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

15F647 - Incorporation of novel brewers' spent grain (BSG)-derived protein hydrolysates and blended ingredient in functional foods for older adults and assessment of health benefits in vivo.

Final Report

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

SUMMARY

Objectives: To optimise the extraction of BSG-derived fractions, i.e., proteins, phenolics, carbohydrates, fibre and lipids. To perform a detailed in vitro/in situ bioactivity analysis on the above extracts and select a test sample(s) for performance of an older adult human intervention trial. To investigate the potential mechanisms of action of the selected test sample(s).

Results: An integrated strategy was developed at laboratory-scale for the extraction of fractions enriched in BSG-derived phenolics, lipids, carbohydrates, and proteins/peptides. The lab- and semi-pilot scale samples were characterised using a range of in vitro and in situ bioassays to characterise their health-related biofunctional potential. The results, in conjunction with the outcomes from a focus group sensory evaluation, allowed identification of a peptide/phenolic-rich fraction (sample 3a) for further characterisation in a human intervention study. The selected sample was shown to be suitable, from a microbiological and toxicological perspective, for utilisation in a human study. While ethical approval was granted for the human intervention study, this was not performed due to the restrictions associated with the covid-19 pandemic. However, detailed in vitro/cell-based studies on sample 3a indicated its potential as a health enhancing ingredient.

Overall impact of project: A biorefinery-based strategy for the fractionation of different BSG components was successfully transferred from lab- to semi-pilot-scale yielding kg quantities of spray-dried prototype ingredients. The health-related value of one fraction (3a) was characterised using a range of in vitro/in situ assays. Advanced training was provided to a number of scientific personnel and the work has been published in peer reviewed papers.

KEYWORDS

Brewers spent grain, peptides, bioactive.

ACRONYM	BSG-NIVO
PROJECT COORDINATOR, INSTITUTION	Prof Richard J. Fitzgerald, University of Limerick.
EMAIL	dick.fitzgerald@ul.ie
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Section 1 - Research Approach & Results

Start Date

01 April 2017

End Date

30 June 2021

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 5: Technology validated in relevant environment.

NRPE Priority area

Food for Health

Total DAFM Award

€689,136.80

Total Project Expenditure

€549,362.27

Rationale for undertaking the Research

Approximately 3.4 million tonnes of brewers' spent grain (BSG) are produced annually in the E.U., including 160,000 tonnes in Ireland. Food Research Ireland 2020 highlighted the importance of exploiting food production side-streams in developing bioactive components and innovative food solutions, while stimulating competitiveness through co-product valorisation. Our previous in vitro studies have shown that BSG-derived protein hydrolysates and polyphenolic cofractions have antioxidant and anti-inflammatory activities. Given the recognised importance of the synergism between bioactive ingredients in functional food research, further investigation of these novel extracts, including their in vivo health effects, was warranted.

The Irish elderly population is projected to rise from 535,393 in 2011 to 1.4 million by 2046 (Central Statistics Office, 2013). The high prevalence of overweight and obesity (72.8% women, 83.5% men), cardiovascular disease risk (82.9% women, 78.9% men) and hypertension (48%) in the Irish elderly population (National Adult Nutrition Survey, 2011) is driving the demand for health-enhancing foods for this cohort. Furthermore, the healthy life year scores are lower for men and women in Ireland at age 65 years, than for other European countries (Dept. of Health and Children, 2010). Therefore, there is a potential market for the development of health enhancing foods/food ingredients for older Irish adults. In addition, this is supported by An Bord Bia's predictions on consumer trends, including responsible living, and health and wellbeing aided by converting food by-products into functional foods. The research proposed in this interinstitutional project therefore aimed to advance research in the area of BSG functional extracts.

Methodology

The research methodologies employed were as follows:

Task 1: Extraction/characterisation of BSG protein, phenolic acids, carbohydrates and lipids.
Involved application of solid-liquid separation procedures including enzyme-aided extraction using specific food-grade carbohydrase and proteinase preparations. Different fractions were obtained depending on treatment, e.g., aqueous soluble phenolic compounds were obtained following centrifugation of aqueous BSG suspensions. The procedures were transferred to semi-pilot scale.

