Food-Grade Biorefinery Processing of Macroalgae at Scale: Considerations, Observations and Recommendations

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Abstract: Using brown seaweed kelp species Saccharina latissima and Laminaria digitata as feedstocks, a set of pilot-scale macroalgae processing batches were conducted (50–200 kg per batch) for the production of a range of food-grade liquid and solid fractions. The aim of this communication is to relay a number of lessons learnt during this period in combination with previous relevant observations and considerations for others who are intending to process macroalgae at scale. The novelty of this paper is thus to form a bridge between academic findings and practical know-how. Considerations covers material diversity; abiotic and biotic impact and variation; and supply chain considerations. Observations covers milling and cutting; equipment requirements; and acids including their effects on heavy metals, especially lead. Recommendations summarises key points from this pilot-scale and previous work. These include: harvest seasonality, water quality and proximity to processing facilities; minimising contaminants within the macroalgae such as stones and shells; considering equipment composition and volume for all steps and processes including final product quality; acid choice and its effects on both the equipment used and the metals bioaccumulated within the macroalgae.

Keywords: bioactives; contaminants; cutting; HACCP; heavy metals; mineral acid; pilot-scale; press; seaweed; stainless steel

1. Introduction

As global populations grow, the demands for land increase. The logical step is to move a greater proportion of production into the water and to diversify foods grown there. Macroalgae (seaweeds) do not require land, fresh water or fertilisers to grow [1] and are naturally rich in minerals, vitamins and polyunsaturated fatty acids [2]. The global market for macroalgae products is increasing annually, with a compound annual growth rate (CAGR) of 5.8% forecast between 2019 and 2027 based primarily on increases of seaweed in food products [3]. Macroalgae demand is being met by supply through cultivation, with nearly twice as much between 2009 (18.7 mt/ USD 7.8 bill) and 2018 (32.4 mt/ USD 13.3 bill) produced this way, making 97.1% macroalgae sold now harvested from cultivated material [4]. Approximately 85% of seaweeds are used either directly or indirectly as food products for human consumption [5], making this the predominant market for macroalgae. This market opportunity has been identified by numerous small and medium enterprises globally, with many lacking previous experience working with macroalgae.

Between September 2020 and January 2021, 1000 kg wet weight brown macroalgae kelps (Saccharina latissima (Linnaeus) and Laminaria digitata (Hudson)) were processed in eight sequential batches. Staff from IBERS, Aberystwyth University project managed and operated pilot-scale ERDF-project BEACON equipment based in a food-grade facility on site. A number of insights considered of value to others intending to process macroalgae in food-grade conditions at scale were learnt during this period and have been combined with previous processing points and macroalgal knowledge of value from academic studies and
2. Methodology

2.1. Resource and Pre-Process Milling

Macroalgae was sourced from three origins for this contract research, with the UK material wild-harvested and the French material from cultivated stock. Weights given below are for milled material used in the processing batches (2.2).

*Laminaria digitata* (200 kg) was harvested from Cornwall, UK in the week commencing 8 June 2020, frozen on collection, and transported to Aberystwyth under frozen conditions before storing at −20 °C. *Saccharina latissima* 1 (400 kg) was harvested in Oban, UK on 23 August 2020 and transported in boxes under ice to Aberystwyth on the 24 August 2020. *Saccharina latissima* 2 was supplied as dried, pre-milled material in 2 × 25 kg sacks (rehydrated weight 400 kg) from Algolesko (Loctudy, France).

*L. digitata* material was removed from −20 °C on 23 August with bags of approx. 20 kg spread on trays and allowed to defrost to room temperature overnight. *S. latissima* 1 and *L. digitata* were milled on 24 August 2020 using a FAM Yuran mill (FAM Stumabo, Birmingham, UK) set to 4 mm² mill size. *L. digitata* was passed through the mill twice to reduce particle size after insufficient reduction in size from the first mill. Once milled, material was transferred to lidded storage boxes holding an average 12.7 kg (*S. latissima* 1) and 15.2 kg (*L. digitata*) per box. Boxes were stored at −20°C before defrosting at room temperature prior to processing.

