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

Food Institutional Research Measure

Final Report

'Technologies for the enrichment and recovery of novel bioactive ingredients from plant food processing wastes (NovTechIng)'

DAFM Project Reference No: 11/F/050

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End Date: 30/09/2018

Principal Coordinator and Institution: Dr. Dilip Rai, Teagasc Food Research Centre Ashtown, Dublin, D15JN3K, Ireland. Email: dilip.rai@teagasc.ie

Collaborating Research Institutions and Researchers:
Prof. James Lyng, University College Dublin, School of Agriculture and Food Science, Belfield, Dublin 4, Ireland. james.lyng@ucd.ie

Prof. Nigel Brunton, University College Dublin, School of Agriculture and Food Science, Belfield, Dublin 4, Ireland. nigel.brunton@ucd.ie

Prof. Nissreen Abu-Ghannam, TU Dublin - School of Food Science and Environmental Health, Grangegorman, Dublin 7, Ireland. nissreen.abughannam@TUDublin.ie

Basic/Fundamental → Applied → Pre Commercial

1	2 x	3	4	5	6	7

Priority Area (s)	(1) I: 'Sustainable Food Production and Processing'. (2) M: 'Processing Technologies and Novel Materials', (3) L: 'Manufacturing Competitiveness'

Key words: *(max 4)*

Glycoalkaloids, β-glucans, chitins, Novel Technologies

1. Rationale for Undertaking the Research

Valorisation of by-products from food processing has long been considered crucial for sustainability, food-security and mitigation of environmental degradation. Post-processing by-products, which constitute as much as one-third of the raw-food, account as a reservoir of valuable nutraceuticals. However, one of the major bottlenecks to exploiting the high value compounds contained in the by-products is the lack of cost effective and sustainable technologies to extract them. This project explored the biological (enzyme-assisted) and novel physical technologies (ultrasound and pulsed electric field) to recover targeted high value molecules (β-glucans from spent grains, glycoalkaloids from potato peels, and chitins from mushroom stalks). Furthermore, stability and toxicity of the extracts were evaluated in order to establish the potential usage in food and phyto-pharmaceutical applications. Last but not the least, a cost-benefit analysis from the raw material stage to the final product (extract) was also performed to assess the feasibility of the process for commercial applications.

2. Research Approach

A number of extraction methodologies were applied and optimised, in particular the biological (i.e. enzymes) and novel physical methodologies (ultrasound and pulsed electric field (PEF)), on stand-alone or in tandem for each of the targeted biomolecules proposed in this project. Response surface methodology (Design-Expert 9.0.2), which is a statistical tool was employed to determine the optimum levels of extraction parameters. These parameters included temperature, pH, the percentage of enzymes for biological assisted extraction; amplitude, time and solid/liquid ratio for the ultrasound assisted extraction; and electric field strength, PEF exposure time, and energy for the PEF-assisted extraction. Additional experiments involving the fluorescent light were performed to demonstrate the maximum effect of physical technologies was on the fresh and not on the freeze-dried samples. Mass spectrometry based methods were adapted (and validated) for measuring the quantity of the target molecules in the different biomass substrates. A previously described spectrophotometric method for the quantification of chitin was also set up in this project. Thermal stability experiments of the target molecules to elucidate whether each of the selected molecules would benefit the use of non-thermal methods to aid in the preservation of foods at high temperatures and different pHs reflecting the conditions that occur during pasteurisation and sterilization of common foodstuffs were also performed. Finally, the safety of the polysaccharide-rich extracts produced from the biological and physical-assisted methods were evaluated employing an arsenal of *in-vitro* assays such as MTT assay, Alamar Blue and Neutral Red assays using a human melanoma cell line (Me45), while the measurement of reactive oxygen species was carried out using fluorescence based dichlorofluorescin diacetate method.

3. Research Achievements/Results

The achievements can be divided into three different scientific areas based on project tasks as outlined below:

Extraction Methods:

- A set of optimized methods for the extraction of β-glucans from brewer's spent grains were developed. Conventional solid-liquid extraction method resulted in a β-glucan extraction yield of 57.98%. Ultrasound assisted extraction gave a higher β-glucan extraction yield (66.9%) at a shorter extraction time; however the β-glucan extracted had a lower molecular weight than the conventional method. The fermentation (biologically-

assisted) method using *Lactobacillus plantarum* increased the β-glucan yield by 3-fold than the conventional extraction method.

