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Novel bioactive seaweed based ingredients and products

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Authors:
Dr. Hörður G. Kristinsson
Rósa Jónsdóttir

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Project participants

ICELAND

Marinox - Project owner
Rósa Jónsdóttir
R&D Manager
marinox@marinox.is

Matís - Project leader
Hörður G. Kristinsson
Research Director
hordur@matis.is

Thorfish
Gunnar Tómasson
General Manager
gt@thorfish.is

Nýland
Guðbrandur Sigurðsson
Manager Director
gs@nyland.is

DENMARK

Technical University of Denmark DTU Food
Charlotte Jacobsen
Senior Scientist
chja@food.dtu.dk

NORWAY

Due Miljö A/S
Eddy Torp
General Manager
Eddy.Torp@duemiljoe.no

Sigurd Folland A/S
Sigbjorn Folland
General Manager
sigfoll@online.no

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Executive summary

Marine seaweeds are a highly underutilized resource in the Nordic region with great potential. Seaweeds are known to contain unique compounds that can find many uses in consumer products. The Nordic countries have a unique position to create significant value from its very abundant seaweed resources. Previous research has demonstrated that the seaweed species bladderwrack (*Fucus vesiculosus*) contains extremely bioactive antioxidants, more than any other seaweed researched. Bladderwrack was therefore the focus of this project.

The overall objective of the project was to create new high value ingredients and products from a highly underutilized Nordic resource, marine seaweeds. The project period was from January 2012 to December 2014.

The specific objectives were:

a. Development of extraction, fractionation and concentration techniques to produce high value bioactive ingredients from marine seaweeds (bladderwrack)

b. Characterize and define the bioactivity and properties of the extracted ingredients

c. Develop and produce new products containing the bioactive marine seaweed based ingredients with active participation of target consumers and users

d. Investigate the market for the ingredients and products and establish a marketing and sales plan

e. Scale-up production processes to produce bioactive marine seaweed based ingredient on a large scale

The ultimate objective of the project was a commercial production of novel bioactive marine seaweed based ingredients and products, leading to a new industry and significant value creation.

All the objectives of the project have been reached. Extraction, fractionation and concentration techniques of bioactive ingredients have been performed resulting in a scaled up process producing bioactive ingredients. The process techniques developed are all environmental friendly and the seaweeds are collected in a sustainable manner.

The bioactivity and properties of the ingredients have been characterized and defined. Results of the project have shown that the extracts are stable and keep their positive properties through storage for over a year at room temperature, cold and frozen storage. Characterisation techniques include HPLC and NMR analysis. Among bioactivities that were measured was ORAC value, a method measuring the antioxidant capacities *in vitro*, where the seaweed extracts showed excellent antioxidant activity in line with other better known natural compounds like rosemary extracts and tea.

The extracts were shown to be very promising ingredients in food products by inhibiting oxidation in different food models like coating of salmon fillets, milk enriched with fish oil and mayonnaise.

Thorough investigation of the market for ingredients and products has been performed. Based on this work a marketing and sales plan have been put in action for two types of products, a line of marine bioactive ultra-rich skincare products named UNA skincare and an extract Marinox™ to be used as ingredient in for example food products and food supplements. Other types of products are in the pipeline for marketing. The project has shown that there is a demand on the market for products with bioactive seaweed ingredients and market potentials are good. This has resulted in a new industry in the Nordic region and significant value creation has been reached beneficial for the BioMarine industry.

The project participants recommend that research parties and companies should aim at utilizing the ample marine areas surrounding the Nordic countries in a sustainable manner. It is important that the utilization is built on thorough research and technology of this natural resource where bioactivity is investigated. Furthermore, it is essential that the work coming out of this project will be followed up with clinical trials as well as more market and consumer based studies. Thereby a successful sustainable new industry based on sound science and technology can be started up, beneficial for both health and business of the Nordic countries.

1. Introduction

Seaweeds are a significant global commodity, valued at about $15 billion annually as a raw material. Research has clearly demonstrated that marine seaweeds contain highly active ingredients which can find many different applications in the food, pharmaceutical and cosmetic industry. Many countries outside Scandinavia have recognized the great potential of seaweed based ingredients, including Ireland, France, China, Japan and Korea to name some leading countries. At the same time the Nordic countries have enormous seaweed resources, probably the most pristine in the world, without a real effort to create value and value added products from the high value ingredients that can be produced from seaweeds. Our preliminary R&D work has demonstrated that the brown seaweed *Fucus vesiculosus* contains extremely bioactive antioxidants, more than any other seaweed species the research group at Matís in collaboration with Marinox have researched. *F. vesiculosus* was therefore the focus of this project.

The overall objective of the project was to create new high value ingredients and products from a highly underutilized Nordic resource, marine seaweeds. The specific objectives were to:

I. Development of extraction, fractionation and concentration techniques to produce high value bioactive ingredients from *F. vesiculosus.*

II. Characterize and define the bioactivity and properties of the extracted ingredients.

III. Develop and produce new products containing the bioactive marine seaweed based ingredients with active participation of target consumers and users.

IV. Investigate the market for the ingredients and products and establish a marketing and sales plan.

V. Scale-up production processes to produce bioactive marine seaweed based ingredient on a large scale.

The ultimate objective of the project was to start a commercial production of novel bioactive marine seaweed based ingredients and products which will lead to a new industry and significant value creation.

2. Results & Discussion

2.1 Development of extraction processes

Extraction and filtration processes were developed in the project with the aim to extract bioactive ingredients. Different extraction methods were evaluated using extraction media of different polarity, varying pH, time and temperature. Different filtration and drying methods were also evaluated.

2.1.1 Extraction

Icelandic brown marine alga, *F. vesiculosus*, was collected four times in Hvassahraun in southwest Iceland, during different times of the year. The algae was washed with seawater to clean most of the sand from the seaweed and then transported to the laboratory. At the laboratory the seaweed was washed with cold tap water and frozen at -18°C until analysed. The polyphenols were extracted using different temperature, pH and time in addition to seaweed/water ratio. For one group of collected samples, the pre- treatment was the same except that the upper part of the seaweed was cut off and collected instead of collecting the whole plant. This part of the project was performed by Matís in collaboration with Marinox.

Different extraction media was tested and different pH, time, and temperature. The extraction was evaluated by measuring the total polyphenol content (TPC) as gram phloroglucinol equivalents per 100 grams seaweed extract (g PGE/100g dry weight extract). Amount of polyphenols was different with extraction media and methods used, ranging from 34 to 89 g PGE/100 g extract. Based on both yield and properties aqueous extract media was selected for further trials and production. The amount of polyphenols varied somewhat within the time of collection.

2.1.2 Fractionation/concentration

Filter fractionation

Several different fractionation fabrics/materials were tested to fractionate and separate out molecules of interest from the seaweed. The suitability of the specific material used depended on the properties of the seaweed extracts. Extracts from one season had low viscosity while extracts from other seasons had high viscosity due to high polysaccharide content. After fraction samples were frozen in portions and then subjected to different concentration/drying methods. The following concentration methods were tested: sieving using different mesh size screens, membrane filtration, using different molecular size cut-offs. The following drying methods were tested: freeze-drying and spray drying.

Membrane fractionation

Work was undertaken to explore an even more advanced molecular filtration using ceramic membrane filtration systems (from partner Due Miljö), where a single group of molecules can be selectively separated from another. Experiments using gel filtration columns and capillary electrophoresis at the Biomolecules laboratory in Matís were done to identify which molecular weight cut-offs were select for the ceramic membranes. By combining microfiltration, ultrafiltration and reverse osmosis the aim was to separate polysaccharides, proteins and polyphenols from the brown algae extract.

2.2 Characterization of ingredients

Selected extracts produced were characterized with regards to chemical composition and antioxidant activity. The aim was to measure the trace elements, i.e. total and inorganic arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) in aqueous extract and the different organic extracts and to develop a High-Performance Liquid Chromatography (HPLC) method in order to characterize the highly active compounds. In addition, the objective was to evaluate the antioxidant activity using chemical based *in vitro* assays (oxygen radical absorbance capacity (ORAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity, reducing power and metal chelating ability) and food model systems. The storage stability of selected extract was also studied.

2.2.1 Trace elements

The inorganic contaminants, i.e. mercury, cadmium and lead, were in very low concentration in all extracts. The content of inorganic arsenic was very low or less than 0.18 mg/kg extract. According to the European Food Safety Authority (EFSA) Panel the inorganic arsenic exposure has been estimated to range from 0.13 to 0.56 µg/kg bodyweight (b.w.) per day for average consumers (EFSA Journal 2009). The content of inorganic arsenic is therefore well below acceptable limits.

