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Executive summary

This deliverable summarises the state-of-art regarding various methods of processing and storage which are, or may be, suitable for use on seaweeds destined for various different commercial uses. Specifically, this paper explores how the processing and end use of seaweed are closely linked, through identification of methods that are able to maintain the desirable characteristics with minimal cost and effort. For some uses, such as bioactives, expensive and time-consuming high-tech methods may be essential, whereas for others such as biofuels, cheap and straightforward solutions are key to storage of seaweed as a low value feedstock. As GENIALG progresses, the authors will augment this review with new experimental and industrial findings, drawn from the consortium's practical work and experience acquired through research projects that specifically investigate the feasibility of any one storage method (e.g. ensilage via UK SEAGAS or Macrobiocrude). This process will inform a discussion aiming at identifying best storage practices, as well as future research priorities.

Desk study report on potential storage methodologies for seaweed biomass

1. Introduction

Most seaweeds are aquatic organisms adapted for a sessile, sedentary life within the marine, or intertidal environment. Ripping them from their home during harvest is very stressful, and unavoidably leads to their death. In fact, interfering with seaweed will result in changes to their physical, chemical and biological characters (Karel et al., 1993). For instance, harvesting and slicing seaweed increases oxygen exposure, triggers wound responses and activates enzymes that can catalyse degradation (Amarowicz et al., 2009). Even cleaning seaweed, often the first processing step, can be damaging, as freshwater exposure can accelerate the degradation of tissues compared to seawater (Liot et al., 1993).

A variety of post-harvest procedures are currently used for seaweed around the world.
The methods so far developed are wide-ranging, and heavily dependent on which characteristic/s of the seaweed are valuable for the end use of the material. Three cases are identified:
1. To be eaten fresh. In this case the seaweed must be kept alive within, their sometimes highly restrictive physiological tolerances, whilst retaining favourable organoleptic characteristics such as texture, smell and taste. This end-use requires only short term storage from a few days to weeks.
2. Stored for longer-term, while retaining maximal quantities of easily degraded chemical constituents. This case encompasses their use as a) highly nutritious food ingredients, where a long shelf life is desired, and favourable organoleptic characteristics must also be maintained or b) for the extraction of refined bioactives. In this case, the seaweed will be stored dead, in stable conditions to limit any changes to the biomass.
3. Stored in volume for large scale industrial processing such as the bulk extraction of chemicals e.g. hydrocolloids, use in animal feeds or biofuels. In this case, only a very few sensory and chemical characteristics of the biomass are important to the final product, whereas high throughput processing and cheap storage are essential to allow business profitability. Therefore, the processing is often low-tech or quite aggressive, causing considerable degradation of the biomass compared to in 1 or 2. Processing not only varies with end-use, but also depending on the species and the scale and technicity of the operations (Radulovich et al., 2015).

2. Drying

Drying is the oldest and most important post-harvest handling procedure used for worldwide agricultural produce (Bonazzi et al., 1996; Fudholi et al., 2011; Gardner and Mitchell, 1953a). Drying has many advantages:

1. Allows storage by limiting the microbial, chemical and enzymatic activity;
2. It removes most of the water and so reduces the weight (and potentially volume) of the crop, so reducing downstream transportation costs;
3. Extends the useable lifetime of the crop;
4. Allows the maintenance of a relatively constant price for the farmer by improve their bargaining position and;
5. Produces a simple to handle ingredient for other products.

Similar to other agricultural productions, drying is also the most important post-harvest procedure for seaweed (Radulovich et al., 2015), particulary since wet seaweed is known to deteriorate fairly quickly (Naylor, 1976) limiting the processing time. A large quantity (numbers) of seaweed is dried each year for the hydrocolloid industry (Porse and Rudolph, 2017), and it is known that these can be stored for years with minimal loss of the gel content (Naylor, 1976).

Various drying methods have been developed, each with their own costs and benefits. During different drying processes, the material can undergo multiple processes that can differentially affect the physical (colour change, rehydration, texture), biochemical (browning reactions, lipid oxidation) and also nutritional (vitamins and antioxidant loss) properties of the material (Bonazzi and Dumoulin, 2011). The final use of the material will therefore strongly influence the method of drying based on the allowable changes to the material. E.g. a shrivelled and sun bleached seaweed may be acceptable for hydrocolloid extraction, but will not be suitable as a food product or for pigment extraction.

The moisture content of raw food products varies widely, from 25-35% in grains, to as high as 90% in fruits or seaweed. This water content needs to be reduced, both to avoid microbial growth and inhibit degradative enzymes (Troller, 2012; Vairappan, 2003). Water activity (a_w) is a better indicator of product stability than the % water content alone; a_w is calculated as the partial vapour pressure of water in a material divided by that of distilled water. The a_w scale runs from 1 (pure water) to 0 (no water). To guarantee food product quality, it is usually recommended to achieve a $a_w<0.6$ (see details in Rahman and Labuza, 1999), as enzymatic activity is greatly inhibited when $a_w<0.75$ and microbial activity is suppressed at 0.6-0.7 (Bonazzi and Dumoulin, 2011). Maximum stability of other biomolecules such as pigments is observed below $a_w=0.12$ (Bonazzi and Dumoulin, 2011). However, while low water activity maybe desirable for many end uses, lipid oxidation, responsible for rancidity and off flavours, is enhanced at very low $a_w<0.15$ (Bonazzi and Dumoulin, 2011). Therefore, water content should be carefully optimised depending on the intended use of the raw material.