2.2. Batch Processing

Batches began with smaller weights and increased through the contract. Batches 1 and 2 were 51 and 49 kg of *S. latissima* 1 and *L. digitata*, respectively. Batches 3–5 were *S. latissima* 1 at 78, 131 and 154 kg, respectively. Batch 6 was *L. digitata* at 162 kg; batches 7 and 8 were *S. latissima* 2 at 25 kg dry weight (approximately 200 kg wet equivalent) each.

Each batch consisted of two water washes with decanter centrifuge separation between and after washes; two dilute sulphuric acid washes with decanter centrifugation as before; particle treatment through maceration (Gamme master MX-DMX-410, Dymanic, Mortagne sur Sevre, France) or cavitation (company prototype, specifics confidential); an enzyme hydrolysis incubation, enzyme denature step and separation through decanter centrifugation; neutralisation of the solid fraction and drying of the solid fraction. An outline processing diagram is shown in Figure 1. Due to the commercially sensitive nature of this work, further specifics cannot be reported on within this paper.

![Diagram of macroalgae processing](image)

**Figure 1.** Schematic of macroalgae processing at scale, showing the main steps of the process. Inverted ‘T’s indicate stirring. Between steps, material was stored at 4 °C (overnight) or at −20 °C (longer term).
3. Pre-Processing Considerations

3.1. Resource Diversity

Macroalgae, or seaweeds, are very generalised terms for organisms which predominantly grow in the marine environment and share some characteristics to one another but which are highly evolutionarily diverse. Frequently divided into colours of red, green and brown within the divisions Rhodophyta, Chlorophyta and the class Phaeophyceae, respectively [6], each algal ‘type’ has different structural and storage compounds to the others. Thus, ‘seaweed processing’ will differ considerably both between and within these divisions. Macroalgae within Rhodophyta contain the hydrocolloid agar (processed from, e.g., Gracilaria and Gelidium genera) or carrageenan (processed from, e.g., Kappaphycus and Eucheuma genera); Phaeophyceae contain the hydrocolloid alginate (processed from, e.g., Laminaria and Macrocystis genera) [7]. Chlorophyta, e.g., Ulva spp. do not contain hydrocolloids but do contain ulvan, a sulphated polysaccharide with low viscosity properties [8]. These hydrocolloids all have identified bioactive properties, such as boosting the immune system and demonstrated anti-cancer properties [9]. Other commercially important compounds with similar or enhanced bioactivities are also ‘type’ specific, with Phaeophyceae containing fucoidan, laminarin and phycarine; and Rhodophyta containing porphyran [9,10]. All seaweeds also contain high concentrations of elements, typically detected through ash quantification [2], and with these different structural and storage molecules can and often do react or handle differently to terrestrial biomass. This is something which is regularly overlooked and can cause significant issues unless initially considered.

3.2. Variation of Feedstock—Abiotic

As with terrestrial crops, the chemical composition of macroalgae varies greatly based on the stage of cultivation and the environment in which it is grown. For example, in L. digitata, the desirable storage carbohydrate laminarin is minimally abundant early in spring but peaks later in the summer as the algae matures. The relative abundance of elements, such as alkali metals, in the algal tissues is inversely related to laminarin content, indicating tradeoffs in physiological requirements throughout the year [11,12]. Alternatively, the presence of some compounds, such as alginic acid, is constant throughout the year [13]. Location is also highly impactful, with macroalgae typically growing better in areas containing high concentrations of dissolved nutrients, low resuspended organic material and sufficient light penetration, such as that seen in deeper coastal waters [14]. Macroalgae are bioaccumulators, meaning they will take up metals, including heavy metals, to concentrations above the surrounding environment and potentially exceeding food safety limits [15]. Harvesting should therefore occur from areas with low heavy metal content, with metal concentrations determined in the macroalgae and their removal considered if required in downstream processing.

Other additional factors include water temperature, light irradiance and salinity concentrations [16]. If the macroalgae are cultivated, set growth depth [17] can be controlled, as can seedling age and density, leading to a more standardised, higher quality ‘crop’ than from wild harvesting with potentially few opportunistic other algae present. Hatchery conditions can also affect off-shore macroalgae composition; for example, in off-shore cultivated U. fenestrata, low seedling density increased the proportion of carbohydrate in the harvested material, but high seedling density was the most important factor for producing high total biomass [18].

