Food Institutional Research Measure

Final Report

‘ProSurf - Controlling surface-activity of protein aggregates for their incorporation into nutritional formulation for optimised processibility’

DAFM Project Reference No: 10RDTMFRC723

Start date: 01/12/2011

End Date: 30/11/2016

Principal Coordinator and Institution:
Dr André Brodkorb, Teagasc Food Research Centre Moorepark
Email: andre.brodkorb@teagasc.ie

Collaborating Research Institutions and Researchers:
Dr Mark Fenelon, Teagasc Food Research Centre Moorepark
Seamus O’ Mahony, School of Food and Nutritional Sciences, University College Cork

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>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4 ×</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report:

<table>
<thead>
<tr>
<th>Priority Area (s)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>L Manufacturing Competitiveness</td>
<td></td>
</tr>
<tr>
<td>M Processing Technologies and Novel Materials</td>
<td></td>
</tr>
<tr>
<td>H Food for Health</td>
<td></td>
</tr>
</tbody>
</table>

Key words: food proteins, processing, whey protein, infant formula
1. **Rationale for Undertaking the Research**

During the last two decades, whey protein products have become major commodity products for Irish ingredient manufacturers. Providing customers with exact product properties regarding stability and processibility in complex food products can be a major competitive advantage. However, unlike caseins, whey proteins are inherently instable with regard to processing stresses (e.g. pH, salt, and shear). Minor inconsistencies in the product characteristics (e.g. mineral content, degree of denaturation or pH) can also be challenging during subsequent incorporation in complex food matrices, such as infant formula, causing the uncontrolled denaturation, aggregation (mainly with caseins) and destabilisation of the food product.

Therefore two questions were identified and addressed by the project:

- Develop and improve process-induced improvements in the heat stability and viscosity of beverages containing whey proteins
- Provide the scientific data of how and why whey proteins can be stabilised by pre-heat treatment

2. **Research Approach**

There are some known approaches to minimise whey protein instability when heated in formulated products. For example, nutritional beverage manufacturers require protein-based ingredients that have been heat treated under controlled conditions prior to use. This thermal pre-treatment of whey proteins causes partial denaturation and controlled self-aggregation. The surface characteristics and reactivity of the proteins/protein particles change, which in turn reduces their affinity to associate with casein and themselves. This study used a systematic approach to minimise the loss of stability in nutritional formulations due to excessive whey protein aggregation and identify key controlling factors for high viscosity developments. It used both lab and pilot-scale heating treatment of whey protein products in the absence and presence of caseins and additional minerals. In addition, the effect of heat treatment on the *in vitro* digestive behaviour was investigated.

3. **Research Achievements/Results**

**Summary**

- The outcome of this project demonstrated the profound effect of pre-heating on the heat stability of whey proteins in whey protein isolates. Heating times of 1-2 min at 75 to 105°C can increase the heat coagulation time at 120°C by up to three times.
- Small amounts of calcium have a negative effect on the heat stability.
- Results showed that the aggregation of whey proteins is more influential than denaturation in determining viscosity development during the HTST treatment of IMF.
- Heat-induced unfolding and aggregation of whey proteins in WPI accelerate the *in vitro* digestion of both β-lactoglobulin and α-lactalbumin.

**Results by tasks:**

This project was divided into two major (tasks 1 and 2) and two minor tasks (3 and 4).

*Task 1* dealt with the fundamentals of whey protein denaturation and aggregation and their subsequent *in vitro* digestion behaviour. Pre-heat treatment of whey proteins can improve the heat stability of whey protein products. This is achieved by controlling the unfolding, denaturation and aggregation into nanoparticles of less than 100nm. Whey proteins were treated at pilot scale in the Biofunctional Engineering
Facility (BFE) Moorepark, hence the results can be easily translated into industrial scale, which is a real strength of this study. Pilot-scale pre-heating of whey protein isolate (WPI) had a profound effect on their subsequent heat stability. For example, heating a 9% (w/w) WPI solution at pH 7 for 1 min at 75, 85, 95 or 105°C can increase the heat coagulation time at 120°C from 17.8±6.8 min for unheated samples to 47.9±2.6, 50.2±5.8, 49.9±1.7 and 58.9±2.9, respectively. All samples had a high degree of denaturation (>60%) and aggregation (30-90nm).