- Amongst the different optimized methods for the extraction of glycoalkaloids from potato peels, the best yield (247mg/kg of dried peels) was from the conventional solid-liquid extraction at 80° in 30 min. While the ultrasound and pulsed electric field could extract between 154 and 159 mg/kg total glycoalkaloids dry weight.
- An industrially relevant extracting protocol for the isolation of chitin from mushroom stipes was developed and optimized. The optimized chemical method involving deproteination and demineralization can extract up to 33% chitin, of which less than 1% was chitosan. Through the application of proteases, the chitin yield increased up to 44%, which was slightly higher than the fermentation process (~40%).
- The application of biological or novel physical methods did not increase yield of chitins from the mushroom stalks when applied alone, and therefore their use in sequence was not examined. In contrast, the use of ultrasound to increase yields of β-glucans from BSG was successful, however PEF delivered no additional benefit. For potato peels, neither PEF alone nor a combination of PEF and US increased yields of glycoalkaloids in comparison to US alone.

Analytical Methods:

- A validated UPLC-MS/MS quantification method of specific glycoalkaloids was developed and published in *Journal of Chromatography* B.
- MALDI-Q-TOF quantification of large polysaccharides (β-glucans, chitins) was limited by the upper limit of the quadrupole (Q) mass analyser, which had the maximum threshold of 24 kDa. Studies on the hydrolysed extracts lacked reproducibility of the quantification data due to lack of homogeneity of matrix.
- Alternative rapid colourimetric methods using Congo red for intact β-glucans (35.6 kDa to 691 kDa) and hydrolysed chitin were also set-up and applied on real samples. However, it was noted that high-purity β-glucan extract devoid of starch contamination and/or free sugars is required for the specific quantification of β-glucans.
- The UPLC-MS/MS quantification methods for hydrolysed β-glucan (monomeric glucose unit) and hydrolysed chitins (monomeric glucosamine unit) were developed. Method development for β-glucan required hydrolysis followed by derivatisation of glucose to i.e. penta-acetyl-glucose-methyloxime.

Bioactivity and Stability of the extracts:

- Cytotoxicity testing showed that both β-glucan and chitin extracts showed no cytotoxic effects on MTT assays involving gastrointestinal cells. The conventional extraction achieved similar impacts or even less of an impact in some cases when compared to both enzymatic and fermentation processes. This is a positive outcome suggesting that green methods of extraction approaches could produce extracts with a higher antitumour activity as compared to conventional solid-liquid extraction methods. Cytotoxicity variations between enzymatic and fermentation approaches were minimal for shorter incubation times (6 hrs.), however, variations became more pronounced on cell viability as incubation time (48 hrs.) increased. Fermentation increases the rate of bioactive peptides, γ -aminobutyric acid and natural phenolics, which are known to possess antitumour activity.
- Reactive oxygen species formation from β-glucan was more than half of that observed for chitin extracts. This is somehow expected as β-glucan has a higher antioxidant capacity than chitin extracts.

- All three target molecules (i.e. β-glucans, chitins and glycoalkaloids) exhibited strong thermal stability when exposed to conditions used to preserve foods using conventional thermal processing.

Cost-Benefit Analysis:

- A comprehensive report (Blueprint) for each matrix has been generated based on the operating costs (both fixed (i.e. maintenance, operating labour, laboratory) and variable (i.e. estimates of raw materials, utilities etc.)). Particular emphasis was placed on accurately calculating energy consumption and efficiency due to the relationship between fossil fuel consumption, pollution and the environment.
- Responses and benefits from the application of novel technologies varied between matrices and were influenced by the scale of production.
- For β-glucans, conventional extraction would appear to be a viable proposition, but only at a medium or larger scale production. The incorporation of ultrasound into the β-glucan extraction process on first impressions seem extremely promising with process time reduced to one-third of conventional process time (10 min. vs 30 min.) and extraction yield increased (+17.5% vs conventional extraction). These benefits appear to be significant and not "marginal gains", therefore the incorporation of an ultrasound step yields many benefits for the processors.
- For glycoalkaloids, medium and larger scale conventional extraction protocols see very viable options for producers. On initial impressions, the incorporation of ultrasound (US) seemed to demonstrate some benefits in terms of reducing extraction time and thermal energy input. This suggests gains in process intensification from this technology. However, these benefits were significantly offset by the fact that the optimal US conditions examined yielded lower glycoalkaloids and when viewed in the context of the overall economic evaluation, the benefits of US incorporation were not enough to conclude that the incorporation of US was profitable at any scale of production. By contrast, pulsed electric field (PEF) was demonstrated to be extremely viable when applied at medium and large-scale throughputs. PEF requires lower energy inputs and gives significantly higher yields and profits at those scales compared to conventional extraction.
- For the chitins, findings from this study and other researchers strongly suggested that the application of either US or PEF would be severely disruptive to the functionality of the chitin. It is unlikely that either technology would have shortened the process time (which was already extremely short), and while they may have negative impact on functionality ruled out their incorporation into the extraction processes. However, on a more positive note, a thorough evaluation of the conventional extraction process did show that this procedure is quite viable at medium and larger scale but not at small scale.

4. Impact of the Research

Industry:

Processors of potatoes and mushrooms, and beverage industries generate a large amount of by-products in the form of peels, stipes (stalks), and spent grains, respectively. The by-products from these industries amount between 70 thousand tons and 39 million tons per year worldwide. As alluded earlier, the by-products can serve as sustainable sources of high value compounds, such as glycoalkaloids from potato peels, chitins from mushroom stalks and β-glucans from brewers' spent grains. For the agri-food processing and beverage industries, the valorisation of the by-products for high value compounds will not only reduce their disposal cost, but also provide added value of the by-products through the downstream processors for food and health applications. This project provides useful information on physical, chemical and biological methods of extraction of these

valuable ingredients, and combination of this knowledge with safety (non-cytotoxicity) and thermal stability of target molecules, and economic evaluation of the extracts present opportunities for food ingredient suppliers and manufacturers of healthy prepared foods. Furthermore, the biological activities studied in this research, in particular the anti-tumour against melanoma cells and anti-inflammatory potentials of spent grain and mushroom-stalk extracts make them highly attractive not only for functional food applications but also with possible pharmaceutical applications that can be further explored.

Similarly, analytical capabilities to detect and quantify potato glycoalkaloids, β-glucans in spent grains, chitins in mushroom stalks, as well as other co-extracted biomolecules will be useful for farmers, food processors and food regulatory agencies. For example, European Food Safety Authority (EFSA) has been turning their attention to monitoring glycoalkaloids in potato cultivars in recent years because of the health risks associated with consumption of potato glycoalkaloids. Already, the validated analytical method developed in this project has been used in screening new seedlings from the Potato Breeding Programme based in Teagasc Oak Park, Carlow. Potato growers and processors can also avail this service should EFSA and/or FSAI introduce maximum glycoalkaloid threshold for consumer's safety.

Consumers:

This was a food and beverage industry focussed project providing innovative solutions for processors to valorise their high volume by-products. Nevertheless, a number of outcomes are relevant to consumers. For example, the project served to inform the consumers the hidden high-valued ingredients in the agri-food processing by-products, which many consumers may not have been aware-of. In addition, potato peels and mushroom stalks examined in the project are also produced in domestic environment. The project, thus served to highlight both the potential value of these discards to consumers and that the nutritional and functional values of the discards. The project outcomes also showed anti-inflammatory and anti-tumour (against melanoma) activities of the extracts rich in β-glucans and chitins, and their possible inclusion in functional foods or pharmaceutical agents would impart positive health benefits on the consumers.

