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

17FP273 – Commercialization of Next Generation Infant Formula

Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

SUMMARY

This FIRMplus proposal was built on the outcomes of a completed FIRM-funded project “Concept Protein Ingredient for Next Generation Infant Formulation”. The latter project highlighted the complexity of altering the protein profile of milk for use in first-stage infant-milk-formula (IMF) and successfully manufactured a protein ingredient containing a casein to whey protein ratio similar to that in human milk through the use of membrane filtration at lab and pilot-scale (300 kg batch size). The microfiltration of skim milk at temperatures < 10°C allowed for the permeation of β-casein into the whey protein stream. The aim of this FIRMplus project was to move this process up to commercialization scale, and to also continue on the journey of ‘humanization’ of bovine milk protein by dephosphorylation of the β-casein fraction. The β-casein enriched whey protein ingredient was successfully manufactured at semicommercial scale in Moorepark Technology Limited (volumes up to 3000 kg). The second step in the process was also successfully achieved through the dephosphorylation of the β-casein fraction; however, the aggregation of the β-casein fraction, while retarded, still occurred to some degree during the evaporation process. Therefore, the decision was made to add a small proportion of the original skim milk back into the novel β-casein enriched whey protein ingredient to provide stability to the β-casein fraction. This small addition of skim milk provided sufficient stability to the product, and evaporation and spray drying was performed successfully. Subsequent amino acid analysis was carried out as well as gastric and intestinal digestion.

This project has highlighted the impact that removing post-translational modifications such as phosphoserine groups did not prevent beta-casein re-micellization but that a low quantity of micellar casein was capable of stabilizing the protein system even through evaporation and spray drying. This is a significant development in the production of a more humanized IMF.

KEYWORDS

Beta-casein, Hydrolysis, Whey Protein Ingredient

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Section 1 - Research Approach & Results

Start Date

01 January 2019

End Date

31 December 2019

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 7: System prototype demonstration in operational environment

NRPE Priority area

Sustainable Food Production and Processing

Total DAFM Award

€97,760.00

Total Project Expenditure

€98,758.25

Rationale for undertaking the Research

The proposal was based on the FIRM project: 'Concept Protein Ingredient for Next Generation Infant Formulation'. The rationale of the original 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 could be directly prepared and dried creating substantial savings to the manufacturer. The philosophy was very new and thus referred to as next generation manufacture. The first step of the project was to optimize β-casein removal from skim milk by low temperature microfiltration. This cold microfiltration (MF) step coupled with diafiltration (DF) facilitated the formulation of a protein base with a casein profile close to human milk. Using this process, it was demonstrated that two major formulation targets, a casein:whey ratio (40:60) and casein profile (βcasein), could be achieved. 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 inprocess proteolysis (lower levels of casein hydrolysis products). However, an issue arose during the concentration and spray drying of this ingredient whereby the β-casein micellized/aggregated and resulted in sedimentation of the protein. Therefore, the current project allowed us to test 2 main theories: 1. would the dephosphorylation of the β-casein reduce protein-calcium interactions and 2. add a minute quantity of natural micellar casein to stabilize the β-casein fraction.

Methodology

Laboratory scale production was performed initially prior to pilot and semi-commercial scale trials using 0.14 µm and 10 kDa molecular weight cut-off membranes. This allowed for relatively small quantities of the β-casein enriched whey protein ingredient (protein profile; Fig.1) to be produced and for trials to be performed on the dephosphorylation Task. Dephosphorylation was performed using bovine alkaline phosphatase across a range of temperatures. This was performed to identify if the enzyme could work at lower temperatures to reduce the risk of microbial growth. The enzyme performed optimally at 37 °C.

Leaving the enzyme to incubate over 24 h at 4 °C resulted in very little dephoshorylation; however, at 10 °C, dephosphorylation started occurring after 7 h. A temperature of 10 °C for 24 h was deemed appropriate for the current work, allowing sufficient dephosphorylation to occur.

