



**An Roinn Talmhaíochta,
Bia agus Mara**
Department of Agriculture,
Food and the Marine

Food Institutional Research Measure

Final Report

Concept Protein Ingredient for Next Generation Infant Formulation

DAFM Project Reference No: 10 RD TMFRC 706

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End Date: 30.06.2016

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Collaborating Research Institutions and Researchers: UCC (Dr. Seamus O'Mahony).

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

Basic/Fundamental	→	Applied	→	Pre Commercial		
1	2	3 X	4	5	6	7

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

Priority Area (s)	Food for Health. Processing Technologies and Novel Materials.
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Key words: (max 4)

Infant formula, membrane separation, protein profile

1. Rationale for Undertaking the Research

With the rapid growth in the world's infant formula market there is an urgent requirement for Ireland to be at the forefront of ingredient research given the dependence of our dairy processors on this sector. The rationale of the current project was to adapt a new approach to manufacturing infant formulations by use of a membrane-based integrated manufacturing system to produce a new concept protein base ingredient from which an infant formulation can be directly formulated and dried creating substantial savings to the manufacturer and environment. The philosophy is new and thus referred to as next generation manufacture.

Infant formula manufacture in Ireland is based on wet processing which is energy intense with extremely high carbon footprint. Double dehydration of ingredients exists, i.e., processing of whey and skim ingredients, followed by, and after transport, rehydration (at a different location), fortification and dehydration for a second time. The resulting final product has high carbon footprint with intense utilisation of energy. Furthermore, the functionality of the final formulation is difficult to predict as the protein ingredients, i.e., skim and whey, have different processing history's and thus when combined in a formulation, their reactivity in the presence of the minerals is difficult to predict. This project aimed to develop the science to make a concept ingredient, with protein profile closer to human milk, which could substantially reduce the cost of manufacture of Infant formula. .

The key to this project is that the concept ingredient will be multifunctional in that it can be used to develop the next generation process or can be used in existing wet process, dryblending or high solids manufacturing processes. The new ingredient will be the first of its kind as it will provide the total protein requirements for the infant with protein profile closer to human milk.

2. Research Approach

The primary objective of the project was to design a new concept 'protein base' ingredient (closer to human milk, i.e., β -casein and α -lactalbumin enriched) with higher quality protein using integrated membrane systems coupled with mineral loading/selectivity to confer broad spectrum stability during processing. The first step of the project was to optimisation of β -casein removal from skim milk by low temperature microfiltration using integration of membrane systems and cold microfiltration of Milk. The results from these experiments were used to develop of protein base with broad spectrum functionality and manufacture an infant formulation using this concept protein base ingredient.

The protein base was 'pre-reacted' to provide optimal performance during formulation. The aim here was to reduce variation in thermal stability during processing, currently caused by interactions between protein ingredients (Skim milk powder and Whey) and minerals, by

adding a pre-conditioned optimized protein base. The protein base was used to build a next generation infant formula manufacturing scenario. The aim was to build a formulation (1st age) using the concept protein base, by adding nutrients (fat, carbohydrate and minerals) to the required solids content suitable for direct drying without intermediate processing such as evaporation, creating a simplified process that can be located at a single, integrated manufacturing facility.

3. Research Achievements/Results

The main findings are as follows:

Cold microfiltration (MF) coupled with diafiltration (DF) facilitated the formulation of a protein base with a casein profile close to human milk using a skim milk feed. Using this process it was demonstrated that two major formulation targets, a casein:whey ratio (40:60) and casein profile (β -casein), can be achieved. This β -casein-containing whey stream has a protein profile closer to human milk. Other potential benefits of the cold MF and DF process were also identified, ranging from a reduction in β -lactoglobulin levels (thus increase in α -lactalbumin levels) and reduced in-process proteolysis (lower levels of casein hydrolysis products). The manufacture of suitable infant formula protein base ingredients using integrated membrane systems will be an important development in next-generation Infant formula manufacture. The process has the advantage of a lower thermal load on the protein ingredients and expected lower carbon emissions. The new approach to infant formula manufacture should be a cost effective process (given the reduced number of processing steps), have good quality, highly native, colour free with minimal processing. A high quality micellar casein side stream has also been generated for further investigation and use in new applications.

