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Novel Biorefinery Process for Extraction of Laminarin, Alginate and Protein from Brown Seaweed Using Hydrodynamic Cavitation

Xianglu Zhu
Teagasc Food Research Centre, Ireland

Laura Healy
Technological University Dublin, laura.healy@tudublin.ie

Rahel Suchintita Das
University College Dublin, Ireland

See next page for additional authors

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Novel biorefinery process for extraction of laminarin, alginate and protein from brown seaweed using hydrodynamic cavitation

Xianglu Zhu [a,b], Laura Healy [a,c], Rahel Suchintita Das [d], M.L. Bhavya [a], Shanmugapriya Karuppusamy [a,b], Da-Wen Sun [b,*,1], Colm O'Donnell [b], Brijesh K. Tiwari [a]

[a] Teagasc Food Research Centre, Ashtown, D15 DY05 Dublin, Ireland
[b] Food Refrigeration and Computerized Food Technology (FRCFT), School of Biosystems and Food Engineering, University College Dublin, National University of Ireland, Belfield, Dublin 4, Ireland
[c] School of Food Science and Environment Health, College of Sciences and Health, Technological University Dublin, D01 K822 Dublin, Ireland
[d] School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

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ABSTRACT

This paper investigates a novel biorefinery process designed for the extraction of valuable compounds from brown seaweed *Alaria esculenta* using hydrodynamic cavitation (HDC). A two-stage process was developed to maximize the value of seaweed biomass by control of the processing time, solvent selection and HDC conditions to extract laminarin, alginate, mannitol and protein in a cascading manner, maximizing the value of seaweed biomass. After the first extraction stage using 0.1 M HCl, membrane ultrafiltration was employed to separate laminarin and mannitol. The purity of the laminarin and mannitol obtained was up to 86.57 ± 3.72 % and 40.49 ± 2.78 % with recovery rates of 55.55 ± 3.10 % and 75.90 ± 4.49 %, respectively. Ethanol precipitation was then carried out to recover sodium alginate after the second extraction stage process using 2 % Na_2CO_3 (w/v). The sodium alginate purity extracted by employing HDC twice (HDC-HDC) was 88.98 ± 4.70 % with a recovery rate of 65.13 ± 5.14 %. The remaining residue after the biorefinery process had an enriched protein content of 17.19 ± 1.33 %. This study demonstrates that an HDC-assisted biorefinery process can significantly ($P < 0.05$) reduce energy consumption. The laminarin extracts were further characterised by antioxidant activity, anti-inflammation activity, FT-IR, and anti-microbial activity. The laminarin extracted in this study was shown to have identical bioactive activities as the commercially available samples.

1. Introduction

Biorefinery was developed using the concept of a fossil oil refinery. Seaweed biorefinery refers to the process of using seaweed as the raw material to produce a range of high-value products through a series of integrated processes [1]. Seaweed biorefinery is an emerging field that offers a promising pathway towards a sustainable and low-carbon economy. Seaweed is a sustainable and renewable resource that has gained increasing attention as a potential feedstock for biorefinery processes due to its abundance, rapid growth, and high productivity [2]. The goal of a seaweed biorefinery is to maximize the value of the seaweed by utilizing as much biomass as possible while minimizing waste. The process typically involves the extraction of valuable components such as carbohydrates (e.g. laminarin, alginate, etc.), proteins and lipids, which can be used for food, feed, pharmaceuticals, cosmetics, and biofuels [3]. Similar biorefinery concept has been introduced into coffee industry. Le et al. have successfully carried out researches on protein, phenolics and dietary fibre from coffee silverskin using ultrasound and microwave assisted extraction techniques [4-6]. Seaweed biorefinery also has the potential to contribute to the development of a circular bioeconomy, where waste streams from one process can be used as a feedstock for another. For example, the residual biomass from laminarin and mannitol extraction can be used for alginate extraction. The residual biomass after alginate extraction can be used as a nutrient source for aquaculture or agriculture. As research and development in this area continue to advance, the potential for seaweed biorefinery to become a significant contributor to the bioeconomy is becoming increasingly apparent.

* Corresponding author.
E-mail address: dawen.sun@ucd.ie (D.-W. Sun).
[1] http://www.ucd.ie/refrig, http://www.ucd.ie/sun.

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Laminarin, alginate, mannitol and proteins are all important compounds that can be extracted from seaweed using a biorefinery approach. Seaweed, especially brown seaweed, is a source of high-value carbohydrates. Laminarin is a type of polysaccharide that is commonly found in brown algae and has been shown in many studies to have strong anti-oxidant, anti-inflammatory activities and cell toxicity to selected cancer cells [7–9]. Alginate is a type of polysaccharide that is commonly found in brown algae and is used as a thickener and emulsifier in food, cosmetic and pharmaceutical applications. Alginates are also used in wound healing and drug delivery due to their ability to form hydrogels [10]. Mannitol is a sugar alcohol that is found in brown and red algae. It is commonly used as a sweetener and bulking agent in food, as well as a cryoprotectant in the preservation of cells and tissues [11]. Proteins are also an important component of seaweed and can be extracted for use in food and animal feed [12]. Seaweed proteins have been found to have various functional properties, including emulsification [13].

