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

17RDSUSFOOD2ERANET2 Innovative (pre)pomace valorization process

Final Report

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

SUMMARY

The overall aim of ImPrOVE (Innovative pre-pomace valorization process) was to valorise pomace. In UCD the main objectives were to test extracted samples using deep eutectic solvents (DES) for bioactivity and examine potential molecular mechanisms. The bioactivity analysis focused on antioxidant capacity and ability to stimulate insulin secretion from pancreatic beta-cells. A range of extraction solvents were examined for extraction of bioactive compounds from apple and olive pomace.

With respect to apple pomace: ChCl:EG(1:4) extracts resulted in high antioxidant capacity. Extracts obtained from the classical systems demonstrated an ability to promote insulin secretion significantly higher than the positive control, $p<0.05$. ChCl:EG(1:4) extracts stimulated insulin secretion to a lesser extent. Olive pomace extracts obtained using classical extraction system and the green solvent systems ChCl:EG, ChCl:citric acid and ChCl:Oxalic acid also exhibited antioxidant properties. Whereas classical olive pomace extracts and extracts using ChCl: levulinic acid and ChCl: Citric acid promoted insulin secretion. The apple and olive pomace extracts that promoted insulin secretion resulted in increased intra cellular calcium and depolarization of plasma membrane potential. At higher concentrations, the extracts from the DES systems resulted in decreased viability of the cells indicating cell toxicity as a limiting factor. Our further analysis revealed that certain DES systems caused mammalian cell death through apoptosis highlighting the need for thorough examination of green solvents prior to use in a food preparation procedure.

In conclusion, the project provides evidence for the potential of DES systems to extract bioactive compounds from apple pomace that have relevance for metabolic health. However, the extraction procedure and type of solvent used should be tailored for the downstream desired bioactivity. Furthermore, care is needed when selecting the green solvent as our work clearly demonstrates a negative impact of certain solvents on mammalian cells.

KEYWORDS

Sustainable, food, systems.

ACRONYM ImPrOVE

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Section 1 - Research Approach & Results

Start Date

01 May 2018

End Date

31 October 2021

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 5: Technology validated in relevant environment

NRPE Priority area

Sustainable Food Production and Processing

Total DAFM Award

€285,800.00

Total Project Expenditure

€274,987.75

Rationale for undertaking the Research

The ImPrOVE (Innovative (pre)POmace Valorization procEss) project addressed a major European wide agro-related problem: pomace resulting from pressing fruit. This high amount of pomace is considered waste, but this 'waste' contains natural and highly functional compounds. Skin and core of fruit contain protecting and functional molecules: antioxidants, stabilizers, colorants, aromas, fibres with potential in high value applications in cosmetics, diets and, as bio-additives in food and beverages.

ImPrOVE set out to fully valorize pomace by using a combination of existing and innovative processes. These should be easy without high energy/cost demands, resulting in access for S(M)E's (economic strategic European targets) with profit redistributed over the whole chain, strengthening Europe's agro and food activities. ImPrOVE designed a generic process flow applicable to most pomace types. Total valorization was achieved in three process clusters: (1) pre-treatment giving rise to aromas and oil from separated seeds; (2) extraction of high value materials from the pre-treated pomace and (3) valorization of the resulting fibrous mass, either directly (functionally designed fibers) or by splitting cellulose-lignin and valorizing both materials physically, enzymatically and/or chemically. Furthermore, ImPrOVE examined the use of bio-based ionic liquids (BIOILs) or natural deep eutectic solvents (DES) as extraction liquids for advanced green solvents.

Methodology

Samples were received from our project partners in Pisa and Belgium. The bioactivity tested were antioxidant capacity and ability to stimulate insulin secretion from pancreatic beta-cells. The antioxidant capacity of extracts was examined using the DPPH and the FRAP assay. Impact of the extracts on cell viability and insulin secretion were examined using the BRIN-BD11 cell line.

The deep eutectic solvents (DES) were prepared by our partners in the University of Pisa as follows: ChCl was dried under vacuum for 6h at 80°C. Briefly, ChCl and the corresponding hydrogen bond donor (oxalic acid or ethylene glycol) were mixed in the proper molar ratio until a homogenous transparent liquid was formed. After the DESs formation, no purification step was needed and the DESs were kept at room temperature in sealed vessels until their use. Composition and purity of DESs was assessed by 1H-NMR analysis. The DES with oxalic acid as HBD was mixed with 25%wt of water to reduce its viscosity. The classical system used was methanol/water.

To assess cell viability the WST-1 colorimetric assay was used initially. Following this more detailed analysis of the impact on cell death was performed using flow cytometry to determine how the cells were dying and assess the levels of intracellular reactive oxygen species.