The water activity of a material is non-linearly related to the water content by its moisture sorption isotherm curve for a given temperature. This allows a prediction of stability over

time in different storage conditions, or in other words, how much moisture it can gain or loss during storage or drying before a_w>0.6, allowing microbial growth.

2.1 Sun drying

2.1.2 Principles and applications outside seaweed

Sun drying is the most ancient and cheapest method of crop preservation, and is still the most commonly used method in tropical and sub-tropical countries, where most seaweed is cultivated to date (Esper and Mühlbauer, 1998). The crop are spread out on a flat surface or left bound in bundles in the field. Short wave energy from the sun is absorbed unevenly by the crop surface, depending on crop colour, while some is reflected. This is converted to long wave thermal energy raising the surface temperature. Moisture and is then lost from the surface in the form of evaporation, in the initial fast stage. Further heating of the surface is conducted towards the interior and helps to mobilise water diffusion towards the surface where it can then evaporate (Sharma et al., 2009). This second stage is far slower, dependent on the thickness of crop layer. This can be accelerated by turning the crop.

Sun drying requires a large open space over a long period, dependent on the availability of sunshine (continuity, day-length and intensity). Problems with such an open system are that the crop is susceptible to contamination with foreign material such as wind-blown debris and are exposed to the activities of rain, rodents, insects, birds and microorganisms which can lead to considerable crop deterioration, loss or contamination (Esper and Mühlbauer, 1998). If the sun is intermittent, crops can also become over/under dry (Murthy, 2009).

The process requires a large area, is labour intensive and exposure to UV radiation often causes characteristic discolouration and a low product quality. The outcome of sun drying is a product of extended shelf-life but drastically reduced quality compared to the fresh material (Ratti, 2001). Sun-dried products generally do not fulfil the international food quality standards, preventing their sale on international markets (Esper and Mühlbauer, 1998). This rudimentary method is still in use in many (mostly tropical) countries, but the introduction of mechanical drying has often been and still remains encouraged by the authorities, resulting in higher quality product that command higher retail prices (Luxton, 1993).

2.1.2 Sun drying seaweed

In the case of seaweeds, sun drying may make place on concrete, tarmac surfaces or hung on racks and can produce material with 30-35% moisture (see e.g. Ling et al., 2015; Radulovich et al., 2015). Seaweeds traditionally dried in this manner include *Kappaphycus/Eucheuma* for carrageenan, *Gracilaria* and *Sargassum* spp. for food/fodder *and Laminaria japonica* for alginate. Due to the propensity of seaweed for fast deterioration (Naylor, 1976), the crop must be attended to over several days, including regular turning to allow even drying (Radulovich et al., 2015). Rain is known to damage the process.

In Scotland, since at least the 1930's *Laminaria hyperborea* were air-dried on walls to 20-30% moisture, and then bulk-shipped for alginate extraction within the establishing industry industry (Gardner and Mitchell, 1953a; Gardner and Mitchell, 1953b; Gardner and Mitchell, 1953c; Reid and Jackson, 1956). Fronds did not dry well and thus were discarded. Trials in the 1940-50s then assessed whether solar drying could be utilised to establish a *Ascophyllum nodosum* harvesting industry, similar to the one in Norway and Iceland (Reid and Jackson, 1956 and references therein). These found that the best method was to use inclined horizontal grids, where air could blow through the seaweed and rain could drain off. 6" bed could then be dried to 50% moisture in 54hrs or 12" beds over 150hr, despite significant rainfall and an ambient humidity generally above >80%. However, the limited success of these trials meant that sun drying never became established.

A comparative studies of sun-, oven- and freeze-drying, found that sun-drying did not affect the total protein or lipid content of *Sargassum hemiphyllum*, but did result in the lower concentrations of ash, mineral and vitamin C (Chan et al., 1997). These losses are reasoned to be due to cellular leaching due to the lower drying rate and antioxidant loss due to UV exposure. A study comparing 7 drying methods on *Kappaphycus alvarezii* (crocodile morphotype) found that sun-dried seaweed had the lowest phytochemical content (anthocyanin, carotenoids, phenolics and flavonoids), scavenging activity and reducing activity (Ling et al., 2015). These finding agree with the literature on higher plants, where UV radiation, light and air and know to lead to degradation of not only anti-oxidants like vitamin C, but also potentially valuable phytochemicals such as tocopherols and carotenoids (Klein and Kurilich, 2000). In a separate study on the *K. alvarezii* (giant morphology), sun-drying resulted in a low phenolic and flavonoid compounds, generally lower antioxidant activity and white bleaching (Neoh et al., 2016); generally considered an indicator antioxidant loss (Ratti, 2001). In both studies sun dried material was measured to have high free radical scavenging ability with only the DPPH antioxidant assay but not the FRAP or ABTS assays (Ling et al., 2015; Neoh et al., 2016). It is considered that this may be a peculiarity of the assay.

