The Effect of Sample Preparation on Yield and Composition of Certified Organic Ethanolic Extracts Produced from Icelandic Marine Algae Species

Daniel James Coaten

M.Sc. thesis

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The Effect of Sample Preparation on Yield and Composition of Certified Organic Ethanolic Extracts Produced from Icelandic Marine Algae Species

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M.Sc. thesis in Environment and Natural Resources
Supervisor: Dr Sesselja Ómarsdóttir
Faculty of Pharmaceutical Sciences
School of Health Sciences, University of Iceland
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Áhrif mismunandi meðhöndlunar sýna á heimtur og efnasamsetningu vottaðra lífrænna etanólúrdrátta úr íslenskum þarategundum

Daniel James Coaten

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Author Mr Daniel James Coaten (B.Sc. Hons.)

Supervisor Dr Sesselja Ómarsdóttir (Ph.D.)
Associate Professor
Faculty of Pharmaceutical Sciences
University of Iceland

Instructors Dr Þórdís Kristmundsdóttir (Ph.D.)
Professor
Faculty of Pharmaceutical Sciences
University of Iceland

Dr Brynhildur Davíðsdóttir (Ph.D.)
Professor / Director of graduate studies
Environment and Natural Resources
University of Iceland

The project was carried out at the research facilities of Alkemistinn ehf, Keilir Institute of Technology and Faculty of Pharmaceutical Sciences, University of Iceland

Útdráttur

Á undanförnum árum hefur áhugi á rannsóknum náttúruefna úr sjávarlífverum aukist verulega. Þá má einkum nefna að sýnt hefur verið fram á að þörungar eru rík uppspretta byggingarlega mismunandi lífvirkra efnasambanda sem hugsanlega er hægt að hagnýta í matvæla-, snyrtivöru- og lyfjaiðnaði.
Þess vegna er markmið þessarar rannsóknar að kanna hvaða áhrif mismunandi meðhöndlun og úrhlutunaraðferðir hafa á heimtur og efnasamsetningu útdrátta úr íslensku þangi. Út frá þessu er unnt að velja hentugar aðferðir til að auka heimtur úr framleiðslunni. Rannsókninni var skipt í tvo hluta. Fyrri hlutinn miðaði að því að finna hentugt svæði til að fá lífræna vottun með það fyrir augum að ná í nægan efnivið af lífrænt vottuðu hráefni til rannsóknarinnar. Fyrir valinu varð Stafnes sem hlaut lífræna vottun frá Vottunarstofunni Túni í desember 2013. Í seinni hluta rannsóknarinnar var ferskum sýnum af *Ascophyllum nodosum*, *Laminaria digitata* og *Saccharina latissima* safnað og sýnin meðhöndluð á fjóra mismunandi vegu þ.e. þau voru ýmist fryst, þurrkuð við stofuhita, frostþurrkuð eða þurrkuð í ofni. Þar á eftir voru öll sýnin úrhlutuð í svokölluðu, vacuum Soxhlet" tæki með mismunandi styrk af lífrænt vottuðu etanóli (96%, 48% eða 0%) í vatni til að útbúa þurra útdrætti.
Í kjölfarið voru gerðar margskonar greiningar á bæði hráefnunum og útdráttunum eins og t.d. meginefnagreining (proximate composition analysis), steinefnagreining (elimental analysis) og jurtaefnagreining (phytochemical analysis).
Niðurstöðurnar sem fengust sýna að bestar heimtur fást ef hráefnið er fryst og af þeim hráefnum sem voru efnagreind var hæsta meðalmagnið af fitu (4,36%) og próteinum (7,51%) í frystu sýni af *Ascophyllum nodosum* og mesta meðalmagn af kolvetnum var í frystu *Saccharina latissima* (45,23%).
Niðurstöður úr efnagreiningum á útdráttum leiddu í ljós sá útdráttur sem innihélt hæsta hlutfall af kolvetnum var útbúinn úr þurrkuðu sýni af *Saccharina latissima*, sem úrhlutað var með hreinsuðu vatni (ultra-pure water).
Náttúruefnagreining sýndi að fryst sýni af *Laminara digitata* sem úrhlutað var með 96% etanóli gaf bestar heimtur af fúkóxanþíni eða 1.96 mg/g af þurrefni.
Að lokum sýndu gæðamælingar (quality control analysis) að engin PCB efni voru í sýnunum í mælanlegum styrk og mesta magn af arseniki mældist 4,9 ppm. Þessar mælingar staðfesta að öll hráefnin og útdrættirnir eru innan þeirra öryggismarka sem sett eru fyrir þessi efni.
Það má því draga þá ályktun að plöntuefnið sem notað var til úrhlutunar hefur bæði áhrif á gæði og efnasamsetningu útdráttanna. Jafnframt skiptir meðhöndlun hráefna og úrhlutunaraðferðir miklu máli ef áhugi er á að auka styrk ákveðinna markefna (targeted compounds) í útdráttum.

Abstract

During recent years, the natural product chemistry of marine organisms has received much attention as a promising new field of study. In particular, marine algae have proven to be a rich source of structurally diverse bioactive compounds with a high potential value to food, cosmetic, and pharmaceutical industries.

Therefore, the main focus of this study was to investigate how preparation and extraction methods effect the yield and chemical composition of the crude extracts obtained from Icelandic marine macroalgae. Insight from this could then be used to increase the efficiency of production methods and so achieve maximum yields.

As such, the research was conducted in two parts. The former focused on the identification of a suitable site for conversion to organic status, in order to secure a supply of certified organic raw material required during the latter part of this study. Such an area was identified in Stafnes, and an application for organic conversion via Vottunarstofan Tún was accepted in December, 2012.

In the second part of the study, fresh samples of *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*, were carefully collected and subjected to the following four types of material preparation: Freezing, air drying, freeze drying and oven drying. Each material type then underwent extraction via the use of a vacuum Soxhlet, using certified organic ethanol (96%, 48% and 0%) and ultra-pure water to produce extract fractions that were subsequently dried.

The following types of analysis were then conducted on both the raw prepared material and the extracts: Proximate composition analysis, elemental analysis and phytochemical analysis.

The results showed that, in almost all cases, the highest constituent contents measured were obtained when they were prepared via freezing. Of the prepared raw material analysed, the highest average values of fat (4.36%) and protein (7.51%) content were obtained from frozen *Ascophyllum nodosum*. Whereas the highest average carbohydrate content (45.23%) was seen in frozen *Saccharina latissima*.

The results from the analysis of the extracts showed that the fraction which contained the highest average carbohydrate content (87.17%) was from freeze dried *Saccharina latissima*, which had been extracted with a low concentration of ethanol (high concentration of ultra-pure water).

The phytochemical analysis of fucoxanthin results revealed that the use of frozen *Laminaria digitata* material extracted with 96% ethanol, provided the highest yield (1.96 mg/g dry weight of extract).

Finally, the quality control analysis showed that there were no PCBs present (within detection limits) in any of the samples tested, and that the highest concentration of arsenic measured was 4.9 ppm, thereby confirming that the raw material, the extracts and the collection site were all within safe limits of these compounds.

In conclusion, the way the plant material is prepared before extraction can have a significant effect on both the yield and composition of the final crude extracts achieved. Furthermore, variations in the methods used also play an important role in the effective extraction of specific target compounds of interest.

This work is dedicated to my wife Bryndís,

and also to my daughter Amelía Molly Tatjana,

whose encouragement and support made this research possible.

Preface

The author of this paper has been a qualified medical herbalist (Phytotherapist) for almost 15 years. During his career he has gain a great deal of experience, not only as a medical practitioner, but also in terms of laboratory work and analysis of medicinal herbs.

Much of his research on herbs has focused on their extraction, and as such, experiments have been conducted using a variety of methods, ranging from the simple traditional to the advanced modern. Over the course of these investigations an interesting observation was made that the majority of the studies done in relation to this field of work centred on the results of the extraction processes themselves in terms of the quantity and quality of the extracts they produced. However, little research had been conducted that specifically looked at how the treatment and preparation of the materials before extraction may affect these end results.

As with other areas of chemistry the end products are only ever as good as the starting materials used. Therefore it was hypothesised that in terms of herbal extraction, the quality of the raw material is of paramount importance, and so anything that could effect this (such as the time of year the material was harvested, the way it was preserved, such as drying, the age of sample, and under what conditions it was stored before extraction), could contribute to inferior results. Therefore, based on this hypothesis, the research project contained within this paper was envisioned.

The emphasis of the research needed to have a focus on a particular plant type with which to conduct experiments upon. As the author's previous research had included the study of both terrestrial plants and fungi, it was therefore decided that a study involving marine macroalgae would be of great interest, and in particular also for the fact that much research has been done recently showing their great potential in affording novel bio-active compounds. This choice was also compounded by the fact that the author was now residing in Iceland, which was seen to harbour a huge variety of high quality material that could be studied.

As a medical herbalist, the author also has interest in environmental issues and ways in which damage to the environment can be minimised or even prevented. In this way, he has always favoured the use of certified organic material for his studies, which not only follows the principles of sustainability and environmental protection, but also due the lack of use of chemical agents in their agriculture, reduces the risk of dangerous contamination of the final extracts.

It was therefore decided that a key part to this study would also involve the identification of a potential collection site, and that an application for organic conversion via Vottunarstofan Tún (organic certification agency in Iceland) would be applied for to secure a supply of material for the research.

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Abbreviations

ACS	American Chemical Society
AOAC	Association of Analytical Communities
BHP	British Herbal Pharmacopoea
COSMOS	Cosmetic Organic Standard
DAD	Photodiode Array Detector
DCB	Decachlorobiphenyl
DCMA	Dry Color Manufacturer's Association
ECD	Electron Capture Device
EDQMH	European Directorate for the Quality of Medicines and Healthcare
EEC	European Economic Community
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organisation of the United Nations
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
M	Molar
MSY	Maximum Sustainable Yield
N	Normal
NPP	Net Primary Production
PCB	Polychlorinated biphenyls
PTFE	Polytetrafluoroethylene (Teflon®)
QAI	Quality Assurance International
R_f	Retardation factor
TCA	Trichloroacetic acid
TLC	Thin Layer Chromatography
USPC	United States Pharmacopeial Convention
UV	Ultra violet
v/v	volume/volume
wt/wt	weight/weight

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I would also like to thank Eydís Mary Jónsdóttir, for her time, expertise and assistance in the collection and positive identification of the fresh marine algae samples used during the course of this study.

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1 INTRODUCTION

Internationally, demands for marine algae have steadily been rising, with the industry sector estimated to be worth €30 million per annum by 2020 (Marine Institute, 2006). Because of this, many countries are now starting to realise the vast economic opportunities associated with this market.

Nowadays, a major impetus of the industry is the search for novel natural substances extracted from these marine organisms (Hardouin *et al.*, 2013). Various examples of marine algae species and their extracts have been shown to have valuable properties with potential applications in a variety of industry sectors (see Appendix 1, Table A1.1).

Historically, Iceland has a long tradition in the utilisation of marine algae. According to the Icelandic Sagas, the marine algae known in Icelandic as "Söl" (*Palmaria palmata)* (Guiry, 1974) has been known to be edible since at least the year 961, and it is also mentioned in Iceland's oldest law book that the right to both collect and eat fresh Söl is allowed without further need for permission from the landowner. Thus, Söl was regarded as being highly valued from around 1118 until the latter part of the 19th Century, and as such was commonly traded with inland farmers in exchange for other goods such as wool and meat (Hallsson, 1961). Other species that were also highly regarded by the Icelanders were *Saccharina latissima* and *Ascophyllum nodosum*, both of which were considered priceless as feedstock to cattle to help them survive the harsh winter months and were also often applied as a green manure to help boost crops. In some cases they were even employed as a source of fuel, and were also useful as a folk medicine (e.g. used for heart ailments, indigestion and wounds), and for dying home spun cloth (Hallsson, 1961), and so could be regarded as playing a key role in the history of Iceland and its national heritage.

Today the algae industry in Iceland is still relatively small and the following six main species are all commercially wild harvested: Rockweed (*Ascophyllum nodosum*), Oarweed (*Laminaria digitata*), Babberlocks (*Alaria esculenta*), Kelp (*Laminaria longicruris*), Dulse (*Palmaria palmata)*, Sea belt (*Saccharina latissima*)[1] and Bladderwrack (*Fucus vesiculosus*)[2,3]. This industry currently produces products for several key sectors such as agriculture/horticulture, food, cosmetic and health supplements; the first two sectors being the most economically important.

[1] Information gathered from two of the main collectors of seaweed in Iceland, Thorverk hf and Seaweed Iceland ehf (30/06/2012).
[2] Matis ohf. http://www.matis.is/english/. Accessed on 08/05/2014.
[3] Marinox ehf. http://www.unaskincare.com/en/story/technology/. Accessed on 08/05/2014.

Research that was sporadically conducted over the late 1930's to the early 1960's suggest that via the use of geothermal energy, a number of industrially important marine algae components (such as sodium alginate, alginic acid, mannitol, laminarin, combined fructose, ash and protein) could be effectively and economically produced in Iceland (Hallsson, 1961). Although the above research seemed favourable, to date, no such production is currently taking place in Iceland. Marine algae are therefore regarded as a relatively under exploited resource in Iceland, but one which shows great potential for further development and expansion.

The main focus of this project is to look at the possibility of using the different species of marine algae which grow in the Suðurnes area of Iceland (practically along the coastline of the area known as the Reykjanes peninsula).

The main target areas of usage which have been chosen are in the form of the food (specifically health food), cosmetic and pharmaceutical industries. In all of these industries various types of marine algae are already regularly used either in their raw form (i.e. generally just cleaned and dried), or as purified extracts (such as thickeners like carrageenan or agar).

There are many traditional processes used to produce commercially available seaweed based extracts. However, many of these methods use chemicals and/or processing techniques which are not tolerated under international organic certification standards.

1. *The first aim of this project is to conduct research to evaluate and compare currently available and compliant extraction methods, and possibly develop new processing techniques which would achieve the production of high quality, functionally active, certified organic phyto-extracts of marine algae species from Iceland.*

It has also been seen that over the last 10 years consumer interest in buying certified organic products (both as food and in the form of skin-care products) has risen considerably making it a multibillion euro industry just in Europe alone (Willer *et al.*, 2014).

This research would therefore provide a unique edge to any organic company who may already be using similar extracts in their non-certified organic form as part of their ingredients/formulations in foods and/or cosmetics, and who would in effect be able to increase the percentage of organic status of their products. This is important to take into consideration as for example, cosmetic products need to have at least 95% of their agricultural ingredients as certified organic in order for the final product as a whole to be allowed to be marketed as being organic (Tún, 2011).

Obtaining organic certification of an area where marine algae grow would mean that the material collected from this site would be classified as certified organic, and would therefore fetch a much higher market price. Not only this, but because of Iceland's reputation as a clean and unspoilt country it makes it one of the few places in the world

which could qualify for certification of their coastline as certified organic (at present there are only two other areas in the West of Iceland where the shoreline has been certified as organic). This in turn would make these products/extracts an even rarer commodity which would dominate this niche market area and sell at premium prices.

> *2. The second aim of this project is to research the possibility of getting the proposed area in which the marine algae species are to be collected to be certified organic and to apply for organic conversion of this site.*

Another advantage of obtaining organic certification in these areas would mean that not only marine algae collected would be certified organic, but also other species of plants/animals/fish etc. present in the designated area would also be eligible for certified organic status. As such, this may help to open up a whole new sector of industry for the local area/s with further development of more companies and more job opportunities.

In order to produce marine algae and their products both profitably and responsibly, it is important that any potential collection site is managed appropriately to accommodate this, just as it is for land agriculture. It is for this reason that an aspect of report will focus upon the possibility of utilising modern aquaculture methods for sustainable marine algae production.

2 MARINE ALGAE AQUACULTURE

The term “aquaculture” is officially defined by the Food and Agricultural Organisation of the United Nations (FAO) as “the farming of aquatic organisms, including fish, molluscs, crustaceans, and aquatic plants” (New, 1992).

Worldwide the aquaculture sector is rapidly growing, with a total production of 79 million tonnes (including aquatic plants and non-food products), which was valued at US$125 billion, in 2010. The major producer of marine algae is China, followed by Indonesia and the Philippines (FAO, 2012).

Recently, efforts have been made to try to establish marine algae aquaculture in North America and Europe (Vásquez, 2008). Significant advances in research and technology and current market trends have fuelled this increased interest, and have led to the creation of new applications and new demands. In the Western hemisphere, cultivation emphasis has focused on the use of high–value low–volume marine algae species, and towards more environmental friendly integrated aquaculture (polyculture) systems comprising finfish, shellfish and marine algae (Stachowicz *et al.*, 2008; Douglas Westwood Ltd, 2005).

In regards to certified organic aquaculture, not only can environmentally friendly production methods and sustainable harvesting be ensured, but because these products fetch higher market prices, it can also help fulfil the high–value low–volume criteria. According to an FAO report, certified organic aquaculture production is estimated to be in the region of 1.2 million tonnes by 2030 (Scialabba and Hattam, 2002).

Currently in Iceland, all the species collected are harvested from the wild; in some cases mechanically[4], but predominantly by hand[5]. This is very energy intensive and time consuming, and could therefore been seen as possible hindrance to the potential expansion of this industry.

As a solution to this, it is suggested that certified organic marine algae aquaculture may provide the most cost–effective method to meet the growing world market demand, by providing it with a high–quality, high-value material for use in specific sectors such as food, cosmetics and biotechnology.

[4] Thorverk hf. http://www.thorverk.is/english/products/material.php. Accessed on 10/11/2012.
[5] Marine algae Iceland ehf. http://www.marine algae.is/hafnot/index.php. Accessed on 10/11/2012.

2.1 Worldwide Production and Market Value Data

The most independent and respected source of data on aquaculture production of marine algae is the Food and Agriculture Organisation (FAO). They publish their annual fisheries statistics and make information available via their web site[6].

According to the FAO's latest annual fisheries statistic report, entitled "The State of World Fisheries and Aquaculture, 2012", worldwide aquatic algae production (by volume) increased at average annual rates of 9.5% in the 1990's and 7.4% in the 2000's, with production increasing from 3.8 million tonnes in 1990 to 19 million tonnes in 2010 (which is comparable with rates for farmed aquatic animals). Also, cultivated algae have overshadowed production of wild-crafted species, which accounted for only 4.5% of total marine algae production in 2010 (FAO, 2012).

The total value of farmed aquatic algae in 2010 was estimated at US$ 5.7 billion (see in Figure 2.1), where only a few species seem to dominate global marine algae culture (FAO, 2012).

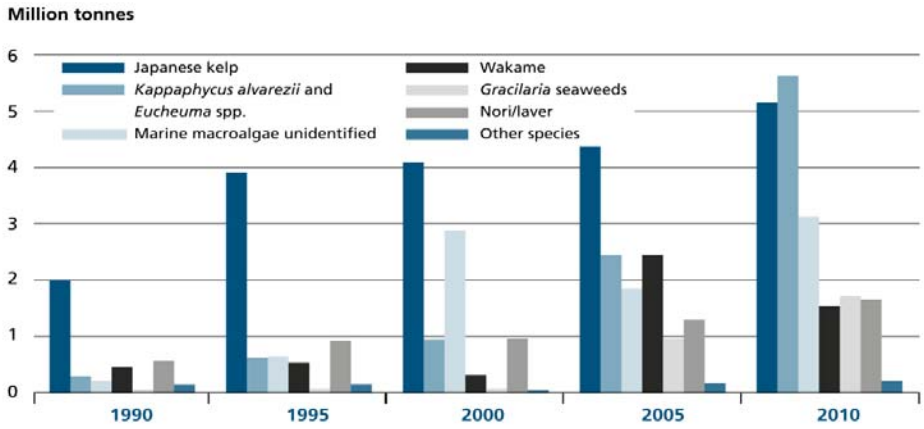


Figure 2.1: World production of farmed aquatic plant (algae) by major species or species group (FAO, 2012).

2.2 Common Types of Marine Algae Selected for Aquaculture

2.2.1 Brown Marine Algae

In general, brown marine algae, and in particular Kelp, account for the most voluminous and valuable production of marine algae globally. Kelp cultivation includes species such as *Laminaria japonica* and *Undaria pinnatifida* (Kraan, 2010).

[6] FAO. http://www.fao.org/fishery/en. Accessed on 09/11/2012.

2.2.2 Red Marine Algae

Examples of commercial cultivated red marine algae are *Eucheuma* ssp. and *Kappaphycus* ssp. (Lüning and Pang, 2003), *Gracilaria* ssp. (Matos, 2006), *Porphyra* ssp. (Lüning and Pang, 2003), *Chondrus crispus* (Matos, 2006), *Asparagopsis* ssp. (Lüning and Pang, 2003), and *Palmaria palmata* (Matos, 2006).

2.2.3 Green Marine Algae

Types of green algae that are commonly grown commercially are *Ulva* ssp*., Monostroma* ssp*.*, *Enteromorpha* ssp. (Lüning and Pang, 2003), and *Caulerpa* ssp. (Nielsen, 1982).

2.3 Species of Potential Commercial Interest

In regards to marine algae selection, it is advisable that open-water aquaculture production be applied only to native species. At least until more research is done regarding the possible consequences of introducing a new (possibly invasive) species to selected site areas. However, in regards to the tank method of aquaculture, exotic species may be grown with minimal risk of escape and/or influence on the surrounding ecosystem (Phillips, 1990; Zamroni and Yamao, 2011). The introduction of new marine algae species depends strongly on advances in research, product development and the economic feasibility of cultivating a particular alga. Various examples, given in Appendix 1, Table A1.1, have been shown to have valuable properties with potential applications in different sectors. For instance, sulphated oligo– and polysaccharides are of major interest for pharmacological applications, cosmetics and nutraceuticals (Mayakrishnan *et al.*, 2013). Carrageenan, heparin–like polysaccharides, laminarans and fucoidans have shown, for instance, anti–inflammatory, anti–bacterial, anti–viral and immuno–stimulant activities (Raveendran *et al.*, 2013; Chennubhotla *et al.*, 2013; Kim, 2014). These properties are already exploited by the cosmetic industry and for the production of food supplements/nutraceuticals (Agatonovic-Kustrin and Morton, 2013).

3 CULTIVATION AND PROCESSING METHODS

3.1 Open-water Cultivation

Open-water aquaculture consists of “seeding” lengths of rope with pre-cultivated plants, and allowing them to grow in designated areas. Therefore, for open-water cultivation to be successful, the establishment of a commercial marine algae hatchery is strongly recommended in order to supply the aquaculture operation with high–quality seed stock (Glenn *et al.*, 1996).

It is essential to develop efficient and reliable methods to produce sufficient seed stock for the main species of interest at times appropriate for out–growth in the sea. In regards to new projects, research should focus on large–scale gametophyte cultivation methods of kelps, artificial induction of sexual reproduction in red marine algae species, and improvement of pre–cultivation methods of seeded ropes (Tseng and Fei, 1987).

Atlantic open-water cultivation of most marine algae is restricted to a growth period from autumn to spring. During the summer months, high light intensities, elevated water temperatures and rapid growth of fouling organisms on culture ropes limit the cultivation of target species (Brinkhuis *et al.*, 1984).

The feasibility of polyculture should also be investigated (see Appendix 1, Table A1.1 for a list of open-water cultured species). Cultivation trials should be carried out in collaboration with fish farmers to grow marine algae in the direct vicinity of fish cages to investigate the potential benefits of co–cultivation (enhancement of marine algae growth, beneficial effects on fish) and evaluate potential negative effects (potential fouling on cage structures, impact on water current). Research should include an assessment of the economic feasibility, and the possibility for sharing work facilities and infrastructure.

3.2 Tank Cultivation

Compared to sea–based open–water cultivation, tank cultivation offers the opportunity of closely controlling and consequently optimising cultivation conditions. It is therefore especially advantageous for algal species which are propagated vegetatively (e.g. by thallus fragmentation, tissue culture), and for obtaining highly homogenous and high–quality raw material. For the cost–efficient cultivation of marine algae in land–based systems, it is essential to develop tank systems that meet the needs for optimal production of marine algae at lowest costs with respect to energy input and space required (Lüning and Pang, 2003).

Unfortunately, not all species can be cultivated in this way, and therefore new projects research should be conducted to ascertain which one will be successful, and tests done

to develop tank systems for large–scale algal production in view of different physiological requirements of different species (e.g. nutrients, salinity, pH etc.). Also, as was mentioned in regards to the open-water cultivation, additional research should focus on integrated polyculture, not only with marine animal species (either directly or indirectly), but also with other macroalgae grown simultaneously (see Appendix 1, Table A1.1 for a list of known tank cultured species).

3.3 Management and Collection

In areas of North America, commercial open-water collection of consumable marine algae species is rapidly growing industry with small operations along with their larger counterparts collectively supply local, national and global markets (Thompson *et al.*, 2010). However, despite this rising trend, little guidance is given to collectors. The result is that damaging collection methods and inappropriate harvesting times are used, rare species may be put at greater risk, and overharvesting is common. Nevertheless, many suppliers of these products often misleadingly market their wares as being “sustainably wildcrafted” (Thompson *et al.*, 2010). In the study by Thompson *et al.* (2010), experiments were conducted in California to try to find the best harvesting techniques which offer the maximum yield with minimum damage to the marine algae *Postelsia palmaeformis*. The results showed that both how the algae was cut as well as the time of year it was harvested had significant effects on its regeneration, growth rates, yield, and quality. Cutting at the stipe was found to be lethal in most cases and could easily lead to extinction of a population. However, frond trimming preserved the meristem, thereby allowing the fronds to regrow. In regards to time of harvesting, it was seen that fronds trimmed in April–June were able to regrow and eventually produce viable spores (although at slightly reduced rates compared to non-trimmed samples). Conversely, fronds that were trimmed after the onset of sporogenesis (i.e. at the end of July), displayed a steep reduction in spore production, and these effects were noted to be similar across the geographic range examined but varied in magnitude. Lastly, recruitment was generally noted to be 38% greater in populations that were not trimmed, compared with a 40 to 50% reduction when they were (Thompson *et al.*, 2010).

In Norway, extensive experience has been gained over many years with regards to managing and collecting marine algae species (specifically *Laminaria hyperborea*) sustainably. Vea and Ask (2011) show how environmental policies and management plans can be instigated and enforced which not only allows the continuation of the lucrative algae trade, but at the same time helps to minimise potential environmental impacts of this industry and conserve marine algae communities.

In order to achieve this goal it is recommended that the following should be done:

> **Careful study and understanding the life cycle of the target species and monitoring of its environment.** In this way limiting factors may be identified along with when the species may be most vulnerable to disturbance, and so harvesting schedules can be adjusted accordingly. Also, potential environmental threats may

be predicted such as raising sea temperature and the increased occurrence and severity of storms due to the effects of climate change, which may severely damage or reduce stocks.

Use of modern technology. The employment of “seaweed trawlers” are able to harvest whole mature plants but leave juvenile ones of around 20 cm or less, and sonar equipment which is able to map harvest sites and give accurate data on stock density and volume.

Use of better techniques. Using 4-5 year (depending on area) rotational harvesting allows sufficient time for effective rejuvenation of collection sites to take place.

Collaboration of governmental bodies and other organisations. This is important in order to instigate effective protective legislation and formulate a long-term management plan. It should be in the form of a committee that includes representatives from (but not limited to) The Directorate of Fisheries, The Directorate for Nature Management, The Institute of Marine Research, The Institute for Nature Research, and The Fishermen’s Association.

One important aspect to sustainable collection is an accurate determination of a species maximum sustainable yield (MSY), and is usually given as a maximum % (often 17%, depending on species) that can be removed from stocks in order for the species to regenerate effectively. Seeley and Schlesinger (2012), state that the current metric for MSY for marine algae is inappropriately narrow and does not match calculations made in regards to Net Primary Production (NPP). They suggest that more studies are needed to accurately assess the MSY for individual species, taking into account not only their services to other organisms in a particular ecosystem, but also the possibility of negative effects of climate change on yields as well. They also warn that if these studies are not done then this could have grave consequences, ultimately resulting in the collapse of traditional fisheries.

NPP is defined as the net flux of carbon from the atmosphere into green plants and refers to a rate process per unit of time (i.e. the amount of vegetable matter produced per day, week, or year). It is a fundamental ecological variable, not only because it measures the energy input to the biosphere and CO_2 assimilation, but also because of its significance in indicating the status of a wide range of ecological processes (Howarth, 1988). In relation to the article mentioned above by Seeley and Schlesinger (2012), in order to try to define exactly what a “sustainable harvest” of marine algae entailed they decided to look at the relationship between NPP and the consumption of biomass by native herbivores in terrestrial ecosystems. What they found was that by using information gained from an earlier study by Cebrian (2002), which reported that about 10% of NPP is consumed in marine macroalgal communities, they were able to calculate that in regards to their study areas in Maine and the Maritime Provinces, where 17% biomass was removed per year (based on MSY calculations and considered as sustainable), this harvesting level should rather be viewed as being more excessive than sustainable. The reason for this is because removing 17% of the biomass really translates as eliminating 34% of NPP (the biomass turnover rate was typically 0.5), and so illustrates just how much of an error can occur when using MSY as a measure of sustainable harvests.

Lastly, there is the question of whether or not to allow mechanical collection. Some studies suggest that mechanical equipment (such as trawler or suction cutter type collectors) can have severe effect on benthic communities, such as disruptions in sediment and populations of other aquatic flora (e.g. sea grass (*Zostera japonica*)), which in turn removes the habitat for other organism such as amphipods (Titlyanov and Titlyanova, 2010), or when other marine species are inadvertently harvested as bycatch (Seeley and Schlesinger, 2012). One study in particular also showed that there was a 20 to 36% increase in plant mortality compared with control sites, reducing overall plant density from 92.6 to 73.6 individuals m^{-2} (Ang *et al.*, 1993). Others studies state that modernised equipment is now specifically designed to minimise environmental impact. From the original sled/trawl type designs of the 60's to the innovative machinery of today, many advances have taken place such as the elimination of the front cutting blade, the ability of whole plant harvesting (including the holdfast) and the selective harvesting of mature species (Vea and Ask, 2011).

It is therefore as of yet still unclear as to which choice to make and more research is needed in order to come to a conclusion. What is clear, however, is that the tide level at the time of harvest, the length of time the machine is operated at one site, the skill of the machine operator, and the sharpness of the machine cutting blades may all contribute to the impact of mechanical harvesting on marine algae and their habitat (Ang *et al.*, 1993). Also, the fact that a huge amount of time and effort are needed to hand harvest marine algae means that it will never be able compete with mechanical harvesting, both in terms of biomass collected and related disturbance to the surrounding biota, and could therefore be regarded as being self-limiting in terms of its environmental impact as compared with mechanical harvesting.

3.4 Processing of Raw Material

Correct storage and processing of algal raw material is of particular importance to avoid spoilage, and therefore innovative, cost–efficient methods are required.

The most common way to preserve harvested marine algae is via air drying on racks (Zamroni and Yamao, 2011). However, although this may be suitable for hotter climates such as Indonesia, this technique would not work as well in colder countries such as Iceland. Instead electric heaters or even dry geothermal steam is used (Arason, 2003). Nevertheless, many other options could be explored (such as freezing, freeze drying, vacuum desiccation etc.).

Due to the fact that some species of marine algae can contain active constituents of anywhere up 69% of their dried biomass (e.g. carbohydrates (Cumashi *et al.*, 2007), of which sulphated complex polysaccharides such as fucoidans can be present up to 48% of this total (Rioux *et al.*, 2007)), therefore further processing and purification techniques, such as solvent extraction, distillation and supercritical fluid extraction are used to make higher value concentrated extracts.

It is advantageous to house the processing facility on or near to the marine algae collection site in the case of wild harvesting or open water aquaculture. In regards to tank cultivation, this can easily be achieved by employing an “all in one facility”, housing both the aquaculture and processing aspects of the operation within the same building.

3.5 Regulatory and Environmental Considerations

In Iceland, the environmental policy for the harvesting and management of wild aquatic plant species falls under “The Nature Conservation Act, No.44/1999”, and more specifically can be found in Chapter III: Right of public access, treatment of the natural environment and outdoor leisure; articles 25 – 27 (Umhverfisstofnun, 1999).

At first the articles seem to be comprehensive in covering most aspects involved in wildcrafting marine algae, such as legal ownership, site location, species type, amount and methods of the harvesting. However, upon closer inspection, a number of ambiguities (such as the definition of “commercial harvesting”, or who is to police this and what legal action, if any, would be taken for not following this article) were noted which could potentially brand these articles ineffective.

3.6 Organic Certification

In order for a collection site and/or facility to achieve certified organic status, the marine algae have to be managed, harvested and processed in accordance to EU Regulations 834/2007 Reg 710/2009 (part of Reg 889/2008); (Regulation C, 2007).
As part of these regulations, the harvesting site must be inspected and declared as meeting organic standards. Examples of such criteria that need to be met are: that the collection site should not be located near any known source of contaminants, accurate records for collection are kept, species are not overharvested, drying and processing is done using environmentally sound methods, and care is taken not to contaminate the products with non-organic materials (Tún, 2011).

In order to ensure that a producer abides to these regulations, and to be able to hold a valid certificate of organic certification, companies must register with a regulated, independent certifying authority (in Iceland the certifying authority is called Vottunarstofan Tún). This authority both monitors the production and traceability methods (via yearly audits and inspections) and also provides updates on changes in European Union organic regulations.

The main advantage of becoming certified organic is that consumers have the assurance that products are grown and/or manufactured in an environmentally friendly way, and that they are guaranteed to be free of undesirable chemicals, such as pesticides, and are not genetically modified. Such products are very sought after by consumers, and as such, naturally can fetch higher market prices, making this a desirable option for marine algae aquaculture (Scialabba and Hattam, 2002).

4 SUITABLE SITE CRITERIA

Selecting the most appropriate certified organic marine algae aquaculture sites involves two key areas of consideration which must be balanced. Firstly, the suitability of a site with respect to requirements of the target marine algae species should be assessed. Secondly, the feasibility of aquaculture development with respect to availability of space and competition with other interest groups and coastal resource users (e.g. shellfish/finfish farmers, fishermen, shipping, yachting, tourism, protected areas) should be studied (New, 2009).

4.1 Light

Light is essential for photosynthesis and consequently growth. The quantitative light demand for photosynthesis and growth depends on the algal species, its morphology and adaptation mechanisms. Species inhabiting the upper euphotic zone (intertidal) are well adapted to exposure to high irradiances and are referred to as “sun plants”. Species of the deeper euphotic zone (subtidal) lack adequate adaptation mechanisms but have developed strategies to cope with low light intensities and overall annual quantities (Lüning, 1990). The type of marine algae (sun plant or shade plant), the season (light intensity), and the turbidity of the water body all must be considered during the design of a cultivation system.

4.2 Nutrients

In a similar way to land plants, not only do nutrients determine productivity and biomass yield of marine algae, but also the abundance of epiphytes in aquaculture systems. Nutrients essential for growth are divided into three main categories: macronutrients (e.g. nitrogen (N), phosphorous (P), carbon (C)), micronutrients or trace elements (e.g. iron (Fe), zinc (Zn), selenium (Se), copper (Cu), manganese (Mn), molybdenum (Mo)) and vitamins (e.g. vitamin B12, thiamine and biotin), which are all required in different concentrations for different species (Lobban and Harrison, 1994).

Micronutrients and vitamins are rarely a limiting factor for marine algae production in coastal waters. The most important nutrients for high productivity are nitrogen (i.e. ammonium (NH_4), and nitrate (NO_3)), and phosphorus (i.e. orthophosphate, PO_4) (Lüning, 1990).