Due to instability with the β-casein enriched ingredient as discussed previously a proportion of the original skim milk was back-added. The thought process was that a number of the native casein micelles in the skim would be able to stabilize the large quantity of β-casein. Therefore, the β-casein enriched whey protein ingredient could be dephosphorylated as before or the novel β-casein ingredient could be mixed directly with a small portion of skim depending on the end-users desire. Therefore, a number of skim milk addition levels were examined, namely 0 (control), 0.1, 0.3 and 0.5% of the total protein in the β-casein ingredient would come from re-added skim milk. Sedimentation profiles were produced using a LumiSizer across a number of temperatures. Particle size measurements were performed using a Mastersizer 3000. Microbial analysis was carried out by an external laboratory (Eurofins, Cork). Eight protein ingredients were subjected to static in-vitro digestion (INFOGEST, 2014). Digestates were sampled at 0, 15, 30, 60, 90 and 120 min.

Project Results

Once the β-casein enriched whey protein ingredient was produced and dephosphorylation (Fig. 2) had took place a range of stability work commenced. Storing the protein ingredient for extended periods of time at 4, 20, 37 and 50 °C was performed to determine if dephosphorylation was sufficient to prevent uncontrollable re-micelleization/aggregation of the β-casein. However, it was seen that β-casein continued to aggregate and re-micelleize, and while dephosphorylation retarded the aggregation it was deemed insufficient, particularly at temperatures of 55°C and above (Fig. 3A and B in attached file). Rheological data showed that the viscosity increased with increasing temperature as a result of aggregation. Also of note is that the protein system contained a high concentration of the milk minerals which will contribute to aggregation but are important from a nutritional point of view. However, whey proteins remained native in the system due to the relatively low temperatures used during the process. Also, initial curd forming properties showed that the dephosphorylated β-casein produced a softer curd compared to non-dephosphorylated β-casein, which is a positive outcome for infant digestion. The results of this task have been submitted in part to a peer-reviewed Journal for consideration for publication.

As dephosphorylation of β-casein was not sufficient to prevent aggregation during concentration (i.e., water removal) but where 0.3% of the total protein content is re-added micellar casein then this stability issue was overcome.

Sedimentation of the control and the sample with 0.1% skim addition increased with increasing temperature. Similarly, visual observations of β-casein enriched ingredients with casein to whey protein ratios of 31:68 were unstable and precipitated during storage at 4 °C as well as upon heating from 20 to 50 °C. However, a major improvement was observed upon 0.3% skim milk protein addition. The particle size of the control sample was significantly higher than samples with added skim milk during the evaporation of the UF retentate. This indicated its tendency for precipitation. The addition of a small portion of the skim milk protein content to the final UF retentate in the range of 0.3 to 0.5% of total protein provided a stabilization effect to the β-casein enriched protein ingredients and was sufficient to allow for successful concentration of the ingredient without any fouling occurring. Note: all evaporation was performed at 60°C to prevent whey protein denaturation.

Section 2 - Research Outputs

Summary of Project Findings

This 1-Year project has developed significant advances in the area of milk protein fractionation and up to now there has been very little work don on dephosphorylation of bovine milk protein; however, for infant milk

formulas to advance and become more 'humanized' this is a requirement as human breast milk is partially dephosphorylated and results in a softer curd and easier digestibility by the infant. This project has allowed us to highlight the issues, solutions and mitigation that can be taken to advance this type if protein chemistry and demonstrated that it can be done at industrial level. A beta-casein ingredient which was highly unstable has been shown that through controlled addition of micellar casein it can be stabilized and converted into a spray dried powder.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
Post Doctorates	1	0	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
Other	3	1. Hailu Y., Fenelon, M.A., McCarthy, N.A. Dephosphorlation of β-casein as a function of temperature and time. A short communication. International Dairy Journal. Manuscript prepared. 2. Hailu Y., McCarthy, N.A. Effects of micellar casein addition on a β-casein enriched whey protein system. International Dairy Journal. Manuscript prepared. 3. Hailu Y., McCarthy, N.A. In-vitro digestion of a novel micellar β-casein protein system. Food Chemistry. Manuscript under preparation.
Peer Reviewed Conference Papers	1	International Conferences: 11th NIZO Dairy Conference, Milk Protein Functionality, 8-11 October 2019, Papendal, The Netherlands (Poster presentation).