2.2 Solar drying

2.2.1 Principle and applications outside seaweed

Some of the disadvantages of sun drying, such as exposure to outside interference, UV-driven bleaching and biochemical deterioration, can be remediated through the use of solar drying, leading to faster production of a higher quality product (Murthy, 2009). In this process, the material to be dried is contained within an enclosed area. Short wave energy from the sun is collected and converted to thermal energy heating the air within the enclosure. In simpler direct driers, a glass roof may allow the light to fall directly onto the crop, carrying a risk of bleaching. In indirect driers, a solar collector, is used to heat air, causing convective flow over the product (Murthy, 2009). Indirect systems are necessarily larger, but also more efficient (Sharma et al., 2009). Air flow within the enclosure causes evaporation from the crop, with the moist hot air vented outside.

By increasing the area of solar collection and trapping the heat generated, solar driers are up to 50% faster and more efficient than sun-drying (Esper and Mühlbauer, 1998; Sharma et al., 2009). Other advantages include the creation of a hygienic environment away from contaminants, the retention of greater nutritional value such as vitamin C and enhanced marketability of the product due to greater consistently and more appeal look (Sharma et al., 2009). Solar driers are therefore suitable for small-scale processing of high quality food products (Sharma et al., 2009). The design of such solar driers can be made from simple materials and does no necessity any mechanical drying equipment. This makes solar drying a very promising application of solar energy (Fudholi et al., 2011; Fudholi et al., 2012), suitable for developing countries, although continued reliance on climate and weather are a disadvantage of such systems.

A number of different forms have been developed including cabinet/ chimney, greenhouse and tunnels (Esper and Mühlbauer, 1998; Murthy, 2009). Several features enhance efficiency or speed up the process, such as including V grove collectors, mechanically forcing (ambient or dehumidified) air circulation. Installing supplementary heating, double pass or thermal storage systems also enable the process to continue off-sunshine, e.g. at night (Chauhan et al., 1996; Fudholi et al., 2010; Murthy, 2009). Further information can be obtained in the excellent reviews by Fudholi et al (2010) and Murthy (2009).

2.2.2 Solar drying of seaweeds

Solar drying of seaweed is currently only conducted on a small scale. Mohammed et al 2009 found that *Gelidium sesquipedale* took between 1-3 hrs to dry in a force air system with auxillary heater, operating in 50-57% humidity. The shortest run time was needed at 60^{o}C, or 50^{o}C with enhanced air flow. In a larger system, Fudholi et al (2014) found that it took 15 hr to dry 40kg of *Kappaphycus alvarezii* (synonym of *Eucheuma cottonii*) to 10% moisture, with the chamber conditions generally 40-60^{o}C and 35-50% relative humidity. In a larger scale experiment, Ali et al (2015) reported that the use of a forced convection solar dryer allowed to half the duration required to dry five tons of fresh *Kappaphycus*, compared to direct drying in the sun. A 50% relative humidity was achieved in roughly two days, leading to time saving of 58%. Similarly, Othman *et al*. (Othman et al., 2012) found that *Graciliaria changii* could be dried within 7hr by a force air system operating with an average temperature of 50^{o}C and 20% humidity.

The effect of the drying method on the composition of *Sargassum muticum* and *Bifurcata bifurcata* dried within a greenhouse in Brittany, France for 72hr (Le Lann et al., 2008), which had an average humidity of 66% and varied between 15-30^{o}C. This direct solar dried seaweed had reduced anti-oxidant capacity and 3-5 times lower total phenolic compounds when compared to the fresh seaweed. It is thought that direct sunlight leads to fast degradation of certain phenolics, which may explain this decline (Lim and Murtijaya, 2007).

2.3 Oven-drying

2.3.1 Principles and applications outside seaweed

Mechanised drying provides reliability, control and product consistency not achievable through solar-dependent methods. Oven-based systems require far less land and are able to dry the products to a standardised final moisture content of <20% (Radulovich et al., 2015) in a fraction of the time, day or night. Some of the constraints are that ovens have a more limited batch capacity and have far higher capital and operating costs, in the form of fuel or electricity. This makes such dryers only suitable for commercial operations which are able to handle and process large volumes of seaweed and generate substantial revenues (Sharma et al., 2009). In addition, heating the material leads to partial loss of nutrients and other thermolabile components (dependent on the set operating temperature and run time); however, this is generally far less than when using solar methods (Murthy, 2009).

2.3.2 Oven drying seaweed

Generally, lowering the oven's humidity and increasing its temperature allow for faster drying, although results are species dependent (Table 1).

