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

Food Institutional Research Measure

Final Report

'Innovative solutions for quality & safety improvement in dairy ingredient manufacture for infant formula - InSo

DAFM Project Reference No: 11/F/052

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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	4 x	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)	Sustainable Food Production & Processing Processing Technologies & Novel Materials

Key words: *(max 4)*
Ingredients, Infant Formula, PAT tools

1. Rationale for Undertaking the Research

Dairy ingredients and infant milk formula (IMF) manufacturers constantly strive to improve the quality and nutritional composition of their products. As a result, quality specifications are extremely high and meeting these demands can be challenging due to variability in composition, environmental and processing parameters which can alter the underlying physicochemical interactions that govern product quality. Typically, dairy manufacturers use time-consuming analytical techniques to determine effect of certain variable parameters on product quality. However, the timeframe in which results become available does not allow vital process interventions to be made in real time. Therefore, in-line process analytical technology (PAT) which can rapidly capture and successfully apply the required intervention is of extreme interest to manufactures as it allows for real-time control of critical process parameters which affect critical quality attributes. PAT tools are already extensively employed in the pharmaceutical sector; they allow manufacturers to produce products with consistent quality and also help to reduce waste & overall costs. However, tools need to be selected according to fitness for purpose in all cases. Developments are taking place in vibrational spectroscopy and data analysis which offer significant potential in food process applications and it is the goal of this project was to evaluate these technologies for use as process analytical tools in dairy ingredient manufacture and in infant formula manufacture.

2. Research Approach

The approach employed in InSo was designed to show the potential of PAT tools across the entire distribution chain of dairy ingredients and IMF i.e. from primary production to consumer. Typical stages within the distribution chain were simulated e.g. powder manufacture, storage etc. and various technologies were investigated as potential PAT tools.

Manufacture/procurement of materials

- Dairy ingredients were manufactured in the Bio-functional Food Engineering (BFE) facility at Teagasc Food Research Centre, Moorepark. Skim milk powder (SMP), demineralised whey powder (DWP) and whey protein concentrate (WPC) powder were chosen for manufacture as they represent the most commonly used sources of protein in IMF recipes and are therefore key determinants of product quality. Various heat treatments were employed during the manufacture of dairy ingredient powders to simulate a range of typical industrial conditions. Critical quality parameters such as soluble protein, whey protein nitrogen index were measured both during the manufacturing process and during subsequent storage to simulate the typical lifetime of powders.
- Model IMF powders were manufactured at Teagasc's BFE facility to simulate the next stage in the lifetime of dairy ingredient powders. As with dairy ingredient manufacture, various heat treatments were applied to produce powders of varying quality. Typical quality parameters for IMF e.g. soluble protein, Maillard browning, surface free fat were measured both during manufacture and subsequent storage.
- Commercial IMF (along with IMF manufactured by the project team) was procured and subjected to a number of storage trials to simulate the post manufacturing lifetime of products.

PAT tool development

Front facing fluorescence (FFFS), Infra-Red (Vis-IR and Mid-IR) and Raman spectroscopy were used to generate spectra at various stages of the manufacture and/or shelf life of products described above. FFFS measurements were carried out using a Cary Eclipse fluorescence spectrofluorimeter (Varian Inc., PaloAlto, CA, USA). Vis-NIR spectra were collected on a NIRSystems 6500 instrument (NIRSystems. Inc., Maryland, USA). Mid-IR spectra were collected on a Bio-rad Excalibur series FTS 3000 FT-IR spectrometer (Analytica Ltd. Dublin, Ireland). Raman spectra were collected on a DXR SmartRaman spectrometer (ThermoFisher Scientific UK Ltd., Loughborough, UK). Focused Beam Reflectance Measurement (FBRM) was also use to monitor dissolution behaviour of dairy ingredients during processing. Each spectrum was matched to a corresponding quality measurement e.g. soluble protein, surface free fat etc. Models were generated using Unscrambler X 10.3 (CAMO, Norway) which allowed the project team to link processing parameters to quality attributes. Models were tested for accuracy by testing the response from validation batches produced under similar conditions. Where necessary, spectral pre-treatments were employed to improve performance of models. The project team focussed on models with applications for

