Food Institutional Research Measure

Final Report

‘Development of novel whey ingredients by protein-carbohydrate conjugation’

DAFM Project Reference No: 10/RD/OptiHydro/UCC/702

Start date: 01/11/2011

End Date: 31/03/2016

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Collaborating Research Institutions and Researchers:
Prof. Daniel Mulvihill, University College Cork, Cork, Ireland
Prof. Yrjo Roos, University College Cork, Cork, Ireland
Dr. Mark Auty, Teagasc Food Research Centre, Moorepark, Fermoy, Ireland

Please place one “x” below in the appropriate area on the research continuum where you feel this project fits

<table>
<thead>
<tr>
<th>Basic/Fundamental</th>
<th>Applied</th>
<th>Pre Commercial</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td>3</td>
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Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report;
### Key words: conjugated protein, protein functionality, emulsion stability, infant formula

1. **Rationale for Undertaking the Research**

The aim of the project was to develop next-generation whey protein ingredients/emulsifiers with significantly enhanced physicochemical functionality for application in premium nutritional beverages and powders. The principle technological hurdles limiting the use of whey protein ingredients in formulation of value-added nutritional beverages and powders are (1) poor solubility of intact whey proteins in high acid ready-to-drink (RTD) beverages - causing turbidity, phase separation and sedimentation (Akhtar and Dickinson, 1997), (2) poor emulsification properties of hydrolysed whey proteins (Singh and Dalgleish, 1998; Agboola et al., 1998) - causing emulsion breakdown and spray drying difficulties (powder stickiness and free fat) during the manufacture of powdered nutritional products and (3) physical instabilities such as aggregation, sedimentation and creaming during processing and shelf-life due to high ionic strength and thermal processing (Yadav et al., 2010). This project involved development of next-generation whey protein ingredients with significantly enhanced physicochemical functionality to overcome the above technological hurdles in optimising formulation, processing and shelf-life performance of such ingredients in value-added nutritional beverages and powders. Recent research has shown that significant improvements in solubility, emulsification, mineral sensitivity and heat stability of dairy proteins can be achieved by conjugation of proteins to carbohydrates using the Maillard reaction (Shepherd et al., 2000; Akhtar and Dickinson, 2007; Augustin et al., 2006; O'Regan and Mulvihill, 2009a). These protein-carbohydrate conjugates are natural and can be prepared readily by dry heating (O'Regan and Mulvihill, 2009a) or wet heating techniques (Augustin et al, 2006; Zhu et al., 2008). This project contributed to ensuring that Ireland retains its position at the forefront of ingredient innovation and bolstered the combined expertise and analytical resources in ingredient science and technology available at UCC and the Teagasc Food Research Centre at Moorepark (TFRCM) which stimulated and supported industry-led innovation in developing natural, clean label, sustainable ingredients and finished food products with superior nutritional, physical and sensory properties.

2. **Research Approach**

Whey protein (intact and hydrolysed) was conjugated with maltodextrin using previously developed dry heating protocols at UCC (O'Regan and Mulvihill, 2009a) and wet heating approaches used by other research groups (Augustin et al, 2006; Zhu et al., 2008). Selected physicochemical properties of the conjugates (e.g., solubility, viscosity, emulsification, heat stability, colour, mineral-induced aggregation etc.) were studied in
Simple model food systems and selected conjugates with optimal formulation, stability and processing performance were used in high value-added food systems – infant nutritional products and ready to drink beverages. Some of the novel approaches employed in this research included combination of analytical techniques such as particle size distribution, rheological assessment of liquid systems and elemental surface composition of powders with advanced microscopic techniques (i.e., confocal laser scanning microscopy and scanning electron microscopy). This approach facilitated generating of comprehensive information about the complex structures of model nutritional systems (i.e., RTF infant formula, powdered infant formula and nutritional beverages) and changes that these systems undergo during formulation, manufacturing processes and during storage.