Species	**Reference(s)**
Brown seaweed	
Macrocystis pyrifera	(Clark et al., 1944; Leyton et al., 2016; Park, 1934; Turrentine, 1924)
Sargassum hemiphyllum, *S. muticum*, *S. henslowianum* & *S. patens*	(Chan et al., 1997; Le Lann et al., 2008; Wong and Cheung, 2001b)
Laminaria cloustoni (now called *L. hyperborea*), *L. digitata*	(Gardner and Mitchell, 1953a; Gardner and Mitchell, 1953b; Gardner and Mitchell, 1953c)
Ascophyllum nodosum	(Chenlo et al., 2017)
Himanthalia elongata	(Gupta et al., 2011)
Bifurcaria bifurcata	(Le Lann et al., 2008; Moreira et al., 2016b)
Fucus vesiculosus	(Jiménez-Escrig et al., 2001; Moreira et al., 2016a; Moreira et al., 2017)
Saccharina latissima	(Sappati et al., 2017)
Hizikia fusiformis	(Kim et al., 2007)
Undaria pinnatifida	(Chenlo et al., 2017; Kim et al., 2007)
Durvillaea antarctica	(Uribe et al., 2017)
Hormosira banksia	(Dang et al., 2017)
Turbinaria turbinata	(Monsur Hammed et al., 2013)
Red seaweed	
Gracilaria cangii, Gracilaria sp.	(Fudholi et al., 2012; Lemus et al., 2008)
Kappaphycus alvarezii	(Djaeni and Sari, 2015; Ling et al., 2015; Neoh et al., 2016)
Gelidium sesquipedale	Mohamed *et al.* 2007; (Hnini et al., 2013; Hnini et al., 2014)

Table 1: Synopsis of oven-drying studies performed on different seaweed species.

In early trials, Gardner & Mitchell (1953a; 1953b), showed it was possible to dry both *Laminaria hyperborea* stipes and fronds in a large Pehrson drier designed for the grass. The seaweed was precut with a chaff cutter to ~1cm^3 stipe pieces and 2x2cm frond squares, then passes through a pneumatic tower 120-190^{o}C, then two rotary drum driers at 70-100^{o}C and 100-200^{o}C (seaweed temperature). Finally it was fed to a hammer mill, and after a total of 20-25 min this resulted in a milled product of <10C moisture content.

More recently, studies have focussed on the alterations, beneficial or not, of the biomass properties during the process, and how the different parameters (temperature, duration, air flow and possibly, oven humidity) could be optimised to reach best results.

The major constituents (carbohydrates, lipids, ash, protein) are generally found to not change substantially during oven drying. However, Uribe *et al.* (Uribe et al., 2017) recently found an apparent increase in ash and crude protein and decrease in carbohydrates in *Durvillaea antarctica* when dried at 30-80^{o}C compared to when fresh. Interestingly however, higher temperatures increased the extractability of some compounds of interest: *F. vesiculosus* dried at 75^{o}C subsequently yielded more alginate than if treated at 35^{o}C, though the overall carbohydrate content of the starting biomass was identical (Moreira et al., 2017). Wong and Cheung (Wong and Cheung, 2001a; Wong and Cheung, 2001b) showed that the protein content of three *Sargassum* spp. was not affected by oven drying at 60^{o}C for hr, in agreement with previous work on *S. hemiphyllum* (Chan et al., 1997) and higher plants (Julkunen-Tiitto, 1985). However the *in vitro* digestibility was higher in oven-dried compared to freeze-dried material, possibly due to a considerable reduction in phenolic compounds, which are known to influence protein digestibility (Hurrell and Finot, 1985).

In contrast, Sappati *et al*. (2017) recently presented a very detailed analysis of the kelp *Saccharina latissima.* They showed that, similar to other highly porous and polysaccharide-rich plants crops, operating at 80^{o}C resulted in greater shrinkage than at 40^{o}C, and led to a drastic change in texture. They concluded that operating at low temperature and low humidity best allowed to preserve the kelp viscoelastic properties. Notably, due to their thick fronds, case hardening was not seen to become a problem, as it can in other materials.

Another potential downside of higher oven temperatures is unwanted colour changes, due to either pigment destruction or browning reactions. For example, *Fucus vesiculosus* powder dried at 75^{o}C became more yellow, while greenness was enhanced at 50-60^{o}C (Moreira et al., 2017).

Finally, antioxidants, phytochemicals such as phenolic and flavonoid compounds are degraded during prolonged or intense thermal treatment. For instance, ascorbic acid and carotenoids in herbs decline by 10 and 2 fold respectively during drying (Capecka et al., 2005). A similar loss of antioxidants (carotenoids, flavonoids and vitamin C) was reported in semni-dried tomatoes, after a comparatively gentle processing at 40^{o}C (Toor and Savage, 2006). In general, higher drying temperatures result in greater physical and chemical degradation of the material, and losses of volatile compounds including flavour and aroma (Fellows, 2000). Therefore, if retention of such compounds is important such as in the food industry, it is necessary to optimise the oven drying protocol to the specific material if

retention of these components is important (Garau et al., 2007). Both the duration and the temperature need being taken into account in this optimisation process, as shorter drying times might give better results than prolonged exposure to lower temperatures; for example, the highest highest antioxidant capacity of orange peel/pulp was retained at 60ºC, compared to longer drying times at 30-40ºC, or rapid drying at 80-90ºC (Garau et al., 2007).

Unsurprisingly, phenolic compounds and antoxidant activity have been reported to reduce in seaweeds during oven-drying, with rapid degradation at temperatures above 40ºC. Jimenez-Escrig *et al.* (2001) found a 98% reduction of phenolics in *Fucus* after 48hr at 50ºC. Phenolics in *Himanthalia elongata* reduced by either 51% or 29% after drying at 25 or 40ºC respectively , while flavonoids reduced by 49 and 30% (Gupta et al., 2011). Likewise, phenolic compounds and antioxidant activity declined linearly with increasing temperature from 35-75ºC in *F. vesiculosus* powder (Moreira et al., 2017). In *Kappaphycus alvarezii*, oven drying at 40 or 80ºC retained the highest quantity of phenolics, flavonoids, anthocyanins and carotenoids and also high scavenging activity, compared to solar-, sun- and freeze-drying (Ling et al., 2015). Neoh *et al.* (2016) also reported a high antioxidant and radical scavenging activity was retained in *K. alvarezii* (giant morphotype) after 60 ± 5 ºC for 29 hours. Phenolics in *Undaria pinnatifida* and *Hizikia fusiformis* also reduced due to oven drying, particularly at 60ºC rather than 40ºC (Kim et al., 2007).

