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

17RDSUSFOOD2ERANET1 - Extraction and characterization of bioactives and carbohydrates from seaweeds and seagrasses for food-related applications

Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

SUMMARY

The overall objective of the project was to explore novel, environmentally friendly and efficient extraction techniques (ultrasound, microwave, enzymes and their combinations), combined with the exploitation of the remaining biomass, rich in bioactive compounds, to sequentially obtain novel carbohydrate-based extracts from seaweeds. Characterisation, structure, technological properties, toxicity and bioactivity of the fractions obtained from the various extraction technologies and a life cycle assessment (LCA) was also performed to prove the sustainability of the procedures.

Agar, alginates and laminaran were extracted from different macroalgae by using novel extraction technologies, i.e., Ultrasound-Assisted Extraction, Microwave-Assisted Extraction, and characterized by a range of different techniques, i.e., FT-IR, TGA, DSC, HPLC-SEC. The development of novel processing technologies for enhancing carbohydrate yields of extraction was established. The use of UAE and EAE enhanced the agar extraction yields compared to conventional extraction, and significantly decreased the time of extraction. Changes in quality characteristics were observed showing an improvement in terms of purity, and other important characteristics for industrial applications. The project outputs demonstrated to improved process efficiency, development of ingredients with high added value from already commercialized seaweed species and from under-exploited sources (seagrasses) which can positively impact in the competitiveness of seaweed, food and non-food companies at EU scale by a better valorisation of raw materials and complete utilisation of all fractions.

KEYWORDS

Seaweeds, alginates, agar, ultrasound and extraction

ACRONYM	BIOCARB-4-FOOD
PROJECT COORDINATOR, INSTITUTION	Prof Brijesh Tiwari, Teagasc.
EMAIL	Brijesh.tiwari@teagasc.ie

COLLABORATORS, INSTITUTION:

Dr Amparo Lopez-Rubio, Institute of Agrochemistry and Food Technology, Spain.

Dr Simon Balance, Nofima AS, Norway.

Mr Timo Enderle, University of Hohenheim.

Dr Anna Ekman Nilson, RISE Research Institutes of Sweeden, Sweeden.

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Section 1 - Research Approach & Results

Start Date

01 May 2018

End Date

30 April 2021

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 3: Experimental Proof of Concept

NRPE Priority area

Sustainable Food Production and Processing

Total DAFM Award

€305,884.40

Total Project Expenditure

€199,786.00

Rationale for undertaking the Research

The BIOCARB-4-FOOD project is integrated by five different partners from Spain (IATA-CSIC), Norway (Nofima AS), Ireland (Teagasc), Germany (University of Hohenheim), and Sweden (RISE) whose expertise consist of carbohydrate extraction knowledge, extract characterization technologies, Life Cycle Assessment (LCA), project management and dissemination. Macroalgae have been widely used as a source of carbohydrates with multiple industrial applications given by their antimicrobial, antitumoral, antiviral, antioxidant, etc, characteristics, especially in Asian countries. Provided that macroalgae are green sources, it is a current research interest of these partners to develop methods that can enhance the extraction of carbohydrates from seaweed without compromising the quality of the extracts. Previous works related to carbohydrate extraction and characterization have been carried out previously by these partners who now have a new focus on innovative extraction technologies which can benefit both research and industry. It is of interest for this project to also integrate the views of third parties with seaweed biopolymer processing expertise, to come up with a common strategy for the development of the seaweed carbohydrate field.

Methodology

Agar and alginates (hydrocolloids) were extracted from the cell-walls of different macroalgae (Gelidium sesquipedale from Spain for agar extraction, and Ascophyllum nodosum from Ireland, Saccharina latissima, and Alaria esculenta Norway) by using novel extraction technologies, i.e., Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Enzyme-Assisted Extraction (EAE), and their combinations, applied during the pre-treatment or the extraction step of the process, and characterized by a range of different techniques, i.e., Fourier Transform Infrared Performance Liquid Chromatography – Size Exclusion Chromatography (HPLC-SEC), gel strength, among others.

