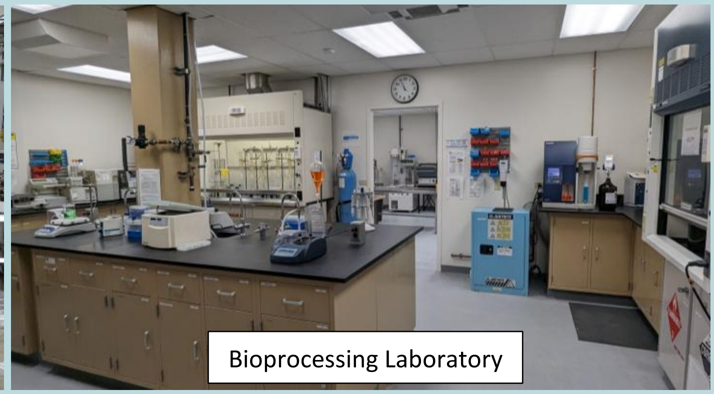

Investigation of nutritive properties of Atlantic seaweed species (*Laminaria longicruris*)

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Investigation of nutritive properties of Atlantic seaweed species (*Laminaria longicruris*)

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EXECUTIVE SUMMARY

Sugar kelp is an excellent source of macro and micronutrients, contains carbohydrates, proteins, lipids and minerals and is used as food worldwide (Dhakal et al., 2024). It also contains bioactive compounds such as phenolics, sulfated polysaccharides, polyunsaturated fatty acids, minerals and carotenoids, which have various health benefits, including anticancer, antimicrobial, antifungal, antiviral, and anti-inflammatory activities and are potential sources of new therapeutic agents (Jiao et al., 2012, Pérez et al., 2016). However, limited information is available on the nutritional composition and bioactive compounds of Newfoundland-grown sugar kelp.

HoldFastNL Seaweed Farm Inc., the first commercial licence holder for sugar kelp cultivation in Newfoundland, aims to understand the nutritional composition and bioactive compounds of cultivated sugar kelp compared to wild-grown sugar kelp. Additionally, the company is interested in the application of high-pressure processing (HPP) treatment to enhance the extraction process of marine phenolics and polysaccharides.

The Centre for Aquaculture and Seafood Development (CASD) research team conducted this study using materials supplied by HoldfastNL. The study included proximate composition, mineral and fatty acid profiling, and phenolic extraction for both the cultivated and wild sugar kelp.

Key findings indicate that cultivated sugar kelp from St. Mary's Bay and wild sugar kelp from Tors Cove differ in proximate composition, fatty acid profiles and macro and mineral content. Cultivated sugar kelp had significantly higher moisture, ash and lipid, while wild sugar kelp contained more carbohydrates. No significant differences were observed in protein content. Cultivated sugar kelp also exhibited higher levels of phospholipids, polar lipids, and free fatty acids as well as higher MUFA and PUFA, including omega-3 EPA and PUFA:SFA ratio compared to the wild sugar kelp. This difference is likely due to the maturity levels of the kelp at the time of harvest, as well as differences in environmental conditions across in growing areas. Cultivated sugar kelp was harvested at a younger stage, exhibiting higher nutrient uptake compared to the wild kelp. A more detailed investigation into environmental factors, nutrient availability, maturity, and physiological stages is necessary to better understand the observed difference between cultivated and wild sugar kelp.

Mineral analysis revealed that cultivated sugar kelp contained higher levels of potassium, calcium, iron, manganese, boron, aluminum and molybdenum, whereas wild sugar kelp had greater amounts of sodium, magnesium, zinc and copper. While both types of sugar kelp contained heavy metals, particularly arsenic, the concentrations remained within the safety limits established for seaweed products available on the market. Published reports suggest that the arsenic present in the sugar kelp is primarily organic, which is considered non-toxic.

The study also examined the effects of HPP treatment on sugar kelp composition. HPP treatment at 550 MPa pressure facilitated extracting protein content and reduced ash content in cultivated sugar kelp, whereas treatment at 450 MPa led to higher protein and ash levels in wild sugar kelp. Following HPP treatment, phenolics and polysaccharides were extracted using a cascading biorefinery approach. HPP enhanced phenolic extraction in both sugar kelp types, with cultivated sugar kelp exhibiting a higher total phenolic content. The water-soluble sulphated polysaccharide, fucoidan, yielded higher in the cultivated sugar kelp than in wild ones, while alginate yield was similar in both sugar kelp. HPP-treated cultivated sugar kelp showed a lower alginate yield than the untreated ones, while the alginate yield was at similar levels among the HPP treatments.

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ACRONYMS/ABBREVIATIONS

AOAC: Association of Official Analytical Chemists

AAS: Atomic Absorption Spectroscopy

CDOM: Chromophoric dissolved organic matter

DHA: Docosahexaenoic

dwb: Dry weight basis

EPA: Eicosapentaenoic acid

HPP: High-pressure processing

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

MPa: Megapascal

MUFA: Monounsaturated fatty acids

PUFA: Polyunsaturated fatty acids

PTEs: Potentially toxic elements

SFA: Saturated fatty acids

wwb: Wet weight basis

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1 BACKGROUND AND INTRODUCTION

The brown macroalga *Saccharina latissima* (formerly known as *Laminaria longicruris*), commonly referred to as sugar kelp or royal kombu, is a cold-water, perennial species that thrives on hard bottom substrates(Sharma et al., 2018). The morphology of the adult sugar kelp sporophyte is defined by three key structures: holdfast, stipe, and blade. Sugar kelp is an excellent source of macro and micronutrients, containing lipids (2-6%), proteins (3-11%), ash (18-35%), and carbohydrates (40-60%) (Dhakal et al., 2024). It also contains bioactive compounds such as phenolic, sulfated polysaccharides, polyunsaturated fatty acids, minerals and carotenoids, which provide various health benefits, including anticancer, antimicrobial, antifungal, antiviral, anti-inflammatory activities and are potential sources of new therapeutic agents (Jiao et al., 2012, Pérez et al., 2016). Most available research on sugar kelp composition focuses on populations from the North Atlantic coastal line in Europe and the Eastern Pacific Ocean, where their cultivation has significantly expanded (Sæther et al., 2024). However, understanding the nutritional composition of sugar kelp along Canada's Atlantic coast, including Newfoundland, is essential for its commercialization and potential use in various industries such as food, feed, functional food, cosmetics, pharmaceuticals, and nutraceuticals. The nutritional composition and bioactivity of these compounds are influenced by several factors, including environmental conditions (light, temperature, salinity), reproductive state, age and processing methods (Pérez et al., 2016, Blikra et al., 2024).

Sugar kelp is often processed by blanching in freshwater or seawater or by steaming to reduce iodine levels and remove non-essential elements, such as arsenic, cadmium, lead, and mercury. However, blanching can lead to the loss of bioactive compounds such as phenolics, minerals, vitamins, carotenoids and other water-soluble and heat-senstivie compounds (Blikra et al., 2024, Trigo et al., 2023, Lafeuille et al., 2023). As an alternative to conventional pretreatment methods, high-pressure processing (HPP) has been explored to extend the shelf life and provide better texture and color to seaweeds. HPP has been applied to sugar kelp cultivated in Sweden and to various green and brown seaweeds in Spain with the goal of retaining essential nutrients (del Olmo et al., 2020, Jönsson et al., 2023). However, research on the use of HPP to enhance the extraction of bioactive compounds in sugar kelp remains limited, particularly in Canada.

Newfoundland and Labrador (NL) have an extensive coastline that serves as a natural habitat for several commercially valuable seaweed species, including sugar kelp. Despite its potential, seaweed resources in the region have remained largely untapped for over 20 years. HoldFastNL Seaweed Farm Inc., the first research-based seaweed company in NL, recently obtained a commercial licence for sugar kelp cultivation along the province's Atlantic coastline. The company cultivated sugar kelp in St. Mary's Bay with the seedlings grown in the laboratory under controlled conditions, with parent stock originating from wild populations. However, the nutritional composition of this newly cultivated sugar kelp remains unknown.

To address this knowledge gap, HoldFastNL Seaweed Farm Inc. (the client) sought to compare the nutritional composition and bioactive compounds of cultivated sugar kelp with those of wild-grown sugar kelp. The client approached the Centre for Aquaculture and Seafood Development (CASD) at the Marine Institute to conduct this investigation. Additionally, the client expressed interest in exploring high-pressure processing (HPP) as a method to enhance the extraction process of phenolics and

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polysaccharides from sugar kelp. In response to these objectives, the CASD research team developed the following research scope and objectives to support the client's interest.

2 SCOPE AND PURPOSE

2.1 Scope of Project

The project's scope was to evaluate nutritional values and bioactive compounds of cultivated and wild sugar kelps grown in Atlantic seawater of Newfoundland and Labrador. Additionally, the use of HPP as a pretreatment in seaweed processing to improve the retention of bioactive compounds after drying was evaluated

2.2 Purpose of Project

The purpose of this project was to conduct a scientific review of the biochemical and nutritional properties of sugar kelp including laboratory assessment of HoldFastNL Seaweed Inc.'s sugar kelp (*S. latissima*) for nutritional composition and bioactive compounds, for the determination of potential market uses.

3 OBJECTIVES

A comprehensive scientific assessment of the Atlantic seaweed species *Laminaria longicruris* (sugar kelp), focusing on its nutritional composition and bioactive compounds was conducted.

The specific objectives included:

1. **Scientific Assessment of *L. longicruris***
   - Conduct an in-depth scientific literature review on the species, including its biochemical and nutritional characteristics.
2. **Comparative Composition Analysis of Farmed and Wild *L. longicruris***
   - Harvest cultivated and wild sugar kelp.
   - Develop a transportation and delivery plan in collaboration with the client.
   - Establish an appropriate pretreatment method before testing.
3. **Nutritional Composition Analysis**
   - Perform proximate analysis, including moisture, ash, protein, lipid, and carbohydrate content.
   - Conduct fatty acid and amino acid profiling using gas chromatography-mass spectrometry (GC-MS).
   - Analyze macronutrient and micronutrient content using inductively coupled plasma spectrometry (ICP-MS).
4. **Investigation of Pretreatment Techniques for Bioactive Compound Extraction**
   - Quantify individual polysaccharides (e.g., fucoidan, laminarin, alginate).
   - Evaluate pretreatment techniques, including high-pressure processing (HPP), based on scientific assessment.
   - Assess the economic feasibility of the proposed pretreatment methods.

