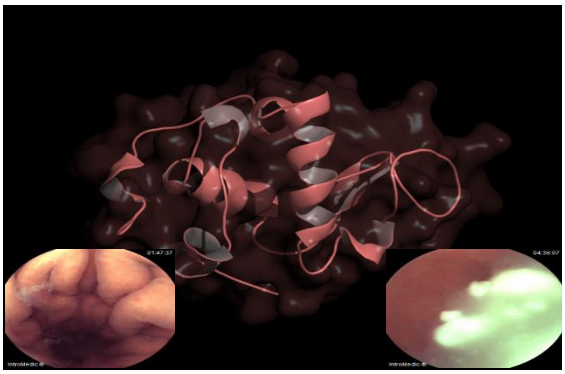
Project number: 6261
Funding source: Dairy Levy Research Trust

Date: March 2018
Project dates: Dec 2011- March 2018

Controlling Milk Protein Aggregation during Processing and Gastro-Intestinal Digestion



Key external stakeholders:

Dairy ingredient manufacturers, Lifestyle beverage manufacturers including infant formula & sports nutrition

Practical implications for stakeholders:

Milk proteins are widely used in the formulation of nutritional, medical and/or sports beverages. However, milk processing, in particular heat treatment of protein ingredients, can affect product stability and gastro-intestinal behaviour of dairy products. Heating whey proteins enriched with κ-casein or caseinomacropeptide can improve the heat stability of whey proteins. β-casein-enriched protein products can be used as a potential ingredient for nutritional beverages; however, the aggregation of β-CN needs to be taken into consideration. Heat treatment or increasing the whey protein to casein ratio can accelerate the gastric digestion of milk or protein ingredients. Static and semi-dynamic digestion models can help the understanding of the mechanism and kinetics of food digestion, in particular dairy products.

Main results:

- Heating in the presence of κ-casein or caseinomacropeptide (CMP; a by-product of cheese manufacture), improved heat stability of whey proteins. The charged glycosylated side chains of κ-casein or CMP play a key role in the chaperone-like stabilisation mechanism.
- The extent of temperature-induced aggregation of β-casein-enriched ingredients was highly dependent on β-casein purity i.e. the presence of other proteins, and the concentration of ionic calcium.
- β-casein-enriched infant formulas (IF) were successfully produced, with similar physicochemical properties to regular first-stage IF.
- A semi-dynamic *in vitro* digestion method based on available physiological data was developed, which can accurately simulate the main dynamic processes during gastric food digestion.
- Milk processing can affect the gastric behaviour and nutrient digestion kinetics of liquid milk.
- The concept of 'slow and fast proteins' in relation to digestion for caseins and whey proteins, respectively, was shown to be due to their gastric behaviour, which controlled the rate of gastric emptying and absorption of milk proteins.

Opportunity/Benefit:

This project helps to understand process-induced changes and interaction of protein ingredients, which can improve processing efficiency, product stability and nutrient delivery.

Collaborating Institutions:

UCC, Quadram Institute, University of Leeds

Contact Andre Brodkorb Email: andre.brodkorb@teagasc.ie.

Teagasc project team: Dr André Brodkorb (PI)
Dr Mark Auty
Dr Mark Fenelon

External collaborators: Dr Seamus O'Mahony, UCC
Prof Alan Kelly, UCC
Prof Alan Mackie (University of Leeds, UK)
Prof Pete Wilde (Quadram Institute, Norwich, UK)

1. Project background:

Controlling the aggregation behaviour of proteins is one of the critical processes that can affect the stability of dairy protein-based products during processing. Fractionated protein ingredients offer opportunities to add physical or nutritional functionalities to milk ingredients or finished products in which they are incorporated. β-casein-enriched protein ingredients can be used by infant formula manufacturers to create a protein profile closer to that of human milk. However, the aggregation behaviour of β-casein differs from that of other milk proteins. Thus, there is a need to understand the mechanisms through which it interacts with itself and other nutrients to improve stability in complex systems such as first stage IF formulations. For example, other ingredients can be used to improve the stability of whey proteins, and in particular the denaturation and aggregation behaviour of the whey protein β-lactoglobulin. Process-induced changes and re-formulations of milk protein products can affect the micro and macrostructures of dairy ingredients or products. Previous research has suggested that dairy structures exhibit different physiological responses. However, their mechanistic behaviour is still poorly understood.

