman, 1996). Depending on the seasons, the EAA ratio of *S. latissima* varies from 0.41 to 0.42 in July to 0.21 in March. Generalising the amino acid profile, *S. latissima* has an amino acid score of 82, which is comparable to that of beef and eggs (maximum of 100; Marinho et al., 2015.). In short, this makes seaweed protein and *S. latissima* a relatively high-value protein when properly isolated and makes protein an interesting target for further valorisation.

1.2.5 Lipids

The value of the lipidome present in any biomass depends mainly on its composition. Steadily increase in global consumption of cheaper vegetable oil rich in n-6 polyunsaturated fatty acids (PUFA) has slowly increased PUFA ratios of n-6 to n-3, also known as omega-6 and omega-3 fatty acids, within the human body (Micha, 2014). Corporeal, n-6 to n-3 ratios greater than two have been associated with increased risk of mortality due to cancer, cardiovascular, inflammatory and autoimmune diseases (Simopoulos, 2008). Numerous studies have shown the nutritional value of n-3 PUFA and the prevention of multiple chronic diseases through its consumption (Bazan et al., 2011). Additionally, n-3 PUFA play a relevant role in the development and functioning of the central nervous system. Having a crucial role during early

brain development and remains relevant in memory formation and neuronal signalling in later stages (Rey et al., 2019; Simopoulos, 2008). Due to these health risks, algae oils are mainly valued for their n-6 to n-3 ratio within the fatty acid profile. Generally, seaweed and other marine sources such as *S. Latissima* present a much higher prevalence of n-3 PUFA or Omega-3 than land vegetables. This gives the lipidome and any *S. Latissima*-derived lipid fractions an elevated omega-3-rich plant-based evaluation, as used in Table 1.

Within the fatty acid profile of the brown seaweed *S. latissima,* C_{16} and C_{18} are the most abundant, representing 29.4% and 15.5% of the total fatty acid profile, respectively. Additionally, relevant PUFA have been registered notably different n-6 and n-3 PUFA, such as C18:2 n-6, C18:3 n-3, C18:4n-3, C20:4n-6 and C20:5n-3 (Figure 5). Of these, C20:5n-3 or Eicosapentaenoic acid (EPA) seems to be specifically effective in promoting many of the health benefits allocated to omega-3 fatty acids (Natural Standard, 2024.). Overall, S. latissima was reported to have a n-6/n-3 ratio of around 0.9 ± 0.1 (Rey et al., 2019). Besides triglycerides-based free fatty acids, which account for around $15.2\% \pm 2.5$ % of the total lipid profile, like any photosynthetic organism, *S. latissima* contains significant amounts of polar lipids. Chiefly among those glycolipids, phospholipids and lesser amounts of betaine lipids, sulfolipids and galactolipids (Rey et al., 2019).

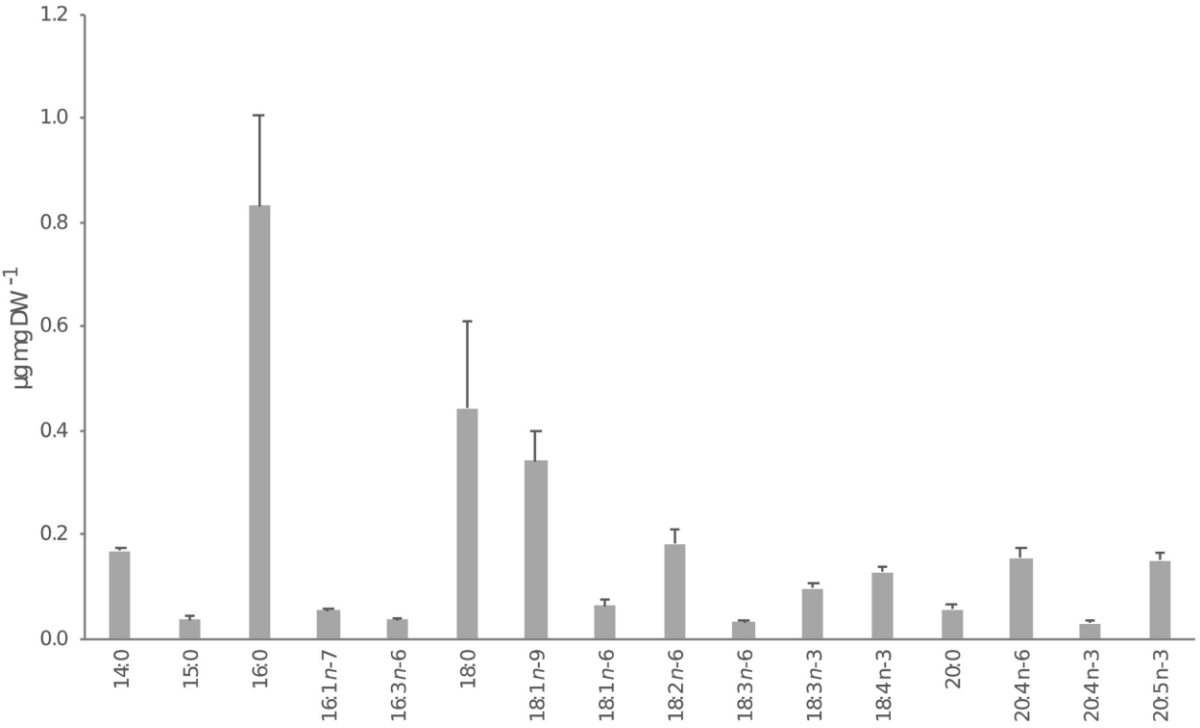

Figure 5: Fatty acid profile of Saccharina latissima samples as µg/mg dry weight−1 (DW). Source: (Rey et al., 2019).

1.2.6 Fucoxanthin

Fucoxanthin, a distinctive carotenoid, exhibits numerous bioactivities. Findings from animal studies indicate its potential in preventing and treating lifestyle-related diseases such as obesity, diabetes, cancer, cardiovascular disease, and other chronic conditions (Ae et al.,2013; Nomura et al., 1997; Figure 6). Notably, fucoxanthin has no adverse effects and can be readily extracted from macroalgae. Through continued examination and clinical research, fucoxanthin stands ready to be developed into secure marine drugs and nutritional products to prevent and treat lifestyle-related diseases (H. Zhang et al., 2015).

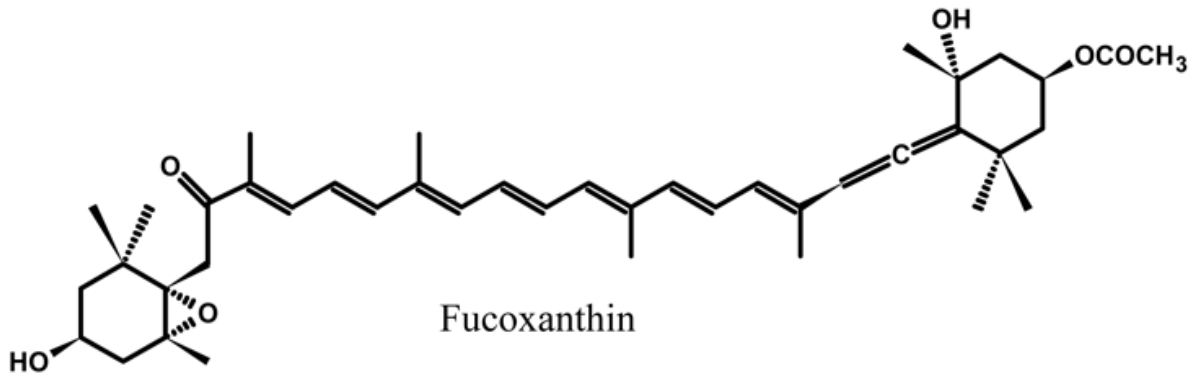

Figure 6: Structure of fucoxanthin. Source: *(Peng et al., 2011)*

These remarkable biological properties are a result of its unique chemical structure. Fucoxanthin consists of oxygenic functional groups such as epoxy, hydroxyl, carboxyl and carbonyl. The epoxy, carbonyl, and an unusual allenic bond make the carotenoid unique and give it elevated antioxidant activity (Sachindra et al., 2007). In summary, multiple biological properties against widespread difficult-to-treat illnesses and conditions, its limited bioavailability, heightened photosensitivity, and degradable nature have given fucoxanthin the highest market evaluation of all the compounds residing in *S. latissima* (Afonso et al., 2022; Pocha et al., 2022).

1.3 Conventional hydrocolloid extraction

Currently, the main holdback to widespread adoption of seaweed-derived products is plain and simple economics. Its biomass is processed in such a way that cultivation of much larger volumes hasn't become viable (Ciravegna et al., 2023.). Thus, the next steps forward are effectively altering the process by increasing extraction efficiency, lowering processing costs and/or creating a multi-product refinery from macroalgae-based biomass. Currently, seaweed process plants often only produce alginate, while the remaining components residing in the

solid residue (SR) are considered a side-product and sold as feed or fertiliser (Fertah, 2017; Saji et al., 2022). The conventional extraction process described in this research is based on the information kindly provided by Algaia^{TM}, simplified in Figure 7. This process and other similar processes will now be referred to as the conventional industrial process. Generally, the extraction and purification processes of alginates are based on the conversion from the insoluble form in the seaweed cell walls to the soluble one, normally the sodium salt, followed by successive dissolutions and precipitations to eliminate impurities (Fernández et al., 2018; Saji et al., 2022).

Current alginate extraction yields based on the current conventional process range between 21-54%, depending on pretreatment and species (Saji et al., 2022). All the while, the remaining majority of fucoxanthin, fucoidan, proteins, lipids, etc., are separated as solid residues (Fertah, 2017; Saji et al., 2022). Any further extraction and purification of this stream depend on its composition. Depending on the source, this stream is rich in cellulose, protein and fucoidans (Bojorges et al., 2023; Fertah, 2017). Hence, further extraction of these valuable compounds in a multi-product refinery can make any macroalgae processing more viable and economically competitive for more sustainable production chains.