2.2.2 Characterisation of seaweed extract

A separation method for HPLC was developed, using Dionex HPLC system Ultimate 3000 with UV-vis detector, and a semi-prep. Inertsil® HILIC column (5 µ, 4.6 x 250 mm). Freeze dried *F. vesiculosus* was methodically extracted and the most bioactive compounds (Table 1) in the seaweed extract were further analysed and structurally characterised. The extract was fractionated on a Sephadex LH-20 column and the most active fraction - using 75% MeOH – was collected. The ORAC value of the fraction was very high or 17960 ± 418 μmol TE/g extract. This fraction was subjected to preparative HPLC for further purification. Two peaks were collected to obtain sub-fractions and their bioactivity was measured by ORAC. Other components were in too low amounts to collect or analyse further. The more active peak was dissolved in deuterium oxide (D_2O) prior to 400 megahertz (MHz) Nuclear magnetic resonance (NMR) analysis that indicate that the compound is Fucodiphlorethol E (Figure 1). Characterization of phlorotannins in a diol-fraction of extract was also performed with Liquid chromatography-mass spectrometry (LC-MS) and some phlorotannins was qualitatively identified.

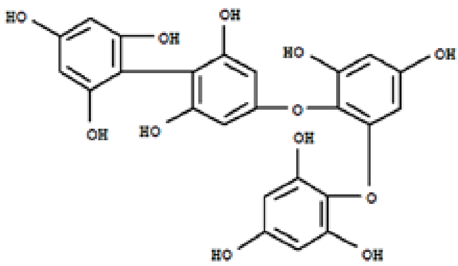


Figure 1. Fucodiphlorethol E.

2.2.3 *In-vitro* antioxidant acitivity

The main results of antioxidant trials were that the TPC of extracts and antioxidant activity measured as ORAC, DPPH, and reducing power value corresponded well (Table 1). As in previous studies have showed the metal chelating ability corresponds neither with ORAC, DPPH or TPC. Extracts with high TPC value have previously show lower metal chelating ability and extracts with lower TPC value have higher metal chelating ability.

Table 1. Chemical based in vitro antioxidant activity tests (ORAC value, trolox equivalent (μmol TE/g extract), DPPH scavenging ability (%), Metal chelating ability (%) and Reducing Power (Ascorbic acid equivalent mg/g extract) in four different extracts.

Extract	TPC (g PGE/100 g extract)	ORAC (μmol TE/g extract)	DPPH Scavenging ability (%)	Metal Chelating ability (%)	Reducing Power (Ascorbic acid equivalent mg/g extract)
1	34.5	2769	54.1	67.4	251
2	39.2	3925	60.2	57.2	293
3	88.9	4732	72.7	20.9	555
4	59.2	3904	54.1	49.5	377

2.2.4 Food models

Antioxidant activity was measured in three different food models: different fish products, in fish oil enriched milk and mayonnaise.

I) Evaluation of antioxidant activity of crude extracts from F. vesiculosus in fish mince

Four different seaweed extracts were tested in different products (minced cod and farmed salmon) in two different concentrations and stored at 4°C for up to 10 days. The extract (0.5%) was blended to approximately 1080 g of minced cod and mixed in a blender for 2 minutes. The control sample was mixed in a blender for 2 minutes without added seaweed extract. Farmed salmon was filleted and deboned one day after slaughter. The fillets were cut into approximately 100 g/pieces. For each group of the salmon samples 600 mL of extract/water solution was added and mixed gently for 2 minutes and afterwards put in small zipper seal bags and stored at 4C.Samples were taken every third day for colour measurement and kept at -80°C until analysed further for oxidation stability (Thiobarbituric acid reactive substances (TBARS), peroxide value (PV), tertiary oxidation compound using fluorescence and Fourier transform near infrared (FT-NIR).

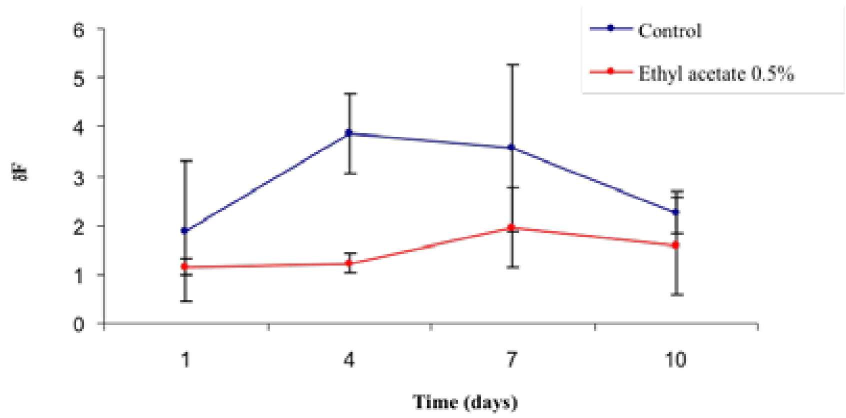


Figure 2. Oxidation in minced cod measured with Fluorescence in samples with no seaweed (blue line) and seaweed extract (red line) after up to 10 days cold storage (4°C).

Figure 2 shows an example of the oxidation analysis done on the minced cod, a very sensitive assay based on fluorescence. The samples treated with seaweed extract show clearly lower oxidation values.

In another trial, extracts were applied to fresh skinless salmon fillet portions by dipping into a solution of extract in water. In those experiments, the seaweed extracts tested clearly reduced both primary and secondary lipid oxidation products. Figure 3 shows the results for the development of secondary oxidation products in untreated salmon (control) and samples treated with seaweed extract. As expected, oxidation was significantly higher in the dark muscle, which was effectively reduced by one of the extract treatment. A simple sensory test (results not included) done on the samples confirmed that the samples treated with seaweed extract had lower perceived lipid oxidation (rancidity).

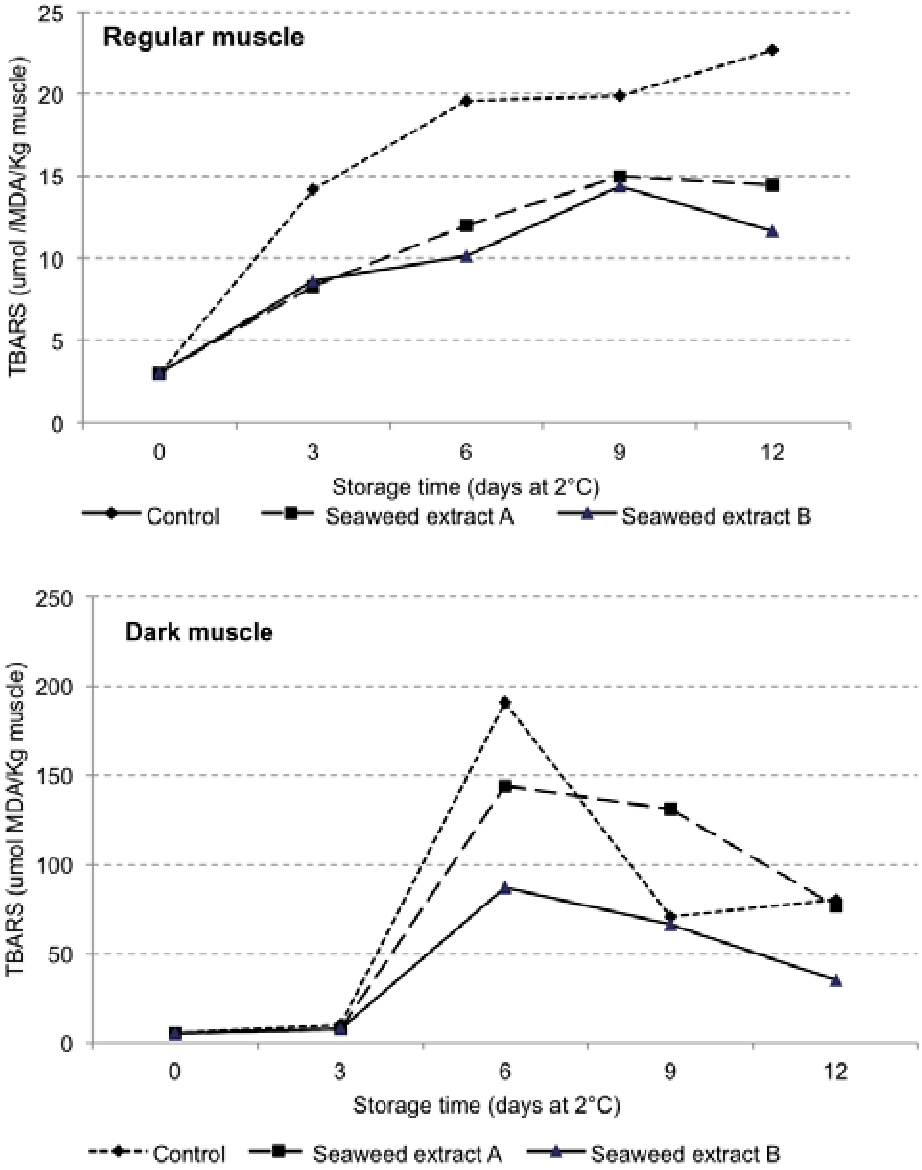


Figure 3. Lipid oxidation in salmon regular and dark muscle at 2°C as assessed by TBARS. Control represents samples with no treatment.