The application of marine algae as biofilters for removing inorganic nutrients from effluents of finfish/shellfish polyculture systems, requires a good knowledge of the ecophysiological demands of a species to identify one with a potential for maximum nutrient removal efficiency that are additionally commercially valuable species for aquaculture.

4.3 Salinity

Fluctuations in salinity can be a critical factor for aquaculture sites located in bays with restricted water exchange and high fresh water inflow, in estuaries and in shallow areas. Most marine algae species grow optimally at salinities around 30% but will tolerate a certain degree of fluctuation (Lüning, 1990).

4.4 Temperature

Each marine algae species has an optimal temperature range for growth and reproduction. For most species native to Iceland the average optimal range for growth is between 10ºC and 15ºC with a survival temperature range between 0ºC and 25ºC (Lüning, 1990). Elevated temperatures, especially in connection with high irradiance, can be critical for some marine algae (e.g. kelps and *Palmaria palmata*) and may lead to deterioration and bleaching of the thalli (Lobban and Harrison, 1994). To avoid this, open-water aquaculture sites should be located in areas with a minimum depth of 4–6 metres and good water exchange.

4.5 Exposure

The demands of the commercially important marine algae with respect to exposure and tidal current vary considerably. Whereas *Alaria esculenta* inhabits very exposed sites, *Palmaria palmata* grows on less exposed sites with a good tidal current. Other algae such as *Saccharina latissima* are found in more sheltered areas (Lüning, 1990). The demands have to be balanced with the feasibility for an aquaculture operation to work efficiently at any season and weather condition and to avoid damage to the farm. Therefore very exposed sites have to be excluded. An increased water velocity at the algal surface enhances nutrient uptake and algal productivity (Hurd, 2000). Water motion is therefore an essential factor for algal growth and also has to be considered in tank cultivation.

4.6 Pollution

Marine algae have the ability to remove nutrients from surrounding waters and also internally accumulate heavy metals (e.g. mercury (Hg), arsenic (As), cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn)), radionuclides (e.g. Caesium–137 and Technetium–99) and other contaminants such as polychlorinated biphenyls (PCBs) (Schramm, 1991). Therefore potential pollution of certain areas has to be considered especially with respect to the production of marine algae (and in particular their concentrated extracts) for use in cosmetics and food products.

As can be seen above, the effect of the environmental factors on the productivity and biomass yield of cultivated marine algae mean that potential aquaculture sites should be examined with these criteria. Preferably, trials should be conducted first to verify if the site is suitable for production of a target species.

4.7 Organic Requirements

To fulfil the requirements of certified organic collection from an open-water site, the following criteria must be met:

1. That beds are not located near any source of radioactive, chemical, or bacteriological contaminants including the following minimum distances: within 3 miles of any commercial boat building facility; within 3 miles of any industrial discharge area; within 2 miles of any city, town or village sewage discharge; within ¼ mile of a small harbour entry, and within 3 miles of a major harbour or thoroughfare.
2. A bed's location relative to prevailing winds and currents and/or major cities and commercial activities may make the distances defined in the first criteria.
3. Collection sites must be adequately mapped, clearly indicating each harvesting bed with a number and/or name.

(Tún, 2011)

In regards to tank aquaculture and organic standards, it is obviously much easier to satisfy the requirements. This is because there is less likelihood of contamination, and much more control over environmental variables.

5 LOCATING A SUITABLE SITE IN ICELAND

According to research conducted by Caram and Jónsson (1972), there are 230 types of benthic algae recorded in Icelandic coastal waters, of which are comprised of an assortment of species, subspecies, and varieties from the following three main families: Rhodophyceae, Phaeophyceae and Chlorophyceae. In regards to the overall distribution of vegetation patterns, and indeed placement of individual species within these communities, it was found that this was largely due to variations in temperature regimes (Munda, 1975). For example, in the south, the influence of the warmer Atlantic waters is at its strongest, whereas the East coastal areas are affected by currents of Arctic origin (Munda, 1972). Also, diverse patterns of algae zonation are noted in each of the main regions (i.e. south, south west, north west, north and east) and are likely due to differences in hydrographic conditions and shore configuration (Figure 5.1 shows areas of Iceland that have already been investigated for marine algae and the general distribution of main sites throughout the island) (Munda, 2004).

Figure 5.1: Areas of marine algae investigation and general distribution of main sites in Iceland (Munda, 2004).

In general, the following marine algae species are examples of those which are typically found along the North coast: *Devaleraea ramentacea*, *Acrosiphonia* sp. (Munda, 1975), *Corallina officinalis* (Munda, 1981), *Ceramium* sp., *Cystoclonium purpureum*, *Rhodomela lycopodioides*, *Mastocarpus stellatus* and *Saccharina latissima* (Munda, 2004). Whereas in the South (more specifically the South-West), the following typical species were noted: *Pelvetia canaliculata*, *Fucus spiralis*, *Fucus vesiculosus, Ascophyllum nodosum*, *Fucus distichus*, *Fucus serratus* (Munda, 1972), *Laminaria digitata*, *Saccharina latissima*, *Alaria esculenta*, *Gigartina stellata*, *Corallina officinalis*, *Ahnfeltia plicata*, *Asperococcus fistulosus*, *Chondrus crispus*, *Cystoclonium purpureum* and *Dumontia contorta* (Munda, 1991).

As the Reykjanes peninsula is situated in the South-West of Iceland, it is hoped that a location site is identified that is naturally populated by some (if not all of the species

mentioned above). Also, because the object of this section of the report is to try to ascertain the feasibility of establishing a certified organic marine algae collection site, it would therefore be advantageous to first try to identify if any similar sites may already established in Iceland, thus a comparison may be made to the one proposed in Reykjanes.

One such example is located in Reykhólar (located in the Westfjords area), and is managed by a company called Thorverk hf, which wild harvests and processes two marine algae species (*Ascophyllum nodosum* and *Laminaria digitata*) to produce bulk products for fodder and fertiliser.

The site covers 200 ha of certified organic coastline (certified by both Vottunarstofan Tún and Quality Assurance International (QAI)), and its processing plant is located along a small outstretch of land in the sea which is almost entirely surrounded by water (see Figures 5.2 and 5.3).

Figure 5.2: Thorverk hf viewed from the North West[7].

Figure 5.3: Thorverk hf viewed from the South East[8].

An assessment was done to compare this site against each of the 7 points present in the "Suitable Site Criteria" section mentioned previously (see Appendix 2, Table A2.1). As a result, it appeared to meet all of the requirements for successful marine algae collection and has good potential for commercial open-water cultivation, particularly in regards to the species already wildcrafted from the area.

However, despite being a successful and well established producer of marine algae products since 1986, all of the raw materials are wild harvested only, and no aquaculture methods are utilised. This means that although it is situated on a large area of privately owned land, with little competition against other interest groups and coastal resource users, the lack of cultivation management may ultimately have an impact on future

[7] Skipti.is http://skip.vb.is/hafnaskra/hofn/53/. Accessed on 10/11/2012.
[8] Vefurinn.is http://nepal.vefurinn.is/opna_mynd.asp?myn_id=15777&vef_id=89. Accessed on 10/11/2012.

expansion if the demand for their products rose sharply. Not only this, but none of the material is processed any further than drying and milling, and therefore no concentrated (higher value) extracts are as of yet produced or marketed.

In regards to finding a potential site in the Suðurnes area that met all of the requirements needed for the project, it was found that by working in partnership with Náttúrustofa Reykjaness a suitable candidate was identified at a location close to Stafnes, an area situated on the Southern Peninsula (see Figure 5.4).

Figure 5.4: Location of the potential conversion site at Stafnes, Reykjanes peninsula[9].

Stafnes is a small area of farm land located between the towns of Sandgerði and Hafnir in Iceland. During the 17th and 18th centuries it was noted as the most populous fishing region in the whole of the Reykjanes peninsula[10], but now the location is mostly home to a handful of permanent residence and summerhouses, and a small (seldom used) harbour.

A map of the proposed site and its surrounding area is shown in Figure 5.5 (Global Positioning System (GPS) co-ordinates 63° 58' 00" N, 22° 45' 00" W[11]), and is located on privately owned land (jointly owned by the inhabitants from Stafnes). The total area covers

[9] Google Maps (2012) http:maps.google.com. Accessed on 24/03/2013.
[10] Visit Iceland. (2010) http://www.visiticeland.com/SearchResults/Attraction/stafnes. Accessed on 24/03/2013.
[11] Geographical names. (2012) http://geographic.org/geographic_names/name.php?uni=-3662226&fid=2359&c=iceland. Accessed on 24/03/2013.

39.5 ha, and facing Southwest, is located just south of the main inhabited area and harbour, and although it appears to be quite open to the wider ocean, its topography allows for the formation of numerous tide pools which help protect populations from strong currents and destructive waves.

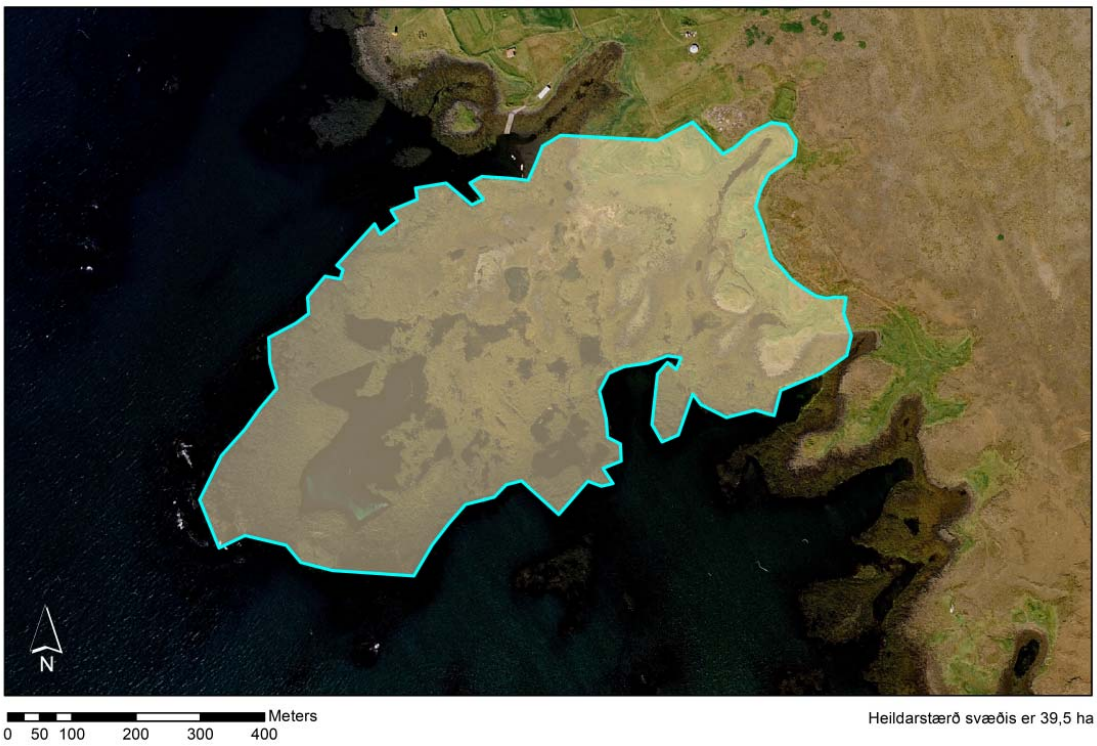


Figure 5.5: Stafnes proposed marine algae collection and organic conversion area (39.5 ha).

In terms of marine algae species naturally occurring in the area, it is seen to be typical of the relatively warm Southern Icelandic coast in that displays a complete fucoid zonation featuring *Pelvetia canaliculata*, *Fucus spiralis*, *Fucus vesiculosus*, *Ascophyllum nodosum*, *Fucus serratus* (Munda, 1972). Also, its upper sublittoral zone is predominantly ruled by *Laminaria digitata* and *Saccharina latissima*, and *Corallina officinalis* was recorded in tide pools of the eulittoral zone along with examples of *Chondrus crispus*, *Mastocarpus stellatus*. Other species recorded in the area were *Palmaria palmata* and *Ahnfeltia plicata* (Munda, 1991).

Through qualitative data collected during previous research at the site (conducted by Náttúrustofa Reykjaness (Jónsdóttir, 2011)), species distribution maps of the area were created (see Figure 5.6).

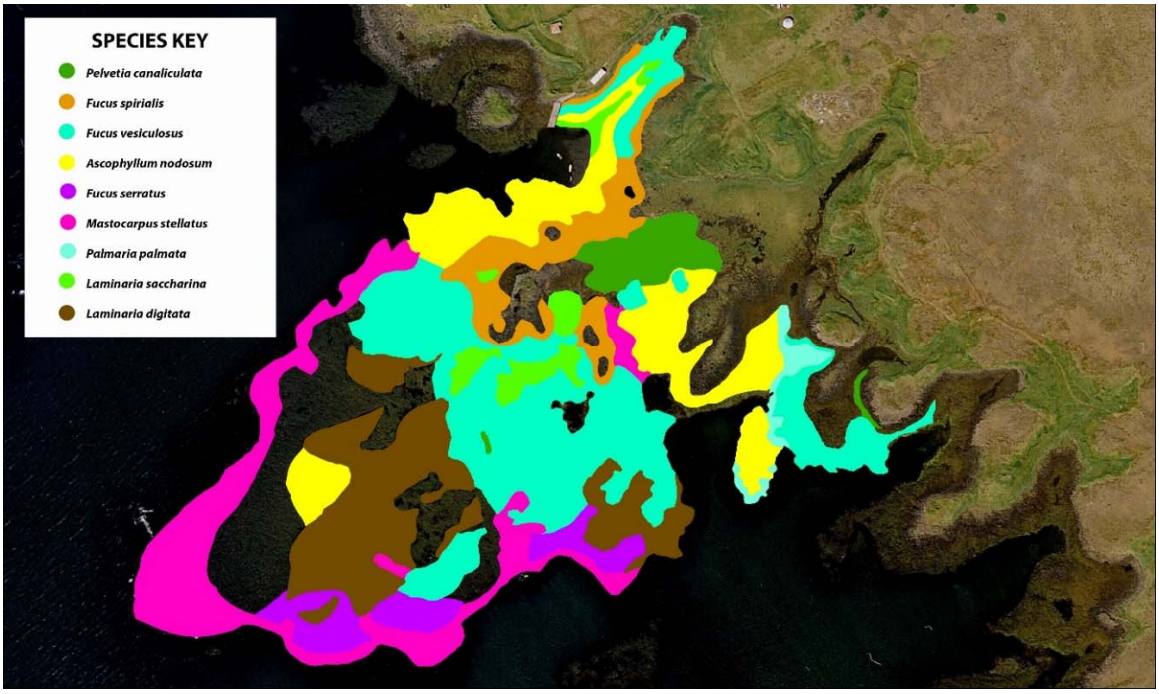


Figure 5.6: Stafnes abundant marine algae species distribution map[12].

The composite map in figure 5.6 shows that the following are examples of abundant species already present in the area: *Pelvetia canaliculata*, *Fucus spirialis*, *Fucus vesiculosus*, *Ascophyllum nodosum*, *Fucus serratus*, *Mastocarpus stellatus*, *Palmaria palmata*, *Saccharina latissima*, *Laminaria digitata*. Using the species distribution maps, percentages of species cover within this site area were then estimated and are shown in Figure 5.7, below.

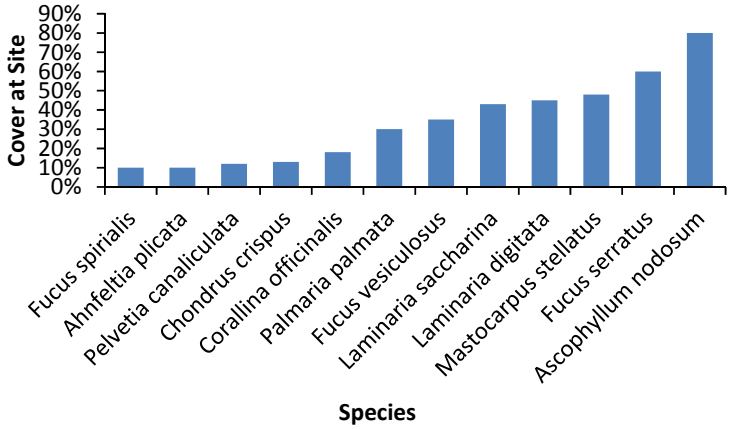


Figure 5.7: Relative percentage of marine algae species cover at the Stafnes site.

[12] Please note that this map shows a composite combination of species and so in some areas there is a complete overlap regarding their relative distribution at the site. Therefore, during sample collection individual maps are used to be able to show a more accurate insight into specific species distribution areas.

As can be seen in this chart, the six most abundant species present at the site were (in order of appearance): *Ascophyllum nodosum*, *Fucus serratus*, *Mastocarpus stellatus*, *Laminaria digitata*, *Saccharina latissima* (formerly *Laminaria saccharina*) and *Fucus versiculosis*.

It was therefore suggested that any laboratory research and analysis done during the latter part of this thesis should focus on the utilisation of abundant species already naturally present at the site, and that of the aforementioned species in particular.

As was done on the Reykólar site, a suitability assessment was also conducted on the proposed Stafnes site to compare it against each of the 7 points present in the "Suitable Site Criteria" section mentioned previously and with that of the Reykólar site (see Appendix 2, Table A2.1). The results showed that the Stafnes site shared many of the same favourable characteristics as the site in Reykhólar, and so it too appeared to meet all of the requirements for successful marine algae collection, with good potential for open-water cultivation of species. It also seemed favourable to meet the requirements needed for successful application of conversion to certified organic status.

Following on from this, an application for organic conversion of the Stafnes site was written and submitted to Vottunarstofan Tún in June, 2012. After a review of the documentation and an official inspection of the site, the proposed area (and the specific marine algae species listed) was awarded organic status in December, 2012 (see Appendix 3, Figure A3.1).

6 MARINE ALGAE SAMPLES

Although the author would have liked to have researched all of the marine macroalgae species present at the Stafnes site during the course of this study, regrettably, due to time and resource limitations, it was decided that only three species would be selected to be the focus of this project.

6.1 Species Selection

The following species were chosen for this research: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

The decision was based on a combination of relative ease of collection and abundance of material present at the site and also the presence of compounds of commercial interest (see Appendix 4, Tables A4.1 - A4.3), for a summary of some of the components identified in these species found in literature), and availability of their corresponding pure analytical standards.

Most notably, fucoxanthin, alginic acid and laminarin were seen to be present in all three chosen species and so were preliminary target compounds of interest in this study.

6.2 Sample Collection

Approximately 3000g of fresh marine algae material was collected from the Stafnes site on 19/08/2013. Each species was botanically positively identified using a marine algae field guide (Braune and Guiry, 2011), and was harvested in a sustainable manor with minimal disturbance to the surrounding environment in accordance with Tún organic standards (Tún, 2011). Upon arrival at the laboratory, the fresh material was immediately prepared as per the methods below.

6.3 Sample Preparation

For each species collected, samples were divided up and accurately weighed into 500g lots. 500g from each species was set aside for air drying, 500g was reserved for oven drying, and 3 lots of 500g were packed into plastic bags, labelled and stored in the freezer (one to remain frozen for experiments, one to be subjected to freeze drying, and the last one was used in proximate composition analysis). The remaining material of each species was also frozen and served as a backup supply.

6.3.1 Freezing of Marine Algae Samples

6.3.1.1 Materials

The following equipment and materials were used: Fridge freezer (Electrolux, model EN3400AOW), 1 roll of plastic freezer bags, laboratory weighing scales (Ohaus “Pioneer”, model PA402), 1 roll of kitchen roll, 5 Inch stainless steel surgical scissors (Whiteley, model 1956SS-5/SB).

The following chemicals were used: 2000ml of ultra-pure distilled water (Millipore, Milli-Q).

The following fresh marine algae species were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

6.3.1.2 Method

Fresh material from each species was cleaned of alien material and thoroughly washed with ultra-pure water. Excess water remaining on the material was removed by carefully padding it dry, before cutting into approximately 1cm strips, weighing and placing into plastic freezer bags.

6.3.1.3 Discussion

Care was taken to remove excess water from the samples which would subsequently freeze and could give error to the weight of the samples used later on. Also, the material was cut into strips to facilitate a more even freezing process and to make taking samples from these batches easier during the subsequent experiments executed.

6.3.2 Air Drying of Marine Algae Samples

6.3.2.1 Materials

The following equipment and materials were used: Laboratory weighing scales (Ohaus “Pioneer”, model PA402), 5 Inch stainless steel surgical scissors (Whiteley, model 1956SS-5/SB), 1 roll of kitchen roll, 6 meters of nylon string, large re-sealable plastic bags.

The following chemicals were used: 2000ml of ultra-pure distilled water (Millipore, Milli-Q).

The following fresh marine algae species were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

6.3.2.2 Method

Approximately 500g of fresh material from each species was cleaned of alien material and thoroughly washed with ultra-pure water. Excess water remaining on the material was removed by carefully padding it dry, before hanging up the material via the use of

the nylon string, and allowing to gently air dry over the course of 1 week at approximately 21°C.

After this period of time, the dried samples were then cut into approximately 1cm strips, were weighed and placing into re-sealable plastic bags and kept in a cool environment and out of direct sunlight.

6.3.2.3 Results

The results and calculations from the air drying process are shown below.

Table 6.1: Air dry weight results for marine algae samples taken from the Stafnes site.

SPECIES	WET WEIGHT	DRY WEIGHT	DRY WEIGHT %†
Ascophyllum nodosum	500.39g	213.87g	42.74%
Laminaria digitata	500.24g	141.87g	28.36%
Saccharina latissima	500.11g	115.73g	23.14%

†Dry weight % was calculated by using the following formula: $\frac{\text{Dry weight}}{\text{Wet weight}} \text{ x } 100$

6.3.2.4 Discussion

As can be seen in table 6.1, *Ascophyllum nodosum* had the highest dry weight, at nearly 50%, whereas both the *Laminaria digitata* and *Saccharina latissima* samples had a dry weight of approximately 20-30%, with *Saccharina latissima* displaying the lowest dry weight of 23.14%.

6.3.3 Freeze Drying of Marine Algae Samples

6.3.3.1 Materials

The following equipment and materials were used: Fridge freezer (Electrolux, model EN3400AOW), laboratory weighing scales (Ohaus “Pioneer”, model PA402), 5 Inch stainless steel surgical scissors (Whiteley, model 1956SS-5/SB), freeze dryer (Scanvac, CoolSafe 110-4 PRO), 3x 1litre plastic tubs, Parafilm®, hypodermic needle head.

The following frozen marine algae species were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

6.3.3.2 Method

Approximately 500g of pre-frozen sample of each species were placed into 1 litre plastic tubs and which were then sealed with parafilm and small holes were carefully pricked into the parafilm using a hypodermic needle head.

The plastic tubs were then placed into the freeze drier that was set at -59°C under a vacuum of $1.0 \text{x} 10^{-3}$ Torr. The samples were allowed to dry for 5 days, before being removed, weighed and stored in re-sealable plastic bags and kept in a cool environment and out of direct sunlight.

6.3.3.3 Results

The results and calculations from the freeze drying process are shown below.

Table 6.2: Freeze dry weight results for marine algae samples taken from the Stafnes site.

SPECIES	WET WEIGHT	DRY WEIGHT	DRY WEIGHT %†
Ascophyllum nodosum	500.40g	148.87g	29.75%
Laminaria digitata	501.50g	111.68g	22.27%
Saccharina latissima	500.80g	83.58g	16.69%

†Dry weight % was calculated by using the following formula: $\frac{\text{Dry weight}}{\text{Wet weight}} \text{ x } 100$

6.3.3.4 Discussion

As can be seen in table 6.2, *Ascophyllum nodosum* again had the highest dry weight, at nearly 30%, whereas both the *Laminaria digitata* and *Saccharina latissima* samples had a dry weight of approximately 15-25%, with *Saccharina latissima* displaying the lowest dry weight of 16.69%.

6.3.4 Oven Drying of Marine Algae Samples

6.3.4.1 Materials

The following equipment and materials were used: Laboratory oven (Binder, model ED23), laboratory weighing scales (Ohaus “Pioneer”, model PA402), 5 Inch stainless steel surgical scissors (Whiteley, model 1956SS-5/SB), 1 roll of kitchen roll, glass desiccator (Isolab, 300 mm).

The following chemicals were used: 2000ml of ultra-pure distilled water (Millipore, Milli-Q), silica gel (Sigma Aldrich - Orange with moisture indicator, free of heavy metals).

The following fresh marine algae species collected were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

6.3.4.2 Method

Approximately 500g of fresh material from each species was cleaned of alien material and thoroughly washed with ultra-pure water. Excess water remaining on the material was removed by carefully padding it dry, before cutting the material into approximately 1cm strips and placing in a laboratory oven that was pre-heated to 40°C, and being allowed to dry over a 24 hours

After this period of time, the hot dried samples were then placed into a dessicator containing silica gel to allow to cool, before being weighed and placing into re-sealable plastic bags and kept in a cool environment and out of direct sunlight.

6.3.4.3 Results

The results and calculations from the oven drying process are shown below.

Table 6.3: Oven dry weight results for marine algae samples taken from the Stafnes site.

SPECIES	WET WEIGHT	DRY WEIGHT	DRY WEIGHT %†
Ascophyllum nodosum	500.32g	187.57g	37.49%
Laminaria digitata	500.10g	91.77g	18.35%
Saccharina latissima	500.00g	112.15g	22.43%

†Dry weight % was calculated by using the following formula: $\frac{\text{Dry weight}}{\text{Wet weight}} \text{ x } 100$

6.3.4.4 Discussion

As can be seen in table 6.3, *Ascophyllum nodosum* again had the highest dry weight, at nearly 40%, whereas both the *Laminaria digitata* and *Saccharina latissima* samples had a dry weight of approximately 15-25%. However, this time it was *Laminaria digitata* which displayed the lowest dry weight of 18.35%.

This could have possibly been due to the presence of higher levels of volatile compounds in the *Laminaria digitata* sample when compared with that of the *Saccharina latissima* sample. Thereby, upon being heated at a higher drying temperature a large proportion of these compounds may have evaporated along with the moisture. However, further investigation would be required in order to confirm this hypothesis.

7 EXTRACTION

According to Cosmetics Organic Standards, in order for an extract from a certified organic raw material to also be eligible for organic status, then any extractions done must be with either water, a third solvent of certified organic plant origin (such as ethyl alcohol, glycerine, vegetable oils, honey) or supercritical CO_2 absorption, on an inert support that conforms to the standard (COSMOS, 2012).

Upon researching the different types of active constituents that are present in marine algae species it was therefore decided that experiments should be conducted favouring ethyl ethanol as the extraction solvent. This is not only because ethyl alcohol has good solvent properties for both polar and non-polar chemicals, but also by varying its dilution with water optimum solvating power for a specific plant material can be achieved. In this way ethanolic extracts (tinctures) are highly regarded by pharmacists and medical herbalists as being one the most effective ways of achieving concentrated levels of a broad-spectrum of active constituents.

7.1 Herbal Tinctures

The preparation of tinctures of vegetable origin is described in the officially recognized European Pharmacopoeia (7th Ed.) and U.S. Pharmacopoeia (2013) for the preparation of herbal medicines and pharmaceutical precursors.

It incorporates the technique of tincture preparation in the old method of the British Herbal Pharmacopoeia (1996), as a basis of the tincture making processes, recognizing the importance of the herb to solvent ratio, the effect of natural plant moisture present in fresh material and the concentration of rectified spirit (ethanol) used for standardising the preparation of tinctures.

The ethanol used is actually a mixture of ethanol (of vegetable origin) and double-distilled water, containing not less than 94.7% volume per volume (v/v) or 92.0% weight per weight (w/w) and not more than 96% v/v or 93.4 % w/w of pure ethanol, with a specific gravity at 20°C of 0.8119–0.8139 (EDQMH, 2010).

Today, herbal tinctures used as medicine are generally still prepared according to the specifications prescribed in the aforementioned pharmacopoeias. Preparation involves two techniques, maceration and percolation. Fresh gummy, mucilaginous vegetable substances which contain moisture are subjected to maceration; dry vegetable substances and xerophytes are subjected to percolation (BHP, 1996).

In maceration, vegetable substances are processed at the earliest opportunity, as moisture is an integral part of the vehicle during preparation of the tincture. Otherwise,

during preservation moisture and other inorganic and organic constituents and volatile oils are lost. Moisture is evaluated by subtracting the quantity of dry substance from a given quantity of fresh moist vegetable substance. The quantity of dry substance in a given quantity of fresh moist vegetable substance is compared with the standard formula for preparation. If the moisture content is in excess of that given in the standard formula, either water is deducted from the alcohol used for preparation or the fresh vegetable substance is subjected to slow drying at a moderate temperature. If the moisture content is less than that given in the formula, the shortage is made up by the addition of purified water (USPC, 2012).

Dry vegetable substances are subjected to percolation according to the formula of respective drug monograph. The ratio of water to strong alcohol is maintained in such a way that the drug strength is finally 1:10 (i.e. 1 part of dry crude drug substance in each 10 parts of the completed tincture), for most vegetable preparations (BHP, 1996).

In either method, the resultant mixture of plant material and ethanol is then left to slowly extract over the period of up to several months with occasional agitation of the contents to facilitate the process (maceration). At the end of this time the liquid extract (menstrum) is then separated from the spent plant material (marc) via the use of a filter press (BHP, 1996).

Tinctures are commonly prepared where the final alcohol percentages are as follows: 25%, 45%, 70% and 90% (BHP, 1996). A higher percentage alcohol is used on plant material that contains more non-polar molecules such as oils, resins and waxes, whereas a lower percentage alcohol (higher percentage water) is used where there are higher levels of polar components, such as mucilaginous substances[13]. However, although some tinctures with lower alcohol concentration have been found to contain extractive medicinal constituents of pharmacological activity, generally speaking alcohol percentages of no lower than 25% are used, as this may otherwise compromise the ethanol's ability to also act as a preservative for the extract (EDQMH, 2010).

7.1.1 Marine Algae Ethanolic Tinctures

In regards to marine algae species, because tinctures of this type are rarely known and used (at least until more recently), there is very little information regarding their standard formulas. However, according to the BHP (1996), one species that is well known and used in tincture form as herbal medicine is *Fucus versiculosis*, and it states that the extraction formula used should be 1:1 (i.e. fluid extract) in 25% ethanol.

Although this traditional method of extraction has been successfully applied for use for many years in both Phytotherapy (herbal medicine) and Pharmacognosy, it was decided that another technique should be used for this research instead. One that may more

[13] Pindari Herb Farm. http://www.pindariherbfarm.com/educate/fpt.htm. Accessed on 21/03/13.

effectively and efficiently extract the broad spectrum of components present so as to improve the accuracy of the subsequent analysis to be done.

7.2 Soxhlet Method of Extraction

Soxhlet extraction has been used since 1879 and is commonly employed as a benchmark for total extractable organic residues in analytical chemistry (Sanghi and Tiwle, 2013). The process itself involves the continual removal and recovery of organic analytes from a permeable solid matrix by means of a solvent which is continually evaporated from a still-pot and condensed in such a manner that it falls into and permeates through the matrix which itself is held in a permeable container in a siphonable chamber (Djenni *et al.*, 2013).

Compared with the more traditional extraction method mentioned previously, generally a greater yield of extract can be achieved in a shorter time using the minimum of solvent. However, one drawback is that extracts are often subjected to high temperatures for many hours over the course of the extraction, which makes this method unsuitable for use with sensitive compounds. To try to overcome this, a variation on the traditional Soxhlet uses an adapted rotary evaporator which enables the interior of the system to be under vacuum. In turn, this facilitates the extraction to be done at much lower temperatures than would otherwise be achieved at atmospheric pressure.

The use of this method in the study would mean that any variation in the yield and/or composition of the extracts would be more likely due to the original preparation of the raw material rather than from the extraction process, thereby improving the accuracy and reliability of analysis results obtained.

7.2.1 Marine Algae Ethanolic Fractions via Vacuum Soxhlet Extraction

7.2.1.1 Materials

The following equipment and materials were used: Fridge freezer (Electrolux, model EN3400AOW), laboratory weighing scales (Ohaus “Pioneer”, model PA402), 18cm vegetable knife (Global, G-5), Rotary evaporator with vacuum Soxhlet attachment (Heidolph, Hei-VAP Value with G6 glassware set), 36x large cellulose extraction thimbles (to fit Soxhlet), 100ml measuring cylinder, 500ml measuring cylinder, 36x500ml glass bottles, vacuum glass desiccator (Isolab, 300 mm), laboratory vacuum pump (KNF Neuberger, model UN810.3FTP), 4x2 meters of rubber tubing (for vacuum and condenser water), 4x100mm watch glass, electronic pH meter (Oakton, pH 700 benchtop meter with probes and stand), glass hydrometer, 36x25ml plastic vial with screwtop lid.

The following chemicals were used: 5500ml of 96% certified organic ethanol (Sacchetto – derived from certified organic wheat), 5500ml of ultra-pure distilled water (Millipore, Milli-Q), silica gel (Sigma Aldrich - Orange with moisture indicator, free of heavy metals).

The following prepared marine algae species were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

7.2.1.2 Method

Approximately 100g (fresh weight or dried equivalent) of prepared material from each species was chopped into finer pieces using a knife before being placed into cellulose extraction thimbles and extracted using a vacuum Soxhlet.

Each of the samples were extracted with 300ml of the following three concentrations of ethanol diluted with ultra-pure water as the solvent: 96%, 48%, 0% (100% H_2O). Therefore each sample was subjected to 3 extractions of decreasing ethanol concentrations; each extraction lasting 6 hours each and was performed under a vacuum of 50 Pabs, with a vapour temperature of no more than 40°C and a rotating 1000ml boiling flask set at 50 rpm.

Ethanolic extract fractions of each concentration were removed between extractions, collected into glass jars, labelled and were then stored in a fridge (4°C) until further processing.

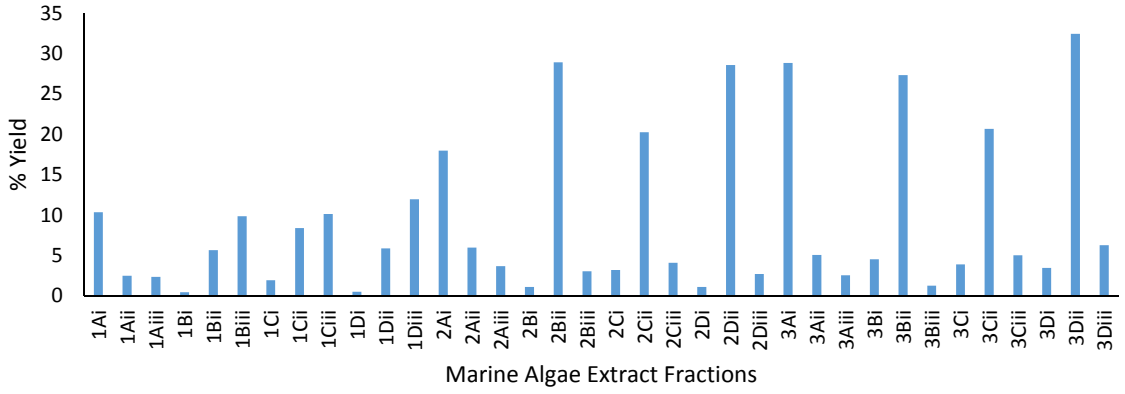
In the meantime, each ethanolic extract was measured for its pH and specific gravity (% alcohol).