Interestingly however, studying the time course of these compounds during the process revealed that the antioxidant activity of *Himanthalia elongata* attributable to phenolics (as measured with the Folin-Ciocalteu reagent) showed an initial increase over the first few hours, with a maximum increase of 41% after 2h at 40ºC (Gupta et al., 2011). The authors considered that this might reflect a wound-like response; however, it is well known that in higher plants antioxidant activity and specifically phenolics can increase due to heat exposure (Nicoli et al., 1999).

Currently, many seaweed food companies currently advocate lower temperature drying to preserve the nutritional content i.e. Algamar dried at <42ºC (website). Where higher throughput is required, such as in the production of seaweed meal for animal feed this is often carried out in rotary driers using hot furnace air at 700-800ºC, with the seaweed reaching 70ºC (Kadam et al 2015).

2.4 Freeze-drying

2.4.1 Principles and applications outside seaweed

Freeze drying , also known as lyophilisation, is a process in which a frozen material, is subjected to low pressures causing the crystallised solvent (usually water) to sublime from solid, directly into a vapour phase. The absence of liquid water during this process limits most deterioration and microbial reactions (Ratti, 2001). The advantage of this process compared to the conventional drying techniques, are that the preserved material retains morphological and biochemical characteristics similar to the fresh material. It also allows the preservation of heat-sensitive biological material and prevents the loss of many volatile

compounds (Liu et al., 2008; Ratti, 2001). The process was originally developed to preserve bioactive molecules, pharmaceutical products and solvent impregnated materials (Kusakabe and Kamiguchi, 2004). It has since become increasingly popular for the preservation of food such as fruits which are temperature sensitive (Ciurzyńska and Lenart, 2011), minimising flavour loss and degradation e.g. protein denaturation, browning and enzymatic reactions. The drawback of freeze-drying is that it is an energy intensive and time-consuming process to complete; the costs of freeze-drying are 4-8x higher than air-drying. A costing analysis by Ratti (2001), showed that the cost of freeze drying becomes very low when working with high value raw materials. This allows economical production where it produces a high quality added value food or biotechnological product biotechnology (Ciurzyńska and Lenart, 2011).

2.4.2 Freeze-drying seaweed

Freeze-drying of seaweed has mostly been examined in the context of food, by a number of comparative drying studies. In foods, it is generally accepted that freeze-dried material retains the highest value for many characteristics such as pigments and antioxidant activity when compared to other drying methods (Ciurzyńska and Lenart, 2011; Ratti, 2001). It is thought that this is due to the reduced mobility of the reactants and reduced oxygen concentrations during the process (Bonazzi and Dumoulin, 2011).This appears to be in agreement with the majority of the seaweed literature.

Chan *et al.* (Chan et al., 1997) found that freeze-dried *Sargassum hemiphyllum* had higher contents of total amino acids, polyunsaturated fatty acids (PUFAs) and vitamin C compared to sun or oven drying. The total phenolic content and antioxidant activity of freeze-dried *Sargassum muticum and Bifurcata bifurcata* was found to be very similar to both fresh and 3 week frozen material by Le Lann *et al*. (Le Lann et al., 2008). In *Hormosira banksia*, Dang *et al.* (Dang et al., 2017) found that freeze-dried samples had the highest content of flavonoid and phenolic content, proanthocyanidins and antioxidant activity compared to various other drying methods. Wong & Cheung (Wong and Cheung, 2001b) also found higher phenolics in three species of *Sargassum* spp. which were freeze dried compared to oven dried. Freeze drying has also been show to retain the antiflammatory activity of the polysaccharide fraction of *Turbinaria turbinata* (Hammed *et al.,* 2013). In *Kappaphycus alvarezii*, a slightly different result was found by Neoh *et al.* (2016); freeze-dried material had lower total phenolics and flavonoids than vacuum oven-dryed and lower antioxidant activity than oven-dried, it also had higher total lipids and underwent a colour change to light green; colour retention is commonly correlated to antioxidant activity (Ratti, 2001).

The use of seaweeds as functional foods is a high value utilisation stream for harvested biomass, but requires careful processing of the material to retain the required bioactivity. Freeze drying may destabilise the native conformation of certain bioactives (Franks, 1998), in general it provides superior preservation compared to other drying methods.