Task 2: Bioactivity screening of BSG-derived fractions.
Carried out using a range of in vitro, e. g., antioxidant (ORAC, FRAP), angiotensin converting enzyme (ACE) inhibitory and dipeptidyl peptidase-IV (DPP-IV) inhibitory assays along with cell-based assays, e.g., reactive oxygen species (ROS) production in HepG2 cells, cytokine release in U937 and Jurkat T cells.

Task 3: Development of a range of functional foods for the elderly cohort.
Different approaches were investigated for the delivery of the selected biofunctional ingredient, i.e., inclusion in beverage and solid formats. A range of analyses were carried out to determine, e.g., impact of BSG ingredient substitution on the sensory and texture properties of muffins.

Task 4. Sensory evaluation of formulated functional foods.
This involved a focus group to determine the acceptability of the selected ingredient in a beverage format.

Task 5. Dietary intervention study to test physiological efficacy of functional foods.
This study planned the assessment of a range of biochemical (blood lipids, fasting glucose, IFN-γ, TNF-α, serum glutathione levels) and functional (weight, BMI, waist circumference, MUAC, blood pressure, muscle strength) indicators in an elderly cohort following consumption of the selected BSG containing test sample.

Task 6. In vitro validation of in vivo results and investigation of mechanisms of action.
This involved in vitro/cell-based studies to characterise the adipogenesis/adipolysis, satiety, immunomodulatory, antioxidative and LDL oxidation effects of the selected BSG ingredient(s)., e.g., release of incretins from STC-1, insulin release in BRIN-BD11 and bioavailability in Caco-2 cells.

Project Results

A detailed study was carried out to develop an integrated strategy for the generation of protein, carbohydrate, phenolic and lipid rich fractions from BSG. This was supported by a detailed proximate compositional analysis of the fractions obtained. These lab-scale protocols were successfully transferred to semi-pilot scale (starting with 400 kg BSG) yielding kg quantities of spray-dried extracts. Conclusion: BSG is an excellent source of protein, carbohydrate and phenolic extracts. The process developed has potential to transform a low value co-product to higher value functional extracts.
The bioactive properties of the extracts were assessed using in vitro and in situ (cell culture) assays. The different extracts obtained at both lab- and semi-pilot scale, along with their in vitro simulated gastrointestinal digests, were shown to display activities relevant to blood pressure control, glycaemic management, and potential anti-obesity effects. These preliminary screening assays allowed the selection of a BSG-derived prototype ingredient, containing proteins/peptides and phenolic compounds, for a human intervention study. Conclusion: the BSG extracts and their digests displayed promising effects on health biomarkers relevant to older adults.

An acute human intervention study was planned to study the physiological effects of a functional beverage with the selected BSG extract as a key ingredient. A thoroughly detailed study protocol was prepared and submitted to the Clinical Research Ethics Committee of the Cork Teaching Hospitals (CREC) in February 2020. A participant information leaflet/ informed consent form was prepared to ensure that all participants in the study had a detailed understanding of the study. Full approval to carry out the study was granted by CREC in April 2020. Conclusion: A specific BSG preparation was selected for a human intervention study which was manufactured under good manufacturing conditions at semi-pilot scale. Microbiological and toxicological assessments demonstrated that this ingredient was suitable for performance of a human study. However, it was not possible to proceed with the human intervention study due to the Covid-19 pandemic restrictions.
A more detailed examination of the bioactive mechanisms of the selected BSG fraction was undertaken on the selected extract in cell culture models by assessing antioxidant, anti-inflammatory and anti-adipogenic effects as well as intestinal absorption, in the absence of data from the human intervention trial. This involved assessment of the extract before and after simulated gastrointestinal digestion. Conclusion: The selected extract reduced the triglyceride content in a mouse cell model of adipogenesis and increased adipolysis indicating that the extract could have the potential to reduce obesity. The extract did not demonstrate any significant anti-inflammatory effects under the conditions of the study. The extract showed some protection against the oxidative effects of glucose and fatty acids in a human liver cell model. Furthermore, the extract demonstrated insulinotropic effects in pancreatic cells while also displaying dipeptidyl peptidase-IV inhibitory effects.

Overall conclusion: The project's outcome was somewhat limited by the absence of data from the human trial. However, extracts of BSG demonstrated antioxidant, anti-adipogenic and glycaemic management effects in cell models and consequently have potential as functional ingredients to benefit the older adult health.