4. Impact of the Research

The project has played a central role in supporting the dairy processing and infant milk formula (IMF) sectors in Ireland through the experiments carried out at Teagasc Food Research Centre, Moorepark and at University College Cork. A strong platform has been established, delivering research capability in core separation technologies and ingredient science to develop innovative solutions for both ingredient and infant formula processors. The project team have transferred new processes, and analytical and quality methodologies, for industrial use. The research has delivered new insights into the consequences of process change that affect hydration of ingredients, stability of concentrates and finished powder functionality in relation to manufacture of infant formula. Many of the techniques developed during course of the project are currently being used for troubleshooting commercial applications and development of new practices in-process. The work has led to significant recognition of Ireland's competency in the area of ingredient development for infant formula internationally; validated by the number of company visits from both national / international origin and the number of invited lectures.

and presentation's at international conferences (see Section 4 b below). The project team acknowledge the significant change that the project represents in regards to manufacturing practices associated with infant formula which has created new interest amongst the commercial and scientific community. The process should generate interest amongst regulatory authorities and policymakers as it represents a viable alternative to current manufacturing practices with a lower carbon footprint. The benefits to consumers are the lower thermal load (minimal processing) on the nutrients within a formulation that the new process could provide if adapted.

The project has provided much needed personnel resources for the Irish dairy ingredient and infant formula industry by way of students and researchers recruited to this project working for/with industry, i.e., Noel McCarthy - Multinational infant formula company and then Research Officer at Teagasc, Moorepark; Henibudi Wijayanti - Dairy Processing Technology Centre, DPTC; Shane Crowley - Lectureship in Food Processing at University College Cork).

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

Fruitful (researcher mobility and co-authored publications) and long lasting collaborative research linkages were developed between the NextGenIMF project team and a number of major international dairy research and development centres such as NIZO food research and the Centre for Dairy Research at the University of Wisconsin, WI, USA.

A significant linkage was developed with a multinational infant formula company with operations in Ireland. The current project methodologies / findings contributed to the preparation of a project proposal which was successfully funded and subsequently completed with significant impact for the company.

Three of the project team (Mark Fenelon, Seamus O Mahony and Shane Crowley) gave oral presentations at the recent International Dairy Federation sponsored International Spray Drying Conference held in Dublin in 2016. All of the Irish dairy processing and infant formula companies were present at this conference in addition to many of the international companies.

Seamus O'Mahony presented the project and the main outputs at the 19th Dairy Ingredients Symposium at Calpoly in Shell Beach, California in Feb 2017 - this annual Symposium was attended by >120 delegates from the 6 US dairy research centres, the major dairy and infant formula processors and the relevant dairy research funding agencies (e.g., US Dairy Export Council, American Dairy Products Institute and the California Dairy Research Foundation).

Shane Crowley also presented results from the project at the Centre for Dairy Research in Wisconsin in the US. For the above reasons and the additional contact / forum brought about by the new Dairy Processing Competency Centre (DPTC) with respect to the Dairy companies, the team agreed that the methods used to disseminate were sufficient

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

A new process based on use of cold microfiltration (MF) coupled with diafiltration (DF) was developed to produce a protein base with a casein profile close to human milk. The process produced ingredient with a casein:whey ratio (40:60) and casein profile enriched in β -casein. The process has less heat compared to traditional processes, i.e., lower thermal load on the protein ingredients.

A technological approach used to stabilise the emulsion made using a β -casein enriched whey ingredient has been developed.

Processes have been developed to generate 'purified' β -casein ingredients and factors influencing their stability in model infant formula systems have been characterised in detail.

A number of Laboratory techniques / methodologies have been developed including model infant formulations for evaluation of ingredients and methods for rehydration of powders (see publication listing under Section 4b)

(ii) Outcomes with economic potential

The project team have been in contact with many commercial partners with regards to the potential of this new process. Both Teagasc and UCC have presented the findings of the project to numerous companies on a confidential basis. The process has been described as innovative and novel from a processing and formulation perspective. While the new process can be implemented in full, many companies will adapt certain aspects of the design and incorporate these into existing processes. The new process is designed to change the way infant formula is made and is applicable to green-field developments. One potential 'limitation or benefit' of the process is the generation of a second stream of micellar casein for utilisation. Many of the interested commercial partners see this stream as a new ingredient with potential for generation of additional formulations other than the targeted 1st age formulation that the new process is targeted at. The novelty of the process has prompted nutritional beverage companies to visit Moorepark/UCC and the possibility of interaction with Irish ingredients manufacturers has been explored. Keeping Ireland at the forefront of innovation in infant formula manufacture is key to the future of the Irish dairy sector; the new process developed in this project is 'next generation'

and can provide many advantages from a sustainability viewpoint. The project team are continuing to build on the findings of this task (in conjunction with previous tasks) and are currently in discussions with ingredient manufacturers on projects related to its deliverables.