As mentioned above, freeze dried material tends to retain their original volume, depending on the temperature during freeze drying (Krokida et al., 1998), usually shrinking by 5-15%, compared to air drying where shrinkage can be 80% in berries (Janković, 1993). This can be a desirable texture for certain food applications e.g. freeze-dried *Sargassum* has a greater

water/oil holding ability and so is more suitable than oven dried as a highly nutritious texturising and bulking ingredient in low calories food products (Wong and Cheung, 2001a). However, freeze-dried materials tend to collapse if heated (Shishehgarha et al., 2002). In addition, the high porosity of freeze dried material allows easy rehydration, causing them to easily collapse in liquid, although this may be avoided using coatings (Ciurzyńska and Lenart, 2011; Ratti, 2001). This high porosity also makes them more susceptible to degradation due to reactions with oxygen, negatively affecting storage stability. This increased susceptibility means that freeze dried material, should be hermetically stored in an inert atmosphere (Bonazzi and Dumoulin, 2011).

2.5 Combined treatments or pre-treatments

The vast majority of studies reviewed here investigate the performance of one approach over the other. However, there have been several attempts to combine several methods, or to add pre-treatments, in order to optimise processing of fresh biomass. Back in 1944, Clark *et al.* patented a procedure for drying chopped *Macrocystis pyrifera* (87% initial water content, down to 5-15% final) in a rotary drier for 20mins at 650-980^{o}C, followed by discharge of a 6-10 cm deep bed of seaweed on a conveyer drier for 30mins at 90-130^{o}C (Clark et al., 1944).

Whereas drying methods usually precede grinding, the possibility to grind the seaweed before, or during the drying process has also been explored. Back in 1956, Booth presented a technique that combined milling and steam drying of several species of brown macroalgae. The main innovation underpinning this process was the introduction of a desingrator allowing to separate the tramp materials (e.g. stones attached to the seaweed) from the product (Booth, 1956). More recently, Bono (Bono et al., 2011) reported that spray-drying as a promising method to process *Kappaphycus* in a very controlled manner.

Also, Garcia & Bueno (1998) described combined convective-microwave drying for high-value products, in this case agar extracted from *Gelidium*. Similar to the food sector, it appears that microwave-assisted drying is usable, but requires significant know-how and initial investment costs, thus hindering its large-scale adoption in the industry (Zhang et al., 2006). Pre-treatment of *Undaria pinnatifida* with ethanol, followed by spray drying, was also described as a method to remove undesirable smell to fucoidans (Cho et al., 2011). It is clear that complex processes can only be envisaged for the production of high-value products, and will probably need to be tailored on a case-by-case basis.

2.6 Long term stability of dried material

While there are quite a few comparative studies on the immediate effects of various drying methods on the biochemical constituents of seaweed, much fewer studies so far have considered the long term impacts of storage. Oxidative deterioration during storage can lead to the destruction of anti-oxidants, vitamins, pigments, amino acids or lipids, leading to the development of off-flavours (Gardner, 1979; St. Angelo and Ory, 1975). Lage-Yusty *et al*. (Lage-Yusty et al., 2014) evaluated the composition of 45^{o}C dried *Himanthalia elongata, Laminaria spp., Undaria pinnatifida, Palmaria palmata* and *Porphyra umbilicalis* stored for 18

months in polypropylene bags at 20-25ºC. All species underwent substantial loss of antioxidant activity and pigments. In all samples vitamin C and E was lost within 3 months, except in *H. elongata* where higher initial values of both vitamins allowed their retention for up to 6 or 18 months, respectively. Chlorophyll *a* was lost within 12 months in the brown algae, whereas it was very low initially in *P. palmata* and lost within 3 mo, or was not detectable (*P. umbilicalis*). Fucoxanthin and antioxidant activity both declined over time to varying degrees, but were all still detectable in all except *P. palmata* where its low initial value was lost within 6 mo. Total polyphenol content declined in all, except *Laminaria* spp. where it did not change over 18mo. The authors concluded that the various bioactives are slowly degraded by oxidative processes during long-term storage, mirroring results found in dried vegetables (see for example Lee and Kader, 2000; Oladele and Aborisade, 2009).

In a second study, the stability of lipids was analysed in freeze dried and ground *P. palmata* and *L.digitata* stored for 22 months in small plastic bags at -20, 4 or 18-20ºC (Schmid et al., 2016). It was shown that -20ºC protected the fatty acids in both species over 22mo, while *L. digitata* was also suitable for storage at 4ºC. Both species showed similar degradation of PUFAs at room temperature.

Finally, Choe and Oh (2013) investigated dried sheets of *Porphyra*, which are vulnerable to oxidation due to their high surface/volume ration. They found that antioxidants decreased significantly during storage for 14 days in the dark and concluded that ensuring the preservation of tocopherol was the most important factor to prolong the quality of dried stored *Porphyra*.

3. Other methods of Dewatering: Screw press and Plasmolysis

Dewatering essentially corresponds to the removal of water by mechanical means such as centrifugation, plasmolysis or compression. Since it does not involve a change of state of water, its energy requirements (and pertaining costs) are typically much lower than those associated with drying. However, the highly hygrophilic nature of jellifying seaweeds make dewatering generally difficult.

A potential dewatering method that has been explored is to use a screw press on fresh seaweed material as an initial processing step. This compresses the material at high pressure, bursting cells and separating a portion of cellular liquid from the material, such as separating oil from seeds. Screw presses are often used in the chemical industry for the production of both alginate and carrageenan. After extraction into solution, the compound is precipitated as a insoluble salt e.g. calcium alginate. These fibres are then screw pressed to remove the majority of the excess water (www.gracesguide.co.uk) .