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4 MATERIALS AND METHODS

The client delivered about 40 lb of fresh wild sugar kelp in a tote pan on June 20, 2024, and 80 lb of cultivated sugar kelp on June 26, 2024. Wild sugar kelps were harvested from Tors Cove NL, and cultivated sugar kelps were harvested from 111 km Southwest St. Mary's Bay. The size of an individual whole wild sugar kelp plant (holdfast, stipe and blade) ranged from 6-11 feet tall and cultivated ones ranged from 2-5 feet tall. The cultivated sugar kelp was lighter in color than the wild one. The seaweed was washed twice with tap water, rocks and other debris were removed, and the holdfast of the plants was removed and vacuum-packed in 1 kg size bags. 1-1.5 wild and 2-3 cultivated whole sugar kelps were packed in a bag and stored at -20 °C until laboratory analysis and HPP treatment were performed.

4.1 Pretreatment using high-pressure processing (HPP)

Frozen seaweed was thawed overnight in a chill room (4 °C) and then placed under cold water for about 2 hours until all ice pellets melted. The thawed sample packs were placed into HPP carrier cylinders, loaded into the HPP chamber and subjected to the pressure of 300, 450 and 550 MPa for 5 min using a pilot-scale NC Hiperbaric Wave 6000/55 HPP equipment (Hyperbaric, Burgos, Spain). All treatments were conducted at ambient temperature (16 °C) using water as a pressure-transmitting medium. Following HPP treatment, the samples were taken from the vessels, chopped into 100-200 cm^2 and transferred to a −18 °C freezer overnight. The samples were dried in a Harvest Right freeze dryer for 50 h.

4.2 Seaweed grinding

Thawed seaweed was minced for 1 min at high speed using a Ninja Blender (Shark Ninja Operating LLC, Ville St. Laurent, QC, Canada). Moisture content was determined following the standard method of the Association of Official Analytical Chemists AOAC 930.15 at 105 °C (AOAC 2012). Approximately 300 g of mince from each sample was dried overnight at 105 °C and then ground into a fine powder (~0.5 mm particle size) using a coffee grinder for the proximate analysis. HPP-treated and non-treated freeze-dried samples were first ground in a Ninja Blender for 30 seconds and then further ground with a coffee grinder. The dried powder was passed through a mesh (Mesh#45; 355 µm) sieve and stored in an air-tight container until laboratory analysis.

4.3 Laboratory analysis

1. Proximate analysis was performed on thawed, HPP-treated and non-treated freeze-dried sugar kelp following the AOAC standard methods (AOAC, 2012). Moisture and ash content were determined following AOAC 930.15 and AOAC 938.08, respectively. The Kjeldahl method (AOAC 954.01) was used for protein content analysis, and the Soxhlet extraction (AOAC 948.15) was used for total lipid content. Carbohydrate content was calculated by difference using the following formula (Rasyid, 2017):

Carbohydrate content (%) = 100 - (moisture % + ash % + protein % + lipid %)

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2. Total lipid class and fatty acid composition of lipids in freeze-dried cultivated and wild sugar kelps were analyzed at the Ocean Science Centre (OSC) of Memorial University of Newfoundland (St. John's, NL) following Parrish and Wells (2021).

3. Mineral and trace element analysis for the freeze-dried cultivated and wild sugar kelp was performed at the Research & Productivity Council (RPC), Fredericton, NB, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (RPC method: SOP IAS-M01). Mercury was analyzed by Cold Vapour Atomic Absorption Spectroscopy (AAS) (RPC: SOP IAS-M52 & SOP IAS-M53).

4. The phenolic content of HPP-treated and non-treated freeze-dried cultivated and wild sugar kelp was extracted following Wang et al., (2009) with some modifications. The total phenolic content in the extract was determined using the Folin-Ciocalteu reagent following Goyali et al., (2013). The detailed methods are available in Appendix A.

5. A three-stage extraction process was used to estimate phenolic compounds and polysaccharides (fucoidan and alginate) in HPP-treated sugar kelps following (Fawzy and Gomaa, 2021, Okolie et al., 2020, Ummat et al., 2024). The detailed methods are available in Appendix A. Laminarin was extracted following Lafeuille et al., (2023). The recovery (%) or the total yield of the bioactive compounds (phenolic extracts, fucoidans and alginates), was calculated following the formula below:

$$\text{Recovery (\%)} = \text{Phenolic extract (\%)} + \text{fucoidan \%} + \text{alginate (\%)}$$

4.4 Statistical analysis

Statistical analysis was performed for the proximate composition between cultivated and wild seaweeds. Proximate tests were carried out in triplicate, and the data were presented as mean ± standard deviation. The data were processed using a one-way analysis of variance (ANOVA), and mean separations were analyzed using Tukey's HSD test using R-Studio.

5 RESULTS AND DISCUSSIONS

5.1 Nutritive properties of Atlantic seaweed (*S. latissima*) (literature review)

5.1.1 Proximate composition of sugar kelp

The proximate composition of the sugar kelp from various coastal regions worldwide is summarized in **Table 1**. Fresh sugar kelp typically consists of approximately 90% water and is a rich source of macro- and micronutrients. Carbohydrates, proteins and ash are principal components of sugar kelp, even though their compositions vary with seasonal fluctuations and growing regions (Sæther et al., 2024, Pandey et al., 2022). In general, sugar kelp is the highest in carbohydrate content, followed by ash, protein and lipids. As a brown macroalga, sugar kelp contains lower concentrations of protein than red and green algae. It can contain up to 17% dwb crude protein and 2.6-5.5% lipids. Protein and mineral contents are generally highest during winter and spring, while carbohydrates and polyphenols tend to peak in summer or autumn (Dhakal et al., 2024, Schiener et al., 2015, Lafeuille et al., 2023).

Table 1. The nutritional composition (dwb) of fresh sugar kelp (*S. latissima*) (adopted from Dhakal et al., (2024)Sæther et al., (2024)

Major group	Item	Content	Reference
Proximate composition	Moisture (%)	77.5 - 89.8	(Bojorges, Fabra, López-Rubio, & Martínez-Abad, 2022; Lytou et al., 2021; Manns, Nielsen, Bruhn, Saake, & Meyer, 2017; Marinho, Holdt, & Angelidaki, 2015; Schiener, Black, Stanley, & Green, 2015)
	Ash (%)	2.7 - 41.1	
	Protein (%)	1.3 – 16.5	
	Crude fat (%)	2.6 - 5.5	
	Carbohydrates (%)	15.3 - 63.1	
Carbohydrates			
Monosaccharides	Mannitol (%)	2.5	(Lafeuille et al., 2023)
	Fucose (%)	4.7	
	Rhamnose (%)	0.1	
	Galactose (%)	0.5	
	Glucose (%)	12.3	
	Xylose (%)	0.4	
	Manose (%)	1.0	
Polysaccharides	Fucoidan (%)	1.8 – 8.8	(A. Bruhn et al., 2017; Lafeuille et al., 2023; Manns et al., 2017; Schiener et al., 2015; Sharma et al., 2018; Vilg et al., 2015)
	Laminarin (%)	3.60 - 39.0	
	Alginate (%)	12.53 - 36.0	
Amino acids	Total amino acids (%)	1.5 - 12.7	(Marinho et al., 2015; Tibbetts, Milley, & Lall, 2016; Trigo et al., 2023; Vilg et al., 2015)
	Non-essential amino acids (%)	0.25 – 2.71	(Konstantin, Anastasia, Nikolay, & Daria, 2023)
	Essential amino acids (%)	0.35 – 3.21	
Fatty acid composition	SFAs (%)	25.5 – 34.2	(Marinho et al., 2015, Konstantin et al., 2023)
	MUFAs (%)	14.1 –23.3	
	PUFAs (%)	31.3 –54.0	
	Omega-3 FA (%)	13.4 –28.8	
	Omega-6 FA (%)	8.9 –22.9	
	Omega-6 : Omega-3	0.56 – 1.2	
	DHA (%)	0.60 – 7.0	
	EPA (%)	4.7 – 13.5	

Saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), eicosapentaenoic (EPA), docosahexaenoic acid (DHA)

5.1.2 Carbohydrates/polysaccharides

Sugar kelp contains several commercially valuable polysaccharides, some of which are water-soluble (fucoidan and laminarin), while others are insoluble (alginate and cellulose). The reported polysaccharide composition of sugar kelp is summarized in **Table 1.** One of the most commercially relevant polysaccharides is alginate, which is primarily extracted from wild-harvest brown algae in Europe (Sæther et al., 2024). With growing global demand and the development of new alginate-based applications, cultivated sugar kelp can be an important future source of alginates. Alginate accounts for up to 36% of dwb in sugar kelp, which depends mainly on source materials and extraction methods. Fucoidans are sulfated fucose-rich polysaccharides predominantly found in the cell walls of sugar kelp. The bioactive properties of fucoidans attract increasing interest from the medico-pharmaceutical industries, which drives an increase in demand (Bruhn et al., 2017). Fucoidan in sugar kelp ranged from 1.8-8.8% dwb, with variations influenced by seasonal fluctuations and environmental factors in the region of harvest. Russian sugar kelp exhibited the highest fucoidan content (8.8% dwb), followed by samples from Denmark, Canada and England (Bruhn et al., 2017). Fucoidan levels were found to correlate with key environmental factors such as irradiance, nutrient availability, salinity and exposure, which can alter the fucoidan content by a factor of 2–2.6. However, the effects of individual environmental factors were not consistent. Laminarins can be co-extracted with fucoidans, which are separated from fucoidans based on molecular size through cross-flow filtration or charge-based ion exchange chromatography. Lafeuille et al., (2023) reported a laminarin concentration of 3.6-4.0% dwb in the sugar kelp cultivated in Paspébiac Bay (Baie-des-Chaleurs, QC, Canada) with no significant seasonal variation. A comparative study between *S. lattisima* and *Fucus evanescens* by Nguyen et al., (2020) identified several monosaccharides in sugar kelp, with glucose being more abundant in sugar kelp than in *F. evanescens*. However, sugar kelp contained lower levels of fucose, galactose and xylose while exhibiting similar levels of mannitol, rhamnose, and mannose.