2. Questions addressed by the project:

- To investigate the factors affecting the aggregation of β-casein-enriched ingredients.
- To understand why and how the aggregation of β-casein-enriched ingredients influences the stability of complex food systems such as nutritional beverages.
- To study the heat stability of whey protein beverages as influenced by the addition of κ-casein or CMP (a peptide derived from the enzymatic cleavage of κ-casein during cheese manufacture).
- To simulate and understand the impact of process-induced changes of proteins and reformulation on gastro-intestinal digestion *in vitro and ex vivo*.

3. Experimental studies:

This study used an integrated approach (labelling techniques, microscopy, spectroscopy, rheology and chromatography) to investigate the effect of β-casein, κ-casein and CMP on the functionality of whey proteins and more complex nutritional formulations.

In addition, a static and semi-dynamic *in vitro* digestion model was developed and tested using protein formulations produced in-house. The method was evaluated in several European laboratories in order to establish an international consensus on the digestion method.

The project resulted in 3 PhD theses.

4. Main results:

The main results can be divided into three parts based on three PhD theses: (i) the effect of κ-casein and caseinomacropeptide on denaturation and aggregation of whey proteins, (ii) the aggregation behaviour of β-casein in pure solutions, emulsions and infant formula and (iii) the effect of processing and formulation on in vitro digestion of dairy products.

i. The outcome of this project demonstrated that κ-casein and caseinomacropeptide could be used to improve the heat stability of whey proteins. The temperature of denaturation and aggregation of whey proteins heated in the presence of caseinomacropeptide increased by up to 3 and 7°C, respectively. The charged glycosylated side chains of κ-casein or CMP play a key role in the stabilisation mechanism.

i. The results demonstrated that the aggregation of β-casein could be controlled by temperature, the ratio of calcium and phosphorus and selected ingredients, which would be beneficial for the application of β-casein-enriched ingredients in nutritional beverages.

i. The processing of milk (heat treatment and homogenisation) has a profound impact on the digestive behaviour of the protein due to gastric restructuring. Lower heat treatment caused the formation of a firm curd during the gastric phase, whereas homogenisation caused phase separation and creaming during the gastric phase. This implies that gastric emptying and the overall digestive kinetics and absorption of

Contact Andre Brodkorb Email: andre.brodkorb@teagasc.ie.

nutrients can be affected by commonly used processing of protein ingredients. In addition, it was clearly demonstrated that *in vitro* digestion methods can be used to understand and explain digestion phenomena such as fast whey proteins vs. slow caseins. In addition, a static in vitro digestion method was developed as part of an international network (INFOGEST). The methods (Minekus et al. 2014, Brodkorb et al. 2019) is now the academic and industry standard to simulate the digestion of food using standard laboratory equipment.

5. Opportunity/Benefit:

The primary stakeholders for this research are manufacturers of nutritional beverages containing milk proteins. The studies present the scientific basis for future application of protein products enriched with κ-casein, CMP or β-casein.

In addition, static and semi-dynamic in vitro digestion models were developed and published as international consensus methods. These methods are particularly suited for the evaluation of digestive behaviour of dairy proteins and can be used as tools to underpin claims for digestive benefits.