Screw presses have been used successfully on various forage crops as part a biorefinery process, where the organic and inorganic compounds of the expressed liquor are available for further processing, such as extraction of proteins or sugar fermentation to lactic acid for

biomaterial production (Takara and Khanal, 2011; Winters et al., 2010). Kamm *et al.* (2016) took this one stage further, suggesting that ensiled winter crop, could be screw pressed, and the liquor chromatographically separated as a source of lactic and acetic acid.

1950s trials on the mechanical dewatering of fresh *L. hyperborea*, found that 40-60% of the cellular fluid could be removed (Reid and Jackson, 1956). They tested 6 different versions, finding that only two were successful, the best being a double-screw press designed for wholemeal which removed 48% of the moisture from stipes or whole plants (along with approximately 35, 50, 60 and 45% of the dry matter, ash, mannitol and nitrogen, respectively). The authors proposed that with modification, 50-60% should be feasible. With other machines, the material tended to either clog or passes through without liquor expulsion. Reid and Jackson (1956), also explored the use of centrifugation batch presses or squeeze rolls, however, these were only successful on stipe which had been finely divided e.g. through mincing. When fronds were pressed alone with either method, they gave poor results, often clogging the machine or producing very little sticky liquor. More recently, Harmsen (2014) was able to express only 25-30 wt% from brown seaweed or ~30-35% of the cellular fluid assuming 85% moisture. Working with *Ulva lactuca*, Bjere et al (2012) were able to express 52 wt%, carry 1/3 of the total ash content.

Gallagher *et al.* (2017), found that acidification using hydrochloric or phosphoric acid, reduced the stickiness allowing greater liquor production from screw pressed *L. digitata*. However, the authors were unable to express juice from live fronds or with a number of other treatments. This was likely due to the frond clogging as originally identified by Reid and Jackson (1956). Finally, Lightfoot & Raghavan (1994) found that dewatering of the kelp *Nereocystis lutkeana* using a combination of mechanic pressure and electric current significantly reduced its ash content thanks to leaching of salts. Though it also decreased its polysaccharide content, proteins, fats and uronic acids were retained. They concluded that introduction of a combine dewatering/plasmolysis step before drying would significantly decrease the energy requirements linked to drying the biomass towards the production of dried kelp meal.

4. Ensilage

Ensilage is a well-established process currently used mainly for the wet preservation of forage crops (McDonald et al., 1991). Pioneering work in the 1950s (Black, 1955) showed that it could potentially be used for cheap long-term storage of seaweed biomass. The principle is that under anaerobic conditions, bacterial conversion of water soluble carbohydrates into organic acids, mainly lactic acid will result in a reduction in pH. Once a certain level is reached (around pH 4), this will inhibit the growth of spoilage microbes such as *Clostridia* or *Enterobacteria* as well as further lactic acid formation.

The fermentation reaction of sugars being converted to lactic acid, maintains a high energy yield within the biomass. For example only $2H_2O$ is generated by the conversion of fructose or glucose to 2 Lactate molecules. Successful ensilage can only undergo <7% energy loss from the biomass (McDonald et al., 1991). It has been shown that the process also makes

the biomass more easily digestable, resulting in an increased methane yield which may fully compensate or exceed storage losses (Herrmann et al., 2011, Seagas consortium unpublished results).

The conditions necessary for ensilage are:

1. Sufficiently high concentrations of water soluble sugars within the biomass;
2. The removal of oxygen;
3. The presence of sufficient lactic acid bacteria (LAB) to dominate the biomass;
4. A rapid pH decline to inhibit other microbes.

If condition 1 is not met, insufficient lactic acid will be produced. This will prevent the pH from reducing far enough allow butyrifying and sulphur reducing anaerobic organism such as *Clostridium* spp. to dominate while the pH will maintain at ~5-6. These produce butyric acid and release CO_2, leading to substantial energy loss. In addition, the production of toxic H_2S can endanger workers. A potential remediation used in land crops, is to partially dry the crop by wilting beforehand, to concentrate the available sugars.

If condition 2 is not met, the biomass will end up covered in mould, some of which are able to grow below pH 4. This lead to energy loss and biomass degradation over time.

If condition 3 is not met, other organisms such as *Clostridium* spp. will be come dominant leading ot degradation. This may be counteracted via initial inoculation.

If pH 4 is not met, it is likely due to insufficient lactic acid being produced, or a high buffering capacity within the biomass. Again this will prevent successful ensilage.

Lactic acid bacteria (LAB) are essential to successful ensilage, however they are often a very low natural levels on seaweeds. Because of this, relying on the natural populations of LAB maybe risky. Natural ensilage has been shown to work quite reliability for *S. latissima* due to its high concentration of sugars (Cabrita et al., 2017; Herrmann et al., 2011, Kerrison et al in prep.), however it has been less successful for other species particularly the red and green species such as *P. palmata* and *Ulva lactuca,* which has been blamed on their low concentrations of easily digestable sugar (Cabrita et al., 2017; Herrmann et al., 2011; Redden et al., 2017). To ensure a successful fermentation, it may be desirable to use additives to encourage the process, either bacterial inoculants or organic acids. Various formulations are available for forage crop ensilage, some of which have been trialled on seaweed (Cabrita et al., 2017; Herrmann et al., 2011, Kerrison et al in prep.).