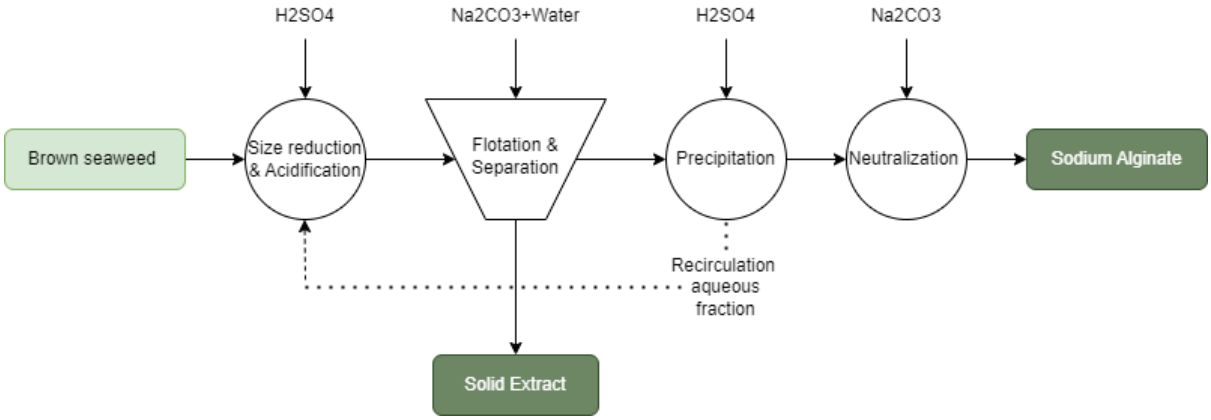

Figure 7: Schematic diagram of the current alginate extraction process. Based on data provided by Algaia^{TM}.

Valorisation and further extraction of this solid residue stream can be based on many different techniques. Conventional extraction methods for algal components, such as polysaccharides, are typically done using hot water and acidic and salt solutions at high temperatures for several hours. Conventional processes, besides their innate high energy demand and use of strong acids and salts, often lack high selectivity and require multiple steps with associated lower yields (Dobrinčić et al., 2020; Figure 7).

Novel extraction methods that have been proven to be effective can be used as sustainable alternatives to improve seaweed utilisation and maintain sustainable production models simultaneously. (Dobrinči´c et al., 2020; Dobrinčić et al., 2020; Fernández et al., 2018).

1.4 Deep Eutectic Solvents

Sustainable chemical processes should obey the 12 principles of green chemistry introduced by Anastas & Warner, 2000. Briefly summarised, these principles call for an efficient process, the use of relatively safe materials that are derived from renewable feedstocks and end streams that are bio-degradable. In 2002, a new class of solvents, called deep eutectic solvents (DESs), that could obey these principles of Green Chemistry were reported. (Abbott et al., 2002; Van Osch et al., 2019).

Eutectic stems from the Greek "ευ" (eu = easy) and "Τήξις" (teksis = melting), hints at the physiochemical properties of these new solvents (Gamsjäger et al., 2008). DES mixtures are substances forming layers that melt and freeze at a single temperature that is significantly lower than the melting points of the separate constituents. The term eutectic reaction is defined as an isothermal reversible reaction of a liquid phase, which is then transformed into two or more different solid phases during the cooling of a system. The eutectic point is a thermodynamic constant of the mixtures and represents the composition and the minimum melting temperature along the two intersecting melting curves. Neglecting the influence of temperature on heat capacities and assuming pure solid phases, classical thermodynamics proposes Equation 1 to describe these melting curves (Martins et al., 2018).

$$ln(x_i y_i) = \frac{\Delta_m H}{R}\left(\frac{1}{T_m} - \frac{1}{T}\right) + \frac{\Delta_m C_p}{R}\left(\frac{T_m}{T} - ln\frac{T_m}{T} - 1\right) \quad (1)$$

By this definition, eutectic solvents are “deep” when they deviate from idealised thermodynamic behaviour in such a matter that the eutectic point is lower than predicted (Figure 8, blue line). Therefore, by this classification only a selection of eutectic systems are deep. Due to a lack of research, the term deep eutectic solvent will be kept as a reference to all eutectic systems further mentioned.

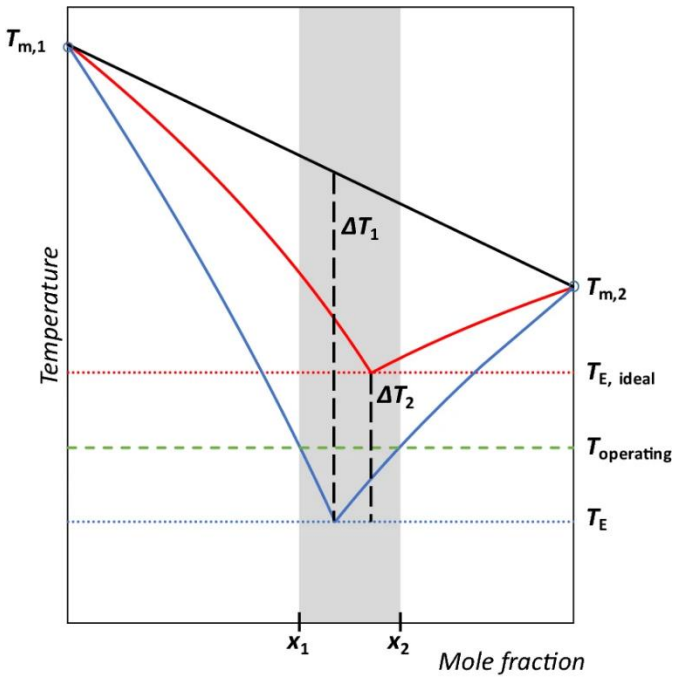

Figure 8: Simple representation of the solid-liquid equilibria of a simple ideal mixture (red line) and a deep eutectic mixture (blue line). Source: (Martins et al., 2018).

Typical DES consists of a hydrogen-bond donor (HBD) and acceptor (HBA) that are dissolved together to form the super-lattice mixture. The hydrogen bonds form a matrix that lowers the liquid melting of the solution to the deep eutectic point (Figure 8). This newly formed matrix readily dissolves a variety of compounds and has been reported to be able to extract almost abundant components present in brown seaweed selectivity, depending on the DES combination (Meng et al., 2023). DES can be claimed as biodegradable, tuneable, simple to prepare and relatively low in cost (El Achkar et al., 2019).

Successful extractions of brown seaweed carbohydrates, e.g. alginate, have been reported using choline chloride as an HBA and glycerol and urea as HBD, but many more combinations could be viable (Ling & Hadinoto, 2022; Meng et al., 2023; Saravana et al., 2018). Besides alginate, DES has effectively extracted valuable secondary underutilised components in brown seaweed, such as fucoxanthin, fucoidan and proteins (Meng et al., 2023). Thus, researching DES extraction on an alginate production side stream shows great promise.

Which specific DES to select highly depends on the hydrophobicity, selectivity and sustainability of your DES and the composition of your stream. Research has primarily focused on hydrophilic DES and hydrophobic DES reported in 2015 in literature for the first time, but have shown extraction rates of higher than 90% of free fatty acids of marine sources and

capable of selectively extracting fucoxanthin from *S. latissima* (Kholany et al., n.d.; Meng et al., 2023; Topal et al., 2023).

One significant obstacle hindering the widespread adoption of DES lies in the challenges associated with isolating extracted compounds and the subsequent recirculation of the solvent in an effective matter. Despite numerous advancements and proposed solutions for efficient isolation, there is still much progress to be achieved. Previous works have indicated that anti-solvent models, such as methanol in semi-hydrophobic DES and choline chloride (CC-L) in hydrophobic Levulinic DES, promote isolation by bi-phasic separation (Kholany et al., n.d.; Lo, 2021).

1.4.1 NaDES

Regarding sustainability, the term natural deep eutectic solvents (NaDES) has been widely used in literature. In general, NaDES are derived from natural sources or are synthesised from low-impact renewable feedstocks and are, therefore, the preferred option when selecting a DES for any extraction process. What truly constitutes a NaDES? The concept of NaDES may be relevant in the context of plant biology or biochemistry, but it has been abused in the field of novel green solvents (Choi et al., 2011; Van Osch et al., 2019). For example, choline chloride can be found in biochemical pathways within living beings. Choline chloride is, however, only a cheap compound because it is produced on a large scale by reacting hydrogen chloride with trimethylamine and ethylene oxide that is used as, e.g. chicken feed or on fracking as a clay-controlling additive (Abranches & Coutinho, 2023). In short, according to the principles of green chemistry, a DES is only a NaDES when it is truly derived from renewable feedstocks (Abbott et al., 2002).

1.5 Aim

This thesis focused on evaluating the extraction of valuable compounds from side streams of seaweed alginate production using Deep Eutectic Solvents. Firstly, the seaweed, solid residue, and remaining streams of the conventional alginate extraction process will be characterised. Secondly, mass balances are set up to determine the fractionation of the compounds. Thirdly, extraction of compounds using DES and assess the quality of the extracted compounds of interest. Finally, the obtained metrics were used to assess the economic viability of the proposed process in combination with the conventional one.

2 Materials and methods

2.1 Biomass

The *S. latissima* biomass was collected at Kamperland, Zeeland, the Netherlands, on the 8th of May 2023. After collection, the biomass was immediately shipped to Wageningen University & Research laboratories, frozen using liquid nitrogen, and stored in airtight conditions at -80 °C.

2.2 Lab-scale conventional process alginate extraction.

A protocol was used to extract alginate on a lab scale based on the current conventional industrial process described by Algaia™. The lab scale protocol was developed by Essers D, (2023) and applied for alginate extraction using *Ascophyllum nodosum* as a raw material. This protocol has been modified in this research to accommodate slightly larger volumes and to limit foaming in the second acidification procedure. These adjustments were made in part by consideration of the differences that might be present between *A. nodosum* and *S. latissima* (Samarasinghe et al., 2021).

Fresh seaweed was combined in a 1:1 (w/w) ratio with Milli-Q H2SO4 0.2 M solution to achieve a pH of 1.9. The particle size of the mixture was reduced for 10 minutes using a kitchen blender. Subsequently, stirring was carried out for 1 hour at 25°C.

Na_2CO_3 was added to the total weight of the mixture in a ratio of 0.0285:1 (w/w). After 2 min initial mixing, the resulting blend was allowed to rest at 55°C for an additional 1 hour. After this step, the solution is diluted 5-fold. This correlates with a viscosity reduction from 12000 cP to 200 cP applied in the industrial process, based on an exponential relationship between alginate dry weight percentages and viscosity (Essers D, 2023).

After dilution, an Allegra-X-30R centrifuge was used for 25 minutes at 4000 xg using 500 ml buckets. The solid fraction, i.e., the solid residues (SR), were separated from the supernatant, and the former was directly weighed.