In addition, in vitro adult digestion clearly showed that the kinetics of the whey proteins is accelerated by the pre-heating of whey proteins.

Task 2 dealt with the application of denaturation and aggregates in more complex systems containing salts and other ingredients commonly used in infant formula. These results can be used for stabilising whey proteins in complex nutritional beverage systems such as infant formula. Small amounts of calcium have a negative effect on the heat stability. Results showed that the aggregation of whey proteins appeared to be more influential than the degree of denaturation in determining viscosity development during the high temperature-short time (HTST) treatment of infant formula. In addition, it was observed that even small amounts of ionic calcium can have a detrimental effect on the heat stability of WPI. Added calcium (2.2mM in 8% WPI at pH 6.8) treated for 2 min at 72, 85 or 85 °C induced significant changes in the viscosity, turbidity and colour in the heated WPI solutions due to extensive Ca-induced aggregation.

In Task 3, innovative labelling techniques were used to visualise specific proteins in complex emulsions. β-casein was covalently labelled with a fluorescent rhodamine-type dye and purified. The complex was used to monitor the interfacial changes in the emulsion in pure model protein emulsions as well as in mixed protein-based model infant formulae (mixed casein/whey protein model infant formulas – first stage IF containing 60% whey proteins/40% casein). Confocal images were recorded in the National Food Imaging Centre Moorepark.

Task 4 dealt with secondary molecules that are able to stabilise whey protein with regard to denaturation and aggregation. Kappa-casein (κ-CN) was found to affect the denaturation behaviour of whey proteins. An experiment of κ-CN in the presence of WPI showed a reduction of denaturation and aggregation of β-lactoglobulin in WPI. Furthermore, caseinomacropeptide (CMP), which is present in cheese whey as a breakdown product of κ-CN by the action of chymosin during cheese production, was investigated as a chaperone-like molecule to control whey protein denaturation and aggregation. Initial results are promising and are currently being further investigated in Teagasc to fully substantiate any claim of a protective role of CMP.

4. Impact of the Research

Pre-heat treatment of whey protein used in the formulation of model infant milk formula (IMF) systems helped to reduce the susceptibility of whey proteins to aggregation when incorporated into a model IMF protein system. This work has shown that the physical state/reactivity of whey protein molecules/particles strongly influences protein-protein interactions and their ability to contribute to viscosity during thermal processing, even at similar levels of denaturation. These findings are important in facilitating an enhanced understanding of protein-protein interactions and their influence on viscosity development and the processing performance of formulated nutritional beverages such as infant formula.

It was also demonstrated that the short-time heating of whey proteins facilitates the proteolysis during in vitro digestion, using an internationally standardised digestion method (INFOGEST) suitable for food.
Overall, the outcome of this project will help end-users of whey protein products, e.g. manufacturers of nutritional beverages (infant formula, sports drinks, nutritional products for the elderly) to understand and improve heat stability, one of the most important physical properties of whey protein products. The study has demonstrated how the short-time pre-heating of whey proteins causes unfolding and subsequent aggregation, which is beneficial for process stability and accelerated digestion.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

Teagasc collaboration:
Aarhus University (Dr Lotte Larsen), desialylation of CMP
University of Leeds (Prof Alan Mackie), development of semi-dynamic in vitro digestion method
University of Valencia (Prof Amparo Lopez-Rubio), small-angle X-ray scattering SAXS (ALBA Synchrotron, Barcelona)

(ii) Outcomes where new products, technologies and processes were developed and/or adopted

None

(iii) Outcomes with economic potential

It was clearly shown how relatively short heating times can be beneficial for stabilising whey protein product in complex formulations, which can prevent expensive down-time during food processing due to blockage or burn-on effect in equipment.