Once ethanolic fractions from all prepared material were obtained they were then each reduced to dryness using a rotary evaporator (again under vacuum, with vapour temperature of no more than 40°C).

The dry extracts obtained were then placed into a vacuum desiccator containing silica gel and were further dried under vacuum for 3 days before then being weighed and stored in plastic vials and kept in the fridge (4°C).

7.2.1.3 Results

The results from the extraction process are shown in Appendix 5, Tables A5.1 – A5.3, an overview of the extraction yields can be seen in figure 7.1, below.



SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Figure 7.1: Extraction yields of the fractions gained from the prepared marine algae samples.

7.2.1.4 Discussion

Preliminary assessment of the extracts showed a large variation in components composition in each fraction both in their liquid and dried forms. This could be clearly noted by the contrast of colours and the bulk of precipitates present in the samples collected. It also appeared that in some fractions a large amount of salt was present along with the crude material, the presence of which was seen to give the dried extract a more crystalline appearance and also added extra volume and weight, giving misleading yields.

Compared to pH 7.0 (the pH measured for all of the solvents mixes before extraction), in all fractions from the *Ascophyllum nodosum* samples, the pH was seen to increase slightly (towards neutral) with decreasing ethanol percentages, indicating a higher extraction of acidic compounds in the earlier fractions. In contrast, all fraction from the *Laminaria digitata* and *Saccharina latissima* samples showed a decreasing pH (away from neutral) with decreasing ethanol percentages, indicating a higher extraction of acidic compounds in the later fractions.

It was interesting to note that final the ethanol percentages of the liquid extracts obtained were much different than those originally applied to the extractions.

In general, earlier fractions were seen to have a lower ethanol percentage, whereas later fractions had higher percentages. This was probably due to the fact that the prepared samples still retained some moisture (especially with the frozen samples) which contributed to the dilution of earlier fractions. On the other hand, the higher ethanol percentages in the later fractions were probably due to the fact that samples were not

completely free of ethanol left over from the previous higher ethanol extraction, which was then carried over into the next extraction.

In regards to the relative dry extract yield percentages from the original raw material, the highest levels were seen in the *Laminaria digitata* extract fractions with an average of 10.03% (the lowest being 1.07% for fraction 2Bi, the highest being 28.92% for fraction 2Bii). The next highest were the *Saccharina latissima* extract fractions with an average of 9.83% (the lowest being 1.22% for fraction 3Biii, the highest being 32.43% for fraction 3Dii). *Ascophyllum nodosum* had the lowest average of 4.97% (the lowest being 0.42% for fraction 1Bi, the highest being 11.94% for fraction 1Diii).

Interpretation of these results suggests that the oven dried material may afford higher yield percentages than the other preparative methods in terms of this extraction method. However, as mentioned previously, the high presence of salt in some of the extracts is likely to be giving a false view of the extraction yields. It is therefore suggested that future research should include the quantification of salt present in these extracts in order to more effectively establish an accurate determination of yields.

8 ANALYSIS

8.1 Proximate Composition Analysis

The following set of analyses are commonly used in industry to estimate the relative amounts of water, ash, lipid, carbohydrate and protein (Gökoðlu and Yerlikaya, 2003), and so were used to analyse and compare these values present in the prepared marine algae samples, and in some cases their extracts as well.

8.1.1 Moisture

Analysis was conducted in accordance with Association of Analytical Communities (AOAC) method 930.04 (AOAC International and Latimer, 2012).

8.1.1.1 Materials

The following equipment and materials were used: 1 roll of re-sealable plastic bags (Ziplock®), laboratory oven (Binder, model ED23), laboratory weighing scales (Ohaus "Pioneer", model PA402), 5 Inch stainless steel surgical scissors (Whiteley, model 1956SS-5/SB), 18cm vegetable knife (Global, G-5), 12 x 30ml ceramic crucibles (Coorstek, model 60108), Borosilicate glass vacuum desiccator (Isolab, 300mm), laboratory vacuum pump (KNF Neuberger, model UN810.3FTP), 1 meter of rubber tubing, heat protective glove.

The following chemicals were used: Silica gel (Sigma Aldrich - Orange with moisture indicator, free of heavy metals).

The following marine algae species were used (9g wet weight (or equivalent) of prepared sample material): *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

8.1.1.2 Method

Each of the ceramic crucibles were labelled and individually weighed, before 3g of finely chopped sample material (total of 3 x 3g of each species) was added to each crucible and weighed again.

All crucible containing the samples were then placed in a laboratory oven set at 100°C, and were left for 24 hours.

Upon completion of the 24 hours, the crucibles were removed from the oven with a heat protective glove and were immediately store in a vacuum desiccator (pre-filled with activated silica gel desiccant) for 1 hour, by which time the samples had sufficiently cooled under vacuum.

Each of the crucibles containing the dehydrated samples were then re-weighed and their percentage moisture (wt/wt) was calculated, and the mean average was then taken of the three results gained for each species analysed.

8.1.1.3 Results

The data and calculations from the moisture analysis are shown in Appendix 6, Tables A6.1 – A6.4, an overview of which can be seen in figures 8.1 and 8.2, below.

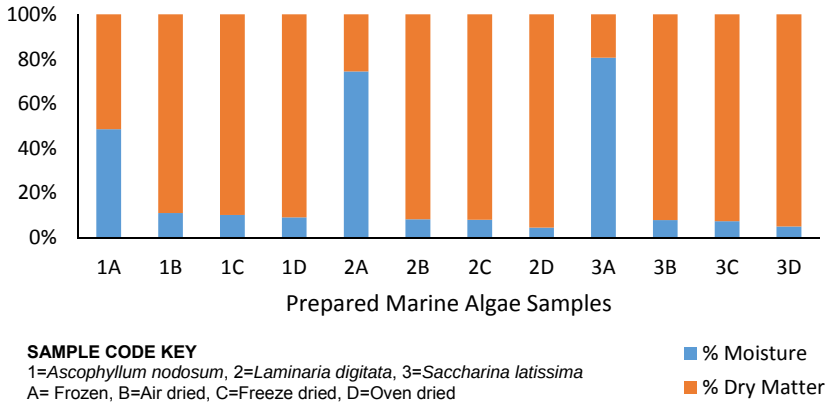


Figure 8.1: Moisture and dry matter percentages for the prepared marine algae samples.

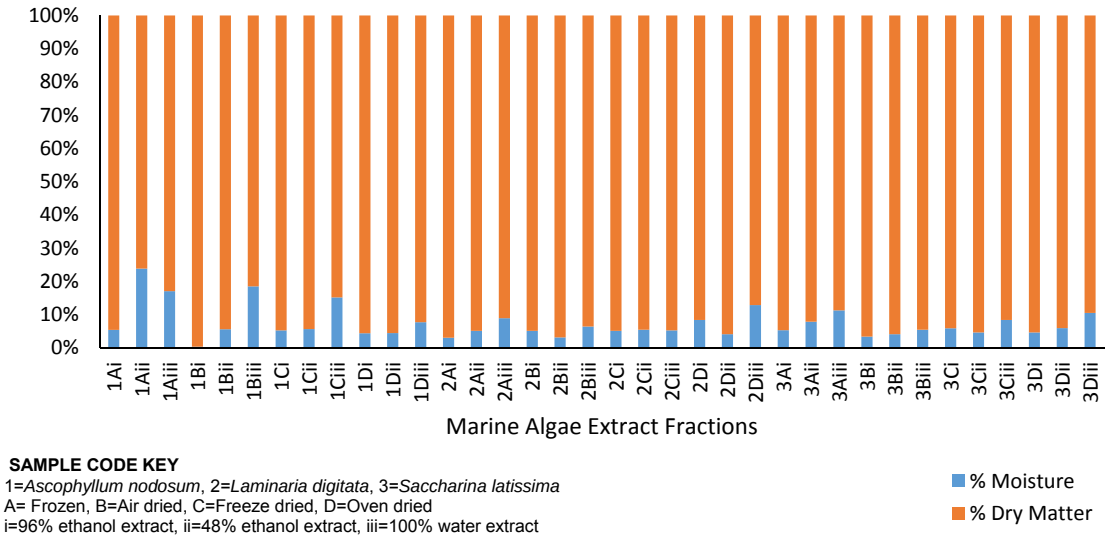


Figure 8.2: Moisture and dry matter percentages for the marine algae extracts.

8.1.1.4 Discussion

In regards to moisture percentages present within the prepared algae samples, it was seen that with each of the species studied the frozen samples contained the highest amounts (this is as to be expected, considering that the other types of prepared material have all undergone some degree of drying in their processing).

The species with the highest moisture content was *Saccharina latissima* with 80.57%, followed closely by *Laminaria digitata* with 74.45%, and *Ascophyllum nodosum* having 48.52%.

For each of the species, it was seen that of the dried material, the air dried material contained the highest amount of moisture, and the oven dried material contained the lowest amount of moisture. Again, this is not surprising as the application of higher temperatures during the drying process is likely to remove more moisture as well as any volatile compounds that may be present.

In regards to the extract fractions analysed, the highest levels of moisture content were seen in the *Ascophyllum nodosum* with an average of 9.39% (the lowest being 0.29% for fraction 1Bi, the highest being 23.78% for fraction 1Aii). The next highest were the *Saccharina latissima* extract fractions with an average of 6.38% (the lowest being 4.03% for fraction 3Bii, the highest being 11.24% for fraction 3Aiii). *Laminaria digitata* had the lowest average of 6.01% (the lowest being 2.98% for fraction 2Ai, the highest being 12.81% for fraction 2Diii).

In relation to the dry matter percentages of each sample/fraction, as to be expected, these were found to be inversely proportional to the moisture percentages.

One interpretation of the results could be that due to the relatively small moisture content present in *Ascophyllum nodosum* a higher dry biomass for this species could be obtained which could possibly result in higher rates of extraction when considering commercial production of isolated marine compounds.

8.1.2 Ash

Analysis was conducted in accordance with Association of Analytical Communities (AOAC) method 930.05 (AOAC International and Latimer, 2012).

8.1.2.1 Materials

The following equipment and materials were used: Laboratory muffle furnace (Thermo Scientific, Thermolyne, model EW-33900-10), laboratory weighing scales (Ohaus “Pioneer”, model PA402), 12 x 30ml ceramic crucibles (Coorstek, model 60108), Borosilicate glass vacuum desiccator (Isolab, 300 mm), laboratory vacuum pump (KNF Neuberger, model UN810.3FTP), 1 meter of rubber tubing, furnace tongs.

The following chemicals were used: Silica gel (Sigma Aldrich - Orange with moisture indicator, free of heavy metals).

The following marine algae species were used (the same sample material was used from previous moisture analysis conducted – see 8.1.1 Moisture): *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

8.1.2.2 Method

The dehydrated samples (still contained within their crucibles), as obtained from the moisture analysis were immediately used in this dry ashing process.

The samples were placed into a laboratory muffler furnace set at 550°C for 18 hours.

Upon completion of the 18 hours, the crucibles were removed from the furnace with a tongs and were immediately store in a vacuum desiccator (pre-filled with activated silica gel desiccant) for 2 hours, by which time the samples had sufficiently cooled under vacuum.

Each of the crucibles containing the ashed samples were then re-weighed and their percentage ash (wt/wt) was calculated, and the mean average was then taken of the three results gained for each species analysed.

8.1.2.3 Results

The results and calculations from the ash analysis are shown in Appendix 7, in Tables A7.1 – A7.3, an overview of which can be seen in figures 8.3 and 8.4, below.

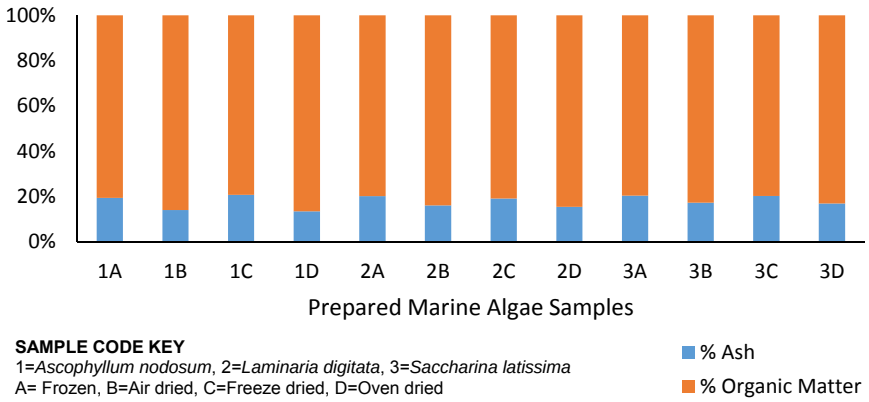


Figure 8.3: Ash and organic matter percentages for the prepared marine algae samples.

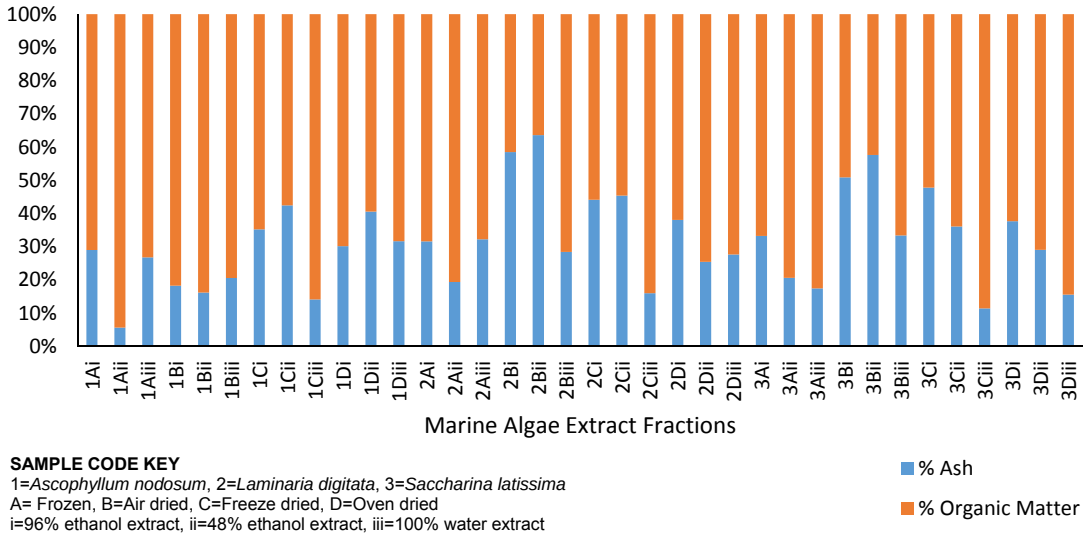


Figure 8.4: Ash and organic matter percentages for the marine algae extracts.

8.1.2.4 Discussion

Looking at the prepared material, the species with the highest ash value was seen to be *Saccharina latissima* with an average of 18.68% (the lowest being 16.92% for sample 3D, the highest being 20.37% for sample 3A), followed closely by *Laminaria digitata* with an average of 17.69% (the lowest being 15.45% for sample 2D, the highest being 20.13% for sample 2A), and then *Ascophyllum nodosum* having an average of 16.89% (the lowest being 13.46% for sample 1D, the highest being 20.72% for sample 1C).

In regards to the extract fractions analysed, the highest levels of ash content were seen in the *Laminaria digitata* with an average of 35.83% (the lowest being 15.98% for fraction 2Ciii, the highest being 63.56% for fraction 2Bii). The next highest were the *Saccharina latissima* extract fractions with an average of 31.71% (the lowest being 11.39% for fraction 3Ciii, the highest being 57.60% for fraction 3Bii). *Ascophyllum nodosum* had the lowest average of 25.86% (the lowest being 5.59% for fraction 2Aii, the highest being 42.39% for fraction 1Cii).

In relation to the organic matter percentages of each sample/fraction, as to be expected, these were found to be inversely proportional to the ash percentages.

One interpretation of the results could be that the relatively small ash content (high organic content) present in *Ascophyllum nodosum* could possibly result in higher rates/yield of extraction when considering commercial production of isolated marine compounds.

8.1.3 Fat

Analysis was conducted in accordance with Association of Analytical Communities (AOAC) method 996.06 (AOAC International and Latimer, 2012).

8.1.3.1 Materials

The following equipment and materials were used: Laboratory weighing scales (Ohaus "Pioneer", model PA402), 3x Soxhlet apparatus (Supelco – 50 ml capacity with 500 ml round bottom flask and Allihn condenser), 36x Soxhlet cellulose extraction thimbles (Whatman, single thickness – 30 mm x 80 mm), digital hotplate stirrer (Stuart, model CD162), fume hood, laboratory vacuum oven (Binder, VDL 23), Borosilicate glass vacuum desiccator (Isolab, 300 mm), laboratory vacuum pump (KNF Neuberger, model UN810.3FTP), 1 meter of rubber tubing, pestle and mortar, laboratory tongs, heat protective gloves.

The following reagents and chemicals were used: Silica gel (Sigma Aldrich - Orange with moisture indicator, free of heavy metals), petroleum ether (Sigma Aldrich - ACS reagent).

The following prepared marine algae species were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

8.1.3.2 Method

Approximately 50g (or equivalent wet weight) of finely chopped prepared material from each of the three species samples was individually and gently ground via the use of a mortar and pestle[14].

Whilst wearing rubber gloves, three pre-dried cellulose extraction thimbles were removed from the vacuum desiccator and were accurately weighed, before 10 g (or equivalent wet weight) of ground sample material (total of 3x10 g - each species/preparation type done in triplicate) were added to individual thimbles and weighed again. A small plug of dried glass wool was then placed into each of the thimbles (holding the sample in place), which were then reweighed once more.

Each of the thimbles were then placed within, and extracted via, a glass Soxhlet apparatus (using approximately 350 ml of petroleum ether as the extraction solvent), and were continuously extracted over a period for 6 hours.

After this time, the apparatus was allowed to cool, before removing the thimbles from the Soxhlet extractor using tongs.

The thimbles were then air dried overnight in a fume hood, and then were dried in a vacuum oven at 70ºC, 635 mm Hg, for 24 hours.

Upon completion of the 24 hours, the thimbles were removed from the oven with heat protective gloves and were immediately store in a vacuum desiccator (pre-filled with

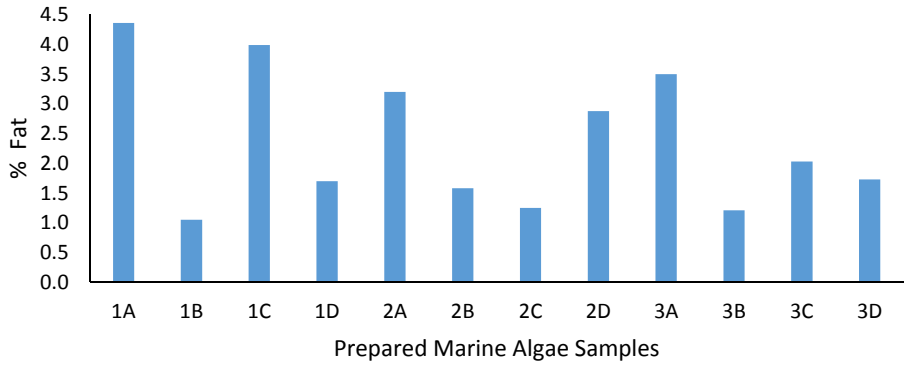
[14] Caution was taken here so as not to excessively grind the material which could have led to loss of fat in the mortar.

activated silica gel desiccant) for 1 hour, by which time the samples had sufficiently cooled under vacuum.

Each of the thimbles containing the defatted samples were then re-weighed and their percentage fat (wt/wt) was calculated, taking care to correct for moisture content using data from the previous moisture analysis (see section 8.1.1 Moisture). The mean average was then taken of the three results gained for each species analysed.

8.1.3.3 Results

The results and calculations from the fat analysis are shown in Appendix 8, Table A8.1, an overview of which can be seen in figure 8.5, below.



SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried

Figure 8.5: Fat percentages found in the prepared marine algae samples.

8.1.3.4 Discussion

The results from the prepared material showed that the species with the highest fat content was seen to be *Ascophyllum nodosum* with an average of 2.78% (the lowest being 1.05% for sample 1D, the highest being 4.36% for sample 1A), next was *Laminaria digitata* with an average of 2.23% (the lowest being 1.25% for sample 2C, the highest being 3.20% for sample 2A), and then *Saccharina latissima* having an average of 2.12% (the lowest being 1.21% for sample 3B, the highest being 3.50% for sample 3A).

Interpretation of the results could be that in order to achieve the highest extraction of fats/lipids from these species (and especially if *Ascophyllum nodosum* is used), then it is best to use frozen starting material.

8.1.4 Carbohydrate

Analysis was conducted in accordance with the method outlined in an article by Saha and Brewer, 1994.

8.1.4.1 Materials

The following equipment and instrumentation was used: Laboratory weighing scales (Ohaus “Pioneer”, model PA402), UV/Visible Spectrophotometer (GE Healthcare Life Sciences - Ultrospec 2100 pro), 1000 ml volumetric flask, fume hood, nylon in-line filters (0.45 µm), 34 x 30 ml test tubes, 20-100 µl micropipette (Gilson - Pipetman Classic™ P100), micropipette tips, vortex test tube mixer (Scientific Industries - Vortex Genie 2), ultrasonic water bath (Cole Parmer - 8891), 3x quarts cuvettes.

The following reagents and chemicals were used: Glucose (Fluka - European Pharmacopoeia (EP) Reference Standard), methanol (Sigma Aldrich - CHROMASOLV®, for HPLC, ≥99.9%), ultra-pure distilled water (Millipore, Milli-Q), Phenol (Sigma Aldrich – loose crystals, ACS reagent, ≥99.0%), sulphuric acid (Sigma Aldrich - ACS reagent, 95.0-98.0%).

The following prepared marine algae species and their related extracts were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

8.1.4.2 Method

Standard curve tubes were prepared using a glucose standard solution (100 mg glucose/L) and ultra-pure water by pipetting aliquots of the glucose standard into clean test tubes (duplicates for each concentration) such that the tubes contained 0–100 µg of glucose in a total volume of 2 ml. The 0 µg glucose/2 ml sample was used to prepare the reagent blank.

The samples to be tested were then dissolved in 50% methanol (50:50 ultra-pure water/methanol) in test tubes, which were filtered with a 0.45 µm nylon in-line filter before analysis. Duplicates were made of each sample that used the prepared marine algae species material. However, due to the limited amount of extract material available, only single samples were made with these.

Marine algae extracts were diluted to 500 µg per 2 ml (250 ppm), and their equivalent prepared material was also diluted to this same concentration using the extract to raw material ratio.

Under a fume hood, to each test tubes containing the samples, standards and blanks, 0.05 ml of 80% phenol was added. Each tube was then thoroughly mixed using a vortex test tube mixer.

5.0 ml concentrated sulphuric acid (H_2SO_4) was then carefully added to each test tube, which was again mixed on a vortex test tube mixer.

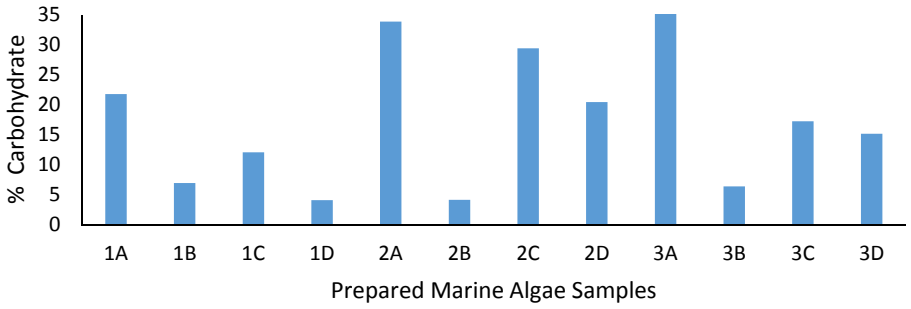
The tubes were allowed to for 10 min before being placed place in a 25°C bath for 10 min (i.e., to cool them to room temperature), and vortex mixed again before their absorbance’s were measured.

In order to read the absorbance, small amounts of each sample was poured into clean quarts cuvettes. The spectrophotometer was then zeroed using the blank, which then remained in the spectrophotometer and was used to calibrate the system after each sample was read.

All samples were read at a wavelength of 490 nm. Firstly the standard curve tubes were recorded from low to high concentration (i.e., 10 µg/ml up to 50 µg/ml), and then the samples were analysed.

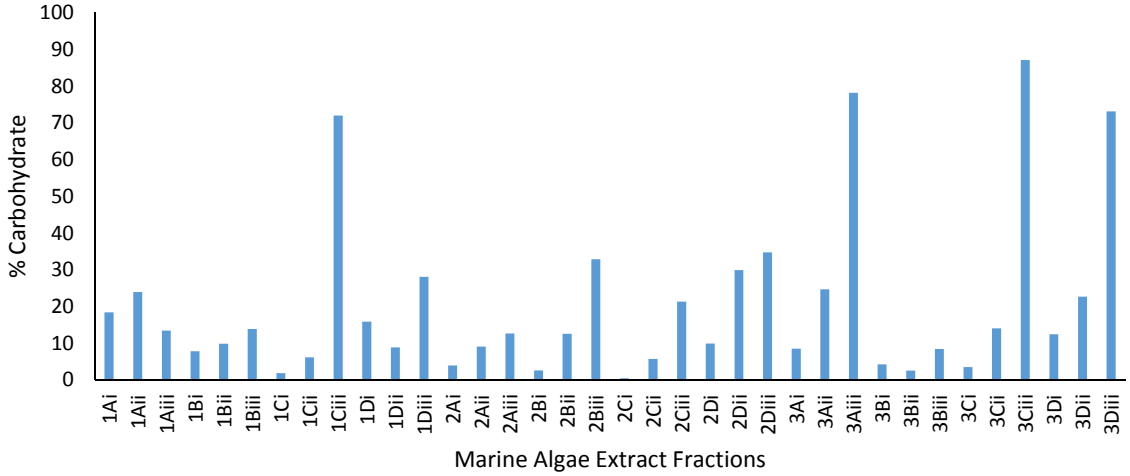
8.1.4.3 Results

The results and calculations from the carbohydrate analysis are shown in Appendix 9, Tables A9.1 – A9.7 and figure A9.1, an overview of which can be seen in figures 8.6 and 8.7, below.



SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried

Figure 8.6: Carbohydrate percentages found in the prepared marine algae samples.



SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Figure 8.7: Carbohydrate percentages measured in the marine algae extracts.

8.1.4.4 Discussion

Starting with the prepared material, the species with the highest carbohydrate value was seen to be *Laminaria digitata* with an average of 21.95% (the lowest being 4.13% for sample 2B, the highest being 33.85% for sample 2A), next was *Saccharina latissima* with an average of 20.98% (the lowest being 6.34% for sample 3B, the highest being 45.23% for sample 3A), and then *Ascophyllum nodosum* having an average of 11.21% (the lowest being 4.07% for both sample 1D, the highest being 21.76% for sample 1A).

In regards to the extract fractions analysed, the highest levels of carbohydrate content were seen in the *Saccharina latissima* with an average of 28.29% (the lowest being 2.55% for fraction 3Bii, the highest being 87.17% for fraction 3Ciii). The next highest were the *Ascophyllum nodosum* extract fractions with an average of 18.34% (the lowest being 1.80% for fraction 1Ci, the highest being 72.02% for fraction 1Ciii). *Laminaria digitata* had the lowest average of 14.64% (the lowest being 0.43% for fraction 2Ci, the highest being 34.73% for fraction 2Diii).

The results clearly show that if carbohydrates are the target extraction compounds, then freeze dried *Saccharina latissima* material should be employed and extracted using a high concentration of ultra-pure water as the solvent.

8.1.5 Protein

Analysis was conducted in accordance with the modified Lowry method outlined in an article by Barbarino and Lourenço, 2005.

8.1.5.1 Materials

The following equipment and instrumentation was used: Laboratory weighing scales (Ohaus “Pioneer”, model PA402), Fridge freezer (Electrolux, model EN3400AOW), Centrifuge (Thermo Scientific - Sorvall™ RC 6 Plus Centrifuge with Fiberlite™ F13-14x50cy fixed angle rotor), UV/Visible Spectrophotometer (GE Healthcare Life Sciences - Ultrospec 2100 pro), 24x25 ml plastic vials with screw top lids, 12 x 50 ml centrifuge tubes, ice bath, 20-100 µl micropipette (Gilson - Pipetman Classic™ P100), 200-1000 µl micropipette (Gilson - Pipetman Classic™ P1000), micropipette tips, vortex test tube mixer (Scientific Industries - Vortex Genie 2), 3x quarts cuvettes.

The following reagents and chemicals were used: Ultra-pure distilled water (Millipore, Milli-Q), sodium hydroxide concentrate (Fluka - 0.1M NaOH (0.1N)), 2-mercaptoethanol (Aldrich, ≥99.0%), trichloroacetic acid (Sigma Aldrich - ACS reagent, ≥99.0%), protein standard (Sigma Aldrich - Micro Standard, 1mg bovine serum albumin in 1ml liquid), Folin & Ciocalteu’s phenol reagent (Sigma Aldrich - 2 N), sodium carbonate (Sigma Aldrich - anhydrous, free-flowing, Redi-Dri™, ACS reagent, ≥99.5%), copper(II) sulfate pentahydrate (Sigma Aldrich - ACS reagent, ≥98.0%), potassium sodium tartrate tetrahydrate (Sigma Aldrich - ACS reagent, 99%).

The following prepared marine algae species were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

8.1.5.2 Method

Of the prepared samples to be tested, 50 mg of each was added to individual plastic vials and was allowed to soak in 4 ml of ultra-pure water for 12 hours.

1 hour before this time period had completed, the samples were hand homogenised for 20 mins using up to 4 ml of ultra-pure water as a rising aid to make sure that all water-ground material is recovered.

Upon completion of the 12 hours, the samples were then centrifuged at 4°C, 15,000 *g* (10,588 rpm) for 20 min, after which, the supernatants were collected, and the pellets were extracted using 1.0 ml 0.1N NaOH with 0.5% β-mercaptoethanol (v/v) at room temperature for one hour with occasional shaking, before being centrifuged at 21°C, 15,000 *g* (10,588 rpm) for 20 min.

Supernatants from this step were then combined with those previously collected to make up a final volume of 9 ml for each extract.

25% of cold (4°C) trichloroacetic acid (TCA) was then added to the extracts in a 2.5:1 ratio (TCA : homogenate, v/v), and was kept in an ice bath for 30 mins before being centrifuged for 20 min at 4 ◦C and 15,000 *g* (10,588 rpm).

Supernatants were discarded, and the pellets were washed with cold 10% TCA (4°C) and centrifuged again.

Pellets formed after the second centrifugation were suspended in 5% TCA at room temperature, in a proportion of 5:1 (5% TCA : precipitate, v/v) and centrifuged at 21°C and 15,000 *g* (10,588 rpm) for 20 min.

Supernatants were again discarded and the resultant precipitated protein was suspended in 4.0 ml 0.1N NaOH in preparation for the protein analysis.

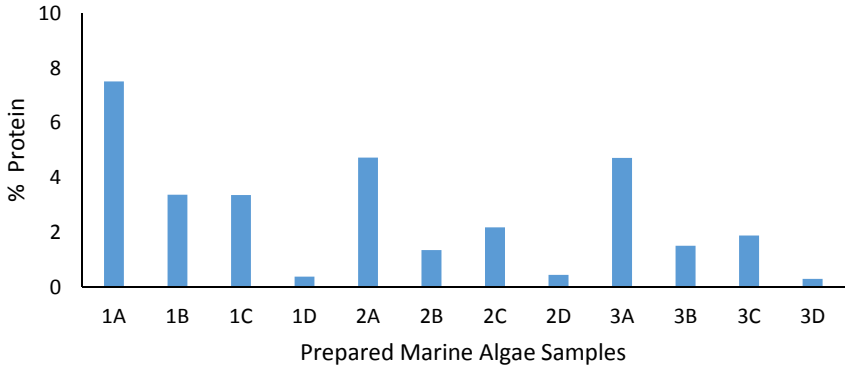
Before the samples were measured, blanks and standard curve tubes were prepared using the protein standard in 0.1N NaOH.

The Folin–Ciocalteu reactive was diluted in two volumes of ultra-pure water (1:2) and 0.5 mL of the diluted reactive was added to 1.0 mL of sample, previously mixed with 5.0 mL of freshly prepared reactive “C” [50 volumes of reactive “A” (2.0% sodium carbonate (Na_2CO_3) + 0.1N NaOH) + 1 volume of reactive “B” (½ volume of 0.5% copper(II) sulfate pentahydrate ($CuSO_4$ $5H_2O$) + ½ volume of 1.0% (potassium sodium tartrate tetrahydrate) $C_4H_4KNaO_6$ $4H_2O$)].

After the addition of each reactive, samples were stirred for 2 s in a vortex mixer, and absorbances were measured at 750 nm using a UV/Visible Spectrophotometer, 35 min after the start of the chemical reaction at room temperature.

8.1.5.3 Results

The results and calculations from the protein analysis are shown in Appendix 10, Tables A10.1 – A10.4, and figure A10.1, an overview of which can be seen in figure 8.8, below.



SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Figure 8.8: Protein percentages found in the prepared marine algae samples.

8.1.5.4 Discussion

The results from the samples used showed that the species with the highest protein content was seen to be *Ascophyllum nodosum* with an average of 3.66% (the lowest being 0.38% for sample 1D, the highest being 7.51% for sample 1A), next was *Saccharina latissima* with an average of 3.04% (the lowest being 0.30% for sample 3D, the highest being 4.72% for sample 3A), and then *Laminaria digitata* having an average of 1.87% (the lowest being 0.45% for sample 2D, the highest being 4.73% for sample 2A).

A trend was noticed throughout all the samples were the air dried material showed the highest levels of proteins, the frozen material showed the second highest, the next highest, and the oven dried the lowest. This may indicate that the last three forms of preparation may have a deleterious effect on proteins present in the original fresh material.

Interpretation of the results show that the highest levels of extracted protein can be expected from the extraction of frozen *Ascophyllum nodosum*.

8.2 Elemental Analysis

The following analysis was used to detect the presence, and determine the relative amounts of inorganic chemicals in the marine algae and their related extracts.

8.2.1 Flame Atomic Absorption Analysis of Prepared Marine Algae and their Ethanolic Extracts

Analysis was conducted in accordance with the method outlined in Nielson, 2010.

8.2.1.1 Materials

The following equipment and instrumentation was used: Flame Atomic Absorption Spectrometer (Perkin Elmer, model AAnalyst 400), lamps for detection of sodium (Na), calcium (Ca), potassium (K) and Magnesium (Mg), all from Perkin Elmer, laboratory weighing scales (Ohaus “Pioneer”, model PA402), fume hood, 60x 75ml digestion tubes, digestion block (Cole Parmer - AIM600 programmable block digestion system), cooling rack, hardened ashless filter paper (Whatman #541), tongs and protective gloves.

The following reagents and chemicals were used: Nitric acid (Sigma Aldrich - puriss. p.a., ACS reagent, reag. ISO, ≥69%), hydrogen peroxide solution (Sigma Aldrich - Green Alternative, contains inhibitor, 30 wt. % in H_2O, ACS reagent), Ultra-pure distilled water (Millipore, Milli-Q), Lanthanum (III) chloride (Aldrich - anhydrous, beads, −10 mesh, ≥99.99% trace metals basis). acetylene (99.998% purity - AGA) was used as the fuel gas in flame atomic absorption spectroscopy.