Alginate precipitation was initiated by gradually adding H_2SO_4 (2.5 M) to the supernatant to decrease the pH to 1.9. Caution was exercised, particularly near a pH of 6, to limit foam formation. For bleaching the fibres, sodium hypochlorite was added as a bleaching compound until the desired transparency was achieved. Removal of alginate was executed through a

subsequent centrifugation step under identical conditions previously described. Both fractions were collected and weighed for analysis.

To halt further hydrolysis and produce sodium alginate, Na_2CO_3 was added to the precipitated fraction at a ratio of 0.05:1 (w/w). For subsequent analysis, all biomass samples and solid residues underwent freeze-drying. All inputs, outputs and losses were weighted and recorded to establish comprehensive component balances of the process.

2.2.1 Freeze drying

The initial macroalgae biomass and the solid residues, collected from the lab-scale conventional process of alginate extraction, were freeze-dried at a minimum temperature of -50°C and a pressure range of 0.5 to 0.05 mbar for 42 hours using wide glass containers.

2.2.2 Rotary vacuum concentrating

The liquid and alginate extract collected from the lab scale conventional alginate extraction process were dried in 12 ml glass tubes using a Christ© rotary vacuum concentrator (RVC) 2-25 CD plus. The tubes were rotated at 1200 RPM at 60°C for 20 hours for each 5 ml of liquid to dehydrate the samples. For overall and residual water content determination, samples were dried another 4-8 hours per 5ml.

2.3 Chemical analysis

2.3.1 Water and Ash Determination

Water and Ash content were determined with fresh biomass and side stream extracts with sample sizes of 2-5 g. The samples were dried at 106 C° for 16 hours overnight. Whereafter the samples were put in the ash oven for 4 hours at 575 C°. The weights after drying and combustion were noted and the water and ash contents were determined.

2.3.2 Crude protein analysis

Protein analysis was done by a modified version of the Kjeldahl method (Rhee, 2001), where the TOC analyser was used according to the Total Organic Carbon Analysis Measurement of Total Nitrogen (TN) for Protein Estimation on the Shimadzu TOC-L with TNM-L. Samples of 50 mg of freeze-dried seaweed and side streams were mixed with 5 ml of 2.5M H_2SO_4 for 3 hours at 96°C. After hydrolysis, 5 ml of 2.5 M NaOH was added for neutralisation. Standards were used with a concentration of Nitrogen 100 ppm and 50 ppm, which was made using 0.722

and 0.361 g/L potassium nitrate in milli-q water. To determine the protein content from this total nitrogen content a conversion factor of 5 was used according to (Angell et al., 2015). Protein hydrolysis was assumed to be 100%.

2.3.3 Crude lipid analysis

The total lipid content was determined according to Folch et al., 1957. In a glass tube, a sample of 200 mg of freeze-dried biomass was mixed with 5 ml of chloroform-methanol mixture (2:1 v/v) and incubated at 60°C for one h. The mixture was centrifugated for 10 min at 4255 xg using a Beckman Allegra-X-30R centrifuge. The supernatant was then transferred to a new pre-weighted glass tube and washed with 2 mL of a 0.9% NaCl solution. The sample was vortexed and let rest for 15 minutes. The top layer was removed, and the bottom phase was dried using a gentle stream of gas nitrogen to evaporate all the solvents. The fraction of lipids was then calculated by dividing the sample's weight after drying over the initial biomass and expressed in $g_{lipid}/g_{biomass}$.

2.3.4 Polysaccharides analysis

The polysaccharide contents were analysed by assessing the concentrations of the monomers D-glucose (Sigma-Aldrich™, 99+% purity), mannitol (Sigma-Aldrich™, ≥98% purity), guluronic acid (BOC Science™, ≥ 98%) mannuronic acid (Sigma-Aldrich™, ≥90% purity) and fucose (Sigma-Aldrich™, ≥95% purity. Freeze-dried biomass and side stream samples of 20 mg were pre-treated by hydrolysation with 11M H_2SO_4 at 37°C for one hour. Whereafter 5 mL of Milli-Q water was added to reach 1.8M and incubated for three h at 100°C. Samples were filtered using 0.22 µm polyvinylidene difluoride (PVDF) filters before analysis. 10 mg of standards of polysaccharide were hydrolysed in parallel to determine hydrolysis efficiencies. Consisting of fucoidan (Sigma-Aldrich™, ≥95% purity, *Undaria pinnatifida),* laminarin (Sigma-Aldrich™, ≥98% purity, *Laminaria digitata*) and sodium alginate (Sigma-Aldrich™, ≥98% purity, species unknown).

High-Performance Liquid Chromatography (HPLC) was conducted using an Agilent Technologies system while separation was performed by a Rezex ROA-Organic acid H+ column (8%, 300x7.8mm). A volume of 10 µL of filtered hydrolysate was injected through the column at 60 °C while the mobile phase consisted of 0.008M H_2SO_4 in MilliQ water flowed at 0.8 mL/min. The detection was performed by a Refractive Index Detector (RID). Every single run lasted 20 min.

Polysaccharide content was calculated using equation 2.

$$Polysaccharides\ \left(\frac{w}{w}\right) = \frac{[Monomer]*V*\left(\frac{MWmono-MWw}{MWmono}\right)}{h.eff.*m} \quad (2)$$

Where [monomer] is the sum of the measured monomer concentrations (g/ml) indicative of the polymer, *V* is the total sample volume (ml), $\frac{\text{MWmono}-MWw}{\text{MWmono}}$ describes the molecular weight (g/mol) differences in monomeric form and within a polysaccharide context, *h.eff,* the hydrolysation efficiency in percentages and *m* the total samples mass (g) administered.

Besides the different monomeric compositions for alginate and fucoidan, a combination of monomers is considered. Guluronic acid, mannuronic acid and solely fucose, respectively. Fucoidan consists of many more monosaccharides (Bilan et al., 2002; H. Zhang et al., 2015). These are not calibrated and are thus indirectly accounted for by the lower hydrolysis efficiency. Lastly, since mannitol is already a monomer, its detected concentrations are not corrected by hydrolysis efficiencies nor by molecular weight changes. These molecular differences give slight alterations to equation above, depending on the polysaccharide in question.

The total carbohydrate concentration was determined by subtracting all the major chemical components from the total dry weight mass. Within this carbohydrate fraction, we deduct the analysed carbohydrates to assess the quantity of the remaining "other carbohydrates".

The "other" carbohydrates fraction mainly consist of consists of cellulose, but also phlorotannins, halogenated carbohydrates and more (Cabrita et al., 2010; Li et al., 2021; Sardari et al., 2020). Due to redundancy for this thesis, the remaining fraction will be grouped in this category.

2.3.5 Fucoxanthin extraction and analysis

For fucoxanthin extraction, a sample of 10 mg freeze-dried biomass of seaweed and solid residues was mixed with 10 ml of methanol for 10 minutes at 25 ºC. The mixture was later centrifuged for 10 minutes at 4255 xg using 12 ml glass tubes in an Allegra-X-30R centrifuge. 10 µl of the supernatant was injected in a Shimadzu UPLC spdM20A system with a Kinetex C:18 column to quantify fucoxanthin and other pigments. Buffer A consisted of 0.5 M ammonium acetate in methanol: milli-Q water (85:15 v/v), Buffer B was acetonitrile: milli-Q water (90:10 v/v), and Buffer C was 100% ethyl acetate. The gradient elution program lasted a total of 53 minutes, details are provided in Appendix A. The pump operated at 1 mL/min, and

a photodiode array detector (PDA) at 448 nm was employed. A standard gradient of fucoxanthin was prepared in methanol with concentrations of 15, 7.5, 1.5, 0.75, 0.375, and 0.1875 µg/ml. The samples consist of 10 mg freeze-dried biomass and solid residues mixed with 10 ml of methanol. DES extracts and blanks were injected into the UPLC system without any dilutions. Fucoxanthin concentrations were calculated using Equation 3.

$$C_{Fucoxanthin} = \frac{C_{UPLC} * V_{sample}}{m_{Dry\ weight}} \quad (3)$$

Where $C_{fucoxanthin}$ (mg/g_{DW}) is the fucoxanthin concentration and C_{UPLC} is the concentration indicated in the samples by the machine based on used standards.

2.4 DES extractions

For each DES selected specific molar ratio where applied: Choline chloride: Levulinic acid (CC:L,1:2, Sigma-Aldrich™, purity ≥98%). Menthol: Thymol (M:T,1:1, L-Menthol Thermo scientific, ≥99% purity, Thymol Sigma-Aldrich™, purity ≥99%), and Menthol: Levulinic acid (M:L,1:2). These molar rates are based on their specific chemical eutectic points and their extraction effectiveness (Lo, 2021; Topal et al., 2023; Van Osch et al., 2019). The mixtures were heated at 60 °C and shaken at a rate of 100 rpm.

A first extraction method was performed with DES (≥4g) of M:T, CC:L and M:L were mixed with freeze-dried solid residue and freeze-dried seaweed samples in a ratio of 0.1 (w/w) with 10% water. The extraction was then performed at 35°C in a shaking water bath at 100 RPM for 1 hour. All samples were centrifugated at 4000 xg for 10 min using an Allegra-X-30R centrifuge after extraction. Supernatants were then separated and prepared for either HPLC or GC analysis. A second DES extraction method was performed in an Eppendorf Thermomixer C - ThermoTop® with a biomass ratio of 0.5 (w/w_{DES}) for 2 hours. For each sample, 75 mg of dried seaweed or solid residue was placed in 2 mL Eppendorf tubes with 1.5 mL of the solvent containing 10% water.

2.5 Fatty acid analysis by gas chromatography

Fatty acid analyses were done using DES Extracts derived from the first extraction method. Extracts consisted of dried solid residue extracts (50 mg), dried seaweed extracts (50 mg) and DES blanks in parallel. DES extracts were dissolved in toluene (0.2 mL containing 0.1% w/v C15:0 methyl ester internal standard). Methylation was performed on the samples with 1.8mL of methanol 5% H_2SO_4 (v/v) at 100 °C for 1 hour. The controls consisted of total lipid extract

obtained after the nitrogen gas drying by using the crude lipid analysis protocol. After cooling the DES extracts, blanks and the controls were diluted with 1 ml of hexane and 3 ml of water. The samples are vortexed for 2 min and rest for 15 min. For analysis, the top hexane layers were isolated in ambient coloured glass vials.

The analysis of fatty acids was performed using the GC-FID Agilent 7890 system with the Supelco Nucol™ 25357 column (30 m length 530 micrometre internal diameter and 1 mm film thickness), H_2 gas as carrier and n-Hexane as solvent with a split ratio of 0.1:1 and split flow of 3.55 mL/min. The oven temperature profile was 90 °C to 200 °C at 44.08 °C/min and held for 7.5 minutes.