Briefly, in order to extract these polysaccharides, an acid or alkali pre-treatment was performed to form the alginic acid, or to reduce the sulphate content of the agar final extract. Then the extraction step followed, which consisted of applying the different novel extraction technologies varying some parameters, i.e., time and amplitude for the UAE, time for MAE, and enzyme type for EAE, in order to test which parameters could lead to the highest yields of extraction, and best physic-chemical extract's characteristics (these technologies were also tested at the pre-treatment step as mentioned previously). Finally, the extracts were freeze-dried by using a freeze-dryer. The extracts were then characterized by FT-IR in order to identify the compounds in the sample by the presence of certain chemical bonds in the spectra, TGA to analyse the thermal decomposition behaviour of the samples, DSC to know the extract's melting point, which is directly linked to the purity of the sample, HPLC-SEC to know the molecular weight (kDa), and gel strength, which is linked to the concentration of agar in the extracts, and therefore, purity. To assess the environmental impact of the selected extraction process developed data was collected a Life Cycle Assessment was performed.

Project Results

1. A range of thermal and non-thermal technologies were employed for seaweed carbohydrate extraction, finding that these were able to enhance the yields of the extraction in the case of UAE, and UAE, and EAE in the case of agar.
2. Non-alkali-based agar extraction allowed to enhance the yields of extraction by means of ultrasound or microwave technologies compared to alkali-based extractions. Ultrasound-Assisted Extraction (UAE) applied for a one-hour treatment increased the agar yields of extraction compared to conventional thermal methods, and innovative thermal methods (Microwave- Assisted Extraction). Furthermore, UAE allowed a significant reduction in the time of extraction (1 h) compared to conventional extraction methods (2-4 h).
3. Non-alkali treated samples reported a high-antioxidant capacity (19 -24 µmol TE/ g extract), and stronger gel strength than alkali treated samples (337–438 g/cm2) compared to (224–311 g/cm2). LCA showed that the non-alkali extraction process was greener compared to alkali extraction process.
4. Enzyme-Assisted Extraction combined with UAE also increased agar extraction yields by 2 to 6-fold compared to the yield of a non-enzyme method (2-3 h). Furthermore, a significant reduction in the time of extraction to less than 1 h was obtained.
5. Agar extracted by EAE using Vyscozyme® reported similar characteristics (FT-IR, apparent viscosity, molecular weight, sulphate content,3,6-anhydrogalactose content, gel strength, compositional analysis and thermogravimetric analysis) to industry standards.
6. Ultrasonic technologies used for 6 h as a pre-treatment and for the solubilisation of sodium alginate enhanced alginate yields of extraction from Alaria esculenta and Saccharina latissima compared to conventional extraction methods, while also keeping the quality of the extracts compared to commercial sodium alginate.
7. Optimization studies using ultrasound, microwave in combination with ultrasound, enzyme assisted alone or in combination were carried out. Extraction methods using alcalase and Viscozyme® are the most promising methods to achieve high yields, especially the methods combined with the US treatment for 30 min.
8. Scale up of selected processes of the extraction process was also carried out, proving that these processes were suitable to extract alginates, and agar.
9. LCA analysis demonstrated that the drying process involved in the production of agar is the most energy intensive process.

Section 2 - Research Outputs

Summary of Project Findings

Key benefits arising from the project include the development and advance the state-of-the art novel extraction technologies, and combination of conventional and novel technologies for carbohydrate extracts. Amongst all technologies investigated UAE and EAE were demonstrated to be effective to enhance agar extraction compared to conventional methods and reported similar quality to industry standards. These technologies allowed to reduce extraction times, and solvent consumption. UAE and EAE have the potential to be up scaled for industrial purposes, and therefore, develop more robust, quicker, greener, and specific (in the case of EAE) processes. The processes developed and investigated has enhanced the scientific capabilities and technical know-how of the processes for industrial uptake.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD Students	0	1	1
Post Doctorates	0	1	1

Summary of Academic Outputs

Research Outputs	Total	Details
Publications in Peer Reviewed Scientific Journals	6	1. Gomez, L. P., Alvarez, C., Zhao, M., Tiwari, U., Curtin, J., Garcia-Vaquero, M., &Tiwari, B. K. (2020). Innovative processing strategies and technologies to obtain hydrocolloids from macroalgae for food applications. Carbohydrate Polymers, 116784. 2. Martínez-Sanz, M., Gomez-Barrio, L. P., Zhao, M., Tiwari, B., Knutsen, S. H.,Ballance, S., ... & López-Rubio, A. (2021). Alternative protocols for the production of more sustainable agar-based extracts from Gelidium sesquipedale. Algal Research, 55, 102254. 3. Li, Y., Zhao, M., Gomez, L. P., Senthamaraikannan, R., Padamati, R. B., O'Donnell, C. P., & Tiwari, B. K. (2021). Investigation of enzyme-assisted methods combined with ultrasonication under a controlled alkali pretreatment for agar extraction from Gelidium sesquipedale. Food Hydrocolloids, 106905. 4. Cabral, E. M., Mondala, J. R. M., Oliveira, M., Przyborska, J., Fitzpatrick, S., Rai,D. K., . . . Devereux, M. (2021). Influence of molecular weight fractionation on the antimicrobial and anticancer properties of a fucoidan rich extract from the macroalgae Fucus vesiculosus. International Journal of Biological Macromolecules, 186, 994-1002. 5. Cabral, E. M., Oliveira, M., Mondala, J. R., Curtin, J., Tiwari, B. K., & GarciaVaquero, M. (2021). Antimicrobials from Seaweeds for Food Applications. Marine Drugs, 19(4), 211.