The stock atomic spectroscopy reference standard solutions (1000 mg L^{-1}) of Na, Ca, K and Mg were all of TraceCERT® grade obtained from Fluka Analytical.

The following marine algae species and their respective extracts were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

8.2.1.2 Method

Approximately 350 mg of each extract (or equivalent raw material) was placed in digestion tubes. Duplicates were made of each sample that used the prepared marine algae species material and for the blanks. However, due to the limited amount of extract material available, only single samples were made with these.

In the fume hood, 5 ml of concentrated nitric acid was pipetted into each tube, before being placed (along with the reagent blanks) into the digestion block, which was set at 175°C to start the pre-digestion. During this time, the samples were gently swirled once or twice using tongs and protective gloves.

Tubes were removed from the block once brown gas started to elute (or when the solution began to steam, if no brown gas was seen), set in a cooling rack, and were allowed to cool for at least 30 min.

After this time, 4 ml of 30% hydrogen peroxide was added to each tube (only doing a few tubes at one time), and the tubes were gently swirled to facilitate the reaction. These tubes were then placed back into the digestion block, still set to 175°C.

The start of the reaction was indicated by the appearance of rapidly rolling bubbles. As soon as this reaction started, the tubes were removed from the block, and the reaction was allowed to continue in the cooling rack.

Once the reaction started to subside, another 4 ml of hydrogen peroxide was added to the tubes and they were once again placed into the digestion block until the reaction started again, before being removed to the cooling rack.

Once the reaction had subsided again, all of the tubes were then placed into the digestion block and were allowed to reduce until approximately 1–1.5 ml of liquid remained, when the tubes were once more allowed to cool.

The samples were then filtered through hardened ashless filter paper, before appropriate dilutions of the samples were then made using ultra-pure water in a volumetric flask and lanthanum (III) chloride ($LaCl_3$) was added to final conc. of 0.1% in order to help inhibit chemical interferences.

Samples were then analysed using the flame atomic absorption spectroscope against corresponding standards (see Appendix 11, Tables A11.1 – A11.24 for concentrations used) and calibrating with the blanks.

8.2.1.3 Results

The results and calculations from the elemental analysis are shown in Appendix 11, Tables A11.1 – A11.24, an overview of which can be seen in figure 8.9, below.

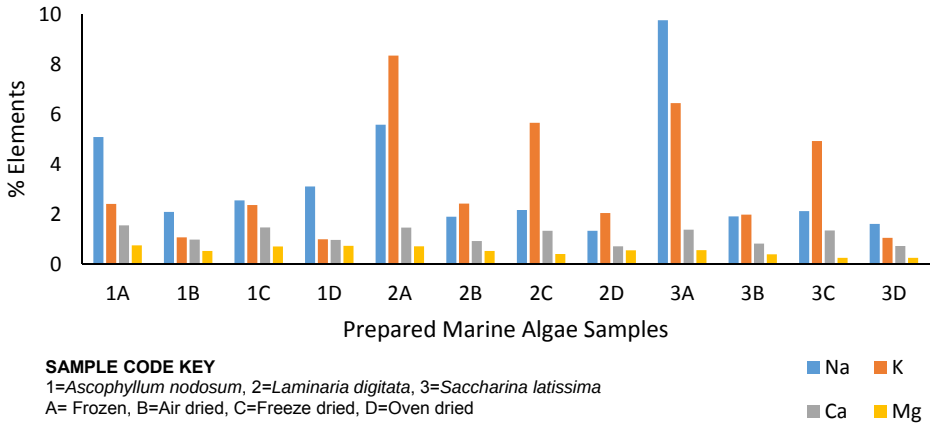


Figure 8.9: Percentages of elements found in the prepared marine algae samples.

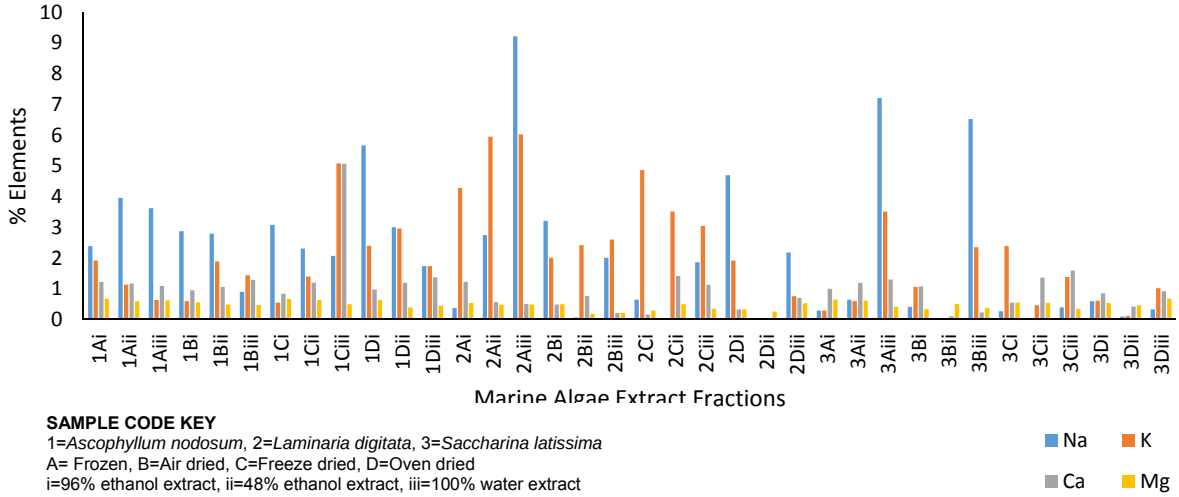


Figure 8.10: Percentages of elements found in the prepared the marine algae extracts.

8.2.1.4 Discussion

Analysis of the prepared material showed that the species with the highest levels of sodium was seen to be *Saccharina latissima* with an average of 3.85% (the lowest being 1.61% for sample 3D, the highest being 9.77% for sample 3A), next was *Ascophyllum nodosum* with an average of 3.21% (the lowest being 2.09% for sample 1B, the highest being 5.09% for sample 1A), and then *Laminaria digitata* having an average of 2.74% (the lowest being 1.33% for sample 2D, the highest being 5.58% for both sample 2A).

In regards to the extract fractions analysed, the highest levels of sodium were seen in the *Ascophyllum nodosum* with an average of 2.87% (the lowest being 0.90% for fraction 1Biii, the highest being 5.66% for fraction 1Di). The next highest were the *Laminaria digitata* extract fractions with an average of 2.25% (the lowest being 0.02% for fraction 2Cii, the highest being 9.21% for fraction 2Aiii). *Saccharina latissima* had the lowest

average of 1.39% (the lowest being 0.01% for fraction 3Bii, the highest being 7.21% for fraction 3Aiii).

Results from the prepared material in terms of the highest levels of potassium showed that *Laminaria digitata* had the highest, with an average of 4.62% (the lowest being 2.04 % for sample 2D, the highest being 8.35% for sample 2A), next was *Saccharina latissima* with an average of 3.60% (the lowest being 1.05% for sample 3D, the highest being 6.45% for sample 3A), and then *Ascophyllum nodosum* having an average of 1.71% (the lowest being 0.99% for sample 1D, the highest being 2.41% for sample 2A and 1A).

The highest levels of potassium found in the extracts were seen in the *Laminaria digitata* with an average of 3.12% (the lowest being 0.03% for fraction 2Dii, the highest being 6.02% for fraction 2Aiii). The second highest were the *Ascophyllum nodosum* extract fractions with an average of 1.81% (the lowest being 0.55% for fraction 1Ci, the highest being 5.08% for fraction 1Ciii). *Saccharina latissima* had the lowest average of 1.15% (the lowest being 0.04% for fraction 3Bii, the highest being 3.51% for fraction 3Aiii).

Analysis of the prepared material showed that the species with the highest levels of calcium was seen to be *Ascophyllum nodosum* with an average of 1.24% (the lowest being 0.97% for sample 1D, the highest being 1.55% for sample 1A), next was *Laminaria digitata* with an average of 1.11% (the lowest being 0.71% for sample 2D, the highest being 1.46% for sample 2A), and then *Saccharina latissima* having an average of 1.07% (the lowest being 0.72% for sample 3D, the highest being 1.38% for sample 3A).

The highest levels of calcium found in the extracts were seen in the *Ascophyllum nodosum* with an average of 1.45% (the lowest being 0.84% for fraction 1Ci, the highest being 5.07% for fraction 1Ciii). The next highest were the *Saccharina latissima* extract fractions with an average of 0.88% (the lowest being 0.11% for fraction 3Bii, the highest being 1.59% for fraction 3Ciii). *Laminaria digitata* had the lowest average of 0.63% (the lowest being 0.05% for fraction 2Dii, the highest being 1.42% for fraction 2Cii).

Assessment of the prepared material showed that the species with the highest levels of magnesium was seen to be *Ascophyllum nodosum* with an average of 0.68% (the lowest being 0.52% for sample 1B, the highest being 0.75% for sample 1A), next was *Laminaria digitata* with an average of 0.55% (the lowest being 0.40% for sample 2C, the highest being 0.71% for sample 2A), and then *Saccharina latissima* having an average of 0.36% (the lowest being 0.25% for sample 3C, the highest being 0.56% for samples 3A).

Lastly, results from the extract fractions analysed showed that the highest levels of magnesium were seen in the *Ascophyllum nodosum* with an average of 0.56% (the lowest being 0.39% for fraction 1Dii, the highest being 0.67% for fractions 1Ai and 1Ci). The next highest were the *Saccharina latissima* extract fractions with an average of 0.50% (the lowest being 0.34% for fraction 3Bi, the highest being 0.68% for fraction 3Diii). *Laminaria*

digitata had the lowest average of 0.39% (the lowest being 0.19% for fraction 2Bii, the highest being 0.54% for fraction 2Ai).

It is interesting to note that contrary to assumptions made by the author that there would be little difference in concentrations of the analysed metals between preparation methods used for the raw material, the results show that in some cases there were instead differences seen of between 0.02% - 8.16%. Also, in each case here it was the frozen material that showed the highest values.

It is uncertain as to exactly why this was, but it is speculated that it may have something to do with inaccuracies that could have occurred in the extract to raw material ratios. To investigate and confirm hypothesis, it is therefore suggested that for future reference the whole analysis be done again and the results compared with those of this one.

8.3 Phytochemical Analysis

The following set of analyses was used to detect the presence, and determine the relative amounts of fucoxanthin in the marine algae extracts.

8.3.1 Thin Layer Chromatography Analysis of Marine Algae Ethanolic Extracts

8.3.1.1 Materials

The following equipment and materials were used: Laboratory weighing scales (Ohaus "Pioneer", model PA402), nylon in-line filters (0.45 µm), 1 ml plastic syringes, ultrasonic water bath (Cole Parmer - 8891), UV cabinet (VWR – Jencons, viewing box with wavelengths 254 and 366 nm), fume hood, Thin layer Chromatography (TLC) sheets (Merk – 20x20cm Silica gel 60 F_{254} on aluminium), plastic ruler, scalpel knife, pencil, 36x glass Pasteur pipettes, 3x chromatography chamber (Camag, twin trough for 20 x 10 cm plates), 100 ml measuring cylinder, glass beakers, TLC hotplate, glass reagent sprayer (Supelco – 250 ml), rubber bulb.

The following reagents and chemicals were used: Ultra-pure distilled water (Millipore, Milli-Q), dichloromethane (Sigma Aldrich - CHROMASOLV®, for HPLC, ≥99.9%), methanol (Sigma Aldrich - CHROMASOLV®, for HPLC, ≥99.9%), hexane (Sigma Aldrich - CHROMASOLV®, for HPLC, ≥97.0%), ethyl acetate (Sigma Aldrich - CHROMASOLV®, for HPLC, ≥99.8%), p-anisaldehyde (Aldrich – 98%), acetic acid (Sigma Aldrich - ACS reagent, ≥99.7%), sulphuric acid (Sigma Aldrich - ACS reagent, 95.0-98.0%), Fucoxanthin (Fluka - Analytical standard, all-trans).

Extracts from the following marine algae species were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

8.3.1.2 Method

Samples and standard were prepared by dissolving each of the extracts first in warm (40°C) sonicated ultra-pure water for 30 mins (ensuring that as much of the solid particles

were dissolved as possible), and then adding methanol to achieve a concentration of 1mg per ml in 50% methanol. Each of these were then filtered through a nylon in-line filter via the use of a plastic syringe, and were stored in labelled glass vials.

Nine TLC sheets were cut to a length of 10 x 20 cm. Start lines were then faintly drawn 17 mm up from the bottom of the plate that included 12 dots to mark the sample points (each of which were 10 mm apart), and a further dot (40 mm from the last sample point) to mark the standard point. Finish lines were then scored on the plates, 76 mm from the start line.

The liquid extracts were then applied to the TLC sheets (one species per sheet in triplicate), being added dropwise and in order starting from left to right (i.e. 1Ai, 1Aii, 1Aiii etc.) via the use of clean glass Pasteur pipettes, before being allowed to dry.

In the meantime, the following solvent mixes were made up: dichloromethane : methanol (80:20), hexane : ethyl acetate (1:1) and dichloromethane : methanol (97:3).

In the fume hood, each of the three sets of prepared sheets were then developed one after the other using these three solvent systems in glass, rectangular, chromatography chambers.

In every case, the time taken for the solvent to reach the finish line from the start line was recorded, and sheets were allowed to dry before being processed further.

Spots that could be seen with the naked eye were marked with pencil on the sheets. Sheets were then viewed under UV light (at wavelengths of 254 and 366 nm) and visualised spots were again marked with pencil.

The following visualisation reagent was then freshly prepared help detect the presence of phenols, sugars, steroids and terpenes: 0.5 ml of p-anisaldehyde in 50 ml of glacial acetic acid and 1 ml of 97% sulphuric acid (Waksmundzka-Hajnos *et al.*, 2008).

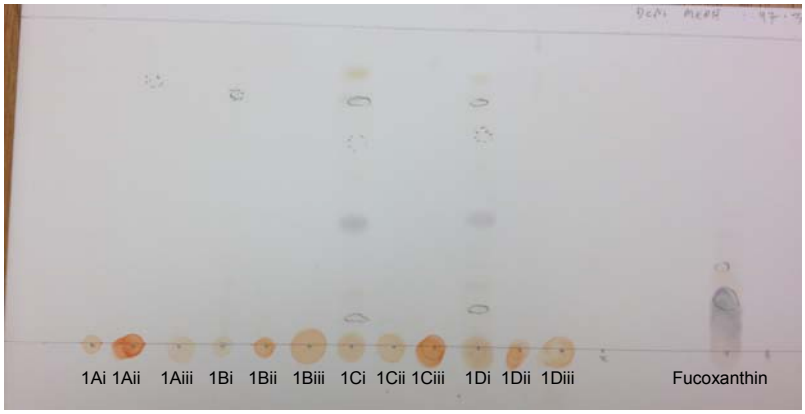
Sheets were carefully sprayed with this reagent in the fume hood and were allowed to dry at 105°C before spots were visualised and marked with pencil (see figures 8.10 – 8.12 in 8.3.1.3 Results).

Retardation factor (R_f) values for each spot visualised were then calculated.

8.3.1.3 Results

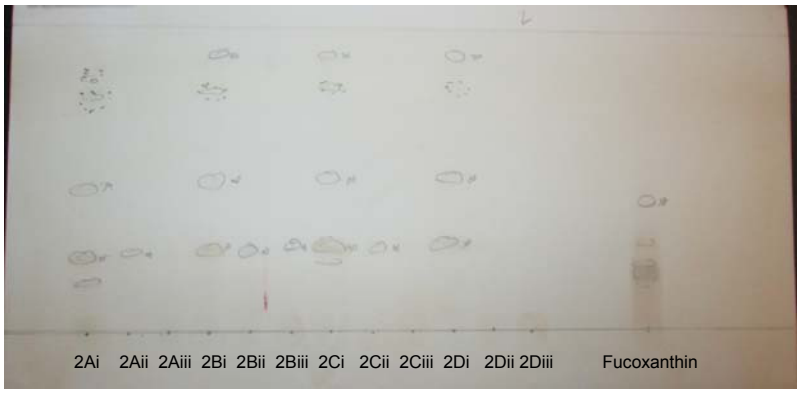
The results from the TLC analysis are shown in Appendix 12, Tables A12.1 – A12.3.

The following figures show photographs of the TLC sheets selected.



SAMPLE CODE KEY
1=*Ascophyllum nodosum*
2=*Laminaria digitata*
3=*Saccharina latissima*
A= Frozen
B=Air dried
C=Freeze dried
D=Oven dried
i=96% ethanol extract
ii=48% ethanol extract
iii=100% water extract

Figure 8.11: Developed TLC plate showing the analysis of the extraction fractions from *Ascophyllum nodosum*[15].



SAMPLE CODE KEY
1=*Ascophyllum nodosum*
2=*Laminaria digitata*
3=*Saccharina latissima*
A= Frozen
B=Air dried
C=Freeze dried
D=Oven dried
i=96% ethanol extract
ii=48% ethanol extract
iii=100% water extract

Figure 8.12: Developed TLC plate showing the analysis of the extraction fractions from *Laminaria digitata*.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*
2=*Laminaria digitata*
3=*Saccharina latissima*
A= Frozen
B=Air dried
C=Freeze dried
D=Oven dried
i=96% ethanol extract
ii=48% ethanol extract
iii=100% water extract

Figure 8.13: Developed TLC plate showing the analysis of the extraction fractions from *Saccharina latissima*.

[15] Note: In each case, the marine algae fractions were applied to the TLC plate from left to right and in order (i.e 1Ai, 1Aii, 1Aiii etc). The material on the far right was the fucoxanthin, which was used as the main biomarker in this research. Visualised spots were gently marked with a pencil so that the R_f values could be calculated.

8.3.1.4 Discussion

The visualisation of the spots on the sheets showed that there in many of the samples tested contained one or more of the following compounds: phenols, terpenes, sugars and steroids. Although further analysis would be required in order to more accurately determine which compounds were present, and in what quantities.

In many of the extract fractions a positive identification of the presence of fucoxathin was seen when tested against the equivalent standard.

The results also showed that the best solvent system to use for TLC analysis of these extracts was the dichloromethane : methanol (97:3) mix. This was because of the highly polar nature of the fucoxanthin.

However, in relation to choosing an appropriated High Performance Liquid Chromatography (HPLC) column for further analysis of these extracts it is recommended that a reverse phase type should be selected, along with an appropriate mobile phase mix to support adequate elution of the fucoxathin molecule.

8.3.2 High Performance Liquid Chromatography Analysis of Marine Algae Ethanolic Extract

Analysis was conducted in accordance with the method outlined in Zhen *et al*., 2012, using fucoxanthin as the biomarker.

8.3.2.1 Materials

The following equipment and instrumentation was used: HPLC apparatus (Dionex - model U-3000 HPLC equipped with a quaternary pump, autosampler injector, column thermostat and photodiode array detector (DAD)), HPLC column (GLSciences - Inertsil® “Herbal Medicine” C18 reverse phase column, 5 µm particle size, 260 x 4.6 mm ID), 1-10 µl micropipette (Gilson - Pipetman Classic™ P10), 2-20 µl micropipette (Gilson - Pipetman Classic™ P20), 20-100 µl micropipette (Gilson - Pipetman Classic™ P100), 200-1000 µl micropipette (Gilson - Pipetman Classic™ P1000), micropipette tips, 60x1.5 ml injection vials with PTFE screwtop caps, nylon in-line filters (0.45 µm), 1 ml plastic syringes.

The following reagents and chemicals were used: Ultra-pure distilled water (Millipore, Milli-Q), methanol (Sigma Aldrich - CHROMASOLV®, for HPLC, ≥99.9%), 96% certified organic ethanol (Sacchetto – derived from certified organic wheat), acetonitrile (CHROMASOLV® Plus, for HPLC, ≥99.9%), ethyl acetate (Sigma Aldrich - CHROMASOLV®, for HPLC, ≥99.8%), Fucoxanthin (Fluka - Analytical standard, all-trans).

Liquid extracts of the following marine algae species obtained from the TLC analysis were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*.

8.3.2.2 Method

Duplicate standards of fucoxanthin in the following serial dilutions were prepared in 1ml of 50% methanol (with ultra-pure water) and then filtered through a nylon in-line filter via the use of a plastic syringe: 0.01 µg, 0.1 µg, 0 µg, 1 µg, 10 µg, 100 µg, 200 µg, 400 µg, 600 µg, 800 µg and 1000 µg.

To rule out the possibility of interference from the solvents used, vials of ethanol with water were also prepared in the following concentrations: 0% (100% H_2O), 12%, 48% and 96%.

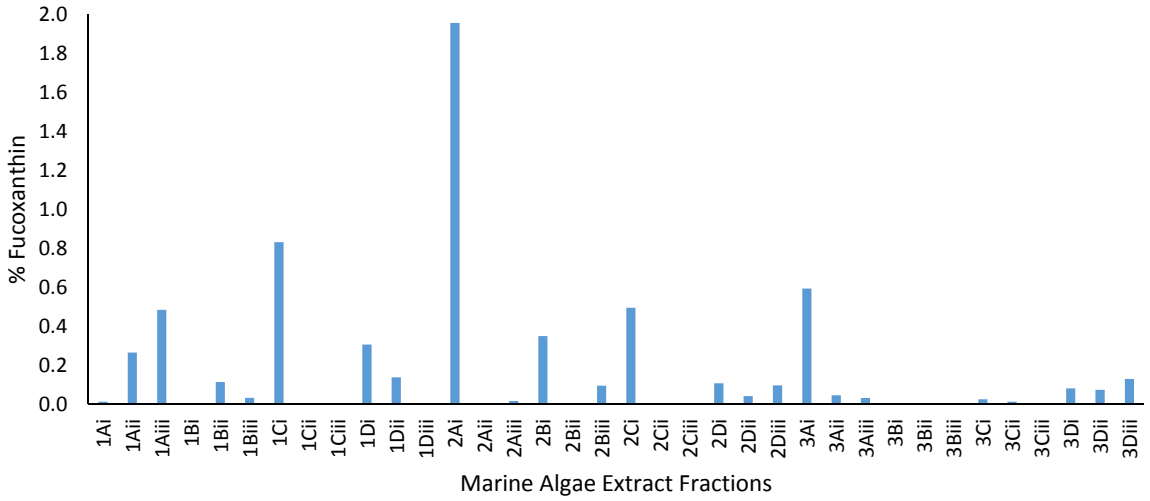
All standards, blanks and samples were then analysed using the HPLC apparatus and Herbal Medicine column using the following parameters:

Mobile phase: acetonitrile : water (9:1 v/v) (eluent A) and 100% ethyl acetate (eluent B).
Program: Gradient elution - initially 100% A and 0% B, 0–20 min linear gradient to 100% B, 20–22 min 100% B, 22–23 min return to 100% A, 23–25 min 100% A for re-equilibration before the next injection.
Column temperature: 20°C.
Injection volume: 20 µl.
Injection wash: 10% methanol between each injection.
Flow rate: 1 ml min^{-1}.
Detection wavelength: 445 nm.

Results from the standards were used to make a standard curve (see Appendix 13, figure A13.1), with which concentrations of fucoxanthin within the samples were determined.

8.3.2.3 Results

The data and calculations from the HPLC analysis are shown in Appendix 13, Tables A13.1 – A13.4, and figure A13.1, an overview of which can be seen in figure 8.14, below, and an example of one of the HPLC chromatograms obtained in figure 8.15.



SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Figure 8.14: Relative percentages of fucoxanthin gain from the marine algae extract fractions analysed.

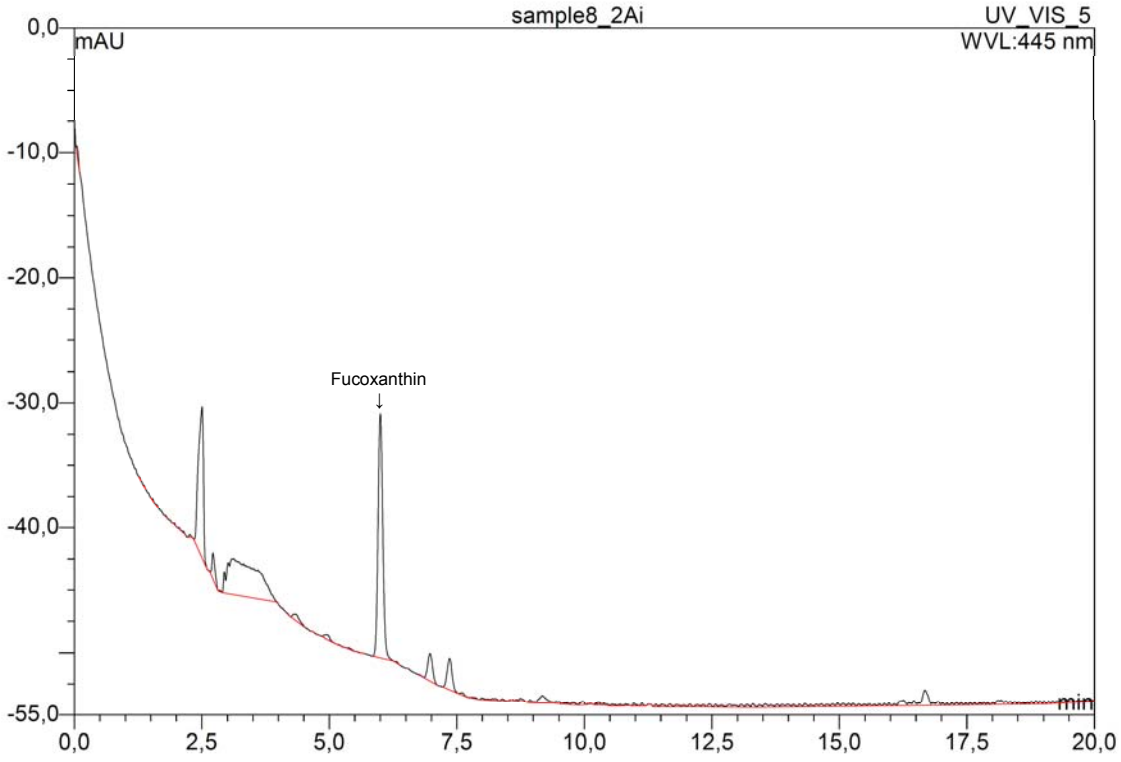


Figure 8.15: Chromatogram of fraction 2Ai, taken of frozen *Laminaria digitata*, extracted using 96% ethanol.[16]

[16] Note that the peak present at the 6 min mark was positively identified as fucoxanthin by comparing to a standard. The other peaks present are unknown but are likely to have a similar structure (possibly astaxanthin?)

8.3.2.4 Discussion

Analysis of the blanks proved that there was no interferences present on the fucoxanthin chromatograph from either the dilution solvent or traces of the original extraction solvent used.

Of the extract fractions analysed, the highest levels of fucoxanthin were seen in the *Laminaria digitata* with an average of 0.26% (the lowest being 0% for fraction 2Cii, the highest being 1.96% for fraction 2Ai). The next highest were the *Ascophyllum nodosum* extracts with an average of 0.18% (the lowest being 0% for fractions 1Ciii and 1Diii, and the highest being 0.83% for fraction 1Ci). *Saccharina latissima* had the lowest average of 0.08% (the lowest being 0% for fractions 3Bi, 3Biii and 3Ciii, the highest being 0.59% for fraction 3Ai).

Calculations were made using these figures to determine the relative amounts of fucoxanthin present in each of the prepared material types. The result of which showed that the material that had the highest concentration was *Laminaria digitata* with an average of 0.29% (the lowest being 0.02% for the 2B sample, the highest being 0.89% for the 2A sample). The second highest was *Saccharina latissima* with an average of 0.09% (the lowest being 0% for the 3B sample, the highest being 0.24% for the 3A sample). The lowest was *Ascophyllum nodosum* with an average of 0.07% (the lowest being 0.02% for the 1B sample, the highest being 0.12% for the 1A sample).

Conclusion of these results suggests that the use of frozen *Laminaria digitata* material provided the highest yield of fucoxanthin (0.89% of dry weight material). Furthermore, extraction of this material using 96% ethanol provided a fraction containing a total fucoxanthin concentration of 1.96%.

Comparing the results obtained to marine algae fucoxathin levels found in literature, it was found that in a study by Peng *et al.*, 2011, concentrations measured in a related species (*Laminaria japonica*) were seen to be 0.03 mg/g^{-1} fresh weight of material.

8.4 Environmental and Quality Control Analysis

As was mentioned previously in the site suitability section, one of the main concerns of marine algae collection from the wild is the possibility of the presence of high levels of contaminants (such as heavy metals, bacterial infections and persistent organic pollutants (POPs)) in the material, which could cause possible harm if used in food and/or health products. These compounds may be absorbed from the natural environment that the marine algae grow in and bioaccumulate within the plant tissues. If the material is then processed into concentrated extracts, the levels of these contaminants may then also become concentrated to unacceptable or even dangerous levels within the final products.

It is therefore the aim of this section to provide analytical information on the possible contamination of the samples collected from the Stafnes site, as part of a quality control and safety assessment.

According to an article by McCormic and Cairns, 1994, it is suggested that due to fact that the marine algae naturally act as biofilters for the environment that they reside in, it is suggested that analysis of them could be used as as an environmental indicator of pollution levels. In particular, substances such as PCB and organophosphates seem to have a high aiffinity for algae tissues. It is therefore an important aspect of this study to analyses the harvested marine algae in this way in order to try to determine whether or not some of these persistent pollutants are present (both in the material studied and the at the collection site).

8.4.1 PCB Screening of Marine Algae Samples via Gas Chromatography

Analysis conducted was adapted from the United States Environmental Protection Agency (USEPA) method of detecting levels of PCBs in soils (EPA Method 8082A[17] via EPA Method 3540C[18] – Soxhlet extraction), a summary of which is written below. In this instance, soil samples were substituted with fresh marine algae samples[19], the rest of the procedure, however, remained the same.

8.4.1.1 Materials

The following equipment and instrumentation was used: Fridge freezer (Electrolux, model EN3400AOW), 1 roll of plastic freezer bags, laboratory oven (Binder, model ED23), laboratory weighing scales (Ohaus “Pioneer”, model PA402), 5 Inch stainless steel surgical scissors (Whiteley, model 1956SS-5/SB), 12 x 30 ml ceramic crucibles (Coorstek, model 60108), Soxhlet apparatus (Supelco – 100 ml capacity with 250 ml round bottom flask), digital hotplate stirrer (Stuart, model CD162), Kaderna-Danish apparatus (Supelco), solvent recovery condenser (Supelco), fume hood, steam bath (Thermo Scientific), Supelco® Dioxin Prep System with florisil adaptor (Supelco), laboratory vacuum pump (KNF Neuberger, model UN810.3FTP), 2 meters of rubber tubing, gas chromatograph (Perkin Elmer, model Clarus 500); using a SLB-5ms column (30 m x 0.25 mm I.D., 0.25 µm), and fitted with an Electron Capture Device (Perkin Elmer), 5 x 10 ml glass amber sample bottles with PTFE lined septum cap.

The following reagents and chemicals were used: Sodium sulphate (Sigma Aldrich - ACS reagent, ≥99.0%, anhydrous powder), acetone (Sigma Aldrich - CHROMASOLV®, for HPLC, ≥99.9%), hexane (Sigma Aldrich - CHROMASOLV®, for HPLC and GC, ≥97.0%).

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[17] US Environmental Protection Agency. (2007) polychlorinated biphenyls (PCBs) by gas chromatography Method 8082A. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8082a.pdf. Accessed on 23/05/2012.
[18] US Environmental Protection Agency. (1996) Soxhlet Extraction Method 3540C. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3540c.pdf. Accessed on 23/05/2012.
[19] Fresh marine algae species samples for use in this analysis were taken from the extra material that was collected from the Stafnes site on 19/08/2013 for the extraction research previously discussed (see section 6).

A stock analytical standard PCB mixture “DCMA PCB mixture” (10 PCBs dissolved in hexane at various concentrations, including the surrogate spike used: Decachlorobiphenyl (DCB)), and analytical standard DCB (200 µg/mL in acetone – used for external calibration) was obtained from Supelco.

Helium (99.998% purity - AGA) was used as the carrier gas in the analysis.

The following fresh marine algae species were used: *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima.*

8.4.1.2 Method

Frozen marine algae material was used from the stock collected from the Stafnes site on 19/08/2013. This material had been stored in a freezer for approx. 5 months until until which time the main extraction work of this analysis was conducted.

In the meantime, the dry weight of each species selected was determined. This was achieved by taking 3 x 5g of fresh, cut, material samples of each species and placing them in crucibles, before being gently dried over a 24 hour period in a laboratory oven at 40°C.

Each sample was then reweighed, and the average dry weight and dry weight % of each species was then calculated (see 8.4.1.3 Results, Table 8.1).

These values were then used to ascertain the dry weight of each species in order to accurately determine potential concentration levels of contaminants present.

For each species sample taken, 10 g of the frozen, crushed marine algae material was mixed well with 10 g of sodium sulphate before then being added to a Soxhlet cellulose extraction thimble. Decachlorobiphenyl (DCB) was then added to the sample (5 ppm in relation the sample dry weight) for external calibration and also to confirm the efficiency of the extraction method. The thimble was put into a glass Soxhlet extractor and a mixture consisting of 100 ml hexane and 100 ml acetone was added to the round bottom flask of the Soxhlet extractor to be used as the solvent for the extraction. The extraction was performed in a fume hood on a high flow rate whilst the bottom flask was heated/stirred in an oil bath at 250°C at 200 rpm using a small magnetic stir bar. At the same time, cold water continually flowed through the reflux condenser. Each extraction ran for 24 hours.

After completion of the extraction, the obtained liquid extract was dried through a column of sodium sulphate before then being transferred to a glass Kuderna-Danish apparatus, where it was to be concentrated down to 2 ml via gentle distillation over a steam bath.

To facilitate a solvent exchange, hexane was added to the concentrated liquid extract to make its volume up to 10ml, before being reduced again to 2 ml in the Kuderna-Danish apparatus.

The resultant extract was then added to a 10 ml glass amber sample bottle, and more hexane was used to rinse the inside of the apparatus before being added to the sample bottle to make up the volume again to 10 ml. The samples were stored in a fridge at 4°C until they were processed further (24 hours).

Each sample was then cleaned via the use of a Supelco® Dioxin Prep System with florisil adaptor[20], where each sample was allowed to run first through a multi-layer silica gel column, and then a florisil micro tube using 100ml of hexane as a solvent under vacuum. This method was designed to clean out traces of compounds which could cause interference with the gas chromatography analysis.

The cleaned samples were then concentrated down to 10 ml once more using the Kuderna-Danish apparatus, before being injected into a Gas Chromatograph (GC) fitted with an Electron Capture Device (ECD), to determine the concentration of PCBs in the samples.

The following parameters were used for the GC-ECD:
Oven program: 100 °C for 2 min., then raise by 15 °C/min to 330 °C, where it is held for 3 min.
Injector port temperature: 250 °C
Detector: ECD, held at a constant temperature of 330 °C.
Carrier gas: helium, with a constant flow of 25 cm/sec.
Injection: 1µl (splitless, with 0.75 min. delay).

In order that all the data produced could be compared equally, and so a high degree of accuracy could be obtained, the GC was internally calibrated with standard samples of the tested PCBs as well as the surrogate spike used (DCB).

8.4.1.3 Results

The data and calculations from the dry weight determination used in the PCB analysis are shown in Appendix 14, Table A14.1. The following table below shows an overview of the dry weight results below.