2.6 Techno-economic analysis

SuperPro Designer® version 13 build 2 academic edition was used to simulate the different processes to estimate the feasibility of DES extraction used for the solid residues. The conventional process was modelled after data given by Algaia™. Component splits were based on mass balancing and data obtained by this thesis.

Based on the second method, M-L conditions and data, DES extraction parameters were used. This translates to a residence time of 2 h, an operating temperature of 35 °Celsius, and a stirring power consumption of 3 kW/m^3.

Combining three different protocols for effective DES recirculation and component isolation (Kholany M et al., n.d.; Lo, 2021), Figure 9 illustrates the proposed model. After M-L extraction, the model starts by mixing CC-L with the M-L extract in a 6:1 ratio, and is succeeded by a biphasic separation system. Separating the light fraction rich in menthol, methanol is mixed as an antisolvent in a 1:1 ratio. Another two-phase system forms where the heavier lipid (algae oil) layer is isolated, and the lighter methanol M-L layer is further distilled for MeOH and DES recycling. Water is added in a 1:1 ratio to the heavy fraction derived from the first biphasic system. A relatively small fucoxanthin-rich lipid layer is separated hereafter, and the DES water fraction is treated using reverse osmosis to recircle the CC-L.

Some important parameters used are a refreshment DES percentage of 20%, where every cycle 20% of the whole is reused, Levulinic acid ratio splits based on DES molar ratios, the recovery ratio of 50% for the membrane retentate and a 99.9% methanol separation in the distillate. Assumptions used in this model are a high purity of the lipid isolates with a total lipid fraction of 0.95, effective DES recovery and separation by the biphasic models, and full MeOH

separation and recirculation after distillation. These assumptions are based on qualitative tests and electrochemical differences between components residing in the streams. For simplicity's sake, the lipids and fucoxanthin we sold at market price, as listed in Table 1, are based on their content within the sold streams.

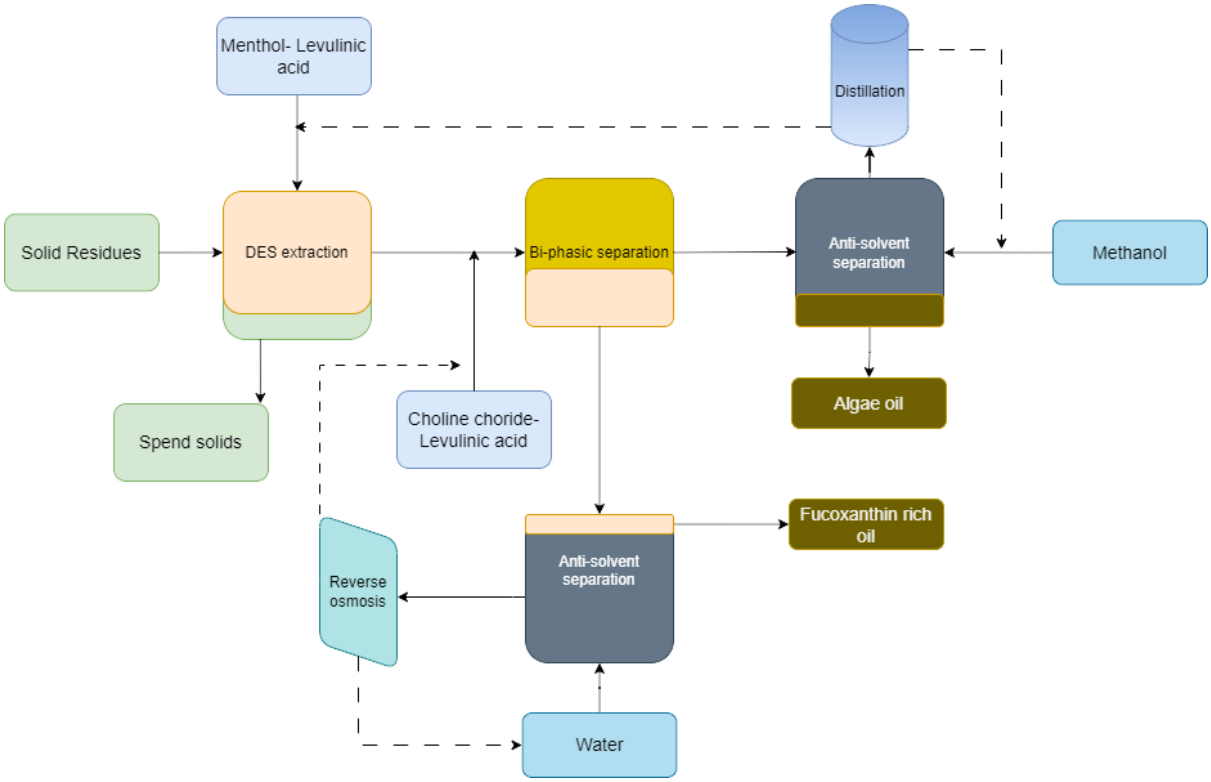

Figure 9: Proposed DES extraction bi-phasic separation and double anti-solvent isolation with solvent recirculation.

3 Results

3.1 Seaweed composition

The full seaweed characterisation was performed on the first batch of *S. latissima* harvested in May at Kamperland, the Netherlands. Both lipid and the total "other" carbohydrates fraction had dry weight percentages of 8.7±2.5% and 13.3%, respectively (Figure 10). Furthermore, this particular batch consisted of 10.8±0.6 %DW protein and 40±6.4 %DW total carbohydrates. Dry weight values of the polysaccharides were as follows: alginate 9±0.9%, laminarin 7.4±3.5%, fucoidan 7.2±2.5% and mannitol 3.3±1.2%. Finally, ash levels of 40.3±5.4% and fucoxanthin concentration of 0.25 ± 0.014 mg/g DW were also recorded.

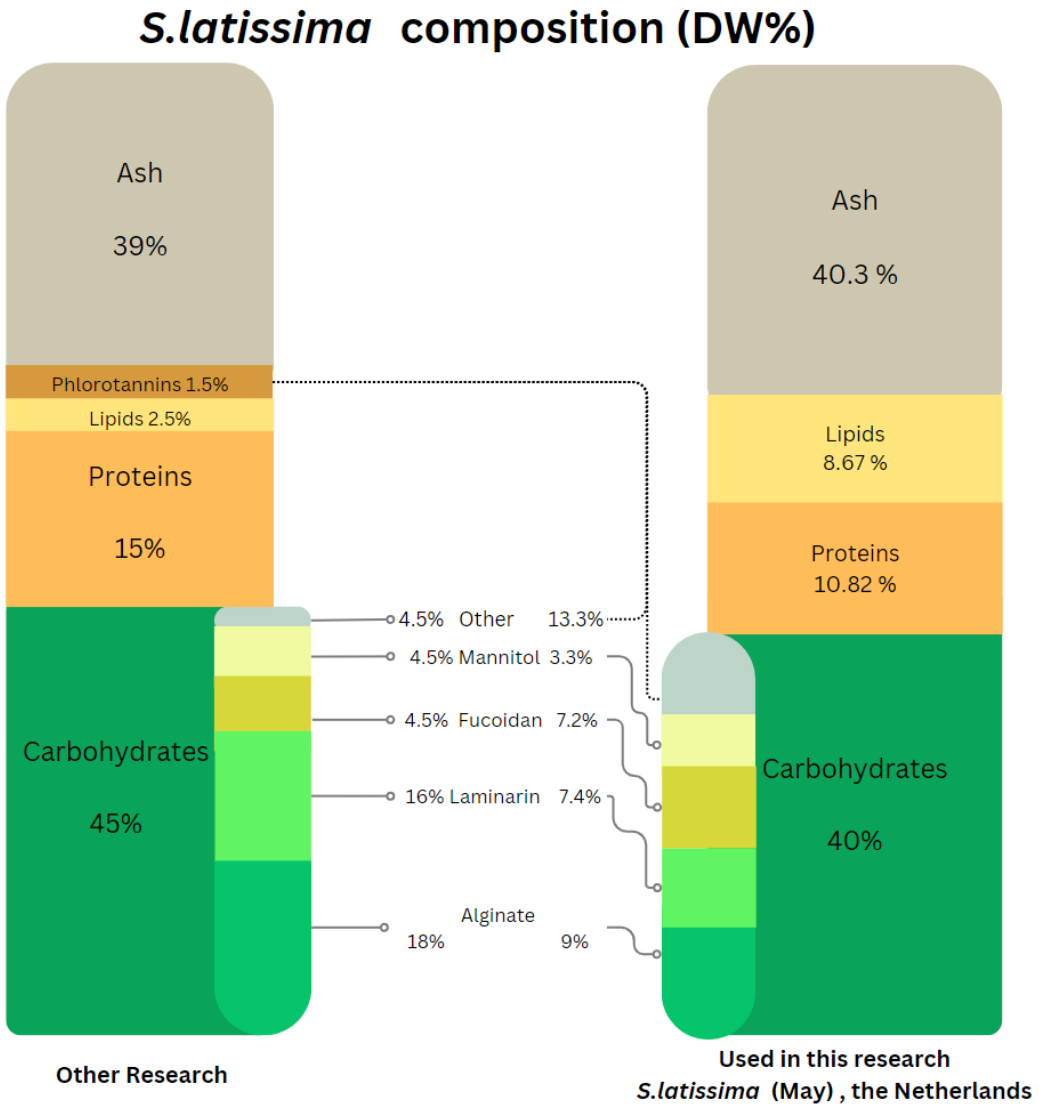

Figure 10: Comparative stacked dry weight bar chart of detected chemical compounds by previous studies (left) and this thesis (right).

3.2 Mass balances in alginate extraction

In order to have a clear birds-eye view of the process of mass balancing, a thorough analysis of the two remaining streams as a crucial component was completed, summarised in Figure 11. As the name suggests, the element most present within the alginate extract was alginate (14% DW), excluding water and minerals. With a weight fraction of 0.59 (g_{stream}/g_{total}) compared to the starting total, correlates to an average alginate extraction efficiency of 59% of the process using *S. latissima*. Besides alginate, this stream's significant components are fucoidan 5.4 and some other carbohydrates 2.7 (DW%).

As shown in Figure 12 in DW%, weight fractions found in the SR amounts give a different perspective. In this regard, the most significant weight fractions are fucoxanthin 66, lipids 65, other carbohydrates 37, fucoidan 25 and protein 22 (w%) in relation to the starting seaweed.