5.1.3 Fatty acid and amino acid composition

Although the lipid content in sugar kelp is relatively low, the quality of its fatty acids is high, particularly in terms of the PUFA:SFA and omega-6:omega-3 ratios, with a notably high concentration of EPA. The proportion of PUFA in sugar kelp is consistently higher compared to other species (**Table 1**). According to Konstantin et al. (2023), the fatty acid content in sugar kelp varies depending on the harvest season and location. The average lipid content in sugar kelp was relatively low, ranging from 1.78% to 2.11% in May and June, respectively. The amino acid and fatty acid composition of sugar kelp reported previously are presented in **Table 1.** Konstantin et al. (2023) found that essential amino acids (EAA) accounted for the majority of the protein content in sugar kelp, ranging from 52-62%. The harvesting month significantly impacts the amino acid composition of macroalgae, with the percentage of EAA being lowest in the cold season (33%) and the highest in the spring and summer (52%) (Konstantin et al., 2023).

5.1.4 Minerals

Sugar kelp contains varying levels of macrominerals (calcium, magnesium, sodium, and potassium) and microminerals (iron, zinc, selenium, manganese, chromium, copper, molybdenum, boron, cobalt, aluminum, arsenic, and tin) (**Table 2**). The four major metal cations in the sugar kelp are found in the following order: potassium > sodium > calcium > magnesium (Pandey et al., 2022, Schiener et al., 2015). The cultivated sugar kelp in Paspébiac Bay, QB, Canada (Lafeuille et al., 2023) had lower sodium compared to that reported by Trigo et al., (2023) for sugar kelp cultivated on the Westcoast in Sweden. Mineral concentrations in sugar kelp vary depending on growing location and nutrient availability (Konstantin et al., 2023).

Table 2. Elemental composition (mg/kg dwb) of fresh sugar kelp (*S. latissimal*) and total phenolic content thereof (Dhakal et al., 2024)

Item	Content	Reference
Minerals		
Potassium	40000 - 70000	(A. Bruhn et al., 2017; Lafeuille et al., 2023; Nguyen et al., 2020; Schiener et al., 2015; Sharma et al., 2018; Trigo et al., 2023)
Sodium	20000 - 33000	
Calcium	8000 - 17000	
Magnesium	5000 - 7000	
Iron	16.0 - 1280.0	
Aluminum	11.0 – 1877.0	
Arsenic	5.49 – 92.5	
Cadmium	0.02 – 1.77	
Mercury	0.02 – 0.1	
Lead	0.2 – 2.2	
Hallogens		
Iodine	1700 - 7977	
Bromine	400 - 1500	
Chlorine	67700 - 178000	
Total phenolic content		
TPC (mg PGE/g)	4.3 - 16.5	(Pandey et al., 2022; Tibbetts et al., 2016; Vilg et al., 2015)
TPC (mg GAE/g)	0.7 – 7.0	(Schiener et al., 2015; Sharma et al., 2018)

TPC: total polyphenol content; PGE: Phloroglucinol equivalent; GAE: gallic acid equivalent

5.1.5 Pigments and phenolic compound

Pigments and phenolic compounds are one of the major constituents of sugar kelp. Compared to *L. digitata*, sugar kelp generally has higher levels of phenolic compounds, with variation observed across seasons. The total phenolic content in sugar kelp ranges from 0.7-7.0 mg GAE/g as reported by Schiener et al., (2015) and Sharma et al., (2018) (**Table 2)**. These variations are attributed to seasonal fluctuations.

5.2 The nutritional composition of sugar kelp (Present study)

5.2.1 Thawed sugar kelp

The proximate composition of both thawed cultivated and wild sugar kelp was analyzed and recorded on a wet weight basis (wwb) and a dry weight basis (dwb). For simplicity, only the dwb composition is presented in **Table 3**. Thawed sugar kelp contained over 90% of water. The moisture, ash and lipid content

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in the cultivated sugar kelp was significantly higher compared to wild sugar kelp. Carbohydrates were higher in wild sugar kelp than cultivated ones, while there was no significant difference in protein content between the two types of seaweeds studied. These findings differ from previous studies (Krook et al., 2024; Trigo et al., 2023). Krook et al., (2024) reported that cultivated sugar kelp had higher solids (low moisture), including carbohydrate content and less ash and protein compared to wild ones. Ash content (29.9%) of cultivated sugar kelp in the Swedish west coast was higher compared to the current study (Trigo et al., 2023). The difference in the proximate composition of the sugar kelp observed in this study compared to published data may be attributed to the variation in maturity stage, nutrients in the water and environmental factors such as sunlight, temperature, and salinity. Konstantin et al., (2023) reported that the proximate composition varied due to seasonal variation, harvest location and nutrient availability.

Table 3. Proximate composition (dwb) of thawed cultivated and wild sugar kelps (n=3).

Proximate composition (%)	Cultivated	Wild
Moisture	93.6±0.4a	90.2±1.9b
Ash	24.8±0.9a	18.5±4.5b
Protein	6.9±0.4a	7.1±1.3a
Lipid	0.7±0.1a	0.4±0.0b
Carbohydrate	67.6±0.6b	74.0±5.8a

Different letters in the same row showed significant differences ($p < 0.05$) between cultivated and wild sugar kelp.

5.2.2 Pretreated freeze-dried sugar kelps

The moisture content in freeze-dried sugar kelps ranged from 2.8%-9.5%. Samples subjected to HPP treatment at 300 MPa and 450 MPa pressures had higher moisture content than untreated and 550 MPa HPP-treated samples (Error! Reference source not found.). This increase in moisture may be attributed to the drying conditions—specifically, the 300 MPa and 450 MPa samples were dried simultaneously and remained in the freeze dryer in standby mode for an additional 72 hours, potentially absorbing moisture from the freeze dryer chamber, resulting in higher moisture. All the freeze-dried samples, including two with high initial moisture content, were completely dried at 105 °C overnight for moisture determination. The fully dried samples were used for proximate composition (ash, protein, lipid and carbohydrate) analyses. Therefore, the proximate composition on a dry weight basis (dwb) was not influenced by the initial moisture content of the samples..

he ash content in HPP-treated cultivated sugar kelp varied from 24.3% at 550 MPa to 28.5% in untreated samples, while in the wild sugar kelp, it ranged from 17.9% at 550 MPa to 19.9% at 450 MPa pressure (Figure 2). The ash content observed in this study was significantly lower than previously reported for cultivated sugar kelp in Sweden by Jönsson et al. (2023), where values ranged from 41.9%-44.9% dwb with the higher levels found in the HPP-treated (400 MPa) samples. Overall, freeze-dried cultivated sugar kelp exhibited higher ash content than the wild counterparts, regardless of HPP- treatment. This trend aligns with observations in fresh or thawed seaweed, where cultivated sugar kelp also showed higher ash content (**Table 3**). The difference in the ash content (non-combustible inorganic minerals and substances) between cultivated and wild sugar kelp may be attributed to variations in maturity stages and influenced

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by environmental factors such as temperature, chlorophyll level and chromophoric dissolved organic matter (CDOM) in seawater at the harvested sites (Lafeuille et al., 2023)

Proximate analysis showed that HPP-treated sugar kelps appeared to have a slightly higher protein content than untreated samples (**Figure 2**). The cultivated seaweed samples treated with 550 MPa pressure contained the highest level of protein (7.2% dwb) followed by untreated (6.5%), HPP-treated at 300 MPa (6.5%) and 450 MPa pressure (6.2%). Meanwhile, the HPP treatment at 450 MPa pressure resulted in higher protein (6.9% dwb) in the wild sugar kelp. The lipid content in the cultivated sugar kelp did not change during the HPP treatments, while 450 MPa and 550 MPa reduced the lipid levels in the wild sugar kelps. Carbohydrates are the primary component of the sugar kelps, representing 64%-68% dwb in cultivated sugar kelps and 72%-75% dwb in wild ones. The HPP treatment at 550 MPa enhanced carbohydrate extraction by disrupting the cell wall matrix, including the microfibril network leading to a high carbohydrate content in both cultivated (67.8%) and wild (75.6%) sugar kelps (**Error! Reference source not found.**). The proximate composition of cultivated sugar kelp obtained in this study (ash 24%–29% dwb, protein 6.2%–7.2% dwb and carbohydrates 64%–68% dwb) was compared with analysis performed on the same species in the Swedish National Park, Kosterhavet in 2021 (Jönsson et al., 2020, Jönsson et al., 2023). Ash and protein content in the sugar kelp at Newfoundland were lower than those in sugar kelp from Sweden, while carbohydrates were much higher. However, the nutritional values obtained in this study fall within the general range for sugar kelp (ash 2.7%–41% dwb, carbohydrates 15%-63% dwb, and protein 1.3%–11.2% dwb) as reported by Dhakal et al. (2024). To the best of our knowledge, only one prior study has analyzed the nutritional composition of sugar kelp after HPP treatment (Jönsson et al., 2020). They observed a minor protein and carbohydrate content variation in HPP-treated sugar kelp at pressures of 200, 400 and 600 MPa. The slightly higher protein content in HPP-treated samples, compared to untreated ones, was likely due to natural variances in biomass (Jönsson et al., 2020).