II) Evaluation of antioxidant activity of crude extracts from F. vesiculosus in fish oil enriched milk

Two different seaweed extracts were tested in milk enriched with 0.5% fish oil. Fish oil enriched milk was produced by adding 0.5% fish oil to milk (1% fat) giving a total of 1.5% fat. The dried extracts were added in four different concentrations and mixed into the fish oil enriched milk. The milk was then homogenised (Niro Soavi table homogeniser) at 25/250bar and recirculated 4 times.

The milk samples were stored in the dark at 5°C for 12 days. The oxidative stability of the samples was analysed throughout the storage at 5 different time points (day 0, 3, 6, 9 and 12) by determination of the peroxide value and secondary volatile oxidation products by dynamic headspace, and by performing a simple sensory test in which different off-odours, caused by lipid oxidation, were determined.

The results of this study showed that the formation of secondary oxidation products increased throughout the storage, but the content of these compounds was lower in milk containing the *F. vesiculosus* extract after 12 days of storage (Figure 4 and 5). Especially the content of 2-butenal, 1-penten-3-one, 1-penten-3-ol, hexanal, heptanal, 2,4-heptadienal and heptanal was lower after 12 days of storage compared to the reference milk with no extract added. The main conclusion was that the two crude extracts did show antioxidant activity in the milk enriched with fish oil when studying the development in lipid oxidation during 12 days of storage.

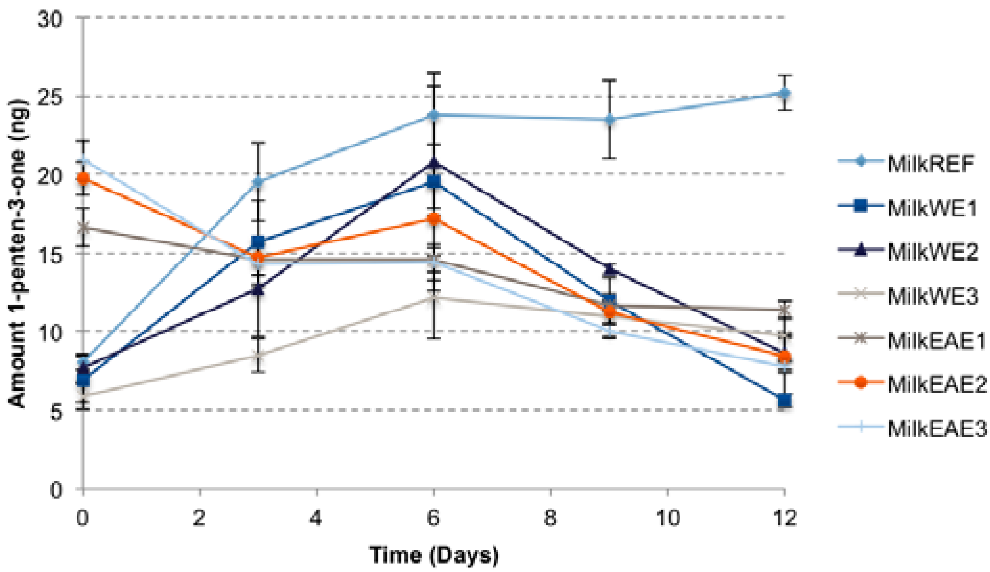


Figure 4. Oxidation measured as 1-penten-3-one in Fish-Oil-Enriched milk without seaweed extract (reference - light blue line on top) and with different F. vesiculosus extracts after up to 12 days cold storage.

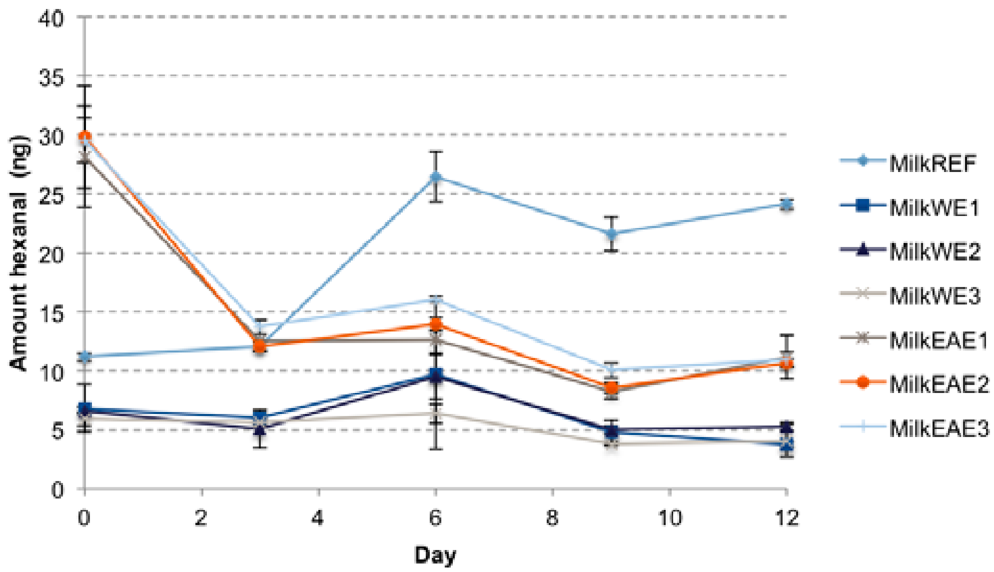


Figure 5. Oxidation measured as development of hexanal in Fish-Oil-Enriched milk without seaweed extract (light blue line on top) or with different F. vesiculosus extracts after up to 12 days cold storage.

III) Evaluation of antioxidant activity of extracts from F. vesiculosus in fish oil enriched mayonnaise

The results from the first storage experiment with fish oil enriched milk gave rise to new ideas of how to apply the extracts from *F. vesiculosus* in other food model systems. Therefor the extracts were tested in mayonnaise as well, since mayonnaise is a different system than milk – for example the fat content is higher, the pH is lower and there is a higher content of pro oxidants, such as iron. Therefore, it would be interesting to look at this food system and how the extracts would act in this type of food system. The aim of the study was to evaluate the antioxidant activity of different concentrations of two types of crude Icelandic *F. vesiculosus* extracts in mayonnaise enriched with fish oil. Two separate trials were made.

Based on the concentration used to obtain the *in vitro* antioxidant assay results, four different concentrations (0, 1, 1.5 and 2 g dried extract/kg mayonnaise) of the two extracts was used in this study with 2 g/kg being the maximum concentration.

A storage experiment was performed where the two extracts from *F. vesiculosus* were added to fish oil enriched mayonnaise in 3 different concentrations. At five time points (day 0, 7, 14, 21 and 28) samples were taken and analysed. Lipid oxidation during storage was followed by determination of peroxide value (PV), tocopherol content, fatty acid composition, and development of secondary oxidation products was determined by dynamic headspace. Furthermore, simple sensory test was performed to detect different off-odours, caused by lipid oxidation.