Hydrodynamic cavitation (HDC) processing is a type of physical processing that involves high-intensity cavitations in a liquid medium. Cavitation is the formation of cavities (small bubbles or voids) in a liquid due to changes in pressure, which can occur when the liquid is subjected to high levels of mechanical agitation or turbulence [14]. Hydrodynamic cavitation, serving as a scalable counterpart to ultrasound cavitation, has emerged as a promising extraction technique. This is in recognition of the efficiency demonstrated by ultrasound extraction in extracting bioactive compounds [15-17]. A hydrodynamic cavitator has a speciality reactor that contains a series of nozzles, orifices, and other flow-modifying elements designed to generate a pressure difference. The high-energy cavitation bubbles can cause physical, chemical, and biological changes in the liquid, including the generation of heat, shock waves, and free radicals. These effects can be harnessed to achieve a range of processing goals, such as emulsification, mixing, homogenization, and extraction [18,19]. Thus HDC can be employed to extract bioactive compounds from seaweed, such as polysaccharides, proteins, and pigments. The high-intensity cavitation bubbles generated during the process can cause the breakdown of cell walls and facilitate the release of intracellular compounds. In addition, seaweed biorefinery processes can generate wastewater streams that contain high levels of organic matter and nutrients. Hydrodynamic cavitation can be used to degrade pollutants in the wastewater and reduce its environmental impact [20]. Hydrodynamic cavitation processing has several advantages over traditional processing techniques, such as reduced processing times, lower energy requirements, and higher product yields. It is also a relatively simple and scalable process that can be easily integrated into existing production lines.

Hydrodynamic cavitation was investigated as a method of processing seaweed for product extraction in a biorefinery method and compared with conventional methods which do not account for waste byproducts. We employed a cascading biorefinery approach for seaweed processing, which is a novel way to extract high-value seaweed bioactives including laminarin, sodium alginate and mannitol and enrich the protein content in the residual biomass. In this study, we aimed to provide valuable insights into the potential benefits and viability of implementing hydrodynamic cavitation in seaweed biorefinery processes by reducing waste and also exploring the scale-up potential of the approach. Biorefinery efficiency parameters including extraction yield, purity, quality and energy efficiency were measured to evaluate the potential and benefits of the biorefinery process designed. Keeping the theme of sustainability in mind, we also measured the energy consumption of the process and compared it with conventional methods of extraction, to give an overall picture of the novelty of the approach.

2. Materials and methods

2.1. Seaweed sample and chemicals

Seaweed biomass was obtained from two locations in Ireland. Fresh *A. esculenta* (moisture content of 73.08 ± 0.29 g/100 g, w.b.) was wild harvested from Kerry, Ireland in November 2019. Fresh *A. esculenta* (moisture content of 87.11 ± 0.18 g/100 g, w.b.) was harvested from an aquaculture site in Blacksod Bay, Ireland in February 2020. Fresh seaweed samples were washed thoroughly with tap water to remove salt and surface impurities. Samples were then wiped with tissue to remove surface water and were then ground until approximately 1 to 2 cm in length. All samples were stored at −30 °C prior to further processing.

All reagents (laminarin, acetone, potassium bromide) were purchased from Sigma-Aldrich, USA. Maximum recovery diluent CM0733 (MRD) was purchased from Oxoid, UK.

2.2. Seaweed biorefinery process

The seaweed biorefinery process designed is shown in Fig. 1. Two main process steps were developed to extract laminarin, mannitol and alginate. Enriched protein and fibre were obtained in the final residue.

2.2.1. Conventional process (Con)

A conventional thermal process was carried out using a Firex crucifix mixer with temperature control (Sedico, Italy). After the rehydration of seaweed powder (1 kg) mixed with the extraction solvents, 50 rpm (reps per minute) was selected for both the extraction steps at 70 °C for 2.5 h and 60 °C for 3 h, respectively.

2.2.2. Hydrodynamic cavitation process (HDC)

The customized hydrodynamic cavitation (HDC) assisted extraction employed a reactor (CaviMax, UK) is illustrated in Fig. 2, which consists of a hydraulic pump, a substrate pump, a cavitator pump as well as two storage tanks of 50 L volume. One kilograme of seaweed powder was dispersed in extraction solvents in the storage tank. The mixed sample was then processed using the hydrodynamic cavitator operating at a pump speed of 50 Hz, a rotacav speed of 50 Hz and a flow rate of 800 L/h. Fifty passes were used for both steps. Samples were then stored at 4 °C until further study.

2.2.3. Laminarin recovery process

A. esculenta was combined with 0.1 M HCl (1,15, w/v) for both conventional and HDC. After filtration, ethanol precipitation (absolute ethanol, 1:3 v/v), freeze-drying directly and membrane ultrafiltration were selected to further stabilize the extracts. Laminarin and mannitol were obtained from the supernatant using membrane ultrafiltration. Supernatant was ultra-filtrated successively using ultrafiltration membranes with a molecular weight cut-off of 10 kDa (Millipore Co., Ltd.). Before use, each membrane was soaked in ultrapure water overnight and then installed in a membrane filtration cell (Amicon 8200, USA) possessing the liquid with an effective membrane surface area of 28.7 cm^2. At least 500 ml of ultrapure water was then filtered through the membrane at a constant pressure of 5 bar to remove and collect the retentate from the membrane unit. The retentate (laminarin fraction) and permeate (mannitol fraction) were collected and freeze-dried. Freeze dried powders from both fractions were stored until further analysis.