*Schematic representation of research approach for OptiHydro project*
3. Research Achievements/Results

- Assays for physicochemical/functional characterisation of conjugates developed
- Conjugates prepared and functional characterisation of these ingredients completed
- The influence of enzymatic hydrolysis of whey proteins (WPH) on their ability to be conjugated with carbohydrates was assessed thoroughly in direct comparison with that of intact whey proteins
- The relationship between degree of hydrolysis and physicochemical functionality of the resultant conjugates was established
- Detailed mechanistic understanding of the influence of hydrolysis of whey protein on susceptibility to conjugation, and functionality of the resultant conjugates was developed using assays for measuring protein/peptide molecular weight, structure/conformation, reactivity, surface hydrophobicity etc.
- Model ready-to-feed (RTF) infant formula (IF) emulsions stabilised by conjugated WPH displayed the best stability to a range of heat treatments (75-100°C) and to storage stability
- Conjugate-based emulsions displayed superior stability and processing performance
- Lowest thermal stability was observed for the WPH system with no pre-treatment (i.e., no pre-heating or conjugation) due to the reduction in the number of available sulphydryl groups
- Conjugation of WPH with MD improved thermal stability of liquid systems formed with that ingredient

4. Impact of the Research

Arising from this highly successful project next-generation whey protein-maltodextrin conjugates with improved functionality in high-value food products were developed. These novel ingredients displayed superior functionality in model infant nutrition products and nutritional beverage applications. Functionality and application performance data for these novel conjugates in value-added nutritional products was actively disseminated with the Irish and international dairy industry. New product development opportunities for whey ingredients, especially hydrolysed whey ingredients were generated in this project; this mainly dealt with strategies aimed at improving the heat stability (i.e., by controlled pre-heating, blocking of free thiol groups and controlled conjugation) of WPH ingredients. The research outputs of this project identified a viable opportunity of providing the consumer with natural, dairy-based alternatives to synthetic emulsifiers. It was shown that (non-dairy) low molecular weight emulsifiers can be replaced by conjugated whey protein ingredients, resulting in nutritional product with a clean label and enhanced stability. New scientific information on the role of emulsion interfacial layer composition/structure in determining the oxidative stability and shelf life of fat-filled foods was generated in the
project. A significant advancement in the knowledge of the role of the interfacial layer of oil globules in emulsions on the physicochemical properties, processing performance and stability was achieved in this project. Novel analytical approaches were developed in this project to assist the dairy industry with product development and innovation, with a focus on the evaluation and improvement of ingredient functionality in complex food matrices. The Irish dairy industry was provided with 2 PhD graduates trained in the areas of ingredients, formulation, functionality, processing performance and shelf-life assessment.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research
The existing collaborative links between UCC and Teagasc, through the Strategic Alliance in Food Research, were developed further (e.g., analysis of volatile compounds in conjugated ingredients) and new international collaborations were established through this project. Specifically, a collaboration centred around analysis of Maillard reaction products in formulated nutritional products was established with Prof MaryAnne Drake at the Department of Food Science, Bioprocessing and Nutritional Sciences, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC 27695, USA. A second collaboration was established with Prof Luisa Pellegrino at the Department of Food, Environmental and Nutritional Sciences, University of Milan, Italy on analysis of Maillard reaction products in whey protein-carbohydrate mixtures. The project team hosted an internship food engineering student from INRA, Rennes, France for 6 months in UCC to support the 2 PhD students working on this project. Additional collaborations were established with Ghent University, Belgium and the university of Wisconsin-Madison, Madison, Wisconsin on development of analytical capability to characterise interfacial properties of proteins and molecular weight profile of maltodextrin ingredients respectively. Prof Paul van der Meeren from the University of Ghent, Belgium visited UCC and gave a seminar on the use of lecithin to improve thermal stability of milk protein based systems. Collaborative analytical research was performed in conjunction with Prof John Lucey at the University of Wisconsin, Madison, Wisconsin, whereby the molecular weight profile of the starch hydrolysis products used in this project were determined using a state of the art molecular weight profiling method.

(ii) Outcomes where new products, technologies and processes were developed and/or adopted
New analytical approaches were investigated and established for rapid heat stability screening of emulsion-based systems using a controlled stress rheometer and laser light diffraction analysis capability. This capability was used in the project and was a central part of one of the high-impact scientific publications from the project, in addition to being transferred to two Irish, and two international, dairy ingredient companies to support their ingredient development.
activities. Processes were also developed in the project for rapid physical stability and shelf-life testing of dairy protein beverages using the novel Lumisizer accelerated physical stability analyser instrument.