Regulatory authorities:

Amended Directive (EU) 2018/851 on the EU waste management (2008/98/EC) puts strong emphasis on producers' and organisational responsibility to contribute to waste prevention through reusability and recyclability of the products. A 50% reduction on food waste generation along the production and supply chains is aimed to meet the UN Sustainable Development Goal by 2030. The directive also highlights examples of incentives to apply the waste hierarchy, such as landfill and incineration charges and pay-as-you-throw schemes. Segregation of high volume organic waste by food processors would also make it more suitable for application of some of the approaches put forward here to allow processors to not only comply with legislation but to do so in a profitable manner.

Recently on April 2022, the EU commission on monitoring the presence of glycoalkaloids in potatoes and potato-derived products has adopted the recommendations (EU) 2022/561 that each EU Member State with the active involvement of food business operators should monitor glycoalkaloids in potatoes and potato products. The recommended methods of glycoalkaloid analysis are liquid chromatography with ultraviolet photodiode-array detection (LC-UV-DAD) or liquid chromatography mass spectrometry (LC-MS). To this, the project has developed and validated an LC-MS quantification method for the glycoalkaloids, and the analysis service can be accessed by potato growers and processors as well as state regulatory authorities (i.e. FSAI).

In addition, the information on the impact of novel processing on biomolecules in this matrices serve as scientific base for the processors and policy makers to frame guidelines on the use of novel processing technologies.

Scientific community:

Selection of the most sustainable extraction technique for food by-products depends on the type of the biomass and the high value compound to be recovered from it. This project showed the viability and benefits of conventional extraction methods when applied to side streams at certain scales. It also showed the gains that can be achieved from the incorporation of either biological or novel technologies into such extraction processes. However, the project also is unique in providing a rigorous evaluation of their potential application of these extractions at commercial scale.

The project developed methods for detection and quantification of glycoalkaloids, glucosamine (monomeric unit of chitins), β-glucans and polyphenols. High throughput UPLC-MS/MS quantification method for glycoalkaloids has been validated and has been used as and when required by the Potato Breeding Programme. The analytical methods set-up have been used to assess the impact of sequential novel processing technologies such as pulsed electric field and ultrasound targeted high-value biomolecules will have on the co-extracted compounds. Furthermore, the co-extracts rich in β-glucans and chitins demonstrated anti-inflammatory activity. In line with other research studies, the MALDI-Q-TOF quantification of targeted β-glucans and chitins has been challenging due to matrix inhomogeneity.

It is critical that food ingredients proposed to be incorporated in food are primarily safe, especially if obtained under extraction conditions that are novel and are not considered as common practice. There is little reporting on cytotoxicity testing methodologies of such applications in literature specifically for food. To this, the project has established relevant protocols, in addition to knowledge and skills, and so leveraging the capacity to participate in future work related to cytotoxicity of food ingredients.

To date very little research has been conducted on the thermal stability of β-glucans, glycoalkaloids and chitins. This is most likely due to the fact that most researchers have assumed based on the structure of these molecules that they were thermally stable, however, to the best of our knowledge this has not been proven. The outcomes of this milestone have therefore been the first to provide critical evidence for the thermal stability of these molecules when exposed to conditions used to preserve foods using conventional thermal processing.

Two post-docs, one research assistant and two PhD students in the project were trained in the research methodologies.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

The project facilitated the formation of collaborative links with Dr. Denis Griffin, a principal research officer and expert in the cultivation and breeding in potatoes. The collaboration arose because of the need to monitor the levels of glycoalkaloids in the research trials of new potato cultivars at Teagasc Oak Park. More than 100 samples have been analysed so far from the Potato Breeding Programme.

In recognition of our work on the recovery of chitin from mushroom stalks in this project the group was contacted by Prof. Kevin O'Connor who was leading a bid to fund an SFI Centre grant on the Bioeconomy. This collaboration eventually lead to successful funding for the BiOrbic SFI centre. In this project, members of group worked on a targeted project with an industrial partner (Co-Operative Mushroom Producers) on the development for the recovery of valuable components from

fungal mycelia. The success of BiOrbic 1 has led to the confirmation of finding for the second phase and members of the project group are part of the successful consortium to be granted funding.