6. Dissemination:

AWARDS

Young Scientist Best Paper AWARD for Meng Li: Food Structure and Functionality Symposium: From Molecules To Functionality in Amsterdam (April 2014); Li, M. "The effect of covalent labelling techniques on dairy protein stabilised emulsions"

TEAGASC PRIZE for the Best Poster by Sophie Gaspard at the 44th Annual Irish Food Research Conference held in the Teagasc Food Research Centre Moorepark, Fermoy Co. Cork (Dec 2015) "Thermal Stability of Heat-Induced κ-Casein/Whey Protein Aggregates Isolated from Milk Protein Concentrate"

AWARD for the Best Poster by Anabel Mulet-Cabero, Society of Dairy Technology Spring Conference-Advances in nutritional dairy products and ingredients, Cork, Ireland (April 2017); Mulet-Cabero, A. "How can food structure affect gastric digestion?"

PHD THESIS

Mulet-Cabero, A.-I., "Effect of Dairy Structures on Gastric Behaviour and Nutrient Digestion Kinetics using a Semi-Dynamic Model", Quadram Institute of Bioscience, Norwich, UK. 2/2019, Quadram Institute: Norwich.

Gaspard, S.B., "Controlling the denaturation and aggregation of whey proteins using κ-casein and caseinomacropeptide", School of Food and Nutritional Sciences. National University of Ireland, Cork, Ireland. 4/2020

Li, M., "Aggregation behaviour of beta-casein in pure and complex nutritional beverages", School of Food and Nutritional Sciences., National University of Ireland, Cork, Ireland. 7/2020

PEER-REVIEWED PUBLICATIONS

Gaspard, S. J., Sunds, A. V., Larsen, L. B., Poulsen, N. A., O'Mahony, J. A., Kelly, A. L., & Brodkorb, A. (2020). Influence of desialylation of caseinomacropeptide on the denaturation and aggregation of whey proteins. Journal of Dairy Science, 103(6), 4975–4990.

Mulet-Cabero, A.-I., Egger, L., Portmann, R., Ménard, O., Marze, S., Minekus, M., . . ., Brodkorb, A, Mackie, A. (2020). A standardised semi-dynamic in vitro digestion method suitable for food – an international consensus. Food & Function, 11, 1702-1720.

Mulet-Cabero, A.-I., Mackie, A. R., Brodkorb, A., & Wilde, P. J. (2020). Dairy structures and physiological responses: a matter of gastric digestion. Critical Reviews in Food Science and Nutrition, 1-16.

Mulet-Cabero, A.-I., Torcello-Gómez, A., Saha, S., Mackie, A. R., Wilde, P. J., & Brodkorb, A. (2020). Impact of caseins and whey proteins ratio and lipid content on in vitro digestion and ex vivo absorption. Food Chemistry, 319, 126514.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., . . . Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. Nature Protocols, 14(4), 991-1014.

Li, M., Auty, M. A. E., Crowley, S. V., Kelly, A. L., O'Mahony, J. A., & Brodkorb, A. (2019). Self-association of bovine β-casein as influenced by calcium chloride, buffer type and temperature. Food Hydrocolloids, 88, 190-198.

Li, M., O'Mahony, J. A., Kelly, A. L., & Brodkorb, A. (2019). The influence of temperature- and divalent-cation-mediated aggregation of β-casein on the physical and microstructural properties of β-casein-stabilised emulsions. Colloids and Surfaces B: Biointerfaces, 187, 110620.

Contact Andre Brodkorb Email: andre.brodkorb@teagasc.ie.

Picone, G., De Noni, I., Ferranti, P., Nicolai, M. A., Alamprese, C., Trimigno, A.,Brodkorb, A., . . . El, S. N. (2019). Monitoring molecular composition and digestibility of ripened bresaola through a combined foodomics approach. Food Research International, 115, 360-368.

Gaspard, S. J., Auty, M. A. E., Kelly, A. L., O'Mahony, J. A., & Brodkorb, A. (2017). Isolation and characterisation of κ-casein/whey protein particles from heated milk protein concentrate and role of κ-casein in whey protein aggregation. International Dairy Journal, 73, 98-108.

