



Department of
**Agriculture,
Food and the Marine**
An Roinn
**Talmhaíochta,
Bia agus Mara**

Food Institutional Research Measure

Final Report

'Profiling and Optimising chemical composition of red Sea Vegetables
for enhanced bioactive yields' - 'Pro-SeaVeg'

DAFM Project Reference No: 13/F/536

Start date: 01/03/2014

End Date: 31/05/2017

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Collaborating Research Institutions and Researchers: Professor Dick FitzGerald, University of Limerick

Please place one "x" below in the appropriate area on the research continuum where you feel this project fits

Basic/Fundamental	→	Applied	→	Pre Commercial		
1	2 x	3	4	5	6	7

Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report:

Priority Area (s)	Marine Origin Foods/Food for Health
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Key words: (max 4) Seaweed, food, composition, bioactivity,

1. Rationale for Undertaking the Research

This section should outline the rationale for carrying out the research and identify the need / problem to be addressed

Marine macroalgae contain many compounds with potential health benefits, and have been identified as likely sources of new functional food ingredients. However, to fully realise their potential in food applications, a detailed assessment and characterisation that takes into account natural seasonal and spatial variation within and between populations, is required. This project undertook a detailed profiling of bioactives in two red sea vegetables currently available to consumers in Ireland. This project built upon previous research of the project partners, and focused on the bioactive profiling of the two Irish red seaweeds to underpin their future development by Irish SMEs as high-value food species. Specific isolated strains of both species are currently cultivated abroad and represent the basis of significant international industries. However their potential for high value applications in Ireland has not been fully exploited, although local populations of these species are available and products are commonly sold in local markets. While these natural seaweed populations exhibit considerable compositional variability, their detailed nutritional and bioactivity profiles have been poorly described. This project thus addressed an existing gap with regard to the natural variability in chemical composition of two promising macroalgal food species from Ireland; bioactivity was related to chemical composition with particular emphasis on peptides with anti-diabetic, antioxidant and cardio-protective activities; mechanisms of optimising of chemical profiles (e.g. protein and bioactive peptide levels, antioxidant capacity of high value compounds) were explored during lab-scale experiments by carefully altering growth conditions.

The well-documented high chemical diversity of algae meant that existing culture techniques could only be applied to a limited extent to new algal species, and a dedicated research effort was needed to develop their potential for high value food applications. Furthermore, previous recent research by the project partners had revealed that the variability protein and bioactivity in other red seaweeds were greater than anticipated which warranted further investigation. Therefore, the studies herein were extended to other macroalgal food species.

2. Research Approach

Specify the research methodologies employed, emphasising novel techniques and also outline any modifications from the original approved project proposal

Based on successful previous research conducted within the laboratories of the project partners, the project team conducted a detailed, targeted assessment of composition and impact on bioactivity of new red algal food species of international value which are not currently cultivated (but harvested locally) in Ireland. Seasonal and spatial variation in biochemical composition which directly relates to nutritional value was assessed in seaweeds from locations where commercial harvesting for food uses currently occurs; related biochemical composition was optimised in cultivation in an attempt to achieve higher levels of bioactivity with food applications. The methods used to profile bioactive compounds in seaweeds included GC-MS, HPLC and spectrophotometry based on in-house

method developed and optimised at NUI Galway (see Guiheneuf et al 2018) as part of this project. Assessment of *in vitro* bioactivities using different antioxidant assays (ORAC, FRAP, ABTAS and DPPH), cardio-protective capacity using ACE inhibition assays and anti-diabetic capacity using dipetidyl peptidase IV inhibition assays were undertaken at UL.

There were no modifications from the original project proposal. However, additional work was undertaken as part of the project no-cost extension. This involved investigating the stability of selected bioactive compounds during processing.

3. Research Achievements/Results

Outline main results achieved

The work was attributed to 5 different workpackages (Tasks) which had specific outputs relating to the assessment of seasonal and spatial profiling of high value compounds in selected seaweed species (Tasks 1, 2), the relevant profiling of bioactivity associated with natural samples (Task 3), the identification of drivers of changes in biochemical composition as well as processing impacts (Task 1-4), and the identification optimum conditions for the production of specific compounds, as well as associated bioactivities for single and multiple (Task 4) components. Task 5 related to management and dissemination, outputs of which are reported below under Sections 4 and 5 (Impact and Outcomes, respectively).