2.2.4. Alginate recovery process

The leftover biomass after laminarin recovery was remixed with 2 % sodium carbonate using a solid: solvent ratio of 1:20, w/v. After the process shown in Fig. 1 for both conventional and HDC, the supernatant was obtained for spray drying (S-300, Buchi, Ireland) and ethanol precipitation to recover sodium alginate. The residue was freeze-dried and stored prior to nitrogen measurement. The recovery rate for laminarin, sodium alginate and mannitol were calculated using the following Eq. (1).

$$\text{Recovery rate}\,(\%) = \frac{\text{Purity of the extract}^{*}\text{freeze dried extract obtained}\,(\text{g})}{\text{Extract content in seaweed sample's dry weight}\,(\text{g})} \times 100 \quad (1)$$

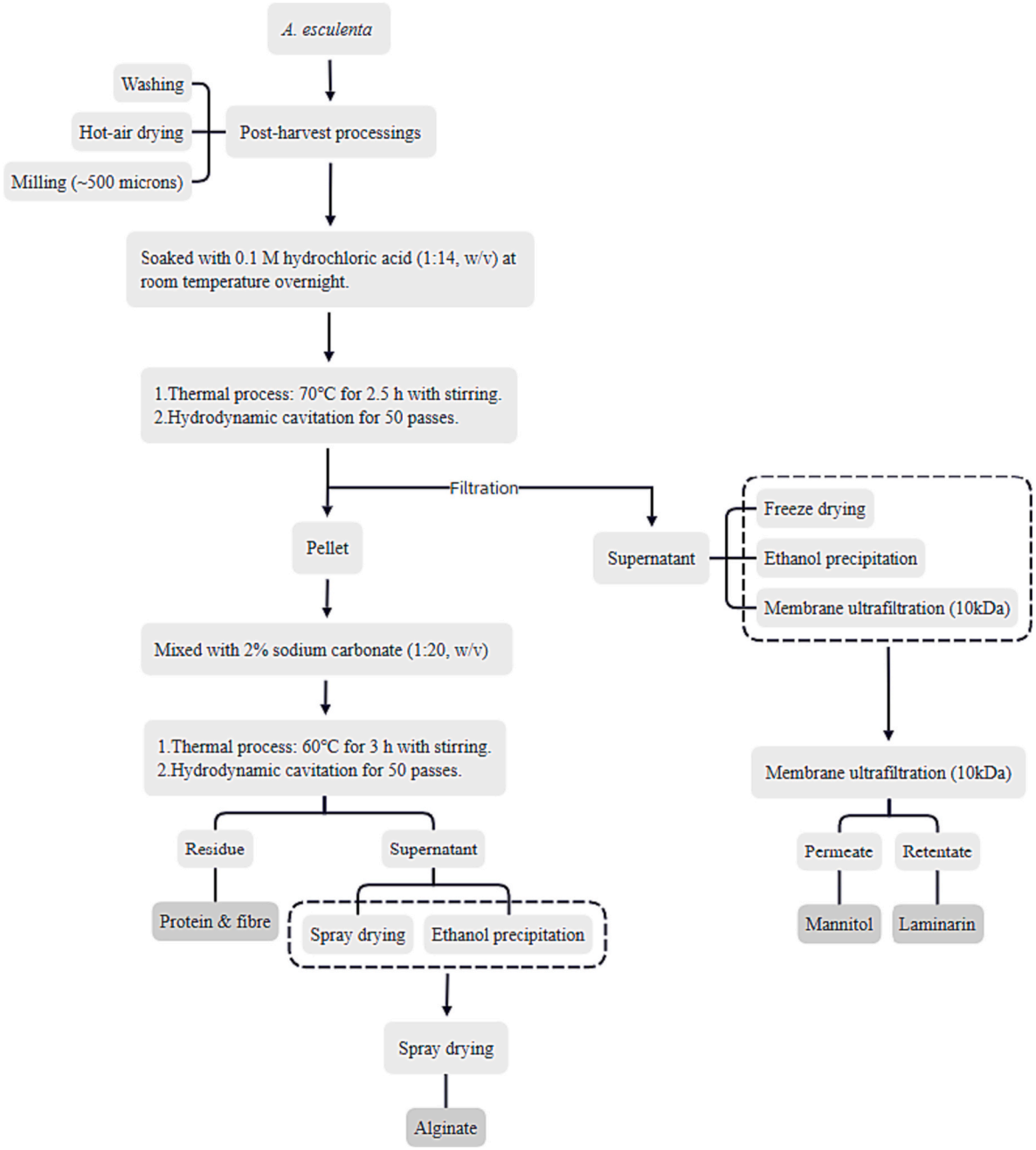

Fig. 1. Schematic flow chart of brown seaweed biorefinery process.

2.3. Quantification and molecular weight of extracts

The molecular weight determination and laminarin quantification methods were carried out using a High-performance liquid chromatography (HPLC, Agilent 1200 LC system, Agilent Technologies, Santa Clara, California, USA) fitted with a refractive index detector connected with a guard column (OHpak SB-G 6B, 8 × 50 mm) and a Shodex OHpak SB-804 HQ with 6 % cross-linked HPLC carbohydrate column with 8 × 300 mm (length × I.D.) (Shodex, Japan). All samples were prepared with a concentration of 2 mg/mL with the running solvent and filtered through 0.45 μm filters (Econo Filter, PTFE, Agilent).

2.3.1. Determination of molecular weight

All samples were dissolved in 0.1 % sodium chloride. The mobile phase was 0.1 % sodium chloride aqueous solution at 40 °C with a flow rate of 0.5 mL/min and an injection volume of 20 μL. The molecular weights of the extracted laminarin were calculated using calibration curves obtained from various pullulan standards with defined molecular weights (0.342, 1.26, 6.6, 9.9, 23, 50.6, 115, 202, 343, and 805 kDa).

