Environment, Natural Resources and Agriculture Strategic Research Programme 2022-2027

RESAS
Rural & Environmental Science and Analytical Services

END OF PROJECT REPORT

Purpose of End of Project Report

SRP 2022-27 projects provide quarterly progress reports and annual narrative summaries as well as research outcomes throughout the term of the project via the Researchfish platform. This end of project report provides additional information when a project finishes that can be used to summarise what the project has delivered, lessons learned and next steps. This report will be published on the SRP 2022-27 project webpages of SEFARI Gateway or on the Scottish Government website.
All sections must be completed

Project Researchfish ID	RI-B7-02		
Project Name	Revalorisation potential of agricultural waste materials into a sustainable source of health-promoting dietary fibre		
Principal Investigator	Petra Louis		
Start Date	1st April 2022	**Completion Date**	31st March 2024

Purpose of the project

This project addressed Theme B Topic B7 RQ1 '*Please set out your plans for researching the components of healthy diets and their effects. How can we develop our understanding of the physiological effects of interventions on dietary health?*'

The aim of this project was to establish the suitability of agricultural side stream materials as a source of novel dietary fibre ingredients by applying a new technology to improve its microbial fermentability. If successful, this opens up new opportunities for waste revalorisation into potential new ingredients for food products with clear health benefits.

The main drivers for this proposal were at the intersection of two major societal drivers, Climate change and promoting health and well-being by becoming a Good Food Nation. Agricultural and food production side stream materials represent an under-utilised resource that has great revalorisation potential. Plant-based materials are a potential novel source of dietary fibre, an essential component of a healthy diet. Fibre intake in the UK is below current recommendations and development of novel fibre sources would aid in the formulation of healthy food products.

Dietary fibre is not digested in the upper intestine and constitutes the main nutrient source for the resident microbes in the large intestine, the gut microbiota. An appropriate supply of fibre is important for health maintenance, as its microbial fermentation leads to the production of metabolites that play an important role in maintaining gut and systemic health. Thus, short-chain fatty acids produced by the gut microbiota have been implicated in the prevention of several diseases, including colorectal cancer, inflammatory diseases such as Ulcerative Colitis and metabolic syndrome/type 2 diabetes. Soluble fibre is largely removed during food production processes, such as during the manufacture of juices. The human microbiota has limited capacity to efficiently break down the residual complex insoluble material in its native state. However, the

way the material is processed is highly likely to have a major impact on its fermentability by the gut microbiota, as the surface structure of the fibre particle will dictate how easily the microbes can attach and gain access to the different fibre components to initiate their deconstruction. The objective of this project was therefore to assess the gut microbial fermentability of agricultural side stream materials that have been processed to optimise their surface structure for microbial degradation.

Objectives achieved/not achieved

We have achieved the objective of this project to assess the gut microbial fermentability of agricultural side stream materials that have been processed to optimise their surface structure for microbial degradation.

Five plant materials (brewery spent grain, oat husks, blackcurrant skins, broccoli and cauliflower) were prepared as flours of identical particle sizes (75-150 μm) in two forms differing in their surface characteristics based on the mode of micronisation. The materials were subjected to simulated upper gut digestion by exposing them to the conditions and enzymes present in the mouth, stomach and small intestine. After freeze-drying, their fermentability was assessed with a model gut microbial community of twelve different bacteria. Only minor differences in microbial fermentability, microbiota composition and metabolite production between the two grinding techniques were observed for all of the assessed plant materials.

Major differences between the two grinding techniques had previously been observed during pilot experiments with brewery spent grain in the absence of upper gut digestion treatment of the plant flours. Furthermore, the digestion procedure is not routinely being applied to assess colonic microbial fermentability and further work was required to assess if technical issues were responsible for the limited effect of fibre structure on microbial fermentability. We therefore tested different approaches to avoid drying of digested plant material ahead of microbial fermentation, as this would not happen during the passage of food through the intestinal tract in the human body and as the drying process may affect fibre structure. We assessed the suitability of ethanol precipitation and dialysis to remove digestion products that would be absorbed in the small intestine. Ethanol precipitation proved unsuccessful, as it was impossible to completely remove the ethanol by evaporation. A dialysis protocol was established and different flour-types of two different plant materials (brewery spent grain and blackcurrant skins) were subjected to the new upper gut digestion protocol, followed by microbial fermentation by the model gut community of twelve bacteria. Similar to the first experiment, only relatively minor differences in overall fermentability, microbial community and metabolite changes were observed, however, microbial community compositional differences between the two flour types were more pronounced.

Outcomes

i. Using laboratory-based model systems, **we did not confirm our hypothesis that different methods of micronisation of plant agricultural side stream materials have a major impact on lower intestinal fermentability**, in terms of total fermentation capacity, changes

in the overall composition of the microbial community, or metabolite production. Trends were, however, observed for the flour structure affecting certain bacteria. For example, we observed the trend that the cellulose-degrading bacterium *Bacteroides cellulosilyticus* had higher relative abundance on traditionally ball-milled flours, whereas the pectin degrader *Lachnospira eligens* had a trend towards higher relative abundance on micronised flours with a larger surface area.

ii. **We identified potential technical limitations of laboratory-based upper gut digestion procedures** for the production of materials for simulation of lower gut microbial fermentation. Dialysis of the digested materials to remove digestion products that would be absorbed in the small intestine appears to be a suitable method to avoid drying of the material after digestion. However, the digestion components, especially the pancreatin that supplies small intestinal digestive enzymes, constitute a very large proportion of the insoluble material at the end of the procedure, which may influence microbial access to the material. While control experiments showed no major inhibitory effect of the digestion protocol ingredients on our model microbiota, scanning electron microscopy of digested plant fibres revealed that the fibres had a tendency to clump together, and string-like material was observed coating the fibre particles that was not seen before digestion.

iii. We are currently in the process of preparing an **academic paper** to disseminate the results to the research community.

Project Insights

Despite our promising pilot studies suggesting a major effect of fibre structure on gut microbial fermentability ahead of the start of the project, differences seen were only minor after we applied the upper gut digestion procedure ahead of microbial fermentation. We therefore changed the project to investigate potential technical confounders rather than moving on to assess fibre structure effects in human faecal microbiota incubations as originally planned.

Laboratory-based protocols for the simulation of the whole intestine are crucial to allow investigation of whole plant-based food ingredients. We followed the Infogest digestion procedure, which is well established for assessment of upper gut digestion processes, but it does no detail how to prepare materials further for lower gut fermentation. Our work showed that potential technical issues remain to be resolved as detailed above.
Furthermore, we also found technical limitations in working with certain plant materials, namely broccoli and cauliflower. It proved difficult to separate insoluble fibres from the microbial culture by centrifugation, as the fibres retain a large amount of liquid. This made it difficult to assess the bacterial distribution between those that had attached to fibres and free-swimming (planktonic) ones. Therefore, different methods for an effective separation of fibres need to be established to facilitate the assessment of fibre-attached bacteria for certain plant materials.

Next Steps/ Future Plans

<What are the next steps or future plans for research from this project?>

While we did show only a relatively minor effect of fibre structure on gut microbial fermentability, it would be worthwhile to investigate this area by exploring other micronisation techniques and flour particle sizes, and we will seek further funding to continue this research.

This project also provided valuable technical insights on the appropriate setup of laboratory-based model systems for whole intestinal digestion and fermentation of food stuffs, which will inform other projects, that utilise those models. This includes three other projects within the SRP and an Innovate UK project working with Scottish Bakeries to incorporate nutritious, sustainable and locally produced crops into acceptable and affordable everyday food products.