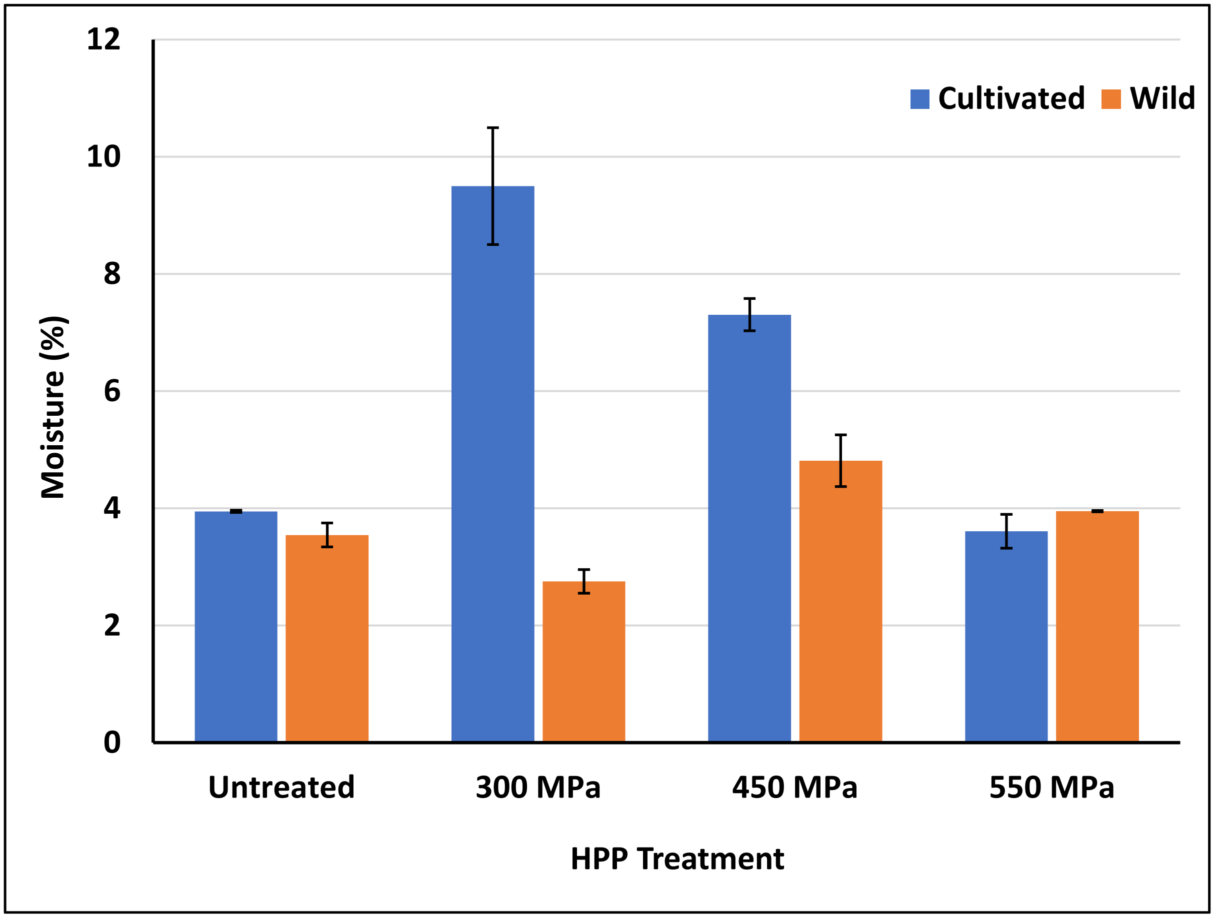

Figure 1. Moisture content in HPP-treated freeze-dried sugar kelps (n=3)

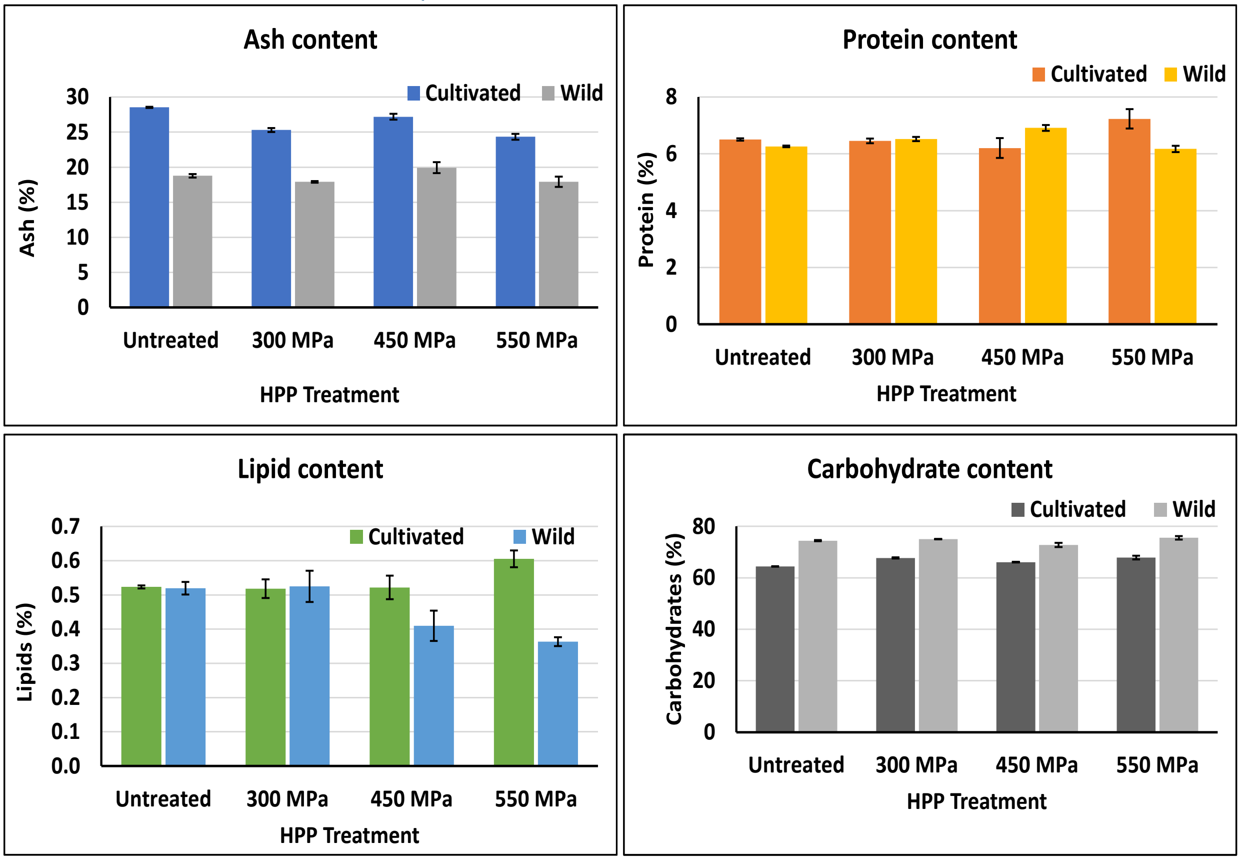

Figure 2. Proximate composition (dwb) of HPP-treated freeze-dried sugar kelps (n=3)

5.2.3 Lipid class

The lipid class determined using thin-layer chromatography is presented in **Table 4**. Total lipids measured in this technique were lower in both cultivated and wild sugar kelps compared to those estimated by the Soxhlet method. The total lipids in cultivated sugar kelp (26.3 ± 2.8 mg/g dwb) were higher than in wild samples (21.6 ± 3.7 mg/g dwb). Phospholipids were the dominant lipid class in cultivated and wild sugar kelps, at 64.1% and 72.5% of total lipids, respectively. This suggests that both groups prioritize phospholipids for membrane formation, which is critical for cell integrity and function. However, cultivated samples had a higher absolute amount of phospholipids (17.0 ± 3.3 mg/g dwb) than wild samples (15.8 ± 3.9 mg/g dwb), possibly due to higher metabolic activity in the early growth stages of cultivated sugar kelp. Polar lipids were found at higher levels in cultivated seaweed (16.3 ± 4.1%) compared to wild samples (8.9 ± 1.2%). Triacylglycerols, which are typically reserved for energy storage, were present in higher concentrations in wild samples (13.1 ± 2.6%) than in cultivated samples (10.0 ± 0.9%). This may reflect the energy storage needs of wild sugar kelp, which is typically more mature with thicker blades, facilitating fatty acid storage. Free fatty acid levels were higher in cultivated sugar kelp (4.8 ± 0.4%) compared to wild ones (2.3 ± 0.3%). This could be due to higher metabolic activity in cultivated seaweed, which may be actively synthesizing fatty acids during rapid growth. As the cultivated sugar kelp was young and growing quickly, it likely synthesized more fatty acids.

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Phospholipids and sterols act as natural antioxidants and have preventive impact against coronary heart disease, inflammation or cancer (Küllenberg et al., 2012). The high phospholipid content of sugar kelp suggests that it may offer valuable nutrients and bioactive molecules with potential applications in the nutraceutical, pharmaceutical and cosmeceutical industries (da Costa et al., 2021).

Table 4. Lipid classes in cultivated and wild sugar kelps*

Lipid class	mg/g dwb		Relative percentage (%)	
	Cultivated	Wild	Cultivated	Wild
Hydrocarbons	0.01±0.0	0.0±0.0	0.1±0.0	0.0±0.0
Steryl esters	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Ethyl esters	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Ethyl ketones	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Methyl ketones	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Glyceryl ethers	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Triacylglycerols	2.6±0.0	2.8±0.1	10.0±0.9	13.1±2.6
Free fatty acids	1.2±0.0	0.5±0.0	4.8±0.4	2.3±0.3
Alcohols	0.1±0.2	0.0±0.0	0.5±0.5	0.2±0.3
Sterols	2.2±0.0	1.5±0.0	8.4±0.8	6.9±1.3
Diacylglycerols	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Polar lipids	4.2±0.6	1.9±0.1	16.3±4.1	8.9±1.2
Phospholipids	17.0±3.3	15.8±3.9	64.1±5.7	72.5±5.6
Total lipids	**26.3±2.8**	**21.6±3.7**		

**Lipid class analysis was conducted by the Department of Ocean Science, MUN and is reported on a dwb*

5.2.4 Fatty acid composition

The fatty acid composition is a crucial indicator of seaweed suitability for food. The fatty acid composition of cultivated and wild sugar kelps is presented as mg/g dwb and relative percentage (%) to the total fatty acids in **Table 5**. Total fatty acids were higher in cultivated (17.5±2.2 mg/g dwb) sugar kelp compared to the wild counterparts (15.2±2.8 mg/g dwb). Polyunsaturated fatty acids (PUFAs) accounted for the highest fraction among the major fatty acid classes, followed by saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) in both cultivated and wild sugar kelps. SFAs, MUFAs, and PUFAs (mg/g dwb), including Omega-3 and Omega-6, were slightly higher in cultivated sugar kelp than in wild samples. In terms of the relative percentage of specific fatty acids, both cultivated and wild samples had similar proportions of SFAs, MUFAs, and PUFAs, with PUFAs making up the highest percentage in both groups.

PUFA:SFA ratio is a key indicator when evaluating the lipid quality of foods. The PUFA:SFA ratios of cultivated and wild sugar kelp were 2.2 and 2.1, respectively (**Table 5**). In general, a PUFA:SFA ratio above 0.45 is recommended in human diets to prevent the development of cardiovascular diseases and some chronic conditions such as cancer (Health, 1994, Wołoszyn et al., 2020). Foods with PUFA:SFA ratios below 0.45 have been considered undesirable for the human diet because of their potential to increase cholesterol levels in the blood (Wołoszyn et al., 2020). Both cultivated and wild sugar kelp had PUFA:SFA ratios higher than the recommended minimum value of 0.45, with cultivated sugar kelp showing a slightly higher ratio than the wild samples. This suggests that the lipids in the sugar kelp are of high quality.