3.3. Variation of Feedstock—Biotic

Biotic factors, such as sea snails and other herbivores grazing over the algae, cause algal tissue wounding. This can create cracks in the underlying tissues leading to mechanical damage such as a reduction in stiffness, strength or extensibility, in turn leading to an increased propensity in breaking and subsequent biomass loss [19]. Differently sourced seaweeds may also generate different responses to biotic and abiotic stresses; for example,
the Phaeophyceae *Silvetia compressa* from northern California responded to low levels of herbivore grazing whereas *S. compressa* from southern California did not, even after 24 days of acclimatization [20]. Epiphyte presence and abundance is another consideration, both for the health and yield of the cultivated seaweed which could be negatively affected [21]; and for contamination in subsequent processing.

### 3.4. Supply Chain

Large scale farmed macroalgae biorefinery processing is dependent not only on the availability of macroalgae feedstock but also that the supplied macroalgae is of the correct quality and price. Proximity of farmed material to processing location is also a strong consideration, as harvested macroalgae is subject to rapid microbial degradation [22] and composition losses, e.g., carbohydrate and polyphenol losses in kelps [23]. If no steps are taken to stabilise fresh macroalgae, microbiological counts of spoilage bacteria (aerobic colony count) very rapidly exceed the upper limit of determination, and yeasts and moulds start to be problematic [24]. Unpublished industry-led studies [25] have shown that microbial degradation is critically dependent on both seawater temperature and storage temperature after harvest. Sea temperatures for Scotland are 6–9 °C in winter, 12–15 °C in summer; elsewhere round the UK, typical minima could be as low as 4 °C but in summer typical maxima could be up to 19 °C. These variations are strongly dependent on depth and weather [26] as well as tidal mixing [27] and with regional and local variation occurring outside these ranges. Unpublished industry-led studies [25] have determined a number of options to manage stability during and after harvest.

#### 3.4.1. Initial Processing

1. **Temperature control during harvest.** Macroalgae can be held floating in the sea in nets using the water as a coolant and isonicot storage facilities to delay deterioration for extended periods. For example, deterioration of algae was only detected after two weeks in French *L. hyperborea* fragments [28].

2. **Temperature control on land.** Keeping the macroalgae cool prior to processing is critical to reduce or prevent spoilage; simple steps such as using insulated bulk containers, packing nets of macroalgae with ice, or keeping out of strong sunlight all help reduce heating.

3. **Stabilisation prior to processing.** There are a range of stabilisation options which will depend on downstream processes and end products. For short-term stabilisation: fresh macroalgae should be held between 1–8 °C before processing. If chopping macroalgae, storing the pieces in insulated tanks slows degradation as does anaerobic storage. For medium-term stabilisation, blast freezing is recommended. For long term storage, drying to <10 % moisture is recommended.

4. **Stabilisation during processing.** Use Hazard Analysis and Critical Control Points (HACCP) analysis or other national food standard guidance [29] to identify critical control points to control microbial load during processing, including possible kill steps.

#### 3.4.2. Final Product

Almost all macroalgae biorefinery processing will include the manufacture of food products, with European uses focussed mainly on the production of hydrocolloid thickeners [2]. The processor must control all of the food safety hazards that are associated with macroalgae, from concentrations of iodine and heavy metals [15] to those from the macroalgae environment, including foreign bodies, pathogenic microbes, allergens including crustaceans, and environmental contaminants such as polyaromatic hydrocarbons [30]. These hazards should be managed by standard food safety prerequisites (e.g., hygiene controls and pest control), and through a comprehensive food standard plan such as HACCP. This plan needs to be built into the whole supply chain, i.e., the farmer should also implement a plan for their role in the macroalgae production. The processor and farmer...
should work together to establish a traceability system which ensures 100% traceability of all macroalgae from harvest through to finished products. Biorefinery processing critical control points will depend on the processes being used, but are likely to include ‘kill’ steps to eliminate pathogenic bacteria, and steps to reduce heavy metals and iodine. Processing must be supported by a programme of monitoring to ensure product compliance with regulations and in-house specification, enabling the product to be sold [25].

