Department of Agriculture, Fisheries and Food
An Roinn Talmhaíochta, Iascaigh agus Bia

Food Institutional Research Measure

Final Report

Expanding the potential for whey protein-based encapsulation

DAFF Project Ref No: 11/FP/404

Start date: 01/01/2013

End date: 30/04/2014

Principle Coordinator: Dr André Brodkorb, Teagasc Food Research Centre (TFRC), Moorepark, Fermoy, Co. Cork
Email: Andre.Brodkorb@teagasc.ie

Other Principle Collaborating Researchers: n/a

Please tick below the appropriate area on the research continuum where you feel this project fits

BASIC/FUNDMENTAL →		APPLIED/PRE COMMERCIAL
		√

Key words: Encapsulation, microbeads

NDP NATIONAL DEVELOPMENT PLAN

1. Rationale for Undertaking the Research

Encapsulation of bioactive ingredients, organisms, flavours or bio-medical compounds in dairy-based proteins is of interest to the food industry, as the ability to protect such compounds during processing, storage and gastric transit in order to deliver them to the target site intact is essential for incorporation into foods. A method for producing whey protein-based microbeads for encapsulation was recently developed and patented at Teagasc. To date this method had been applied to probiotic LGG bacteria and allowed for survival of the bacteria through the stomach and targeted release in the intestinal tract. The method also provides a platform for the encapsulation of a wide range of bioactive compounds such as other sensitive food or pharma ingredients like vitamins, bioactive peptides, phytochemicals and antigens.

However, the main application would appear to be within the food, feed and ingredient sector; feedback from end-users has been largely positive but some technical and scientific hurdles for commercialisation need to be addressed, namely:

1. Cost of encapsulating materials
2. Ability to dry encapsulated materials
3. Diversification of bioactives encapsulated

By refining the encapsulating materials and process, the technology is going to be ready within short time for commercialisation for encapsulating a range of compounds.

2. Research Approach

Task 1: Title: Modification of encapsulation matrix

MS1.1. Selection of suitable ingredients:

The following ingredients were selected; Proteins (Whey protein isolates, heat treated milk protein isolates, skim milk powder), Polysaccharides (Sodium Alginate) and Bioactive Compounds, Water soluble (Folic Acid).

MS1.2. Formulation and evaluation of Polymer matrices using selected ingredients:

WPI (Isolac), heat treated milk protein isolates (MPI) and skim milk powders (SMP) was sourced from a local Irish Company and used to formulate matrices to make micro beads by extrusion methods.

Aqueous solutions of mixture of WPI (4 -10%) and SMP (2 -6%) with various ratios of solid content were prepared.

Heat treated milk protein isolate (MPI) solutions (5.0-8.0%, w/w) and sodium alginate solution (2.0% w/w) were also prepared in distilled water. Normally MPI solution (≥ 5.0% w/w) was viscous and it formed gel structure immediately after mixed with alginate solution. So, MPI solutions were centrifuged before mixing with alginate solution. The amount of MP

was 2.7, 3.1, 3.5 and 4.1% in supernatant of 5.0, 6.0, 7.0, and 8.0% MPI solutions respectively after centrifugation. These MP solutions were used for formulation of most suitable polymer matrix solution by combining with alginate solution with ratios of 75/25, 70/30, 65/35, and 60/40 to make polymer matrix solutions.

MS1.3. Make microbeads and test for efficacy:
Micro-beads were prepared by extrusion method using the Inotech IE-50R Encapsulator® (Inotech AG, Dottikon, Switzerland). Each polymer solution mentioned in milestone 2 of Task 1 was extruded through a vibrating in a calcium chloride (0.2 M) gelling bath with continuous slow stirring. The procedure adopted is described.

The formulation of MP (3.1%) and alginate (2.0%) solutions with ratios of 75/25, 70/30, 65/35, and 60/40 were used for successful encapsulation of sensitive bioactive component, such as folic acid and their encapsulation efficiency into the beads. Folic acid was added at a final concentration of 1 mM/L in each polymer solution.

The size and morphology of micro-beads were analysed using an optical microscope equipped with a digital camera (BX51 light microscope, Olympus, Essex, UK). The amount of folic acid encapsulates in micro-beads was determined using reverse-phase HPLC with C18 column. Encapsulation efficiency (EE) of folic acid was calculated as follows:

$$\text{EE}\ (\%) = \frac{\text{Amount of folic acid in micro}-\text{beads}}{\text{Amount of folic acid loaded in polymer solution}} \times 100$$

Task 2: Title: Drying micro-beads.

