Project number: 6560
Funding source: Enterprise Ireland
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Pre-commercial scale-up of biologically active milk protein hydrolysates

Key external stakeholders:

Food for Health Ireland (FHI) is an industry-led Enterprise Ireland Technology Centre co-funded by four major Irish dairy manufacturers Dairygold, Glanbia, Kerry and Carbery. FHI was initiated in 2008 and the project detailed here is part of its second phase of existence (2013 – 2018). FHI is governed by a consortium agreement drawn-up in conjunction with all participants which set out protocols for the uptake of results.

Practical implications for stakeholders:

- Successful pre-commercial scale-up protocols were developed which retained the biological activity of milk protein hydrolysates identified at lab-scale. This allowed the industry-led consortium to:
  - Further validate hydrolysates through human clinical trials
  - Prepare documentation and know-how to aid commercialisation of hydrolysates
  - Determine cost-effectiveness of manufacture and where necessary reduce number of processing steps
- Novel technologies and monitoring approaches were investigated and successfully applied to enzymatic hydrolysis of milk proteins:
  - A novel process was developed utilising enzymatic membrane bioreactors (EMBR) to improve selective hydrolysis of β-lactoglobulin from whey protein solutions
  - Fluorescence spectroscopy was developed as a rapid, accurate assay for monitoring hydrolysis

Main results:

- No. of trials at pre-commercial level: 40
- No. of bioactives scaled-up: 6
- EMBR was shown to be an effective method for termination of selective hydrolysis of β-lactoglobulin in whey streams
- Monitoring of intrinsic fluorescence proved to be a suitable and effective method for real-time analysis of degree of hydrolysis

Opportunity / Benefit:

As per the FHI consortium agreement Industry Partners have priority right of access to outputs with commercial potential. The IP associated with this work is firstly made available to the FHI industry partners before being put on general release. Outside of this, scale-up and characterization of milk protein hydrolysates and their fractions is a service offered by Teagasc.

Collaborating Institutions:

University of Limerick
University College Dublin
University College Cork
Food for Health Ireland

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1. Project background:

Food for Health Ireland (FHI) is a consortium of academic and industry partners initiated in 2008 with a strong focus on creating a critical scientific mass to test scientific hypotheses surrounding the preparation and efficacy of bioactive peptides from milk. As part of the second phase of FHI (2013-2018) this project sought to build upon pre-existing work of research groups from Phase 1, in particular the Kelly research group, to scale-up up a select number of key ingredients. The ultimate goal of this work was to support the commercialization of hydrolyzed milk ingredients through design of robust processes which retain bioactivity and are industrially feasible. Complementary research relating to design of novel processes and tracking mechanisms for hydrolysis of milk proteins was also undertaken. In particular, enzyme membrane bioreactors (EMBRs) were investigated as means of terminating selective hydrolysis of β-lactoglobulin over α-lactalbumin in whey protein isolate.

2. Questions addressed by the project:

- Can bioactivities identified at lab scale be retained in commercially viable processes?
- Can EMBRs be utilized to terminate selective hydrolysis of β-lactoglobulin in a more efficient manner compared to heat or pH inactivation?
- What are the combined effects of ultrasonication and heat-treatment on hydrolysis?
- Can fluorescence spectroscopy be used as a rapid, non-destructive tool to track hydrolysis?

3. The experimental studies:

The project consisted of two distinct classes of study:

- Scale-up studies – laboratory hydrolysis protocols shown to generate bioactivity were transferred to the project team. The scale-up procedure replicated and, where necessary, modified laboratory protocols in a sequential fashion, beginning at 150L scale and progressing to 1000L scale. At each step of the sequence, confirmation by the FHI bioassay testing platform that the applied hydrolysis conditions resulted in the desired bioactivity was required to progress to the next step. In addition, feedback from the FHI industry partners relating to the overall industrial feasibility of scaled-up protocols was taken into account, which in some cases resulted in further process modification to reduce process complexity, increase cost effectiveness etc. The resultant materials were then transferred to FHI partner institutes for clinical trials and other studies.
- Technological studies – a number of technologies and monitoring approaches were investigated:
    - Traditional heat inactivation and pH approaches for termination of selective hydrolysis of β-lactoglobulin in a whey protein isolate (WPI) substrate.
    - Enzymatic membrane bioreactors (EMBR) as a tool to facilitate scale-up of selective hydrolysis of β-lactoglobulin
    - The effect of selective heat treatments and ultrasonication on physical properties of hydrolysates i.e. viscosity, turbidity, solubility and degree of hydrolysis
    - Use of fluorescence spectroscopy as tool for monitoring degree of hydrolysis across a range of proteases

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4. Main results:

- Scale-up studies:
  - 6 scaled-up bioactive dairy hydrolysates
  - A number of studies with FHI partners utilized the scaled-up hydrolysates, yielding the following results:
    - A casein hydrolysate generated within the project was found to significantly reduce postprandial glucose response. Within the study a low number of test subjects were classified as responders, which highlighted the variability in efficacy of dietary interventions and the requirement for precision nutrition (Curran et al. 2019).
    - A casein hydrolysate generated within the project was shown to increase GHSR-1a-mediated intracellular calcium signaling in vitro. This provided novel data supporting the potential of the casein hydrolysate as an appetite-enhancing bioactive (Howick et al. 2018).
    - High molecular weight and low molecular weight hydrolysates generated within the project were incorporated at various levels in beverages and bitterness of the beverages was assessed. Hydrolysates with higher molecular weight could be included in beverages to a much greater extent compared to lower molecular weight hydrolysates (Murray et al. 2019).
- Technological studies:
  - Optimization of selective hydrolysis of β-lactoglobulin in whey protein isolate:
    - Optimum enzyme-to-substrate ratio for hydrolysis was determined to be 1:50, with an incubation time ranging from 3 h to 7 h, which resulted in ~ 90% hydrolysis of β-lactoglobulin and minimal conversion of α-lactalbumin. However, termination of the hydrolysis reaction through the conventional means of heat or pH resulted in considerable digestion of α-lactalbumin and downstream processing issues, respectively.
  - Development of EMBRs as a tool to facilitate scale-up of the selective hydrolysis of β-lactoglobulin and subsequent recovery of α-lactalbumin and peptides:
    - A number of configurations of membranes and molecular weight cut-offs were assessed for separating intact α-lactalbumin and peptides from enzyme and unreacted substrate. The optimum configuration was found to be spiral-wound 20 kDa membranes with internal recirculation and diafiltration. Subsequent recovery of α-lactalbumin from peptides was possible using spiral wound 10 kDa membrane.
  - Effect of selective heat treatments and ultrasonication on physical properties of hydrolysates:
    - Ultrasonication in combination with pre-heat treatment has a direct effect on the physical and chemical properties of pancreatin hydrolysates of whey protein isolate. Continuous ultrasonication during hydrolysis combined with pre-heat treatment improved degree of hydrolysis. However, there was no effect when ultrasonication was performed before hydrolysis.
  - Use of fluorescence spectroscopy as a rapid, non-destructive tool for monitoring degree of hydrolysis:
    - Changes in fluorescence were studied over four-hour hydrolysis using five different enzymes. The fluorescence measurements of maximum wavelength as well as intensity were highly correlated with conventional TNBS and pH-stat methods for determination of degree of hydrolysis.

5. Opportunity/Benefit:

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6. Dissemination:

Conferences & presentations:

1. L. Sáez, A. Fernández, R.J. FitzGerald, P.M. Kelly (2016), Front phase fluorescence as a novel tool for the determination of the degree of hydrolysis during enzymatic break-down of whey proteins. In IDF, Parallel Symposia 11-12 th, April 2016
2. L. Sáez, E. Murphy, R.J. FitzGerald, P.M. Kelly (2016), Application of enzymatic hydrolysis-based membrane bioreactor (EH-MBR) to whey proteins as means of enzyme recovery from the targeted released peptides. In SDT, Society of Dairy Technology Conference, April 2017, UCC, Cork, Ireland Dublin, Ireland

Main publications:

1. Sáez, L., Murphy, E., FitzGerald, R. J., & Kelly, P. (2019). Exploring the Use of a Modified High-Temperature, Short-Time Continuous Heat Exchanger with Extended Holding Time (HTST-EHT) for Thermal Inactivation of Trypsin Following Selective Enzymatic Hydrolysis of the β-Lactoglobulin Fraction in Whey Protein Isolate. Foods, 8(9), 367.
2. Sáez, L., (2019). The application of novel technologies and process monitoring to the enzymatic hydrolysis of whey protein isolate. PhD thesis. University of Limerick.
3. Murray, N. M., Jacquier, J. C., O'Sullivan, M., Hallihan, A., Murphy, E., Feeney, E. L., & O'Riordan, D. (2019). Using rejection thresholds to determine acceptability of novel bioactive compounds added to milk-based beverages. *Food quality and preference*, *73*, 276-283.
4. Curran, A. M., Horner, K., O'Sullivan, V., Nongonierma, A. B., Le Maux, S., Murphy, E., ... & Brennan, L. (2019). Variable glycemic responses to intact and hydrolyzed milk proteins in overweight and obese adults reveal the need for precision nutrition. *The Journal of nutrition*, *149*(1), 88-97.
5. Howick, K., Wallace-Fitzsimons, S. E., Kandil, D., Chruścicka, B., Calis, M., Murphy, E., ... & Ryan, A. M. (2018). A dairy-derived ghrelinergic hydrolysate modulates food intake in vivo. *International journal of molecular sciences*, *19*(9), 2780.

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