- Determination of severity of heat treatment and protein denaturation
- Discrimination between dairy ingredients for recipe verification
- Prediction of age and storage temperature profile
- Product verification at destination (anti-counterfeiting)

3. Research Achievements/Results

Task 1: *Procurement and controlled storage of dairy ingredients for infant formula and development of analytical methods*

As described in Section 2, 36 dairy ingredients and 12 model infant formulas were produced in the Moorepark. 118 commercial infant formulas were procured from local supermarkets. These samples were stored at 15 °C and 37 °C and analysed at different time points for up to one year. Databanks were generated relating to effect of storage on physicochemical properties of powders. Trends observed during both manufacture and storage provided valuable insights into powder behaviour which can be used to leverage funding for novel projects.

Task 2: *Applying Focused Beam Reflectance Measurement and Particle Vision Microscope process analytical technologies to monitor reconstitution / dissolution behaviour of dairy ingredients and infant formula*

Robust Focused Beam Reflectance Measurement (FBRM) experimental protocols were developed using commercial milk samples which were evaluated under a range of relevant experimental conditions (including shear, concentration, temperature) using an FBRM probe. Power ultrasound was demonstrated to enhance dissolution of commercial dairy ingredients. Higher ultrasound amplitude and longer treatment times further decreased the average

particle size. FBRM was demonstrated to be a potential PAT tool that can provide real time and in situ particle counting and sizing data during dissolution processes. For IMF, higher preheat temperature, longer storage time and higher storage temperatures were shown to increase FBRM unweighted median chord length and square-weighted chord length.
FBRM is a powerful tool that has the potential to monitor particle size. Only a limited number of FBRM studies have been reported in dairy applications, and none for infant formula manufacture. This task adds to the research base through demonstration applications in dairy ingredient and infant formula processing. This work will facilitate industry in adopting FBRM to achieve enhanced process efficiencies.

Task 3: *Application of fluorescence for rapid determination of impact of (i) heat treatment on dairy ingredients and infant formula and (ii) impact of storage on infant formula*

This task addressed the key research challenges necessary to develop and validate fluorescence based PAT tools for quality assessment and process control in dairy ingredient and IMF manufacture. This was the first detailed study to:

- Investigate FFFS as a rapid and reliable tool for discrimination of selected dairy ingredient types and preheat temperatures.
- Investigate a FFFS PAT tool for the assessment of selected model IMF quality and compositional parameters in both powder and rehydrated liquid states.
- Develop chemometric models for commercial IMF samples using a range of fluorescence excitation and emissions spectra for brand authentication, selected quality and compositional parameters.
- Evaluate the performance of the fluorescence-based Amaltheys II analyser at-line PAT tool for dairy ingredient and IMF applications.

Front-facing fluorescence spectroscopy (FFFS)
The initial study within this successfully demonstrated the potential of FFFS coupled with chemometrics as a PAT tool to discriminate between dairy ingredients (SMP, WPC and DWP) and pre-drying heat treatment temperatures, and, to monitor protein denaturation and Maillard browning in dairy ingredient manufacture. The best models developed achieved 100 % discrimination (i) between dairy ingredients and (ii) between pre-drying heat treatment temperatures. This was followed by a study which demonstrated the successful deployment of FFFS to simultaneously estimate pre-drying heat treatment temperature and storage time and predict soluble protein and surface free fat in model IMF samples; partial least squares-discriminant analysis was also found to be an interesting tool for differentiating based on storage temperature. A final study investigated the use of FFFS as a PAT tool combined with chemometric methods for rapid assessment of commercial IMF quality parameters and brand authentication. Models were developed using appropriate data pre-treatment and spectral variable selection methods on FFFS data obtained from a range of fluorophores (n = 500). Tryptophan was found to be a suitable fluorophore for development of models allowing for discrimination between commercial IMF powder and prediction of storage time.