MS2.1. Establishing the conditions most suitable for drying the microbeads using the 3 methods.
For long time storage and transportation of micro-beads produced from the formulation of MP (3.1%)/alginate (2.0%) with ration of 65/35 in task 1 was dried by three different techniques:
Spray-drying: Micro-beads with and without folic acid were spray-dried using a laboratory spray-drier (Anhydro A/S, Copenhagen, Denmark). Malto-dextrin solution (12%, w/v) was used as a carrier medium for spray-drying of micro-beads.

Freeze-drying: Micro-beads were frozen at -20°C for 12 hrs and subsequently at -80°C for 24 hrs. Frozen micro-beads were dried using a freeze-dryer (Labconco, stoppering tray freeze dryer, USA) for 70 hours.
Modification:

Drum drying: it was initially proposed to include drum drying (to be outsourced) as one of the drying techniques. However, after consultation with some of the relevant companies it

proved more difficult than anticipated and it was instead decided to concentrate on the much easier, better and cheaper to oven-dry technique, using a variety of drying aids.

Vacuum oven drying: Micro-beads in petri dishes were dried in oven (OV-12, JEIO Tech, Korea) at 50°C under vacuum for 24 hrs.

MS2.2. Production of dried beads containing suitable bioactives:
Micro-beads with folic acid were successfully spray-dried using maltodextrin solution as carrier media in Moorepark using facilities of FIRM-funded bio-functional engineering facility and freeze-dried without any carrier media.

MS2.3. Beads incorporated into a test food product, evaluation of processibility:
MP/alginate (65/35) micro-beads with folic acid were stored in cranberry and orange juices at 4°C and 25°C for up to 28 days to investigate the release pattern of folic acid from micro-beads during storage.

MS2.4. Testing of the beads for bioactive retention and activity upon rehydration: Dried micro-beads (spray-dried and freeze-dried) were rehydrated in water and fruit smoothie and evaluated for its size and retention capacity of folic acid.

Task 3: Title: Diversification of encapsulated bioactives

MS3.1. Selection of emulsions which are most suitable to use for encapsulation:
MP solution (3.1% protein) prepared in task 1 and MP (3.1%) solution with 5% α-lactose monohydrate (ALM) were used for preparation of emulsion using 5.0% (w/w) sunflower oil. Coarse emulsions with composition of 95% protein solution and 5% oil (w/w) were prepared by pre-homogenizing for 5 min using high speed UltraTurax T25 (IKA, Staufen, Germany). Coarse emulsions were then homogenized for 3 cycles by a single stage valve lab homogenizer (APV 2000, Albertslund, Denmark) with pressure of 150 bars. Oil droplet size in the emulsions was measured immediately after preparation using a laser light scattering instrument (MasterSizer hydro MV 3000, Malvern Instruments Ltd, Worcestershire, UK).

MS3.2. Encapsulation of hydrophobic bioactives:
Emulsions with hydrophobic coloured bioactive compound such as β-carotene were prepared using the formulations mentioned in milestone 1 of Task 3. β-carotene was encapsulated in micro-beads prepared with the mixture of emulsions and alginate solution with ratio of 65/35 (w/w).
MS3.3. Determination of loading required to deliver required dose of bioactive to the gut:
Loading 1mM β-carotene into the microbeads appeared to be sufficient for delivery of the necessary dose.