Project Results

We examined the bioactivity of extracts from apple pomace obtained by non-conventional green extraction methods (DES systems). Bioactivity was antioxidant capacity and ability to stimulate insulin secretion from pancreatic beta-cells. The antioxidant capacity of extracts was examined using the DPPH and the FRAP assay. Impact of the extracts on cell viability and insulin secretion were examined using the BRIN-BD11 cell line. Extraction of polyphenolic compounds from two different apple pomace types, Jonagold and Bittersweet mix using DES was performed to develop a sustainable and green process to separate and recover high added value compounds from food waste biomass.

ChCl:EG(1:4) extracts resulted in high antioxidant capacity in the DPPH assay (80.1% inhibition versus 11.3%). Extracts obtained from the classical systems demonstrated an ability to promote insulin secretion significantly higher than the positive control, $p<0.05$. ChCl:EG(1:4) extracts stimulated insulin secretion to a lesser extent. As ChCl:EG(1:4) was the best performing DES system, different extraction times (2h, 1h and 30 min) were screened to evaluate and improve the properties of the extracts which showed observable difference in the profile of phenolic extracts obtained after 6h.

Olive pomace extracts obtained using classical extraction system and the green solvent systems ChCl:EG, ChCl:citric acid and ChCl:Oxalic acid also exhibited antioxidant properties. Whereas classical olive pomace extracts and extracts using ChCl:levulinic acid and ChCl:Citric acid promoted insulin secretion. The apple and olive pomace extracts that promoted insulin secretion resulted in increased intra cellular calcium and depolarization of plasma membrane potential.

At higher concentrations, the extracts from the DES systems resulted in decreased viability of the cells indicating cell toxicity as a limiting factor. Our further analysis of this through Flow Cytometry revealed that certain DES systems caused mammalian cell death highlighting the need for thorough examination of green solvents prior to use in a food preparation procedure. Further detailed analysis revealed that exposure to certain DES systems and their components were causing cell death via apoptosis and enhanced intracellular reactive oxygen species production.

Overall, the data provides evidence for the potential of DES systems to extract bioactive compounds from apple pomace that have relevance for metabolic health. However, the extraction procedure and type of solvent used should be tailored for the downstream desired bioactivity. Furthermore, care is needed when selecting the green solvent as our work clearly demonstrates a negative impact of certain solvents on mammalian cells.

Section 2 - Research Outputs

Summary of Project Findings

The project provides evidence for the potential of DES systems to extract bioactive compounds from pomace that have relevance for metabolic health. However, the extraction procedure and type of solvent used should be tailored for the downstream desired bioactivity. Furthermore, care is needed when selecting the green solvent as our work clearly demonstrates a negative impact of certain solvents on mammalian cells.

Our results are available to the wider susfood consortium and the issues with mammalian toxicity associated with the green solvents were highlighted. This will be important going forward for the potential use of such solvents in the food industry.

For the scientific community, a range of extraction solvents were examined for extraction of bioactive compounds from apple and olive pomace. With respect to apple pomace: ChCl:EG(1:4) extracts resulted in high antioxidant capacity. Extracts obtained from the classical systems demonstrated an ability to promote insulin secretion significantly higher than the positive control, $p<0.05$. ChCl:EG(1:4) extracts stimulated insulin secretion to a lesser extent. Olive pomace extracts obtained using classical extraction system and the green solvent systems ChCl:EG, ChCl:citric acid and ChCl:Oxalic acid also exhibited antioxidant properties. Whereas classical olive pomace extracts and extracts using ChCl: levulinic acid and ChCl: Citric acid promoted insulin secretion. The apple and olive pomace extracts that promoted insulin secretion resulted in increased intra cellular calcium and depolarization of plasma membrane potential.

In conclusion, the project provides evidence for the potential of DES systems to extract bioactive compounds from apple pomace that have relevance for metabolic health. However, the extraction procedure and type of solvent used should be tailored for the downstream desired bioactivity. Furthermore, care is needed when selecting the green solvent as our work clearly demonstrates a negative impact of certain solvents on mammalian cells.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
Post Doctorates	0	1	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	1	Moni Bottu, H.; Mero, A.; Husanu, E.; Tavernier, S.; Pomelli, C.S.; Dewaele, A.; Bernaert, N.; Guazzelli, L.; Brennan, L. The ability of deep eutectic solvent systems to extract bioactive compounds from apple pomace. 2022. Food Chemistry, vol 386, 132717. DOI: 10.1016/j.foodchem.2022.132717

Intellectual Property

N/A

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Other	Our results are available to the wider susfood consortium and the issues with mammalian toxicity associated with the green solvents were highlighted. This will be important going forward for the potential use of such solvents. The KEY POLICY RECOMMENDATIONS that emerged from the overall project were: 1. Provide clear messages to consumers to increase the trust in food. 2. Harmonize the nutritional and sustainability regulations and labels. 3. Support the uptake of new technologies and side stream valorization. 4. Create a forum to collect the evidence and discuss regulatory aspects for new technologies.