Fluorescence-based Amaltheys II analyser

A fluorescence-based Amaltheys II analyser was evaluated for rapid measurement of soluble protein and whey protein nitrogen index, using tryptophan to measure structural changes to the protein. The instrument performed rapid measurements which were correlated (R^2 > 99 %) with the more time consuming reference methods. However, rehydration time was found to be an important parameter affecting repeatability of results. This was especially true for non-agglomerated powders which, due to relatively slower rehydration kinetics, exhibited more variability than agglomerated powders. Therefore, arising from this work it is recommended that defined sample preparation and rehydration protocols be developed to ensure instrument repeatability and inter-lab reproducibility.

Task 4: Application of vibrational spectroscopic techniques to verify ingredient quality and infant formula integrity

This task focused on the utilisation of Infra-Red (Vis-IR and Mid-IR) and Raman spectroscopy as a PAT tool. A number of statistical approaches and spectral pre-treatments were employed to generate both discriminative and predictive models for use in dairy ingredient and IMF manufacture.

Dairy ingredients
MIR and Raman spectroscopy in combination with spectral pre-treatments and chemometrics produced models which were capable of 100% discrimination between SMP, DWP and WPC based on product type and intensity of heat-treatment applied during processing. Initially, preheat treatment discrimination models had good discrimination accuracy (86–100%) however, they employed a high number of factors (8–9 for the best model). The use of the Martens uncertainty test successfully reduced the number of factors employed (3–4 for the best models) showing the importance of spectral pre-treatments in PAT development. Similarly, Vis-NIR was successful at providing discriminatory models for dairy ingredients, but was also capable of producing partial least squares regression models for prediction of pre-heat treatment temperatures.

IMF
Vis-MIR, Mid-IR and Raman spectroscopic techniques were shown to be successful qualitative and quantitative approaches for a) discrimination between IMF preheat and storage temperatures, and b) prediction of key quality attributes. The study showed that high levels of accuracy were possible for discriminatory preheat temperature and storage temperature models i.e. accuracies of between 92.3–100% and 91.7–100%, respectively.
Furthermore, predictive models were also generated for key quality attributes: soluble protein, fat content, storage time, FAST index and surface free fat content.

In summary, Tasks 3 and 4 developed and validated novel spectroscopic PAT approaches for (i) rapid determination of heat treatment impacts on dairy ingredients and IMF, (ii) monitoring selected quality control parameters and (iii) product/brand authentication. The research outcomes will facilitate dairy ingredient and IMF manufacturers to employ a process analytical control philosophy in their processing plants and to exploit the significant

benefits of PAT adoption, including increased process efficiency and improved final product quality.

Task 4: Dissemination

See section 4(b)

4. Impact of the Research

This research project has demonstrated the suitability of a multitude of spectroscopic techniques for application in IMF and dairy ingredient quality control. For manufacturers of these products, such approaches can improve specification control to avoid costly out-of-specification batches. In the case of IMF, this is of extreme importance due to potential impacts of poor quality products on extremely sensitive end-users. For exporters/importers of products, the potential use of spectroscopic PAT techniques to identify and discriminate between products can be used to verify product authenticity and remove counterfeit product from the market. Ultimately, use of PAT methodologies developed in this project will be beneficial to end-users/consumers as the cumulative effect of employing spectroscopic PAT tools across the distribution chain will result in better, safer products.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research:

- Links between the participant RPOs (Teagasc and UCD) have been strengthened and built upon for new ideas and projects.
- A collaborative study was undertaken with Spectralys the supplier of the fluorescence based Amaltheys analyser. This resulted in a report that was circulated to Irish industry and several discussions were held regarding industrial implementation
- A workshop was organised at Teagasc, Moorepark, on 12/3/15 for dissemination of FIRM-funded projects in conjunction with the Society of Dairy Technology. Two presentations were made by members of the InSo project team, along with a demonstration of the Amaltheys analyser by Spectralys. Several Irish and multi-national companies were present and as a result visits to several companies took place to discuss the technology.

