



13F511 – Seaweeds as a source of non-digestible complex polysaccharide components for the development of novel prebiotic ingredients for the functional food industry

Final Report

SUMMARY

Seaweeds are high in fibre and harbor a diverse range of polysaccharides (including xylan, fucoïdan, alginate, laminarin, carrageenan and ulvan), which may have potential as prebiotic functional food ingredients. The main objectives are to develop marine based prebiotic ingredients, using low-cost industrial scale extraction processes for polysaccharide extraction from a range of sustainable Irish seaweeds and ex vivo bioassays were used to assess prebiotic activity of extracts. Based on positive results, selected extracts were used in the development of organoleptically tested food products – the most promising of which were validated for prebiotic activity in humans. Sufficient seaweed extracts were produced on a laboratory scale for bioactivity assessment. Multiple depolymerisation methods were carried out to produce a range of seaweed extracts with differing molecular weights, analysed by HPSEC. Crude and polysaccharide rich extracts obtained in T1M2 underwent a simulated in vitro gastro-intestinal digestion and ex vivo batch culture fermentation. Irish *Palmaria palmata* seaweed was the selected extract, and for pilot-scale production, was purchased from Irish Seaweeds (Belfast) and Ocean Harvest Technology (Co. Galway, Ireland), then up-scale production of the candidate seaweed extract took place at Moorepark Technology Limited (MTL). Culture-dependent, culture-independent, and targeted metabolomics were used to determine whether extracts could modulate gut microbial composition and metabolic activity in vivo, as a prebiotic. Irish Seaweeds (Belfast) is positive for links with the developing Irish seaweed sector, while MTL highlights knowledge transfer e.g. use of dairy processing technologies to produce non-dairy functional food ingredients. The expertise of staff at the UU Department of Hospitality& Tourism Management developed two food vehicles suitable for human intervention studies, over coming problems with masking taste of the seaweed-based ingredient. Two human intervention studies (acute and chronic) were conducted at University of Coleraine which successfully validated prebiotic activity of the candidate seaweed extract.

KEYWORDS

Seaweeds, polysaccharides, prebiotics

ACRONYM

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Section 1 - Research Approach & Results

Start Date

01 July 2014

End Date

30 November 2018

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 3: Experimental Proof of Concept

NRPE Priority area

Food for Health

Total DAFM Award

€600,326.04

Total Project Expenditure

€533,398.90

Rationale for undertaking the Research

Prebiotics are defined as “non-digestible and selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confer benefits on host well-being and health”. Prebiotic strategies predominately aim to stimulate the growth and/or activity of lactobacilli and bifidobacteria, thereby positively altering the balance of the human colonic microbiota leading to improved host health. This study established the prebiotic activity in vitro, of a range of Irish seaweed derived polysaccharide extracts and subsequently validated that activity in humans. The seaweed harvesting plan indicates optimum periods for sustainable harvesting of *Laminaria digitata*, *Fucus vesiculosus*, *Palmaria palmata*, and *Ulva intestinalis* at Spiddal, Co. Galway. Gut health foods are identified as a major opportunity for the food industry, and this project has potential to generate novel scientific information on the health benefits of marine Ingredients for gut health and their stable incorporation into functional/medical foods. Such information would provide Ingredient suppliers and the Health Professionals with essential knowledge relating to the impact of seaweed derived ingredients for gut health and human nutrition. The resulting functional food products developed in this project, have potential application in the area of gut health (including IBS & IBD), obesity, diabetes & (low calorie fat replacement) and hyperlipidemia. This work is relevant to the food industry for development of functional foods enriched in prebiotic ingredients for enhancing gut health.