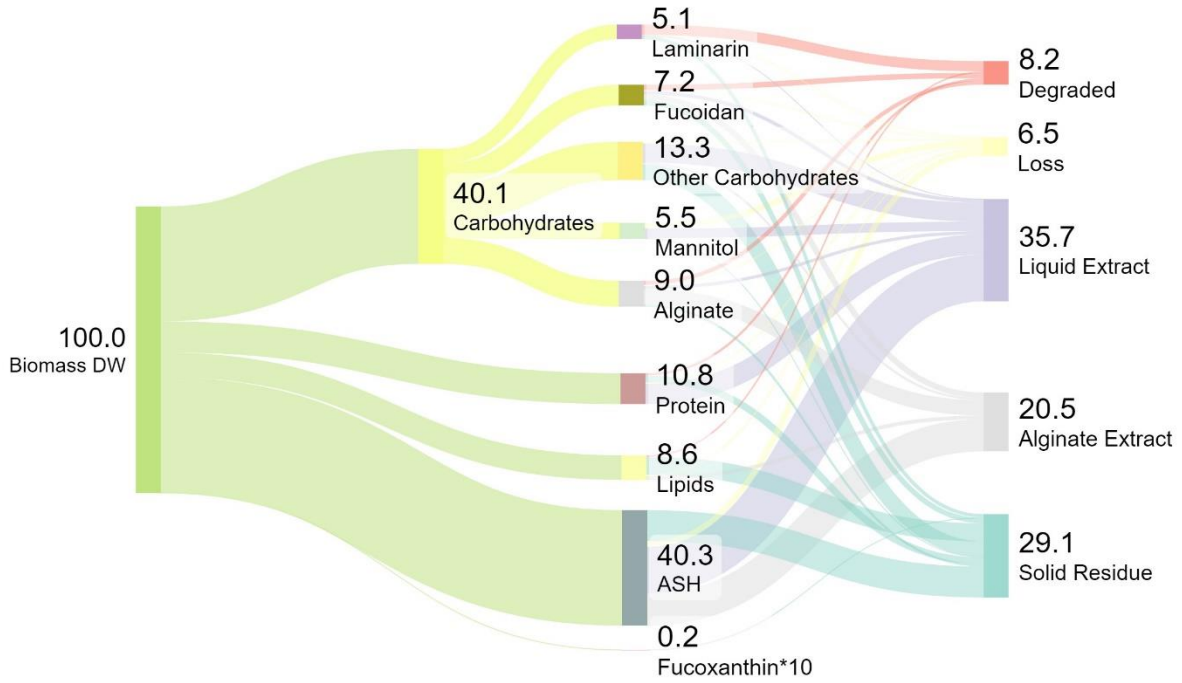

Figure 11: Shankey diagram of the mass balances of the conventional lab-scale alginate process. Starting with 100% DW S. latissima feedstock ending in the 5-ending stream, each analysed chemical component is indicated in the middle.

A fixed amount of loss was calculated based on total mass losses between the processing steps and applied uniformly to all components. Any measurable remaining discrepancies between the starting biomass and the final weights were labelled “degraded”, emblematic of the undeviating smaller total content measured. The degraded fraction does, in reality, not exist but

remains nonetheless unmeasured. Most prominently in the case of laminarin were 92 w% as well for fucoidan 30 w% and alginate 9 w%, but to lesser extents.

As the last stream to be discussed, although non-existent within the large-scale conventional process, mannitol was almost exclusively present in the aqueous stream (liquid extract) with a weight percentage of 81% relative to the starting amount. Most other residual mannitol 19% ended up in the solid residues.

Likewise, most of the proteins (66 w%) in total measured nitrogen ended up in the liquid extract. For visualisation, in Figure 15, the assumption is made that the ash within the solid residues consists of no sulfuric acid nor the disodium carbonate added during the process. In large part due to the high amount of water added, for simplification the added minerals are computed as going in their entirety to the liquid extract, with the remainder deriving from the seaweed. Concerning an exact visualisation of the ash, a mineral analysis would be required to retrace the minerals originating from the starting seaweed biomass.

3.3 Solid residue composition

Fucoxanthin, the total lipid fraction, along with the remainder of "other" carbohydrates, are all concentrated within the solid residue stream. Resulting in values of 0.83±0.15 (µg/gDW), 25.1±0.9 (DW%), and 17,3 (DW%) respectively (Figure 12). The compounds that remained relatively stable in concentration were fucoidan 6.3±0.4, proteins 8.4±0.3, mannitol 2.2±1.1 and ash 37.6±3.9 (%DW). These dry weight concentrations differed no more than 20% from the starting seaweed biomass. Lastly, the alginate and laminarin dry weight percentages reduced significantly to 2.8±0.87 and 0.3. Important to note that in total mass, the solid residues consisted of only a fraction of 0.3 of the total starting biomass, meaning that all components, even the ones that concentrated, had nonetheless significant losses in absolute numbers. In the

end, Fucoxanthin was the component most relatively present within the solid residue, with a weight percentage of 66% relative to the starting amount(Figure 13).

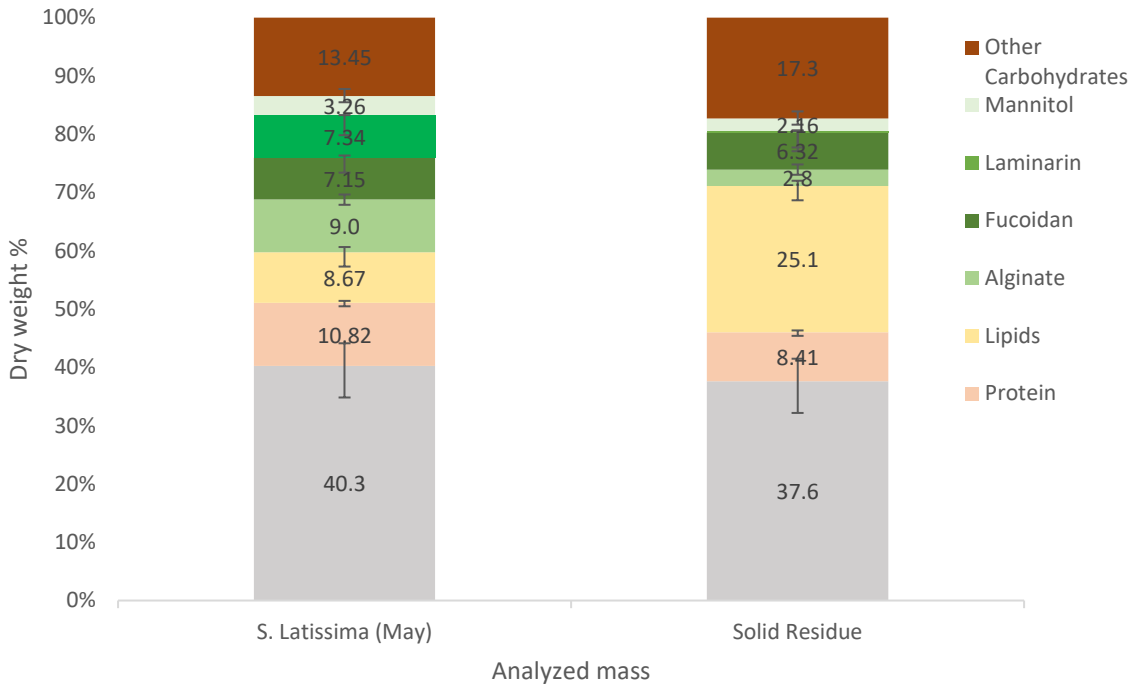

Figure 12: Stacked bar chart of the chemical composition of the starting biomass S. latissima (left) and the subsequent derived solid residue (right) with standard deviation as error bars.

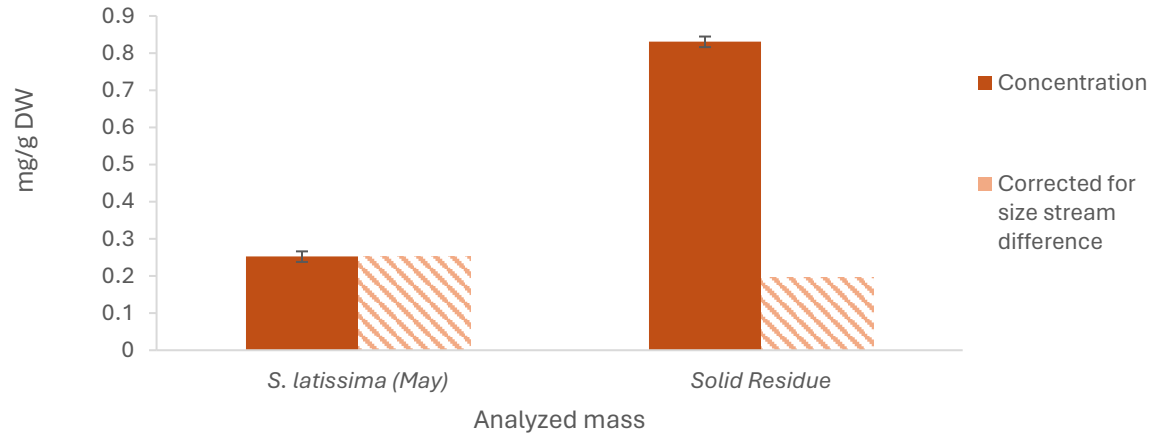

Figure 13: Fucoxanthin concentrations within S. latissima and the solid residue after alginate extraction

Based on these composition numbers, a relative evaluation similar to the one done in Figure 2 indicates that the three most valuable compounds are fucoxanthin 55%, lipids 21.9 % and

fucoidan 21.5% of relative market value within the whole stream. This relative value percentage remains only valid as long as chemical integrity is preserved. For fucoxanthin as a pigment, this was straightforwardly assessed by analysing the overall absorbance spectrum simultaneously during the quantification. Fucoxanthin absorbance shifted maximally 2 nm after extraction from the different biomasses, having a maximum absorbance within the range 448 - 450nm. Indicating that the detected fucoxanthin concentration had no alteration in the length of the conjugation, which in the case of fucoxanthin practically means chemical integrity since the majority of the skeletal structure of the carotenoids is conjugated (Figure 6).