Table 8.1: Dry weight results for the PCB analysis of the marine algae samples taken from the Stafnes site.

SPECIES	WET WEIGHTS AVERAGE (g)†	DRY WEIGHTS AVERAGE (g)†	DRY WEIGHT %
Ascophyllum nodosum	5.06 SD±0.0208	2.15 SD±0.0917	42.49 SD±1.8339
Laminaria digitata	5.02 SD±0.0058	1.47 SD±0.4451	29.28 SD±8.8909
Saccharina latissima	5.03 SD±0.0058	1.66 SD±0.0751	33.00 SD±1.4941

[20] Supelco (2011). How to use the multi-layer silica gel column. http://www.sigmaaldrich.com/etc/medialib/docs/Supelco/.../t704007.pdf. Accessed on 30/05/2012.

8.4.1.4 Discussion

The final result of this quality control analysis showed that none of the following PCBs were present in any of the species samples above the limits of detection of 200 ppt (Jaouen-Madoulet *et al.*, 2000):

2-chlorobiphenyl
3,3-dichlorobibiphenyl
2,4,5-trichlorobiphenyl
2,2,4,4-tetrachlorobiphenyl
2,3,4,5,6-pentachlorobiphenyl
2,2,3,3,6,6-hexachlorobiphenyl
2,2,3,4,5,5,6-heptachlorobiphenyl
2,2,3,3,4,4,5,5-octachlorobiphenyl
2,2,3,3,4,4,5,5,6-nonachlorobiphenyl

8.4.2 Heavy Metal Screening of Marine Algae Samples via Flame Atomic Absorption Spectroscopy

Analysis was conducted in accordance with the method outlined in Nielson, 2010.

8.4.2.1 Materials

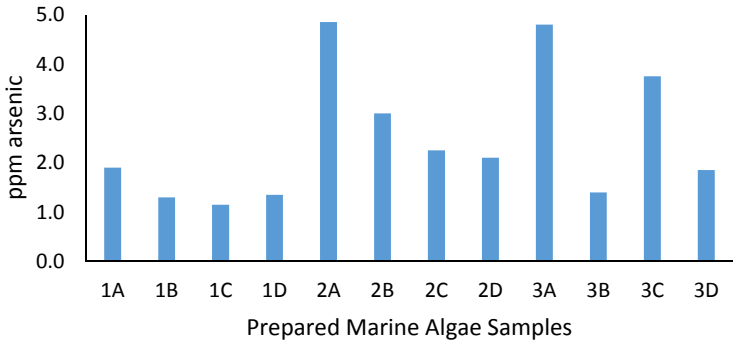
These were the same as was previously mentioned for the elemental analysis of the marine algae and extracts (see 8.1.6.1 Materials), with the exception that Arsenic standard (Fluka - TraceCERT®, 1000 mg/L As in nitric acid) and lamp was used.

8.4.2.2 Method

The procedure was the same as has been previously mentioned for the elemental analysis of the marine algae and extracts (see 8.1.6.2 Method of this paper). The calibration concentrations used can be seen in Appendix 15, Tables A15.1 – A15.6.

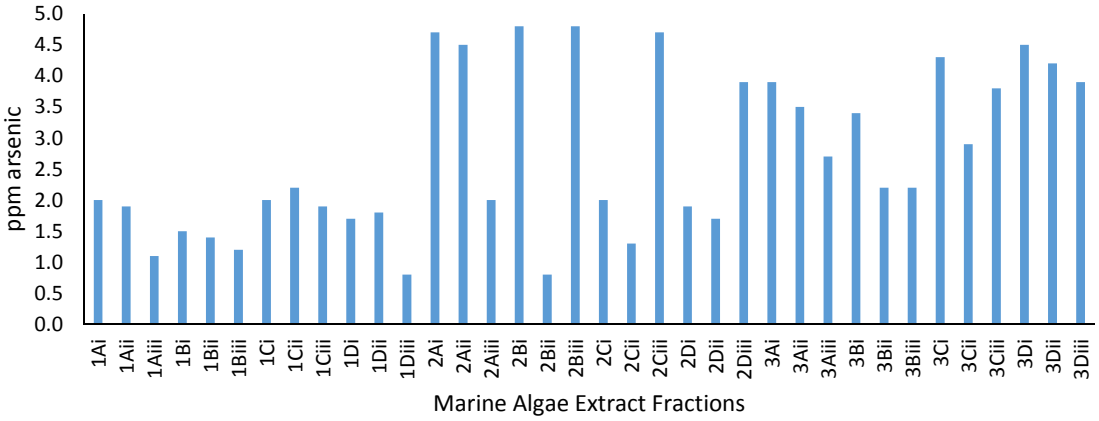
8.4.2.3 Results

The results and calculations from the protein analysis are shown in Appendix 15, Tables A15.1 – A15.6, an overview of which can be seen in figures 8.14 and 8.15, below.



SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried

Figure 8.16: Concentrations of arsenic measured in the prepared marine algae material.



SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Figure 8.17: Concentrations of arsenic measured in the marine algae extracts.

8.4.2.4 Discussion

Analysis of the prepared material showed that the species with the highest levels of arsenic was seen to be *Laminaria digitata* with an average of 3.05 ppm (the lowest being 2.1 ppm for sample 2D, the highest being 4.9 ppm for sample 2A), next was *Saccharina latissima* with an average of 2.95 ppm (the lowest being 1.4 ppm for sample 3B, the highest being 4.8 ppm for sample 3A), and then *Ascophyllum nodosum* having an average of 1.43 ppm (the lowest being 1.15 ppm for sample 1D, the highest being 1.9 ppm for samples 1A).

The results of this analysis give a good indication of arsenic concentrations present in the environment at the Stafnes site. Arsenic levels present in seawater can be anywhere from 1-3 ppm in unpolluted waters. However, depending on the geographic area of where the sample was taken, levels can be higher (Correia *et al.*, 2010). It is therefore likely that the presence of underwater volcanic activity (as is commonly seen in Iceland) may be contributing to the higher levels of arsenic found in some samples.

With regards to the extract fractions analysed, the highest levels of arsenic were seen in the *Saccharina latissima* with an average of 3.46 ppm (the lowest being 2.2 ppm for fractions 3Bii and 3Biii, the highest being 4.5 ppm for fraction 3Di). The next highest were the *Laminaria digitata* extract fractions with an average of 3.09 ppm (the lowest being 0.8 ppm for fraction 2Bii, the highest being 4.8 ppm for fractions 2Bi and 2Biii). *Ascophyllum nodosum* had the lowest average of 1.63 ppm (the lowest being 0.8 ppm for fraction 1Diii, the highest being 2.2 ppm for fraction 1Cii).

The results also show that the levels of arsenic present in the original raw material seem to be coming through and becoming concentrated in the corresponding extracts and so awareness of this should be kept in mind when producing extracts commercially on a larger scale.

Again, as with the previous analysis of metals done, there were differences in concentration between prepared material types. Here differences of between 0.3 – 4.0 ppm were seen. Also, as before, in each case the frozen material showed the highest values, and so further investigation is needed in terms of the analysis of this element as well.

9 CONCLUSION

The result of the research conducted in the former part of this study was that an ideal macroalgae collection area was located in Iceland, and that an application for organic conversation of the site was accepted.

Results from the latter part of the study are concisely summarised in tables A16.1 – A16.4, in Appendix 16.

The subsequent extraction of the three species harvested from the site (i.e. *Ascophyllum nodosum*, *Laminaria digitata* and *Saccharina latissima*) appeared to show a large variation in constituents present between fractions. This was evident both by the extract's colour and consistency, and also differences measured in pH. In some cases, large amounts of what appeared to be salt was also present, which may have contributed to a misleading variation in yield obtained.

Constituent analysis of the prepared material showed that the species which had the highest average moisture content (80.57%) was *Saccharina latissima*, and the species with the highest average dry matter content (51.48%) was *Ascophyllum nodosum*.

Measurements showed that freeze dried *Ascophyllum nodosum* had the highest average ash value (20.72%). Whereas, conversely, the sample with the highest average organic content (86.54%) was seen to be the oven dried *Ascophyllum nodosum*.

Analysis of the prepared material showed that the sample which contained the both the highest average fat (4.36%) and protein (7.51%) content was from frozen *Ascophyllum nodosum*. Whereas the sample with the highest average carbohydrate content (45.23%) was seen to be frozen *Saccharina latissima*.

Of the samples that underwent elemental analysis, it was seen that the sample with the highest average levels of sodium (9.77%) was from frozen *Saccharina latissima*, of potassium (8.35%) was from frozen *Laminaria digitata*, and of calcium (1.55%) and magnesium (0.75%) was frozen *Ascophyllum nodosum*.

Constituent analysis of the extracts showed that the fraction which had the highest moisture content (23.78%) was that of the frozen *Ascophyllum nodosum* extracted with 48% ethanol, and the fraction with the highest dry matter content (99.71%) was from air dried *Ascophyllum nodosum* extracted with 96% ethanol.

Measurements taken from air dried *Laminaria* digitata, which had been extracted with 48% ethanol showed the highest ash value (63.56%). Whereas, conversely, the fraction

with the highest organic content (94.41%) was seen to be from frozen *Ascophyllum nodosum* extracted with 48% ethanol.

It was found that in all cases, the highest constituent contents measured from the raw material were obtained when they were prepared via freezing.

Analysis showed that the fraction which contained the highest average carbohydrate content (87.17%) was from freeze dried *Saccharina latissima* extracted with high concentration of ultra-pure water.

Of the extracts that underwent elemental analysis, it was seen that the fraction with the highest levels of sodium (9.21%) was from frozen *Laminaria digitata* extracted with ultr-pure water, of potassium (6.02%) was frozen *Laminaria digitata* extracted with a high content of ultra-pure water, and of calcium (5.07%) and magnesium (0.67%) was both from *Ascophyllum nodosum* that had been respectively frozen and freeze dried, and extracted with 96% ethanol.

The result of the phytochemical analysis suggests that many of the extracts contained fucoxanthin. However, the use of frozen *Laminaria digitata* material extracted with 96% ethanol, via the use of a vacuum Soxhlet, provided the highest yield (1.96 mg/g dry weight of extract). The current market price for this product in pure form was seen to be £7,890 (GBP) per gram[21], which could potentially make this a profitable manufacture route to this product.

Lastly, the quality control analysis done showed that there were no PCBs present in any of the samples tested, and that of the prepared samples, frozen *Laminaria digitata* contain the highest concentration of arsenic (4.9 ppm), and out of the extracts, air dried *Laminaria digitata* extracted with both 96% ethanol and ultra-pure water contained the highest concentration of arsenic (4.8 ppm). However, on average the levels of arsenic present in the samples were within usual and safe limits.

In summary, it can clearly be seen that the way raw plant material is prepared before extraction can have a significant effect on both the yield and composition of the final crude extracts achieved. Not only this, but variations in the method used for extraction can also play an important role in helping to isolate specific target compounds of interest.

The application of these findings may not only just be relevant to the species and material types researched here, and so it is likely that the implications of this study may also apply on wider level to other compounds that are commonly extracted from nature. As such, further research based upon these findings may afford a greater understanding of these processes which may lead to more efficient manufacturing processes. One consequence of this improved production output could be that less raw material would need to be

[21] Sigma Aldrich. Fucoxanthin. http://www.sigmaaldrich.com/catalog/product/sigma/f6932?lang=en&region=GB. Accessed on 18/04/2014.

collected to achieve the same yields. In turn, this could help to reduce the environmental damage and biodiversity loss caused by overharvesting from the wild.

Following on from this study, it is likely that future research would consist of an expansion upon the current parameters discussed here. Further analysis of the samples would include comparison of the results with those obtained from using fresh samples, the production of more extract material in order to do the tests for fat and protein content in the fractions, the identification and quantification of other active constituents not covered in this paper (such as complex polysaccharides, polyphenols and phlorotannins), and the presence of similar type compounds that were seen to be present in the extracts (possibly astaxanthin) also need to be further investigated and confirmed via HPLC against the use of standards. It may also include the analysis of other marine algae species with which to compare results, as well as research into the possible effects of using other methods of extraction on the crude extract quality and yields, and their corresponding target biomarkers.

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APPENDIX 1

Table A1.1: Marine algae species established in aquaculture and their utilisation, and species with novel applications currently under investigation.

		INDUSTRY SECTOR						
Species	**Cultivation Method***	**Human Consumption**	**Animal Feed**	**Health**	**Cosmetics**	**Agrochemicals**	**Biotechnology**	**Biomedicine**
Alaria esculenta	O	●	●	●				
Ascophyllum nodosum	U					●		
Asparagopsis armata	O				●			
Brown algae	U				●	●	●	
Ceramium spp.	U							●
Chondrus crispus	O,T	●		●	●			
Delesseria sanguinea	U							●
Dumontia contorta	T							●
Fucus spp.	U					●	●	
Gracilaria spp.	U							●
Laminaria digitata	O					●	●	●
Saccharina latissima	O	●		●			●	
Laurencia spp.	U							●
Palmaria palmata	O,T	●	●	●	●			
Porphyra spp.	O,T	●		●				
Ulva spp.	T	●	●					
Undaria pinnatifida	O,T	●						
Red algae	U		●		●	●	●	

● Denotes species use or potential use in that sector (Mayakrishnan *et al.*, 2013; Raveendran *et al.*, 2013; Chennubhotla *et al.*, 2013; Kim, 2014; Agatonovic-Kustrin and Morton, 2013).

* Cultivation Method: O = Open-water sea cultivation, T = Tank cultivation. U = Unknown - more research needed. (Kraan, 2010; Lüning and Pang, 2003; Matos, 2006).

APPENDIX 2

Table A2.1: Open-water aquaculture suitability analysis for both Reykhólar and Stafnes sites.

SITE CRITERIA	REYKHÓLAR SITE †	STAFNES SITE †
Light	Good access for specific species	Good access for specific species
Nutrients	Further analysis required to confirm	Further analysis required to confirm
Salinity	Situated in a bay - susceptible to fluctuations	Within acceptable range (30%)
Temperature	Within range, and depth is 6 meters	Within range, and depth is 4-5 meters
Exposure	Situated in a sheltered bay with good tidal current	Predominantly exposed with good tidal current
Pollution	No, but may need further analysis to confirm	No, but may need further analysis to confirm
Certified Organic	Yes, by Tún and Quality Assurance International	No, but good candidate for conversion application

†Analysis done via information collected from the sites and personal communication with site owners (12/12/2012)

APPENDIX 3

Certificate

CERTIFIED ORGANIC
TÚN ICELAND

Vottunarstofan Tún ehf.
(EN45011 – ISAC Accreditation No. 11)
certifies that:

ALKEMISTINN EHF.
Reykjanes, Iceland

has satisfied the requirements for inspection, operating procedures and production methods as specified in the **Tún Standards for Organic Production** for the following:

TYPE OF OPERATION:	**Agriculture:** Collection of Wild Terrestrial and Marine Plants
CERTIFIED LAND:	**Organic:** 31.5 ha **In conversion:** 8 ha
CERTIFIED PRODUCTS:	Wild Collected Herbs and Marine Plants
CERTIFICATE RENEWAL DATE	31.12.2013
LICENCE NUMBER:	IS-1 TUN-104

Signed on behalf of Vottunarstofan Tún ehf.

Vottunarstofan Tún ehf. • Bildshöfdi 12 • IS-110 Reykjavík • Iceland
Tel: +354 511 1330 • Fax: +354 511 1331 • tun@tun.is • www.tun.is

Figure A3.1: Stafnes site organic status certificate awarded by Vottunarstofan Tún.

APPENDIX 4

Table A4.1: Marine algae constituent analysis table for *Ascophyllum nodosum*.

SPECIES	MAJOR CONSTITUENTS	CHEMICAL CLASS	KNOWN USES	REFERENCES
Ascophyllum nodosum	Ascophyllan.	Fucoidan like	Immunostimulant	Jiang *et al.*, 2011.
	420kDa Fucoidan (L-fucose 52.1%, glucose 21.3%, galactose 6.1%, and xylose 16.5%). 556kDa Fucoidan (38.7% L-fucose and 33.7% sulphate). 556kDa Fucoidan (L-fucose 31.3%, sulphate 26.1%, uronic acid 5.7%). 516kDa Fucoidan (L-fucose 35.8%, sulphate 18.4%, and uronic acid 11.6%). 600kDa Fucoidan (L-fucose 43.2%, sulphate 35.3%, and uronic acid 1.7%). 25kDa Fucoidan (L-fucose 47%, sulphate 30%, and uronic acid 6%). 18.6kDa Fucoidan (L-fucose 39.7%, sulphate 27%, and uronic acid 4.1%). 417kDa Fucoidan (L-fucose 45.4%, sulphate 22.1%, and uronic acid 9.9%). 1,323kDa Fucoidan (L-fucose 28.4%, sulphate 19.4%, anduronic acid 5.8%). 13kDa Fucoidan (L-fucose 42%, sulphate 31%, and uronic acid 5%).	Fucoidan	Antithrombotic Anti-inflamatory Intestinal mucosal protective	Foley *et al.*, 2011; Kloareg *et al.*, 1986; Nardella *et al.*, 1996; Daniel *et al.*, 1999; Haroun-Bouhedja *et al.*, 2000; Rioux *et al.*, 2007; Berteau *et al.*, 2002.
	Myristic acid (3,027+/-395mcg/g). Palmitic acid (3,693+/-682mcg/g). Stearic acid (240+/-79mcg/g). Butyric acid.	Carboxylic acid	Co-emulsifier	van Ginneken *et al.*, 2011; Rayirath *et al.*, 2009.
	Linoleic acid (4884+/-236mcg/g). Gamma-Linoleic acid (235+/-42mcg/g). Arachidonic acid (4592+/-2986mcg/g). Eicosapentaenoic acid (1569+/-127mcg/g).	Omega-6 fatty acid	Anti-inflammatory	van Ginneken et al., 2011.

SPECIES	MAJOR CONSTITUENTS	CHEMICAL CLASS	KNOWN USES	REFERENCES
Ascophyllum nodosum	Oleic acid (23193+/-4833mcg/g). 18:1 monounsaturated fat (unknown position of double bond; 120+/-42mcg/g).	Monounsaturated fatty acid	Anti-inflammatory	van Ginneken *et al.*, 2011.
	Phlorotannins (5% of dry weight). 1,2,3,5-tetrahydroxybenzene 2,5-disulfate ester.	Tannin	Anti-diabetic Anti-oxidative Antibacterial Rdioprotective	Blanc *et al.*, 2011. Jensen and Ragan, 1978.
	Phenolics (2.5% of dry weight).	Phenols/ Polyphenols	Astringent	Audibert *et al.*, 2010.
	Alginates	Anionic Polysaccharide	Thickener Emulsifier	Haug *et al.*, 1967.
	Betaine. Choline. Laminine.	Quaternary Ammonium compounds	Hepatoprotective	DaSilva and Jensen, 1973; Blunden *et al.*, 1985.
	Iodine (553+/-186mcg/g).	Halogen	Antiseptic	Kundel *et al.*, 2012.
	Vanadium (seasonally related levels of 0.6-1.4mg/kg (summer to early winter) or 1.5-2.2mg/kg (winter to spring)).	Transition Metal	Nutrient	Hartung *et al.*, 2008.
	Fucosterol (up to 50% of lipophilic components).	Sterol	Anti-diabetic activity	Rayirath *et al.*, 2009; Jung *et al.*, 2013.

Table A4.2: Marine algae constituent analysis table for *Laminaria digitata*.

SPECIES	MAJOR CONSTITUENTS	CHEMICAL CLASS	KNOWN USES	REFERENCES
Laminaria digitata	Protein (19+/-6% w/w).	Protein	Nutrient	Reith *et al.*, 2005.
	Lipids (4+/-0.92% w/w).	Lipid	Nutrient	Reith *et al.*, 2005.
	Cellulose (9+/-3% w/w).	Polysaccharide		Reith *et al.*, 2005.
	Alginates (30+/-17% w/w).	Anionic Polysaccharide	Thickener Emulsifier	Reith *et al.*, 2005. MacArtain *et al.*, 2007.
	Laminarin (14% w/w). Phycarine.	Glucan	Immunostimulant	Reith *et al.*, 2005. MacArtain *et al.*, 2007. Mayer *et al.*, 2007
	Fucoidan (5.5% w/w).	Fucoidan	Antithrombotic Anti-inflamatory Intestinal mucosal protective	Reith *et al.*, 2005. MacArtain *et al.*, 2007.
	Mannitol (18.25+/-7% w/w).	Carbohydrate	Vaso-dilative	Reith *et al.*, 2005. MacArtain *et al.*, 2007.
	β-carotene (63, 336mg kg−1 of dry weight (=ppm))	Carotenoids	Provitamin A activity	Jensen, 1966. Morrissey *et al.*, 2001. Astorg, 1997.
	Fucoxanthin (468mg kg−1 of dry weight (=ppm))	Xanthophylls	Anti-oxidant Anti-inflammatory Antinociceptive	Jensen, 1966. Maeda *et al.*, 2008b. Maeda *et al.*, 2008a.

Table A4.3: Marine algae constituent analysis table for *Saccharina latissima*.

SPECIES	MAJOR CONSTITUENTS	CHEMICAL CLASS	KNOWN USES	REFERENCES
Saccharina latissima	Total Polysaccharides (38-61% dry weight)	Polysaccharides	Anti-tumor action Anti-herpetic Decrease in LDL-cholesterol in rats	Wen *et al.*, 2006. Morrissey *et al.*, 2001. Rioux *et al.*, 2007. Dawczynski *et al.*, 2007. Murata and Nakazoe, 2001. Ye *et al.*, 2008. Athukorala *et al.*, 2007. Ghosh *et al.*, 2009. Amano *et al.*, 2005.
	Total protein (3-21% dry weight)	Protein	Nutrient	Jensen and Haug, 1956. Haug and Jensen, 1954. Dawczynski *et al.*, 2007. McHugh, 2003. Rupérez and Saura-Calixto, 2001. Rioux *et al.*, 2007. Marsham *et al.*, 2007. Wen *et al.*, 2006.
	Total fatty acid (0.3-2.1% dry weight)	Fatty acids	Nutrient	Jensen and Haug, 1956. Marsham *et al.*, 2007. Morrissey *et al.*, 2001. Haug and Jensen, 1954. Dawczynski *et al.*, 2007. Wen *et al.*, 2006. McHugh, 2003. Rioux *et al.*, 2007.
	Algins/alginic acid (17–33%, 18% dry weight)	Anionic Polysaccharide	Antibacterial	Haug and Jensen, 1954. Morrissey *et al.*, 2001. Hennequart, 2007.
	Mannitol (2–19%, 14% dry weight)	Carbohydrate	Vaso-dilative	Haug and Jensen, 1954. Morrissey *et al.*, 2001.
	Laminaran / Laminarin (0-33%, 16% dry weight)	Polysaccharides	Immunostimulant	Haug and Jensen, 1954. Morrissey *et al.*, 2001.

SPECIES	MAJOR CONSTITUENTS	CHEMICAL CLASS	KNOWN USES	REFERENCES
Saccharina latissima	Iodine (23-1200mg per $100g^{-1}$ of dry weight)	Halogen	Antiseptic	van Netten *et al.*, 2000. Mabeau and Fleurence, 1993. Haug and Jensen, 1954. Rupérez, 2002. Wen *et al.*, 2006. Arasaki and Arasaki, 1983. Jensen and Haug, 1956. Morrissey *et al.*, 2001.
	Phenolics (0.2-5.3% of dry weight). Total phenolic content (20.9 ± 0.3mg/100g dried material in water extract and 35.4 ± 1.3mg/100g dried material in ethanolic extract). Gallic acid (1.4 ± 0.0mg/g in ethanolic extract, 1.6 ± 0.0mg/g in water extract). Protocatechuic acid (9.5 ± 0.0mg/g in ethanolic extract, 1.2 ± 0.0mg/g in water extract). Gentisic acid (18.3 ± 0.0mg/g in ethanolic extract). Hydroxybenzoic acid (2.7 ± 0.0mg/g in water extract). Chlorogenic acid (0.4 ± 0.0mg/g in water extract). Vanilic acid (0.2 ± 0.2mg/g in water extract). Syringic acid (0.3 ± 0.0mg/g in water extract).	Phenols/ Polyphenols	Astringent	Connan *et al.*, 2004. Connan *et al.*, 2006. Rupérez and Saura-Calixto, 2001. Hammerstrom *et al.*, 1998. Horn, 2000. Sabeena Farvin and Jacobsen, 2013.
	Tyramine	Alkaloid	Central nervous system stimulant	Kneifel *et al.*, 1977.

APPENDIX 5

Table A5.1: Results from extraction of *Ascophyllum nodosum*.

SPECIES	% ETHANOL	PREPARATION METHOD			
		FROZEN	**AIR DRIED**	**FREEZE DRIED**	**OVEN DRIED**
Ascophyllum nodosum	96%	Fraction code: **1Ai** Sample weight: **100.09g** DM sample weight: **51.53g** Liquid extract % ethanol : **76%** pH at 25°C: **5.50** Extract weight: **5.48g** DM extract weight: **5.32g** % yield: **5.48%** DM % yield: **10.32%**	Fraction code: **1Bi** Sample weight: **42.74g** DM sample weight: **38.06g** Liquid extract % ethanol : **72%** pH at 25°C: **5.64** Extract weight: **0.16g** DM extract weight: **0.16g** % yield: **0.37%** DM % yield: **0.42%**	Fraction code: **1Ci** Sample weight: **29.75g** DM sample weight: **26.75g** Liquid extract % ethanol : **80%** pH at 25°C: **5.42** Extract weight: **0.54g** DM extract weight: **0.51g** % yield: **1.82%** DM % yield: **1.91%**	Fraction code: **1Di** Sample weight: **37.49g** DM sample weight: **34.10g** Liquid extract % ethanol : **75%** pH at 25°C: **5.40** Extract weight: **0.17g** DM extract weight: **0.16g** % yield: **0.45%** DM % yield: **0.47%**
	48%	Fraction code: **1Aii** Sample weight: **100.09g** DM sample weight: **51.53g** Liquid extract % ethanol : **48%** pH at 25°C: **6.81** Extract weight: **1.66g** DM extract weight: **1.27g** % yield: **1.66%** DM % yield: **2.46%**	Fraction code: **1Bii** Sample weight: **42.74g** DM sample weight: **38.06g** Liquid extract % ethanol : **56%** pH at 25°C: **5.42** Extract weight: **2.26g** DM extract weight: **2.14g** % yield: **5.29%** DM % yield: **5.63%**	Fraction code: **1Cii** Sample weight: **29.75g** DM sample weight: **26.75g** Liquid extract % ethanol : **52%** pH at 25°C: **5.32** Extract weight: **2.37g** DM extract weight: **2.24g** % yield: **7.97%** DM % yield: **8.37%**	Fraction code: **1Dii** Sample weight: **37.49g** DM sample weight: **34.10g** Liquid extract % ethanol : **54%** pH at 25°C: **5.56** Extract weight: **2.09g** DM extract weight: **1.99g** % yield: **5.57%** DM % yield: **5.84%**
	0% (100% H_20)	Fraction code: **1Aiii** Sample weight: **100.09g** DM sample weight: **51.53g** Liquid extract % ethanol : **10%** pH at 25°C: **7.00** Extract weight: **1.45g** DM extract weight: **1.20g** % yield: **1.45%** DM % yield: **2.33%**	Fraction code: **1Biii** Sample weight: **42.74g** DM sample weight: **38.06g** Liquid extract % ethanol : **12%** pH at 25°C: **7.02** Extract weight: **4.6g** DM extract weight: **3.75g** % yield: **10.76%** DM % yield: **9.85%**	Fraction code: **1Ciii** Sample weight: **29.75g** DM sample weight: **26.75g** Liquid extract % ethanol : **11%** pH at 25°C: **6.98** Extract weight: **3.19g** DM extract weight: **2.71g** % yield: **10.72%** DM % yield: **10.13%**	Fraction code: **1Diii** Sample weight: **37.49g** DM sample weight: **34.10g** Liquid extract % ethanol : **12%** pH at 25°C: **6.99** Extract weight: **4.41g** DM extract weight: **4.07g** % yield: **11.76%** DM % yield: **11.94%**

Table A5.2: Results from extraction of *Laminaria digitata*.

SPECIES	% ETHANOL	PREPARATION METHOD			
		FROZEN	**AIR DRIED**	**FREEZE DRIED**	**OVEN DRIED**
Laminaria digitata	96%	Fraction code: **2Ai** Sample weight: **100.09g** DM sample weight: **25.57g** Liquid extract % ethanol : **84%** pH at 25°C: **5.43** Extract weight: **4.74g** DM extract weight: **4.59g** % yield: **4.74%** DM % yield: **17.95%**	Fraction code: **2Bi** Sample weight: **28.36g** DM sample weight: **26.14g** Liquid extract % ethanol : **86%** pH at 25°C: **5.56** Extract weight: **0.30g** DM extract weight: **0.28g** % yield: **1.06%** DM % yield: **1.07%**	Fraction code: **2Ci** Sample weight: **22.27g** DM sample weight: **20.48g** Liquid extract % ethanol : **96%** pH at 25°C: **5.32** Extract weight: **0.75g** DM extract weight: **0.71g** % yield: **3.37%** DM % yield: **3.19%**	Fraction code: **2Di** Sample weight: **18.35g** DM sample weight: **17.53g** Liquid extract % ethanol : **90%** pH at 25°C: **5.08** Extract weight: **0.21g** DM extract weight: **0.19g** % yield: **1.14%** DM % yield: **1.08%**
	48%	Fraction code: **2Aii** Sample weight: **100.09g** DM sample weight: **25.57g** Liquid extract % ethanol : **52%** pH at 25°C: **5.97** Extract weight: **1.60g** DM extract weight: **1.52g** % yield: **1.60%** DM % yield: **5.94%**	Fraction code: **2Bii** Sample weight: **28.36g** DM sample weight: **26.14g** Liquid extract % ethanol : **50%** pH at 25°C: **5.54** Extract weight: **7.8g** DM extract weight: **7.56g** % yield: **27.50%** DM % yield: **28.92%**	Fraction code: **2Cii** Sample weight: **22.27g** DM sample weight: **20.48g** Liquid extract % ethanol : **55%** pH at 25°C: **5.42** Extract weight: **4.39g** DM extract weight: **4.15g** % yield: **19.71%** DM % yield: **20.26%**	Fraction code: **2Dii** Sample weight: **18.35g** DM sample weight: **17.53g** Liquid extract % ethanol : **50%** pH at 25°C: **5.79** Extract weight: **5.22g** DM extract weight: **5.01g** % yield: **28.45%** DM % yield: **28.58%**
	0% (100% H_20)	Fraction code: **2Aiii** Sample weight: **100.09g** DM sample weight: **25.57g** Liquid extract % ethanol : **10%** pH at 25°C: **6.99** Extract weight: **1.02g** DM extract weight: **0.93g** % yield: **1.02%** DM % yield: **3.64%**	Fraction code: **2Biii** Sample weight: **28.36g** DM sample weight: **26.14g** Liquid extract % ethanol : **11%** pH at 25°C: **7.00** Extract weight: **0.84g** DM extract weight: **0.79g** % yield: **2.96%** DM % yield: **3.02%**	Fraction code: **2Ciii** Sample weight: **22.27g** DM sample weight: **20.48g** Liquid extract % ethanol : **12%** pH at 25°C: **6.97** Extract weight: **0.88g** DM extract weight: **0.83g** % yield: **3.95%** DM % yield: **4.05%**	Fraction code: **2Diii** Sample weight: **18.35g** DM sample weight: **17.53g** Liquid extract % ethanol : **10%** pH at 25°C: **7.03** Extract weight: **0.54g** DM extract weight: **0.47g** % yield: **2.94%** DM % yield: **2.68%**

Table A5.3: Results from extraction of *Saccharina latissima*.

SPECIES	% ETHANOL	PREPARATION METHOD			
		FROZEN	**AIR DRIED**	**FREEZE DRIED**	**OVEN DRIED**
Saccharina latissima	96%	Fraction code: **3Ai** Sample weight: **100.08g** DM sample weight: **19.45g** Liquid extract % ethanol : **88%** pH at 25°C: **5.38** Extract weight: **5.92g** DM extract weight: **5.61g** % yield: **5.92%** DM % yield: **28.84%**	Fraction code: **3Bi** Sample weight: **23.14g** DM sample weight: **21.33g** Liquid extract % ethanol : **90%** pH at 25°C: **4.96** Extract weight: **0.99g** DM extract weight: **0.96g** % yield: **4.28%** DM % yield: **4.50%**	Fraction code: **3Ci** Sample weight: **16.67g** DM sample weight: **15.44g** Liquid extract % ethanol : **96%** pH at 25°C: **4.85** Extract weight: **0.64g** DM extract weight: **0.60g** % yield: **3.84%** DM % yield: **3.87%**	Fraction code: **3Di** Sample weight: **22.43g** DM sample weight: **21.31g** Liquid extract % ethanol : **94%** pH at 25°C: **4.66** Extract weight: **0.77g** DM extract weight: **0.73g** % yield: **3.43%** DM % yield: **3.43%**
	48%	Fraction code: **3Aii** Sample weight: **100.08g** DM sample weight: **19.45g** Liquid extract % ethanol : **49%** pH at 25°C: **6.08** Extract weight: **1.06g** DM extract weight: **0.98g** % yield: **1.06%** DM % yield: **5.04%**	Fraction code: **3Bii** Sample weight: **23.14g** DM sample weight: **21.33g** Liquid extract % ethanol : **50%** pH at 25°C: **5.25** Extract weight: **6.08g** DM extract weight: **5.83g** % yield: **26.28%** DM % yield: **27.33%**	Fraction code: **3Cii** Sample weight: **16.67g** DM sample weight: **15.44g** Liquid extract % ethanol : **56%** pH at 25°C: **5.53** Extract weight: **3.34g** DM extract weight: **3.19g** % yield: **20.04%** DM % yield: **20.66%**	Fraction code: **3Dii** Sample weight: **22.43g** DM sample weight: **21.31g** Liquid extract % ethanol : **52%** pH at 25°C: **5.27** Extract weight: **7.34g** DM extract weight: **6.91g** % yield: **32.72%** DM % yield: **32.43%**
	0% (100% H_20)	Fraction code: **3Aiii** Sample weight: **100.08g** DM sample weight: **19.45g** Liquid extract % ethanol : **9%** pH at 25°C: **6.98** Extract weight: **0.55g** DM extract weight: **0.49g** % yield: **0.55%** DM % yield: **2.52%**	Fraction code: **3Biii** Sample weight: **23.14g** DM sample weight: **21.33g** Liquid extract % ethanol : **11%** pH at 25°C: **6.99** Extract weight: **0.27g** DM extract weight: **0.26g** % yield: **1.17%** DM % yield: **1.22%**	Fraction code: **3Ciii** Sample weight: **16.67g** DM sample weight: **15.44g** Liquid extract % ethanol : **13%** pH at 25°C: **7.02** Extract weight: **0.84g** DM extract weight: **0.77g** % yield: **5.04%** DM % yield: **4.99%**	Fraction code: **3Diii** Sample weight: **22.43g** DM sample weight: **21.31g** Liquid extract % ethanol : **10%** pH at 25°C: **7.00** Extract weight: **1.49g** DM extract weight: **1.33g** % yield: **6.64%** DM % yield: **6.24%**

Note: DM is dry matter weight, and was calculated by using the following formula: Sample (or extract) weight x %DM (as calculated from the moisture analysis).