Our knowledge and skills from the work on the brewer's spent grains have leveraged the funding of Teagasc Walsh Scholarship and collaborated with Prof. Peter Wilde at Food Innovation and Health Programme, Quadram Institute, Norwich, UK. The Walsh Scholar recently defended a PhD thesis entitled "Enrichment of brewer's spent grain polyphenols and assessment of their role in inhibition of cholinesterases, amylase and glucosidase" from the University of East Anglia, Norwich. Similarly, this also has leveraged an on-going Teagasc Research Leaders Fellowship co-funded by Teagasc and the EU Marie Skłodowska-Curie grant on the research project entitled "Innovative plant-based functional beverages from brewers´, where the Marie-Curie fellow was hosted by Prof. Rossana Coda at University of Helsinki.

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

As a task of the project, we developed a validated method for the quantification of glycoalkaloids in potato peels. This method was published in the peer-reviewed journal as detailed below:

- Hossain, M. B., Rai, D.K., Brunton, N.P. (2015). Optimisation and validation of ultra-high performance liquid chromatographic-tandem mass spectrometry method for qualitative and quantitative analysis of potato steroidal alkaloids. *Journal of Chromatography* B 997:110-115.

The establishment of a validated method for the quantification of potato glycoalkaloids is key importance to potato producers in Ireland as it can be used to ensure the safety of this crop by ensuring that it does not contain toxic glycoalkaloids at a level above the toxic dose for humans.

(iii) Outcomes with economic potential

A number of novel protocols for the recovery of components with potential economic value were developed as part of the project. For example, in the publication below a method for the recovery of potato glycoalkaloids using pressurised liquid extraction was developed. This has potential economic value as the recovered glycoalkaloids could be used as the raw material for the manufacture of value phyto-pharmaceuticals.

- Hossain, M.B., Rawson, A., Aguiló-Aguayo, I., Brunton, N.P., Rai, D.K. (2015). Recovery of steroidal alkaloids from potato peels using pressurized liquid extraction. *Molecules* 13;20(5):8560-73.

We have also developed methods for the recovery of valuable components from various matrices using novel technologies as outlined in the publications below. These technologies increase the sustainability of the recovery methods, which is very much in line with Ireland's ambition to develop a vibrant circular bioeconomy.

Glcoalkaloids:

- Hossain, M.B., Tiwari, B.K., Gangopadhyay, N., O'Donnell, C.P., Brunton, N.P., Rai, D.K. (2014). Ultrasonic extraction of steroidal alkaloids from potato peel waste. *Ultrasonics sonochemistry* 21(4):1470-1476.

Polyphenols:

- Frontuto, D., Carullo, D., Harrison, S. M., Brunton, N. P., Ferrari, G., Lyng, J. G., Pataro, G. (2019). Optimization of pulsed electric fields-assisted extraction of polyphenols from potato peels using response surface methodology. *Food and Bioprocess Technology* 12, 1708-1720.
- Kumari, B., Tiwari, B.K., Hossain, M.B., Rai, D.K., Brunton, N.P. (2017). Ultrasound-assisted extraction of polyphenols from potato peels: profiling and kinetic modelling. *International Journal of Food Science and Technology* 52(6), 1432-1439.
- Kumari, B., Tiwari, B.K., Walsh, D., Griffin, T.P., Islam, N., Lyng, Brunton, N.P., Rai, D.K. (2019). Impact of pulsed electric field pre-treatment on nutritional and polyphenolic contents and bioactivities of light and dark brewer's spent grains. *Innovative Food Science & Emerging Technologies*. 54:200-210.

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

The method for quantification of potato glycoalkaloids could be used to put together a National monitoring plan for glycoalkaloids in potatoes (such as those that exist for other valuable commodities) thus protecting Irish potato producers and industries (e.g. potato crisp and chip industries).