Levi, C. S., Alvito, P., Andrés, A., Assunção, R., Barberá, R., Blanquet-Diot, S., . . . Brodkorb, A, Lesmes, U. (2017). Extending in vitro digestion models to specific human populations: Perspectives, practical tools and bio-relevant information. Trends in Food Science & Technology, 60, 52-63.

Mulet-Cabero, A.-I., Rigby, N. M., Brodkorb, A., & Mackie, A. R. (2017). Dairy food structures influence the rates of nutrient digestion through different in vitro gastric behaviour. Food Hydrocolloids, 67, 63-73.

Li, M., Auty, M. A. E., O'Mahony, J. A., Kelly, A. L., & Brodkorb, A. (2016). Covalent labelling of β-casein and its effect on the microstructure and physico-chemical properties of emulsions stabilized by β-casein and whey protein isolate. Food Hydrocolloids, 61, 504-513.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., . . . Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. Food & Function, 5(6), 1113-1124.

PUBLICATIONS / ABSTRACTS INCLUDING THOSE PRESENTED AT CONFERENCES

Gaspard, S. J., Auty, M.A.E., Kelly, A.L., O'Mahony, J.A., Brodkorb, A., Isolation and characterization of κ-casein/whey protein particles from heated milk protein concentrate. Oral presentation held at the 10th NIZO Dairy conference, Innovations in Dairy Ingredients on the1st-3rd October, 2017. Papendal, The Netherlands.

Li, M., Auty, M.A.E., Crowley, S.V., O'Mahony, J.A., Kelly, A.L. and Brodkorb, A. Effect of source, temperature and calcium chloride on the aggregation behaviour of bovine β-casein. Oral presentation at the NIZO Dairy Conference- Asia Pacific on the 8-10 November 2016 in Singapore.

Mulet-Cabero, A.-I., Mackie, A., Wilde, P., Fenelon, M. A., & Brodkorb, A. Oral presentation at 3rd Food Structure and functionality forum symposium and 3rd IDF symposium on microstructure of dairy products, June 2018 Montreal, Canada. "Homogenisation and heat treatment of milk affect in vitro gastric digestion".

Brodkorb, A. Invited Plenary Speaker at the 81st Annual Meeting and International Conference of Korean Society of Food Science and Technology (KoSFoST) in Gwangju, Republic of Korea, 25-27 August 2014

Brodkorb, A. Invited speaker at the Annual Meeting of the Danish Dairy Science and Technology Society, 23 January 2017

Brodkorb, A. Plenary Opening Lecture at The 19th Gums & Stabilisers for the Food Industry Conference in Berlin, 1July 2017

Brodkorb, A. Invited Plenary Speaker at the 32nd EFFoST International Conference, Nantes, France; 6-8 November 2018

OTHER PUBLICATIONS

Irish Times article (2/1/2014). "When the experimenters become the experiment"; featuring A. Brodkorb, written by Claire O'Connell.

Irish Times article (31/8/2017). "Food proteins offer plenty to digest - Research lives": A. Brodkorb, written by Claire O'Connell.

TV APPEARANCES

A. Brodkorb featured on the RTE programme "Big week on the farm" on April 6, 2017. Dr Brodkorb explained how whey-based sports drinks are produced and how they are digested. For this they went to the gastroenterology lab in the Mercy Hospital in Cork where Shane O'Donnell, Clare All-Ireland Senior Hurler6261, drinks a whey sports drink and the digestion is followed real-time by wireless endoscopy.

SOCIAL MEDIA

A. Brodkorb featured on the Teagasc podcast "The Research Field" (1/2020)

A. Brodkorb runs a YouTube channel on "*In Vitro* Food Digestion" with over 32,000 views since 2014

7. Compiled by: Dr André Brodkorb

Contact Andre Brodkorb Email: andre.brodkorb@teagasc.ie.