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

- Chemometric models for spectroscopic techniques were developed which may form the central structure of in-process PAT tools in the future
- An at-line fluorescent based PAT tools was adopted/developed for use in IMF and dairy ingredient quality control. This could potentially form a key part of commercial quality assurance protocols in the future

(iii) Outcomes with economic potential

- There are clear economic benefits associated with utilisation of spectroscopic PAT tools across the whole distribution chain of dairy ingredient and IMF production. These include:
    i. Ingredient authentication – ensuring the correct raw material specification upon receipt can reduce losses associated with out of specification end-products and unexpected behaviour during processing
    ii. Process control – Correct use of in-line PAT tools can allow for real-time optimisation of processing costs while ensuring product quality specifications are met
    iii. Reduction of laborious and time consuming analytical methods

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

- Dairy ingredients and IMF are required to be of the high nutritional and physical quality. This is particularly true for IMF and other sectors where the end-users are extremely vulnerable and must be protected from out of speciation products. This can be achieved through PAT assisted quality control process developed in this project:
    i. Verification of ingredient quality
    ii. Verification of in-process heat –treatments for microbial reduction
    iii. Product authentication at destination for anti-counterfeiting purposes

4 (b) Summary of Research Outputs

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

- S.Shaikh, C.O'Donnell (2017) Applications of fluorescence spectroscopy in dairy processing: a review. Current Opinion in Food Science, 17, 16-24
- X. Wang, C. Esquerre, G. Downey, L. Henihan, D.M. O'Callaghan, C.P. O'Donnell (2018) Assessment of infant formula quality and composition using Vis-NIR, MIR and Raman process analytical technologies. Talanta, 183, 320-328
- X. Wang, C. Esquerre, G. Downey, L. Henihan, D.M. O'Callaghan, C.P. O'Donnell (2017) Feasibility of discriminating dried dairy ingredients and preheat treatments using mid-infrared and Raman spectroscopy. Food Analytical Methods, 11, 1380-1389
- Lisa E. Henihan, Colm P. O'Donnell, Carlos Esquerre, Eoin G. Murphy, and Donal J. O'Callaghan (2019). Quality assurance of model infant milk formula using a front face fluorescence process analytical tool. Food and Bioprocess Technology, 7, 1402-1411
- Henihan, L. E., O'Donnell, C. P., Esquerre, C., Murphy, E. G., & O'Callaghan, D. J. (2019). Fluorescence-based analyser as a rapid tool for determining soluble protein content in dairy ingredients and infant milk formula. Innovative Food Science & Emerging Technologies, 52, 75-79.

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

- Henihan, L., O'Donnell, C. and O'Callaghan, D. The effects of heat treatment on Model Infant Formula powder using front-face fluorescence spectroscopy 43rd Annual Food Research Conference, UCD, Dublin Ireland (10 – 11 December 2014)
- Henihan, L., O'Donnell, C. and O'Callaghan, D. Use of front-face fluorescence spectroscopy for analysing the effects of heat treatment on rehydrated skim milk powder 9th NIZO Dairy Conference Milk Protein Functionality. Papendal, Netherlands. (30 September 2015 – 02 October 2015)
- Henihan, L., O'Donnell, C. and O'Callaghan, D. Analysing the effects of heat treatment on dairy powders & infant formula. IDF Concentrated & Dried Milk Products Symposium, Dublin (11-13 April 2016)
- Henihan, L., O'Donnell, C. and O'Callaghan, D. Predictive modelling of pre-heat treatment, protein denaturation and Maillard browning of skim milk powders using front-face fluorescence spectroscopy. Society of Dairy Technology Conference - UCC, Cork (10-12 April 2017)
- O'Callaghan, D. The surface characteristics and functional properties of formulated dairy powders. IDF Concentrated & Dried Milk Products Symposium, Dublin (11-13 April 2016)
- O'Donnell, C. Development of process analytical technology(PAT) tools for enhanced quality and safety in food processing Concentrated & Dried Milk Products Symposium, Dublin (11-13 April 2016)