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

The research officers who worked on this (Noel McCarthy and HeniBudi Wijayanti) have both taken up employment in the dairy research area; Noel is now a permanent researcher within Teagasc and HeniBudi took up a role contract research worker with the EI funded industry led Dairy Processing Technology Centre (DPTC). The project has also contributed to the decision to purchase new membrane technology at Teagasc in conjunction with Moorepark Technology limited (MTL; as part of a 3.5 million strategic equipment fund). The project has inputted into the design of the projects within the new DPTC, more specifically in Pillar 3 project 5.

Shane Crowley, PhD student based at UCC on this project, took up employment as a Lecturer in Food Processing at UCC in January 2017 and, building on his experiences in this project, is developing a new research group at the interface of Food Science and Process Engineering. Shane is also now an Associate Editor at the International Journal of Dairy Technology.

The new process that has been developed in this project has potential to generate significantly lower carbon emissions than conventional processes. The process supports the use of fresh milk in the manufacture of infant formula, and given the close proximity of Irish dairy processors to infant formula manufactures and the strategic direction of the major Irish dairy processors manufactures, it could potentially provide a unique advantage to Ireland in relation to production of sustainable and minimally processed infant formula.

4 (b) Summary of Research Outputs

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

1. Crowley S. V., Megemont M., Gazi, I., Kelly, A.L., Huppertz, T., O'Mahony J.A. (2014). Heat stability of reconstituted milk protein concentrate powders. *International Dairy journal*. 37, 104-110.
2. McCarthy, N.A., P.M. Kelly, P.G. Maher and M.A. Fenelon. 2014. Dissolution of milk protein concentrate (MPC) powders by ultrasonication. *Journal of Food Engineering* 126:142-148
3. Crowley, S. V., Desautel, B., Gazi, I., Kelly, A. L., Huppertz, T., and O'Mahony, J. A. (2015). Rehydration characteristics of milk protein concentrate powders. *Journal of*

- Food Engineering*, 149, 105-113.
4. Crowley, S. V., Gazi, I., Kelly, A. L., Huppertz, T., and O'Mahony, J. A. (2014). Influence of protein concentration on the physical and flow properties of milk protein concentrate powders. *Journal of Food Engineering*, 135, 31-38.
 5. Crowley, S. V., Caldeo, V., McCarthy, N. A., Fenelon, M. A., Kelly, A. L., and O'Mahony, J. A. (2015). Processing and protein-fractionation characteristics of different polymeric membranes during filtration of skim milk at refrigeration temperatures. *International Dairy Journal*, 48, 23-30.
 6. Crowley S.V., Kelly, A.L. and O'Mahony, J.A. (2014). Fortification of reconstituted skim milk powder with different calcium salts: Impact of physicochemical changes on stability to processing. *International Journal of Dairy Technology* Vol 67, No. 4 November 2014, 474-482
 7. Crowley, S. V., O'Callaghan, T. F., Kelly, A. L. Fenelon, M. A., and O'Mahony, J. A. (2015). Use of ultrafiltration to prepare a novel permeate for application in the functionality testing of infant formula ingredients. *Separation and Purification Technology*, 141, 294-300.
 8. Crowley, S. V., Jeantet, R., Schuck, P., Kelly, A. L., and O'Mahony, J. A. (2016). Rehydration and solubility characteristics of high-protein dairy powders, in, *Advanced Dairy Chemistry: Volume 1: Proteins, Part B, Applied Aspects*, 4th edn., P. L. H. McSweeney and J. A. O'Mahony (eds.), New York: Springer, pp. 99-131.
 9. Crowley, S. V., Boudin, M., Chen, B., Gazi, I., Huppertz, T., Kelly, A. L., and O'Mahony, J. A. (2015). Stability of milk protein concentrate suspensions to in-container sterilisation heating conditions. *International Dairy Journal*, 50, 45-49.
 10. Crowley, S. V., Dowling, A. P., Caldeo, V., Kelly, A. L., and O'Mahony, J. A. (2016). Impact of α -lactalbumin: β -lactoglobulin ratio on the heat stability of model infant milk formula protein systems. *Food Chemistry*, 194, 184-190.
 11. Crowley, S. V., and O'Mahony, J. A. (2016). Drying: Effect on Nutrients, Composition and Health, in, *Encyclopedia of Food and Health, Volume 2*, 1st ed., B. Caballero, P. M. Finglas, F. Toldrá (eds.), Oxford: Elsevier, pp. 439-445.
 12. Vos, B., Crowley, S. V., O'Sullivan, J., Evans-Hurson, J., McSweeney, S., Krüse, J., Ahmed, M. R., Fitzpatrick, D., and O'Mahony, J. A. (2016). New insights into the mechanism of rehydration of milk protein concentrate powders determined by Broadband Acoustic Resonance Dissolution Spectroscopy (BARDS). *Food Hydrocolloids*, 61, 933-945.
 13. Crowley, S. V., Kelly, A. L., Lucey, J. A., and O'Mahony, J. A. (2017). Potential applications of non-bovine mammalian milk in infant nutrition, in, *Handbook of Milk of Non-Bovine Mammals*, 2nd ed., Y. W. Park, G. F.W. Haenlein and W. L. Wendorff (eds.). John Wiley & Sons, Ltd, Oxford, UK.
 14. Crowley, S. V., O'Mahony, J. A., and Fox, P. F., (2017). The proteins of milk, in, *Achieving Sustainable Production of Cow's Milk, Volume 1: Safety and Quality*, N. van Belzen (ed.). Burleigh Dodds Science publishing, Philadelphia, US.
 15. McCarthy, N.A., H.B. Wijayanti, S.V. Crowley, J.A. O'Mahony and M.A. Fenelon. 2017. Pilot-scale ceramic membrane filtration of skim milk for the production of a protein base ingredient for use in infant milk formula. *International Dairy Journal* 73 (2017) 57-62.