		6. Garcia-Vaquero, M., O'Doherty, J. V., Tiwari, B. K., Sweeney, T., & Rajauria, G. (2019). Enhancing the extraction of polysaccharides and antioxidants from macroalgae using sequential hydrothermal-assisted extraction followed by ultrasound and thermal technologies. Marine Drugs, 17(8), 457.
Peer Reviewed Conference Papers	3	1. Gomez, L. P., Alvarez, C., Tiwari, U., Curtin, J., & Tiwari, B. K. (2020) Ultrasound and microwave assisted agar extraction from macroalgae, 4th International Caparica Conference on Ultrasonic-based Applications: from analysis to synthesis 2020, Caparica, Portugal (Online) - Awarded best presentation award. https://ultrasonics2020.bioscopegroup.org/index.html 2. Zhao, M., Vaquero,M.G., Sivagnanam, S., Tiwari, B.K. (2019). The development of analytical methods for the purity determination of fucoidan extracted from Fucus vesiculosus. FucoSan – from Science to Innovation Day, Kiel, Germany. https://www.fucosan.eu/en/news/from-science-to-innovation-day-2019/ 3. Li, Y., Zhao, M., Tiwari, B. K. O'Donnell, C. P. (2020). Ultrasound and enzyme assisted agar extracted from gelidium sesquipedale. Biosystems and food engineering research review 25. (UCD Biosystems Engineering Seminar)
Other	1	Gomez, L. P., Ming, Z. & Tiwari, B. K. (2020). Clean and Green, T-Research Vol 14 (4), 26- 27.
Other	1	Gomez, L., Tiwari, B. K., Garcia-Vaquero, M. (2020). Chapter 9 - Emerging extraction techniques: Microwave-assisted extraction, Editor(s): Maria Dolores Torres, Stefan Kraan, Herminia Dominguez, In Advances in Green Chemistry, Sustainable Seaweed Technologies, Elsevier, 207-224. (Book Chapter).

Intellectual Property

An Invention Disclosure Form will be requested through Teagasc's Technological Transfer Office for the sodium alginate extraction by means of ultrasound-assisted extraction for a six-hour treatment. This process has proved to be able to extract sodium alginate while meeting industry standards for the quality of the extracts. Furthermore, the main advantage of this process is that the whole extraction process is shorter compared to industry's alginate extraction which implies soaking the seaweed for long periods of time (i.e., overnight), also avoiding formalin soaking, and final bleaching steps, which leads to a more sustainable overall process.

Summary of other Project Outputs

Project Outputs	Details	Total No.
New Processes	1. Development of novel processing technologies i.e., clean and green process for enhancing laminarin yields. 2. Enhanced recovery of alginates by employing ultrasound technology.	2

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Environmental Sustainability	1. Environmental/social: As a part of the project, LCA analysis of aquaculture production of seaweeds i.e., Dulra Marine (Co Mayo), This is the first time carried out on Irish seaweed farm and further research work is ongoing. 2. The development of new processes and improvement in current processes adopted for seaweed processing has initiated renewed interest amongst industry and other seaweed processors.
Industry	New processes were generated and currently these processes are under evaluation for enhancing TRL level via additional funding secured via SFI BiOrbic in collaboration with two Irish industries.
Other	Scientific: High impact peer reviewed publications obtained demonstrates the international leadership in the area of novel technologies applicable to seaweed processing.