(iii) Outcomes with economic potential
Many of the analytical techniques and model formulations developed as part of this project have been transferred to Irish and international dairy industry partners and are being actively used in the targeted and expedited development and functional evaluation of newer generation whey protein-based ingredients for use in nutritional beverage applications (e.g., infant formula, clinical nutrition). The possibility to remove non-protein low molecular weight emulsifiers from the formulation of infant formula and nutritional beverages based on WPH by promoting conjugation of WPH during the manufacturing process has commercial potential. This could directly reduce ingredient-related costs involved in the manufacture of these products. Strategies aimed at improving the functionality of WPH developed in this project could directly benefit the manufacturers of these ingredients by allowing them supplying their customers with an improved product/ingredient with higher specification and at a premium price.

(iv) Outcomes with national/policy/social/environmental potential
These outcomes of the project are numerous and may be summarised as follows:

- Extending functionality of dairy proteins to increase global competitiveness of Irish dairy ingredients and products.
- Development of viable strategies for manufacture of clean label dairy products.
- Replacement of non-dairy imported emulsifiers with locally produced dairy-based functional ingredients.
- Training and professional development of Irish food industry personnel by academic and research students/staff working on this project through the UCC Food Industry Training Unit.

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications, International Journal/Book chapters.


(ii) Popular non-scientific publications and abstracts including those presented at conferences


(iii) National Report

Not applicable.

(iv) Workshops/seminars at which results were presented

Thermal Processing Training Course, 25-26 April 2017, Food Industry Training Unit, University College Cork

FS4023 (5th Year Food Science programme module) lecture series on emulsifiers and emulsions, February-March 2017, University College Cork

FS6106 (MSc class on Advanced Dairy Biochemistry) interfacial properties of milk proteins, April 2017, University College Cork

(v) Intellectual Property applications/licences/patents

Not applicable.

(vi) Other

5. Scientists trained by Project

Total Number of PhD theses: 2

Kamil P. Drapala, University College Cork, Processing and Stability of Infant Formula-Based Emulsions as Affected by Emulsifier Type, submitted May 2017.


Total Number of Masters theses: 0
6. Permanent Researchers

<table>
<thead>
<tr>
<th>Institution Name</th>
<th>Number of Permanent staff contributing to project</th>
<th>Total Time contribution (person years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>University College Cork</td>
<td>3</td>
<td>2.13</td>
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<tr>
<td>Teagasc Moorepark</td>
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<td>0.18</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>4.00</strong></td>
<td><strong>2.31</strong></td>
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7. Researchers Funded by DAFM

<table>
<thead>
<tr>
<th>Type of Researcher</th>
<th>Number</th>
<th>Total Time contribution (person years)</th>
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</thead>
<tbody>
<tr>
<td>Post Doctorates/Contract Researchers PhD students</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Masters students</td>
<td></td>
<td></td>
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<tr>
<td>Temporary researchers</td>
<td></td>
<td></td>
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<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2</strong></td>
<td><strong>8</strong></td>
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8. Involvement in Agri Food Graduate Development Programme

<table>
<thead>
<tr>
<th>Name of Postgraduate / contract researcher</th>
<th>Names and Dates of modules attended</th>
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<tbody>
<tr>
<td>Kamil Drapala</td>
<td>Next-Generation Food Formulation (February 2012)</td>
</tr>
<tr>
<td></td>
<td>Industrial Scale R&amp;D (June 2012)</td>
</tr>
<tr>
<td></td>
<td>Analysis and Interpretation of Experimental Data with Mathematical and Statistical Tools (October 2012)</td>
</tr>
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<td></td>
<td>Farm to Fork – Sustainability in the Bioeconomy (November 2012)</td>
</tr>
<tr>
<td></td>
<td>Innovation in the Bioeconomy (February 2013)</td>
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<td></td>
<td>National and Global Food Sector May 2013)</td>
</tr>
<tr>
<td>Eve Mulcahy</td>
<td>Next-Generation Food Formulation (February 2012)</td>
</tr>
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</table>
9. Project Expenditure

Total expenditure of the project: €318,882

Total Award by DAFM: €348,053

Other sources of funding including benefit in kind and/or cash contribution(specify):
There were no other sources of funding.