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

1. Crowley, S. V. (2016). Next generation milk protein ingredient isolation and functionality. IDF Parallel Symposia: Concentrated and Dried Milk Products, Dublin, Ireland, April 11-13th, 2016.
2. Crowley, S. V., Vos, B., O'Sullivan, J., Evans-Hurson, J., McSweeney, S., Krüse, J., Ahmed, M. R., Fitzpatrick, D., and O'Mahony, J. A. (2016). New insights into the mechanism of rehydration of milk protein concentrate powders determined by Broadband Acoustic Resonance Dissolution Spectroscopy (BARDS). IDF Parallel Symposia: Concentrated and Dried Milk Products, Dublin, Ireland, April 11-13th, 2016.
3. Jeantet, R., Crowley, S. V., and Auty, M. (2016). The rehydration dilemma: Panel discussion. IDF Parallel Symposia: Concentrated and Dried Milk Products, Dublin, Ireland, April 11-13th, 2016.
4. Crowley, S. V., Molitor, M., Kalscheuer, R., Lu, Y., Kelly, A. L., O'Mahony, J. A., and Lucey, J. A. (2016). Use of a liquid-solid hydrocyclone in the recovery of milk minerals from acid whey. IDF Parallel Symposia, Dublin, Ireland, April 11-13th, 2016.
5. Crowley, S. V., Vos, B., O'Sullivan, J., Evans-Hurson, J., McSweeney, S., Krüse, J., Ahmed, M. R., Fitzpatrick, D., and O'Mahony, J. A. (2016). New insights into the mechanism of rehydration of milk protein concentrate powders determined by Broadband Acoustic Resonance Dissolution Spectroscopy (BARDS). IDF Parallel Symposia, Dublin, Ireland, April 11-13th, 2016. Selected for oral presentation.
6. Fenelon et. al. 2016. Oral Presentation: Lead Speaker: "Next Generation Infant Formula Processing. IDF Parallel Symposia: Concentrated and Dried Milk Products, Dublin, Ireland, April 11-13th, 2016.
7. Fenelon et. al., Invited Speaker. Impact of Protein Aggregation on In-process and Finished Product Stability of Infant Formula. 28/06/2017. ADSA, Pittsburgh, 2017.
8. O'Mahony, J.A., Kelly, A.L., McCarthy, N. M., Fenelon, M.A., 2015. Invited Speaker. Development of Next Generation of Infant Formula Ingredients using Integrated Membrane Filtration. 2nd International Conference on Separation + Drying Technologies for milk and whey. Cologne, March 23-24, Anuga FoodTec.