Section 2 - Research Outputs

Summary of Project Findings

The project has led to the elucidation of an integrated strategy to extract different fractions from BSG which was scaled up to semi-pilot scale. To date, the literature mainly focuses on the extraction of single ingredients from BSG and does not adopt a biorefinery approach to value addition. This added value arises by way of extracting different fractions enriched in phenolics, proteins/peptides and carbohydrates for which specific market outlets exist. This has significant potential from an end-user perspective and represents the elucidation of a sustainable approach for industry to extract additional value from BSG. Novel preliminary information has been generated in relation to the health related biofunctional value of different BSG fractions, i.e., in terms of their antioxidant and potential anti-obesity effects. Peer reviewed papers have been published expanding our knowledge of the biofunctional value of BSG-derived components. The knowledge generated has potential to inform policymakers on the scientific feasibility of valorising different health enhancing fractions from BSG.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
Post Doctorates	0	2	2
MSc Students	0	1	1

Summary of Academic Outputs

Research	Total	Details
Peer Reviewed Conference Papers	11	1. Connolly, A., Cermeño, M., Crowley, D., O'Callaghan, Y., O'Brien, N. M., & FitzGerald, R. J. Characterisation of the in vitro bioactive properties of alkaline and enzyme extracted brewers' spent grain protein hydrolysates. Food Research International 2019, (121), 524-532. https://doi.org/10.1016/j.foodres.2018.12.008) 2. Cermeño, M., Connolly, A., O' Keeffe M., Flynn C., Alashi A., Aluko & FitzGerald, R. J. Identification of bioactive peptides from brewers' spent grain and contribution of Leu/Ile to bioactive potency. Journal of Functional Foods 2019, (60) 103455. 3. Connolly, A.; Cermeño, M.; Alashi, A. M.; Aluko, R. E.; FitzGerald, R. J. Generation of phenolic-rich extracts from brewers’ spent grain and characterisation of their in vitro and in vivo activities. Innovative Food Science and Emerging Technologies 2021 (68), 102617. 4. Cermeño, M.; Dermiki M.; Kleekayai T.; Cope L.; McManus R.; Ryan C.; Felix M.; Flynn C.; FitzGerald, R. J. Effect of enzymatically hydrolysed brewers’ spent grain supplementation on the rheological, textural and sensory properties of muffins. Future Foods 2021 (4) 100085 5. Cermeño, M.; Connolly, A.; Kleekayai, T.; FitzGerald, R. J., Encapsulation of enzymatic-aided phenolic acid rich extracts from

		brewers' spent grain with different wall materials and assessment of their stability and bioactivities. In preparation. 6. Cermeño, M.; Santos-Hernandez, M.; FitzGerald, R. J., Brewers' spent grain peptides with antidiabetic potential. In preparation. 7. Cermeño, M., Connolly, A., O' Keeffe M., Alashi A., Aluko & FitzGerald, R. J. Identification of bioactive peptides from brewers' spent grain and contribution of Leu/Ile to bioactive potency. Abstract of an oral presentation presented at the 2ND INTERNATIONAL SYMPOSIUM ON BIOACTIVE PEPTIDES, May 22-24th 2019 in Valencia, Spain. 8. Cermeño, M., Dermiki M.; Kleekayai T.; Cope L.; McManus R.; Ryan C.; FitzGerald, R. J. Incorporation of brewers' spent grain ingredients into muffins and assessment of their rheological, textural and sensory properties. Abstract of a poster presentation presented at the NIZO Plant Protein Functionality Conference 21-22 October 2020 (Online). 9. Cermeño, M., Connolly, M., McCarthy A., Aluko & FitzGerald, R. J. Generation and characterisation of phenolic rich extracts from brewers' spent grain with hypotensive properties. Abstract of an oral presentation presented at the XX EuroFoodchem Conference, June 16-19th 2019 in Porto, Portugal. 10. Cermeño, M., O' Keeffe & FitzGerald, R. J. Cell-based assessment of the bioavailability of brewers' spent grain derived peptides. Abstract of an oral presentation presented at the ISNFF Conference, December 1-5th 2019 in Kobe, Japan. 11. O'Brien, S., O'Halloran, F., McCarthy, A.L. (2018) Comparing the antioxidant properties of digested BSG-derived protein and phenolic fractions and investigating synergistic effects of digestate combinations. 47th Annual Food Science and Technology Conference, 6-7 December 2018, UCC, Cork
Masters Theses	1	Suzanne O'Brien graduated from MTU in June 2021, with an MSc entitled 'Investigating the bioactivity of novel BSG-derived extracts and their potential as ingredients in functional foods for older adults'. The thesis details investigations of the antioxidant, immunomodulatory and anti-obesogenic properties of novel ingredients derived from brewer's spent grain, using cellular and non-cellular assays.