2.3.2. Identification and quantification of alginate, laminarin and mannitol

The mobile phases used for alginate and laminarin/mannitol were 0.05 M Na_2SO_4/0.01 M EDTA (pH 7) and ultrapure water, respectively. All samples were dissolved in their own mobile phase. The mobile phase was used for isocratic elution at room temperature. The flow rate and injection volume were set to 0.6 mL/min and 10.0 μL, respectively. Temperatures of 60, 80 and 40 °C were maintained for alginate, laminarin and mannitol, respectively. The purities of extracted samples were calculated using a commercial standard curve with different concentrations (0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25 and 2.5 mg/ml).

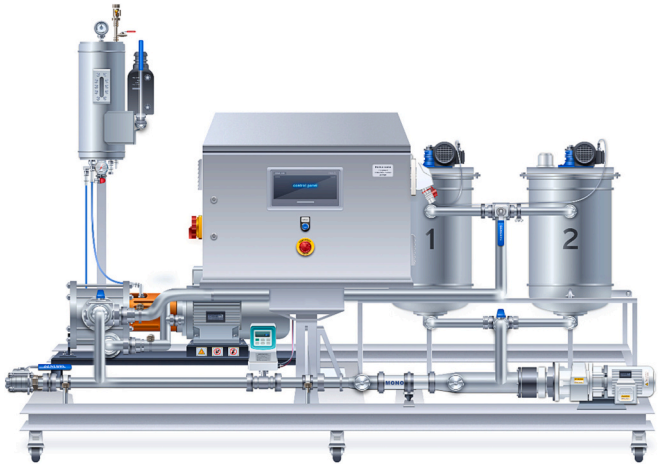

Fig. 2. Hydrodynamic cavitator designed for brown seaweed biorefinery process.

2.4. Protein content

The protein content (%) of the freeze-dried seaweed residues was weighed and protein content (%) was analysed by using a nitrogen analyser (FP-328 Leco Instrument, Leco Corporation, USA) based on the Dumas principle (method 968.06, [21] and conversion factor 6.25).

2.5. Antioxidant activity

2.5.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

Seaweed extracts as samples and control (ascorbic acid) were prepared to a concentration of 1 mg/mL in 0.1 M citrate phosphate buffer with 0.3 % of Triton X-100. Samples were prepared in triplicates. Then, 10 μL of a 2 mM methanolic DPPH solution was added to each sample. Further, the reaction mixture was incubated at room temperature in the dark for 30 min and read against the blank at 515 nm before and after the reaction with the DPPH solution using a UV–Vis spectrophotometer. The inhibition percentage of DPPH scavenging activity was calculated using Eq. (2) below.

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{Abs_{Blank} - Abs_{Sample}}{Abs_{Blank}} \times 100 \quad (2)$$

where "Abs_{Blank}" is the absorbance of the Blank (DPPH solution without sample/standard), "Abs_{Sample}" is the absorbance of the test sample (DPPH solution plus test sample/standard).

2.5.2. Ferric-reducing antioxidant power (FRAP)

Triplicate samples were prepared at 1 mg/mL concentration. Trolox was used as a standard and triplicate standards were prepared at concentrations in the range of 15–420 μM as triplicates. The FRAP solution was freshly prepared in the ratio of 10:1:1:1.4 with 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride, 10 mM 2,4,6-Tripyridyl-s-Triazine (TPTZ) in 40 mM HCl and Milli Q water. Then, 280 μL of a FRAP working solution was added to 20 μL of sample/standard and incubated at 37 °C in the dark for 30 min. The absorbance was read at 593 nm using a UV–Vis spectrophotometer. The FRAP antioxidant activity values are expressed as μM Trolox equivalents (TE) per mg of extract.

2.6. Fourier-transform infrared spectroscopy (FT-IR) measurement and multivariate data analysis

Before FT-IR measurements, the freeze-dried powder of each sample (ca. 0.4 g) was compressed into a pellet using a 15-ton hydraulic press (Specac Ltd., Orpington, UK). Two pellets were prepared from each sample. Sample pellets were placed on the surface of a diamond crystal attenuated total reflectance (ATR) accessory (iD7 ATR, Thermo Scientific, Madison, WI, USA), and spectral measurements were carried out using a Fourier transform mid-infrared spectrometer (Nicolet™ iS5, Thermo Scientific, Madison, WI, USA). Single-beam reflectance spectra were collected and converted into absorbance spectra in the wavelength range of 450–4000 cm^{-1} with a resolution of 2 cm^{-1}. Air blank background calibration was carried out before each measurement. 64 scans were performed on each measurement to acquire the averaged spectral data. Spectral data acquisition was managed using the supplied OMNIC software v 9.2.98 (Thermo Fisher Scientific Inc., USA). Each sample pellet was measured in quadruplicate at two different surface areas on both sides of the pellet. Principal component analysis (PCA) was also performed on the pre-treated spectral data (450–4000 cm^{-1}) to analyse the principal components to identify the sample variations that relate to the extraction strategies used in the current study.

2.7. Anti-inflammatory activities of the bioactive compounds against COX-1, and 2 enzymes

The inhibition of Cyclooxygenase (COX) -1 and -2 enzymatic inhibition activity was analysed in duplicate using the COX inhibitor screening assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Laminarin extracts were prepared in DMSO and tested at a concentration of 1 mg/mL.