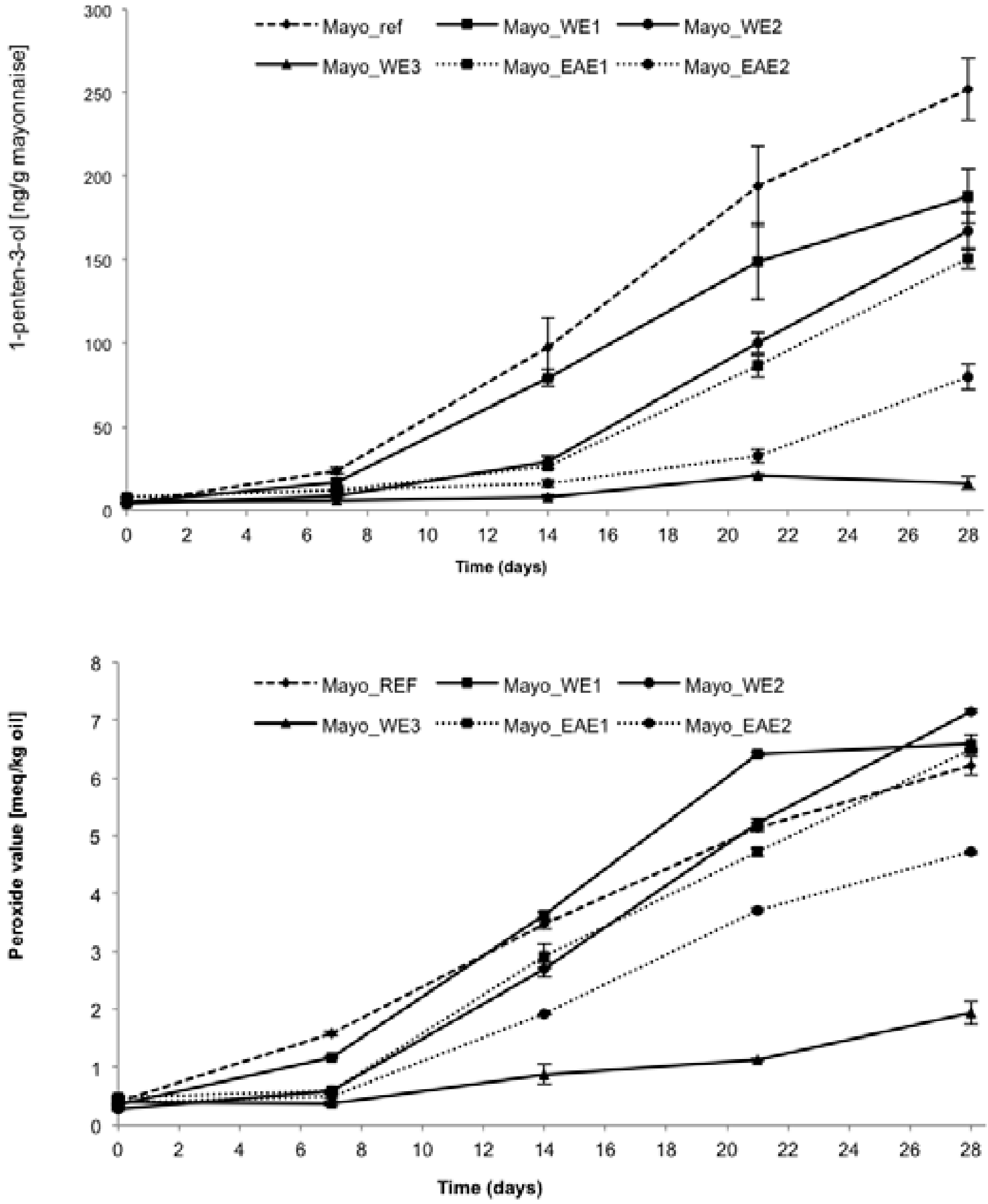


Figure 6. Mayonnaise with water and ethyl acetate extracts (WE and EAF) in three concentrations; 1.0 (1), 1.5 (2) and 2.0 (3) g dry extract/kg mayonnaise. Concentration of peroxides measured as PV [meq. peroxides/kg oil] (a) and development of secondary oxidation product, 1-penten-3-ol [ng/g mayonnaise] Mayonnaise was stored for up to 28 days at 20°C. Error bars indicate SD of the measurements (n = 2 for PV and n = 3 for volatiles compounds).

The main conclusion of the task was that the extracts in some concentrations were found to be able to inhibit the oxidation of docosahexaenoic acid (DHA) and eicosahexaenoic acid (EPA) in mayonnaise, when compared to mayonnaise without added extract (Figure 6). Extracts in the highest concentration were able to decrease the formation of primary oxidation products in fish oil enriched mayonnaise. Furthermore, the formation of some secondary oxidation products was decreased when extracts were added. The highest concentration was the most efficient to decrease the formation of secondary oxidation products in fish oil enriched mayonnaise.

2.2.5 Stability

The storage stability of *F. vesiculosus* extracts from two different seasons were studied at different storage temperatures (-80°C, -18°C, 4°C and room temperature) for 64 weeks and stored for 64 weeks. Ascorbic acid was added to two samples at the level of 1000 mg/kg and stored at -18°C with the aim to increase the storage stability. The samples were stored in closed plastic containers and samples taken every 8-10 weeks and subject to following *in vitro* bioactivity tests: ORAC and DPPH activity, iron chelation capacity and reducing power. Furthermore, total polyphenol content of the seaweed extracts were also measured. In addition, chemical (water, protein, fat, salt, and ash) and microbial (Total viable count, *E. coli*, Salmonella and *Staphylococcus aureus*) analysis were only performed at the beginning of the storage time (Table 2).

Table 2. Chemical composition and microbial analysis of seaweed extracts from season 1 and 2.

	SW1*	SW2*
Water (%)	3.1%	5.1%
Protein (%)	6.5%	4.1%
Fat (%)	0.13%	0.22%
Ash (%)	31.4%	21.1%
NaCl (%)	16.24%	9.1%
Total viable count	40000	9000
Salmonella	Negative	Negative
E. coli	<10	<10
Staphylococcus aureus	<20	<20

* Seaweed from season 1
**Seaweed from season 2

The total polyphenol content of seaweed extract 2 was higher compared to seaweed extract 1, indicating seasonal variations. Seaweed extract 2 had a higher antioxidant power, measured as ORAC values and reducing power, compared to seaweed extract 1.

Neither temperature nor storage time did affect the antioxidant power except seaweed extract 2, kept at room temperature, which had lower ORAC and reducing power after 64 weeks compared to samples kept at 4°C or -18°C. The antioxidant power was similar at the initial stage of the study and after 64 weeks. No significant effect of adding ascorbic acid to increase storage stability was seen in samples kept at -18°C.

2.3 Product development

Product development was performed with four different tasks, i.e. (I) ingredient production, (II) production of food supplement, (III) production of food antioxidant and (IV) production of cosmetics products.

2.3.1 Ingredient production

The aim was to run a pilot scale in order to produce sizable amounts of ingredients for food supplements, food antioxidants and cosmetics. The aim was also to verify their activity and microbial status of the extracts was confirmed, chemical analysis was carried out and bioactivity tests were performed to confirm the quality and bioactivity of the seaweed extracts. The most suitable production processes for the production of seaweed extract was selected based on results from the extraction development performed in the project. Three different ways of the production have been performed; (I) small scale production for cosmetic products, (II) pilot scale production of crude extract and (III) pilot scale production of different fractions (phlorotannins and fucoidans).

(I) Small scale production

Seaweed extract, as bioactive ingredient in cosmetic products was produced on small scales at Marinox facilities. Each batch produced for cosmetic products was measured for total polyphenol content and ORAC value.

(II) Pilot scale production of crude extract

Several pilot trials productions on crude extract were performed by Marinox in collaboration with Matís in Iceland. Microbial status of the extracts was confirmed, chemical analysis was carried out and bioactivity tests were performed to confirm the quality and bioactivity of the seaweed extracts.

(III) Pilot scale production of different fractions (Phlorotannins and fucoidans)

DUE MILJØ's contractor performed one pilot scale production for food supplement and food antioxidant applications. The main conclusion was that by combining microfiltration, ultrafiltration and reverse osmosis, an attempt to separate polysaccharides, proteins and polyphenols from brown algae extract was performed. The sugars were mainly retained by microfiltration at 0.1µm (equivalent to 500kDa) but the active polysaccharides from *F. vesiculosus* are fucoidans, their molecular weight (MW) usually being below 500kDa (maximum 100kDa in literature, and average 20kDa). The ultrafiltration steps at 50 then 8 kDa did not allowed segregation of specific MW sugars. As expected, phlorotannins passed through the process completely and were retained by reverse osmosis (RO) membrane like mineral salts. This process was very efficient to concentrate polyphenols. A simpler process was proposed including microfiltration (MF) and nano filtration (NF) separation only which should allow the recovery of the same components but without ultra filtration (UF) filtration steps. A detailed report with those results was presented with status report at the end of year two of the project (Brown Algae Extract Fractionation. Process development for DUE MILJOE/MARINOX by Stanislas Baudouin, SEPROSYS, France, 2014).

2.3.2 Food supplement

In the project the Marinox™ extract was developed and marketed as ingredient for production of food supplements. The extract can be sealed in a capsule or worked into a tablet, and taken as a supplement. This would allow the consumer to gain the benefits of the extract, while controlling the precise amount imbibed. Based on analysis on trace elements and other results the maximum recommended daily dose is 6.75 g

2.3.3 Food antioxidant

Extracts are furthermore marketed as possible food ingredient due to its high antioxidant values. Lyophilized Marinox™ extract may be added to recipes to increase the ORAC value and health benefits of the food in question, and to increase food shelf-life. Table 3 shows the amount of extract needed based on estimated recommended daily intake (RDI) (%) of ORAC in three examples of food categories.

