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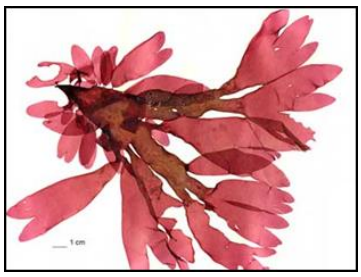
PREMARA:
Seaweeds as a source of non-digestible complex polysaccharide components for the development of novel prebiotic ingredients for the functional food industry



Key external stakeholders:
DAFM, dairy and other functional and medical foods companies, medical profession.

Practical implications for stakeholders:
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. In this project, we developed marine based prebiotic ingredients, using low-cost industrial scale extraction processes for polysaccharide extraction from a range of sustainable Irish seaweed and evaluated the most promising of these for prebiotic activity in humans.

Main results:

- Seaweed harvesting plan developed
- *Palmaria palmata* was found to be a potential prebiotic candidate owing to its bifidogenic capacity and ability to increase short chain fatty acid production when compared to commercially available controls including inulin
- Up-scale production of a polysaccharide-rich extract of Irish-sourced Palmaria palmata was then completed at Moorepark Technology Limited (MTL).
- Evidence from the longer-term intervention study suggests that Palmaria palmata may have effects on lipid metabolism and appears to mobilise triglycerides. Furthermore, participants taking the seaweed extract reported improvements which are indicators of improvements in GUT health (e.g. stool frequency).

Opportunity / Benefit:
Gut health foods are identified as a major opportunity for the food industry, and this project has generated novel scientific information on the health benefits of marine Ingredients for gut health and their stable incorporation into functional/medical foods.

Collaborating Institutions:
Teagasc, NUIG, UU (Coleraine)

Teagasc project team:	Prof. Catherine Stanton (Project Leader)
External collaborators:	Dr. Emeir McSorely, Dr. Philip Allsopp, University of Ulster, Coleraine, Prof. Dagmar Stengel, NUIG

1. Project background:
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

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and bifidobacteria, thereby positively altering the balance of the human colonic microbiota leading to improved host health. 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.

2. Questions addressed by the project:

- To confirm whether seaweed extract exhibit prebiotic activity and impact on gut microbiota composition in an ex-vivo batch culture model system and in vivo in human intervention study.
- To develop a sensory acceptable seaweed extract based functional food ingredient for assessment in a human intervention study.

3. The experimental studies:

Several depolymerisation protocols were assessed for optimum molecular weight reduction. Depolymerisation with citric acid was selected as the optimum approach and the remaining 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.

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. The acute study is complete, and the chronic study will be completed by 31st October 2018.

Human intervention studies were conducted at University of Ulster, Coleraine to confirm the prebiotic activity of candidate seaweed extracts.

4. Main results:

The seaweed harvesting plan indicates optimum periods for sustainable harvesting of *Laminaria digitata*, *Fucus vesiculosus*, *Palmaria palmata*, and *Ulva intestinalis* at Spiddal, Co. Galway. This is relevant to anyone wishing to sustainably hand-harvest a small quantity of these seaweeds; for research or personal use. Further research into successful aquaculture/farming of these seaweeds should be carried out if large quantities of seaweed are required.

Sufficient seaweed extracts were produced on a laboratory scale for bioactivity assessment, with the quantities of initial raw material and extraction yield recorded. This included desalination of the aqueous extract via membrane separation technologies at TMFRC, usually used in dairy processing. The use of membrane separation technologies such as electrodialysis and ultrafiltration/nanofiltration to desalinate, and isolate polysaccharide components (based on molecular weight cut off), could be investigated further on a laboratory scale, for their application during marine bioactive extraction processes.

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; however, three additional stages to the extraction protocol were required. This should be considered during cost-benefit analysis of up-scale extraction.

Reference compounds for characterisation of laminarins, alginates, ulvans and xylans were purchased and used throughout characterisation studies as standards. The molecular weight distribution of each extract was established using HPSEC at IT Sligo. This involved method development and will be disseminated in future publications. The NUIG Postgraduate (MSc) student was trained in widely used

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analytical techniques applicable to characterisation studies. The seasonal impact on polysaccharides molecular weight and total content was achieved using size-exclusion gel permeation chromatography and phenol-sulfuric acid assay, respectively. 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 obtained in T1M2 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. Based on these data, alongside mineral analysis and commercial availability, *Palmaria palmata* was selected as the most promising the prebiotic candidate owing to its bifidogenic capacity and ability to increase short chain fatty acid production. The above screening methods can be employed by other researchers interested in prospective prebiotics, which now include non-dietary fibre components such as polyphenols, fatty acids, and other phytochemicals such as carotenoids – all of which are found in seaweeds.

Irish *Palmaria palmata* seaweed was purchased from Irish Seaweeds (Belfast, UK) and Ocean Harvest Technology (Co. Galway, Ireland), then up-scale production of the candidate seaweed extract took place at Moorepark Technology Limited (MTL). The former is positive for links with the developing Irish seaweed sector, while the latter 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, 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 optimised organoleptic properties to meet consumer acceptability/palatability.