Intellectual Property

Powder samples produced in Moorepark Technology Limited were requested and sent to laboratories in September 2019 for further testing and a feasibility exercise was performed. Industry showed an interest in the β-casein depleted micellar casein ingredient (i.e., rich in αs1- and αs2casein). Further discussions and potential trials were to take place in 2020, but unfortunately due Covid19 restrictions and limited staff numbers allowed on-site this has been delayed, but we are confident that once normal working procedures return this collaboration can be continued.

One of the major infant formula companies has approached Teagasc also about the enrichment of beta-casein in a whey stream to be potentially manufactured by a third party but a discussion with the Teagasc Technology Transfer Office must be set up prior to this meeting.

While this 1-year project has finished there is a continued interest in commercializing the technology.

Summary of other Project Outputs

Project Outputs	Details	Total Number

New Products	From a nutritional standpoint this ingredient produces a more suitable protein source for infants and the manuscripts soon to be published highlight Ireland's focus on novel scientific developments in the area of dairy science and infant formula.	1

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Other	This project has furthered the career of the Post-Doctoral Researcher (Dr. Yonas Hailu). The Post-Doctoral Researcher applied for a Marie Curie CareerFit Plus scholarship and was awarded a three year term with one of the project partners. This position started in January 2021.
Industry	The technology and know-how to produce this ingredient is available to the Irish dairy and infant formula industry and can be sought through contract with the project team and Teagasc Technology Office.

Dissemination Activities

Activity	Details
Seminars at which results were presented	International Conferences: 11th NIZO Dairy Conference, Milk Protein Functionality, 8-11 October 2019, Papendal, The Netherlands (Poster presentation).
Workshops at which results were presented	Meting was held subsequently at Teagasc Moorepark (Tuesday 2nd July 2019). Attendees included industry representatives, Seamus O'Mahony (UCC), Mark Fenelon (Teagasc), John Tobin (Teagasc), Yonas Hailu (Teagasc).

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	Both the membrane filtration and the means of stabilizing β-casein so that evaporation and spray drying can be successfully produced and is highly novel. This is a significant output from the project.
Identify any knowledge transfer activities executed within the project.	The pre-commercial trials carried out in MTL pilot-plant were attended to by potential industry partners. Powder samples were sent for further analyses in laboratories. Trials were planned when the membrane filtration plants would start up in the Spring of 2020 but unfortunately with Covid 19, this has seen a delay. We hope later this year that further collaboration can take place.
List any impacts resulting from the knowledge transferred during the project.	During the production of the beta-casein enriched whey there is a substantial quantity of beta-casein depleted micellar casein produced which must find an application. It was discussed that this ingredient has potential in high value formulations for the elderly and sports nutritional market.

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Non Exchequer National Funding	€256,050.00	As mentioned in the previous section the project has furthered the career of its Post-Doctoral Researcher. The Post-Doctoral Researcher applied fir a Marie Curie CareerFit Plus scholarship. The position started in January 2021 after some delays due to Covid 19.

Future Strategies

The working relationship on parts of the project were set to continue but due to Covid19 they have been postponed, we hope to resume with these in the coming months. Depending on industry interactions we also have 2 more manuscripts which could possibly be published. This still has to be decided.

Project Publications

Over the 1 year time frame of the project 3 manuscripts were prepared:

1. Hailu Y., Fenelon, M.A., McCarthy, N.A. Dephosphorylation of β-casein as a function of temperature and time. A short communication. International Dairy Journal. Manuscript prepared.
2. Hailu Y., McCarthy, N.A. Effects of micellar casein addition on a β-casein enriched whey protein system. International Dairy Journal. Manuscript prepared.
3. Hailu Y., McCarthy, N.A. In-vitro digestion of a novel micellar β-casein protein system. Food Chemistry. Manuscript under preparation.