(iv) Outcomes with national/ policy/social/environmental potential

The consensus for an internationally standardised digestion methods, the INFOGEST method (Minekus et al. 2014), which was coordinated by A. Brodkorb, is now the academic and industry standard for the simulation of food digestion. It also formed the basis for new EFSA methods for the evaluation of the allergenicity of proteins. The INFOGEST method was also put forward as an ISO/IDF method to screen/predict protein digestibility.

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

Poster presentations


5. A. Brodkorb. A standardised static in vitro digestion method suitable for food- an international consensus. Poster presented at the 4th International Conference on Food Digestion, Naples/Italy, 17-19 March 2015 (all travel expenses paid by COST1005)


7. Poster presentation presented by Aoife M. Joyce at the International Dairy Federation (IDF) 6th International Symposium of Dairy Dried Products (SDDP), Dublin, 11-13th April 2016 entitled ‘Controlling aggregation of whey proteins to optimise their physicochemical functionality during the manufacture of infant nutritional products’

8. Poster at the EFFOST conference in Athens 9-12 November 2016, A.Brodkorb A standardised static in vitro digestion method suitable for food- an international consensus

Oral presentations


5. Aoife M. Joyce, Alan L. Kelly, André Brodkorb and James A. O’Mahony ‘Pre-denaturation/aggregation of whey proteins to optimise their physico-chemical functionality in infant nutritional products’ presented at the 44th Annual Food Research Conference on the 14th December 2015 held in Teagasc, Moorepark, Fermoy, Co. Cork.

6. Oral presentation presented on the work of research MSc student, Aoife Joyce, by Dr. James A. O’Mahony at the International Dairy Federation (IDF) 6th International Symposium of Dairy Dried Products (SDDP), Dublin, 11-13th April 2016, entitled ‘Separation of the effects of denaturation and aggregation on whey-casein protein interactions during the manufacture of a model infant formula’.

7. Work included as part of an oral presentation presented by Dr. James A. O’Mahony at the 19th Dairy Ingredients Symposium, Cal Poly, California, 22-23rd February, 2017 entitled, ‘Perspectives on current and future protein ingredients for infant nutritional product formulation’.


(iii) National Report
none

(iv) Workshops/seminars at which results were presented
none

(v) Intellectual Property applications/licences/patents
none

(vi) Other
A TV program with Dr André Brodkorb as part RTE’s Big week on the farm was aired in April 6, 2017 entitled “what’s in milk”, featuring Claire hurler Shane O’Donnell swallowing a wireless endoscopy camera and seeing live footage of him digesting commercial (Irish) whey proteins (fast protein) vs. whole milk

5. Scientists trained by Project

Total Number of PhD theses: 0

Total Number of Masters theses: 1
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>Teagasc</td>
<td>2</td>
<td>1.411</td>
</tr>
<tr>
<td>UCC</td>
<td>1</td>
<td>1.25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3</strong></td>
<td><strong>2.661</strong></td>
</tr>
</tbody>
</table>

7. **Researchers Funded by DAFM**

<table>
<thead>
<tr>
<th>Type of Researcher</th>
<th>Number</th>
<th>Total Time contribution (person years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post Doctorates/Contract Researchers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PhD students</td>
<td>3</td>
<td>5.083</td>
</tr>
<tr>
<td>Masters students</td>
<td>1</td>
<td>2.000</td>
</tr>
<tr>
<td>Temporary researchers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>4</strong></td>
<td><strong>7.083</strong></td>
</tr>
</tbody>
</table>

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>
</tr>
</thead>
<tbody>
<tr>
<td>Aoife Joyce</td>
<td>AFDGP Bio-Economy training, Dublin2014</td>
</tr>
<tr>
<td>Sandeep Kanniganti</td>
<td>Writing Skills, Cork 2014</td>
</tr>
</tbody>
</table>
9. **Project Expenditure**