2.8. Anti-microbial activities

Bacillus subtilis (DSM 618), *Escherichia coli* (K12 DH5α), and *Saccharomyces cerevisiae* (DSM 70,449) were obtained from the microbiology stock cultures at Teagasc Food Research Centre (Ashtown, Dublin), and stored in 20 % glycerol at −80 °C. Vials containing frozen stock cultures were reactivated and maintained in sterile brain heart infusion (BHI) broth and yeast extract peptone dextrose (YPD) broth media for bacteria and yeast at 37 and 30 ± 2 °C, respectively for 24 h under static conditions. The cell suspension was centrifuged at 5000 × *g* for 10 min and washed using maximum recovery diluent (MRD). Bacterial and yeast suspensions in BHI and YPD broth, respectively at an initial concentration of ~10^6 colony-forming units (CFU)/mL were used for further studies. The effects of extracts containing laminarin on the growth curve of bacteria and yeast were determined by standard two-fold micro broth dilution methodology as described by Bhavya et al. with slight modifications [22]. A stock solution of 10 mg/mL was added to a 96-well microtiter plate with BHI broth (200 μL) to obtain a concentration of 2.5 and 1.25 mg/mL and to which 5 μL of standardized inoculum was added to each well. The wells containing BHI broth, inoculated broth and antibiotic-added broth served as media, negative and positive controls, respectively. Further, the microtiter plate was incubated for 24 h at 37 and 30 ± 2 °C for bacteria and yeast, respectively. The optical density at 600 and 660 nm was measured for bacteria and yeast, respectively with an interval of 30 min for 24 h using Spark® 10 M Multimode Microplate Reader (Tecan, Mannedorf, Switzerland). The growth rate and lag time were determined by plotting the OD reading against incubation time. The data were fitted using the DMFit excel based tool according to the modified Gompertz model as described by Baranyi and Roberts [23] and the results are represented as the mean of four replicates.

2.9. Statistical analysis

Triplicates measurements performed for all drying and quality parameters unless otherwise stated. Effects of storage methods were evaluated using one-way analysis of variance (ANOVA) with post Hoc-Tukey test, using SPSS (v20.0.0, IBM, U.S.A.). The significance level was defined as $P < 0.05$. All assays were performed in triplicate for the anti-cancer studies, independently of each other with a minimum of three

replicates per experiment. Prism version 9.1.0 (GraphPad Soft-wares, Inc.) was used to carry out curve fitting and statistical analysis. Dose-response curves were measured using nonlinear regression. Data are presented as a percentage and error bars of all figures were presented using the standard error of the mean (SEM), and multiple comparison analyses were performed using Post Hoc Tukey's test.

3. Results and discussion

3.1. Extraction efficiency and product purity

This experiment followed the flowchart in Fig. 1 and aimed to establish a two-step biorefinery process, comparing four treatments: HDC-HDC, Con-Con, Con-HDC, and HDC-Con. Various separation and purification methods, including ethanol precipitation, membrane ultrafiltration, and freeze drying, were employed. In the first step, laminarin and mannitol were extracted, while the second step yielded sodium alginate as the primary product. FT-IR was carried out to characterize and evaluate the structural changes of laminarin and sodium alginate. The anti-microbial, anti-oxidant and anti-inflammatory activities of laminarin were further studied. Additionally, the protein content of the leftover residues was evaluated.

The purity and recovery rate of target compounds are the key efficiency measurable parameters in the biorefinery process. As shown in Table 1, membrane ultrafiltration and ethanol precipitation were selected for laminarin and sodium alginate recovery, respectively. Among these, laminarin content was 86.57 ± 3.72 %, and sodium alginate content was 88.98 ± 4.70 %. Rajauria et al. reported similar results for laminarin content (54.26 ± 0.20 %) that was precipitated using ethanol (54.90 ± 0.28 % in this study by HDC) but a higher purity (90.66 ± 0.00 %) for extraction using hydrothermal assisted extraction followed by membrane (10 kDa) ultrafiltration [9].

In this study, although both the highest purities of laminarin and sodium alginate were extracted using the HDC-HDC process, they were not significantly higher than other treatments. Similar results were also found for the recovery rate. However, it is noted that the protein content of the final residues was significantly ($P < 0.05$) higher in HDC-HDC samples than for other treatments. The protein content was enriched in the final residue compared to the raw *A. esculenta* powder. In addition, both the HDC processes for laminarin (1.83 ± 0.12 kWh) and sodium alginate (5.08 ± 0.37 kWh) extraction process showed a significant ($P < 0.05$) reduction in energy consumption compared to conventional extraction (10.75 ± 0.57 and 20.46 ± 1.69 kWh). Many other studies on a large-scale process using HDC also have the same findings [24–26].

The molecular weights of laminarin and sodium alginate shown in Table 1 were calculated using the standard curve with the square of regression of value 0.99. Both conventional and HDC-extracted laminarin had relatively smaller molecular weights compared to commercial laminarin from Sigma, but not significantly ($P > 0.05$) different. They were all around 5 kDa, which is similar to those values reported by Rajauria et al. [9]. Membrane filtration for laminarin recovery is considered a more efficient and easily scalable method when compared to ethanol precipitation, but the conditions for membrane filtration need to be optimized. A systemic study on the impact of membrane filtration parameters (membrane porous size, pressure and temperature) on laminarin purification was carried out by Sterner and Gröndahl indicated that a prefilter (50 kDa) followed by a 5 kDa membrane filtration at a high feed velocity of 5 m/s and low pressure of 2 bar had the highest laminarin recovery rate [27]. However, the membrane size used should depend on the molecular weight of laminarin which is highly correlated to the degree of polymerisation of glucose moieties [28]. Glucose moieties can vary depending on the seaweed species and extraction conditions including solvent type, extraction time and extraction method. The molecular weight of laminarin extracted from *Saccharina longicruris* was reported to be around 3 kDa [29]. Deville et al. reported that the molecular weight of laminarin was higher for extraction by HCl compared to extraction by H_2SO_4 [30]. According to a study by Benito-Román et al., normally, the longer extraction time decreases the molecular weights of laminarin extracted from plants [31]. In this study, hydrodynamic cavitation is employed for the extraction process. When compared with conventional thermal extraction, HDC reduces the processing time and temperature. In addition, cavitation breaks down a certain amount of molecules. It did not affect the molecular weight of laminarin but significantly reduced the molecular weight of sodium alginate. The treatments that included HDC showed significant ($P < 0.05$) lower molecular weights than Con-Con (Conventional – Conventional) treatment (215.75 ± 2.51 kDa). Among these, only Con-HDC (173.43 ± 3.25 kDa) and HDC-HDC (169.64 ± 2.77 kDa) samples'

Table 1
Purity and molecular weight of laminarin and alginate.