Methodology

Several depolymerisation protocols were assessed for optimum molecular weight reduction. Depolymerisation with citric acid was selected as the optimum approach and polysaccharide rich powders were depolymerised, neutralised, and freeze dried with variation in success of depolymerisation due to differences in polysaccharide structure. The final depolymerised extracts then underwent in-vitro digestion and ex-vivo batch culture analysis for prebiotic screening in vitro. Whole seaweed, polysaccharide rich, and depolymerised extracts underwent simulated in vitro gastrointestinal digestion and ex-vivo batch culture prebiotic screening, using synergy1 as a positive control (a commercially available prebiotic) and cellulose as a negative control (a poorly fermented, non-digestible carbohydrate). Changes in microbial composition following fermentation was analysed using culture-based methods and RT-qPCR, whilst short chain fatty acid (SCFA) composition, indicative of microbial metabolic

activity, was analysed using gas chromatography-mass spectrometry. 16S Illumina MiSeq next generation sequencing was carried out to elucidate differences in microbial composition at 0, 10, and 24 hours of batch culture fermentation. The *Palmaria palmata* polysaccharide extract was selected as the most efficacious, based on bifidogenic capacity and ability to increase health-associated SCFA production.

Up-scale production of a polysaccharide-rich extract of Irish-sourced *Palmaria palmata* was then completed at Moorepark Technology Limited (MTL). The ingredient passed food safety tests and was included into food vehicles suitable for the human intervention studies to assess the impact of short term (acute) and long term (chronic) consumption. Both acute and chronic studies were completed at University of Ulster, Coleraine confirming the prebiotic activity of the candidate seaweed extract in vivo.

Project Results

Seaweed extracts were produced on a laboratory scale using membrane separation technologies electrodialysis and ultrafiltration/nanofiltration to desalinate, and isolate polysaccharide components for bioactivity assessment. Multiple depolymerisation methods were carried out to produce a range of seaweed extracts with differing molecular weights, analysed by HPSEC. Chemical depolymerisation using citric acid (food-grade) was the most successful approach. Reference compounds for characterisation of laminarins, alginates, ulvans and xylans were purchased and used throughout characterisation studies as standards. Proteins, lipids, sulphate, and polyphenols were analysed using multiple spectrophotometric assays. All extracts were analysed for mineral and heavy metal content (Aluminium, Chloride, Copper, Mercury, Lead, Arsenic, Cadmium, Iodine, Sodium and Zinc), where the percentage of reference nutrient intake was compared with WHO/EFSA recommendations. This contributes to understanding the nutritional composition of the four edible seaweeds and re-iterates GRAS status for human consumption. Crude and polysaccharide rich extracts underwent a simulated in vitro gastro-intestinal digestion and ex vivo batch culture fermentation. Culture-dependent, culture-independent, and targeted metabolomics were used to determine whether extracts could modulate gut microbial composition and metabolic activity as pertained to a prebiotic. Fermentation of seaweed extracts did not result in increased populations of deleterious gut microbial species compared to cellulose and synergy 1. *Palmaria palmata* was selected as the most promising prebiotic candidate owing to its bifidogenic capacity and ability to increase short chain fatty acid production. For scale-up production, Irish *Palmaria palmata* seaweed was purchased from Irish Seaweeds (Belfast, UK) and Ocean Harvest Technology (Co. Galway, Ireland), and extract was produced at pilot-scale at Moorepark Technology Limited (MTL) for use in the development of two food vehicles for human intervention studies, overcoming problems with masking taste of the seaweed-based ingredient. The first human intervention study was conducted to validate prebiotic activity of candidate seaweed extracts. 20 subjects were randomised to consume either placebo (soup without seaweed) and *P. palmata* extract-containing soup (5g). Both groups received each of the two treatments interspersed by 2-week washout periods. Participants arrived fasting to the Human Intervention Studies Unity (HISU), UU, Coleraine, and had a cannula fitted by a nurse. Blood samples were obtained over a 3-hour period (0, 15, 30, 45, 60, 90, 120 and 180 mins) for the measurement of glucose, insulin, and the appetite hormones ghrelin and leptin to investigate changes in biomarkers associated with ingestion of dietary fibre, including antioxidant status (ferric acid reducing antioxidant potential assay (FRAP), lipid profile (cholesterol and triglycerides) and inflammatory status (C-reactive protein). The second study involved 3 groups of 20 subjects randomised to consume a placebo control, maltodextrin (2.35g); *Palmaria palmata* extract (5g) or Orafti® Synergy1 (5g) for 4-weeks. After 4 weeks supplementation with *Palmaria palmata* fibre extract resulted in favourable changes to lipid profiles with a reduced LDL:HDL ratio, and a non-significant increase in CRP and triglyceride concentration along with lower FRAP, data which suggest that *Palmaria palmata* may have effects on lipid metabolism and appears to mobilise triglycerides. More research is needed in individuals with dyslipidaemia to fully elucidate these effects.