Intellectual Property

At this juncture, the integrated biorefinery-based strategy which has been elucidated for the extraction of phenolic, protein/peptide and carbohydrate enriched fractions is retained as confidential knowhow. The project partners are open for discussion with parties interested in licensing this knowhow while the patentability is being assessed.

Summary of other Project Outputs

Project Outputs	Details	Total No.
Patents	To date, no patents have arisen from this project, however, confidential know how has been developed which is available for examination by interested parties.	0

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Socio-Economic	The findings in this project demonstrate the industrial feasibility of upscaling the integrated extraction process developed within the project. The approach followed for the extraction makes use of so-called green technologies (e.g., enzymatic aided extraction) which are considered environmentally friendly. The adoption of this approach has potential to add significant value to BSG, a low value co-product of the brewing industry. The fact that the starting material is plant based, i.e., barley, has the potential to allow the generation of plant derived ingredients to serve the growing market for plant-based diets. This in turn is considered in keeping with UN SDGs. Furthermore, the BSG extracts generated herein have demonstrated potential health benefits through anti-oxidant, anti-obesity and glycaemic management effects and may find usage as functional ingredients for foods targeted to older consumers.

Dissemination Activities

Activity	Details
Seminars at which results were presented	The main outcomes from this project have been presented at internal and external conferences, i.e., at the annual Food Science and Technology conference at the UL Biological Sciences Research Day, and at international conferences, i.e., in Portugal, Spain, Canada and Japan

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	Knowledge outputs include (a) development of a strategy to biorefine fractions having different compositions from BSG, (b) development of protocols which can be successfully scaled from lab- to semi pilot-scale and (c) the development of preliminary information indication that a BSG fraction containing peptides and phenolics may have applications in, e.g., obesity management, e.g., via anti-adipogenic and via adipolytic activities. An acute human intervention study was designed and all the preparatory steps for the trial were completed, although it was not possible to proceed with the human trial, the methodology developed can be applied to future projects.
Identify any knowledge transfer activities executed within the project.	Knowledge transfer activities within the project include transfer, in strict confidence, of the detailed protocol for the generation of the different BSG fractions to the project's industrial partner. This has facilitated the semipilot-scale generation of different BSG fractions under GMP manufacturing conditions. Options now exist to transfer this knowledge to interested parties given that this research was public good funded research.
List any impacts resulting from the knowledge transferred during the project.	The impact of transferring the knowledge of the fractionation process to the industrial partner has facilitated the GMP manufacture of BSG prototype ingredients for further studies including the development of prototype ingredients for in vivo studies.

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Non-Exchequer National Funding	€95,000.00	UL has been invited to become a partner in the DAFM-funded U-Protein project. The knowledge generated during the BSG focused project and the associated publications can be utilised during the extraction and characterisation of proteins from other plant crops grown in Ireland in U-Protein. This additional funding currently supports an MSc student.

Future Strategies

Preliminary information shows that BSG-derived fractions have potential to modulate markers associated with noncommunicable diseases. Additional studies are required to further validate these findings. Therefore, it is envisaged that future funding applications from the consortium will address the valorisation of the health beneficial effects, initially during the course of small animal studies. Furthermore, optimisation of the generation of different bioactive fractions can be addressed using in vitro/in situ bioassays along with confirmatory studies in small animals. Human intervention studies will then be targeted at valorisation of that BSG fraction displaying highest potential to enhance health in an older population grouping.