Extracts	Treatment	Purity (%)	Molecular weight (kDa)	Energy consumption (kWh)	Recovery rate (%)
Laminarin	Con-FD	47.23 ± 1.97a			
	HDC-FD	46.40 ± 0.59a			
	Con-EtOH	43.67 ± 0.98a			
	HDC-EtOH	54.90 ± 0.28a			
	Con-membrane	80.36 ± 6.90b	5.14 ± 0.01a	10.75 ± 0.57a	54.98 ± 4.75a
	HDC-membrane	86.57 ± 3.72b	5.13 ± 0.05a	1.83 ± 0.12b	55.55 ± 3.10a
	Sigma		5.24 ± 0.01a		
Mannitol	Con-membrane	37.77 ± 3.41a			54.53 ± 1.58a
	HDC-membrane	40.49 ± 2.78a			75.90 ± 4.49b
Sodium alginate	Con-Con-FD	33.36 ± 4.94a			
	Con-HDC-FD	58.01 ± 7.18a,b			
	HDC-HDC-FD	34.06 ± 10.67a			
	HDC-Con-FD	35.37 ± 11.94a			
	Con-Con-EtOH	68.6 ± 13.34b,c	215.75 ± 2.51a	20.46 ± 1.69[a]	64.84 ± 3.71a
	Con-HDC-EtOH	78.29 ± 9.64b,c	173.43 ± 3.25b,c	13.71 ± 0.93[b]	56.85 ± 4.88a
	HDC-HDC-EtOH	88.98 ± 4.70c	169.64 ± 2.77c	5.08 ± 0.37[c]	65.13 ± 5.14a
	HDC-Con-EtOH	67.18 ± 0.78b,c	188.98 ± 5.62b,d	13.75 ± 1.24[b]	62.34 ± 2.97a
	Sigma		190.80 ± 1.18d		
Protein	Alaria powder	4.94 ± 0.31a			
	Con-Con	13.13 ± 0.11b			
	Con-HDC	12.90 ± 0.19b			
	HDC-HDC	17.19 ± 1.33c			
	HDC-Con	10.73 ± 0.66b			

Note: Data in the same column with the same letter are not significantly different ($P > 0.05$); all the readings were analysed by three replicates. Con-Con: conventional followed by conventional methods; Con-HDC, HDC-HDC, HDC-CON are different combinations of hydrodynamic cavitation and conventional method.

molecular weight were significantly ($P < 0.05$) smaller than the standard pullulan (190.80 ± 1.18 kDa).

Hydrodynamic cavitation has been demonstrated by other researchers to produce lower molecular weight bioactive compounds. Yan et al. employed hydrodynamic cavitation to degrade chitosan and obtained lower molecular weight products [32]. Hydrodynamic cavitation was also employed by Muley et al. to produce lower molecular weight peptides from isolated whey protein [33]. The mechanisms for molecular weight reduction may be due to both mechanical effects and chemical effects. The shear forces generated by cavitation can physically break down large molecules, including polysaccharides [34]. The shear forces cause the polysaccharide chains to be fragmented into smaller molecular segments. In addition to the mechanical forces, cavitation-induced sonoluminescence can produce highly reactive species, such as hydroxyl radicals (•OH) and hydrogen radicals (•H). These radicals can initiate chemical reactions, leading to the cleavage of covalent bonds in the sodium alginate polymer chains and reducing its molecular weight [35].

3.2. FT-IR

The raw FT-IR absorbance spectra of extracted laminarin and sodium alginate collected over the wavelength range of 400–3800 cm^{-1} are shown in Fig. 3. It can be observed that the spectra of the samples extracted using the conventional method have similar spectral profiles to those extracted using the HDC method but have a relatively higher absorbance intensity in general. The spectral features of the averaged spectra were depicted as absorbance peaks at the wavelengths of 3700 cm^{-1} and 3000 cm^{-1}, which corresponds to the O—H and C—H stretching of polysaccharides [36]. Laminarin exhibited anisomeric stretching, which was detected by the band around 1648 cm^{-1}. The presence of a carboxyl group was identified by the band near 1380 cm^{-1}. The bands at 1420 cm^{-1} and 1253 cm^{-1} are related to C—H bending of saccharides and the C—N bending of peptides that might be extracted in a minor amount during the extraction processes [37]. The bands of 1071 cm^{-1} and 1035 cm^{-1} are associated with the CC and CO stretching vibrations of glycosidic bonds and pyranoid ring of saccharides that are reported by Rajauria et al. [9]. In terms of sodium alginate, the observed bands at 1604 cm^{-1} and 1409 cm^{-1} were attributed to asymmetric and symmetric stretching vibrations of the COO- groups, respectively, and are specific to the ionic binding [38]. The peak of C—C stretching (1035 cm^{-1}) shows a higher intensity, suggesting a stronger O—H binding vibration and a larger molecular weight of sodium alginate extracted by conventional treatment than the HDC process. The band at around 1081 cm^{-1} can be attributed to the complex vibrations involving the stretching of the C6-O6 bond and the deformation vibration of the C4-C5 bond [39].