4 (b) Summary of Research Outputs

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

1. Hossain, M.B., Rai, D.K., Brunton, N.P. (2015). Optimisation and validation of ultra-high performance liquid chromatographic-tandem mass spectrometry methods for qualitative and quantitative analysis of potato steroidal alkaloids. *Journal of Chromatography* B 997, 110-115.
2. Hossain, M.B., Rawson, A., Aguiló-Aguayo, I., Brunton, N.P., Rai, D.K. (2015). Recovery of steroidal alkaloids from potato peels using pressurized liquid extraction. *Molecules* 13;20 (5):8560-73.
3. Hossain, M.B., Brunton, N.P., Rai, D.K. (2016). Effect of drying methods on the steroidal alkaloid content of the potato peels, shoots and berries. *Molecules* 21(4), 403.
4. Kumari, B., Tiwari, B.K., Hossain, M.B., Rai, D.K., Brunton, N.P. (2017). Ultrasound-assisted extraction of polyphenols from potato peels: profiling and kinetic modelling. International Journal of Food Science and Technology 52(6), 1432-1439.
5. Dellarosa, N., Frontuto, D., Laghi, L., Dalla Rosa, M., Lyng, J.G. (2017). The impact of pulsed electric fields and ultrasound on water distribution and loss in mushrooms stalks. *Food Chemistry* 236, 94-100.
6. Kumari, B., Tiwari, B.K., Hossain, M.B., Brunton, N.P., Rai, D.K. (2018). Recent advances on application of ultrasound and pulsed-electric-field technology in the extraction of bioactives from agro-industrial by-products. *Food and Bioprocess Technology*, 11(2), 223-241.
7. Ravindran, R., Jaiswal, S., Abu-Ghannam, N., Jaiswal, A.K. (2018). A comparative analysis of pretreatment strategies on the properties and hydrolysis of brewers' spent grain. *Bioresource Technology* 248, 272-279.
8. Abu-Ghannam, N., Balboa, E. (2018). Biotechnological, food, and health care applications. In Sustainable Recovery and Reutilization of Cereal Processing By-Products, ed. C. M. Galanakis, Woodhead Publishing, pp 253-278.

9. Kumari B, Tiwari, B.K., Walsh D., Griffin P.G., Islam N., Lyng J.G., Brunton, N. P., Rai, D.K. (2019). Impact of pulsed electric field pre-treatment on nutritional and polyphenolic contents and bioactivities of light and dark brewer's spent grains. *Innovative Food Science and Emerging Technologies* 54, 200-219.
10. Frontuto, D., Carullo, D., Harrison, S. M., Brunton, N. P., Ferrari, G., Lyng, J. G., Pataro, G. (2019). Optimization of pulsed electric fields-assisted extraction of polyphenols from potato peels using response surface methodology. *Food and Bioprocess Technology* 12, 1708-1720.

(ii) Popular non-scientific publications and abstracts including those presented at conferences

1. Jaiswal, A.K. & Abu-Ghannam, N. (2014). 'Enzymatic Extraction of High-value Ingredients from Food Waste' at Waste not Want not - Recovering value from food waste workshop, Teagasc Food Conference Centre Ashtown, Dublin, 7th February 2014.
2. Jaiswal, A.K. & Abu-Ghannam, N. (2014). Optimization of Enzymatic Hydrolysis of Cellulose and Hemicellulose from Brewers' Spent Grain. 7th International conference and Exhibition on Nutraceuticals and functional foods, Istanbul, Turkey. 14-17 October 2014.
3. Kumari, B., Hossain, M.B., Tiwari, B.K., Rai, D.K., Brunton, N.P. (2014). Ultrasound Assisted Extraction of Bioactive Polyphenols from Potato Processing By-products. 43rd Annual Food Research Conference, UCD, Dublin, IRELAND, 10-11 December 2014.
4. Kumari, B., Hossain, M.B., Brunton, N.P., Rai, D.K. (2015). Effect of novel extraction technology on phenolics of commercially processed potato-peel. 7th International Conference on Polyphenols, Tours, France, 27-30 October 2015.
5. Kumari, B., Hossain, M.B., Tiwari, B.K., Rai, D. K., Lyng, J., & Brunton, N. (2016). Novel approaches for effective valorisation of agro industrial wastes: Extraction of polyphenols from potato peel and brewers' spent grain assisted by ultrasound and pulsed electric fields. International Union of Food Science and Technology (IuFoST).21-25 August 2016.Royal Dublin Society (RDS), Dublin 4, Ireland (Oral).
6. Kumari, B., Islam, N., Walsh, D., Rai, D.K., & Brunton, N. (2016). Pulse electric field (PEF) assisted extraction of novel bioactive preparations from brewer's spent grain (BSG): 1st International Conference on Food Bioactives and Health (FBHC), 13th to15th September, Norwich, UK. (Travel Grant awarded by FBHC).
7. Kumari, B., Hossain, M., Brunton, N., Rai, D.K. (2017). Analysis of β-glucan from brewers' spent grain by UHPLC-MS/MS method" 2nd International Summer School on Natural Products (ISSNP), 3rd to 7th July, Naples, ITALY. (Oral).
8. Kumari, B., Brunton, N.P., Rai, D.K., Tiwari, B.K. (2017). Ultrasound assisted extraction of bioactive enriched fractions from button mushroom stalks by-product. 17th International Conference on Food & Nutrition (ICFN), 22nd to 24th May, Nevada, USA.(Oral)
9. Patra, N., Abu-Ghannam, N. (2018). Statistical optimization of chitin extraction from mushroom wastes by lactic acid fermentation in a stirred tank bioreactor. Biotech France 2018 International Conference and Exhibition, Paris – France, 27-29 June 2018.