Summary Task 1. Results from this task provided a first insight into the significant natural variability in valuable chemicals in three food species (seavegetables) from three sites on the Irish west coast. For the first time, following detailed profiling, considerable local and temporal differences between natural samples from selected commercial harvesting sites were documented. Such results are of direct relevance to the Irish seaweed industry, as well as consumers of Irish seavegetables which represent a growing market. Products from these sites are available on local markets and are offered in local restaurants. The potential health benefits and nutritional value of Irish seavegetables have experienced significant attention but had not previously been studied systematically in Irish commercial food species. Research included the development of a new methodology which allowed the isolation and application of new chemical standards. Results from this task highlight the importance of identification of source, as well as processing procedures of Irish seaweed products to ensure quality control.

Summary Task 2. Results produced in this task involved a detailed protein and peptide profiling of Irish seaweed samples of commercial relevance, complementing the results obtained in Task 1. Extraction and purification methods were specifically developed by the team at UL to optimise yields from different species. Protein and peptide profiles were related to the results from Task 1 regarding the environmental drivers of chemical composition and formed the basis of bioactivity assessments conducted under Task 3.

Summary Task 3. Dedicated efforts allowed the identification of seasonal and spatial variation in bioactivity (antioxidant, cardioprotective and anti-diabetic) of seaweed species currently available for food use in Ireland. The results highlighted significant differences between species, sampling location and sampling time; the impact of these results directly relate to consumer choice, with products on local markets at different times representing different food values and qualities. This is the first time that a connection between sampling time and location and associated bioactivity, and thus potential health benefits, has been clearly revealed for these species.

Summary Task 4. This task focused on the targeted optimisation of chemical composition to achieve higher value biomass enriched with nutritional components for human consumption. Selected seaweed species with current food applications and commercial value were grown under controlled conditions to specifically enhance bioactive compounds through environmental stimulation. Such an approach is directly applicable to commercial cultivation, specifically to on-land tank cultivation which is the preferred method for human food applications as it allows greatest control of growth and chemical composition. The biochemical profiles and related bioactivities were enhanced through the application of different nutrient, temperature and light regimes, and combinations thereof. The results are directly relevant to industry wishing to enhance the composition and stability of high value compounds, and thus product quality, of seaweatables through optimised cultivation methods.

4. Impact of the Research

A summary of the tangible impact of the research project should be provided under the outcomes' and 'outputs' heading below. In addition, please provide a short narrative synopsis of the benefits / improvements the research has made to the area under investigation particularly as regards end users, e.g. industry, consumers, regulatory authorities, policymakers, the scientific community, etc

Industry and consumers

The research outputs are directly relevant to Irish seaweed producers, as well as consumers of Irish seaweed products, and were widely disseminated at national and international industry-related events; regular updates were well-received by industry representatives. Research demonstrated the large natural variability in nutritional value, as well as bioactivity, and thus quality, in Irish seaweed species that are commercially harvested and sold locally. The research has identified more favourable locations, as well as seasons, for harvesting commercially valuable seaweed food species (sea vegetables); this information can be employed to underpin current claims made by industry (seaweed processors and vendors), and enable consumers choice and health.

Additionally the research has also highlighted the potential for the optimised production of Irish seaweed species for high value applications, the potential of which can be exploited by Irish SMEs in the future. Although there was no formal industry

participation, numerous informal contacts with industry have been established and the significant potential identified will encourage future industry collaborations.

Regulatory authorities and policy makers

The results are of significance to regulatory bodies and policy makers with regard to the potential regulation of seaweed harvesting, specifically with respect to site selection and sustainable utilisation of natural resources. The project has highlighted the importance of scientific research in this field, underpinning the development, demonstrating the need for careful assessment and profiling of chemical composition and associated bioactivity of seaweeds as food products. The research is directly relevant to current activities at European level through bodies such as the European Standards Authority (and national efforts through the NSAI) for the standardisation of algae and algal projects with regard to chemical contents and labelling. The project has been recognised by national bodies such as the Marine Institute and BIM as a case study for research towards the development to the Irish seaweed sector, outlining the potential for higher value applications in the Food for Health arena.