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The concentration of omega-3 and omega-6 fatty acids was higher in cultivated sugar kelp, possibly due to differences in nutrient availability at the cultivation site. The growing conditions for the cultivated and wild sugar kelp may vary due to environmental factors such as water temperature, salinity, irradiance and inorganic nitrogen and phosphorus levels (Marinho et al., 2015). Cultivated sugar kelp was harvested from a St. Mary's Bay farm, while wild sugar kelp was collected from Tors Cove, NL. The two sites are approximately 110 km apart.

According to the World Health Organization (WHO) recommendations, the dietary Omega-6:Omega-3 ratio should not exceed 10. The Omega-6:Omega-3 ratio in cultivated (0.9) and wild sugar (1.0) kelp was well below the recommended maximum. These low ratios suggest that consuming sugar kelp may help achieve a more balanced dietary Omega-6:Omega-3 intake, which benefits human health (Marinho et al., 2015, Schmid et al., 2014). In many Western diets, the Omega-6:Omega-3 ratio is around 15 (Schmid et al., 2014), which has been linked to the development of various diseases, including cardiovascular disease, inflammatory, cancer, and autoimmune diseases. In contrast, a lower Omega-6:Omega-3 ratio, with increased levels of omega-3 PUFAs, has been shown to have protective effects against these conditions (Simopoulos, 2002). So, including sugar kelp grown in NL in the diet could significantly improve the Omega-6:Omega-3 ratio and potentially reduce the risk of chronic diseases (Simopoulos, 2002). Additionally, sugar kelp contains other beneficial compounds, such as minerals, polyphenols and sulfated polysaccharides, which offer further health benefits.

Table 5. Summary of the fatty acid composition in the freeze-dried cultivated and wild sugar kelp*

Fatty acid types	mg/g dwb		Relative percentage (%)	
	Cultivated	Wild	Cultivated	Wild
SFAs	4.8±0.7	4.2±0.5	28.4±0.4	28.9±1.9
MUFAs	2.6±0.4	2.3±0.4	15.3±0.5	15.5±0.2
PUFAs	9.9±1.1	8.6±1.9	59.0±0.9	58.3±2.3
PUFA: SFA ratio	2.2±0.1	2.1±0.2	2.2±0.1	2.1±0.2
Omega-3 FA	5.2±0.4	4.3±1.0	30.9±1.6	29.6±1.2
Omega-6 FA	4.6±0.7	4.1±0.9	27.6±0.7	28.2±1.2
Omega-6 : Omega-3	0.9±0.1	1.0±0.0	0.9±0.1	1.0±0.0
DHA	0.02±0.0	0.0±0.0	0.1±0.0	0.0±0.0
EPA	2.2±0.1	1.8±0.4	13.3±0.9	12.1±0.5
Total FA	**17.5±2.2**	**15.2±2.8**		

SFAs: Saturated fatty acids; MUFAs: Monounsaturated fatty acids; PUFAs: Polyunsaturated fatty acids; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; **Fatty acid compositional analysis was conducted by the Department of Ocean Science, MUN and is reported on a dwb.*

EPA content was higher in cultivated sugar kelps, reflecting the higher overall omega-3 content in these seaweeds. Overall, sugar kelp is a good source of beneficial fatty acids, with cultivated samples appearing to have a slightly superior omega-3 profile, particularly eicosapentaenoic acid (EPA), and a more favourable lipid profile in terms of PUFA:SFA ratio. This suggests that cultivated sugar kelp may offer a more advantageous fatty acid composition compared to wild kelp. However, this could be due to the difference in the maturity levels and nutrient availability at the growing sites. So, a detailed study is needed on the growing environments. DHA was either absent or present in tiny amounts in both cultivated and wild sugar kelps, consistent with the findings of Marinho et al., (2015), who reported that DHA is either undetectable or found in very low proportions in seaweed.

Overall, the sugar kelp in this study has a fatty acid composition rich in PUFA, especially EPA, its precursor arachidonic acid (ARA) and stearidonic acid (SDA) (Appendix B), which are not found in common vegetables such as cabbage and lettuce (Marinho et al., 2015). It also exhibits nutritionally balanced or even superior PUFA:SFA and Omega-6:Omega-3 ratios. Based on these findings, sugar kelp can be recommended as a valuable seafood option. However, to meet the recommended daily intake of 250 mg of EPA & DHA (Lagiou et al., 2009), 112 g dwb cultivated and 138 g wild sugar kelp would be required. A previous study by Marinho et al., (2015) estimated that 166–244 g dwb of sugar kelp would be needed to meet the daily intake recommendation. Given these figures, relying solely on sugar kelp to meet omega-3 PUFA needs is not practical. However, it remains an excellent source of minerals.

5.2.5 Macro and trace elements

Macrominerals or elements, such as calcium, magnesium, sodium, and potassium, are typically required in amounts greater than 100 mg/day (Farag et al., 2023). In contrast, micro or trace minerals, including iron, zinc, selenium, manganese, chromium, copper, molybdenum, boron, cobalt, aluminum, arsenic, and tin, are needed in smaller amounts, typically less than 100 mg/day. The macro and microelement concentrations in the cultivated and wild sugar kelps are presented in **Table 6**. The four primary macroelements in the sugar kelps followed this order: potassium > sodium > calcium > magnesium, which aligns with findings from Lafeuille et al., (2023) cultivated sugar kelp in Paspébiac Bay (QB, Canada) and Schiener et al., (2015) for wild sugar kelp. Cultivated sugar kelp had higher potassium, calcium, iron, manganese, boron, aluminum and molybdenum content than the wild ones, while wild sugar kelp had high sodium, magnesium, zinc and copper compared to cultivated ones. Krook et al., (2024) reported higher potassium, less sodium, magnesium, and calcium in wild sugar kelp compared to cultivated ones. In the current study, cultivated sugar kelp exhibited lower levels of potassium, sodium and iron but higher levels of calcium and magnesium compared to previous studies of sugar kelp cultivated in Canada and on the Westcoast of Sweden (Trigo et al., 2023, Lafeuille et al., 2023). The difference in the mineral content between cultivated and wild sugar kelp may be influenced by environmental factors such as temperature, chlorophyll level and chromophoric dissolved organic matter (CDOM) in seawater. Lafeuille et al., (2023) reported that the iodine, potassium and iron levels in cultivated sugar kelps varied between May and June, likely due to the changes in temperature, chlorophyll and CDOM levels. The two collection sites in this study were 111 km apart. A more detailed investigation into environmental factors, nutrient availability, maturity, and physiological stages is needed to better understand the observed difference between cultivated and wild sugar kelp.

The United States and Canada jointly developed the Dietary Reference Intakes (DRIs) for the macrominerals for the healthy population (Health Canada, 2023), with the values for males and females listed in **Table 7.** The macrominerals in both cultivated and wild sugar kelps were found to be much higher than the DRIs. As such, dried sugar kelp could serve as a potential source of macroelements in the diet. Given its high potassium content, sugar kelp could be a valuable addition to human diets as a potassium source.

Table 6. Macro and micro/trace minerals in freeze-dried cultivated and wild seaweed (n=2)*

Minerals (mg/kg)	Cultivated	Wild
Macrominerals		
Potassium	100450.0±5253.9	52200.0±2655.8
Sodium	20200.0±461.9	23800.0±692.8
Calcium	11500.0±115.5	10900.0±346.4
Magnesium	7910.0±115.5	8680.0±207.8
Microminerals		
Strontium	906.5±15.6	674.5±16.7
Boron	226.5±4.0	168.0±5.8
Iron	106.0±2.3	48.0±2.3
Rubidium	30.1±0.2	18.4±0.5
Aluminum	24.5±0.2	9.4±0.3
Zinc	12.2±0.1	14.2±0.3
Barium	7.3±0.1	6.4±0.2
Manganese	7.1±0.1	2.6±0.1
Copper	3.9±0.2	6.5±0.1
Vanadium	1.2±0.0	0.5±0.0
Nickel	0.5±0.1	< 0.2
Chromium	0.3±0.0	0.3±0.1
Lithium	0.3±0.0	0.4±0.0
Molybdenum	0.2±0.0	0.1±0.0
Uranium	0.1±0.0	0.2±0.0
Cobalt	0.1±0.0	0.0±0.0
Selenium	< 0.2	< 0.2
Bismuth	< 0.2	< 0.2
Antimony	< 0.02	< 0.02
Thallium	< 0.02	< 0.02
Tellurium	< 0.02	< 0.02
Silver	< 0.02	< 0.02
Beryllium	< 0.02	< 0.02

Mean ±SD (n=2); Values recorded as < 0.02 are below the detection limit of the equipment

**Analyses conducted by the Research and Productivity Council (Fredericton, NB).*

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Table 7: Dietary reference intake (DRI) values of macro elements for males and females aged 19-50 (Canada, 2023; National Academies of Sciences, 2019)

Macro elements	Male (mg/day)	Female (mg/day)
Potassium	3400	2600
Sodium	1500 (AI[1])	1500 (AI)
Calcium	1000-1200 (UL=2500)	1000 (UL=2500)
Magnesium	400-420 (UL[2]=350)	310-320 (UL=350)

[1] AI=Adequate intake; [2] UL = Upper limit

5.2.6 Heavy metals or potentially toxic elements (PTEs)

The levels of heavy metals or potentially toxic elements (PTEs): arsenic, cadmium, lead, mercury and tin in the freeze-dried cultivated and wild sugar kelps are presented in **Table 8**. These elements are treated as toxic when they exceed the recommended limit for human or other animal consumption. The cultivated sugar kelp contained higher arsenic, cadmium and lead than the wild counterparts. However, the values of these PTEs in either cultivated or wild sugar kelp were in the comparable range of reported values for the same species (*S. latissima)* Bruhn et al., 2019, Krook et al., 2024, Afonso et al., 2021, Dhakal et al., 2024). Sugar kelps in this study had a similar range of total arsenic, higher cadmium, lead and less mercury compared to sugar kelp cultivated in the Westcoast in Sweden (Trigo et al., 2023). This difference might be due to the differences in the growing environment, like nutrient availability, temperature, and tidal current (Lafeuille et al., 2023).