4. Observations

4.1. Milling and Cutting

Previous experience has shown that different seaweed types mill and handle differently. Brown macroalgae contain the hydrocolloid alginate, which not only acts as a thickener and gelling agent once extracted [7] but also when processing. This also means that putting brown seaweeds such as the kelp *L. digitata* through a screw press yields almost no liquid [1] unless other additives such as dilute hydrochloric acid are included [31] despite their high moisture content. In contrast, screw pressing the red seaweed *Palmaria palmata* gives a good juice yield [32], as does screwpressing green *Ulva* spp. [33]. This does offer opportunities for brown macroalgae processing to either use juicing systems such as a screw press for particle size reduction, or to use cutting machines without concern regarding significant moisture or bioactive loss.

In our scaled processing, one key observation was that when using commercial cutting machines, it is imperative that the macroalgae material is clean, taken either from rope cultivation or hand-harvested with the holdfasts left at sea. Even a few kelps taken from the surrounding environment with foreign bodies within their haptera, such as stones or toughened shells, e.g., top shells (*Steromphala* spp.), cause damage such as dents and nicks to arise on cutting blades, causing incomplete cutting to subsequent samples.

4.2. Equipment Considerations

In previous lab-based studies, glassware has been typically used with autoclaving as the sterilising route of choice. As processing scale expands, glassware and autoclaving will become unviable, with vessels typically replaced by plasticware, especially if disposable; or metal containers, potentially with liners; with cleaning conducted in situ using steam or disinfection regimes. Equipment has to comfortably take all batch volumes for all relevant steps to be held within it.

For food-grade processing, a typical clean-in-place regime consists of a pre-rinse, caustic wash, water rinse, acid wash, water rinse, and then sterilisation in place. Sterilisation can be by chemical disinfectant or steam. Chemical disinfectants should be effective, safe and easy to use, easy to rinse away, and should leave no toxic residues on surfaces [34]. Steam sterilisation is a reliable method of sterilisation; however, with it comes a high energy demand and high capital cost, as equipment must be designed to withstand the required steam pressure [35].

Glass vessels for small-scale work have a range of advantages, including being corrosion-resistant, non-toxic and transparent, allowing easy inspection of its contents [36]. However, at a large scale, glass vessels are unsuitable for in-situ steam sterilisation as any damage or cracks in the glass could result in breakage; ideally if a vessel is too large to fit in an autoclave, it should not be made of glass, although chemical disinfection can be used [35]. At a larger scale, disposable plastic process bags for liquid storage and single-use bioreactors with disposable bags have the advantage of no cleaning or sterilisation requirements, which, along with quick and easy deployment, shortens overall processing time. However, disadvantages include limited options for mixing contents and size limitation, with most single bioreactors built up to 2000 L and the largest size of single use bioreactors currently available at only 6000 L [37]. Stainless steel vessels are widely used in bioprocessing, making them well established and understood; advantages over single-use plastic reactors include larger capacities and better mixing, heat transfer and oxygen transfer [36].
Disadvantages include higher maintenance requirements and for cleaning and sterilisation in place to be built in, resulting in high utility costs and longer processing times.

In our scaled processing, we had to calculate all the weights and volumes needed to complete all steps of the process, then review, as these occasionally resulted in unanticipated bottlenecks. For smaller batches (<100 kg macroalgae), initial washes were conducted in 250 L stainless steel vessels with integrated scales, allowing highly accurate liquid addition as a proportion of macroalgae present. For batches >100 kg wet weight, water and acid washes were conducted in a high-density polyethylene intermediate bulk container (IBC). For both vessel and IBC, a stainless steel overhead stirrer was used to provide mixing of the slurries. For subsequent processing steps, containers and/or mixing devices were composed of stainless steel or non-degrading purpose-made plastics.

Sample quality was principally maintained through a short processing schedule and storage at 4 °C overnight between steps. Due to the acidic nature of the material for much of the processing and a high-temperature enzyme denaturing step, microbial degradation was not a major concern. Equipment cleaning was primarily conducted using pressurised hot water and physical cleaning; again, due to the low pH of the macroalgae, a partial clean-in-place treatment was occurring during processing. For non-accessible regions such as tubing and pipework, additional cleaning regimes were applied involving circulation of hot detergent solutions. Equipment was also reserved for this feedstock only during the processing of these batches to prevent contamination from other sources.