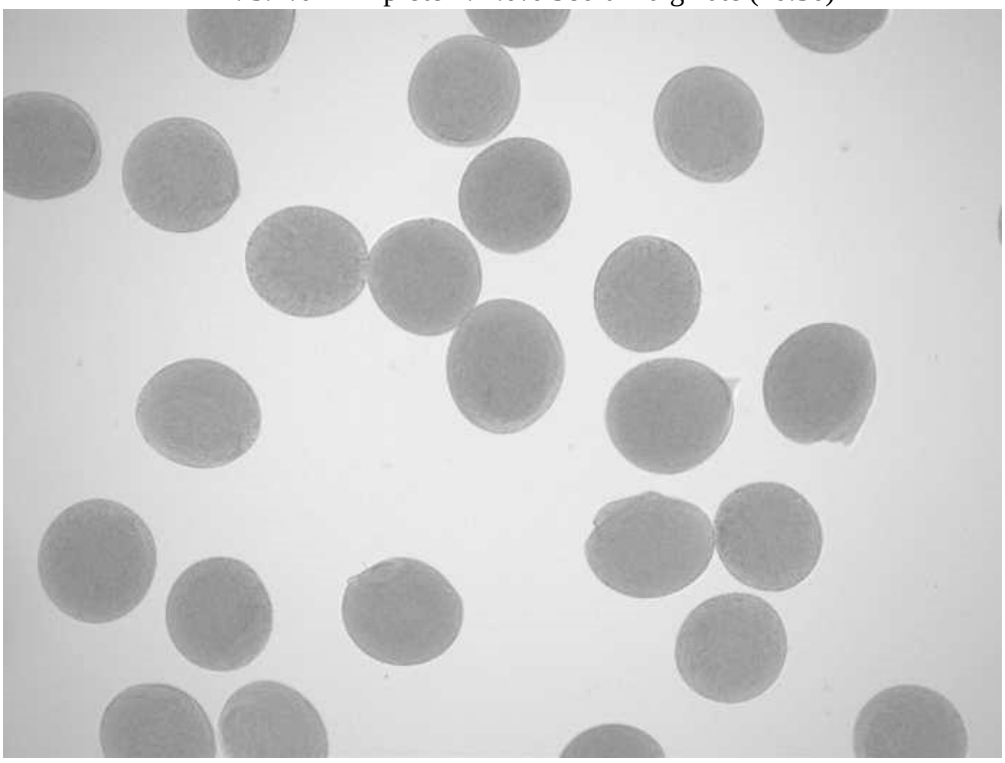
3. Research Achievements

Task 1: Title: Modification of encapsulation matrix

MS1.2. Formulation and evaluation of Polymer matrices using selected ingredients: Polymer solutions were prepared with various combination of WPI (4-6%) and SMP (2-6%) generated weak gel structure, which were too brittle to make gel beads, whereas solutions prepared with combination of WPI (8-10%) and SMP (3-6%) with different ratios were too rigid after heat denaturation of whey proteins. Relatively better matrix media were generated from combination of WPI (7.0-7.5%) and SMP (2.0-2.5%), which were suitable to make gel micro-beads. However, shape of beads produced was not spherical and large size variation was observed. Even micro-beads produced from these matrix solutions were not reproducible.

Therefore, relatively cheaper heat treated MPI was used as noble ingredient to produce suitable matrix solution by combining with alginate polysaccharide for preparation of gel micro-beads. Polymer matrix solutions formulated using mixture of MP solutions (from 2.7 to 4.1%) and alginate (2.0%) with ratios of 75/25 and 70/30 generated weak and brittle gel structure, whereas matrix solutions from mixture of MP solutions (from 3.5 to 4.1%) and alginate with ratios of 65/35 and 60/40 generated rigid gel structure immediate after mixing. Matrix solutions from mixture of protein solutions (from 3.5 to 4.1%) and alginate with ratios of 75/25 and 70/30 were easy to extrude through 200 µm vibrating nozzle but discontinuous stream of droplets were extruded with ratios of 65/35 and 60/40. Mixtures of MP solutions (from 2.7 to 3.1%) and alginate with ratio of 65/35 and 60/40 generated suitable matrix polymer. A steady stream of matrix droplets was extruded through the nozzle from these solutions representing the preliminary requirement to prevent flocculation of polymer droplets upon contact with curing media.

A. 3.1% Milk protein: 2.0% Sodium alginate (70:30)



Light microscope images of micro-beads made from a novel protein-based formulation

MS1.3. Make microbeads and test for efficacy:

The shape and size of micro-beads measured by light microscopy were varied according to composition of polymer solutions. The most homogeneous and spherical beads were obtained from formulation of MP (2.7%)/alginate (2.0%) and MP (3.1%)/alginate (2.0%) with ratios of 65/35. Entrapment of folic acid was overall high in micro-beads prepared from the formulation of 65/35 and 60/40 (70%) compared to the formulation of 75/25, 70/30 (64-65%). By considering the presence of protein content (as high as possible) but able to form spherical beads with high encapsulation efficiency by instantaneous gelation in collection bath only formulation of MP (3.1%)/alginate (2.0%) polymer solution with ratio of 65/35 was used for further study.

Deliverable1.1. Several SOPs for methods for the production of microbeads with lower cost material that WPI:

SOP's for the production of microbeads using different protein formulations were produced and subsequently summarized in the invention report submitted to the TTO of Teagasc. Therefore, SOP's are not included in this report.

Task 2: Title: Drying micro-beads.

MS2.1. Establishing the conditions most suitable for drying the microbeads using the 3 methods.

Spray-drying: Free flowing maltodextrin powder containing micro-beads without any particle agglomeration was achieved by spray-drying. Spray-dried particles were spherical shaped without any dent on the particle surface. Dried micro-beads were kept in air tight brown container for further analysis.