The scientific community

A large number of scientific project outputs (see below, 4b) demonstrated the relevance of the results to the scientific community, both nationally and internationally. A number of peer-reviewed articles have already been published, with others in progress. Research has documented the significant seasonal and spatial variability within Irish macroalgal food species, and identified the drivers of the chemical composition observed. The project also facilitated the training of 2 MSc students, as well as the early career development of a number of postdoctoral researchers at both institutions. All researchers made significant contributions to national and international conferences through poster and oral presentations. The wide dissemination of project outputs at national and international (including industry-focused) conferences where the project has attracted significant attention, promoted the visibility of the team, and Irish research and SME-related activities generally, internationally.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

The existing collaborations between the project partners derived from previous projects have been consolidated. The recent Marine Institute-funded Marine Bioresources project builds upon these linkages, and two further joint project applications were submitted.

(ii) Outcomes where new products, technologies and processes were developed and/or adopted

The project focused mainly on fundamental research but as part of the research, new technologies and standards were developed which are now available to the team and can be applied in further projects that underpin industry-focused research. These include novel

extraction methods adopted for specific seaweed species, isolated chemical standards, as well as novel cultivation methodologies.

(iii) Outcomes with economic potential

Research outcomes have demonstrated the significant potential that exists for the use of selected seaweed species for bioactive production; this can be utilised either directly as a food product or indirectly, through the future development of food ingredients, provided that sustainable biomass production can be achieved.

(iv) Outcomes with national/ policy/social/environmental potential

The project has led to the development of new scientific insights into the environmental control of bioactive compound production by selected seaweed species; this has impacts from a national seaweed harvesting perspective, where recommendations on most suitable harvesting locations and sites can be made. In general the research underpins the development of the marine/seaweed sector which is predominantly located in remote areas in western parts of Ireland. The research outputs have further highlighted the potential to cultivate seaweeds under optimised conditions which reduces environmental pressure on natural seaweed populations.

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications, International Journal/Book chapters.

Stack J, Tobin PR, Gietl A, Harnedy PA, Stengel, DB and FitzGerald RJ (2017). Seasonal variation in nitrogenous components and bioactivity of protein hydrolysates from *Porphyra dioica*. J Appl Phycol. DOI 10.1007/s10811-017-1063-0

Guihéneuf, F., Gietl, A. & Stengel, D.B. J Appl Phycol (2018). Temporal and spatial variability of mycosporine-like amino acids and pigments in three edible red seaweeds from western Ireland. doi.org/10.1007/s10811-018-1436-z

Stack, J., Le Gouic, A., Tobin, P., Guihéneuf, F., Stengel, D., & FitzGerald, R. (2018). Protein extraction and bioactive hydrolysate generation from two microalgae, *Porphyridium purpureum* and *Phaeodactylum tricornutum*. Journal of Food Bioactives 1:153-165

(ii) Popular non-scientific publications and abstracts including those presented at conferences - none

(iii) National Report - none

(iv) Workshops/seminars at which results were presented

- Stengel D.B. (2014). Bioactives from marine algae - overview of some research activities at NUI Galway. 18-20 November Inverness, Scotland, oral presentation at Natural Product Biotechnology Inverness, UK, November 2014 (invited talk)
- Stengel, D.B. (2014). The Algal Biorefinery Concept. The European Marine Biotechnology ERA-NET Stakeholders' Meeting, October 2014, Lisbon, Portugal. Invited keynote address.
- Stengel, D.B. (2014). Exploring the value of algal resources - what is the role of marine biotechnology? Marine ERA-NET Steering group meeting, Galway, September 2014. Invited lecture.
- Stengel D.B. (2014). Variability in algal bioactives - the good, the bad and the unknown International Society of Applied Phycology, Sydney, invited symposium presentation, June 2014.
- Gietl, A., Varela-Álvarez, E., and Stengel, D.B. (2015). Fatty acid and pigment composition in edible red macroalgae from western Ireland, Ryan Institute Research Day, NUI Galway, September 25th (Poster)
- Tobin, P., Stack, J., and FitzGerald, R.J. (2015). Quantification and characterisation of nitrogenous components of three native Irish seaweeds, NUI Galway/UL research day, April 21st (Poster).
- Gietl, A., and Stengel, D. (2015). Environmental impacts on the bioactive composition of red algae. NUI Galway School of Natural Sciences Post-grad colloquium, December 4th, 2015
- Gietl, A., E. Varela-Álvarez, and Stengel D.B. (2015). Fatty acid and pigment composition in edible red macroalgae from western Ireland. EPC6. London August 2015
- Gietl, A., E. Varela-Álvarez, and Stengel, D.B. (2015). Profiling and Optimising the bioactive composition of Irish sea vegetables. NutraMara conference. RDS, Dublin
- Stengel, D. B. (2015). Applications of marine algae in food and health - recent developments and remaining challenges. NutraMara Conference. Dublin.
- Stengel, D. B. (2015). Bridging the gap between algal ecology and biotechnology - more than just learning a new language. 6th European Phycological Congress (EPC6) London.