3.3. Antioxidant activities of laminarin

The DPPH radical-scavenging activity of HDC laminarin extracts (76.44 %) was similar to those obtained for Sigma laminarin (87.78 %) in a concentration-dependent manner but a significant ($P < 0.05$) higher compared with conventional laminarin (51.14 %). Sodium alginate extracts from both methods showed no significant difference ($P > 0.05$) in DPPH compared to Sigma sodium alginate.

Ferric-reducing antioxidant power (FRAP) values were measured in laminarin and alginate extracts for their ability to reduce TPTZ–Fe (III) complex to the TPTZ–Fe (II) complex. The results showed significant differences among FRAP values ranging from 0.41 ± 0.06 to 0.68 ± 0.02 per mg DW of extracts (Table 2). The highest FRAP values were obtained in Con laminarin extract (0.68 ± 0.02 mM TE/mg). Previous studies reported similar results and attributed the high antioxidant activity in seaweed extracts could be due to the presence of both polyphenols and polysaccharide compounds [2,40].

3.4. Antimicrobial activities

The effect of laminarin extracts on the growth curve of bacteria and yeast was studied using the modified Gompertz model which has been reported to be suitable to model key microbial growth parameters such as growth rate and lag phase [41,42]. The obtained growth rate (μmax, OD unit/h) and lag time (λ, h) are presented in Table 3 along with the calculated accuracy of the model using R^2 values. It was noticed that the seaweed extracts did not significantly affect the growth rate and lag obtained from the Gompertz model for *E. coli*. While, in the case of *B. subtilis*, the growth rate was significantly altered by the extracts

Table 2
DPPH and FRAP of laminarin and sodium alginate.

Treatment	DPPH (%)	FRAP (mM TE/mg)
Sigma Laminarin	87.78 ± 3.23a	0.66 ± 0.02a
Con Laminarin	51.14 ± 2.44b	0.68 ± 0.02a
HDC Laminarin	76.44 ± 2.99a	0.46 ± 0.05b
Sigma Sodium Alginate	85.04 ± 2.75a	0.65 ± 0.01a
Con Alginate	62.21 ± 8.16a	0.41 ± 0.06b
HDC Alginate	65.63 ± 8.89a	0.44 ± 0.10b

Note: Data in the same column with the same letter are not significantly different ($P > 0.05$); all data are the means from 3 replicates.

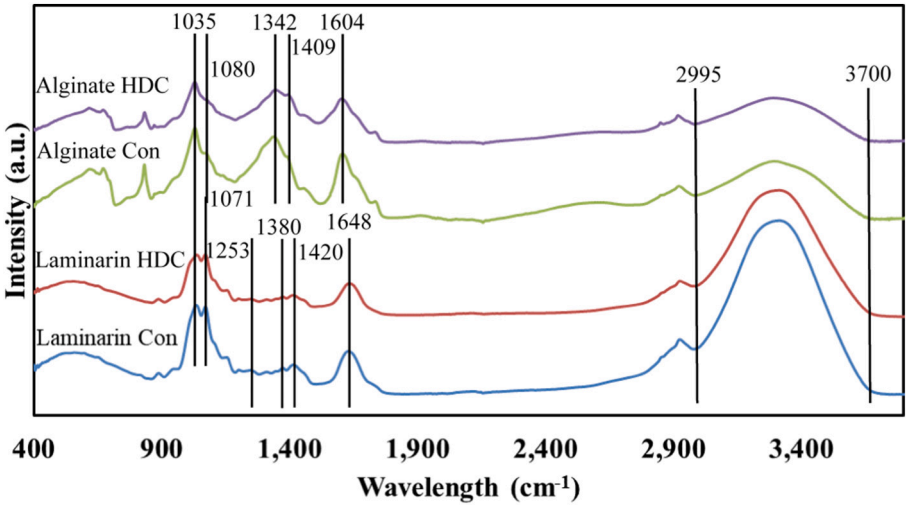

Fig. 3. FT-IR spectra (400–3800 cm^{-1}) of the laminarin and alginate extracts.

Table 3
Growth rate and lag of *B. subtilis*, *E. coli* and *S. cerevisiae* of laminarin extracts.

Treatment	Concentration	*B. subtilis*			*E. coli*			*S. cerevisiae*		
		μmax	λ	R^2	μmax	λ	R^2	μmax	λ	R^2
Control	–	0.137 ± 0.04^{c}	5.515 ± 0.95^{c}	0.805	0.047 ± 0.005^{ab}	1.073×10^{-8a}	0.907	0.332 ± 0.05^{c}	12.094 ± 2.56^{b}	0.999
HDC		0.071 ± 0.02^{a}	3.156 ± 0.52^{a}	0.981	0.053 ± 0.003^{ab}	2.473×10^{-8a}	0.921	0.224 ± 0.09^{a}	8.570 ± 1.73^{a}	0.989
Conventional		0.086 ± 0.01^{ab}	3.667 ± 0.21^{ab}	0.991	0.051 ± 0.007^{b}	1.238×10^{-8a}	0.922	0.237 ± 0.02^{ab}	8.413 ± 0.87^{a}	0.998
Sigma	2.5 mg/mL	0.109 ± 0.01^{b}	5.094 ± 0.10^{c}	0.881	0.041 ± 0.006^{a}	6.896×10^{-9a}	0.930	0.299 ± 0.04^{bc}	12.670 ± 1.63^{b}	0.990
HDC		0.083 ± 0.01^{ab}	3.795 ± 0.26^{ab}	0.979	0.050 ± 0.006^{ab}	1.352×10^{-8a}	0.897	0.274 ± 0.07^{abc}	9.102 ± 1.09^{a}	0.997
Conventional		0.088 ± 0.01^{ab}	3.841 ± 0.30^{b}	0.974	0.054 ± 0.012^{b}	3.495×10^{-8a}	0.918	0.249 ± 0.05^{ab}	9.847 ± 2.76^{a}	0.998
Sigma	1.25 mg/mL	0.104 ± 0.02^{b}	5.074 ± 0.19^{c}	0.916	0.041 ± 0.007^{a}	5.785×10^{-9a}	0.906	0.490 ± 0.03^{d}	12.522 ± 1.57^{b}	0.999