APPENDIX 6

Table A6.1: Moisture analysis results for all of the prepared marine algae samples.

SPECIES	PREPARATION TYPE	CODE	STARTING WEIGHTS				FINISHING WEIGHTS				MOISTURE		DM %†††
			SAMPLE 1 (g)	SAMPLE 2 (g)	SAMPLE 3 (g)	AVERAGE (g)†	SAMPLE 1 (g)	SAMPLE 2 (g)	SAMPLE 3 (g)	AVERAGE (g)†	CONTENT (g)	%††	
Ascophyllum nodosum	Frozen	1A	2.984	2.981	2.982	2.982 SD±0.0015	1.383	1.554	1.670	1.535 SD±0.1444	1.447 SD±0.1455	48.52 SD±4.8591	51.48 SD±4.8591
	Air Dried	1B	3.040	3.060	3.045	3.048 SD±0.0104	2.644	2.711	2.788	2.714 SD±0.0721	0.334 SD±0.0707	10.96 SD±2.3257	89.04 SD±2.3257
	Freeze Dried	1C	2.800	3.200	2.970	2.990 SD±0.2007	2.721	2.774	2.569	2.688 SD±0.1064	0.302 SD±0.1935	10.10 SD±6.1124	89.90 SD±6.1124
	Oven Dried	1D	2.981	3.028	3.032	3.013 SD±0.0284	2.698	2.794	2.732	2.741 SD±0.0487	0.272 SD±0.0343	9.03 SD±1.1527	90.97 SD±1.1527
Laminaria digitata	Frozen	2A	3.043	3.064	3.052	3.053 SD±0.0105	0.762	0.964	0.615	0.780 SD±0.1752	2.273 SD±0.1687	74.45 SD±5.6729	25.55 SD±5.6729
	Air Dried	2B	2.999	3.007	3.023	3.009 SD±0.0122	2.581	2.730	2.972	2.761 SD±0.1973	0.248 SD±0.1851	8.24 SD±6.1785	91.76 SD±6.1785
	Freeze Dried	2C	3.059	3.070	3.055	3.061 SD±0.0078	2.800	2.766	2.879	2.815 SD±0.0579	0.246 SD±0.0649	8.04 SD±2.1028	91.96 SD±2.1028
	Oven Dried	2D	3.010	3.000	2.998	3.002 SD±0.0064	2.865	2.868	2.872	2.868 SD±0.0035	0.134 SD±0.0097	4.46 SD±0.3137	95.54 SD±0.3137
Saccharina latissima	Frozen	3A	3.020	3.023	3.053	3.032 SD±0.0182	0.538	0.572	0.657	0.589 SD±0.0613	2.443 SD±0.0436	80.57 SD±1.9019	19.43 SD±1.9019
	Air Dried	3B	3.025	3.043	3.018	3.028 SD±0.0129	2.699	2.745	2.929	2.791 SD±0.1217	0.237 SD±0.1295	7.82 SD±4.2639	92.18 SD±4.2639
	Freeze Dried	3C	3.012	3.001	2.993	3.002 SD±0.0096	2.740	2.712	2.892	2.781 SD±0.0969	0.221 SD±0.1039	7.36 SD±3.4516	92.64 SD±3.4516
	Oven Dried	3D	2.889	3.118	3.008	3.005 SD±0.1145	2.675	2.799	3.092	2.855 SD±0.2141	0.150 SD±0.2079	4.99 SD±7.3744	95.01 SD±7.3744

†Average starting and dry weights were calculated by using the following formula: $\frac{\text{Sample 1+Sample 2+Sample 3}}{\text{Number of Samples}}$

††Moisture % was calculated by using the following formula: $\frac{\text{Moisture content}}{\text{Average starting weight}} \text{ x } 100$

†††(DM) Dry matter % was calculated using the following formula: 100% - Moisture %

Table A6.2: Moisture analysis results for the *Ascophyllum nodosum* extracts.

SPECIES	PREPARATION TYPE	FRACTION CODE	SAMPLE STARTING WEIGHT (g)	SAMPLE FINISHING WEIGHT (g)	MOISTURE		DM %††
					CONTENT (g)	%†	
Ascophyllum nodosum	Frozen	1Ai	0.0561	0.0531	0.0030	5.35	94.65
		1Aii	0.0540	0.0411	0.0128	23.78	76.22
		1Aiii	0.0594	0.0493	0.0101	17.00	83.00
	Air Dried	1Bi	0.0344	0.0449	0.0001	0.29	99.71
		1Bii	0.0544	0.0514	0.0030	5.51	94.49
		1Biii	0.0461	0.0399	0.0085	18.44	81.56
	Freeze Dried	1Ci	0.0536	0.0514	0.0028	5.22	94.78
		1Cii	0.0522	0.0493	0.0029	5.56	94.44
		1Ciii	0.0568	0.0482	0.0086	15.14	84.86
	Oven Dried	1Di	0.0507	0.0485	0.0022	4.34	95.66
		1Dii	0.0614	0.0587	0.0027	4.39	95.61
		1Diii	0.0589	0.0544	0.0045	7.64	92.36

†Moisture % was calculated by using the following formula: $\frac{\text{Moisture content}}{\text{Starting weight}} \text{ x } 100$

††(DM) Dry matter % was calculated using the following formula: 100% - Moisture %

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A6.3: Moisture analysis results for the *Laminaria digitata* extracts.

SPECIES	PREPARATION TYPE	FRACTION CODE	SAMPLE STARTING WEIGHT (g)	SAMPLE FINISHING WEIGHT (g)	MOISTURE		DM %††
					CONTENT (g)	%†	
Laminaria digitata	Frozen	2Ai	0.0503	0.0488	0.0015	2.98	97.02
		2Aii	0.0497	0.0471	0.0025	5.03	94.97
		2Aiii	0.0419	0.0382	0.0037	8.83	91.17
	Air Dried	2Bi	0.0519	0.0493	0.0026	5.01	94.99
		2Bii	0.0654	0.0634	0.0020	3.06	96.94
		2Biii	0.0504	0.0472	0.0032	6.35	93.65
	Freeze Dried	2Ci	0.0494	0.0469	0.0025	5.06	94.94
		2Cii	0.0536	0.0507	0.0029	5.41	94.59
		2Ciii	0.0535	0.0507	0.0028	5.23	94.77
	Oven Dried	2Di	0.0516	0.0473	0.0043	8.33	91.67
		2Dii	0.0570	0.0547	0.0023	4.04	95.96
		2Diii	0.0523	0.0456	0.0067	12.81	87.19

†Moisture % was calculated by using the following formula: $\frac{\text{Moisture content}}{\text{Starting weight}} \text{ x } 100$

††(DM) Dry matter % was calculated using the following formula: 100% - Moisture %

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A6.4: Moisture analysis results for the *Saccharina latissima* extracts.

SPECIES	PREPARATION TYPE	FRACTION CODE	SAMPLE STARTING WEIGHT (g)	SAMPLE FINISHING WEIGHT (g)	MOISTURE		DM %††
					CONTENT (g)	%†	
Saccharina latissima	Frozen	3Ai	0.0571	0.0541	0.0030	5.25	94.75
		3Aii	0.0500	0.0461	0.0039	7.80	92.20
		3Aiii	0.0498	0.0442	0.0056	11.24	88.76
	Air Dried	3Bi	0.0513	0.0496	0.0017	3.31	96.69
		3Bii	0.0521	0.0500	0.0021	4.03	95.97
		3Biii	0.0503	0.0476	0.0027	5.37	94.63
	Freeze Dried	3Ci	0.0498	0.0469	0.0029	5.82	94.18
		3Cii	0.0526	0.0502	0.0024	4.56	95.44
		3Ciii	0.0517	0.0474	0.0043	8.32	91.68
	Oven Dried	3Di	0.0504	0.0481	0.0023	4.56	95.44
		3Dii	0.0494	0.0465	0.0029	5.87	94.13
		3Diii	0.0497	0.0445	0.0052	10.46	89.54

†Moisture % was calculated by using the following formula: $\frac{\text{Moisture content}}{\text{Starting weight}} \text{ x } 100$

††(DM) Dry matter % was calculated using the following formula: 100% - Moisture %

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

APPENDIX 7

Table A7.1: Ash analysis results for all of the prepared marine algae samples.

SPECIES	PREPARATION TYPE	CODE	STARTING WEIGHTS				FINISHING WEIGHTS				ASH %††	ORGANIC MATTER %†††
			SAMPLE 1 (g)	SAMPLE 2 (g)	SAMPLE 3 (g)	AVERAGE (g)†	SAMPLE 1 (g)	SAMPLE 2 (g)	SAMPLE 3 (g)	AVERAGE (g)†		
Ascophyllum nodosum	Frozen	1A	1.383	1.554	1.670	1.535 SD±0.1444	0.286	0.320	0.285	0.297 SD±0.0199	19.35 SD±2.0616	80.65 SD±2.0616
	Air Dried	1B	2.644	2.711	2.788	2.714 SD±0.0721	0.352	0.360	0.433	0.381 SD±0.0446	14.04 SD±1.2903	85.96 SD±1.2903
	Freeze Dried	1C	2.721	2.774	2.569	2.688 SD±0.1064	0.505	0.618	0.550	0.557 SD±0.0569	20.72 SD±1.9454	79.28 SD±1.9454
	Oven Dried	1D	2.698	2.794	2.732	2.741 SD±0.0487	0.389	0.360	0.359	0.369 SD±0.0170	13.46 SD±0.8214	86.54 SD±0.8214
Laminaria digitata	Frozen	2A	0.762	0.964	0.615	0.780 SD±0.1752	0.164	0.155	0.152	0.157 SD±0.0062	20.13 SD±4.3669	79.87 SD±4.3669
	Air Dried	2B	2.581	2.730	2.972	2.761 SD±0.1973	0.440	0.523	0.366	0.443 SD±0.0785	16.05 SD±3.5041	83.95 SD±3.5041
	Freeze Dried	2C	2.800	2.766	2.879	2.815 SD±0.0579	0.569	0.533	0.512	0.538 SD±0.0288	19.11 SD±1.2749	80.89 SD±1.2749
	Oven Dried	2D	2.865	2.868	2.872	2.868 SD±0.0035	0.367	0.444	0.520	0.443 SD±0.0765	15.45 SD±2.6481	84.55 SD±2.6481
Saccharina latissima	Frozen	3A	0.538	0.572	0.657	0.589 SD±0.0613	0.101	0.135	0.126	0.120 SD±0.0176	20.37 SD±2.6783	79.63 SD±2.6783
	Air Dried	3B	2.699	2.745	2.929	2.791 SD±0.1217	0.467	0.479	0.495	0.480 SD±0.0140	17.20 SD±0.2847	82.80 SD±0.2847
	Freeze Dried	3C	2.740	2.712	2.892	2.781 SD±0.0969	0.554	0.615	0.518	0.562 SD±0.0490	20.21 SD±2.3832	79.79 SD±2.3832
	Oven Dried	3D	2.675	2.799	3.092	2.855 SD±0.2141	0.456	0.489	0.505	0.483 SD±0.0249	16.92 SD±0.5752	83.08 SD±0.5752

†Average starting and finishing weights were calculated by using the following formula: $\frac{\text{Sample 1+Sample 2+Sample 3}}{\text{Number of Samples}}$

††Ash % was calculated by using the following formula: $\frac{\text{Average finishing weight}}{\text{Average starting weight}} \text{ x } 100$

†††Organic matter % was calculated using the following formula: 100% - Ash %

Table A7.2: Ash analysis results for the *Ascophyllum nodosum* extracts.

SPECIES	PREPARATION TYPE	FRACTION CODE	SAMPLE STARTING WEIGHT (g)	SAMPLE FINISHING WEIGHT (g)	ASH %†	ORGANIC MATTER %††
Ascophyllum nodosum	Frozen	1Ai	0.0531	0.0154	29.00	71.00
		1Aii	0.0411	0.0023	5.59	94.41
		1Aiii	0.0493	0.0132	26.77	73.23
	Air Dried	1Bi	0.0449	0.0082	18.26	81.74
		1Bii	0.0514	0.0083	16.15	83.85
		1Biii	0.0399	0.0082	20.55	79.45
	Freeze Dried	1Ci	0.0514	0.0181	35.21	64.79
		1Cii	0.0493	0.0209	42.39	57.61
		1Ciii	0.0482	0.0068	14.11	85.89
	Oven Dried	1Di	0.0485	0.0146	30.10	69.90
		1Dii	0.0587	0.0238	40.55	59.45
		1Diii	0.0544	0.0172	31.62	68.38

†Ash % was calculated by using the following formula: $\frac{\text{Finishing weight}}{\text{Starting weight}} \text{ x } 100$

††Organic matter % was calculated using the following formula: 100% - Ash %

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A7.3: Ash analysis results for the *Laminaria digitata* extracts.

SPECIES	PREPARATION TYPE	FRACTION CODE	SAMPLE STARTING WEIGHT (g)	SAMPLE FINISHING WEIGHT (g)	ASH %†	ORGANIC MATTER %††
Laminaria digitata	Frozen	2Ai	0.0488	0.0154	31.56	68.44
		2Aii	0.0471	0.0091	19.32	80.68
		2Aiii	0.0382	0.0123	32.19	67.81
	Air Dried	2Bi	0.0493	0.0288	58.42	41.58
		2Bii	0.0634	0.0403	63.56	36.44
		2Biii	0.0472	0.0134	28.39	71.61
	Freeze Dried	2Ci	0.0469	0.0207	44.14	55.86
		2Cii	0.0507	0.0230	45.36	54.64
		2Ciii	0.0507	0.0081	15.98	84.02
	Oven Dried	2Di	0.0473	0.0180	38.05	61.95
		2Dii	0.0547	0.0139	25.41	74.59
		2Diii	0.0456	0.0126	27.63	72.37

†Ash % was calculated by using the following formula: $\frac{\text{Finishing weight}}{\text{Starting weight}} \text{ x } 100$

††Organic matter % was calculated using the following formula: 100% - Ash %

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A7.4: Ash analysis results for the *Saccharina latissima* extracts.

SPECIES	PREPARATION TYPE	FRACTION CODE	SAMPLE STARTING WEIGHT (g)	SAMPLE FINISHING WEIGHT (g)	ASH %†	ORGANIC MATTER %††
Saccharina latissima	Frozen	3Ai	0.0541	0.0180	33.27	66.73
		3Aii	0.0461	0.0095	20.61	79.39
		3Aiii	0.0442	0.0077	17.42	82.58
	Air Dried	3Bi	0.0496	0.0252	50.81	49.19
		3Bii	0.0500	0.0288	57.60	42.40
		3Biii	0.0476	0.0159	33.40	66.60
	Freeze Dried	3Ci	0.0469	0.0224	47.76	52.24
		3Cii	0.0502	0.0181	36.06	63.94
		3Ciii	0.0474	0.0054	11.39	88.61
	Oven Dried	3Di	0.0481	0.0181	37.63	62.37
		3Dii	0.0465	0.0135	29.03	70.97
		3Diii	0.0445	0.0069	15.51	84.49

†Ash % was calculated by using the following formula: $\frac{\text{Finishing weight}}{\text{Starting weight}} \text{ x } 100$

††Organic matter % was calculated using the following formula: 100% - Ash %

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

APPENDIX 8

Table A8.1: Fat analysis results for all of the prepared marine algae samples.

SPECIES	PREPARATION TYPE	CODE	STARTING WEIGHTS				FINISHING WEIGHTS				MOISTURE CONTENT (g)††	FAT	
			SAMPLE 1 (g)	SAMPLE 2 (g)	SAMPLE 3 (g)	AVERAGE (g)†	SAMPLE 1 (g)	SAMPLE 2 (g)	SAMPLE 3 (g)	AVERAGE (g)†		CONTENT (g)	%†††
Ascophyllum nodosum	Frozen	1A	10.005	10.022	10.010	10.012 SD±0.0087	4.645	4.802	4.704	4.717 SD±0.0793	4.858 SD±0.0343	0.437 SD±0.0363	4.36 SD±0.3866
	Air Dried	1B	4.273	4.269	4.301	4.281 SD±0.0174	3.525	4.111	3.667	3.767 SD±0.3057	0.469 SD±0.0343	0.045 SD±0.2787	1.05 SD±6.5033
	Freeze Dried	1C	3.453	2.662	2.843	2.986 SD±0.4144	2.326	2.529	2.840	2.565 SD±0.2638	0.302 SD±0.0621	0.119 SD±0.5528	3.99 SD±15.7704
	Oven Dried	1D	3.730	3.612	3.922	3.754 SD±0.1565	3.331	3.538	3.184	3.351 SD±0.1778	0.339 SD±0.0299	0.064 SD±0.3020	1.70 SD±7.6283
Laminaria digitata	Frozen	2A	10.005	10.004	10.002	10.003 SD±0.0016	2.267	2.218	2.225	2.236 SD±0.0265	7.447 SD±0.0189	0.320 SD±0.0065	3.20 SD±0.0671
	Air Dried	2B	2.877	2.832	2.852	2.853 SD±0.0225	2.559	2.586	2.574	2.573 SD±0.0135	0.235 SD±0.0029	0.045 SD±0.0331	1.58 SD±1.0878
	Freeze Dried	2C	2.234	2.236	2.235	2.235 SD±0.0010	1.999	2.019	2.063	2.027 SD±0.0327	0.180 SD±0.0026	0.028 SD±0.0298	1.25 SD±1.3364
	Oven Dried	2D	1.794	1.844	1.891	1.843 SD±0.0485	1.770	1.645	1.711	1.708 SD±0.0625	0.082 SD±0.0043	0.053 SD±0.0917	2.88 SD±4.9016
Saccharina latissima	Frozen	3A	10.003	10.010	10.007	10.006 SD±0.0035	1.550	1.645	1.590	1.595 SD±0.0477	8.061 SD±0.0356	0.350 SD±0.0086	3.50 SD±0.0915
	Air Dried	3B	2.333	2.304	2.295	2.310 SD±0.0199	2.092	2.096	2.094	2.094 SD±0.0020	0.188 SD±0.0017	0.028 SD±0.0197	1.21 SD±0.7748
	Freeze Dried	3C	1.677	1.662	1.682	1.673 SD±0.0104	1.549	1.521	1.478	1.516 SD±0.0358	0.123 SD±0.0029	0.034 SD±0.0377	2.03 SD±2.2124
	Oven Dried	3D	2.260	2.423	2.065	2.249 SD±0.1792	2.098	2.133	2.063	2.098 SD±0.0250	0.112 SD±0.0072	0.039 SD±0.1371	1.73 SD±5.6739

†Average starting and finishing weights were calculated by using the following formula: $\frac{\text{Sample 1+Sample 2+Sample 3}}{\text{Number of Samples}}$

††Moisture content was calculated by multiplying the Average starting weights by their relevant moisture % value. This was then subtracted from the average finishing weights to achieve the fat content weights.

†††Fat % was calculated by using the following formula: $\frac{\text{Fat content}}{\text{Average starting weight}} \text{ x } 100$

APPENDIX 9

Table A9.1: Carbohydrate analysis results for the standard curve tubes.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE	A_{490}
Blanks		Ba	0	2.00	0	0
		Bb	0	2.00	0	0
Glucose standards	20µg in 1ml	S20a	1:50	2.00	0.040	0.306
		S20b	1:50	2.00	0.040	0.325
	40µg in 1ml	S40a	1:25	2.00	0.080	0.509
		S40b	1:25	2.00	0.080	0.487
	60µg in 1ml	S60a	1:16.66	2.00	0.120	0.647
		S60b	1:16.66	2.00	0.120	0.774
	80µg in 1ml	S80a	1:12.5	2.00	0.160	0.832
		S80b	1:12.5	2.00	0.160	0.838
	100µg in 1ml	S100a	1:10	2.00	0.200	1.037
		S100b	1:10	2.00	0.200	1.147

CODE KEY
B=Blank, S=Standard
Number corresponds to amount in µg
a=first duplicate sample,
b=second duplicate sample

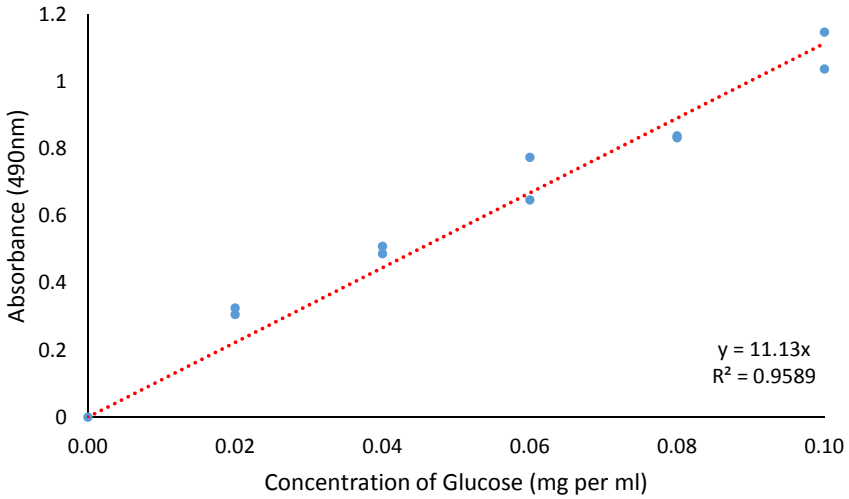

Figure A9.1: Standard curve of known glucose concentrations.

Table A9.2: Carbohydrate analysis results for the prepared *Ascophyllum nodosum* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	A_{490}	GLUCOSE EQUIVALENT					
							mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM†††	AVERAGE % IN DM
Ascophyllum nodosum	Frozen	1Aa	1:400	2.00	1.455	0.905	0.162	0.163 SD±0.0014	11.13	11.20 SD±0.0989	21.63	21.76 SD±0.1838
		1Ab	1:400	2.00	1.455	0.911	0.164		11.27		21.89	
	Air Dried	1Ba	1:400	2.00	0.761	0.304	0.046	0.047 SD±0.0014	6.04	6.18 SD±0.1909	6.79	6.94 SD±0.2051
		1Bb	1:400	2.00	0.761	0.309	0.048		6.31		7.08	
	Freeze Dried	1Ca	1:400	2.00	0.609	0.407	0.066	0.066 SD±0.00	10.83	10.83 SD±0.00	12.05	12.05 SD±0.00
		1Cb	1:400	2.00	0.609	0.401	0.066		10.83		12.05	
	Oven Dried	1Da	1:400	2.00	0.703	0.195	0.026	0.026 SD±0.00	3.70	3.70 SD±0.00	4.07	4.07 SD±0.00
		1Db	1:400	2.00	0.703	0.202	0.026		3.70		4.07	

†Sample in tube weight calculated by equivalent raw material per 0.50mg of extract.

††mg of glucose in tube was calculated by applying the absorbance values to the standard curve.

†††% in DM was calculated using the following formula: $\frac{\text{mg of glucose in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A9.3: Carbohydrate analysis results for the prepared *Laminaria digitata* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	A_{490}	GLUCOSE EQUIVALENT					
							mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM†††	AVERAGE % IN DM
Laminaria digitata	Frozen	2Aa	1:400	2.00	1.699	0.825	0.148	0.147 SD±0.0014	8.71	8.65 SD±0.0849	34.08	33.85 SD±0.3253
		2Ab	1:400	2.00	1.699	0.822	0.146		8.59		33.62	
	Air Dried	2Ba	1:400	2.00	0.397	0.145	0.016	0.015 SD±0.0014	4.04	3.79 SD±0.3606	4.40	4.13 SD±0.3889
		2Bb	1:400	2.00	0.397	0.141	0.014		3.53		3.85	
	Freeze Dried	2Ca	1:400	2.00	0.463	0.705	0.124	0.125 SD±0.0014	26.81	27.03 SD±0.3041	29.15	29.39 SD±0.3394
		2Cb	1:400	2.00	0.463	0.710	0.126		27.24		29.63	
	Oven Dried	2Da	1:400	2.00	0.385	0.445	0.074	0.075 SD±0.0014	19.25	19.51 SD±0.3677	20.14	20.42 SD±0.3889
		2Db	1:400	2.00	0.385	0.454	0.076		19.77		20.69	

†Sample in tube weight calculated by equivalent raw material per 0.50mg of extract.

††mg of glucose in tube was calculated by applying the absorbance values to the standard curve.

†††% in DM was calculated using the following formula: $\frac{\text{mg of glucose in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A9.4: Carbohydrate analysis results for the prepared *Saccharina latissima* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	A_{490}	GLUCOSE EQUIVALENT					
							mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM†††	AVERAGE % IN DM
Saccharina latissima	Frozen	3Aa	1:400	2.00	1.662	0.819	0.146	0.146 SD±0.00	8.79	8.79 SD±0.00	45.23	45.23 SD±0.00
		3Ab	1:400	2.00	1.662	0.822	0.146		8.79		45.23	
	Air Dried	3Ba	1:400	2.00	0.394	0.181	0.022	0.023 SD±0.0014	5.58	5.84 SD±0.3606	6.06	6.34 SD±0.3889
		3Bb	1:400	2.00	0.394	0.189	0.024		6.09		6.61	
	Freeze Dried	3Ca	1:400	2.00	0.433	0.425	0.070	0.069 SD±0.0014	16.18	15.95 SD±0.3253	17.47	17.22 SD±0.3536
		3Cb	1:400	2.00	0.433	0.418	0.068		15.72		16.97	
	Oven Dried	3Da	1:400	2.00	0.292	0.283	0.042	0.042 SD±0.00	14.38	14.38 SD±0.00	15.14	15.14 SD±0.00
		3Db	1:400	2.00	0.292	0.282	0.042		14.38		15.14	

†Sample in tube weight calculated by equivalent raw material per 0.50mg of extract.

††mg of glucose in tube was calculated by applying the absorbance values to the standard curve.

†††% in DM was calculated using the following formula: $\frac{\text{mg of glucose in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A9.5: Carbohydrate analysis results for the *Ascophyllum nodosum* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE	A_{490}	GLUCOSE EQUIVALENT		
							mg IN TUBE††	% IN ORIGINAL SAMPLE	% in DM†††
Ascophyllum nodosum	Frozen	1Ai	1:20	2	0.465	0.288	0.044	9.46	18.38
		1Aii	1:20	2	0.487	0.373	0.060	12.32	23.93
		1Aiii	1:20	2	0.502	0.375	0.060	11.95	13.42
	Air Dried	1Bi	1:20	2	0.489	0.238	0.034	6.95	7.81
		1Bii	1:20	2	0.498	0.290	0.044	8.84	9.83
		1Biii	1:20	2	0.497	0.388	0.062	12.47	13.88
	Freeze Dried	1Ci	1:20	2	0.488	0.120	0.008	1.64	1.80
		1Cii	1:20	2	0.501	0.208	0.028	5.59	6.14
		1Ciii	1:20	2	0.500	0.538	0.092	18.40	72.02
	Oven Dried	1Di	1:20	2	0.493	0.173	0.020	4.06	15.88
		1Dii	1:20	2	0.491	0.268	0.040	8.15	8.88
		1Diii	1:20	2	0.497	0.723	0.128	25.75	28.07

†0.50mg of extract was taken from a 1mg per 1ml stock solution in 50:50 (methanol : ultrapure water).

††mg of glucose in tube was calculated by applying the absorbance values to the standard curve.

†††% in DM was calculated using the following formula: $\frac{\text{mg of glucose in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A9.6: Carbohydrate analysis results for the *Laminaria digitata* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE	A_{490}	GLUCOSE EQUIVALENT		
							mg IN TUBE††	% IN ORIGINAL SAMPLE	% in DM†††
Laminaria digitata	Frozen	2Ai	1:20	2	0.496	0.155	0.018	3.63	3.95
		2Aii	1:20	2	0.504	0.278	0.042	8.33	9.06
		2Aiii	1:20	2	0.497	0.370	0.060	12.07	12.64
	Air Dried	2Bi	1:20	2	0.487	0.123	0.012	2.46	2.58
		2Bii	1:20	2	0.492	0.128	0.012	2.44	12.55
		2Biii	1:20	2	0.501	0.235	0.032	6.39	32.87
	Freeze Dried	2Ci	1:20	2	0.499	0.080	0.002	0.40	0.43
		2Cii	1:20	2	0.493	0.203	0.026	5.27	5.72
		2Ciii	1:20	2	0.496	0.573	0.098	19.76	21.33
	Oven Dried	2Di	1:20	2	0.501	0.303	0.046	9.18	9.91
		2Dii	1:20	2	0.486	0.780	0.138	28.40	29.89
		2Diii	1:20	2	0.497	0.910	0.164	33.00	34.73

†0.50mg of extract was taken from a 1mg per 1ml stock solution in 50:50 (methanol : ultrapure water).

††mg of glucose in tube was calculated by applying the absorbance values to the standard curve.

†††% in DM was calculated using the following formula: $\frac{\text{mg of glucose in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A9.7: Carbohydrate analysis results for the *Saccharina latissima* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE	A_{490}	GLUCOSE EQUIVALENT		
							mg IN TUBE††	% IN ORIGINAL SAMPLE	% in DM†††
Saccharina latissima	Frozen	3Ai	1:20	2	0.498	0.270	0.040	8.03	8.48
		3Aii	1:20	2	0.493	0.638	0.112	22.72	24.64
		3Aiii	1:20	2	0.504	1.868	0.350	69.44	78.24
	Air Dried	3Bi	1:20	2	0.487	0.173	0.020	4.11	4.25
		3Bii	1:20	2	0.491	0.123	0.012	2.44	2.55
		3Biii	1:20	2	0.503	0.268	0.040	7.95	8.40
	Freeze Dried	3Ci	1:20	2	0.488	0.150	0.016	3.28	3.48
		3Cii	1:20	2	0.493	0.403	0.066	13.39	14.03
		3Ciii	1:20	2	0.498	2.115	0.398	79.92	87.17
	Oven Dried	3Di	1:20	2	0.489	0.368	0.058	11.86	12.43
		3Dii	1:20	2	0.478	0.595	0.102	21.34	22.67
		3Diii	1:20	2	0.501	1.758	0.328	65.47	73.12

†0.50mg of extract was taken from a 1mg per 1ml stock solution in 50:50 (methanol : ultrapure water).

††mg of glucose in tube was calculated by applying the absorbance values to the standard curve.

†††% in DM was calculated using the following formula: $\frac{\text{mg of glucose in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

APPENDIX 10

Table A10.1: Protein analysis results for the standard curve tubes.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE	A_{490}
Blanks		Ba	0	1.00	0	0
		Bb	0	1.00	0	0
Protein standards (BSA)	20µg in 1ml	S20a	1:50	1.00	0.020	0.060
		S20b	1:50	1.00	0.020	0.062
	40µg in 1ml	S40a	1:25	1.00	0.040	0.122
		S40b	1:25	1.00	0.040	0.121
	60µg in 1ml	S60a	1:16.66	1.00	0.060	0.209
		S60b	1:16.66	1.00	0.060	0.212
	80µg in 1ml	S80a	1:12.5	1.00	0.080	0.293
		S80b	1:12.5	1.00	0.080	0.290
	100µg in 1ml	S100a	1:10	1.00	0.100	0.345
		S100b	1:10	1.00	0.100	0.349
	200µg in 1ml	S200a	1:5	1.00	0.200	0.697
		S200b	1:5	1.00	0.200	0.704
	400µg in 1ml	S400a	1:2.5	1.00	0.400	1.290
		S400b	1:2.5	1.00	0.400	1.343
	600µg in 1ml	S600a	1:1.66	1.00	0.600	1.748
		S600b	1:1.66	1.00	0.600	1.774
	800µg in 1ml	S800a	1:1.25	1.00	0.800	2.112
		S800b	1:1.25	1.00	0.800	2.104
	1000µg in 1ml	S1000a	0	1.00	1.000	2.321
		S1000b	0	1.00	1.000	2.339

CODE KEY
B=Blank, S=Standard
Number corresponds to amount in µg
a=first duplicate sample,
b=second duplicate sample

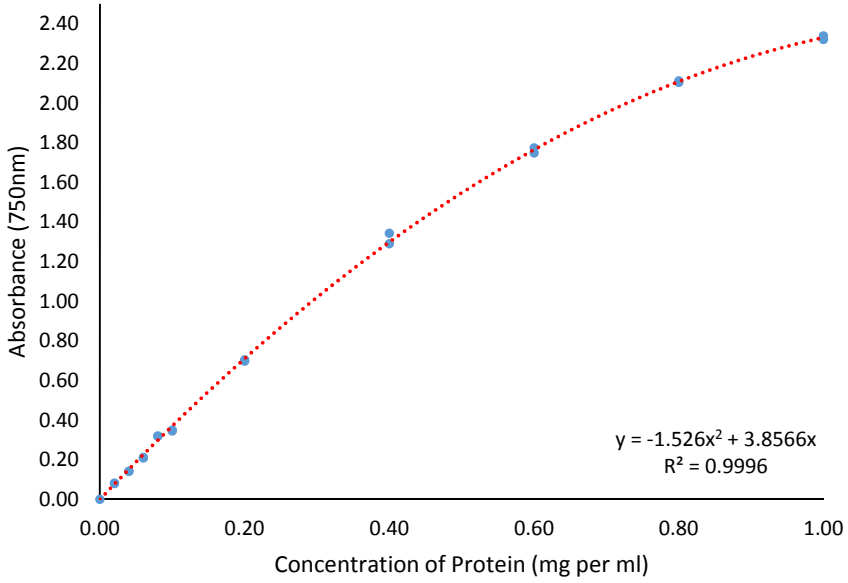

Figure A10.1: Standard curve of known protein (BSA) concentrations.

Table A10.2: Protein analysis results for the prepared *Ascophyllum nodosum* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	A_{750}	PROTEIN EQUIVALENT					
								mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM†† †	AVERAGE % IN DM
Ascophyllum nodosum	Frozen	1Aa	1:4	1.00	12.40	12.47 SD±0.0919	1.286	0.474	0.482 SD±0.0106	3.82	3.86 SD±0.0565	7.43	7.51 SD±0.1061
		1Ab	1:4	1.00	12.53		1.325	0.489		3.90		7.58	
	Air Dried	1Ba	1:4	1.00	10.45	10.48 SD±0.0354	0.935	0.333	0.314 SD±0.0269	3.19	3.00 SD±0.2687	3.58	3.37 SD±0.2969
		1Bb	1:4	1.00	10.50		0.839	0.295		2.81		3.16	
	Freeze Dried	1Ca	1:4	1.00	14.35	14.20 SD±0.2121	1.174	0.429	0.429 SD±0.0007	2.99	3.02 SD±0.0424	3.33	3.36 SD±0.0424
		1Cb	1:4	1.00	14.05		1.171	0.428		3.05		3.39	
	Oven Dried	1Da	1:4	1.00	14.15	14.05 SD±0.1414	0.277	0.070	0.049 SD±0.0304	0.49	0.34 SD±0.2121	0.54	0.38 SD±0.2333
		1Db	1:4	1.00	13.95		0.169	0.027		0.19		0.21	

†mg of protein in tube was calculated by applying the absorbance values to the standard curve.