- Tobin, P., Stack, J., and FitzGerald, R.J. (2015). Quantification and characterisation of nitrogenous components of three native Irish seaweeds, Department of Life Sciences Research Day, May 8 (Oral presentation).
- Tobin, P., Stack, J., and FitzGerald, R.J. (2015). Quantification and characterisation of nitrogenous components of three native Irish seaweeds, Nutramara Conference, 29th - 30th June (Poster).
- Tobin, P., Stack, J., and FitzGerald, R.J. (2015). Quantification and characterisation of nitrogenous components of three native Irish seaweeds, Teagasc Food Research Conference, December 14 (Poster).
- Stack, J., Le Gouic, A.V., Guihéneuf, F., Tobin, P., Stengel, D.B. and FitzGerald, R.J. (2015). Antioxidant activity of protein hydrolysates from *Porphyridium purpureum* and *Phaeodactylum tricornutum*, NutraMara Conference, 29th-30th June (Poster).
- Tobin, P., Stack, J., Gietl, A., Harnedy, P., Stengel, D.B. and FitzGerald, R.J. (2016). Quantification and characterisation of nitrogenous components of three native Irish seaweeds, Environ, 22nd-24th March (Oral presentation).
- Stack, J., Tobin, P., Gietl, A., Harnedy, P., Stengel, D.B. and FitzGerald, R.J. (2016). Seasonal Variation in Nitrogenous Components and Bioactivity of Protein Hydrolysates from *Porphyra dioica*. International Seaweed Symposium Copenhagen, June 2016 (oral presentation).
- Stengel, D.B., Gietl, A., Varela Alvarez, E., Tobin, P., Stack, J., FitzGerald, D. (2016) Pro-SeaVeg - Profiling and enhancing bioactive yields in Irish red sea vegetables. International Seaweed Symposium Copenhagen, June 2016 (oral presentation)
- Tobin, P., Stack, J., Gietl, A., Harnedy, P., Stengel, D.B. and FitzGerald, R.J. (2016). Spatial and Seasonal Variation in Nitrogenous Components of the Red Seaweeds, *Palmaria palmata* and *Chondrus crispus*. International Seaweed Symposium Copenhagen, June 2016 (poster).
- Varela Alvarez, E., and Stengel, D.B. (2016). Optimization of the chemical composition of edible red seaweeds as a source of functional foods. International Seaweed Symposium Copenhagen, June 2016 (poster)
- Tobin, P. and FitzGerald, R.J (2016) "Quantification and Characterisation of Nitrogenous Components of Three Native Irish Seaweeds" Annual UL research day (oral presentation).

Stengel, D.B. (2016). Seasonal and spatial variation in red seaweed biochemical composition and bioactivity profiles - 'Pro-SeaVeg': a case study from Ireland. 57th meeting of the Czech Phycological Society, Prague, Sept. 2016 (oral presentation).