Dissemination Activities

Activity	Details
Workshops at which results were presented	1. Tiwari, B.K. (2020). Keynote address at International Conference on Maritime Studies and Marine Innovation (MSMI 2020), Thailand, Title: Novel disruptive technologies for clean label high value ingredients for the food industry. http://www.maritime.cmu.ac.th/MSMI2020 2. Teagasc Technology Gateway Stand for Irish Agri-Food Industry, Moorepark 2019
Seminars at which results were presented	Tiwari, B.K. (2021). UN FAO-WHO Expert review committee member for seaweed safety current status and future perspectives.
Other	Tiwari, B.K. (2019). Novel food processing technologies and their application in Agri-food industry, Invited lecture to MSc students in Agri Bioscience Institute, NUIG, Galway.

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	Novel extraction technologies have proved to enhance the different polysaccharide extraction, and therefore, these have become a suitable alternative to conventional extraction methods. This finding has the potential to modify current industrial processes into more sustainable and more efficient processes, if industries decide to start using novel extraction technologies.
Identify any knowledge transfer activities executed within the project.	• Ultrasonics 2020 Conference, Caparica, Portugal (Online) • Teagasc Gateways event in Moorepark 2019 • Technological University Dublin Online Twitter Poster Competition
List any impacts resulting from the knowledge transferred during the project.	An Invention Disclosure Form is currently in process to secure the IP via Teagasc's Technological Transfer Office for the sodium alginate extraction by means of ultrasound assisted extraction for a six-hour treatment.

Section 3 – Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding	Funding €	Summary
Non-Exchequer National Funding	€511,938.00	The following two projects were funded based on the outputs arising from the funding as a part of SFI Bioeconomy Research Centre: 1. Value addition to Organic Aquaculture Seaweeds for Novel Functional Food Development (Euro 216,496; 2020 - 2023) Industry (40% funding) 2. Macro-algae for the production of high-quality proteins and polysaccharides (Euro 295442; 2020 - 2022) Industry (40% funding)

Future Strategies

Future strategies is to attract additional industry funding to scale up the processes developed for commercial adoption.

Additional project to EU Horizon Europe was submitted for the call HORIZON-CL6-2021-CIRCBIO-01 (Innovation Action) involving 9 industries and 8 academic partners. Title of the project: CARBON NEGATIVE BIOREFINERY APPROACH FOR SUSTAINABLE ALGAL BIOMASS PRODUCTION AND PROCESSING

Project Publications

1. Gomez, L. P., Alvarez, C., Zhao, M., Tiwari, U., Curtin, J., Garcia-Vaquero, M., & Tiwari, B. K. (2020). Innovative processing strategies and technologies to obtain hydrocolloids from macroalgae for food applications. Carbohydrate Polymers, 116784.
2. Martínez-Sanz, M., Gomez-Barrio, L. P., Zhao, M., Tiwari, B., Knutsen, S. H., Ballance, S., ... & López-Rubio, A. (2021). Alternative protocols for the production of more sustainable agar-based extracts from Gelidium sesquipedale. Algal Research, 55, 102254.
3. Li, Y., Zhao, M., Gomez, L. P., Senthamaraikannan, R., Padamati, R. B., O'Donnell, C. P., & Tiwari, B. K. (2021). Investigation of enzyme assisted methods combined with ultrasonication under a controlled alkali pre-treatment for agar extraction from Gelidium sesquipedale. Food Hydrocolloids, 106905.
4. Cabral, E. M., Mondala, J. R. M., Oliveira, M., Przyborska, J., Fitzpatrick, S., Rai, D. K., . . . Devereux, M. (2021). Influence of molecular weight fractionation on the antimicrobial and anticancer properties of a fucoidan rich extract from the macroalgae Fucus vesiculosus. International Journal of Biological Macromolecules, 186, 994-1002.
5. Cabral, E. M., Oliveira, M., Mondala, J. R., Curtin, J., Tiwari, B. K., & Garcia-Vaquero, M. (2021). Antimicrobials from Seaweeds for Food Applications. Marine Drugs, 19(4), 211.
6. Garcia-Vaquero, M., O'Doherty, J. V., Tiwari, B. K., Sweeney, T., & Rajauria, G. (2019). Enhancing the extraction of polysaccharides and antioxidants from macroalgae using sequential hydrothermal-assisted extraction followed by ultrasound and thermal technologies. Marine Drugs, 17(8), 457.
7. Garcia-Vaquero, M., Rajauria, G., & Tiwari, B. (2020). Conventional extraction techniques: Solvent extraction. In Sustainable Seaweed Technologies (pp. 171-189): Elsevier.
8. Gomez, L., Tiwari, B., & Garcia-Vaquero, M. (2020). Emerging extraction techniques: microwave-assisted extraction. In Sustainable Seaweed Technologies (pp. 207-224): Elsevier.