††% in DM was calculated using the following formula: $\frac{\text{mg of protein in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A10.3: Protein analysis results for the prepared *Laminaria digitata* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	A_{750}	PROTEIN EQUIVALENT					
								mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM† ††	AVERAGE % IN DM
Laminaria digitata	Frozen	2Aa	1:4	1.00	13.03	12.97 SD±0.00)	0.492	0.156	0.157 SD±0.0007	1.20	1.21 SD±0.0141	4.69	4.73 SD±0.0495
		2Ab	1:4	1.00	12.90		0.495	0.157		1.22		4.76	
	Air Dried	2Ba	1:4	1.00	13.73	13.69 SD±0.00)	0.528	0.171	0.170 SD±0.0021	1.25	1.24 SD±0.0141	1.36	1.35 SD±0.0141
		2Bb	1:4	1.00	13.65		0.522	0.168		1.23		1.34	
	Freeze Dried	2Ca	1:4	1.00	13.45	13.57 SD±0.00)	0.783	0.273	0.272 SD±0.0014	2.03	2.01 SD±0.0353	2.21	2.18 SD±0.0424
		2Cb	1:4	1.00	13.68		0.780	0.271		1.98		2.15	
	Oven Dried	2Da	1:4	1.00	14.13	14.22 SD±0.00)	0.254	0.061	0.061 SD±0.00	0.43	0.43 SD±0.00	0.45	0.45 SD±0.00
		2Db	1:4	1.00	14.30		0.255	0.061		0.43		0.45	

†mg of protein in tube was calculated by applying the absorbance values to the standard curve.

††% in DM was calculated using the following formula: $\frac{\text{mg of protein in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A10.4: Protein analysis results for the prepared *Saccharina latissima* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	A_{750}	PROTEIN EQUIVALENT					
								mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM† ††	AVERAGE % IN DM
Saccharina latissima	Frozen	3Aa	1:4	1.00	12.45	12.49 SD±0.0566	0.389	0.115	0.115 SD±0.0007	0.92	0.92 SD±0.0070	4.75	4.72 SD±0.0495
		3Ab	1:4	1.00	12.53		0.386	0.114		0.91		4.68	
	Air Dried	3Ba	1:4	1.00	12.23	12.29 SD±0.0849	0.530	0.171	0.171 SD±0.00	1.40	1.39 SD±0.0141	1.52	1.51 SD±0.0141
		3Bb	1:4	1.00	12.35		0.529	0.171		1.38		1.50	
	Freeze Dried	3Ca	1:4	1.00	11.95	11.94 SD±0.0141	0.615	0.205	0.208 SD±0.0042	1.72	1.75 SD±0.0354	1.85	1.88 SD±0.0424
		3Cb	1:4	1.00	11.93		0.629	0.211		1.77		1.91	
	Oven Dried	3Da	1:4	1.00	12.78	12.73 SD±0.0707	0.192	0.028	0.028 SD±0.00	1.22	1.22 SD±0.00	0.30	0.30 SD±0.0071
		3Db	1:4	1.00	12.68		0.188	0.028		1.22		0.29	

†mg of protein in tube was calculated by applying the absorbance values to the standard curve.

††% in DM was calculated using the following formula: $\frac{\text{mg of protein in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

APPENDIX 11

Table A11.1: Sodium elemental analysis results for the prepared *Ascophyllum nodosum* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	Na EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM†† †	AVERAGE % IN DM
Ascophyllum nodosum	Frozen	1Aa	1:250	50.00	1.364	1.403 SD±0.0552	0.732	0.0366	0.0367 SD±0.0001	2.68	2.62 SD±0.0919	5.21	5.09 SD±0.1768
		1Ab	1:250	50.00	1.442		0.735	0.0368		2.55		4.96	
	Air Dried	1Ba	1:250	50.00	1.581	1.575 SD±0.0085	0.535	0.0268	0.0293 SD±0.0035	1.70	1.86 SD±0.2263	1.90	2.09 SD±0.2616
		1Bb	1:250	50.00	1.569		0.634	0.0317		2.02		2.27	
	Freeze Dried	1Ca	1:250	50.00	1.508	2.263 SD±0.0014	0.586	0.0293	0.0346 SD±0.0075	1.94	2.29 SD±0.4949	2.16	2.55 SD±0.5515
		1Cb	1:250	50.00	1.510		0.797	0.0399		2.64		2.94	
	Oven Dried	1Da	1:250	50.00	1.292	1.324 SD±0.0453	0.698	0.0349	0.0377 SD±0.0039	2.70	2.84 SD±0.1979	2.97	3.11 SD±0.1979
		1Db	1:250	50.00	1.356		0.807	0.0404		2.98		3.25	

†%in DM was calculated using the following formula: $\frac{\text{mg of Na in tube}}{\text{mg sample in tube x \% DM of sample}} \times 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.2: Sodium elemental analysis results for the prepared *Laminaria digitata* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	Na EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM† ††	AVERAGE % IN DM
Laminaria digitata	Frozen	2Aa	1:250	50.00	1.598	1.610 SD±0.0169	0.503	0.0252	0.0229 SD±0.0032	1.58	1.43 SD±0.2121	6.17	5.58 SD±0.8344
		2Ab	1:250	50.00	1.622		0.414	0.0207		1.28		4.99	
	Air Dried	2Ba	1:250	50.00	1.364	1.329 SD±0.0488	0.496	0.0248	0.0232 SD±0.0023	1.82	1.75 SD±0.1061	1.98	1.90 SD±0.1131
		2Bb	1:250	50.00	1.295		0.432	0.0216		1.67		1.82	
	Freeze Dried	2Ca	1:250	50.00	1.299	1.320 SD±0.0297	0.529	0.0265	0.0262 SD±0.0005	2.04	1.98 SD±0.0849	2.22	2.16 SD±0.0919
		2Cb	1:250	50.00	1.341		0.516	0.0258		1.92		2.09	
	Oven Dried	2Da	1:250	50.00	1.347	1.362 SD±0.0205	0.370	0.0185	0.0172 SD±0.0018	1.37	1.27 SD±0.1485	1.44	1.33 SD±0.1626
		2Db	1:250	50.00	1.376		0.318	0.0159		1.16		1.21	

†%in DM was calculated using the following formula: $\frac{\text{mg of Na in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.3: Sodium elemental analysis results for the prepared *Saccharina latissima* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	Na EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM† ††	AVERAGE % IN DM
Saccharina latissima	Frozen	3Aa	1:250	50.00	1.402	1.397	0.536	0.0268	0.0265	1.91	1.89	9.84	9.77
		3Ab	1:250	50.00	1.392	SD±0.0071	0.524	0.0262	SD±0.0004	1.88	SD±0.0212	9.69	SD±0.1061
	Air Dried	3Ba	1:250	50.00	1.283	1.305	0.483	0.0242	0.0229	1.89	1.76	2.05	1.91
		3Bb	1:250	50.00	1.327	SD±0.0311	0.432	0.0216	SD±0.0018	1.63	SD±0.1838	1.77	SD±0.1979
	Freeze Dried	3Ca	1:250	50.00	1.303	1.357	0.556	0.0278	0.0266	2.13	1.97	2.30	2.12
		3Cb	1:250	50.00	1.411	SD±0.0764	0.507	0.0254	SD±0.0017	1.80	SD±0.2333	1.94	SD±0.2546
	Oven Dried	3Da	1:250	50.00	1.324	1.357	0.423	0.0212	0.0207	1.60	1.53	1.69	1.61
		3Db	1:250	50.00	1.389	SD±0.0459	0.415	0.0201	SD±0.0008	1.45	SD±0.1061	1.52	SD±0.1202

†%in DM was calculated using the following formula: $\frac{\text{mg of Na in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.4: Sodium elemental analysis results for the *Ascophyllum nodosum* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	Na EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	% in DM††
Ascophyllum nodosum	Frozen	1Ai	1:31.25	50.00	0.614	0.279	0.0139	2.26	2.39
		1Aii	1:31.25	50.00	0.186	0.112	0.0056	3.01	3.95
		1Aiii	1:31.25	50.00	0.163	0.099	0.0049	3.01	3.62
	Air Dried	1Bi	1:31.25	50.00	0.042	0.023	0.0012	2.86	2.87
		1Bii	1:31.25	50.00	0.592	0.311	0.0156	2.64	2.79
		1Biii	1:31.25	50.00	1.203	0.179	0.0088	0.73	0.90
	Freeze Dried	1Ci	1:31.25	50.00	0.202	0.118	0.0059	2.92	3.08
		1Cii	1:31.25	50.00	0.889	0.387	0.0194	2.18	2.31
		1Ciii	1:31.25	50.00	0.784	0.276	0.0138	1.76	2.07
	Oven Dried	1Di	1:31.25	50.00	0.048	0.052	0.0026	5.42	5.66
		1Dii	1:31.25	50.00	0.624	0.358	0.0179	2.87	3.00
		1Diii	1:31.25	50.00	1.315	0.422	0.0211	1.60	1.74

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of Na in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A11.5: Sodium elemental analysis results for the *Laminaria digitata* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	Na EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	% in DM††
Laminaria digitata	Frozen	2Ai	1:31.25	50.00	0.528	0.039	0.0019	0.36	0.37
		2Aii	1:31.25	50.00	0.176	0.091	0.0046	2.61	2.75
		2Aiii	1:31.25	50.00	0.112	0.187	0.0094	8.39	9.21
	Air Dried	2Bi	1:31.25	50.00	0.118	0.071	0.0036	3.05	3.21
		2Bii	1:31.25	50.00	3.078	0.044	0.0022	0.07	0.07
		2Biii	1:31.25	50.00	0.329	0.155	0.0078	2.37	2.01
	Freeze Dried	2Ci	1:31.25	50.00	0.374	0.045	0.0023	0.61	0.65
		2Cii	1:31.25	50.00	2.205	0.007	0.0004	0.02	0.02
		2Ciii	1:31.25	50.00	0.442	0.155	0.0078	1.76	1.86
	Oven Dried	2Di	1:31.25	50.00	0.128	0.109	0.0055	4.30	4.69
		2Dii	1:31.25	50.00	3.184	0.018	0.0009	0.03	0.03
		2Diii	1:31.25	50.00	0.326	0.123	0.0062	1.90	2.18

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of Na in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A11.6: Sodium elemental analysis results for the *Saccharina latissima* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	Na EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	% in DM††
Saccharina latissima	Frozen	3Ai	1:31.25	50.00	0.662	0.036	0.0018	0.27	0.29
		3Aii	1:31.25	50.00	0.118	0.013	0.0007	0.59	0.64
		3Aiii	1:31.25	50.00	0.061	0.078	0.0039	6.39	7.21
	Air Dried	3Bi	1:31.25	50.00	0.477	0.038	0.0019	0.40	0.41
		3Bii	1:31.25	50.00	2.941	0.005	0.0003	0.01	0.01
		3Biii	1:31.25	50.00	0.128	0.158	0.0079	6.17	6.52
	Freeze Dried	3Ci	1:31.25	50.00	0.429	0.021	0.0011	0.26	0.27
		3Cii	1:31.25	50.00	2.243	0.005	0.0003	0.01	0.01
		3Ciii	1:31.25	50.00	0.563	0.040	0.0020	0.36	0.39
	Oven Dried	3Di	1:31.25	50.00	0.384	0.043	0.0022	0.57	0.60
		3Dii	1:31.25	50.00	3.664	0.068	0.0034	0.09	0.10
		3Diii	1:31.25	50.00	0.742	0.043	0.0022	0.30	0.33

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of Na in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A11.7: Potassium elemental analysis results for the prepared *Ascophyllum nodosum* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	K EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM†† †	AVERAGE % IN DM
Ascophyllum nodosum	Frozen	1Aa	1:250	50.00	1.364	1.403 SD±0.0552	0.343	0.0172	0.0175 SD±0.0004	1.26	1.25 SD±0.0212	2.44	2.41 SD±0.0424
		1Ab	1:250	50.00	1.442		0.354	0.0177		1.23		2.38	
	Air Dried	1Ba	1:250	50.00	1.581	1.575 SD±0.0085	0.325	0.0163	0.0151 SD±0.0018	1.03	0.96 SD±0.1061	1.15	1.07 SD±0.1131
		1Bb	1:250	50.00	1.569		0.276	0.0138		0.88		0.99	
	Freeze Dried	1Ca	1:250	50.00	1.508	1.509 SD±0.0014	0.612	0.0306	0.0323 SD±0.0024	2.03	2.14 SD±0.1556	2.26	2.37 SD±0.1485
		1Cb	1:250	50.00	1.510		0.679	0.0340		2.25		2.47	
	Oven Dried	1Da	1:250	50.00	1.292	1.324 SD±0.0453	0.215	0.0108	0.0121 SD±0.0018	0.83	0.91 SD±0.1061	0.91	0.99 SD±0.1131
		1Db	1:250	50.00	1.356		0.265	0.0133		0.98		1.07	

†%in DM was calculated using the following formula: $\frac{\text{mg of K in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.8: Potassium elemental analysis results for the prepared *Laminaria digitata* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	K EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM† ††	AVERAGE % IN DM
Laminaria digitata	Frozen	2Aa	1:250	50.00	1.598	1.610 SD±0.0169	0.677	0.0339	0.0344 SD±0.0006	2.12	2.14 SD±0.0212	8.29	8.35 SD±0.0778
		2Ab	1:250	50.00	1.622		0.696	0.0348		2.15		8.40	
	Air Dried	2Ba	1:250	50.00	1.364	1.329 SD±0.0488	0.553	0.0277	0.0294 SD±0.0024	2.03	2.22 SD±0.2616	2.21	2.42 SD±0.2899
		2Bb	1:250	50.00	1.295		0.622	0.0311		2.40		2.62	
	Freeze Dried	2Ca	1:250	50.00	1.299	1.320 SD±0.0297	1.448	0.0724	0.0686 SD±0.0054	5.57	5.19 SD±0.5303	6.06	5.66 SD±0.5728
		2Cb	1:250	50.00	1.341		1.294	0.0647		4.82		5.25	
	Oven Dried	2Da	1:250	50.00	1.347	1.362 SD±0.0205	0.486	0.0243	0.0266 SD±0.0033	1.80	1.95 SD±0.2121	1.89	2.04 SD±0.2121
		2Db	1:250	50.00	1.376		0.577	0.0289		2.10		2.19	

†%in DM was calculated using the following formula: $\frac{\text{mg of K in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.9: Potassium elemental analysis results for the prepared *Saccharina latissima* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	K EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM† ††	AVERAGE % IN DM
Saccharina latissima	Frozen	3Aa	1:250	50.00	1.402	1.39 SD±0.0071	0.341	0.0171	0.0176 SD±0.0006	1.22	1.26 SD±0.0495	6.26	6.45 SD±0.2687
		3Ab	1:250	50.00	1.392		0.359	0.0180		1.29		6.64	
	Air Dried	3Ba	1:250	50.00	1.283	1.305 SD±0.0311	0.445	0.0223	0.0239 SD±0.0022	1.73	1.82 SD±0.1273	1.88	1.98 SD±0.1344
		3Bb	1:250	50.00	1.327		0.507	0.0254		1.91		2.07	
	Freeze Dried	3Ca	1:250	50.00	1.303	1.357 SD±0.0764	1.355	0.0678	0.0617 SD±0.0087	5.20	4.57 SD±0.8980	5.61	4.93 SD±0.9687
		3Cb	1:250	50.00	1.411		1.109	0.0555		3.93		4.24	
	Oven Dried	3Da	1:250	50.00	1.324	1.357 SD±0.0459	0.318	0.**0159**	0.0136 SD±0.0033	1.20	1.00 SD±0.2828	1.26	1.05 SD±0.2969
		3Db	1:250	50.00	1.389		0.223	0.0112		0.80		0.84	

†%in DM was calculated using the following formula: $\frac{\text{mg of K in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.10: Potassium elemental analysis results for the *Ascophyllum nodosum* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	K EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	% in DM††
Ascophyllum nodosum	Frozen	1Ai	1:31.25	50.00	0.614	0.223	0.0112	1.82	1.92
		1Aii	1:31.25	50.00	0.186	0.032	0.0016	0.86	1.13
		1Aiii	1:31.25	50.00	0.163	0.017	0.0009	0.52	0.63
	Air Dried	1Bi	1:31.25	50.00	0.042	0.005	0.0003	0.60	0.60
		1Bii	1:31.25	50.00	0.592	0.211	0.0106	1.78	1.89
		1Biii	1:31.25	50.00	1.203	0.283	0.0142	1.18	1.44
	Freeze Dried	1Ci	1:31.25	50.00	0.202	0.021	0.0011	0.52	0.55
		1Cii	1:31.25	50.00	0.889	0.235	0.0118	1.32	1.40
		1Ciii	1:31.25	50.00	0.784	0.676	0.0338	4.31	5.08
	Oven Dried	1Di	1:31.25	50.00	0.048	0.022	0.0011	2.29	2.40
		1Dii	1:31.25	50.00	0.624	0.353	0.0177	2.83	2.96
		1Diii	1:31.25	50.00	1.315	0.422	0.0211	1.60	1.74

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of K in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A11.11: Potassium elemental analysis results for the *Laminaria digitata* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	K EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	% in DM††
Laminaria digitata	Frozen	2Ai	1:31.25	50.00	0.528	0.439	0.0220	4.16	4.28
		2Aii	1:31.25	50.00	0.176	0.199	0.0100	5.65	5.95
		2Aiii	1:31.25	50.00	0.112	0.123	0.0062	5.49	6.02
	Air Dried	2Bi	1:31.25	50.00	0.118	0.045	0.0023	1.91	2.01
		2Bii	1:31.25	50.00	3.078	1.444	0.0722	2.35	2.42
		2Biii	1:31.25	50.00	0.329	0.202	0.0101	3.07	2.60
	Freeze Dried	2Ci	1:31.25	50.00	0.374	0.345	0.0173	4.61	4.86
		2Cii	1:31.25	50.00	2.205	1.463	0.0732	3.32	3.51
		2Ciii	1:31.25	50.00	0.442	0.255	0.0128	2.88	3.04
	Oven Dried	2Di	1:31.25	50.00	0.128	0.045	0.0023	1.76	1.92
		2Dii	1:31.25	50.00	3.184	0.018	0.0009	0.03	0.03
		2Diii	1:31.25	50.00	0.326	0.043	0.0022	0.66	0.76

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of K in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A11.12: Potassium elemental analysis results for the *Saccharina latissima* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	K EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	% in DM††
Saccharina latissima	Frozen	3Ai	1:31.25	50.00	0.662	0.036	0.0018	0.27	0.29
		3Aii	1:31.25	50.00	0.118	0.013	0.0007	0.55	0.60
		3Aiii	1:31.25	50.00	0.061	0.038	0.0019	3.11	3.51
	Air Dried	3Bi	1:31.25	50.00	0.477	0.098	0.0049	1.03	1.06
		3Bii	1:31.25	50.00	2.941	0.023	0.0012	0.04	0.04
		3Biii	1:31.25	50.00	0.128	0.057	0.0029	2.23	2.35
	Freeze Dried	3Ci	1:31.25	50.00	0.429	0.193	0.0097	2.25	2.39
		3Cii	1:31.25	50.00	2.243	0.199	0.0100	0.44	0.46
		3Ciii	1:31.25	50.00	0.563	0.142	0.0071	1.26	1.38
	Oven Dried	3Di	1:31.25	50.00	0.384	0.045	0.0023	0.59	0.61
		3Dii	1:31.25	50.00	3.664	0.081	0.0041	0.11	0.12
		3Diii	1:31.25	50.00	0.742	0.136	0.0068	0.92	1.02

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of K in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 0.5ppm, 1ppm, 5ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A11.13: Calcium elemental analysis results for the prepared *Ascophyllum nodosum* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	Ca EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM†† †	AVERAGE % IN DM
Ascophyllum nodosum	Frozen	1Aa	1:50	50.00	6.820	7.015 SD±0.2758	1.115	0.0558	0.0560 SD±0.0003	0.82	0.80 SD±0.0283	1.59	1.55 SD±0.0566
		1Ab	1:50	50.00	7.210		1.124	0.0562		0.78		1.51	
	Air Dried	1Ba	1:50	50.00	7.905	7.875 SD±0.0424	1.496	0.0748	0.0655 SD±0.0132	0.95	0.84 SD±0.1626	1.06	0.93 SD±0.1838
		1Bb	1:50	50.00	7.845		1.123	0.0562		0.72		0.80	
	Freeze Dried	1Ca	1:50	50.00	7.540	7.545 SD±0.0071	1.999	0.1000	0.0997 SD±0.0004	1.33	1.33 SD±0.0070	1.47	1.47 SD±0.0071
		1Cb	1:50	50.00	7.550		1.987	0.0994		1.32		1.46	
	Oven Dried	1Da	1:50	50.00	6.460	6.620 SD±0.2263	1.115	0.0558	0.0581 SD±0.0032	0.86	0.88 SD±0.0212	0.95	0.97 SD±0.0212
		1Db	1:50	50.00	6.780		1.205	0.0603		0.89		0.98	

†%in DM was calculated using the following formula: $\frac{\text{mg of Ca in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 1ppm, 2.5ppm, 5ppm, 10ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.14: Calcium elemental analysis results for the prepared *Laminaria digitata* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	Ca EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM† ††	AVERAGE % IN DM
Laminaria digitata	Frozen	2Aa	1:50	50.00	7.990	8.050	0.589	0.0295	0.0302	0.37	0.38	1.44	1.46
		2Ab	1:50	50.00	8.110	SD±0.0849	0.615	0.0308	SD±0.0009	0.38	SD±0.0070	1.48	SD±0.0283
	Air Dried	2Ba	1:50	50.00	6.820	6.648	0.998	0.0499	0.056	0.73	0.85	0.80	0.92
		2Bb	1:50	50.00	6.475	SD±0.2439	1.238	0.0619	SD±0.0085	0.96	SD±0.1626	1.04	SD±0.1697
	Freeze Dried	2Ca	1:50	50.00	6.495	6.600	1.736	0.0868	0.0809	1.34	1.23	1.45	1.33
		2Cb	1:50	50.00	6.705	SD±0.1485	1.497	0.0749	SD±0.0084	1.12	SD±0.1556	1.21	SD±0.1697
	Oven Dried	2Da	1:50	50.00	6.735	6.808	0.873	0.0437	0.046	0.65	0.68	0.68	0.71
		2Db	1:50	50.00	6.880	SD±0.1025	0.966	0.0483	SD±0.0033	0.70	SD±0.0354	0.73	SD±0.0354

†%in DM was calculated using the following formula: $\frac{\text{mg of Ca in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 1ppm, 2.5ppm, 5ppm, 10ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.15: Calcium elemental analysis results for the prepared *Saccharina latissima* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	Ca EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM† ††	AVERAGE % IN DM
Saccharina latissima	Frozen	3Aa	1:50	50.00	7.010	6.990 SD±0.0354	0.367	0.0184	0.0187 SD±0.0004	0.26	0.27 SD±0.0070	1.35	1.38 SD±0.0354
		3Ab	1:50	50.00	6.960		0.378	0.0189		0.27		1.40	
	Air Dried	3Ba	1:50	50.00	6.415	6.525 SD±0.1556	0.996	0.0498	0.0495 SD±0.0005	0.78	0.76 SD±0.0283	0.84	0.82 SD±0.0283
		3Bb	1:50	50.00	6.635		0.982	0.0491		0.74		0.80	
	Freeze Dried	3Ca	1:50	50.00	6.515	6.785 SD±0.3818	1.751	0.0876	0.0844 SD±0.0045	1.34	1.25 SD±0.1344	1.45	1.35 SD±0.1485
		3Cb	1:50	50.00	7.055		1.623	0.0812		1.15		1.24	
	Oven Dried	3Da	1:50	50.00	6.620	6.783 SD±0.2298	0.876	0.0438	0.0462 SD±0.0034	0.66	0.68 SD±0.0283	0.70	0.72 SD±0.0283
		3Db	1:50	50.00	6.945		0.972	0.0486		0.70		0.74	

†%in DM was calculated using the following formula: $\frac{\text{mg of Ca in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 1ppm, 2.5ppm, 5ppm, 10ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.16: Calcium elemental analysis results for the *Ascophyllum nodosum* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	Ca EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	% in DM††
Ascophyllum nodosum	Frozen	1Ai	0	50.00	19.20	4.423	0.2212	1.15	1.22
		1Aii	0	50.00	5.80	1.032	0.0516	0.89	1.17
		1Aiii	0	50.00	5.10	0.919	0.0460	0.90	1.09
	Air Dried	1Bi	0	50.00	1.30	0.245	0.0123	0.94	0.95
		1Bii	0	50.00	18.50	3.711	0.1856	1.00	1.06
		1Biii	0	50.00	37.60	7.883	0.3942	1.05	1.29
	Freeze Dried	1Ci	0	50.00	6.30	0.999	0.0500	0.79	0.84
		1Cii	0	50.00	27.80	6.285	0.3143	1.13	1.20
		1Ciii	0	50.00	24.50	21.076	1.0538	4.30	5.07
	Oven Dried	1Di	0	50.00	1.50	0.282	0.0141	0.94	0.98
		1Dii	0	50.00	19.50	4.453	0.2227	1.14	1.19
		1Diii	0	50.00	41.10	10.422	0.5211	1.27	1.37

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of Ca in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 1ppm, 2.5ppm, 5ppm, 10ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A11.17: Calcium elemental analysis results for the *Laminaria digitata* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	Ca EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	% in DM††
Laminaria digitata	Frozen	2Ai	0	50.00	16.50	3.939	0.1970	1.19	1.23
		2Aii	0	50.00	5.50	0.599	0.0300	0.54	0.57
		2Aiii	0	50.00	3.50	0.323	0.0162	0.46	0.51
	Air Dried	2Bi	0	50.00	3.70	0.345	0.0173	0.47	0.49
		2Bii	0	50.00	96.20	14.444	0.7222	0.75	0.77
		2Biii	0	50.00	10.30	0.402	0.0201	0.20	0.21
	Freeze Dried	2Ci	0	50.00	11.70	0.345	0.0173	0.15	0.16
		2Cii	0	50.00	68.90	18.463	0.9232	1.34	1.42
		2Ciii	0	50.00	13.80	2.955	0.1478	1.07	1.13
	Oven Dried	2Di	0	50.00	4.00	0.245	0.0123	0.31	0.33
		2Dii	0	50.00	99.50	1.018	0.0509	0.05	0.05
		2Diii	0	50.00	10.20	1.243	0.0622	0.61	0.70

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of Ca in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 1ppm, 2.5ppm, 5ppm, 10ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A11.18: Calcium elemental analysis results for the *Saccharina latissima* extracts.

TUBE CONTENTS		**TUBE CODE**	**DILUTION FACTOR†**	**ml LIQUID IN TUBE**	**mg SAMPLE IN TUBE†**	**Ca EQUIVALENT**			
						PPM IN TUBE	**mg IN TUBE**	**% IN ORIGINAL SAMPLE**	**% in DM††**
Saccharina latissima	Frozen	3Ai	0	50.00	20.70	3.936	0.1968	0.95	1.00
		3Aii	0	50.00	3.70	0.813	0.0407	1.10	1.19
		3Aiii	0	50.00	1.90	0.438	0.0219	1.15	1.30
	Air Dried	3Bi	0	50.00	14.90	3.098	0.1549	1.04	1.08
		3Bii	0	50.00	91.90	1.923	0.0962	0.10	0.11
		3Biii	0	50.00	4.00	0.177	0.0089	0.22	0.23
	Freeze Dried	3Ci	0	50.00	13.40	1.393	0.0697	0.52	0.55
		3Cii	0	50.00	70.10	18.199	0.9100	1.30	1.36
		3Ciii	0	50.00	17.60	5.142	0.2571	1.46	1.59
	Oven Dried	3Di	0	50.00	12.00	1.945	0.0973	0.81	0.85
		3Dii	0	50.00	114.50	8.981	0.4491	0.39	0.42
		3Diii	0	50.00	23.20	3.836	0.1918	0.83	0.92

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of Ca in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 1ppm, 2.5ppm, 5ppm, 10ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A11.19: Magnesium elemental analysis results for the prepared *Ascophyllum nodosum* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	Mg EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM† ††	AVERAGE % IN DM
Ascophyllum nodosum	Frozen	1Aa	0	50.00	341.00	350.50	25.114	1.2557	1.3559	0.37	0.39	0.72	0.75
		1Ab	0	50.00	360.50	SD±13.7889	29.122	1.4561	SD±0.1417	0.40	SD±0.0212	0.78	SD±0.0424
	Air Dried	1Ba	0	50.00	395.25	393.75	34.487	1.7244	1.8152	0.44	0.47	0.49	0.52
		1Bb	0	50.00	392.25	SD±2.1213	38.118	1.9059	SD±0.1283	0.49	SD±0.0354	0.55	SD±0.0424
	Freeze Dried	1Ca	0	50.00	377.00	377.25	45.786	2.2893	2.3436	0.61	0.63	0.68	0.69
		1Cb	0	50.00	377.50	SD±0.3536	47.956	2.3978	SD±0.0767	0.64	SD±0.0212	0.71	SD±0.0212
	Oven Dried	1Da	0	50.00	323.00	331	44.012	2.2006	2.1801	0.68	0.66	0.75	0.73
		1Db	0	50.00	339.00	SD±11.3137	43.189	2.1595	SD±0.0291	0.64	SD±0.0283	0.70	SD±0.0354

†%in DM was calculated using the following formula: $\frac{\text{mg of Mg in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 10ppm, 50ppm, 100ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.20: Magnesium elemental analysis results for the prepared *Laminaria digitata* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	Mg EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM† ††	AVERAGE % IN DM
Laminaria digitata	Frozen	2Aa	0	50.00	399.50	402.50	14.567	0.7284	0.7261	0.18	0.18	0.71	0.71
		2Ab	0	50.00	405.50	SD±4.2426	14.476	0.7238	SD±0.0033	0.18	SD±0.00	0.70	SD±0.0071
	Air Dried	2Ba	0	50.00	341.00	332.38	28.698	1.4349	1.5784	0.42	0.48	0.46	0.52
		2Bb	0	50.00	323.75	SD±12.1975	34.435	1.7218	SD±0.2029	0.53	SD±0.0778	0.58	SD±0.0849
	Freeze Dried	2Ca	0	50.00	324.75	330.00	14.965	0.7483	1.2155	0.23	0.37	0.25	0.40
		2Cb	0	50.00	335.25	SD±7.4246	33.654	1.6827	SD±0.6607	0.50	SD±0.00)	0.55	SD±0.2121
	Oven Dried	2Da	0	50.00	336.75	340.38	34.723	1.7362	1.7651	0.52	0.52	0.54	0.55
		2Db	0	50.00	344.00	SD±5.12652	35.877	1.7939	SD±0.0408	0.52	SD±0.00	0.55	SD±0.0071

†%in DM was calculated using the following formula: $\frac{\text{mg of Mg in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 10ppm, 50ppm, 100ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.21: Magnesium elemental analysis results for the prepared *Saccharina latissima* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	AVERAGE mg SAMPLE IN TUBE	Mg EQUIVALENT						
							PPM IN TUBE	mg IN TUBE††	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	% in DM† ††	AVERAGE % IN DM
Saccharina latissima	Frozen	3Aa	0	50.00	350.50	349.25	8.129	0.4065	0.3777	0.12	0.11	0.60	0.56
		3Ab	0	50.00	348.00	SD±1.7678	6.976	0.3488	SD±0.0408	0.10	SD±0.0141	0.52	SD±0.0566
	Air Dried	3Ba	0	50.00	320.75	160.38	14.456	0.7228	1.1689	0.23	O.36	0.24	0.39
		3Bb	0	50.00	331.75	SD±7.7782	32.297	1.6149	SD±0.6308	0.49	SD±0.1838	0.53	SD±0.2051
	Freeze Dried	3Ca	0	50.00	325.75	162.88	18.087	0.9044	0.7686	0.28	0.23	0.30	0.25
		3Cb	0	50.00	352.75	SD±19.0919	12.654	0.6327	SD±0.1921	0.18	SD±0.0707	0.19	SD±0.0778
	Oven Dried	3Da	0	50.00	331.00	339.13	14.453	0.7227	0.7908	0.22	0.24	0.23	0.25
		3Db	0	50.00	347.25	SD±11.4905	17.176	0.8588	SD±0.0962	0.25	SD±0.0212	0.26	SD±0.0212

†%in DM was calculated using the following formula: $\frac{\text{mg of Mg in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 10ppm, 50ppm, 100ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A11.22: Magnesium elemental analysis results for the *Ascophyllum nodosum* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	Mg EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	% in DM††
Ascophyllum nodosum	Frozen	1Ai	0	50.00	19.20	2.424	0.1212	0.63	0.67
		1Aii	0	50.00	5.80	0.53	0.0265	0.46	0.60
		1Aiii	0	50.00	5.10	0.521	0.0261	0.51	0.62
	Air Dried	1Bi	0	50.00	1.30	0.144	0.0072	0.55	0.56
		1Bii	0	50.00	18.50	1.71	0.0855	0.46	0.49
		1Biii	0	50.00	37.60	2.882	0.1441	0.38	0.47
	Freeze Dried	1Ci	0	50.00	6.30	0.798	0.0399	0.63	0.67
		1Cii	0	50.00	27.80	3.282	0.1641	0.59	0.63
		1Ciii	0	50.00	24.50	2.077	0.1039	0.42	0.50
	Oven Dried	1Di	0	50.00	1.50	0.18	0.0090	0.60	0.63
		1Dii	0	50.00	19.50	1.454	0.0727	0.37	0.39
		1Diii	0	50.00	41.10	3.421	0.1711	0.42	0.45

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of Mg in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 1ppm, 5ppm, 10ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A11.23: Magnesium elemental analysis results for the *Laminaria digitata* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	Mg EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	% in DM††
Laminaria digitata	Frozen	2Ai	0	50.00	16.50	1.74	0.0870	0.53	0.54
		2Aii	0	50.00	5.50	0.497	0.0249	0.45	0.48
		2Aiii	0	50.00	3.50	0.313	0.0157	0.45	0.49
	Air Dried	2Bi	0	50.00	3.70	0.349	0.0175	0.47	0.50
		2Bii	0	50.00	96.20	3.456	0.1728	0.18	0.19
		2Biii	0	50.00	10.30	0.403	0.0202	0.20	0.21
	Freeze Dried	2Ci	0	50.00	11.70	0.645	0.0323	0.28	0.29
		2Cii	0	50.00	68.90	6.463	0.3232	0.47	0.50
		2Ciii	0	50.00	13.80	0.915	0.0458	0.33	0.35
	Oven Dried	2Di	0	50.00	4.00	0.244	0.0122	0.31	0.33
		2Dii	0	50.00	99.50	5.018	0.2509	0.25	0.26
		2Diii	0	50.00	10.20	0.943	0.0472	0.46	0.53

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of Mg in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 1ppm, 5ppm, 10ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A11.24: Magnesium elemental analysis results for the *Saccharina latissima* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	Mg EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	% in DM††
Saccharina latissima	Frozen	3Ai	0	50.00	20.70	2.536	0.1268	0.61	0.65
		3Aii	0	50.00	3.70	0.413	0.0207	0.56	0.61
		3Aiii	0	50.00	1.90	0.138	0.0069	0.36	0.41
	Air Dried	3Bi	0	50.00	14.90	0.98	0.0490	0.33	0.34
		3Bii	0	50.00	91.90	8.923	0.4462	0.49	0.51
		3Biii	0	50.00	4.00	0.277	0.0139	0.35	0.37
	Freeze Dried	3Ci	0	50.00	13.40	1.393	0.0697	0.52	0.55
		3Cii	0	50.00	70.10	7.199	0.3600	0.51	0.54
		3Ciii	0	50.00	17.60	1.142	0.0571	0.32	0.35
	Oven Dried	3Di	0	50.00	12.00	1.245	0.0623	0.52	0.54
		3Dii	0	50.00	114.50	9.981	0.4991	0.44	0.46
		3Diii	0	50.00	23.20	2.836	0.1418	0.61	0.68

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††%in DM was calculated using the following formula: $\frac{\text{mg of Mg in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.1ppm, 1ppm, 5ppm, 10ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

APPENDIX 12

Table A12.1: TLC analysis results for the *Ascophyllum nodosum* extracts.