SINTEF Materials and Chemistry in collaboration with SES has investigated acid preservation of *Saccharina* for use as feedstock for production of biofuels (unpublished). For use of seaweed as carbon source for fermentation, the main aim of the preservation will be to maintain the carbohydrates. Since silage fermentation will consume sugars, addition of acids was the selected approach. Due to the large volumes and low product price, emphasis was on cheap mineral acids. However, organic acids, like formic, acetic and lactic acid have an antimicrobial effect that enhances the preservation. Combinations of mineral and organic

acids were therefore also included in the study, in order to reduce the total amount of acid required.
Wild biomass with approximately 10 % laminaran and 10 % mannitol of dw, harvested in November-December, was applied. The biomass was efficiently preserved at pH below 3.7 obtained by addition of sulphuric acid, with no reduction in mannitol or laminaran content after 6 months' storage. With a combination of sulphuric acid and formic acid, pH up to at least 4.0 could be applied. These values are upper limits, and for practical applications, a safety margin should be considered. pH should not be too low, as the solubility of the biomass components is reduced at pH below 3. The amount of acid needed to decrease pH to 3-4 depends on the biomass dry weight, and also the alginate content due to the buffering effect of alginate. For the biomass batches applied in the current work, 0.35-0.4 mole H_2SO_4 per kg dw, corresponding to 0.7-0.8 mole of a monoprotic acid, was required. The viscosity, the solubility and the availability of laminaran for enzymatic hydrolysis were similar for biomass that had been stored for 6 months at pH 3.1-3.7 and for fresh, unpreserved, but acid-treated biomass at pH ~3.5. The additional storage period at low pH had therefore minimal effect on these properties.

5. Chemical preservation

By treating fresh seaweed in chemicals which are toxic to both algae and microbial organisms, all cellular activity can be arrested, preserving the composition for later use. These processes were pioneered by Black (Black, 1955) who showed that the preservatives potassium metabisulphate, trichlorophenol, sodium o-phenylphenoxide, pentachlorophenol could be used, as could a 20% solution of sodium chloride. However, as would be expected, the cells ruptured, leading to the loss of soluble consitutents into the liquid media. For some industries such as alginate production, this is not a problem because the required raw material is an insoluble cell wall component. So, such chemical treatment has been trialled and adopted within the hydrocolloid industry for the preservation of fresh seaweed such as *Sargassum* spp. (Radulovich et al., 2015), *L. hyperborea* (Jensen, 1998) and *Eucheuma* (Marinalg International, 2012), where formaldehyde or glutaldehdye is currently used. However, no information on dosing rates could be accessed for the preparation of this report.

6. Fresh storage in seawater or air

The fresh storage of seaweed is problematic due to their fast rate of deterioration (Naylor, 1976). The seaweeds need to be stored in a live and physiologically ‘happy’ state; otherwise the quality of the biomass can begin to deteriorate. The death of seaweed may be accompanied by the extrusion of cellular fluid which in *Saccharina latissima* and *Alaria esculenta* is often 30ml of fluid per 100g (Kerrison unpub results). This can occur within 24hr of a 2min 70% ethanol soak or 24-48hr of gas-tight storage due to suffocation (Kerrison unpub results).

It appears that the common cause of death during fresh storage is suffocation due to lack of oxygen. In lab trials, it has been observed that kelps sealed in containers of seawater died

more quickly than those sealed in containers in only air, accompanied by a characteristic rotten eggs smell of anoxic sulphur reduction (Kerrison unpub results). This apparently contradiction, of increased survival out of water, is that oxygen concentrations within seawater (22.4 $mg \cdot L^{-1}$ at 0ºC) are many less those in air (301 $mg \cdot L^{-1}$ at STP). What this highlights is that it is essential to maintain an adequate supply of oxygenation to support the respiration rate of the stocked biomass. In the dark, respiration rates are reduced and so dark storage with oxygenation may allow survival long term.

In lit storage tanks, photosynthesis will be able to occur, potentially allowing self-oxygenation, however, other problems may occur, particularly at high density stocking a) limitation of available inorganic carbon for photosynthesis b) suffocation when the lights are turned off c) fouling or overgrowth by other photosynthetic contaminant organisms.

To counteract problem a), it may be possible to add organic buffers which will maintain the availability of CO_2 by converting the abundant seawater bicarbonate into CO_2. Many seaweeds are able to do this themselves using the enzyme carbonic anhydrase or carbon concentrating mechanisms. However this is accompanied by an increase in the pH, which can reach a compensation point where no further CO_2 conversion is possible. Organic buffers may be able to keep to the pH from rising this far; however, they are known to interfere with CCMs (refs).

Le Pepe et al 2002, reported that *P. palmata* could be stored for up to 15d at 4ºC in an artificial seawater, although this was likely at a low stocking density, allowing adequate gas exchange. Radulovich et al. (2015)found that cleaned and plastic-bagged material from most species retained freshness through refrigeration for at least 2 weeks, with the exception of Caulerpa racemosa (a variety of sea grapes), which lasted only 5 days. This is similar to the authors experience (Kerrison pers. obs.), where a variety of intertidal and subtidal seaweed could be stored in plastic bags in a fridge for over a week.

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