A human intervention study was conducted at University of Coleraine to validate prebiotic activity of candidate seaweed extracts. An application to the Ulster University Ethical Approval Research Governance Filter Committee was approved for both the acute (cannulation study) [REC/17/0027] and chronic (4 week crossover) [REC/17/0051] human intervention studies.
Cannulation study: A total of 20 subjects were randomised into 2 groups of 10. Subjects were randomised to consume a placebo control (soup without seaweed) and soup containing *P. palmata* extract (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. Volunteers also completed a subjective appetite questionnaire using 100 mm Visual Analogue Scales (VAS) at 0, 15, 30, 45, 60, 90, 120 and 180 minutes, to compare measures of appetite and satiety. Insulin, GLP-1, glucagon, and leptin were determined using the electrochemiluminescence based MescoScale Discovery platform. Ghrelin was determined by ELISA (Tebu-Bio, UK), and glucose was measured on the ILAB 650 clinical analyser. Stool and faecal water samples from visits 1, 2, 3, 4, 5 and 6 (V1 – V6) were sent to TFRCM for 16S rRNA sequencing and SCFA analysis, respectively, under a Human Tissue Transfer Agreement. V1 – V6 blood samples were stored at UU for future analysis including: antioxidant status (ferric acid reducing antioxidant potential assay), lipid profile (cholesterol and triglycerides), and inflammatory status (C-reactive protein). During the cannulation study, subjects were asked to complete subjective appetite questionnaires using 100 mm Visual Analogue Scales (VAS) to record their response to feelings of satiety, hunger, fullness, prospective food consumption, thirst, well-being and desire to eat something fatty, sweet, salty or savoury. Blood samples taken at 0, 15, 30, 45, 60, 90, 120 and 180 minutes were measured for serum biomarkers including glucose (Ilab 650 clinical analyser), insulin (ELISA) and the appetite hormone ghrelin (ELISA). This was to determine whether the seaweed extract evoked any effects associated with acute dietary fibre intake. Subjects on the prebiotic study completed a 4-day food diary to and the composition of subjects' dietary intake was determined using the NETWISP software. Bowel habit questionnaire were also completed on a weekly basis to monitor stool frequency and consistency (constipation, hard, soft or diarrhoea), abdominal pain

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(none, mild, moderate or severe), intestinal bloating (none, mild, moderate or severe) and flatulence (none, mild, moderate or severe) to inform tolerability of the seaweed extract. The blood samples taken in the prebiotic study were used to investigate changes in biomarkers associated with chronic 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).

Chronic study: A total of 60 subjects were randomised into 3 groups of 20 subjects randomised to consume a placebo control, maltodextrin (2.35g); *Palmaria palmata* extract (5g) or Orafti®Synergy1 (5g) as a prebiotic comparator for a 4-week period. All participants received each of the three supplements interspersed by 4-week washout periods. Faecal, urine and fasting blood samples were taken from subjects before and after each 4-week supplementary period. Out of the 60 subjects recruited onto the study, a total of 9 dropped out. The remaining subjects completed the study by 31 October 2018. Supplementation for 4 weeks with *Palmaria palmata* fibre extract resulted in favourable changes to lipid profiles with a reduced LDL:HDL ratio, however, intention-to-treat univariate ANCOVA identified no significant difference between the treatment groups over time on any of the lipid profile markers. A non-significant increase in CRP and triglyceride concentration along with lower FRAP was also observed with Palmaria palmata fibre extract supplementation. Evidence from this study suggests 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. Stool samples and faecal water samples were used for DNA extraction/16S rRNA sequencing, and SCFA analysis, respectively.

5. Opportunity/Benefit:

Gut health foods are identified as a major opportunity for the food industry, and this project has generated novel scientific information on the health benefits of marine Ingredients for gut health and their stable incorporation into functional/medical foods.

The information generated in this project will provide Ingredient suppliers and the Health Professionals with essential knowledge relating to the impact of nutrition on depression.

This work is relevant to the food industry for development of functional foods enriched in prebiotic ingredients.

6. Dissemination:

Main publications:

1. Cherry P; Yadav S; Strain CR; Allsopp PJ; McSorley EM; Ross RP; Stanton C (2019). Prebiotics from seaweeds: An ocean of opportunity? Marine Drugs 17(6). pii: E327. doi: 10.3390/md17060327.
2. 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. Nat Rev Gastroenterol Hepatol, 14(8):491-502.
3. 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.

Popular publications:

APC Microbiome Institute Symposium, Cork, 2016.
Natural Product Biotechnology 2014, 18-20 November 2014, Inverness, Scotland.
ESAI's Environ 25: Sustainability and Opportunities for Change. IT Sligo, Sligo. 8-10th April 2015.
NutraMara Conference 2015: Harnessing Marine Bioresources for Innovations in the Food Industry. Royal Dublin Society, Dublin, Ireland. 29-30th June 2015.

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