A targeted mineral survey of 989 seaweed products and other ready-to-eat foods was conducted by the Canadian Food Inspection Agency (CFIA) in 2011-2020 to establish baseline surveillance data on the level of metals in these products (CFIA, 2024). The survey revealed that cadmium was present in most of the products, with seaweed products showing the highest cadmium levels. The average cadmium level in seaweed products was 1.48 mg/kg, ranging from the limit of detection (LOD) to 6.4 mg/kg. The arsenic, lead and mercury levels were 28-120, 0.1-11 and 0.1-.06 mg/kg, respectively. The range of these PTEs varied significantly (**Table 8**). Despite these variations, Health Canada concluded that none of the food or seaweed products tested posed a health risk to humans. Furthermore, most of the arsenic found in sea plants is in its organic form, which is considered virtually non-toxic (NRC, 2005). Trigo et al., (2023) reported 0.11 mg/kg dwb inorganic arsenic in unprocessed sugar kelp, while the total arsenic level was 60 mg/kg dwb. So, sugar kelps are safe for human consumption even without any treatments. However, a high iodine level in the sugar kelp (1700 – 10000 mg/kg dwb) may limit its use as a food source (Krook et al., 2024, Lafeuille et al., 2023).

Table 8. Heavy metals or potentially toxic elements (PTEs) in freeze-dried cultivated and wild sugar kelps* and previously reported values in *S. latissima* and other seaweed products

Elements	Sugar kelps (mg/kg dwb)		Literature values[1] (mg/kg dwb)	Seaweed products[2]
	Cultivated	Wild		
Total arsenic	58.6±0.6	45.5±0.9	28-120	28-120
Inorganic arsenic			0.06-0.11	<LOD-181.5
Cadmium	1.1±0.0	0.8±0.0	0.2-4.6	<LOD-6.4
Lead	0.4±0.0	0.1±0.0	0.1-11	<LOD-2.2
Mercury	< 0.2	< 0.2	0.01-0.06	<LOD-0.09
Tin	< 0.2	< 0.2		

Mean ±SD (n=2); < 0.02: below determination limit;
**Analyses conducted by the Research and Productivity Council (Fredericton, NB);* LOD = limit of detection; [1]Literature values are from (Afonso et al., 2021; Annette Bruhn et al., 2019; Dhakal et al., 2024; Krook et al., 2024; Lafeuille et al., 2023; Trigo et al., 2023); [2](CFIA, 2024).

5.3 Effect of pre-treatment on bioactive compound extraction

Sugar kelp was treated with HPP at 300, 450 and 550 MPa pressures and phenolic and polysaccharides were extracted using a cascading biorefinery approach.

5.3.1 Phenolic extract yield

A stepwise extraction process was employed to extract phenolics, pigments, and bioactive polysaccharides (fucoidan, laminarin, alginate, and cellulose) from cultivated and wild-harvested seaweed, aiming to minimize waste generation and maximize seaweed utilization by incorporating the principles of a cascading biorefinery. The crude phenolic extract content was higher under HPP treatment in cultivated sugar kelp, particularly at 300 MPa and 450 MPa pressures, with contents of 38.6 ± 3.0% and 39.2 ± 1.0%, respectively (**Table 9**). This indicates that HPP can enhance the extraction of phenolic compounds, likely due to the disruption of cellular structures, including microfibrillar networks embedded in matrices of various polysaccharides and proteins (Shao & Duan, 2022), which facilitates the release of more metabolites. At 550 MPa pressure, phenolic content decreased to 34.3 ± 2.3%, suggesting that extremely high pressures may lead to certain phenolic compounds' degradation or reduced solubility. Polyphenols are a diverse group of bioactive components, many exhibiting health-promoting effects. Isolated marine polyphenols have shown diverse bioactivity, including anti-tumor, anti-cholesterol, antioxidant, antimicrobial, and anti-inflammatory activities, making them attractive for use in nutraceuticals and pharmaceuticals (Zheng et al., 2022, Pereira and Cotas, 2023). The extract yield (34-39%) of freeze-dried sugar kelp could provide a valuable source of beneficial phenolics if incorporated into diets, with HPP treatment serving to increase the yield of phenolics.

Table 9. Yields (dwb) of crude phenolic and polysaccharide extract from wild (untreated) freeze-dried and HPP-treated freeze-dried cultivated sugar kelps (n=3)

Seaweed types	HPP Treatment	Phenolic extract (%)	Fucoidan (%)	Alginate (%)	Recovery (%)	Residues (%)	Laminarin (%)
Wild	Untreated	31.4±4.5	4.2±1.1	47.1±4.5	79.8±9.6	9.5±1.5	-
Cultivated	Untreated	30.3±4.8	4.8±1.4	47.7±3.5	79.6±7.7	16.0±2.1	4.1±0.2
	300 MPa	38.6±3.0	3.7±0.6	33.9±0.8	68.9±3.8	14.8±0.5	3.7±0.4
	450 MPa	39.2±1.0	4.6±0.9	32.9±1.5	71.1±1.0	13.3±0.1	3.9±0.6
	550 MPa	34.3±2.3	5.3±0.8	36.1±1.9	72.9±1.5	14.9±0.8	3.5±0.9

5.3.2 The phenolic content

The effect of HPP treatment on the phenolic content of the cultivated and wild sugar kelps is presented in **Figure 3.** The polyphenol level in the cultivated sugar kelp (4.6 ± 0.2 mgGAE/g dwb) was higher than in the wild (3.0 ± 0.0 mgGAE/g dwb), whether treated with or without HPP. The total phenolic content of untreated cultivated sugar kelp is aligned with the previous report by Pandey et al., (2022) and Schiener et al. (2015) but higher than the values reported by Lafeuille et al., (2023)in sugar kelp cultivated in the marine farm at Paspébiac Bay (QB, Canada) and less than Blikra et al., (2024) studies.

HPP treatment increased the phenolic content in both cultivated and wild sugar kelp. In the cultivated seaweed, a higher phenolic content was observed under HPP treatment with 450 MPa pressure than 300 MPa, which was similar to 550 MPa. Higher phenolics were observed in wild sugar kelp when treated with 550 MPa pressure compared to 300 MPa and 450 MPa.

The wild sugar kelp in this study was older, with thicker and darker blades than the cultivated sugar kelp. Sugar kelp typically reaches maturity between 2 and 4 years of age, with blades growing up to 16 feet long and 7 inches wide. The height of the wild sugar kelp provided by the client ranged from 6-11 feet, whereas the cultivated kelp was 2-4 feet tall. Cultivated sugar kelp grew in seawater for approximately 8 months (September to June), and its blades were softer and lighter in color. The thicker, more mature blades of the wild sugar kelp may require higher pressure to break the cell walls, which could explain the lower phenolic content in wild sugar kelp compared to cultivated ones. Additionally, extraction at higher temperatures can degrade polyphenols, as they are temperature-sensitive (Blikra et al., 2024). This issue can be mitigated by using HPP, as the high pressure breaks the cell walls, releasing bioactive compounds without the need for heat.

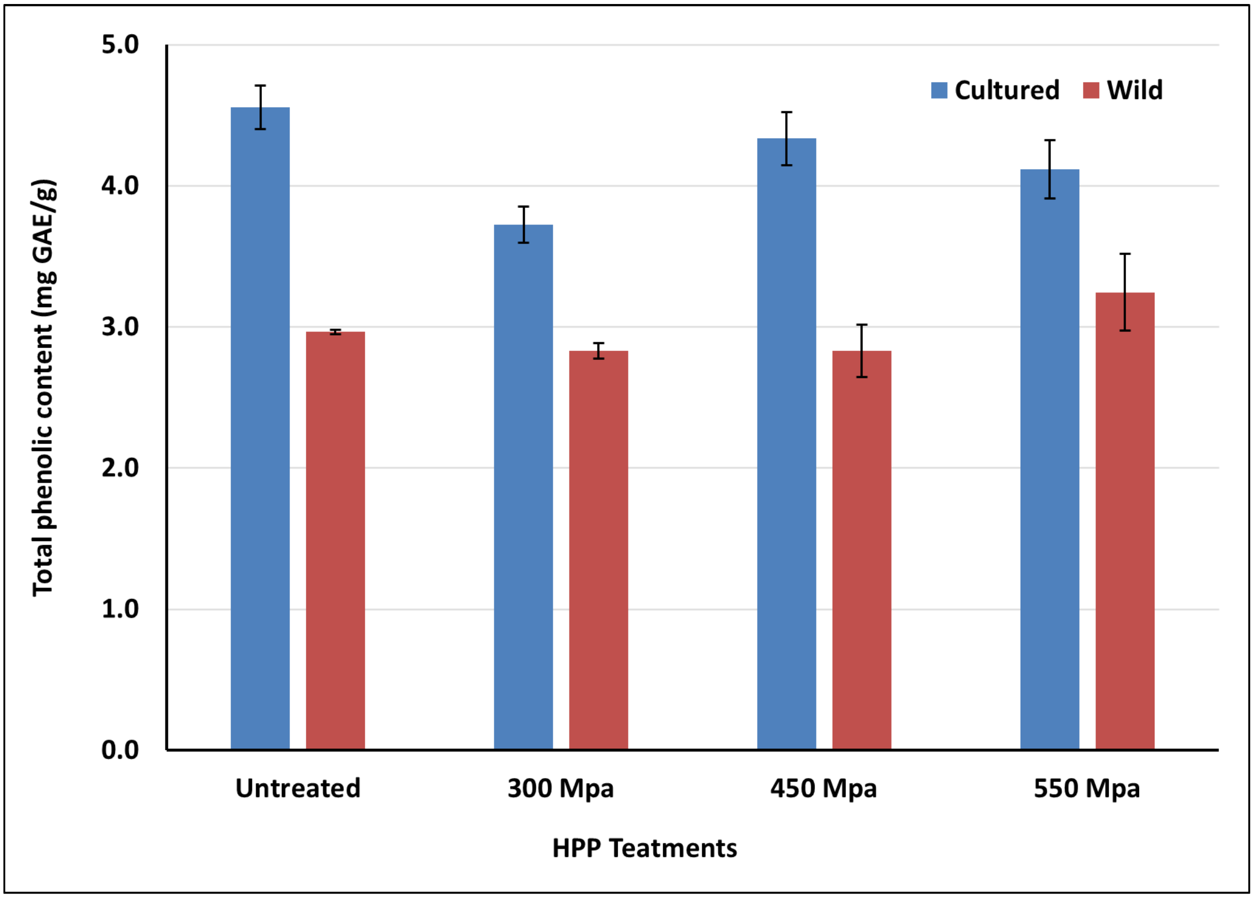

Figure 3. Total phenolic content (g GAE/g dwb) of HPP-treated and non-treated freeze-dried sugar kelps

5.3.3 Polysaccharides

Brown macroalgae have a complex and dynamic cell wall rich in polysaccharides like alginate, fucoidan, laminarin and cellulose (Jönsson et al., 2020). Wild seaweed contained 4.2±1.1% fucoidan and 47.1±4.5% alginate, and 9.5±1.5% residues, mainly cellulose. In comparison, untreated cultivated sugar kelp exhibited slightly higher fucoidan (4.8±1.4%) and similar alginate content (47.7±3.5%). However, cultivated seaweed had a higher residue (16.0±2.1%). Visual observation revealed slight differences in intensity of the color of fucoidan and alginate (**Figure 4**). The color fucoidan and alginate in cultivated sugar kelp appeared slightly darker than in their wild counterparts. This could be attributed to higher levels of phenolics particularly, phlorotannins or carotenoids such as fucoxanthin and neofucoxanthin in the cultivated sugar keep. However, structural color patterns observed in brown algae are complex and research on this topic is still in its early stages (Monteiro Vasconcelos, Vollet Marson, Turgeon, Tamigneaux, & Beaulieu, 2024).