Total expenditure of the project: €307,206

Total Award by DAFM: €333,840

**Breakdown of Total Expenditure**

<table>
<thead>
<tr>
<th>Category</th>
<th>Teagasc</th>
<th>UCC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contract staff</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temporary staff</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post doctorates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post graduates</td>
<td>76,500.00</td>
<td>79,772.47</td>
<td>156,272.47</td>
</tr>
<tr>
<td>Consumables</td>
<td>29,945.55</td>
<td>32,698.88</td>
<td>62,644.43</td>
</tr>
<tr>
<td>Travel and subsistence</td>
<td>12,452.27</td>
<td>4,943.31</td>
<td>17,395.58</td>
</tr>
<tr>
<td><strong>Sub total</strong></td>
<td><strong>118,897.82</strong></td>
<td><strong>117,414.66</strong></td>
<td><strong>236,312.48</strong></td>
</tr>
<tr>
<td>Durable equipment</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Overheads</td>
<td>35,669.35</td>
<td>35,224.40</td>
<td>70,893.74</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>154,567.17</strong></td>
<td><strong>152,639.06</strong></td>
<td><strong>307,206.22</strong></td>
</tr>
</tbody>
</table>

10. **Leveraging**

During the lifetime of the project, Dr André Brodkorb became a work group leader of COST Action FA1005 INFOGEST (2011-2015, [https://www.cost-infogest.eu/](https://www.cost-infogest.eu/)). Besides the substantial travel funding provided by COST, it resulted in an international consensus method for in vitro digestion of food. The publication Minekus et al. 2014 *Food & Function* (Highly Cited Paper status for agricultural sciences in Web of Science, >1000 citation by Google Scholar), coordinated by A. Brodkorb, is now the recognized academic and industry standard for static in vitro digestion of food. It also resulted in a high impact publication: Brodkorb et al. 2019 *Nature Protocols* ([https://rdcu.be/brEMd](https://rdcu.be/brEMd)).

Other projects resulting from ProSurf and previous related FIRM projects coordinated by A. Brodkorb e.g. FIRM BioALac, FIRM WheyEnCaps:
- Enterprise Irelands Innovative Partnership (IP 2018 0711) Coordinator A. Brodkorb GlutDigest 2018-2020, €194,000
- Enterprise Irelands Innovative Partnership (IP 2016 0515) Coordinator A. Brodkorb, Infant Digest 2017-2020, €433,000
- SFI Research Centre VistaMilk 2019-2024; A. Brodkorb is a PI in TP7 and 8, related to protein functionality and in vitro digestion
- Travel funding for A. Brodkorb and S. O’Mahony to various workshops and conferences by COST action INFOGEST FA1005, of Korean Society of Food Science and Technology and International Dairy Federation (IDF).
11. **Future Strategies**

Denaturation and aggregation of whey proteins remains an important scientific area due to its relevance to the increasing interest in industrial processing of food products or ingredients containing whey proteins. Three research projects developed out of ProSurf:

1. Further investigation into chaperone-like molecule to control whey protein denaturation and aggregation. Initial results on the protective effect of caseinomacropeptide (CMP) are promising. The role of the prosthetic side chains (phosphorylation and glycosylation) will be further examined. Collaborator: Dr Lotte Larsen, Aarhus University

2. Examination of early unfolding processes of whey proteins in the presence of CMP using advanced methods such as small-angle X-ray scattering SAXS (Synchrotron) and high resolution NMR. Collaborators: Prof Amparo Lopez-Rubio, University of Valencia and Dr Ken H. Mok, TCD

3. Development of improved *in vitro* digestion method e.g. semi-dynamic, for the study of the digestive behaviour of protein ingredients for nutritional beverages. Collaborators: Prof Alan Mackie, University of Leeds

Point 3 (*in vitro* digestion) has been and will further attract significant interest from the Irish and multi-national food industry.