Freeze-drying: Dried beads were free flowing with flake like structure. There was no particle agglomeration occurred in freeze-dried beads. Dried micro-beads were kept in air tight brown container for further analysis.

Vacuum oven drying: Oven drying of micro-beads was not successful. An agglomerated particle with collapsed structure was achieved by oven drying.

MS2.2. Production of dried beads containing suitable bioactives:

Encapsulation efficiency of folic was 75-78% in spray-dried micro-beads, whereas this value was 98-99% in freeze-dried micro-beads

MS2.3: Beads incorporated into a test food product, evaluation of processibility: MP/alginate (65/35) micro-beads with folic acid were stored in cranberry and orange juices at 4°C and 25°C to investigate the release pattern of folic acid from micro-beads during storage. Data showed that a small amount of folic acid was released in both fruit juices during 28 days storage at 4°C with no significant change in beads shape and size. Further results are described. After 28 days storage, a total of 10.2% and 15.3% folic acid was released at 4°C, whereas 22.4% and 28.3% folic acid was released at 25°C in cranberry and in orange juices respectively. Although micro-beads had slightly better storage capacity of folic acid in cranberry juice but there was a disadvantage for storage of beads in cranberry juice because beads absorbed colour of cranberry juice during storage. Therefore, incorporation of micro-beads with folic acid in cranberry juice might not be suitable to enhance the bioactivity of cranberry juice.

MS2.4: Testing of the beads for bioactive retention and activity upon rehydration

Dried micro-beads (spray-dried and freeze-dried) were rehydrated in water and fruit smoothie and evaluated for its size and retention capacity of folic acid. Spherical shaped with slight swelling of spray-dried micro-beads was observed on rehydration in water. Although better folic acid encapsulation (75-78%) was obtained in dry particles but majority of encapsulated folic acid (55-57%) was released on rehydration in water. On the other hand, flake like structure with very low swelling (2%) of freeze-dried beads was observed on rehydration in water. A small amount of encapsulated folic acid (3.0-3.5%) was released on rehydration in water, whereas no release of folic acid with slight swelling of beads (2%) was detected on rehydration in fruit smoothie at pH 3.0 during storage at 4°C for 14 days. Encapsulated folic acid was slightly released (3.8%) with swelling (4%) of beads after 14 days of storage in fruit smoothie at 25°C. By considering swelling and

retention of encapsulated folic acid in water or fruit smoothie, it could be concluded that freeze-drying process could be more feasible for drying of micro-beads.

Task 3: Title: Diversification of encapsulated bioactives.

MS3.1. Selection of emulsions which are most suitable to use for encapsulation:
Both milk protein and milk protein-ALM formed relatively stable emulsions containing fat soluble bioactive such as β-carotene with droplet size of 2-3 µm. However slight flocculation of oil droplets occurred after one day of storage in both emulsions.

MS3.2. Encapsulation of hydrophobic bioactives:
Encapsulation efficiency was nearly the same (89%) in both formulations mentioned in milestone 1 of Task 3.

MS3.3. Determination of loading required to deliver required dose of bioactive to the gut:
Loading 1mM β-carotene into the microbeads appeared to be sufficient for delivery of the necessary dose.
Deliverable 3.1. Methods for encapsulating several different bioactives for targeted delivery to the gut:
Two model compounds were selected, the water-soluble folic acid and the fat soluble βcarotene. The methods for encapsulating the bioactives was developed and shown for one compound (the most promising) β-carotene.

4. Impact of the Research

The aim of the present study was to develop milk protein-based micro-beads using encapsulator device and to encapsulate water soluble and fat-soluble bioactive components as delivery vehicles in wet and dry systems. Suitable polymer matrix with instant gelation property was obtained from heat treated MPI solution (3.1% MP) with mixture of sodium alginate (2.0%) in ratio of 65/35. Relatively stable micro-beads with encapsulation efficiency of about 70% for water soluble bioactive such as folic acid and about 89% for fat soluble bioactive such as β-carotene were obtained from this formulation.

Micro-beads were successfully spray-dried and freeze-dried in Moorepark using facilities of FIRM-funded bio-functional engineering facility. Micro-beads obtained from spraydrying were free flowing spherical shape, whereas freeze-dried beads were free flowing non-agglomerated flake-like structure. A slight release of encapsulated folic acid (3.0-3.5%) was measured on rehydration of freeze-dried beads in water, whereas no release of folic acid was detected on rehydration in fruit smoothie at pH 3.0 during storage at 4°C for 14 days. Release of encapsulated folic (3.8%) was detected in fruit smoothie when storage at 25°C for the same time.