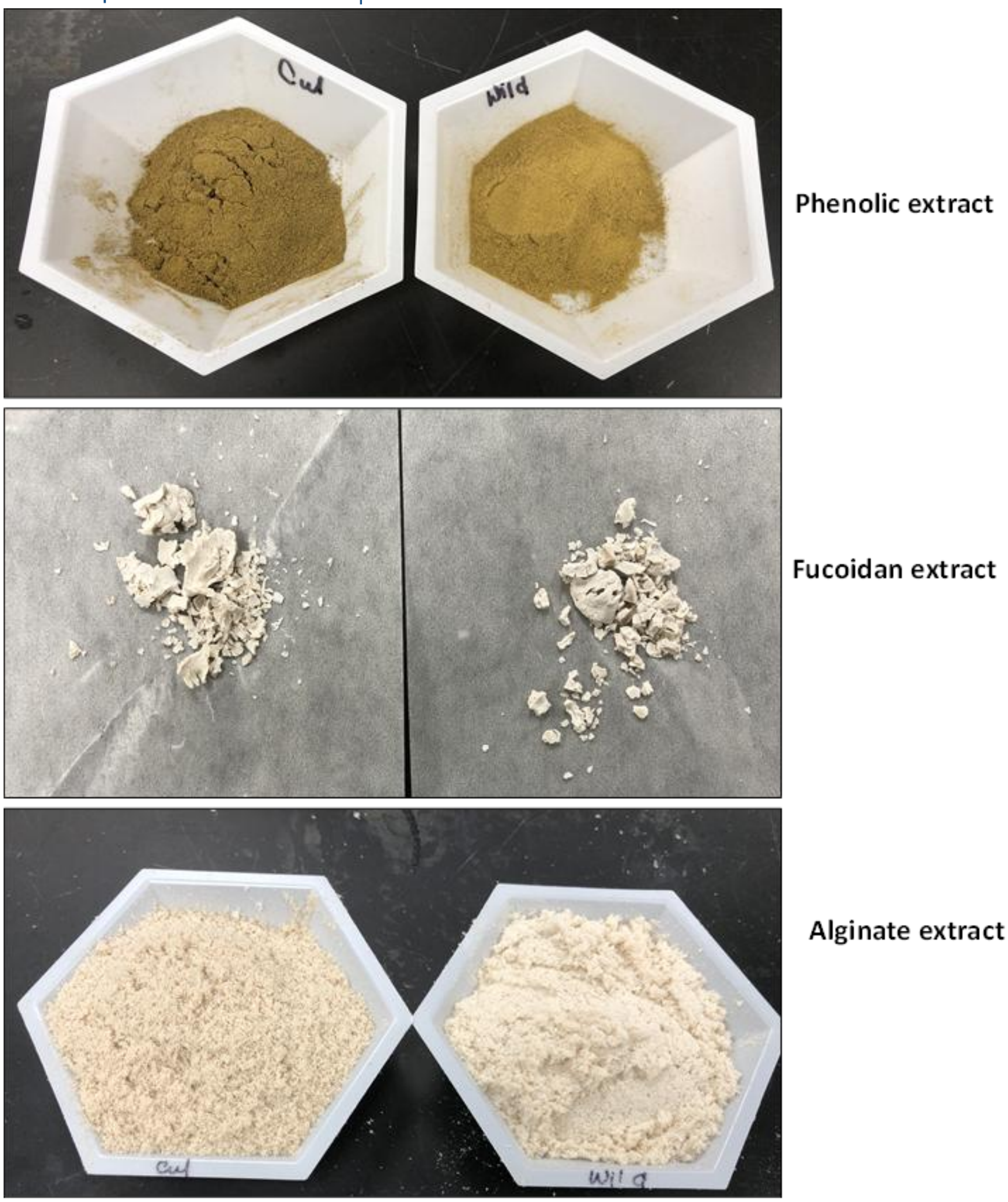

Figure 4. Freeze-dried crude phenolics, fucoidan and alginate extracted from cultivated (left) and wild (right) sugar kelp following cascading biorefinery approach

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5.3.4 Effect of pretreatment on polysaccharide extraction

The fucoidan content of the sugar kelp varied across different HPP treatments. Under HPP treatment, fucoidan yield initially decreased compared to the untreated samples, but it progressively increased from 3.7 ± 0.6% at 300 MPa to 5.3 ± 0.8% at 550 MPa (**Table 9**). The differences suggest that HPP at various pressures influenced the extraction of water-soluble sulfated polysaccharides, with fucoidan yield potentially increasing at higher pressures. The fucoidan yield observed in this study was similar to that reported by Lafeuille et al., (2023) in cultivated sugar kelp.

The alginate content decreased with HPP treatment compared to untreated sugar kelp. In cultivated seaweed, alginate content declined from the untreated value of 47.7±3.5% to lower levels following HPP treatment, with the lowest recorded at 450 MPa (32.9±1.5%). However, at 550 MPa, the alginate yield increased to 36.1±1.9%, higher than that at 300 MPa and 450 MPa. A study by Jönsson et al. (2023) reported a similar trend, where HPP-treated sugar kelp had lower carbohydrate content compared to untreated samples. They suggested that the variation in carbohydrate concentrations between untreated and HPP-treated sugar kelp could be attributed to natural variances in the biomaterials. A slight increase in carbohydrate content at higher pressures (200 > 400 > 600 MPa) may be due to the disruption of carbohydrate structures, resulting in gelatinization, which enhances the extraction process (Jönsson et al., 2023). Alginate is the predominant carbohydrate in sugar kelp, more so than fucoidan or laminarin. The alginate yield in cultivated sugar kelp from this study was significantly higher compared to sugar kelp cultivated in the marine farm at Paspébiac Bay (QB, Canada) (Lafeuille et al., 2023).

Laminarin was extracted from the HPP-treated sugar kelp, with yields ranging from 3.5% at 550 MPa to 3.9% at 450 MPa, which aligns with the findings of Lafeuille et al. (2023) for unprocessed cultivated sugar kelp (**Table 9**). It has been reported that HPP treatment did not significantly affect the biochemical properties of the sugar kelp but did alter its texture, microbial activity, and shelf-life. These texture changes are likely due to high pressure disrupting the special arrangements of proteins and carbohydrate biopolymers, causing gelatinization.

Fucoidan and laminarin are primarily valued for their biological activities, while alginates have multiple applications in food and medical industries as thickeners, emulsifiers, stabilizers, and pharmaceutical additives (Abka-Khajouei et al., 2022). The high alginate yield from the sugar kelp grown by the client suggests the potential for its commercial extraction.

After HPP treatment, the cascading biorefinery approach was applied to the sugar kelp, enabling the step-by-step extraction of bioactive compounds, including crude phenolics, fucoidan, and alginate. Approximately 80% of the seaweed samples were recovered as bioactive crude extracts, with around 15% remaining as cellulose, which could be further processed and used as dietary fibre. HPP treatment did not notably influence the residue content, which remained consistent between 13.3±0.1% and 14.9±0.8%.

The polysaccharide extraction process was carried out at a laboratory scale using 5g of dried seaweed, providing an estimate of the polysaccharide content in cultivated sugar kelp from Newfoundland. However, a key limitation of these results is that the extraction was performed on a very small sample size, which may not accurately reflect outcomes at a larger scale. Variability in extraction efficiency and yield could occur during scale-up. However, there was no significant increase in yields with HPP treatments.

5.3.5 Assess the economic feasibility of pretreatment.

Cost analysis of the HPP treatment at the lab scale was challenging, as phenolics and polysaccharides were extracted from very small sample sizes—no more than 5 g per treatment. As a result, the HPP system could not operate at full capacity or for extended durations (treatments lasted only a few minutes), making it difficult to estimate actual usage and costs. However, the initial investment for installing HPP equipment at a commercial scale may be impractical due to the high cost of the machinery and associated technical staffing expenses.

While the biorefinery approach could be applied on a pilot scale, a bioreactor at the CASD marine bioprocessing pilot plant could facilitate this process. Fucoidan and laminarin are mainly used for their biological activities, while alginates, which make up about 47% of the dry weight of sugar kelp, have several uses in food and medical industries (Abka-Khajouei et al., 2022). The findings of this study show that the biochemical composition of sugar kelp is influenced by HPP treatment, with specific effects on fucoidan, alginate, and phenolic compounds. The decrease in alginate content suggests potential structural disintegration, while moderate pressures enhance the extraction of phenolics. The variations in fucoidan content indicate that different pressures may either induce or suppress its release. Further research is needed to clarify the pressure-induced structural changes and optimize the extraction of fucoidan, a sulfated polysaccharide with anticoagulant, anticancer, anti-inflammatory, and immunomodulatory properties (Pereira and Cotas, 2023).

6 CONCLUSIONS

The sugar kelp cultivated in Atlantic waters at St. Mary's Bay and wild-harvested from Tors Cove exhibited differences in proximate composition, fatty acid profiles, and mineral content. Cultivated sugar kelp had significantly higher moisture, ash, and lipid content compared to wild sugar kelp, while carbohydrates were more abundant in the wild variety. There was no significant difference in protein content between the two groups.

Cultivated sugar kelp exhibited higher levels of phospholipids, polar lipids, and free fatty acids, along with increased monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA)—including omega-3 eicosapentaenoic acid (EPA)—and a more favorable PUFA:SFA ratio compared to wild sugar kelp. In terms of mineral content, cultivated sugar kelp contained greater amounts of potassium, calcium, iron, manganese, boron, aluminum, and molybdenum, whereas wild sugar kelp had higher concentrations of sodium, magnesium, zinc, and copper. Although both types contained trace amounts of heavy metals, particularly arsenic, the levels remained within safe limits. Importantly, the arsenic present in sugar kelp is predominantly in its organic, non-toxic form.