Table 3. Seaweed extract need (%) based on different recommended daily intake (RDI) (%) of ORAC.

Food category examples	Recommended portion size (g)	RDI (%) of ORAC (2500)			
		100%	50%	33%	25%
Cereals	60	2.1	1.0	0.7	0.5
Dairy	150	0.7	0.3	0.2	0.2
Seafood	250	0.5	0.3	0.2	0.1

When adding the extract to recipes it is both possible to add the lyophilized extract directly, or to first dissolve the extract in water. If a very smooth consistency is required, it may be better to dissolve the extract beforehand.

2.3.4 Cosmetic products

The aim of the task was to develop cosmetic products using seaweed extract as a bioactive ingredient. Based on first market information it was decided to develop two types of facial crèmes as a start-up. Different types of bases were tested and different concentration of seaweed extracts in addition to other ingredients were tested and number of stability tests (testing e.g. colour, texture and odour) testing of the efficacy of antimicrobial preservation and consumer tests. Figure 7 shows a prototype of the day crème (left) and sample preparation for storage stability test (right).

Figure 7. Prototype of the day crème (left) and sample preparation for storage stability test (right).

A lot of effort was also put into the product design and early in the process, the brand name UNA skincare was born. Finally, in June 2012, two types of skin care products with seaweed extract as a bioactive ingredient were launched i.e. revitalizing day crème and regenerating night crème. In 2013 an ultra-rich eye crème was launched. Detailed information about the products are given on the company's website www.unaskincare.com and Facebook homepage. Below are several snapshots from the UNA skincare homepage, Facebook, and from Duty Free at Keflavik airport (Figure 8 to Figure 11).

Figure 8 – UNA skincare homepage.

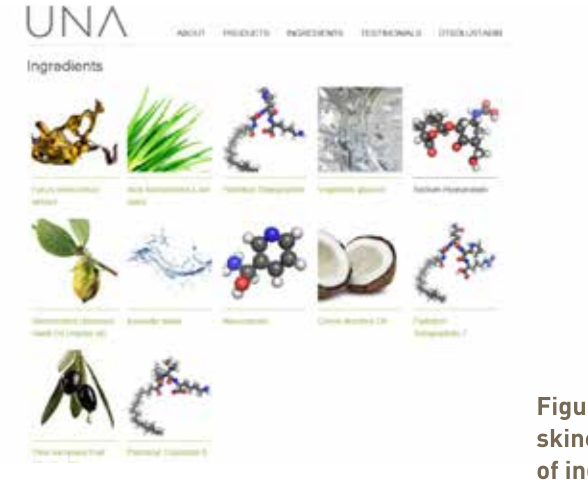


Figure 9 – From UNA skincare homepage, list of ingredients.

Figure 10 – From UNA Facebook page.

Figure 11
– From Keflavik airport.

2.4 Application and consumer tests

2.4.1 Consumer tests and re-evaluation

Consumer testing and focus work was employed in the development of the first two skincare products. The results provided valuable input into product development. The results also showed very high customer satisfaction with more than 90% of users claiming they would recommend the products to a friend (Figure 12). For the consumer testing a considerable amount of day and night creams was produced, packaged with non-descriptive labelling for separation purposes only. Introductory material and questionnaires were prepared. Close to 60 participants, 30 years of age and older (average age 47) were recruited for a 4 week testing of the skincare products, beginning in the middle of January 2012. At the end of the period 41 of the participants returned their completed questionnaires and participated in focus groups to improve the products (Figure 13). The skincare products contain high levels of natural and organic ingredients and instead of undesirable parabens only conservatives acceptable in natural products are used. The products were subjected to challenge testing by inoculation with a known quantity of different microbial germs and bacteria, e.g. *Staphylococcus aureus*, to ascertain the viability of them in the products. The results showed without doubt a concentration dependent antimicrobial effect of the seaweed extract (Figure 14).

During the developments of the facial crèmes all feedbacks from trials and costumers were used to improve the products.

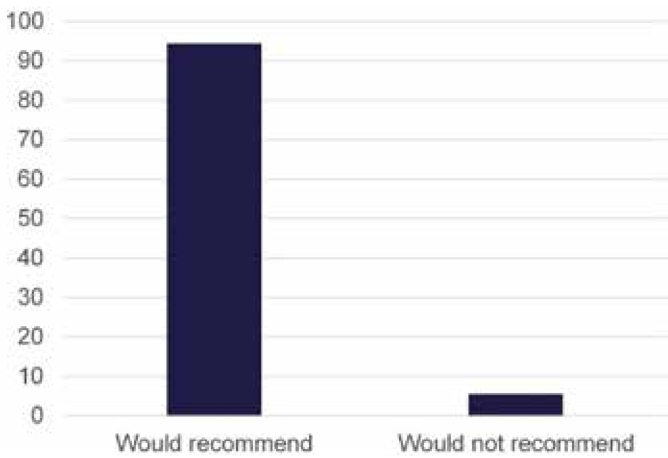


Figure 12. Consumer testing

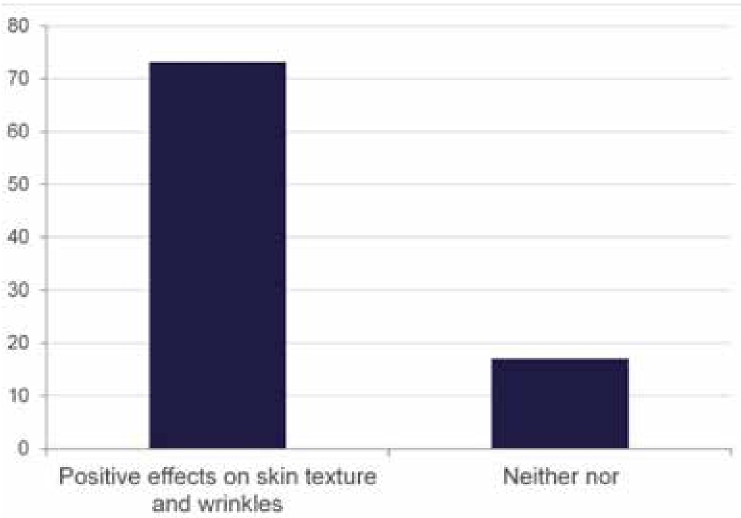


Figure 13. Consumer testing

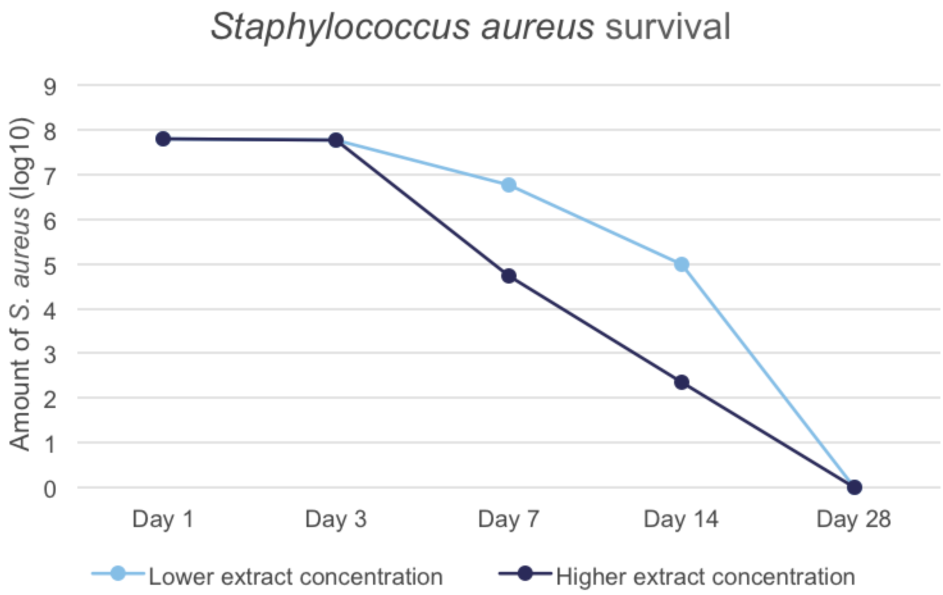


Figure 14. Dose dependent antimicrobial effect of seaweed extract on the survival on *S. aureus*.

2.5 Market studies

Market analysis was performed within the project and based on the results a marketing and promotional plan was formulated.

