Proof of Concept Project Outcomes 2015

**Supercritical CO\textsubscript{2} extraction & enzyme-modification of leaf waxes from brassica waste to produce novel film antitranspirants for increasing crop drought tolerance**

**Award Holder:** Peter Kettlewell, Harper Adams University  
**Academic Partners:** Ray Marriott, Bangor University  
**Industrial Partner Company:** Freshtime

**Project Outcome Summary**

Drought is a massive global problem reducing food crop yield. One way of minimizing drought damage to crop yield could be to apply antitranspirants. These are currently high-cost polymers used to reduce water loss and drought damage, but can only be economically justified on high-value, mainly ornamental, plants. If a lower-cost polymer could be produced, this might enable antitranspirants to be used on food crops to reduce drought damage. Plant leaves have evolved several mechanisms to reduce water loss, and accumulating a layer of wax on the leaves and stems is one such mechanism. The aim of this project was to determine the feasibility of producing a lower-cost antitranspirant by extracting leaf wax from cabbage and cauliflower processing leaf waste, and applying it to crop plants to temporarily block the leaf pores through which water vapour is lost. Brassica leaf trimming waste has not previously been explored as a source of waxes. It is currently disposed of as livestock feed with no value to fresh produce processors.

Leaf wax was extracted from leaf waste using a solvent created by pressurising and heating carbon dioxide gas to liquefy it. An experimental antitranspirant based on the extracted leaf wax was compared with a commercially-available antitranspirant in experiments on oilseed rape plants and wheat plants grown in pots in glasshouses. In general the leaf wax antitranspirant was as effective as the high-cost commercial polymer in reducing water loss from the plants, and responses were broadly similar in both well-watered and drought-stressed plants. These results are sufficiently promising to justify further research.

**Assessment of Ice Cream Waste as a Feedstock for Higher Value Applications**

**Award Holder:** Neil Bruce, University of York  
**Industrial Partner Companies:** Unilever R&D (Port Sunlight)

**Project Outcome Summary**

Industrial fermentation feedstocks typically incorporate food-grade materials, such as sugars and vegetable oils, in varying amounts to produce the desired products. This project has evaluated a range of strains across commercially relevant biological production platforms to see if ice cream waste - a source of simple sugars and oils - could be used in place of the usual food grade feedstocks.

During the study, multiple analyses of the ice cream waste were conducted, the composition varying depending on the products being manufactured at the time. In the fermentation experiments conducted, a comparison was made of the full waste, sugar mixes (e.g. various blends of ice cream waste with optimal pure sugars) and a pure optimal sugar feedstock, to determine the viability of using ice cream waste as a source of sugars and various other growth mediating components.
The results from this proof of concept work have shown that ice cream waste can be utilised by a range of micro-organisms as a fermentation feedstock, including those used as gene expression platforms and others as producers of various alcohols and industrially significant organic acids. The growth of the different micro-organisms, and production of materials of value from the ice cream waste were found to vary. One gene expression strain exhibited best growth using the ice cream waste, some strains showed an equivalent growth and production of organic acids or alcohols compared with the other sugar sources, while other strains showed reduced growth and yields when ice cream waste alone was used, indicating the presence of inhibiting compounds.

This proof of concept project has demonstrated the potential for turning waste ice cream & other similar materials into high value bio-based materials through fermentation.

Assessment of Food Waste Feedstocks for the Production of Novel Antifungal Compounds through Fermentation

Award Holder: Jared Cartwright, University of York
Industrial Partner Companies: Croda Europe

Project Outcome Summary

This project addressed the industrial biotechnology challenges of producing fine and specialty chemicals from biomass by evaluating the potential of converting specific food waste streams into novel antifungal products using microbial fermentation. The industrial partner had developed a microbial strain which is able to produce significant quantities of novel antifungal compounds from a pure sugar source. Building on this, the project investigated whether the fermentation feedstock being used could be switched from standard pure sugar-based media to carbohydrate-rich food waste streams. The main focus of this piece of work was to carry out fermentation screening experiments in which pure sugar sources were replaced with different food wastes to determine their suitability. Extensive research was carried out to identify food waste feedstocks which could feasibly be used in industrial fermentations. Once identified the following practical work was carried out:

- Characterisation of feedstocks
- Evaluation and optimisation of fermentations using the food waste feedstocks
- Development and use of extraction and analytical methods to quantify the levels of antifungal compounds produced

The results obtained from this proof of concept work were promising and highlighted two potential food waste stream that would suitable as fermentation feedstocks for the production of novel antifungal compounds.
Viability of obtaining biopharmaceuticals from eggshell waste

Award Holder: Carole Perry, Nottingham Trent University
Industrial Partner Companies: Avgo Biotech Ltd

Project Outcome Summary

The chicken eggshell comprises an inorganic calcified shell and organic membranes which are essential in the formation and function of the eggshell. The major constituents of the organic component of the eggshell and eggshell membrane is protein with smaller amounts of carbohydrate and lipids. Eggshell membranes are primarily composed of fibrous proteins such as collagen type I. However, eggshell membranes have also been shown to contain glycosaminoglycans (GAGs) such as hyaluronic acid and sulphated glycosaminoglycans such as dermatan sulphate and chondroitin sulphate. Other components identified in eggshell membranes include sialic acid, desmosine and isodesmosine, proteins such as ovotransferrin, lysyl oxidase, lysozyme, and β-N-acetylglucosaminidase and hexosamines such as glucosamine.

These components and specifically chondroitin sulphate have been identified as potentially valuable biologicals which have significant commercialisation potential if they could be isolated from an existing waste valorisation process. This PoC study looked at determining the amount and value of these biomolecules within the waste stream as well as optimising the existing process for their recovery and identifying further processing requirements to meet existing commercial specifications identified during the course of the project.

The project obtained evidence that high molecular weight GAGs can be isolated from eggshell waste using a modification of the existing process on a laboratory scale but to date a thoroughly evaluated process on this scale has not been achieved. As such, costs associated with transferring the process to a commercial scale are not yet fully understood. The priorities for future work have been identified as the following:

- Explore how different processing conditions adversely influence the specification of commercial grade chondroitin sulphate, hyaluronic acid and collagen starting with a known grade of material and processing under different conditions.
- Progress enzymatic processing approaches to eggshell membrane to understand the merits of such an approach and how it could be incorporated into the biomolecule purification process. Gain data to assess the economic viability of this processing approach.
- Further trials at a scale of 1000 L or greater to understand the operational costs of membrane filtration technologies.
- Explore scaling anion exchange and size exclusion chromatography from analytical to production levels.
- Explore a range of different lectin molecules for their suitability for an affinity approach and investigate the feasibility for using affinity chromatography using such molecules on a commercial scale.

To conclude:

- The eggshell waste stream represents a potentially valuable source of biologicals for the pharmaceutical industry for which the extraction technology exists.
- Further work will be required to determine if such a process can be scaled commercially.