In the case of the lipids' chemical integrity, an additional in-depth analysis of the fatty acids was executed (Figure 14). The omega-6/omega-3 fatty acid ratio (n-6/n-3) and the C20:4 n-3 amounts are especially important here. These ratios were 0.74 and 0.55 for the macroalgae and the solid residue, respectively Table 2. The lower n-6/n-3 ratio within the solid residues is in large part due to a higher presence of larger omega-3 fatty acids, specifically C18:3 n-3 and C20:4 n-3. These constituents concentrated in the residue relative to the starting biomass from 2.1% to 2.7% for C18:3 n-3 and 3.6% to 8.4% for C20:4 n-3 as a percentage of the total lipids.

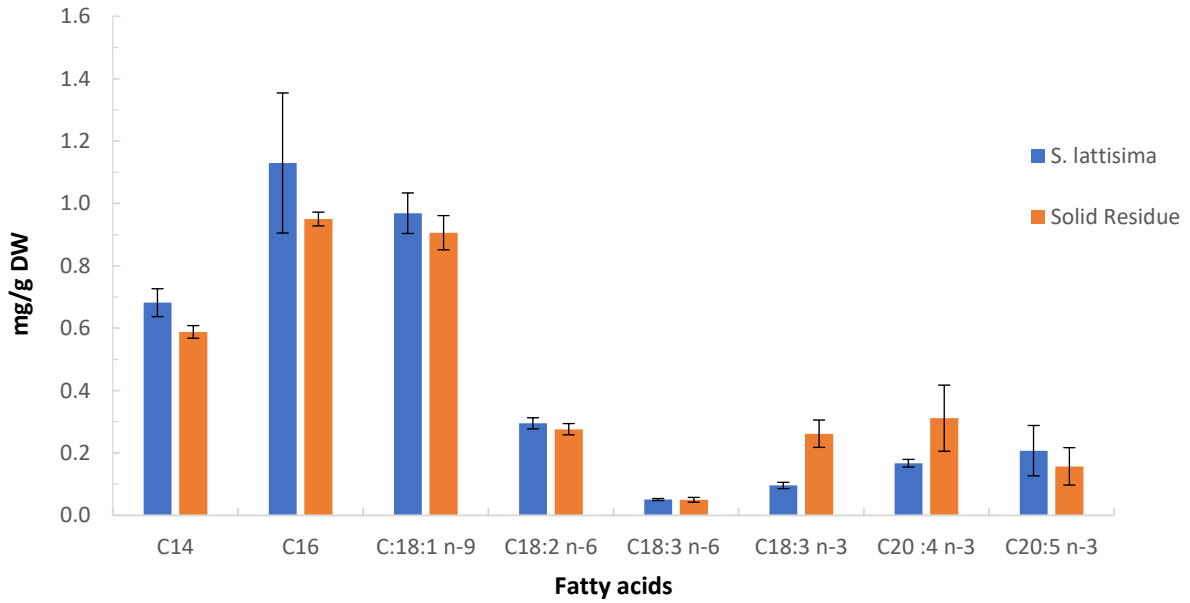

Figure 14: Fatty acid profile in the seaweed and the solid residue.

Eight different fatty acids were persistently present in all samples and summed up as the free fatty acid (FFA) content (Figure 14). These ones being: myristic acid (C14), palmitic acid (C16), oleic acid (C18:1 n-9), linoleic acid (C18:2, n-6), γ-linolenic acid (C18:3 n-6), α-linolenic acid (C18:3 n-3), eicosatetraenoic acid (20:4 n-3) and eicosapentaenoic acid (EPA,

20:5 n-3). Palmitic acid and oleic acid have the highest fatty acid concentrations with percentages of 30%-25% and 26%-23% respectively relative to the total FFA content (Table 2). Summed up, the free fatty acid fraction accounted for 15.1% in *S. latissima* and 14.3% in the solid residue of the total lipid content. These biomass lipid extracts were obtained using the crude lipid protocol and were set as a control for the DES extracts.

3.4 DES extractions

Based on the most relevant fraction found in the solid residues, a set of DES consisting of Menthol-Thymol (M-T), Menthol-Levulinic acid (M-L), and Choline chloride-Levulinic acid (CC-L) have been further investigated for lipid and fucoxanthin extraction. The menthol-based solvents extracted most of the fatty acids and, in the case of M-L, almost constantly superseded relative concentrations seen in the controls (Figure 14).

Remarkably, DES extracts derived from the solid residues had consistently higher FFA content than the seaweed-based extracts. The solid residue had an increase in FFA content by a factor of 2.24 to 1.08 depending on the fatty acid. This was true for each type of DES tested, with the highest relative difference in FFA detected in the CC-L extracts. This observation contrasts the MeOH-based controls, where FFA content remains at relatively stable levels regardless of the type of biomass.

In absolute terms, menthol-based extracts consistently have the greatest detectable FFA content, giving it an FFA content within the total lipid fraction of 19% for the seaweed and 33% for the solid residues. Menthol-thymol FFA concentrations remain similar to the control concentrations regardless of the type of fatty acid. In both relative and absolute terms, the more polar DES CC-L had, the lowest dissolved fatty acid concentrations of all other solvents utilised. CC-L seemed to be selectivity for smaller chained fatty acids since their relative presence is a lot higher. It is most likely due to the low concentrations making larger fatty acids undetectable. Remarkably, C20 within each DES extract was persistently present, albeit in small concentrations. In the controls C20 was also present, but in the final results its concentration was nullified due to non-constant detection.

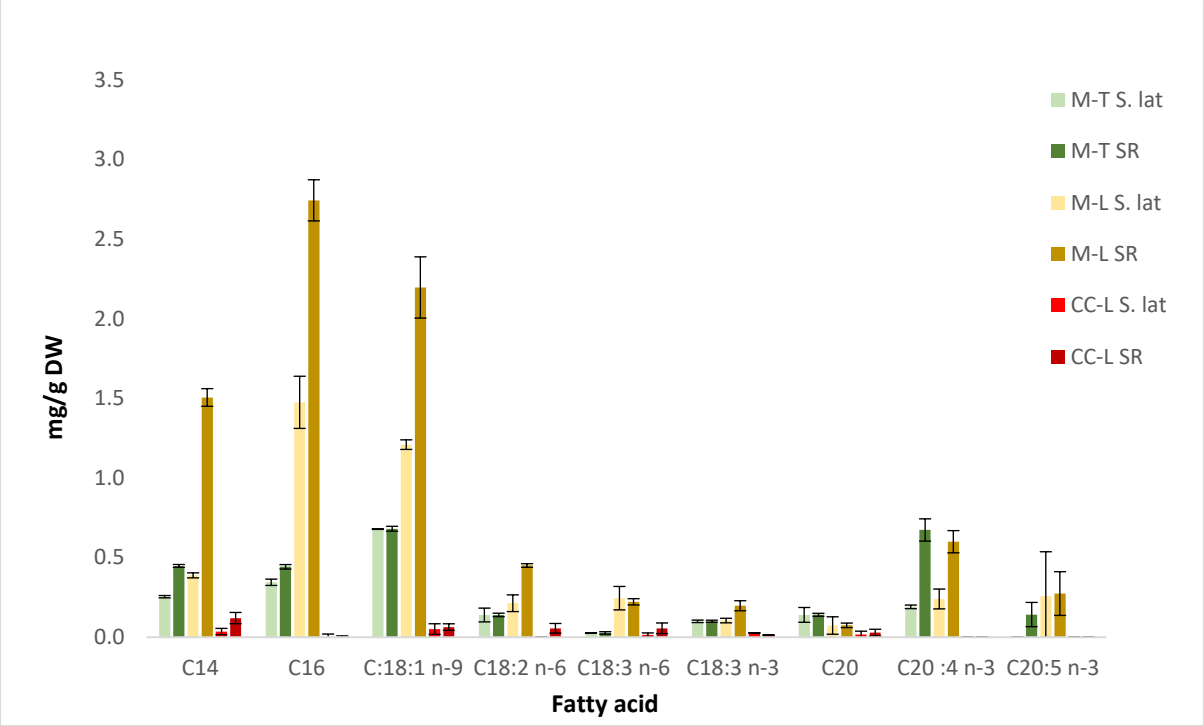

Figure 15: Free fatty acids profile within the different DES extracts derived from S. latissima (S. lat) and solid residue (SR) feedstocks.

The n-6/n-3 ratios within the DES extracts ranged from 0.76 to 0.18 (Table 2). Due to undetectable insignificant concentrations, the outlier within these ratios is the CC-L SR sample with a ratio of 8.6. The solid residue extracts had lower n-6/n-3 ratios than the seaweed itself in both the controls and the extracts.

Due to its proven bioactivity and health benefits eicosapentaenoic acid or EPA/C20:5n-3 concentrations were used as a quality indicator (Swanson et al., 2012). These were again highest in M-L extracts (Table 2). Once again, due to the limited detectability of the low starting concentrations, the EPA levels were relatively variable and, in the cases of M-T seaweed extract and CC-L based, non-existent.

Table 2: FFA quantitative and qualitative parameters of the different biomass extracts.

Extract	n-6/n3 ratio	EPA concentration (mg/g DW)	FFA content (mg/g DW)
Control *S. lat*	0.74	0.21	3.6
Control SR	0.45	0.16	3.5
M-T *S. lat*	0.57	-	1.9
M-T SR	0.18	0.14	2.8
M-L *S. lat*	0.76	0.26	4.2
M-L SR	0.62	0.27	8.3
CC-L *S. lat*	0.55	-	0.1
CC-L SR	8.62	-	0.3

Highly coveted fucoxanthin extracted using DES was performed using the two different extraction strategies. The second method had a clear advantage over the first in terms of fucoxanthin yield, with an average factor of increase of **9.0** over the first method. The first method is identical to the one used in the fatty acid analysis, and the second has been optimised regarding fucoxanthin extraction efficiency (Kholany M et al., n.d.).

The extraction efficiency of fucoxanthin by different DES indicates similar trends to those shown in the fatty acid analysis. Primarily, M-L is the most effective compared to the other DES in terms of total fucoxanthin and FFA extraction. Secondly, the solid residue extracts have higher concentrations of the compounds of interest than the *S. latissima* based ones by a factor of 2.1. Finally, DES M-L extracted on average more than the MeOH standard. However, when excluding the SR M-L method 2 extract, there is actually a relative loss of 38±6% compared to the MeOH standard (Figure 16). The MeOH controls, however, clearly diverged from the specific trend and previous results (Figure 13) by having higher fucoxanthin concentrations within the seaweed extract than in the residue. When corrected for total stream size, this total fraction relative to the total remains within the range (0.52-0.66) of the previously stated results. Meaning due to the componential different batch of *S. latissima* compared to values stated in Figure 13 resulted in a 2.7x larger residue stream in terms of dry weight, diluting the fucoxanthin within the SR.