4.3. Acids

In addition to cleaning and practical issues, the material that equipment is constructed from has further impacts regarding the chemicals used. The initial step (and typical first step) in macroalgae processing is that of fresh water washes. These are conducted to remove contaminating particulates but concurrently extract soluble components such as laminarin and minerals [38]. Subsequent washes with acidic solutions further solubilise compounds and metals [39], necessary for reducing any potential heavy metal concentrations in the macroalgae product. The primary objective of acidic washes for Phaeophyceae, though, is to convert insoluble calcium alginate (with trace quantities of strontium alginate and magnesium alginate also present [40,41]) to alginic acid. This is an intermediate step in the conversion to soluble sodium alginate [7]. In order to achieve the primary ion exchange reaction where alginate’s divalent metal counterions are replaced with hydrogen ions, it is important that a strong acid is used, and that the concentrations used are sufficient to maximise the displacement of the divalent elements [7]. Hydrochloric acid and sulphuric acids are both suitable strong acids for converting divalent cation salts of alginate to alginic acid.

However, these acids differ in their effect on larger-scale metal equipment. Hydrochloric acid is a reducing acid without the oxidising properties required by stainless steels to maintain their surface layer, meaning that common stainless steels are considered non-resistant to hydrochloric acid at any temperature or concentration, and higher grade stainless steels provide only a limited resistance against it [42]. Sulphuric acid is more complex, with most stainless steel types resistant to corrosion at low or high concentrations but becoming damaged at intermediate concentrations. This can occur inadvertently, as sulphuric acid has a high affinity for water. This means that a safely concentrated acid can become diluted enough to display corrosive properties following water absorption from the surrounding environment [43], and rigorous controls and monitoring will need to be adhered to.

A further issue regarding sulphuric acid is that unpublished, industry-led research [25], confirmed through these scaled-up batches, has identified an increase in the relative concentration of lead (Pb) in the remaining solid. In previous small-scale and in these pilot-scale trials it was shown to raise the lead concentration above 0.5 ppm, which is at or above the regulatory limit for the majority of listed foodstuffs [44]. Lead cations have been shown to have a higher affinity to brown algal biomass than other heavy metals such as...
cadmium and nickel, causing precipitation on the cell wall matrix [45]. It appears that this occurs because lead sulphate (PbSO$_4$) is almost insoluble in water [46] and it is therefore ‘trapped’ in the solid and not extracted. In contrast, hydrochloric acid-derived lead chloride (PbCl$_2$) is more soluble, with the solubility increasing if high-concentration chloride is used as higher-order chlorocomplexes are formed [47]; collectively, this means that lead is removed during hydrochloric acid washes. Previous small-scale and these pilot-scale studies on kelp did not see an increase in other heavy metals tested (including cadmium (Cd), mercury (Hg) and inorganic arsenic (iAs)) indicating this is an issue predominantly affecting lead over other elements [25].

5. Recommendations

Processing at pilot scale has highlighted a number of issues not foreseen in lab-scale processing. For those considering scaling up processing, below are several recommendations for consideration which have either arisen during recent pilot-scale processing or are from previous experiences but considered of value.

5.1. Macroalgae Feedstock

- Optimise the condition of material, harvesting proximity and time of year harvested for the main extraction product of interest.
- Minimise level of contamination to acceptable levels, both for snails and stones and for epiphytes.

5.2. Equipment

- Consider the robustness and specifics of all equipment to be used, including vessels, centrifuges and pumps, to identify potential weak points or bottlenecks.
- Review alternatives, e.g., using plastic and non-metal presses, retrofitting existing equipment if needed. Review whether this will affect cleaning or processing regimes.
- Review working volumes for processing, including for future scale-up. One large vessel is often preferable to replicating steps using a smaller vessel.

5.3. Acid

- Consider the impact of selected acid on both the equipment used (especially if constructed of stainless steel or other metals) and the metals within the macroalgae, taking into account the final product use.

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