(ii) National Report

None.

(iii) Workshops/seminars at which results were presented

1. PEF School on Food Processing 21st-24th March 2016 – Dublin (UCD). http://foodpefschool.ucd.ie/
2. Expertise on analytical methodologies presented at the Teagasc Gateways event held at Teagasc Moorepark, Fermoy, Cork on the theme: "Advanced Methodologies for the Food Industry" on 16th November 2017.
3. Following blueprints were presented at the stand at the Teagasc Food Gateways event- Beyond Brexit - Making Food Innovation Go Further, Ashtown, Dublin on 12th June 2018:
    a. Lyng J. and Brunton N. (2018). Valorisation of mushrooms by-products by isolating chitin from mushroom stipes: optimization of extraction parameters.
    b. Abu-Ghannam N. (2018). β-glucan extraction from brewers' spent grain, the main brewery by-product.
    c. Rai, D.K. (2018). Glycoalkaloids in potatoes: a validated quantification method.

(iv) Intellectual Property applications/licences/patents

None.

(v) Other

None.

5. Scientists trained by Project

Total Number of PhD theses: 2

Kumari, B. 'Investigation of the effect of novel extraction technologies on the level of phytochemicals in potato peels, brewer's spent grains and mushroom stalks'. Thesis awarded by University College Dublin, January 2019.

Frontuto, D. 'The use of ultrasound and pulsed electric fields for the extraction of bioactive compounds from plant food processing waste'. University College Dublin – Not submitted.

Total Number of Masters theses: 0

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
Teagasc	Dilip Rai	0.88
UCD	James Lyng	0.40
UCD	Nigel Brunton	0.40
UCD	Michael Cooney	0.40
UCD	Eamon Power	0.20
TUD (DIT)	Nissreen Abu-Ghannam	0.50
Total	**6**	**2.78**

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	9	10.57
PhD students	2	4.05
Masters students	-	-
Temporary researchers	-	-
Other	-	-
Total	**11**	**14.62**

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended
Bibha Kumari	Science writing for Agri Food research – April 2016.
Bibha Kumari	New Idea to New Venture in the Food Sector: Entrepreneurship and Innovation
Bibha Kumari	Leadership skills for the Agri-food sector – January 2016.
Bibha Kumari	Hot Topics – Bio-encapsulation.
Daniele Frontutu	Leadership skills for the Agri-Food Sector – January 2017
Daniele Frontutu	Statistics for Agri-Food Researchers – August 2017