Dissemination Activities

Activity	Details
Seminars at which results were presented	Conferences/seminars/symposiums 1. L. Brennan. (2019). Can metabotyping help deliver the promise of personalized nutrition? 118th Abbott Nutrition research Conference. 7-8th October, Columbus, Ohio. (Keynote speaker) L. 2. Brennan. (2019). Potential of Precision Nutrition to improve Health – Insights from biomarker approaches. FENS, October 15-18th, Dublin. (Invited speaker) 3. L. Brennan. (2019). Metabotyping for Precision Nutrition. Food Nutrition Conference Expo, 26-29 October, Philadelphia, US (invited speaker) 4. L. Brennan. (2019). Metabolomics-based dietary biomarkers in nutritional epidemiology - current status and future opportunities. NuGOWeek 2019. September 9-12, Bern, Switzerland. (Invited speaker) 5. L. Brennan. (2019). Metabolomics in Nutrition: a solution to major challenges in the field. British Mass Spec Society, September 3-5th, Manchester, UK (Keynote speaker) 6. Heleena moni. Bottu. (2021). Insulinotropic effects of apple pomace extracts in Deep Eutectic systems. 7. 2nd International conference on Deep Eutectic Systems. 15-17th June, Online conference, Lisbon, Portugal. (Oral presentation) 8. L. Brennan (2021). Quantitative metabolite measurements: what can we learn from them? 11-12th May, IMSS 2021, online. (Invited speaker) 9. L. Brennan (2021). Metabolomics-based dietary biomarkers - current status and future opportunities. Webinar: Dietary biomarkers for measuring food habits - possibilities and challenges, 26-27th April.

	10. Organised by the Swedish Network in Nutritional Epidemiology (NEON); The National Committee in Nutrition and Food Sciences, The Royal Swedish Academy of Sciences, and EpiHealth. (Invited speaker). Conferences/Seminars 11. L. Brennan (2021). ICDAM Feb 8-10 Metabolomics-based dietary biomarkers in nutritional epidemiology current status and future opportunities 12. L Brennan (2021) 2021 ANNUAL SYMPOSIUM- Stanford Metabolic Health Feb 26 Metabolomics in nutrition research: Current opportunities and developments
Workshops at which results were presented	L Brennan (2021) Food Biomarker Workshop Imperial College L Brennan Challenges for the future, 14th Jan and 21st Jan 7. L. Brennan. (2020). Dietary Biomarkers Overview, Dietary biomarkers - where to next? October 27-28, Dublin. (Keynote speaker)

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	• Photo competition (https://susfood-db-era.net/main/sites/default/files/Photo%20Competition_projects_2.pdf) • A website was established (https://improveproject.it/) for the project which has a YouTube video of our results (https://susfood-db-era.net/main/projects-presentation). • A stakeholder workshop was held, and a summary report produced (https://susfood-dbera.net/main/node/29510)
Identify any knowledge transfer activities executed within the project.	• A website was established (https://improveproject.it/) for the project which has a YouTube video of our results (https://susfood-db-era.net/main/projects-presentation). • A stakeholder workshop took place on the 27 October 2021 (summary report: https://susfood-db-era.net/main/node/29510).
List any impacts resulting from the knowledge transferred during the project.	• The stakeholder workshop indicated follow up steps for the project that will enable transfer of a technology to different concerns. • A number of young researchers including Dr Helenna Bottu Moni worked and developed new skills during this project.

Section 3 – Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding	Funding €	Summary
Other	€283,000.00	Prof. Brennan in collaboration with Kerry Food Group were successful in obtaining an MSCA-Career Fit with Dr Janine Wirth the postdoctoral research fellow on the project 'Examine the impact of timing of protein intake on health outcomes and muscle function'

Future Strategies

Further work is needed to examine potential modifications to green solvents to reduce their toxicity.

Project Publications

(1) Moni Bottu, H.; Mero, A.; Husanu, E.; Tavernier, S.; Pomelli, C.S.; Dewaele, A.; Bernaert, N.; Guazzelli, L.; Brennan, L. The ability of deep eutectic solvent systems to extract bioactive compounds from apple pomace. 2022. Food Chemistry, vol 386, 132717. DOI: 10.1016/j.foodchem.2022.132717