Stengel, D.B. (2016). Algal resources and applications Second Marine Biotechnology. ERA-NET Stakeholder Meeting 'Marine Biotechnology - Enabling Future Innovations' Session 'Marine biotechnology, an industry perspective'. 14th October 2016, Brussels (invited keynote)

Stengel, D.B. (2017). Applications of Algal Biomass. BioProsp. 8th International Conference on Marine Bioprospecting. Unlocking the commercial potential of biomolecules from marine environments. University of Tromsø, Tromsø, Norway. March 8-10 2017 (invited keynote)

(v) Intellectual Property applications/licences/patents - none

(vi) Other - none

5. Scientists trained by Project

Total Number of PhD theses:
(no PhD students funded) n/a

Total Number of Masters theses: 2

Gietl, A. (2016). Seasonal and spatial variation in biochemical composition of red algae from western Ireland. NUI Galway, August 2016. Supervisor: Dr Dagmar Stengel.

Tobin, P. (Dec 2018, in prep). University of Limerick. Characterisation and quantification of the nitrogenous components in red Irish seaweeds. Supervisor: Prof Dick FitzGerald.

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
NUI Galway	1	0.11
University of Limerick	1	0.3
Total	2	0.41

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	8	6.912
PhD students		
Masters students	2	3.99
Temporary researchers		
Other		
Total	10	10.902

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended
na	

9. Project Expenditure

Total expenditure of the project: €575,634.64

Total Award by DAFM: €578,522.41

Other sources of funding including benefit in kind and/or cash contribution(specify): na

Breakdown of Total Expenditure

Category	NUI Galway	University of Limerick	Total
Contract staff			
Temporary staff			
Post doctorates	142,940.10	147,013.99	289,954.09
Post graduates	38,562.00	42,898.91	81,460.91
Consumables	24,510.23	28,738.59	53,248.82
Travel and subsistence	11,565.83	6,109.44	17,675.27
Sub total			
Durable equipment	0	593.82	593.82
Other	0		
Overheads	65,273.45	67,428.28	131,609.75
Total	282,851.61	292,783.03	575,634.64

10. Leveraging

Summarise any additional resources'/funding leveraged by this award from other sources e.g. Additional Staff, National/EU funding secured, EI Commercialisation Fund, etc.

Significant additional research funding has been leveraged by the research time which builds upon and expands the impact, scientifically and industry-related, of the research outputs.

2017-2020. A National Marine BioDiscovery Laboratory in Ireland (NMBLI). Marine Institute, Dr Stengel and Prof FitzGerald Collaborators.

2017-2021. DAFM/FIRM SMI-BIO: Seaweed-Microbe Interactions to enhance bioactive yields for food applications. Department of Agriculture, Food and the Marine (FIRM). Dr Stengel Lead PI.

2016-2018. Marine Biotechnology ERA-net 'NEPTUNA' Novel Extraction Processes for multiple high-value compounds from selected Algal sources materials. FP7, Marine Biotechnology ERA-NET and Marine Institute. Dr Stengel Lead PI.

2015-2018 DAFM/FIRM: AsMARA: Arsenic in Marine Macroalgae and Implications for Commercial Uses. 14/SF/860. Department of Agriculture, Food and the Marine. Dr Stengel Collaborator.

2014-2015. NutraMara (Marine Functional Foods Initiative, Optimised cultivation of macro- and microalgae for improved bioactive recovery. Department of Agriculture, Food and the Marine / Marine Institute. Dr Stengel local PI.

2013-2016 DAFM/FIRM: 'SMART FOOD- Science Based 'Intelligent'/Functional and Medical Foods for Optimum Brain Health, Targeting Depression and Cognition'; total €599,920 (led by C. Stanton, Teagasc Moorepark); Collaborator. Department of Agriculture, Food and the Marine. Dr Stengel Collaborator.

2013-2016. DAFM/FIRM: 'PREMARA. Seaweeds as a source of non-digestible complex polysaccharide components for the development of novel prebiotic ingredients for the functional food industry'. (led by P. Ross, Teagasc Moorepark/UCC); Department of Agriculture, Food and the Marine. Dr Stengel Collaborator with Dr Popper.

11. Future Strategies

Outline development plans for the results of the research.

The research outcomes have opened a number of avenues for further research as well as collaborations with industry who may wish to exploit the results. Both strategies are currently pursued. A number of peer-reviewed publications are currently in progress which will attract further attention from the international scientific community as well as industry. In addition to recently arisen opportunities for further collaboration through funded initiatives funded by the Marine Institute, the team aim to secure further funding through appropriate calls.