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Health promoting bioactives from cider yeast.

Key external stakeholders:

Food manufacturers, dairy industry, pharmaceutical companies, research communities; public health agencies and health professionals; policymakers.

Practical implications for stakeholders:

Beta glucan is a bioactive polysaccharide which has FDA approval for the reduction of cardiovascular risk, the leading cause of death and morbidity in the EU. A cardioprotective diet enriched in dietary fiber, and in particular beta glucan is recommended to protect against the development of cardiovascular disease. Furthermore, food-derived ACE (Angiotensin-I-converting enzyme)-inhibitory peptides have been shown to reduce peripheral blood pressure and exert an antihypertensive effect *in vivo* following ingestion. In this project, bioactive components (ACE inhibitory/antihypertensive peptides and beta glucan) were isolated and characterised from Natural Yeast, which was a by-product of the cider production process.

Main results:

- Laboratory scale trials, involving autolysis and hydrolysis of spent cider yeast, were optimised for production of yeast extracts, enriched in free amino acids, flavour-enhancing components and bioactive ACE-inhibitory peptides.
- Pilot scale trials were performed but further technical trials are required.
- Economic and financial analysis of the prototype products developed in this project were undertaken, and results indicated that the process for their production (involving spray drying at 20%) was not commercially viable, with further technical trials required to overcome this difficulty.

Opportunity / Benefit:

The opportunity exists to further investigate the potential waste stream of Cider production in collaboration with industrial personnel. The research group benefited from improved links with industry (Cybercolors).

Collaborating Institutions:

Cybercolors

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1. Project background:

A previous Innovation Partnership project had shown that bioactive ACE inhibiting peptides can be generated from Cider Yeast by enzymatic hydrolysis. Bioactive peptides are defined as 'food-derived components that in addition to their nutritional value, exert a physiological effect in the body'. Biological activities associated with bioactive peptides include immunostimulatory, antibacterial, antihypertensive and opioid-like properties. The Cell wall fraction produced as a result of autolysis of Cider Yeast Cells is also a source of naturally occurring beta glucan - a bioactive polysaccharide with cholesterol-lowering properties.

2. Questions addressed by the project:

- Could yeast extracts containing bioactive peptides be produced through hydrolysis with commercially available proteolytic enzymes?
- Could anti-ACE peptides be identified using *in-vitro* assays for anti-hypertension?
- Could the ACE-inhibitory peptides and yeast beta glucan be characterized using Reverse-Phase High Performance Liquid Chromatography (RP-HPLC), Molecular Size profiling, Mass Spectrometry and Sequencing?
- Could the products be evaluated for stability during processing?
- Could naturally occurring beta glucan and other galactomannans in Natural Apple Yeast be isolated and measured?
- Could the process be scaled up to pilot production of fractions with promising bioactive properties, for production of pre-commercial product?

3. The experimental studies:

- Method development for the release of yeast bioactive peptides through hydrolysis with commercially available proteolytic enzymes.
- ACE-inhibition assays to identify anti-ACE peptides for anti-hypertension from cider yeast.
- Reverse-Phase High Performance Liquid Chromatography (RP-HPLC), Molecular Size profiling, Mass Spectrometry and Sequencing for characterization of yeast-derived bioactives peptides.
- Method development for the isolation and measurement of naturally occurring beta glucan and other galactomannans in Natural Apple Cider Yeast.
- Pilot scale production of fractions exhibiting bioactive properties.

4. Main results:

This project involved the isolation and characterisation of bioactive components (ACE-inhibitory (antihypertensive) peptides and beta glucan from Natural Yeast) recovered as a by-product of the cider production process. The bioactive nature of ACE-inhibitory peptides was stimulated as a result of release via hydrolysis of the yeast substrate using proteolytic enzymes. It was then proposed to produce bioactive fractions exhibiting bioactive properties at pilot scale and their incorporation into functional foods and beverages. In addition, the concentrated cell wall by-product fraction of the bioactive peptide extraction process was investigated as a source of beta glucan, a bioactive polysaccharide which has FDA approval for the reduction of cardiovascular risk. During the course of this project, a protocol for autolysis/hydrolysis of spent cider yeast was developed and optimised at laboratory scale. Hydrolysis was performed with two commercial proteolytic enzymes in order to generate yeast extracts rich in free amino acids, flavour-enhancing components and bioactive peptides to pilot-scale.

- Laboratory scale trials involving autolysis and hydrolysis of spent cider yeast were optimised for production of yeast extracts, enriched in free amino acids, flavour-enhancing components and bioactive ACE-inhibitory peptides
- Lab-scale trials were undertaken, in which yeast substrate (natural apple yeast) was autolysed and hydrolysed using a range of proteolytic enzymes for release of cell wall, and cell-free supernatant fractions, which were stabilised by freeze drying
- Beta glucan yields were quantified in cell wall, and cell-free dried yeast powders and detected at high concentrations in the cell wall fraction, with similar concentrations as present in the commercially available reference beta-glucan sample

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- Mass balance for beta-glucan isolation completed, and process design for recovery from Natural Apple Yeast
- Scale up and optimisation of process technologies for the large scale production of beta glucan rich fractions from autolysed/hydrolysed cider yeast based on decanting centrifugation at pilot-scale
- Pilot scale trials were performed using decanting technology to fractionate the cell wall and various yeast extract supernatants (arising from the different proteolytic enzyme combinations)
- Spray drying of the beta glucan rich cell wall retentates yielded inferior powders, due to the high water binding capacity of the cell wall material, thus leading to a highly viscous liquid, which could not be further concentrated by evaporation
- Further lab scale trials were undertaken, in which various commercial enzymes were used in attempts to reduce viscosity, with reduction in viscosity obtained using carbohydrate-degrading enzymes Biocellulose and Depol 667P
- Prototype cell wall powders were produced and assessed for economic and financial feasibility and assessed for market acceptability, with encouraging results and commitment of customers to taking 500-1000 kg of product, following testing and approval
- Economic and financial analysis of the prototype products developed in this project were undertaken, and results indicated that the process for their production (involving spray drying at 20%) was not commercially viable, with further technical trials required to overcome this difficulty

5. Opportunity/Benefit:

The opportunity is the availability of bioactive components which have positive impact on human health, such as anti-ACE peptides and beta glucan from the waste stream of Cider production. The exploitation of these bioactive substances as functional food ingredients, using optimized fermentation technology, initially at lab scale, followed by optimized large scale production were the ultimate goals of the technical phase of this project. Protocols were developed for optimising yields of beta glucan yields in cell wall, and cell-free dried yeast powders (with similar concentrations recovered, as present in the commercially available reference b-glucan products). The economic prospects were promising for the beta glucan rich fractions arising from this project. Prototype cell wall powders produced within this project were assessed for economic and financial feasibility and market acceptability, with encouraging results and commitment of customers to taking 500-1000 kg of product, following testing and approval.

6. Dissemination:

The research findings have been made available to the Industrial partner and to stakeholders, particularly the Irish food industry for applications.

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