2.5.1 Market analysis

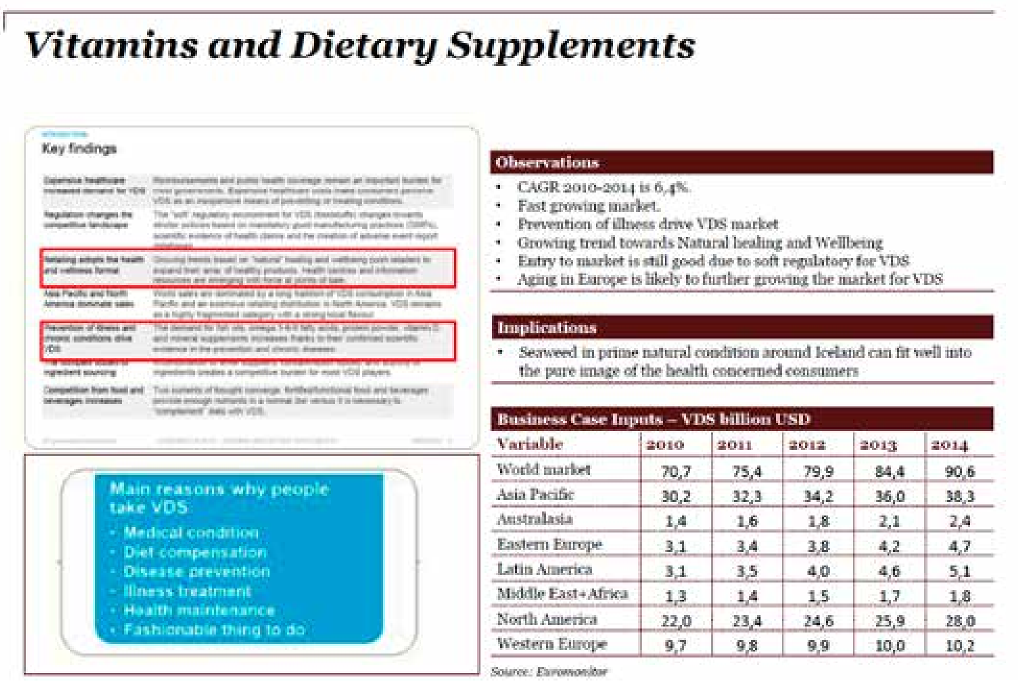
Market analysis was done via available market reports, targeted questionnaires and direct communications with consumers/user focus groups.

PwC (commissioned by project partner Nyland) in cooperation with Marinox performed a comprehensive market analysis. The purpose was to provide a broad-scope vision of the markets were seaweed products are already present or are not present but might be sought after. In addition, to assess the most valuable market sectors for selling high value consumer seaweed-based products or seaweed ingredients. Highest growth market segments within desirable categories were also identified to recognize opportunities for seaweed on the market.

Different ways of market entry were assessed and a plan formed for new Marinox products. Through market analysis, a strategy was formed in product development and business plans were revised based on new information.

The report from PwC includes comprehensive market analysis on (see an example of data sheet in Figure 15):

- Vitamins and Dietary Supplements
- Cosmetics
- Beauty and personal care
- Home care
- Health and Wellness
- Natural and organic
- Food and drink
- Botanicals and bioactives
- Preservatives/Antioxidants



Vitamins and Dietary Supplements

Key findings

Main reasons why people take VDS
- Medical condition
- Diet compensation
- Disease prevention
- Illness treatment
- Health maintenance
- Fashionable thing to do

Observations
- CAGR 2010-2014 is 6,4%.
- Fast growing market.
- Prevention of illness drive VDS market
- Growing trend towards Natural healing and Wellbeing
- Entry to market is still good due to soft regulatory for VDS
- Aging in Europe is likely to further growing the market for VDS

Implications
- Seaweed in prime natural condition around Iceland can fit well into the pure image of the health concerned consumers

Business Case Inputs – VDS billion USD

Variable	2010	2011	2012	2013	2014
World market	70,7	75,4	79,9	84,4	90,6
Asia Pacific	30,2	32,3	34,2	36,0	38,3
Australasia	1,4	1,6	1,8	2,1	2,4
Eastern Europe	3,1	3,4	3,8	4,2	4,7
Latin America	3,1	3,5	4,0	4,6	5,1
Middle East+Africa	1,3	1,4	1,5	1,7	1,8
North America	22,0	23,4	24,6	25,9	28,0
Western Europe	9,7	9,8	9,9	10,0	10,2

Source: Euromonitor

Figure 15. Example from the PwC report.

2.5.2 Marketing plan

Distribution channels were explored and business partners identified and contacted. Based on market analysis a pricing structure was created and a product portfolio with information sheets put together, as well as Material Safety Data Sheet (MSDS) and Technical Design Specification (TDS) for the products. Short and long presentations have been created introducing the company and its products, with company values, vision and mission statements, introduction of staff, research and development and unique selling points of both company and products. Company and product brochures have been designed, printed and distributed; a new company website has been designed and aired. Also Twitter and Facebook pages have been created and are frequently updated. See: www.marinox.is / www.unaskincare.com / www.facebook.com/UNAskincare and https://twitter.com/Marinox_ehf

On the following pages snapshots of Marinox™ extract application notes and Marinox information brochure can be seen.

MARINOX

Marinox™ extract application notes

Fucus vesiculosus, or bladderwrack, is a seaweed which is commonly found on the coasts of Iceland. The Icelandic coastline, famous for its black beaches and unspoiled nature, is the ideal place to collect *F. vesiculosus* in its purest form. Icelandic *F. vesiculosus* is a sustainable, natural resource which has been extensively studied for many years. The research was led by the founders of Marinox, and has culminated in the creation of the F. vesiculosus Marinox™ extract. The high bioactivity of the Marinox™ extract has been studied and verified by several Universities and Research Institutes. The scientists at Marinox use a proprietary and highly advanced process to obtain the natural, bioactive extract.

Marinox™ extract Properties

- Powerful antioxidant
- Fights cancer cells
- Anti-inflammatory
- Anti-diabetic activity
- Naturally increases food shelf-life
- Antimicrobial
- Stimulates collagen production
- Prevents breakdown of the extracellular matrix

Due to the numerous properties of the Marinox™ extract, it has an array of potential uses. Its applications can be sorted into three main categories.

1. Cosmetic ingredient
2. Food ingredient
3. Food supplement

Within these categories a vast amount of application possibilities exist.

Application of Marinox™ extract in cosmetics

Description: Marinox™ extract is derived from Fucus vesiculosus, a brown macro algae. Does not contain preservatives. Light green/brown extract stabilized in in water and glycerin. Characteristic fresh seaweed/beach odor (not transferable to product in quantities used). Water soluble.

INCI Labelling: Glycerin, Aqua, Fucus vesiculosus extract

Properties: The Marinox™ extract solution can be added to skin creams and lotions to serve as an antioxidant and for its anti-ageing and anti-inflammatory effect. It increases collagen production in skin cells and prevents extracellular breakdown.

Use: Add to water phase of formulas. Stable when kept in a closed non-transparent container at 3° - 5°C.

Application: Topical application. Skin lotions/creams, serums, hair care products, anti-ageing products, masks, special skin treatments, sunscreen, and more.

Examples of product that successfully use the Marinox™ extract: UNA skincare cosmetics (www.unaskincare.com).

The Marinox™ extract is a powerful, scientifically verified option for your cosmetics with a proven track record in cosmetics products.

Food ingredient

Marinox™ extract has properties that are very attractive to health conscious consumers. A powerful antioxidant with cancer-fighting qualities, it is anti-inflammatory and anti-diabetic.

Lyophilized Marinox™ extract may be added to recipes to increase the ORAC value and health benefits of the food in question, and also to increase food shelf-life.

The following table shows amount of extract needed based on different % RDI of ORAC in three examples of food categories.

Table 1. % seaweed extract need based on different % RDI of ORAC

Food category examples	Recommended portion size (g)	RDI (%) of ORAC (2500)			
		100%	50%	33%	25%
Cereals	60	2.1	1.0	0.7	0.5
Dairy	150	0.7	0.3	0.2	0.2
Seafood	250	0.5	0.3	0.2	0.1

When adding the extract to recipes it is both possible to add the lyophilized extract directly, or to first dissolve the extract in water. If a very smooth consistency is required it may be better to dissolve the extract beforehand.

Food Supplement

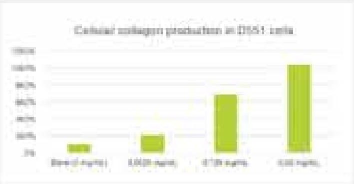
Capsule or Tablet

The extract may also be sealed in a capsule, or worked into a tablet, and taken as a supplement. This would allow the consumer to gain the benefits of the extract, while controlling the precise amount imbibed.

Maximum Daily Dose: 6.75 g

Stimulates collagen production

Collagen is the main component of connective tissue. It is essential when it comes to giving skin its elasticity and strength. Marinox™ extract can increase collagen production dramatically.



Cellular collagen production in D551 skin cells exposed to varying amounts of Marinox™ extract (0.025 – 0.25 mg/mL) compared to a blank.