The observed differences in proximate composition, mineral content, and fatty acid profiles between cultivated and wild sugar kelp in this study may be attributed to variations in maturity stage, nutrient availability in the water, and environmental factors such as sunlight, temperature, and salinity.

High-pressure processing (HPP) had a minor impact on the proximate composition, with inconsistent effects across the two types of sugar kelp. Cultivated sugar kelp treated with HPP at 550 MPa exhibited higher protein content and lower ash content, whereas treatment at 450 MPa resulted in increased levels of both protein and ash in wild sugar kelp. HPP increased protein and decreased ash content in cultivated sugar kelp, whereas 450 MPa treatment resulted in higher protein and ash content in wild sugar kelp.

HPP treatment enhanced phenolic extract yields in both cultivated and wild sugar kelp, with cultivated sugar kelp exhibiting higher total phenolic content. HPP disrupted cellular structures, including microfibrillar networks embedded in matrices of polysaccharides and proteins, facilitating the release of secondary metabolites like phenolic compounds This resulted in higher extraction yields and increased phenolic content.. In the stepwise extraction process, the yield of fucoidan was higher in cultivated sugar kelp, while overall alginate and fucoidan yields were reduced under HPP treatment.

This study highlights the biochemical differences between cultivated and wild sugar kelp. However, differences in protein and ash content should be interpreted with consideration of the maturity stage and environmental factors, which can significantly influence the biochemical composition of sugar kelp.. It demonstrates HPP's potential to enhance the extraction of phenolic compounds. The effects on polysaccharides require further investigation..

7 RECOMMENDATIONS

Polysaccharide Extraction and Utilization

The lab-scale stepwise polysaccharide extraction process demonstrated that approximately 80% of dried seaweed can be recovered as phenolic compounds and bioactive polysaccharides, such as fucoidan and alginate. These crude extracts show potential for use in the food, nutraceutical, and cosmeceutical industries. Further purification of these extracts and analysis of their physicochemical characteristics is recommended to optimize their application.

Residue Utilization

After fucoidan and alginate extraction, the remaining residues were not processed or analyzed. These residues could be a valuable source of dietary fibre, with potential applications in both human and animal nutrition. Further steps should be taken to recover additional beneficial components from these residues, thereby reducing biological waste and increasing resource utilization.

Cascading Biorefinery at Larger Scales

The application of the cascading biorefinery principle to sugar kelp grown in Newfoundland shows promise. It is recommended that environmentally friendly chemicals be used in the extraction process. A more thorough analysis of the yield and quality of the extracts is necessary to refine the process.

Scaling the stepwise polysaccharide extraction model to a pilot level would provide a clearer understanding of the commercial viability and efficiency of the process, as well as its potential for large-scale application.

Biostimulant Production

Given sugar kelp's high concentration of carbohydrates macro- and micro-minerals, it is well-suited for processing into biostimulants. The CASD's pilot-scale bioreactor presents an opportunity to explore this application. Sugar kelp could serve as a valuable biostimulant in agricultural practices, promoting sustainable agricultural solutions and adding value to sugar kelp cultivation.

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APPENDIX A – DETAILED LABORATORY ANALYSIS PERFORMED FOR THE PROJECT

1. Phenolic content determination

The total phenolic content of fine powder of freeze-dried HPP treated and untreated sugar kelps was extracted following Ambigaipalan et al., (2016)with few modifications. About 1 g fine powder was homogenized with 70% acetone at a 1:30 ratio (w/v) in a 50 ml polypropylene centrifuge tube, vortex for one minute and incubated the mixture in a platform Shaker (Thermo-Scientific Max Q 6000, Marietta, Ohio, USA) overnight at 200 rpm speed at room temperature. The extract was collected after centrifugation at 4,000 g for 10 min. 20 ml of acetone was mixed with precipitate by vortex and centrifuged. The supernatant was combined with the previous one and stored at -20°C until the phenolics were determined. The extract stock was diluted 4-6 times (depending on absorbance reading), and the determination of the phenolic compound was carried out following Singleton and Rossi, (1965) modified by(Goyali, Igamberdiev, & Debnath, 2013). In brief, 100µL Folin-Ciocalteu reagent and 1.5 ml deionized water were added with 100µL of extract. The mixture was incubated in the dark at room temperature after the vortex. After 5 min, 200µL of saturated sodium carbonate was added, vortexed thoroughly and placed in the dark at ambient temperature. After 2 h, the mixture was centrifuged at 4000 g for 5 min and the absorbance was read at 760 nm with a Spectrophotometer against the appropriate blank. Total polyphenolic content was determined in triplicates and calculated from the gallic acid calibration curve as milligrams of gallic acid-equivalent (GAE) phenolic compounds per gram of seaweed powder (g GAE/ g).

2. Polysaccharides extraction

Polysaccharides of the HPP-treated and untreated sugar kelp were extracted following a three-stage extraction process. First, the polyphenolic compounds of sugar kelp were extracted by using 70% acetone (v/v) (Fawzy & Gomaa, 2021). 70% acetone was added to 5g of fine powder at a 1:25 (w/v) ratio and incubated in an incubator shaker at 200 rpm and room temperature overnight. The phenolic extract was separated from the residual biomass by vacuum filtration using medium-fast Whatman-1 filter paper. Acetone was evaporated using a rotary evaporator and phenolic residue was freeze-dried for at least 48 hours. The following formula calculated phenolic extract yield:

$$Phenolic\ extract\ yield\ (\%) = \frac{\text{Weight of freeze dried phenolic extract}}{\text{Weight of seaweed powder}} x100$$

In the second stage, fucoidan was extracted by soaking the residual biomass from phenolic extraction in 0.1N hydrochloric acid (HCl) solution at a 1:20 (v/w) with shaking at 200 rpm and 80 °C for 2 hours (Ummat et al., 2024). The extract was separated from the residual biomass using vacuum filtration with Whatman-4 filter paper. The residual biomass was collected from filter paper for alginate extraction. The supernatant was mixed with 2% (w/v) calcium chloride and stored at 4 °C overnight to precipitate the alginate present in the fucoidan extract. The fucoidan extract was filtered through the Whatman-1 paper. 2x absolute ethanol was added to fucoidan extract and stored at 4°C for 24 hours to precipitate fucoidan. The fucoidan was separated in a vacuum filtration system using preconditioned filter paper (oven-heated to remove moisture and pre-weighed) and freeze-dried for 24 h. The weight of the freeze-dried fucoidan was recorded, and the yield was calculated by the following formula:

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$$Crude\ fucoidan\ yield\ (\%) = \frac{\text{Weight of freeze dried fucoidan extract}}{\text{Weight of seaweed powder}} x100$$

In the third stage, double distilled water was added to the residual biomass from the 2nd stage at 1:25 (w/v). 10% (w/v) sodium carbonate was added to the mixture under stirring on a hot plate (45-50 °C) to make pH 9.5-10 and wait for 10 min to confirm the stable pH. The mixture was incubated in an incubator shaker at 200 rpm and 70 °C for 3 hours (Okolie, Mason, Mohan, Pitts, & Udenigwe, 2020). The alginate extract was separated by vacuum filtration using preconditioned filter paper (oven-heated to remove moisture and pre-weighed). The residues were dried at 50 °C for 24 hours, and the residue yield was calculated as below:

$$Residues\ yield\ (\%) = \frac{\text{Dry weight of residues}}{\text{Weight of seaweed powder}} x100$$

2x absolute ethanol was added to the alginate extract and stored at 4°C overnight. The alginate was separated in a vacuum filtration system using preconditioned filter paper (oven-heated to remove moisture and pre-weighed) and freeze-dried for 48 hours. The weight of the freeze-dried alginate was recorded, and the yield was calculated by the following formula:

$$Alginate\ yield\ (\%) = \frac{\text{Dry weight of alginate}}{\text{Weight of seaweed powder}} x100$$

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APPENDIX B- TOTAL FATTY ACID COMPOSITION (mg/g) OF FREEZE-DRIED CULTIVATED AND WILD SUGAR KELPS

Systematic Name	Isomer	Culture	Wild
Myristic acid	14:00	1.37	1.25
Myristoleic acid	14:01	0.01	0.01
	i15:0	0.05	0.04
	ai15:0	0	0
Pentadecanoic acid	15:00	0.07	0.04
	i16:0	0.02	0.01
	ai16:0	0.01	0
Palmitic acid	16:00	2.62	2.24
	16:1w11	0	0
Cis-7 hexadecenoic acid	16:1w9	0.04	0.03
Palmitoleic acid	16:1w7	0.41	0.33
	16:1w5	0.04	0.04
	i17:0	0.08	0.09
	ai17:0	0.05	0.05
	16:2w6	0.03	0.04
Heptadecanoic acid	17:00	0.02	0.02
	16:3w4	0.03	0.03
Heptadecenoic acid	17:01	0.07	0.13
	16:4w1	0.03	0.04
Stearic acid	18:00	0.33	0.3
Oleic acid	18:1w9	1.79	1.53
Vaccenic acid	18:1w7	0.03	0.02
	18:1w5	0.01	0.01
Linoleic acid	18:2w6	2.03	1.83
	18:2w4	0.02	0
Gamma-linolenic acid	18:3w6	0.15	0.13
Linolenic acid	18:3w4	0.01	0
Alpha-linolenic acid	18:3w3	0.9	0.88
Stearidonic acid	18:4w3	1.72	1.42
Alpha-parinaric acid	18:4w1	0	0
Arachidic acid	20:00	0.13	0.12
Gadoleic acid	20:1w11	0.02	0.01
Gondoic acid	20:1w9	0.03	0.03
Paullinic acid	20:1w7	0.01	0.01

Eicosadienoic acid	20:2w6	0.02	0.04
Dihomo-gamma-linolenic acid	20:3w6	0.08	0.1
Arachidonic acid	20:4w6	2.15	1.84
	20:3w3	0	0
Eicosatetraenoic acid	20:4w3	0.18	0.16
Eicosapentaenoic acid	20:5w3	2.12	1.7
Behenic acid	22:00	0.01	0.01
Catoleic acid	22:1w11(13)	0.02	0
Docosahexaenoic acid	22:6w3	0.02	0
Total		16.8	14.6