Breakdown of Total Expenditure

<table>
<thead>
<tr>
<th>Category</th>
<th>UCC</th>
<th>MFRC</th>
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<tr>
<td>Contract staff</td>
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<tr>
<td>Temporary staff</td>
<td></td>
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<tr>
<td>Post doctorates</td>
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<td></td>
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</tr>
<tr>
<td>Post graduates</td>
<td>167,557</td>
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<tr>
<td>Consumables</td>
<td>45,079</td>
<td>4,177</td>
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<tr>
<td>Travel and subsistence</td>
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<tr>
<td>Sub total</td>
<td>232,362</td>
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<tr>
<td>Durable equipment</td>
<td>11,381</td>
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<tr>
<td>Other</td>
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<tr>
<td>Overheads</td>
<td>69,709</td>
<td>1,253</td>
<td>70,962</td>
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<tr>
<td>Total</td>
<td>313,452</td>
<td>5,430</td>
<td>318,882</td>
</tr>
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10. Leveraging

On the basis of the quality and relevance of the technical understanding of physicochemical functionality of whey protein ingredients developed as part of this project, several industry partner-specific projects were initiated and completed during the course of, and after, the OptiHydro project.
During the course of this project, the scientific outputs and reputation developed by the project team with the main Irish dairy companies and funding agencies was instrumental in maximising the representation of the UCC Food Ingredients Research Group in the Dairy Processing Technology Centre (DPTC). Dr Seamus O’Mahony leads the DPTC Pillar 2 focused on Next Generation Dairy Processing Science and Technology and both PhD students on the OptiHydro project subsequently worked with Dr O’Mahony as Research Assistants/Postdoctoral researchers on the DPTC at UCC. The UCC budget for DPTC is €2.2m over 5 years.

A funding proposal developed jointly between Dr Seamus O’Mahony and an international collaborator (INRA, France) is currently under evaluation, which would allow continuation of some of the more fundamental elements of spray drying of conjugated whey proteins to be extended.

11. Future Strategies

This research identified a number of potential research avenues that would be interesting to follow and which could provide high level innovation with a significant economic benefit to the Irish dairy industry which is currently working on expanding its portfolio to include high-end dairy nutritional products (e.g., IF, tailored nutrition products). The research avenues identified could be pursued through DAFMs Competitive national Programme or through innovation partnerships via Enterprise Ireland. Some of these potential research avenues are discussed below.

Harnessing the strong steric stabilisation potential of WPH-MD conjugates can provide significant potential for production of novel IF products, engineered to closer resemble interfacial structures present in human milk, where oil globules are predominately populated by polar lipids and sterically stabilised by glycoproteins (i.e., milk fat globule membranes). Research into the area of mimicking the MFGM structures is a hot topic at the moment and major international dairy players (e.g., Nestle, Danone, Mead Johnson and Fonterra) are currently actively working in that space.

Fractionation technologies to enrich/purify conjugates: as excess unconjugated carbohydrate may not be desirable in such applications with low carbohydrate (<10% carbohydrate) sports beverages are more popular than their high carbohydrate counterparts. Further scope exists to determine if any unreacted carbohydrate can be removed from the conjugated whey protein-carbohydrate systems by membrane filtration, e.g., ultrafiltration.

Hypoallergenic whey proteins with improved sensory attributes: extensively hydrolysed whey proteins are associated with very poor sensory attributes, such as extreme bitterness, conjugation of the proteins with carbohydrates may have the potential to mask the immunogenic epitope sites. As taste is the key driver for consumer acceptability of
food products, it would be advantageous to improve the sensory attributes of hypoallergenic whey proteins.

In addition to the future research recommendations detailed above, knowledge and expertise generated from this project leveraged funding for related projects from industry which has led to exciting and high profile research collaborations and highlighted industry relevant research areas that will be pursued through national funding schemes:

- International collaboration with INRA, France: 1 year project proposal has been submitted for the scheme Programme d’Attractivité Post-Doctorale / Département AAL, Campagne 2017 to work on a project titled: Effect of surface composition and rheological properties of air-water surfaces of particles on the drying kinetics and techno-functional properties of spray-dried nutritional dairy-based powders.

- Plans to seek research funding through SFI, EU and Horizon 2020 calls.