(iii) National Report

(iv) Workshops/seminars at which results were presented

1. Crowley et al. 2016. Presentation at workshop. Microfiltration temperature influences the solubility of micellar casein concentrate powders. DPTC Pillar 2 Workshop: Powder Technology, Rehydration and Heat Stability, University College Cork, Ireland, September 29th, 2016.
2. Fenelon, M.A., 2016 Invited speaker. Infant Formula Processing: Alpha summit, TwinFalls, Idaho, 4th Aug, 2015
3. Fenelon, M.A., 2016 Invited speaker. Chemistry and Functionality of α -Lactalbumin during Processing: Alpha summit, Singapore 20th Oct, 2016

4. Fenelon, M.A., Workshop on achieving sustainability for the Irish dairy processing industry (Challenges of the Irish Dairy Processing Industry). University of Galway, 2016.

(v) Intellectual Property applications/licences/patents

While the project has generated substantial know-how which has largely been disseminated through many channels including industry forums, individual company meetings, publications etc. no patent applications have been filed to-date on the project. The project team are in discussions with industry at present in relation to collaboration in the area. The team are also submitting a project proposal into the 2017 Competitive Call for Research Proposals, i.e., 'Research Plus - under call funding instrument VII'.

(vi) Other

Fenelon, M.A., McCarthy, N., Tobin, J. Teagasc's research infrastructure supporting the infant formula sector. Teagasc Research highlights 2016

5. Scientists trained by Project

Total Number of PhD theses: 1

Physicochemical characterisation of protein ingredients prepared from milk by ultrafiltration or microfiltration for application in formulated nutritional products. PhD thesis, University College Cork. Crowley, S. V. (2016). 30/07/2016 (Status: Graduated)

Total Number of Masters theses: 0

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
TEAGASC	3	0.634
UCC	1	0.229
Total	4	0.863

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract	2	2.041

Researchers		
PhD students	1	4.000
Masters students		
Temporary researchers		
Other		
Total	3	6.041

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended
Shane Crowley	Farm to Fork - Sustainability in the Bioeconomy, 12-14th November 2012

9. Project Expenditure

Total expenditure of the project:	€284,539
Total Award by DAFM:	€296,193
Other sources of funding including benefit in kind and/or cash contribution(specify):	€

Category	Name TMFRC	Name UCC	Name Institution 3	Name Institution 4	Total
Contract staff					
Temporary staff					
Post doctorates	78,669				78,669
Post graduates		82,955			82,955
Consumables	28,489	18,349			46,838
Travel and subsistence	4,780	5,634			10,414
Sub total	111,938	106,938			218,876
Durable equipment					
Other					
Overheads	33,581	32,082			65,663
Total	145,519	139,020			284,539

10. Leveraging

Elements of the chemistry and membrane separation based methodologies have been adapted / incorporated into the Industry Led Dairy Processing Technology Centre (DPTC). Specifically, Projects 1 and 5 in Pillar 3 and Project 1 in Pillar 2.

The project provided important contributions to the business case made to the Department of Agriculture for the appointment of a Dairy Scientist - Process Engineering (with emphasis on membrane technology). This role has been filled and the new scientist is developing a central area for the Irish Dairy Industry in Separation technology.

A proposal was submitted to the Dairy Research Institute (DRI) in the USA to secure additional funds for scale-up work related to manufacture of the protein base and continuation of work related to charged membranes by an MSc student (2 yr.). This application was successful and the student has been recruited

11. Future Strategies

The results of the project are currently (at the time of writing this Report) been prepared for submission (further funding) under the 2017 Competitive Call for Research Proposals, i.e., 'Research Plus - under call funding instrument VII'

The team are in confidential discussions with a number of companies as to possible adaption of some of the findings for development of a new process for manufacture of infant formula / ingredients for infant formula.

The team at both Teagasc and University College Cork have continued to optimise the processing and chemistry associated with the development of the ingredient with protein profile closed to human milk.

Selected aspects of this project (e.g., membrane fouling and cleaning) have been revisited and studied in greater fundamental detail through a number of 4th year Food Science student projects at UCC over the last 2-3 years with a view to addressing some of the queries about this new technological approach to making infant formula received from industry partners and equipment manufacturers and with a longer term view to developing the feasibility information required for building strong future funding proposals.

Additional research articles co-authored by researchers from University College Cork and University of Wisconsin-Madison are in advanced stages of preparation for submission to peer-reviewed journals in mid-late 2017.

The findings of the research have also informed two MSc projects at UCC of practical relevance to the stability of next generation protein ingredients. One focused on the stability of beta-casein to indigenous enzymes (complete) and the other on the stability of beta-casein/milk protein concentrate mixtures to minerals (expected completion in September 2017)