Prevents extracellular material breakdown

Elastin is a protein which, like collagen, is abundantly present in connective tissue. It allows tissues to resume their previous form after being stretched. Elastase is an enzyme which breaks elastin down. Marinox™ extract reduces the production of elastase in a dose dependent manner.

Metalloproteases (MMPs) are known to break down the extracellular matrix of the skin. D551 fibroblast cells that were subjected to the Marinox™ extract expressed markedly less MMPs.

MMP-1 activity in D551 fibroblasts subjected to different concentrations of MarinoxTM extract (0.0625 – 0.25 mg/mL).

MARINOX

Vision

Our vision is to create value from underutilized natural resources, to apply scientific and technical excellence to developing marine algae bioactives and new applications for them, in a socially and environmentally responsible way for the benefit of current and future generations.

Mission

Marinox aims at becoming a leader in the development and production of the highest quality bioactives extracted from marine algae harvested from the cleanest oceans in the world.

Marinox
Vinlandsleid 14, 113 Reykjavik, Iceland
Tel: +354 4 500 100

Contact

Brynhildur Ingvarsdottir, CEO
brynhildur@marinox.is

Dr. Hordur G. Kristinsson, CSO
hordur@marinox.is

MARINOX

Marinox

Marinox is an innovation company located in Iceland, specializing in the extraction of bioactives from marine algae. Only algae of the highest standard, harvested from the cleanest waters of the world, are used in our products. Marinox marine algae extracts therefore have a unique, pure quality. Marinox extracts are available for use in the health, nutrition and personal care markets.

A natural and sustainable bioactive resource

Fucus vesiculosus, or bladderwrack, is a seaweed which is commonly found on the coasts of Iceland. The Icelandic coastline, famous for its black beaches and unspoiled nature, is the ideal place to collect *F. vesiculosus* in its purest form. Icelandic *F. vesiculosus* is a sustainable, natural resource which has been extensively studied for many years. The research was led by the founders of Marinox, and has culminated in the creation of the *F. vesiculosus* Marinox™ extract. The high bioactivity of the Marinox™ extract has been studied and verified by several Universities and Research Institutes. The scientists at Marinox use a proprietary and highly advanced process to obtain the natural, bioactive extract.

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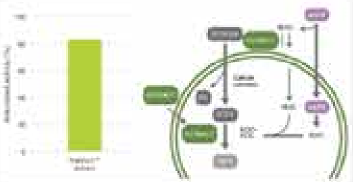
MARINOX

A potent antioxidant

Studies have shown that Marinox™ extract is much more powerful than traditional antioxidants.

Relative antioxidant activity, based on the ORAC values of Marinox™ extract, Vitamin C, Green tea and Vitamin E.

The Marinox™ extract displayed high cellular antioxidant activity when tested in HepG2 cells.



Cellular Antioxidant Activity of the Marinox™ extract in HepG2 cells.

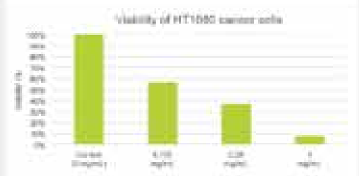
Marinox™ extract outperforms recognized sources of natural antioxidants when it comes to polyphenol content.

Extract	Total polyphenols (g GAE/100g dry extract)
Seaweed extract	52,7 ± 2,6
Green tea extract	24,4 ± 1,4
Pomegranate extract	13,4 ± 3,4
Rosemary extract	7,5 ± 0,6

Polyphenol levels in Marinox™ extract compared with other natural antioxidant compounds.

Fights cancer cells

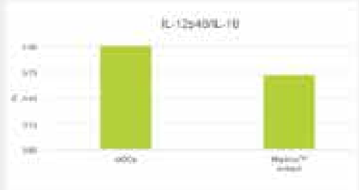
The viability of various cancer cells is radically reduced due to Marinox™ extract.



Viability of HT1080 cancer cells 24 hours after the addition of varying amounts of Marinox™ extract (0,125-1 mg/mL).

Gives the immune system a boost

Dendritic cells (mDCs) were cultured with Marinox™ extract and levels of inflammatory cytokines such as IL-12p40 and IL-10 were measured. The results show that Marinox™ extract has an anti-inflammatory effect.



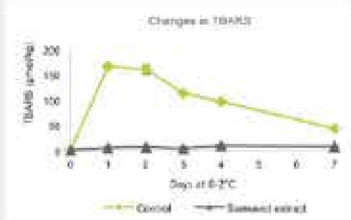
Ratio of IL-12p40 and IL-10 produced by dendritic cells cultured with or without Marinox™ extract. An IL-12p40/IL-10 ratio of less than 1 demonstrates anti-inflammatory activity.

Anti-diabetic activity

Studies have shown that Marinox™ extract inhibits alpha-glucosidase and alpha-amylase. Inhibiting these enzymes is an important part of preventing the development of type II diabetes.

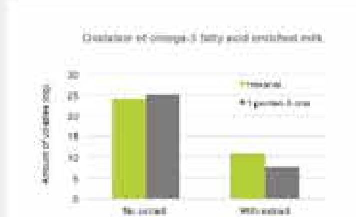
Increases food shelf-life

Thiobarbituric acid reactive substances (TBARS) are degradation products of fats and oils. In fish mince with Marinox™ extract there was no increase in TBARS measured.



Changes in the amount of TBARS measured over time in fish mince with or without Marinox™ extract.

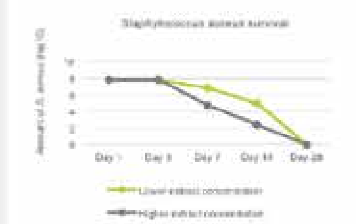
The same findings are seen with other foods, e.g. omega-3 fatty acid enriched foods. The Marinox™ extract was highly effective in preventing oxidation of omega-3 fatty acids in milk.



Volatile off-odour oxidation products in omega-3 fatty acid enriched milk with or without Marinox™ extract. Starting level of volatiles at day 0 was 5-7 ng.

Antimicrobial effect

Cosmetic creams with varying concentrations of Marinox™ extract were cultured with high levels of *Staph. aureus* and its survival measured. Results showed that Marinox™ extract has a strong antimicrobial effect.



Staphylococcus aureus survival over time in skincare creams containing different concentrations of Marinox™ extract.

2.6 Scale-up

The scale up process was divided into two tasks handling ingredients and products:

1. Ingredients: Procedures in WP1 were scaled-up to be able to produce significant volumes of bioactive ingredients. Activity and stability were verified and processes adjusted as needed.

2. Products: Manufacturing of supplements and food antioxidants will be scaled up with appropriate drying and packaging equipment while cosmetics will be scaled up using appropriate blenders and packing dispensers.

2.6.1 Ingredients

Pilot plant processes were adjusted and rerun with good results and Marinox has now established their seaweed extraction methods to be able to process ingredients in sufficient quantity for their further production, UNA skincare products and Marinox™ *Fucus vesiculosus* extract.

2.6.2 Products

Production of UNA skincare products and the Marinox™ *Fucus vesiculosus* extract has been scaled up in sufficient manner to meet their current market needs. In line with their future increased market share – bigger productions lines will be necessary.

3 Conclusions

Based on the scientific work in the project extraction methods have been developed, activity and composition of extracts and their stability has been analysied and tested. This has resulted in a broad know how in manufacturing and use of the seaweeds.

Thorough investigation of the market for ingredients and products has been performed. Based on this work a marketing and sales plan has been put in action for two types of products, a line of marine bioactive ultra rich skincare products named UNA skincare and Marinox™ extracts. Other types of products are in the pipeline waiting for marketing. The project has shown that there is a demand on the market for products with bioactive seaweed ingredients and market potentials are good.

In this way the project has resulted in a new industry in the Nordic and significant value creation has been reached beneficial for the BioMarine industry.

Experience of the project

– and how it will affect the participants in the future work

The consortium of the project consisted of the research companies Matís and DTU, Due MILJØ a company specialising in fractionation and separation of ingredients, Marinox a marine bioactive ingredients producing and selling company, Nyland a company specialising in marketing and possible end-users of a an antioxidant product in fish processing the fish companies Thorfish and Sigurd Folland. In this manner, the consortium covered the whole process from research and development to production, end-use and marketing.