	ASCOPHYLLUM NODOSUM												Fucoxanthin (standard)
	FROZEN			AIR DRIED			FREEZE DRIED			OVEN DRIED			
	1Ai	1Aii	1Aiii	1Bi	1Bii	1Biii	1Ci	1Cii	1Ciii	1Di	1Dii	1Diii	
R_f values and info		0.816[366] Florescent					0.868[vr] Green		0.895[vr] Red	0.855[vr] Green	0.987[vr] Red		
				0.776[366] Red			0.764 Green			0.763 Brown			
							0.632[366] Florescent			0.658[366] Florescent			
				0.382[vr] Violet			0.382[vr] Violet			0.408[vr] Violet			0.355[vr] Red
													0.237[vr] Blue
					0.145[vr] Grey		0.158[vr] Grey			0.184[vr] Grey			0.171[vr] Violet

R_f value were calculated using the following formula: $\frac{\text{migration distance of substance}}{\text{migration distance of solvent front}}$

[366] means that the components were visualised under UV light.

[vr] means that a visualisation reagent was used in order to visualise these components. In this case phenols, sugars, steroids and terpenes are shown as violet, blue, red, grey or green.

Note: the time taken for the solvent to reach the finish line was 10mins.

Table A12.2: TLC analysis results for the *Laminaria digitata* extracts.

	LAMINARIA DIGITATA												**Fucoxanthin (standard)**
	FROZEN			**AIR DRIED**			**FREEZE DRIED**			**OVEN DRIED**			
	2Ai	2Aii	2Aiii	2Bi	2Bii	2Biii	2Ci	2Cii	2Ciii	2Di	2Dii	2Diii	
R_f values and info	0.842[366] Red			0.908[vr] Blue			0.908[vr] Blue			0.908[vr] Blue			
	0.750[366] Red			0.776[vr] Red			0.776[vr] Red			0.776[366] Florescent			
	0.461[vr] Green			0.487[vr] Green			0.487[vr] Green			0.487[vr] Green			0.355[vr] Red
	0.237[vr] Blue	0.250[vr] Blue		0.263[vr] Blue	0.263[vr] Blue	0.263[vr] Grey	0.263[vr] Blue	0.263[vr] Grey		0.263[vr] Blue			0.237[vr] Blue
	0.145[vr] Violet												0.171[vr] Violet

R_f value were calculated using the following formula: $\frac{\text{migration distance of substance}}{\text{migration distance of solvent front}}$

[366] means that the components were visualised under UV light.

[vr] means that a visualisation reagent was used in order to visualise these components. In this case phenols, sugars, steroids and terpenes are shown as violet, blue, red, grey or green.

Note: the time taken for the solvent to reach the finish line was 10mins, 25seconds.

Table A12.3: TLC analysis results for the *Saccharina latissima* extracts.

	SACCHARINA LATISSIMA												Fucoxanthin (standard)
	FROZEN			AIR DRIED			FREEZE DRIED			OVEN DRIED			
	3Ai	3Aii	3Aiii	3Bi	3Bii	3Biii	3Ci	3Cii	3Ciii	3Di	3Dii	3Diii	
R_f values and info	0.868[366] Red	0.868[366] Red					0.921[366] Red			0.776[366] Red			
	0.658[366] Florescent			0.658[366] Florescent			0.658[366] Florescent			0.658[366] Florescent			
	0.447[vr] Green			0.447[vr] Green			0.447[vr] Green			0.447[vr] Green			
													0.355[vr] Red
													0.237[vr] Blue
							0.197[vr] Violet			0.197[vr] Violet	0.197[vr] Violet		0.171[vr] Violet

R_f value were calculated using the following formula: $\frac{\text{migration distance of substance}}{\text{migration distance of solvent front}}$

[366] means that the components were visualised under UV light.

[vr] means that a visualisation reagent was used in order to visualise these components. In this case phenols, sugars, steroids and terpenes are shown as violet, blue, red, grey or green

Note: the time taken for the solvent to reach the finish line was 10mins, 40 seconds.

APPENDIX 13

Table A13.1: HPLC analysis results of the fucoxanthin standard.

VIAL CONTENTS		VIAL CODE	DILUTION FACTOR	µl LIQUID INJECTED	µg SAMPLE IN INJECTION	PEAK RETENTION TIME (MIN)	PEAK AREA (mAU*min)
Blank		Ba	0	20	0	0	0
		Bb	0	20	0	0	0
Fucoxanthin (standard)	0.01µg in 1ml	S0.01a	1:100000	20	0.0002	6.00	0.002
		S0.01b	1:100000	20	0.0002	6.00	0.003
	0.1µg in 1ml	S0.1a	1:10000	20	0.002	6.01	0.010
		S0.1b	1:10000	20	0.002	6.00	0.012
	1µg in 1ml	S1a	1:1000	20	0.02	6.00	0.050
		S1b	1:1000	20	0.02	6.02	0.080
	10µg in 1ml	S10a	1:100	20	0.2	6.02	0.250
		S10b	1:100	20	0.2	6.03	0.248
	100µg in 1ml	S100a	1:10	20	2	6.05	1.777
		S100b	1:10	20	2	6.03	1.804
	200µg in 1ml	S200a	1:5	20	4	6.02	10.654
		S200b	1:5	20	4	6.00	10.599
	400µg in 1ml	S400a	1:2.5	20	8	6.01	50.274
		S400b	1:2.5	20	8	6.02	51.867
	600µg in 1ml	S600a	1:1.66	20	12	6.01	104.856
		S600b	1:1.66	20	12	6.00	100.234
	800µg in 1ml	S800a	1:1.25	20	16	6.03	174.861
		S800b	1:1.25	20	16	6.01	179.349
	1000µg in 1ml	S1000a	0	20	20	6.03	275.711
		S1000b	0	20	20	6.02	273.533

CODE KEY
B=Blank, S=Standard
Number corresponds to amount in µg
a=first duplicate sample,
b=second duplicate sample

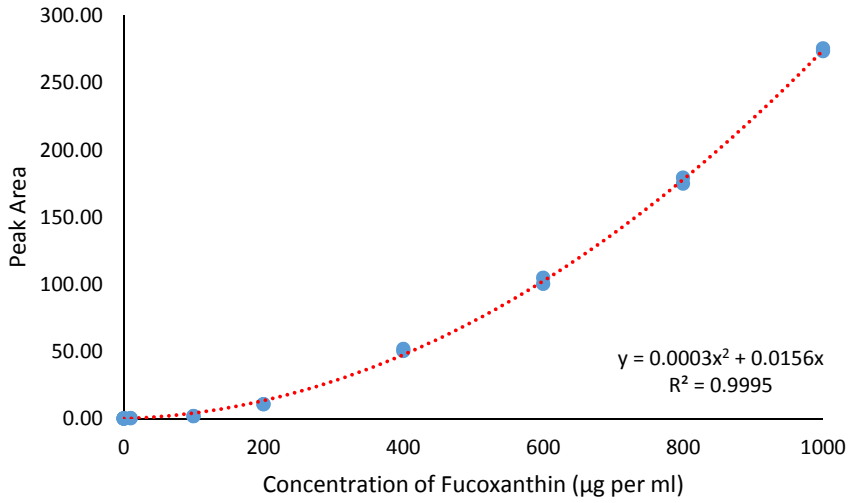

Figure A13.1: Standard curve of known fucoxanthin concentrations.

Table A13.2: Fucoxanthin analysis results for the *Ascophyllum nodosum* extracts.

VIAL CONTENTS		VIAL CODE	µl LIQUID IN INJECTION	µg SAMPLE IN INJECTION	PEAK RETENTION TIME (min)	PEAK AREA (mAUx min)	FUCOXANTHIN EQUIVALENT		
							µg IN INJECTION†	% IN ORIGINAL SAMPLE	% in DM††
Ascophyllum nodosum	Frozen	1Ai	20	20	6.02	0.012	0.0022	0.011	0.012
		1Aii	20	20	6.03	0.222	0.0404	0.202	0.265
		1Aiii	20	20	6.02	0.440	0.0801	0.401	0.483
	Air Dried	1Bi	20	20	6.03	0.001	0.0002	0.001	0.001
		1Bii	20	20	6.01	0.117	0.0213	0.107	0.113
		1Biii	20	20	6.02	0.029	0.0053	0.027	0.032
	Freeze Dried	1Ci	20	20	6.00	0.865	0.1575	0.788	0.831
		1Cii	20	20	6.02	0.001	0.0002	0.001	0.001
		1Ciii	20	20	6.01	0.000	0.0000	0.000	0.000
	Oven Dried	1Di	20	20	6.02	0.322	0.0586	0.293	0.306
		1Dii	20	20	6.01	0.144	0.0262	0.131	0.137
		1Diii	20	20	6.02	0.000	0.0000	0.000	0.000

†µg of fucoxanthin in injection was calculated by applying the absorbance values to the standard curve.

††% in DM was calculated using the following formula: $\frac{\text{µg fucoxanthin in injection}}{\text{µg sample in injection x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A13.3: Fucoxanthin analysis results for the *Laminaria digitata* extracts.

VIAL CONTENTS		VIAL CODE	µl LIQUID IN INJECTION	µg SAMPLE IN INJECTION	PEAK RETENTION TIME (min)	PEAK AREA (mAUx min)	FUCOXANTHIN EQUIVALENT		
							µg IN INJECTION†	% IN ORIGINAL SAMPLE	% in DM††
Laminaria digitata	Frozen	2Ai	20	20	6.00	2.085	0.3796	1.898	1.956
		2Aii	20	20	6.01	0.001	0.0002	0.001	0.001
		2Aiii	20	20	6.01	0.016	0.0029	0.015	0.016
	Air Dried	2Bi	20	20	5.99	0.364	0.0663	0.332	0.349
		2Bii	20	20	5.99	0.001	0.0002	0.001	0.001
		2Biii	20	20	6.00	0.097	0.0177	0.089	0.095
	Freeze Dried	2Ci	20	20	6.01	0.515	0.0938	0.469	0.494
		2Cii	20	20	6.03	0.000	0.0000	0.000	0.000
		2Ciii	20	20	6.02	0.003	0.0005	0.003	0.003
	Oven Dried	2Di	20	20	5.99	0.107	0.0195	0.098	0.106
		2Dii	20	20	6.03	0.043	0.0078	0.039	0.041
		2Diii	20	20	6.02	0.092	0.0168	0.084	0.096

†µg of fucoxanthin in injection was calculated by applying the absorbance values to the standard curve.

††% in DM was calculated using the following formula: $\frac{\text{µg fucoxanthin in injection}}{\text{µg sample in injection x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A13.4: Fucoxanthin analysis results for the *Saccharina latissima* extracts.

VIAL CONTENTS		VIAL CODE	µl LIQUID IN INJECTION	µg SAMPLE IN INJECTION	PEAK RETENTION TIME (min)	PEAK AREA (mAUx min)	FUCOXANTHIN EQUIVALENT		
							µg IN INJECTION†	% IN ORIGINAL SAMPLE	% in DM††
Saccharina latissima	Frozen	3Ai	20	20	6.02	0.617	0.1123	0.562	0.593
		3Aii	20	20	6.03	0.046	0.1675	0.042	0.045
		3Aiii	20	20	6.02	0.030	0.1092	0.027	0.031
	Air Dried	3Bi	20	20	6.01	0.000	0.0000	0.000	0.000
		3Bii	20	20	6.00	0.001	0.0036	0.001	0.001
		3Biii	20	20	6.03	0.000	0.0000	0.000	0.000
	Freeze Dried	3Ci	20	20	6.02	0.025	0.0910	0.023	0.024
		3Cii	20	20	6.03	0.013	0.0473	0.012	0.012
		3Ciii	20	20	6.03	0.000	0.0000	0.000	0.000
	Oven Dried	3Di	20	20	6.02	0.085	0.3095	0.077	0.081
		3Dii	20	20	6.02	0.075	0.2731	0.068	0.073
		3Diii	20	20	6.01	0.127	0.4625	0.116	0.129

†µg of fucoxanthin in injection was calculated by applying the absorbance values to the standard curve.

††% in DM was calculated using the following formula: $\frac{\text{µg fucoxanthin in injection}}{\text{µg sample in injection x \% DM of sample}} \text{ x } 100$

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

APPENDIX 14

Table A14.1: Dry weight results for the PCB analysis of the marine algae samples taken from the Stafnes site.

SPECIES	WET WEIGHTS†				DRY WEIGHTS†				DRY WEIGHT %††
	SAMPLE 1 (g)	SAMPLE 2 (g)	SAMPLE 3 (g)	AVERAGE (g)†	SAMPLE 1 (g)	SAMPLE 2 (g)	SAMPLE 3 (g)	AVERAGE (g)†	
Ascophyllum nodosum	5.08	5.05	5.04	5.06 SD±0.0208	2.17	2.05	2.23	2.15 SD±0.0917	42.49 SD±1.8339
Laminaria digitata	5.01	5.02	5.02	5.02 SD±0.0058	1.78	1.67	0.96	1.47 SD±0.4451	29.28 SD±8.8909
Saccharina latissima	5.01	5.02	5.02	5.03 SD±0.0058	1.66	1.74	1.59	1.66 SD±0.0751	33.00 SD±1.4941

†Average wet/dry weights were calculated using the following formula: $\frac{\text{Sample 1+Sample 2+Sample 3}}{3}$

††Dry weight % was calculated using the following formula: $\frac{\text{Average dry weight}}{\text{Average wet weight}} \text{ x } 100$

APPENDIX 15

Table A15.1: Arsenic elemental analysis results for the prepared *Ascophyllum nodosum* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE	AVERAGE mg SAMPLE IN TUBE	As EQUIVALENT						
							PPM IN TUBE	mg IN TUBE	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	PPM in DM†	AVERAGE PPM IN DM
Ascophyllum nodosum	Frozen	1Aa	0	50.00	341.00	350.75	0.0071	0.00036	0.00035	0.00010	0.00009	2.0	1.9
		1Ab	0	50.00	360.50	SD±13.7886	0.0065	0.00033	SD±0.00002	0.00009	SD±0.000007	1.8	SD±0.1414
	Air Dried	1Ba	0	50.00	395.25	393.75	0.0084	0.00042	0.00045	0.00011	0.00012	1.2	1.3
		1Bb	0	50.00	392.25	SD±2.1213	0.0095	0.00048	SD±0.00004	0.00012	SD±0.000007	1.4	SD±0.1414
	Freeze Dried	1Ca	0	50.00	377.00	377.25	0.0102	0.00051	0.00039	0.00014	0.00011	1.5	1.15
		1Cb	0	50.00	377.50	SD±0.3536	0.0056	0.00028	SD±0.00016	0.00007	SD±0.000049	0.8	SD±0.4949
	Oven Dried	1Da	0	50.00	323.00	331.00	0.0082	0.00041	0.00040	0.00013	0.00013	1.4	1.35
		1Db	0	50.00	339.00	SD±11.3137	0.0078	0.00039	SD±0.0001	0.00012	SD±0.000007	1.3	SD±0.0707

†ppm in DM was calculated using the following formula: $\frac{\text{mg of As in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.0001ppm, 0.001ppm, 0.01ppm, 0.1ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A15.2: Arsenic elemental analysis results for the prepared *Laminaria digitata* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE	AVERAGE mg SAMPLE IN TUBE	As EQUIVALENT						
							PPM IN TUBE	mg IN TUBE	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	PPM in DM†	AVERAGE PPM IN DM
Laminaria digitata	Frozen	2Aa	0	50.00	399.50	402.50 SD±4.2426	0.0098	0.00049	0.00050 SD±0.00001	0.00012	0.00012 SD±0.00	4.8	4.9 SD±0.0707
		2Ab	0	50.00	405.50		0.0101	0.00051		0.00012		4.9	
	Air Dried	2Ba	0	50.00	341.00	332.00 SD±12.1975	0.0158	0.00079	0.00091 SD±0.00017	0.00023	0.00028 SD±0.000063	2.5	3 SD±0.7071
		2Bb	0	50.00	323.75		0.0205	0.00103		0.00032		3.5	
	Freeze Dried	2Ca	0	50.00	324.75	330.00 SD±7.4246	0.0136	0.00068	0.00068 SD±0.00000	0.00021	0.00021 SD±0.000007	2.3	2.25 SD±0.0707
		2Cb	0	50.00	335.25		0.0134	0.00067		0.00020		2.2	
	Oven Dried	2Da	0	50.00	336.75	340.38 SD±5.12652	0.0123	0.00062	0.00069 SD±0.00009	0.00018	0.00020 SD±0.000028	1.9	2.1 SD±0.2828
		2Db	0	50.00	344.00		0.0149	0.00075		0.00022		2.3	

†ppm in DM was calculated using the following formula: $\frac{\text{mg of As in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.0001ppm, 0.001ppm, 0.01ppm, 0.1ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table A15.3: Arsenic elemental analysis results for the prepared *Saccharina latissima* samples.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR	ml LIQUID IN TUBE	mg SAMPLE IN TUBE	AVERAGE mg SAMPLE IN TUBE	As EQUIVALENT						
							PPM IN TUBE	mg IN TUBE	AVERAGE mg IN TUBE	% IN ORIGINAL SAMPLE	AVERAGE % IN SAMPLE	PPM in DM†	AVERAGE PPM IN DM
Saccharina latissima	Frozen	3Aa	0	50.00	350.50	349.25 SD±1.7678	0.0062	0.00031	0.00033 SD±0.00002	0.00009	0.000095 SD±0.000000	4.6	4.8 SD±0.2828
		3Ab	0	50.00	348.00		0.0067	0.00034		0.00010		5.0	
	Air Dried	3Ba	0	50.00	320.75	326.25 SD±7.7782	0.0087	0.00044	0.00042 SD±0.00004	0.00014	0.000130 SD±0.000014	1.5	1.4 SD±0.1414
		3Bb	0	50.00	331.75		0.0077	0.00039		0.00012		1.3	
	Freeze Dried	3Ca	0	50.00	325.75	339.25 SD±19.0918	0.0197	0.00099	0.00119 SD±0.00028	0.00030	0.000350 SD±0.000064	3.3	3.75 SD±0.6364
		3Cb	0	50.00	352.75		0.0276	0.00138		0.00039		4.2	
	Oven Dried	3Da	0	50.00	331.00	339.13 SD±11.4905	0.0131	0.00066	0.00060 SD±0.00008	0.00020	0.000175 SD±0.000035	2.1	1.85 SD±0.3536
		3Db	0	50.00	347.25		0.0107	0.00054		0.00015		1.6	

†ppm in DM was calculated using the following formula: $\frac{\text{mg of As in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.0001ppm, 0.001ppm, 0.01ppm, 0.1ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
a=first duplicate sample, b=second duplicate sample

Table.A15.4: Arsenic elemental analysis results for the *Ascophyllum nodosum* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	As EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	PPM IN DM††
Ascophyllum nodosum	Frozen	1Ai	0	50.00	19.20	0.00073	0.000037	0.00019	2.0
		1Aii	0	50.00	5.80	0.00017	0.000009	0.00015	1.9
		1Aiii	0	50.00	5.10	0.00009	0.000005	0.00009	1.1
	Air Dried	1Bi	0	50.00	1.30	0.00004	0.000002	0.00015	1.5
		1Bii	0	50.00	18.50	0.00050	0.000025	0.00014	1.4
		1Biii	0	50.00	37.60	0.00073	0.000037	0.00010	1.2
	Freeze Dried	1Ci	0	50.00	6.30	0.00024	0.000012	0.00019	2.0
		1Cii	0	50.00	27.80	0.00113	0.000057	0.00020	2.2
		1Ciii	0	50.00	24.50	0.00079	0.000040	0.00016	1.9
	Oven Dried	1Di	0	50.00	1.50	0.00005	0.000003	0.00017	1.7
		1Dii	0	50.00	19.50	0.00066	0.000033	0.00017	1.8
		1Diii	0	50.00	41.10	0.00059	0.000030	0.00007	0.8

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††ppm in DM was calculated using the following formula: $\frac{\text{mg of As in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.0001ppm, 0.001ppm, 0.01ppm, 0.1ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A15.5: Arsenic elemental analysis results for the *Laminaria digitata* extracts**.**

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	As EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	PPM IN DM††
Laminaria digitata	Frozen	2Ai	0	50.00	16.50	0.00149	0.00007	0.00045	4.7
		2Aii	0	50.00	5.50	0.00047	0.00002	0.00043	4.5
		2Aiii	0	50.00	3.50	0.00013	0.00001	0.00019	2.0
	Air Dried	2Bi	0	50.00	3.70	0.00034	0.00002	0.00046	4.8
		2Bii	0	50.00	96.20	0.00145	0.00007	0.00008	0.8
		2Biii	0	50.00	10.30	0.00092	0.00005	0.00045	4.8
	Freeze Dried	2Ci	0	50.00	11.70	0.00045	0.00002	0.00019	2.0
		2Cii	0	50.00	68.90	0.00163	0.00008	0.00012	1.3
		2Ciii	0	50.00	13.80	0.00122	0.00006	0.00044	4.7
	Oven Dried	2Di	0	50.00	4.00	0.00014	0.00001	0.00018	1.9
		2Dii	0	50.00	99.50	0.00330	0.00017	0.00017	1.7
		2Diii	0	50.00	10.20	0.00069	0.00003	0.00034	3.9

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††ppm in DM was calculated using the following formula: $\frac{\text{mg of As in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.0001ppm, 0.001ppm, 0.01ppm, 0.1ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A15.6: Arsenic elemental analysis results for the *Saccharina latissima* extracts.

TUBE CONTENTS		TUBE CODE	DILUTION FACTOR†	ml LIQUID IN TUBE	mg SAMPLE IN TUBE†	As EQUIVALENT			
						PPM IN TUBE	mg IN TUBE	% IN ORIGINAL SAMPLE	PPM IN DM††
Saccharina latissima	Frozen	3Ai	0	50.00	20.70	0.00153	0.000077	0.00037	3.9
		3Aii	0	50.00	3.70	0.00024	0.000012	0.00032	3.5
		3Aiii	0	50.00	1.90	0.00009	0.000005	0.00024	2.7
	Air Dried	3Bi	0	50.00	14.90	0.00098	0.000049	0.00033	3.4
		3Bii	0	50.00	91.90	0.00392	0.000196	0.00021	2.2
		3Biii	0	50.00	4.00	0.00017	0.000009	0.00021	2.2
	Freeze Dried	3Ci	0	50.00	13.40	0.00109	0.000055	0.00041	4.3
		3Cii	0	50.00	70.10	0.00390	0.000195	0.00028	2.9
		3Ciii	0	50.00	17.60	0.00122	0.000061	0.00035	3.8
	Oven Dried	3Di	0	50.00	12.00	0.00104	0.000052	0.00043	4.5
		3Dii	0	50.00	114.50	0.00915	0.000458	0.00040	4.2
		3Diii	0	50.00	23.20	0.00160	0.000080	0.00034	3.9

†Amount of extract used was calculated as equivalent 350mg of corresponding raw material.

††ppm in DM was calculated using the following formula: $\frac{\text{mg of As in tube}}{\text{mg sample in tube x \% DM of sample}} \text{ x } 100$

Note: The standard was used in the following concentrations to calibrate the Atomic absorption instrument: 0.0001ppm, 0.001ppm, 0.01ppm, 0.1ppm.

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

APPENDIX 16

Table A16.1: Summary of all analysis results for the all prepared marine algae samples.

SAMPLES		CODE	MOISTURE % (Average)	ASH % (Average)	ORGANIC MATTER % (Average)	ORGANIC CONTENT (Average)			INORGANIC CONTENT (Average)					PHYTOCHEMICAL
						FAT %	CARBOHYDRATE %	PROTEIN %	Na %	K %	Ca %	Mg %	As ppm	FUCOXANTHIN %†
Ascophyllum nodosum	Frozen	1A	48.52 SD±4.8591	19.35 SD±2.0616	80.65 SD±2.0616	4.36 SD±0.3866	21.76 SD±0.1838	7.51 SD±0.1061	5.09 SD±0.1768	2.41 SD±0.0424	1.55 SD±0.0566	0.75 SD±0.0424	1.9 SD±0.1414	0.12
	Air Dried	1B	10.96 SD±2.3257	14.04 SD±1.2903	85.96 SD±1.2903	1.05 SD±6.5033	6.94 SD±0.2051	3.37 SD±0.2969	2.09 SD±0.2616	1.07 SD±0.1131	0.98 SD±0.1838	0.52 SD±0.0424	1.3 SD±0.1414	0.02
	Freeze Dried	1C	10.10 SD±6.1124	20.72 SD±1.9454	79.28 SD±1.9454	3.99 SD±15.7704	12.05 SD±0.00	3.36 SD±0.0424	2.55 SD±0.5515	2.37 SD±0.1485	1.47 SD±0.0071	0.7 SD±0.0212	1.15 SD±0.4949	0.08
	Oven Dried	1D	9.03 SD±1.1527	13.46 SD±0.8214	86.54 SD±0.8214	1.70 SD±7.6283	4.07 SD±0.00	0.38 SD±0.2333	3.11 SD±0.1979	0.99 SD±0.1131	0.97 SD±0.0212	0.73 SD±0.0354	1.35 SD±0.0707	0.05
Laminaria digitata	Frozen	2A	74.45 SD±5.6729	20.13 SD±4.3669	79.87 SD±4.3669	3.20 SD±0.0671	33.85 SD±0.3253	4.73 SD±0.0495	5.58 SD±0.8344	8.35 SD±0.0778	1.46 SD±0.0283	0.71 SD±0.0071	4.85 SD±0.0707	0.89
	Air Dried	2B	8.24 SD±6.1785	16.05 SD±3.5041	83.95 SD±3.5041	1.58 SD±1.0878	4.13 SD±0.3889	1.35 SD±0.0141	1.90 SD±0.1131	2.42 SD±0.2899	0.92 SD±0.1697	0.52 SD±0.0849	3.0 SD±0.7071	0.02
	Freeze Dried	2C	8.04 SD±2.1028	19.11 SD±1.2749	80.89 SD±1.2749	1.25 SD±1.3364	29.39 SD±0.3394	2.18 SD±0.0424	2.16 SD±0.0919	5.66 SD±0.5728	1.33 SD±0.1697	0.4 SD±0.2121	2.25 SD±0.0707	0.18
	Oven Dried	2D	4.46 SD±0.3137	15.45 SD±2.6481	84.55 SD±2.6481	2.88 SD±4.9016	20.42 SD±0.2889	0.45 SD±0.00	1.33 SD±0.1626	2.04 SD±0.2121	0.71 SD±0.0354	0.55 SD±0.0071	2.1 SD±0.2828	0.08
Saccharina latissima	Frozen	3A	80.57 SD±1.9019	20.37 SD±2.6783	79.63 SD±2.6783	3.50 SD±0.0915	45.23 SD±0.00	4.72 SD±0.0495	9.77 SD±0.1061	6.45 SD±0.2687	1.38 SD±0.0354	0.56 SD±0.0566	4.8 SD±0.2828	0.24
	Air Dried	3B	7.82 SD±4.2639	17.20 SD±0.2847	82.80 SD±0.2847	1.21 SD±0.7748	6.34 SD±0.3889	1.51 SD±0.0141	1.91 SD±0.1979	1.98 SD±0.1344	0.82 SD±0.0283	0.39 SD±0.2051	1.4 SD±0.1414	0.00
	Freeze Dried	3C	7.36 SD±3.4516	20.21 SD±2.3832	79.79 SD±2.3832	2.03 SD±2.2124	17.22 SD±0.3536	1.88 SD±0.0424	2.12 SD±0.2546	4.93 SD±0.9687	1.35 SD±0.1485	0.25 SD±0.0778	3.75 SD±0.6364	0.01
	Oven Dried	3D	4.99 SD±7.3744	16.92 SD±0.5752	83.08 SD±0.5752	1.73 SD±5.6739	15.14 SD±0.00	0.30 SD±0.0071	1.61 SD±0.1202	1.05 SD±0.2969	0.72 SD±0.0283	0.25 SD±0.0212	1.85 SD±0.3536	0.11

Note: All organic, inorganic and phytochemical percentages are in terms of ultimate dry matter (DM) of raw material (as calculated from the moisture analysis).

†Fucoxanthin content and % was calculated from the results obtained from the extract analysis by using the following formula $\frac{\text{DM extract weight x \% of fucoxanthin in DM}}{\text{DM sample weight}} \text{ x } 100$

Table A16.2: Summary of all analysis results for the *Ascophyllum nodosum* extracts.

SAMPLES		CODE	EXTRACT YIELD %	MOISTURE %	DRY MATTER %	ASH %	ORGANIC MATTER %	ORGANIC CONTENT	INORGANIC CONTENT					PHYTOCHEMICAL
								CARBOHYDRATE %	Na %	K %	Ca %	Mg %	As ppm	FUCOXANTHIN %
Ascophyllum nodosum	Frozen	1Ai	10.32	5.35	94.65	29.00	71.00	18.38	2.39	1.92	1.22	0.67	2.0	0.012
		1Aii	2.46	23.78	76.22	5.59	94.41	23.93	3.95	1.13	1.17	0.60	1.9	0.265
		1Aiii	2.33	17.00	83.00	26.77	73.23	13.42	3.62	0.63	1.09	0.62	1.1	0.483
	Air Dried	1Bi	0.42	0.29	99.71	18.26	81.74	7.81	2.87	0.60	0.95	0.56	1.5	0.001
		1Bii	5.63	5.51	94.49	16.15	83.85	9.83	2.79	1.89	1.06	0.49	1.4	0.113
		1Biii	9.85	18.44	81.56	20.55	79.45	13.88	0.90	1.44	1.29	0.47	1.2	0.032
	Freeze Dried	1Ci	1.91	5.22	94.78	35.21	64.79	1.80	3.08	0.55	0.84	0.67	2.0	0.831
		1Cii	8.37	5.56	94.44	42.39	57.61	6.14	2.31	1.40	1.20	0.63	2.2	0.001
		1Ciii	10.13	15.14	84.86	14.11	85.89	72.02	2.07	5.08	5.07	0.50	1.9	0.000
	Oven Dried	1Di	0.47	4.34	95.66	30.10	69.90	15.88	5.66	2.40	0.98	0.63	1.7	0.306
		1Dii	5.84	4.39	95.61	40.55	59.45	8.88	3.00	2.96	1.19	0.39	1.8	0.137
		1Diii	11.94	7.64	92.36	31.62	68.38	28.07	1.74	1.74	1.37	0.45	0.8	0.000

Note: All extract yield, organic, inorganic and phytochemical percentages are in terms of ultimate dry matter (DM) of raw material (as calculated from the moisture analysis).

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A16.3: Summary of all analysis results for the *Laminaria digitata* extracts.

SAMPLES		CODE	EXTRACT YIELD %	MOISTURE %	DRY MATTER %	ASH %	ORGANIC MATTER %	ORGANIC CONTENT	INORGANIC CONTENT					PHYTOCHEMICAL
								CARBOHYDRATE %	Na %	K %	Ca %	Mg %	As ppm	FUCOXANTHIN %
Laminaria digitata	Frozen	2Ai	17.95	2.98	97.02	31.56	68.44	3.95	0.37	4.28	1.23	0.54	4.7	1.956
		2Aii	5.94	5.03	94.97	19.32	80.68	9.06	2.75	5.95	0.57	0.48	4.5	0.001
		2Aiii	3.64	8.83	91.17	32.19	67.81	12.64	9.21	6.02	0.51	0.49	2.0	0.016
	Air Dried	2Bi	1.07	5.01	94.99	58.42	41.58	2.58	3.21	2.01	0.49	0.50	4.8	0.349
		2Bii	28.92	3.06	96.94	63.56	36.44	12.55	0.07	2.42	0.77	0.19	0.8	0.001
		2Biii	3.02	6.35	93.65	28.39	71.61	32.87	2.01	2.60	0.21	0.21	4.8	0.095
	Freeze Dried	2Ci	3.19	5.06	94.94	44.14	55.86	0.43	0.65	4.86	0.16	0.29	2.0	0.494
		2Cii	20.26	5.41	94.59	45.36	54.64	5.72	0.02	3.51	1.42	0.50	1.3	0.000
		2Ciii	4.05	5.23	94.77	15.98	84.02	21.33	1.86	3.04	1.13	0.35	4.7	0.003
	Oven Dried	2Di	1.08	8.33	91.67	38.05	61.95	9.91	4.69	1.92	0.33	0.33	1.9	0.106
		2Dii	28.58	4.04	95.96	25.41	74.59	29.89	0.03	0.03	0.05	0.26	1.7	0.041
		2Diii	2.68	12.81	87.19	27.63	72.37	34.73	2.18	0.76	0.70	0.53	3.9	0.096

Note: All extract yield, organic, inorganic and phytochemical percentages are in terms of ultimate dry matter (DM) of raw material (as calculated from the moisture analysis).

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract

Table A16.4: Summary of all analysis results for the *Saccharina latissima* extracts.

SAMPLES		CODE	EXTRACT YIELD %	MOISTURE %	DRY MATTER %	ASH %	ORGANIC MATTER %	ORGANIC CONTENT	INORGANIC CONTENT					PHYTOCHEMICAL
								CARBOHYDRATE %	Na %	K %	Ca %	Mg %	As ppm	FUCOXANTHIN %
Saccharina latissima	Frozen	3Ai	5.92	5.25	94.75	33.27	66.73	8.48	0.29	0.29	1.00	0.65	3.9	0.593
		3Aii	5.04	7.80	92.20	20.61	79.39	24.64	0.64	0.60	1.19	0.61	3.5	0.045
		3Aiii	2.52	11.24	88.76	17.42	82.58	78.24	7.21	3.51	1.30	0.41	2.7	0.031
	Air Dried	3Bi	4.28	3.31	96.69	50.81	49.19	4.25	0.41	1.06	1.08	0.34	3.4	0.000
		3Bii	27.33	4.03	95.97	57.60	42.40	2.55	0.01	0.04	0.11	0.51	2.2	0.001
		3Biii	1.22	5.37	94.63	33.40	66.60	8.40	6.52	2.35	0.23	0.37	2.2	0.000
	Freeze Dried	3Ci	3.84	5.82	94.18	47.76	52.24	3.48	0.27	2.39	0.55	0.55	4.3	0.024
		3Cii	20.66	4.56	95.44	36.06	63.94	14.03	0.01	0.46	1.36	0.54	2.9	0.012
		3Ciii	4.99	8.32	91.68	11.39	88.61	87.17	0.39	1.38	1.59	0.35	3.8	0.000
	Oven Dried	3Di	3.43	4.56	95.44	37.63	62.37	12.43	0.60	0.61	0.85	0.54	4.5	0.081
		3Dii	32.43	5.87	94.13	29.03	70.97	22.67	0.10	0.12	0.42	0.46	4.2	0.073
		3Diii	6.24	10.46	89.54	15.51	84.49	73.12	0.33	1.02	0.92	0.68	3.9	0.129

Note: All extract yield, organic, inorganic and phytochemical percentages are in terms of ultimate dry matter (DM) of raw material (as calculated from the moisture analysis).

SAMPLE CODE KEY
1=*Ascophyllum nodosum*, 2=*Laminaria digitata*, 3=*Saccharina latissima*
A= Frozen, B=Air dried, C=Freeze dried, D=Oven dried
i=96% ethanol extract, ii=48% ethanol extract, iii=100% water extract