(iii) National Report

(iv) Workshops/seminars at which results were presented

- A workshop was organised at Teagasc, Moorepark, on 12/3/15 for dissemination of FIRM-funded projects in conjunction with the Society of Dairy Technology. Two presentations were made by members of the InSo project team, and there was a demonstration of the Amaltheys analyser by Spectralys
- Regular local seminars were held to disseminate and discuss results among the researchers at Teagasc Food Research Centre,Moorepark and UCD's School of Biosystems and Food Engineering.

(v) Intellectual Property applications/licences/patents

(vi) Other

- Henihan, L. E., O'Donnell, C. P., Esquerre, C., Murphy, E. G., & O'Callaghan, D. J. (2018). Comparison of front-face fluorescence spectroscopy and Fourier transform infrared spectroscopy as process analytical tools in dairy ingredient and infant formula manufacture. Biosystems and food engineering research review 23, 57.

- X. Wang, C O'Donnell (2018). Investigating the effect of preheat temperature and solvent type on dissolution properties of whey protein concentrate using FBRM UCD Biosystems and Food Engineering Research Review 2018
- X. Wang and C. O'Donnell. Effect of heat treatment and storage on the reconstitution properties of milk powder. UCD Biosystems Engineering Research Review, 2015, pp. 53-56.

5. Scientists trained by Project

Total Number of PhD theses: __2__

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date

Dr Lisa Henihan (2019)
Development of front face fluorescence spectroscopy as a process analytical technology tool for dairy ingredient and infant formula manufacture. Supervisors: Eoin Murphy, Donal O'Callaghan (Teagasc); Colm O'Donnell, Carlos Esquerre (UCD).

Dr Xiao Wang (2018)
Development of process analytical technology tools for dairy ingredient and infant formula manufacture. Supervisors: Colm O'Donnell, Carlos Esquerre (UCD).

Total Number of Masters theses: ____

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
Teagasc TMFRC	2	1.127
Teagasc TAFRC	1	0.367
UCD	3	1.6
Total		

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers		
PhD students	2	5.604
Masters students	1	1.470
Temporary researchers		
Other	3 (Research Assistants)	1.740
	1 (placement student	0.583
Total		

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended

9. Project Expenditure

Total expenditure of the project:	€441738.49
Total Award by DAFM:	€488376
Other sources of funding including benefit in kind and/or cash contribution(specify):	€0

Breakdown of Total Expenditure

Category	Teagasc, TMFRC	Teagasc, TAFRC	UCD	Total
Contract staff	0	0	69,023.35	69,023.35
Temporary staff	0	0	0	
Post doctorates	93,872.33	0	62,315.60	156,187.93
Post graduates	0	0	0	
Consumables	30,580.80	195.29	2,539.42	33,315.51
Travel and subsistence	14,360.15	5,011.99	8,449.80	27,821.94
Sub total	138,813.28	5,207.28	142,328.17	286,348.73
Durable equipment	2,785.53	42,733.99	0	45,519.52
Other	23.965.62	0	0	23,965.62
Overheads	41,643.98	1,562.18	42,698.45	85,904.62
Total	**207,208.41**	**49,503.45**	**185,026.62**	**441,738.49**

10. Leveraging

11. Future Strategies

InSo developed spectroscopic PAT approaches which serve as promising proofs-of-concept for implementation of these technologies in the Dairy industry. However, for commercial implementation robust commercially viable prediction models are required to better equip dairy processors and allow compliance with increasingly demanding dairy ingredient specifications/requirements of infant formula companies. This requires incorporation of two additional factors not considered in InSo: 1) Model development using in process samples from manufacturer(s) to allow for truly robust model development and 2) design/assessment of appropriate engineering solutions which allow for spectroscopic data to be taken during processing. These aspects are currently under investigation in the DPTC platform (discussed above).