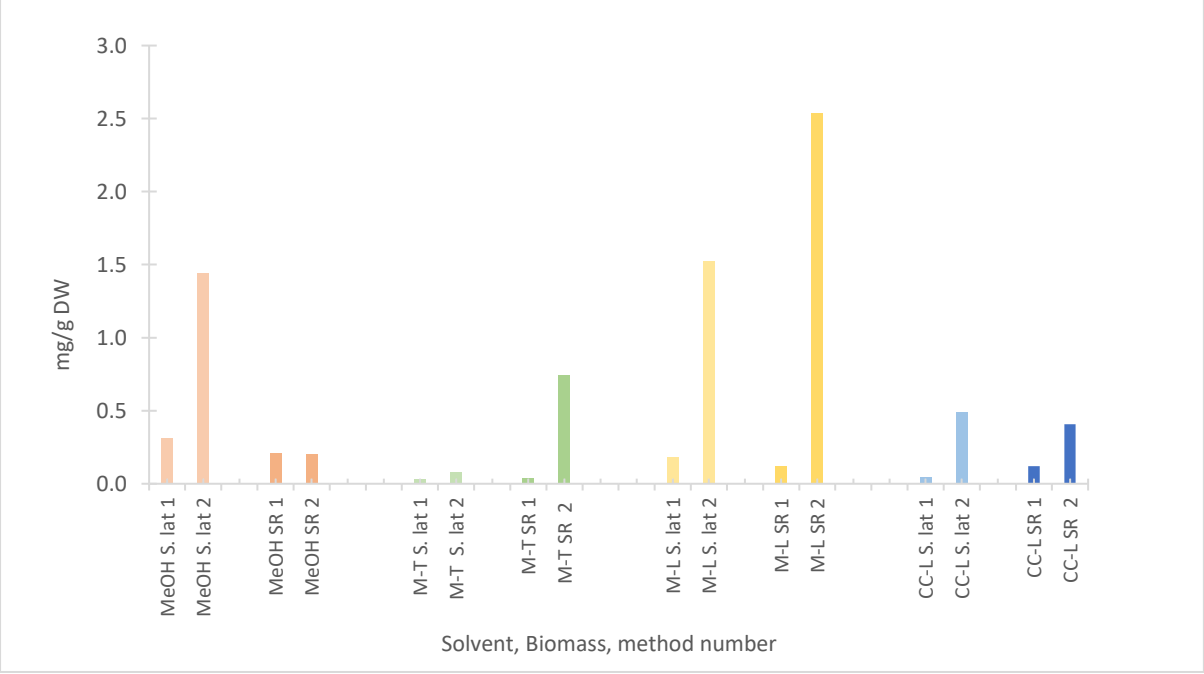

Figure 16: Fucoxanthin concentrations within extracts. Varying in solvent, biomass, and technique applied.

3.5 Techno-economic analysis

With the necessary assumptions and parameters, this extraction model would have a payback time of 4.27 years (Appendix economic evaluation). Here 15% of the annual revenue would come from the fucoxanthin-oil-rich stream and the remaining lipids stream, and the other 85% still come from the conventional extracted alginate. Sized to maximally use all the large bioreactors, this yields a CAPEX of 142 million $, an OPEX of 57 million $/y and processing a total of 18,000 MT of wet *S.latissima* annually. This is paired with an annual revenue of 12.6 million $/y (40 MT/y) for the hydrophobic streams. This makes the DES extraction model break (11.2 million $ OPEX) almost even break even with a 20 % solvent cycle renewal rate. Included in these costs are the thorough DES purification operations such as distillation and reverse osmosis.

Here, the alginate is sold as a gel with a large water fraction of 85 % and an alginate content of 5% DW. The oil stream pricing consisting of 98% fats with some impurities, mainly menthol. Pricing did not take in regard extra purification steps required.

4 Discussion

4.1 Biomass characterisation

The starting amount of the total lipids in *S. latissima* with a dry weight content of 8.7±2.7% has been critical in selecting the DES discussed in this thesis. Previous reports, however, have indicated that these lipids amounts are exceedingly high and rare (Monteiro et al., 2021; Olischläger et al., 2014). Especially for the time of harvest, where other northern Atlantic *S. latissima* stock harvested in May had a lipid content of 2.1±0.6 DW% (Marinho et al., 2013). Despite consistently lower total lipid content in most brown macroalgae-based feedstock (Samarasinghe et al., 2021), using these DES remains relevant within the process in light of fucoxanthin isolation, of which the levels are a lot more consistent (Aslanbay Guler et al., 2019; Jaswir et al., 2012).

For the remaining composition, water, ash, fucoidan, mannitol, and fucoxanthin levels within *S. latissima* showed no signs of relevant standard deviations, and all fell into previously reported content ranges (Bruhn et al., 2017; Handå et al., 2013; Sharma et al., 2018). Protein and total carbohydrate concentrations were slightly lower than average but well within previously reported ranges (Handå et al., 2013; Holdt & Kraan, 2011; Samarasinghe et al., 2021). The alginate and laminarin were significantly lower than other brown seaweed species and other analysed batches (Figure 10) but fell in between previously reported *S. latissima* ranges of 6-23 DW% (Handå et al., 2013). These decreased alginate concentrations compared to *A. nodossum* directly impacted the extraction process, such that less sodium carbonate was necessary to solubilise the polymer.

Regarding polymer content estimations, polysaccharides derived from macroalgae species other than *S. latissima* were used for hydrolysis efficiency estimation (Equation above). Especially in the case of fucoidan and alginate there is significant monomeric heterogeneity possibly altering the hydrolysis process (Draget K. I., 1998; H. Zhang et al., 2015).

In regards to laminarin and mannitol, this is not an issue due to the established homogeneity of these polysaccharides (Li et al., 2021). However, these polymers did have consistent relatively high standard deviations within *S. latissima* (Figure 12). This is under the context of brown macroalgae biology, were laminarin and mannitol serve as a storage molecule and an osmotic regulator respectively (Dobrinčić et al., 2020; Reed et al., 1985).

Laminarin estimation does add another layer of complexity. Since the HPLC organic acid method doesn't distinguish between glucose originating from laminarin or cellulose. Nevertheless, there was a constant underreporting of glucose levels in the conventional process streams but preserved cellulose levels categorised as "other" carbohydrates. Meaning that acidic conditions applied both in HPLC analysis and the conventional process might have a limited effect on the crystalline structure of the cellulose.

4.2 Solid residue characterisation

After processing, the high lipid content of the seaweed trickled down into the solid residue and concentrated into a total lipid DW % of 25.1. Valuation hereafter makes the lipids one of the stream's most significant components. Granted the starting biomass has a significant fraction, which in as stated often the case for *S. latissima* where DW% under 5% is the norm (Khotimchenko, 2005; Monteiro et al., 2021). Nevertheless, a consistent lipid weight fraction within the SR of 65 to 78 (w%) relative to the starting amount was found. This was also true for *S. latissima,* starting with a total lipid content of 2.5±0.1 DW%. Making the conventional future lipid stream assessments predictable regardless of the starting amount.

Likewise, the total fucoxanthin levels remained stable, ranging from 66 to 52 (w%) relative to the total starting amounts. Since fucoxanthin levels within the other than the solid residue streams were not measured due to applied bleaching, it remains unknown whether the remaining fucoxanthin lost its molecular integrity or was diverted to the other process streams.

On a final note regarding fucoxanthin, the pigment seems particularly sensitive in relation to which DES extraction method is used, even when applied to MeOH. In absolute yields, the extractions are particularly effective for the solid residues compared to the seaweed by a factor of 2.2 (Figure 16).

The fatty acid profile of the SR displayed less variability and didn't diverge from the seaweed profile. Herein, differences in FFA levels were not bigger than 5% as a percentage of the total FFA content. Moreover, the biomass lipidomes comprised valuable omega-3 FFA, specifically EPA and had favourable n-6 to n-3 ratios (Table 2). These quality assessments conclude that the solid residue will predictably comprise lipids and pigments in a high ratio in relation to the original biomass and will be composed of high-quality indicative metrics.

Divergently, a previous report stated that fucoidan and proteins were the most significant within the solid residue in terms of content and value, not lipids. These values were derived from a

similar acid pre-treatment alginate extraction process (Fertah, 2017). It is important to note that the aqueous stream, syphoned off in the lab scale process, is non-existent and recirculated within the conventional industrial process. In this scenario, compounds within this stream will accumulate more in the whole system and solid residues as a result. Within this aqueous stream, components' largest weight fraction (w%) relative to the starting amount were mostly proteins 66 in the form of total nitrogen, other carbohydrates 51, mannitol 45 and fucoidan 17. Consequentially, it's highly probable that this concentration will increase within the solids residues derived from the industrial-sized processes compared to ours. In summary, depending on the starting seaweed composition, one should alter valorisation efforts. Flow ratios and valuations in this research can be used to direct valorisation efforts on either hydrophobic or hydrophilic components in the SR.

4.3 DES extractions

Based on the hydrophobic nature of the valuable compounds in the SR, a selection of DES was made. DES Menthol- Thymol (M-T) has shown efficacy in being able to extract 87% of the total lipid extract present in lipid-rich animal biomass with an additional selectivity of fatty acids over polar lipids (Topal et al., 2023). Menthol-Levulinic acid (M-L) has also been reported to be a viable solvent for fucoxanthin and lipids (Essers D, 2023; Xu et al., 2022). These claims were verified, where M-T matched, and M-L even outperformed extraction efficiency compared to the hexane controls (Figure 15). The hexane-based extracts are done after crude lipid isolation by the crude lipid extraction method (Folch et al., 1956). Hereafter, a large pigment fraction remained within the glass tube. Being slightly more hydrophilic than triglycerides based fatty acids, pigments and fatty acids bonded to polar lipids like glycolipids are rich in *S. latissima* (Monteiro et al., 2020; Rey et al., 2019).These compound have elevated levels polar moieties therefore, possibly solubilising better in the slightly more hydrophilic M-L with the carboxylic groups (Lo, 2021; Peng et al., 2011). This would explain the higher M-L FFA yield than the M-T and hexane standards.