Section 2 - Research Outputs

Summary of Project Findings

Seaweeds are high in fibre and harbor a diverse range of polysaccharides (including xylan, fucoidan, alginate, laminarin, carrageenan and ulvan), which may have potential as prebiotic functional food ingredients. This study established the prebiotic activity of a range of Irish seaweed derived polysaccharide extracts and validated that activity in humans. Prebiotics are defined as “non-digestible and selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confer benefits on host well-being and health”. Prebiotic strategies predominately aim to stimulate the growth and/or activity of lactobacilli and bifidobacteria, thereby positively altering the balance of the human colonic microbiota leading to improved host health. Gut health foods are identified as a major opportunity for the food industry, and this project generated novel scientific information on the health benefits of marine Ingredients for gut health and their stable incorporation into functional/medical foods. Such information provide Ingredient suppliers and the Health Professionals with essential knowledge relating to the impact of seaweed derived ingredients for gut health and human nutrition. The resulting functional food products have potential application in the area of gut health (including IBS & IBD), obesity, diabetes & (low calorie fat replacement) and hyperlipidemia. This work is relevant to the food industry for development of functional foods enriched in prebiotic ingredients.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD Students	1	0	1
MSc Students	0	2	2

Summary of Academic Outputs

Research Outputs	Total Number	Details
PhD Theses	1	Cherry P. (2018). PhD Thesis, Seaweed extracts as prebiotic ingredients, University of Ulster.
Masters Theses	2	Charlotte O’Callaghan (2016) NUI Galway. Yvonne Cassidy (2018) University of Ulster, Coleraine.

Intellectual Property

Knowledge generated on a process for production of seaweed extracts.

Knowledge generated on the prebiotic/gut health promoting activity of seaweed extracts.

Development of 2 functional food products with good organoleptic properties with potential application in the area of gut and metabolic health.

Summary of other Project Outputs

Project Outputs	Details	Total Number
New Industry Collaborations Developed	<p>Seaweed extracts were produced using membrane separation technologies electro dialysis and ultrafiltration/nanofiltration to desalinate, and isolate polysaccharide components. Multiple depolymerisation methods were carried out to produce a range of seaweed extracts. Chemical depolymerisation using citric acid (food-grade) was the most successful approach.</p> <p>New industry collaborations were developed with Nutramara, Ltd., on further marine extract development and validation.</p>	1

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Industry	This study established the prebiotic activity of a range of Irish seaweed derived polysaccharide extracts and to validate that activity in humans. The resulting functional food products have potential application in the area of gut health (including IBS & IBD), obesity, diabetes & (low calorie fat replacement) and hyperlipidemia.

Dissemination Activities

Activity	Details
Workshops at which results were presented	<p>Natural Product Biotechnology 2014, 18-20 November 2014, Inverness, Scotland.</p> <p>ESAI's Environ 25: Sustainability and Opportunities for Change. IT Sligo, Sligo. 8-10th April 2015.</p>
Seminars at which results were presented	<p>APC Microbiome Institute Symposium, Cork, 2016.</p> <p>NutraMara Conference 2015: Harnessing Marine Bioresources for Innovations in the Food Industry. Royal Dublin Society, Dublin, Ireland. 29-30th June 2015.</p>
Other	<p>SCIENTIFIC PUBLICATIONS</p> <ol style="list-style-type: none"> Cherry P; Yadav S; Strain CR; Allsopp PJ; McSorley EM; Ross RP; Stanton C (2019). Prebiotics from seaweeds: An ocean of opportunity? <i>Marine Drugs</i>17(6). pii: E327. doi: 10.3390/md17060327. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Reid G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. <i>Nat Rev Gastroenterol Hepatol</i>, 14(8):491-502. Reid G, Abrahamsson T, Bailey M, Bindels LB, Bubnov R, Ganguli K, Martoni C, O' Neill C, Savignac HM, Stanton C, Ship N, Surette M, Tuohy K, van

Hemert S. (2017). How do probiotics and prebiotics function at distant sites? *Benef Microbes*, 8(4): 521-533.