Project Publications

Main publications:

1. Connolly, A., Cermeño, M., Crowley, D., O'Callaghan, Y., O'Brien, N. M., & FitzGerald, R. J. Characterisation of the in vitrobioactive properties of alkaline and enzyme extracted brewers' spent grain protein hydrolysates. Food Research International 2019, (121), 524-532. https://doi.org/10.1016/j.foodres.2018.12.008.
2. Cermeño, M., Connolly, A., O' Keeffe M., Flynn C., Alashi A., Aluko & FitzGerald, R. J. Identification of bioactive peptides from brewers' spent grain and contribution of Leu/Ile to bioactive potency. Journal of Functional Foods 2019, (60) 103455. https://doi.org/10.1016/j.jff.2019.103455.
3. Connolly, A.; Cermeño, M.; Alashi, A. M.; Aluko, R. E.; FitzGerald, R. J. Generation of phenolic-rich extracts from brewers'spent grain and characterisation of their in vitro and in vivo activities. Innovative Food Science and Emerging Technologies 2021 (68), 102617.
4. Cermeño, M.; Dermiki M.; Kleekayai T.; Cope L.; McManus R.; Ryan C.; Felix M.; Flynn C.; FitzGerald, R. J. Effect ofenzymatically hydrolysed brewers' spent grain supplementation on the rheological, textural and sensory properties of muffins. Future Foods 2021 (4) 100085.
5. Cermeño, M.; Connolly, A.; Kleekayai, T.; FitzGerald, R. J., Encapsulation of enzymatic-aided phenolic acid rich extractsfrom brewers' spent grain with different wall materials and assessment of their stability and bioactivities. In preparation, expected submission date early 2023.
6. Cermeño, M.; Santos-Hernandez, M.; FitzGerald, R. J., Brewers' spent grain peptides with antidiabetic potential. Inpreparation, expected submission date early 2023.

Associated publications:

1. Kleekayai, T.; Amigo-Benavent, M.; Khalesi M.; Harnedy P.; Cermeño, M. and FitzGerald, R. J. Enzyme-aided extraction of plant proteins and peptides in Green Protein Processing Technologies from Plants. Springer. Accepted, corrected proof.
2. Bilir, G.; Khalesi, M.; Cermeño, M.; Ekinci, D., I.; FitzGerald, R. J., Extraction and characterisation of protein isolates from Limpet (Patella vulgata) and peptide release following gastrointestinal digestion. Journal of Agricultural and Food Chemistry 2022 70 (36) 11212–11223. https://doi.org/10.1021/acs.jafc.2c00578.
3. Cermeño, M.; Bascon, C.; Amigo-Benavent, M.; Felix M., FitzGerald, R. J., Identification of peptides from edible silkwormpupae (Bombyx mori) with antioxidant potential activity. Journal of Functional Foods 2022 92 (105052). https://doi.org/10.1016/j.jff.2022.105052
4. Santos-Hernandez, M.; Cermeño, M.; Recio, I.; FitzGerald, R. J., In vitro dipeptidyl peptidase IV inhibitory activity and insitu insulinotropic activity of milk and egg white protein digests. Food & Function 2021 12(24): 12372-12380. https://doi.org/10.1039/D1FO00641J
5. Felix, M.; Cermeño, M.; FitzGerald, R. J., Structure and in vitro bioactive properties of O/W emulsions generated withfava bean protein hydrolysates. Food Research International 2021 (150), 110780. https://doi.org/10.1016/j.foodres.2021.110780.
6. Pimentel, F. B.; Cermeño, M.; Kleekayai, T.; Machado, M.; Machado, S.; Fernandes, E.; Alves, R. C.; Oliveira, M. B. P. P.; FitzGerald, R. J., Enzyme-Assisted release of antioxidant peptides from Porphyra dioica conchocelis. Antioxidants 2021 10(2), 249. https://www.mdpi.com/2076-3921/10/2/249.
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10. Pimentel, F. B.; Cermeño, M.; Kleekayai, T.; Harnedy-Rothwell, P. A.; Fernandes, E.; Alves, R. C.; Oliveira, M. B. P. P.;FitzGerald, R. J., Enzymatic Modification of Porphyra dioica-Derived Proteins to Improve their Antioxidant Potential. Molecules 2020, 25 (12), 2838. https://www.mdpi.com/1420-3049/25/12/2838
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