9. Project Expenditure

Total expenditure of the project: €760,162.73

Total Award by DAFM: €799,870.48

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

1. Teagasc Walsh Scholarship fund for Bibha Kumari €22,000/-
2. Travel grant award from FBHC, Norwich, UK. €340.00

Breakdown of Total Expenditure

Category	Teagasc	University College Dublin	Technological University Dublin (erstwhile DIT)	Total
Contract staff	148,541.42	0.00	134,177.50	282,718.92
Temporary staff	0	0	0	0.00
Post doctorates	0	142763.29	0	142,763.29
Post graduates	43,999.98	45,537.37	0	89,537.35
Consumables	13,465.73	14,371.34	23,228.39	51,065.46
Travel and subsistence	4,606.20	8,502.75	5,188.82	18,297.77
Sub total	210,613.33	211,174.75	162,594.71	584,382.79
Durable equipment	0	2,000.00	854.85	2,854.85
Other	0	0	0	0.00
Overheads	60,794.25	63,352.42	48,778.42	172,925.09
Total	**271,407.58**	**276,527.17**	**212,227.98**	**760,162.73**

10. Leveraging

(a) Teagasc Walsh Fellowships Programme has granted €22,000/-, which allowed to upgrade the Masters programme to PhD programme for Ms. Bibha Kumari, after successful stage transfer of her research work on 29th June 2016. Ms. Kumari was based full time in Teagasc Ashtown and continued to carry out research work (especially on co-extracted bioactives) in this project.
(b) Ms. Bibha Kumari, a postgraduate student in this project was successful in securing conference travel grant of €340.00 from the Food Bioactives & Health conference, Norwich, UK.
(c) Ms. Bibha Kumari, a postgraduate student in the project was also granted Scholarship grant for the International Summer School on Natural Products (ISSNP) 2017 in Italy, which covered the cost of registration fee.

The expertise, skills and know-how acquired in this project have helped the grant holders to become recognised experts in the sustainable valorisation of food derived waste raw materials. This has been instrumental in the ability of the research partners to leverage funding from the European Union (under the Biobased Industries call Dr. James Lyng and Dr. Nigel Brunton received funding for a project called AGRIMAX whose aim was also to valorise plant food waste resources. Dr. Dilip Rai, Dr. Lyng and Dr. Brunton are also partners in the Science Foundation Ireland (BioOrbic) project part of which is to develop valuable products from mushrooms and develop new separation processes and analytical methods for bioactives from seaweeds. Other projects include:

- From nature to bedside - algae based bio compound for prevention and treatment of inflammation, pain and IBD (Algae4IBD, project ID 101000501), Horizon 2020. Dr. Rai (Partner)

- Innovative plant-based functional beverages from brewers´ spent grain (BSG-Bev), Teagasc Research Leaders Fellowship co-funded by Teagasc and the EU Marie Skłodowska-Curie grant agreement number 754380. Dr. Rai (PI)
- Exploitation of co-products and wastes from beverage industries to generate functional-food ingredients, renewable chemicals and bio-fuels. (Ref. No. 2014027), Teagasc Walsh Scholar. Dr. Rai (PI)
- Alternate sources of antioxidants (plant polyphenols) from food processing by-products to extend oxidative shelf-life of high value dairy export products (Ref. No. 2016038), Teagasc Walsh Scholar. Dr. Rai and Dr. Brunton (PIs)
- Extraction and characterization of BIOactives and CARBohydrates from seaweeds and seagrasses FOR FOOD-related applications (BIOCARB, 17/RD/SUSFOOD2/ERA-NET/1) H2020 ERA-NET. Dr. Rai (Partner)

11. Future Strategies

The relevant protocols in extraction and analysis of bioactive molecules established in this project, in particular analysis of β-glucans and glycoalkaloids, have had leveraged the capacity to provide service to researchers and stakeholders alike through lab-charging systems.

The results from this project showed that conventional extractions from these waste streams are viable at certain scales, whilst the gains in yields of the targeted compounds could be achieved from the incorporation of novel technologies (i.e. ultrasound and pulsed electric field) into such extraction processes. While the initial findings may appear positive, this project also showed that such proposed processes need to undergo significant desk based feasibility studies by potential end-users before they could be considered at commercial scale. Additional research on these technologies to further improving the yields of the targeted compounds would give better profit margin than the conventional processes. The project team are currently actively pursuing additional funding streams from within Ireland and the EU (Teagasc Walsh Scholarships, Enterprise Ireland, Science Foundation Ireland, DAFM, H2020, etc.).