Contradictory, choline chloride-levulinic acid is polar and due to its synthesis the only one that can’t be considered a NaDES (Abranches & Coutinho, 2023; Anastas & Warner, 2000). It is included solely based on the final conundrum facing hydrophobic DES adoption, being effective isolation. CC-L has been indicated to be a viable antisolvent paired with M-L (Kholany M et al., n.d.). Illustrating with the almost negligible low FFA and fucoxanthin extraction efficiencies that using CC-L as an antisolvent would possibly minimise losses. Even

more so since results indicated that CC-L might be selective for smaller FFA. However, overall fatty acid level detections were too small in the CC-L extracts to exclude the possible presence of larger FA greater than C18 in molecular mass. Henceforward, any further selectivity analysis of CC-L should increase the sample size to determine possible selectivity properties of CC-L conclusively.

The eight different verified lipids within the extract could be only a tiny representation of the total fatty acid profile (Marinho et al., 2013), primarily in the case of stearidonic acid (C18:4 n-3). There was a persistent presence of an unidentifiable methylated hydrophobic compound with a similar relative retention time, based on outside sources, to that of C18:4 n-3 (Youn et al., 2012). Assuming an average response factor of around 1, the biomass extracts would have a total C18:4 n-3 concentration ranging from 0.6-0.1 mg/g DW. Reducing even further the n-6/n-3 ratio to a range of 0.56-0.16 instead of 0.74- 0.18. Nevertheless, no on-site calibration could verify and correctly asses these concentrations.

Other possible regularly detected FFA included small fatty acids caprylic acid (C8), capric acid (C10), and lauric acid (C12). However, there was a lot of background noise withholding any conclusive statements. Likewise, other larger fatty acids were only intermittently detected. This was the case for C20:3 n-6, eicosadienoic acid C20:2 n-6, nonadecylic acid C19, sapienic acid C16:1 and arachidic acid C20 in all the biomass samples. Apart from C8, all have been previously observed in *S. latissima* (Marinho et al., 2013; Rey et al., 2019). As is for the case of pentadecylic acid (C15). This was the internal standard so any relevant concentration originating from the biomass has been made irrelevant. Obliging any further attempts to create a more accurate FFA profile to both increase sample size and the use of a different standard.

Diverging concerning fatty acid extraction, for fucoxanthin extractions, two different protocols were utilised. The second or the “optimised” protocol was applied and indicated a great improvement in extraction yields of fucoxanthin. Similar effects on the FFA extraction yield are plausible for some of the extracts but unreasonable in the case of M-L. Since, for example, M-L solid residue extracts already had an FFA extraction yield of 33% of the total lipids content. This already supersedes some FFA estimations of 21%, not accounting for possible polar lipid-derived fatty acids (Marinho et al., 2013). Fucoxanthin levels in the extracts were also a lot more variable. For example, one extract outlier, SR M-L method 2 (Figure 16), had 4 times higher fucoxanthin yield than its MeOH counterpart, while the other M-L extract had consistently 38±6% lower yields than MeOH.

4.4 Mass balance in alginate extraction

An alginate extraction yield of 59%, although definitely on the high end, is not novel (Bojorges et al., 2023; Fertah, 2017). Higher rates have been reported, but they are highly dependent on the starting biomass and method. Herein have process using similar acidities of 0.2M strong acids with subsequent redissolving Na_3CO_2 methods reported extraction yields of 24-67% (Bojorges et al., 2023).

Combining all the different component mass balances brought to light all the fractionating and specificity of each component for a particular stream within the conventional process. Laminarin was a clear exemption by having the most significant "degraded" fraction of 91 w%, superseding the subsequential highest compound degraded fraction by a factor of 2.9. This means that compared to the starting biomass, the measured laminarin levels within the process final streams had lost almost all of the total laminarin amounts. This could be due to one of two causes. Firstly, during the drying process used for the two more aqueous streams, an insoluble inert pellet formed. This could consist of laminarin and its degradation products, but other monosaccharides like glucose and alginate were detected hereafter. The second potential cause, persistent unidentifiable detection by the RID was observed with retention times differing just 0.1 min compared to the standardised glucose. This could indicate chemically altered glucose derived from the laminarin. Especially since it is known that glucose exposed to highly acidic conditions is more prone to mutarotate from a α-D-glucose configuration to β-D-glucose (Bronsted et al., 1927). Altered standards have to be run in the HPLC to determine if this changes their retention time.

4.5 Techno-economic analysis

Several assumptions have been made in order to make this model work. Most important of all is the relatively high purity of the two oil fractions, which is disputable. Despite this, the extraction model shows promise in that even though the DES is refined every cycle by distillation and membrane filtration, a solvent refreshment rate of 20% is still economically feasible, given the current cost (Appendix economical evaluation). However, this is based on *S. latissima-based* yields and composition analyzed in this research. Meaning a seaweed with a lower total lipid dry weight than 8.7% would put more strain of the feasibility of the DES extraction model.

The process has been size such to make maximally use each bioreactor processing step, giving a payback time of 4.27 years and a seaweed mass flow of 18.060 MT/y. Real processing capacity needed can differ greatly altering the payback time inversely.

5 Conclusion and Recommendations

Flow ratios as stated in this thesis have indicated that the *S. latissima* derived components, lipids and fucoxanthin have the highest weight percentages of over 66% in the solid residue relative to the starting amount after conventional processing. Whereas the seaweeds mannitol 45%, protein 66% and other carbohydrates 51% diverge mostly in the recirculated aqueous stream and lastly alginate 59%, fucoidan 28% to the alginate extract.

Thus, a DES extraction model was selected to valorise these hydrophobic compounds within the solid residues. M-L is highly effective in extracting fucoxanthin and FFA, with yields comparable to or higher than established solvents such as methanol (28% lower) and hexane (78% higher). FFA and fucoxanthin within these DES extracts have no compromised quality in the form of n-6/n-3 ratios of above 1 (0.76 to 0.18), significant EPA levels (0.14-0.27 mg/g DW) and no altered pigment light absorbance.

When extracting fucoxanthin, a longer extraction time of 2 hours, better agitation methods, and a solid-liquid ratio of 0.05 are crucial for elevated yields.

Regarding compound isolation, CC-L has shown low yields of less than 10% for fucoxanthin and FFA. Validating the method of using CC-L as an antisolvent when M-L was used by limited product loss. Combining data parameters makes a hydrophobic DES two biphasic antisolvent model feasible. A DES recirculation percentage of at least 80% is needed in a large-scale model. This gives financial headspace for even thorough refinement of the DES.

Further research in testing used assumptions such as the purity of the anti-solvent isolates and the effectiveness of refinement strategies, which are critical for materialising the model and revealing the concrete possibilities and drawbacks of the M-L extraction and hydrophobic DES overall.

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Appendix

Economic evaluation

Economic Evaluation Report

March 9, 2024

for Start SP Eco. 6-3 DES extraction Recirculation

1. EXECUTIVE SUMMARY (2024 prices)

Total Capital Investment	142,152,000 $
Capital Investment Charged to This Project	142,152,000 $
Operating Cost	57,355,000 $/yr
Main Revenue	72,328,000 $/yr
Other Revenues	12,562,024 $/yr
Total Revenues	84,890,000 $/yr
Cost Basis Annual Rate	3,144,704 kg MP/yr
Unit Production Cost	18.24 $/kg MP
Net Unit Production Cost	18.24 $/kg MP
Unit Production Revenue	26.99 $/kg MP
Gross Margin	32.44 %
Return On Investment	23.39 %
Payback Time	4.27 years
IRR (After Taxes)	16.63 %
NPV (at 7.0% Interest)	95,524,000 $

MP = Total Flow of Stream 'Alginate extract'

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Bleach	0.00	21,184	kg	0	0.00
Choline chlorid	1,180.00	5,083	MT	5,997,969	26.86
H2SO4 (2 M)	0.00	4,796,237	kg	0	0.00
H2SO4 (50% w/w)	0.00	7,179,538	kg	0	0.00
Levulinic Acid	1.45	3,032,931	kg	4,397,750	19.69
Menthol	1,894.00	634	MT	1,200,038	5.37
Methanol	0.24	7,533,349	kg	1,808,004	8.10
Na2CO3(aq)	0.00	1,313,629	kg	0	0.00
S. Latissima	480.00	18,058	MT	8,667,648	38.81
Water	1.23	213,905	MT	263,103	1.18
TOTAL				22,334,512	100.00

10. PROFITABILITY ANALYSIS (2024 prices)

A.	Direct Fixed Capital	132,673,000 $
B.	Working Capital	2,845,000 $
C.	Startup Cost	6,634,000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	142,152,000 $
G.	Investment Charged to This Project	142,152,000 $

H.	**Revenue/Savings Rates**	
	Fucoxanthin rich oil (Revenue)	40,557 kg/yr
	Algae oil (Revenue)	96,250 kg/yr
	Alginate extract (Main Revenue)	3,144,704 kg/yr

I.	**Revenue/Savings Price**	
	Fucoxanthin rich oil (Revenue)	127.00 $/kg
	Algae oil (Revenue)	77.00 $/kg
	Alginate extract (Main Revenue)	23.00 $/kg

J.	**Revenues/Savings**	
	Fucoxanthin rich oil (Revenue)	5,150,801 $/yr
	Algae oil (Revenue)	7,411,223 $/yr
	Alginate extract (Main Revenue)	72,328,196 $/yr
1	Total Revenues	84,890,220 $/yr
2	Total Savings	0 $/yr

K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	57,355,000 $/yr
2	Net AOC (K1-J2)	57,355,000 $/yr

L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	18.24 $/kg MP
	Net Unit Production Cost	18.24 $/kg MP
	Unit Production Revenue	26.99 $/kg MP

M.	Gross Profit (J-K)	27,535,000 $/yr
N.	Taxes (25%)	6,884,000 $/yr
O.	Net Profit (M-N + Depreciation)	33,255,000 $/yr

	Gross Margin	32.44 %
	Return On Investment	23.39 %
	Payback Time	4.27 years

MP = Total Flow of Stream 'Alginate extract'

Gradient elution program fucoxanthin analysis

Solvents:

A: 0.5M ammonium acetate in methanol:MQ water (85:15) -> 425mL Methanol + 75mL MilliQ water + 19.27gr. ammonium acetate, B: acetonitrile:MQ water (90:10) , C: 100% ethylacetate.

Initial 60% A, 40% B, 0% C

Time (min)	Concentration solvent (%)
5	A 60, B 40, C 0
10	A 0, B 100, C 0
40	A 0, B 30, C 70
45	A 0, B 30, C 70
46	A 0, B 0, C 100
47	A 0, B 100, C 0
48	A 60, B 40, C 0
53	A 60, B 40, C 0
53	Pump stop