Note: Data in the same column with the same letter are not significantly different ($P > 0.05$); all data are the means from 3 replicates.

compared to the control. The lag time was unaffected by standard laminarin in comparison to the control for both *B. subtilis* and *S. cerevisiae*. It was inferred that the concentration of the extracts had no significant effect on the growth rate of the bacteria studied, while a significant ($P < 0.05$) effect was observed in commercial laminarin samples on yeast cells. Interestingly, the lag phases of *B. subtilis* and *S. cerevisiae* were reduced by the laminarin extracts obtained from the conventional extraction process and HDC, which could be due to the presence of fucoidan and mannitol residues in the extracts. Fucoidan has been shown to possess an antibacterial effect against *B. subtilis* at a concentration of 12,500–25,000 ppm [42], however, in the present study, laminarin extracts contained trace amounts of fucoidan. In a previous study, Kadam et al. investigated the antibacterial effects of laminarin extracted from Irish brown seaweeds *Ascophyllum nodosum* and *Laminaria hyperborea* against *Listeria monocytogenes* and *Staphylococcus aureus* and reported a minimum inhibition concentration ranging from 2.6 to 66.8 mg/mL [7].

3.5. Anti-inflammatory activity

The screening of anti-inflammatory activities of the various seaweed extracts against COX-1, and -2 was analysed and tabulated (Table 4). The seaweed extracts of HDC had inhibition values of 301.90 ± 0.950 (COX-1) and 294.31 ± 4.362 % (COX-2) of the enzymatic activity at a concentration of 1 mg/mL. Whereas, CON extracts had inhibition values of 305.55 ± 5.375 and 297.95 ± 1.275 %, compared to standard laminarin samples of 296.43 ± 1.875 and 294.65 ± 1.013 %. The inhibition values for the reference standard (diclofenac sodium) were found to be 295.85 ± 0.425 and 293.28 ± 0.137 %.

All the inhibition values were analysed with IC59 and SI values for the standard and samples in the assay. In addition, polysaccharides from brown macroalgae have been reported to have antioxidant, antimicrobial, anti-inflammatory and anticoagulant activities [43–45]. The anti-inflammatory effects of laminarin extracts using different green technologies showed that the extracts had a potential protective inhibition effect in the COX inhibitor screening assay as compared to the standard. These results indicate specific mechanisms of action for specific COX-1 and -2 enzymes. The anti-inflammatory effects of the Laminaria species were described in recent studies [43–45]. The reported anti-inflammatory mechanisms involve enzyme synthesis and signalling transcription factors. COX enzymes are highly involved in prostaglandin (PG) production and inhibition of inflammatory reactions [46]. Seaweed extracts can inhibit these COX enzymes that can easily alleviate symptoms related to human gastrointestinal disorders and other anti-inflammatory health benefits [47]. More specifically, COX-1 and COX-2 enzymes have been widely studied for the detailed anti-inflammatory mechanisms in anti-inflammatory disorders and others associated with pain and fever [48].

Table 4
Screening of anti-inflammatory activities of the bioactive compounds against COX-1, and COX-2.

Samples	%B/B0		IC50		Selectivity index, SI
	COX-1	COX-2	COX-1	COX-2	
HDC	301.90 ± 0.950	294.31 ± 4.362	150.95 ± 0.475	147.16 ± 2.181	1.03 ± 0.012
CON	305.55 ± 5.375	297.95 ± 1.275	152.78 ± 2.688	148.98 ± 0.637	1.03 ± 0.014
STD	296.43 ± 1.875	294.65 ± 1.013	148.21 ± 0.938	147.33 ± 0.506	1.01 ± 0.003
Reference standard	295.85 ± 0.425	293.28 ± 0.137	147.93 ± 0.462	146.64 ± 0.318	1.01 ± 0.031

4. Conclusion

In this study, the potential of a seaweed biorefinery process was demonstrated in an acid process followed by an alkaline process. The target compounds including laminarin, sodium alginate, mannitol and protein were obtained at a high purity and adequate recovery rate. Using HDC improved the biorefinery process resulting in higher extraction efficiency and extracts' purity, recovery rate and significantly lower energy consumption. Laminarin extracted was characterised using HPLC and FTIR and had similar purity and structures to those commercial standards. In addition, the anti-microbial, anti-inflammation and anti-oxidant activities of the laminarin extracts in this study were comparable or higher in terms of their bioactivity to those of the commercial standards. Overall, this research contributes to the development of sustainable and efficient biorefinery processes for the extraction of valuable compounds from renewable resources.

CRediT authorship contribution statement

Xianglu Zhu: Writing - original draft, Formal analysis, Investigation.
Da-Wen Sun: Writing - review & editing, Supervision, Funding acquisition.
Brijesh K Tiwari: Supervision, Funding acquisition, Resources, Writing - review & editing.
All others: Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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