Matís performed the extraction trials in collaboration with Marinox, characterisation of the extracts including the bioactivity tests and finally performed storage studies of food models with added seaweed extracts. DTU tested the antioxidant activities of the extracts in different matrices such as fish oil enriched mayonnaise and fish oil enriched milk. The role of DUE MILJØ's was to perform pilot scale production for food supplement and food antioxidant applications. This work was done in collaboration with their contractor, SEPROSYS. Marinox developed and launched the UNA skincare products in addition to leading the extraction trials. Nyland in cooperation with Marinox performed a comprehensive market analysis. The fish companies participated in and accommodated the trials on the application of the seaweed extract to different fish products. They provided invaluable input into the practicality of applying the extract, as well as participated in the quality evaluation of the products. Thorfish conducted trials and application tests with the extract in different Icelandic seafood products, and the quality and shelf-life of the treated products were then evaluated. Sigurd Folland A/S tested the effect of the effect of the seaweed extract on Norwegian seafood products, and studied the effect on the quality and shelf-life of those products.

The project has been a success for the partners. The R&D companies have deepen their knowledge on extraction, characterisation and use of seaweed extracts and their antioxidant properties. Due MILJØ has acquired knowledge in how their methods and extraction processes perform on the seaweed media, Nyland has increased their know how in market analysis and market strategy planning for new markets. The project has shown that the seaweed extracts are a promising ingredient for the fish producers to increase their product shelf life.

Based on the experience of the project, new product lines have been launched, a line of skincare products and seaweed extracts to be used in production of food ingredients and food supplements. By being a part of this successful story, all the participants have gained intensive experience on how research on molecular bases in a research lab are a good foundation for increased value from the natural ingredients gathered from the nature of the Nordic marine waters.

Scientific presentations and publications as a result of the project

Farvin, S., Larsen, D. B., Jacobsen, C. 2012. Antioxidant effect of seaweed extracts in food emulsion systems enriched with fish oil. ISNFF, December, Hawaii, USA.

Hermund, D. B. 2014. Novel bioactive algae based food ingredients. Presentation at 105th AOCS annual meeting, San Antonio, USA, 4.-14. May 2014: European Section of AOCS travel grant receiver.

Hermund, D. B., Farvin, S., Jacobsen, C. 2013. Antioxidant Effect of Seaweed Extracts in Vitro and in Food Emulsion Systems Enriched With Fish Oil. American Oil Chemists Society Annual meeting and Expo May 2013, Montreal, Canada.

Hermund, D. B., Honold, P., Jónsdóttir, R., Kristinsson, H. G., Jacobsen C. 2014. Seaweed based food ingredients to inhibit lipid oxidation in fish-oil-enriched mayonnaise. Poster at the 12th Euro Fed Lipids Congress, Montpellier, France, 14.-17. September 2014.

Hermund, D. B., Yeşiltaş, B., Honold, P., Jónsdóttir, R., Kristinsson, H. G., Jacobsen, C. 2014. Bioactive compounds extracted from seaweed and application in food systems. Presentation at 2nd International Conference on Algal Biorefinery (ICAB), Lyngby, Denmark, 27. – 29. August 2014.

Hermund, D.B., 2015. Nordic Seaweed extracts as natural antioxidants in omega-3 PUFA enriched granola bars. Presentation at 28th Nordic Lipidforum Symposium, 3. – 6. June 2015. Reykjavík, Iceland. Young Scientist Award Winner.

Hermund, D.B., Karadağ, A., Søgaard Jensen, L.H. Andersen, U., Jónsdóttir, R., Kristinsson H. G, Jacobsen C. 2015. Seaweed based food ingredients to inhibit lipid oxidation in 5% fish-oil-enriched granola bars. Submitted.

Hermund, D.B., Yeşiltaş, R., Honold, P., Jónsdóttir, R., Kristinsson, H.G., Jacobsen, C. 2014. Characterization and antioxidant evaluation of Icelandic F. vesiculosus extracts in vitro and in fish-oil-enriched milk and mayonnaise. Journal of Functional Foods. Available online 4 March 2015.

Honold, P.J., Jacobsen, C., Jónsdóttir, R., Kristinsson, H.G., Hermund, D.B. 2015. Seaweed based food ingredients to inhibit lipid oxidation in fish-oil-enriched mayonnaise. Manuscript.

Ingvarsdóttir B. 2014. Application of seaweed in personal health products. TASTE workshop, Reykjavík, September 16, 2014. TASTE is an EU FP7 SME project See: http://tasteproject.net/

Jónsdóttir R., Sveinsdóttir, K., Pennanen, K., Heiniö, R.L., Martinsdóttir, E. 2013. Consumer perception of seafood products enriched with marine based ingredients. The International marine ingredients conference, 23-24 September 2013 in Oslo.

Jónsdóttir, R. 2015. Novel bioactive ingredients and products derived from marine algae. Presentation at NutraMara Conference, 29. -30. June, Dublin, Ireland.

Jónsdóttir, R. Algae activities in Iceland. The 4th Nordic Algae Network Conference and Workshop. Gothenburg, Sweden, February 28, 2013.

Jónsdóttir, R. Ditte B. Hermund, Charlotte Jacobsen Hordur G. Kristinsson. 2015. The use of natural antioxidants in omega-3 rich food products. Poster at 28th Nordic Lipidforum Symposium, 3. – 6. June 2015. Reykjavík, Iceland.

Kristinsson et al. 2013. Antioxidant Activities of Phenolic Fractions Extracted from the Brown Algae *Fucus vesiculosus* in Washed Minced Tilapia Muscle. Poster presentation. IFT Annual Meeting Chicago, Illinois. July 13 –16.

Kristinsson, H. G. 2012. Functional food from marine resources. 2nd Kiel Food Science Symposium, Max Rubner Institut, Kiel, Germany, May 22-23, 2012,

Kristinsson, H. G. 2012. Functional foods and ingredients from marine resources. Fourth Joint Trans-Atlantic Fisheries Technology Conference – TAFT 2012. Clearwater Beach, Florida, USA.

Kristinsson, H. G. 2012. Icelandic R&D activities on algae. The 1st Nordic Algae Network Conference and Workshop. Reykjavik, Iceland, May 15, 2012.

Kristinsson, H. G. 2013. Global activities in marine biotechnology. Annual Icelandic Seafood Conference. Reykjavik, Iceland. November 21, 2013.

Kristinsson, H. G. 2013. Marine bioactive ingredients and products: An overview. Special IFT Session. IFT Annual Meeting, Chicago, IL, 2013. Abstract 274-01.

Kristinsson, H. G. 2013. Research and development activities with macro algae in Iceland. Macro Algae Conference 2013. Grenaa, Denmark. October 10, 2013.

Kristinsson, H. G. 2013. Value addition of an underutilized macro algal resource. Macro Algae Conference 2013. Grenaa, Denmark. October 9, 2013.

The project was covered in a special technical session at IFT Annual Meeting held in Chicago, Illinois. July 13 -16 led by Dr. Hordur G. Kristinsson, entitled: "Marine bioactive ingredients and products".

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Table of abstract

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Author(s): Hörður G. Kristinsson and Rósa Jónsdóttir			
Organisation(s): Matis and Marinox			
Title: **Novel bioactive seaweed based ingredients and products** Nordic marine 3 innovation programme			
Abstract: Marine seaweeds are a highly underutilized resource in Nordic region with great potential. Seaweeds are known to contain unique compounds that can find many uses in consumer products. Nordic region is in a unique position to create significant value from its very abundant seaweed resources. Our preliminary R&D work has demonstrated that the seaweed species bladderwrack (*Fucus vesiculosus*) contains extremely bioactive antioxidants, more than any other seaweed we have researched. Bladderwrack was therefore the focus of this project and was previously not utilized in Scandinavia. The overall objective of the project was to create new high value ingredients and products from a highly underutilized Nordic resource, marine seaweeds. Furthermore, the ultimate objective of the project was that there would be started a commercial production of novel bioactive marine seaweed based ingredients and products containing them. Thorough investigation of the marked for ingredients and products has been performed. Based on this work a marketing and sales plan has been put in action for two types of products, a line of marine bioactive ultra rich skincare products named UNA skincare and MarinoxTM extract as ingredient for further processing. Other types of products are in the pipeline waiting for marketing. The project has shown that there is a demand on the market for products with bioactive seaweed ingredients and market potentials are good. In this way the project has resulted in a new industry in the Nordic and significant value creation has been reached beneficial for the BioMarine industry.			
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Novel bioactive seaweed based ingredients and products

Marine seaweeds are a highly underutilized resource in the Nordic region with great potential. Seaweeds are known to contain unique compounds that can find many uses in consumer products. The Nordic region is in a unique position to create significant value from its very abundant seaweed resources.

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We stimulate innovation, remove barriers and build relations through Nordic cooperation

NORDIC INNOVATION, Stensberggata 25, NO-0170 Oslo, Norway // Phone (+47) 47 61 44 00 // Fax (+47) 22 56 55 65

info@nordicinnovation.org // www.nordicinnovation.org // Twitter: @nordicinno // Facebook.com/nordicinnovation.org