4. Moroney NC, O'Grady MN, Robertson RC, Stanton C, O'Doherty JV, Kerry JP. (2015). Influence of level and duration of feeding polysaccharide (laminarin and fucoidan) extracts from brown seaweed (*Laminaria digitata*) on quality indices of fresh pork. *Meat Sci*, 99: 132-141.

Knowledge Transfer Activities

<p>Identify knowledge outputs generated during this project.</p>	<p>Several depolymerisation protocols were assessed for optimum molecular weight reduction and depolymerisation with citric acid was the optimum approach.</p> <p>Simulated in vitro gastrointestinal digestion and ex-vivo batch culture prebiotic screening, confirmed prebiotic properties of <i>Palmaria palmata</i> polysaccharide extract, based on bifidogenic capacity and ability to increase health-associated SCFA production. Pilot-scale production of a polysaccharide-rich extract of <i>Palmaria palmata</i> was achieved at Moorepark Technology Limited (MTL). Human intervention studies demonstrated it's potential as gut health enhancing functional food ingredient..</p>
<p>Identify any knowledge transfer activities executed within the project.</p>	<p>Seaweed extract protocols developed at lab scale were transferred to pilot-scale at Moorepark Technology Ltd, for successful production of polysaccharide-rich extract of <i>Palmaria palmata</i>/functional food ingredient for human health application.</p>
<p>List any impacts resulting from the knowledge transferred during the project.</p>	<p>Demonstration of prebiotic activity of Irish seaweed extracts developed in this research, led to increased collaboration with marine Irish SME sector for further development of functional food products with health benefits, addressing gut and metabolic health.</p>

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Exchequer National Funding	€667,000.10	15HDHL3 Longlife 03/01/2016 – 12/31/2019; Source: JPI (EU)Food Processing and Health/Irish Department of Agriculture, Food and Marine (DAFM) Major Goals: To investigate food fermentations for purpose: health promotion and biopreservation, Total €667,000.10.
EU R&I programmes	€8,179,214.89	862957 SMARTProtein 01/01/2020 – 12/31/2023, Source: EU Horizon 2020 Major Goals: To Future-proof alternative terrestrial protein sources for human nutrition encouraging environment

Future Strategies

In this research we demonstrated that Irish *Palmaria palmata* seaweed was useful for development of prebiotic ingredients which were successfully incorporated into two food vehicles suitable for human intervention studies, overcoming problems with masking taste of the seaweed-based ingredient. While inclusion of the seaweed extract into a snack bar holds promise as a future foods ingredient for the growing snack bar market, automated production would be required, and product development would require further research to optimise organoleptic properties to meet consumer acceptability/palatability.

Project Publications

1. Moroney NC, O'Grady MN, Robertson RC, Stanton C, O'Doherty JV, Kerry JP. (2015). Influence of level and duration of feeding polysaccharide (laminarin and fucoidan) extracts from brown seaweed (*Laminaria digitata*) on quality indices of fresh pork. *Meat Sci*, 99: 132-141.
2. Reid G, Abrahamsson T, Bailey M, Bindels LB, Bubnov R, Ganguli K, Martoni C, O' Neill C, Savignac HM, Stanton C, Ship N, Surette M, Tuohy K, van Hemert S. (2017). How do probiotics and prebiotics function at distant sites? *Benef Microbes*, 8(4): 521-533.
3. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Reid G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *NatRev Gastroenterol Hepatol*, 14(8):491-502.
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