In the food and biotechnology sectors some concerns with regard to cost, the ability to diversify the technology to encapsulate water and fat soluble bioactive components and drying of micro-beads were seen as the main hurdles preventing uptake of the existing technology. Even some functional bioactives are inactivated by the harsh environmental conditions during food processing and /or by the conditions in the gastric tract. But the method developed in this research will allow encapsulation of water- and fat-soluble bioactive food components including probiotic bacteria with retention of their activity in low cost dairy protein-based micro-beads, and successful drying techniques (spray-drying and freeze-drying) could solve the existing hurdle in some extent. Irish food industry can be benefited by using this technique for incorporation of encapsulated sensitive food components into the products such as fruit smoothie, milk shake or dairy-based dessert for manufacturing of functional food with enhance nutritional value and health benefit.

Currently there is a trend towards a healthier way of living, which includes a growing awareness by consumers for what they eat and what benefits certain ingredients have in maintaining good health. Therefore, the advantage of using the present developed method is that the micro-beads are made from dairy proteins that consumers are familiar with and consider to be natural, high nutritional value and healthy. Another ingredient used to develop the micro-beads is alginate, which is a natural water-soluble polysaccharide. This process will help to deliver and control release of intact bioactive components to the target site during transport through harsh environmental condition in GI tract. In addition, materials used to develop this process were non-toxic, biodegradable and biocompatible. There was not any EU band food components used in this process. So, there should not have any restriction by Food Safety Authority to use this process by food industry to develop new functional foods by incorporation of bioactive components with present existing products from where consumers can get health benefit beyond the basic nutrition.

5. Exploitation of the Research

At present there is little industrial or technological expertise available in encapsulation within Irish food industry. The patented whey protein micro-beads established in Moorepark have been mainly used for target release of probiotic bacteria in the gut. This method also produces beads which are stored and used in wet conditions. But our developed method in present research will help to encapsulate water- and fat-soluble bioactive components with retention their activity in relatively low-cost dairy-protein based micro-beads. Drying techniques (spray and freeze-drying) established in this project will also help to store, transport, and use encapsulated bioactives in dry conditions along with wet conditions. This technique will allow for the encapsulation of a wide variety of bioactive ingredients outside the probiotic area by the Irish food industries and allowing them to be more innovative and increase their product range in Irish food sector as well as globally.

6. Summary of Research Outputs

(a) Intellectual Property applications/licences/patents

The outcome of the project has been submitted as an internal Invention Disclosure Report to the TTO of Teagasc.

(b) Innovations adopted by industry

(c) Number of companies in receipt of information

The technology was offered to 1 company. Teagasc is currently negotiating an evaluation agreement with 1 company.

(d) Outcomes with economic potential

The outcome of the study is suitable for both highly specialised start-up companies (as the case with the previous encapsulation technology Patent Brodkorb & Doherty (2010) Method of Producing Microbeads. Ireland Patent WO2010119041 (A2)) or larger multinational companies.

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

None to date

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

None to this date

(g) Scientific abstracts or articles including those presented at conferences

None

(h) National Report

None

(i) Popular non-scientific publications

None

(j) Workshops/seminars at which results were presented (excluding those in (g))

None

7. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (months)	Average time contribution per permanent staff member
Teagasc Food Research Centre	1	3.96 (0.33 x year)	3.96 (0.33 x year)
Total	**1**	3.96 (0.33 x year)	3.96 (0.33 x year)

8. Researchers Funded by FIRM

Type of Researcher	Number	Total Time contribution (months)	Average time
Post Doctorates Contract Researchers PhD postgraduates Masters postgraduates Temporary researcher Other	1	12	12
Total	**1**	**12**	**12**

9. Postgraduate Research

Total Number of PhD theses: None

Total Number of Masters theses: None

10. Involvement in Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names of modules attended

11. Project Expenditure

Total expenditure of the project:	€76,425.54
Total Award by FIRM	€99,780.00
Other sources of funding (specify)	€

Breakdown of Total Expenditure

Category	Name Institution 1	Name Institution 2	Name Institution 3	Name Institution 4	Total
Contract staff	TFRC				40,673.51
Temporary staff					0.00
Post doctorates					0.00
Post graduates					0.00
Consumables	TFRC				11,469.66
Travel and subsistence	TFRC				6,645.71
Sub total					58,788.88
Durable equipment					0.00
Other					0.00
Overheads	TFRC				17,636.66
Total					**76,425.54**

12. Future Strategies

One manuscript is written and will be submitted for publication in a peer-reviewed journal once approved by the Teagasc TTO, most likely after filing of the patent application

13. Industry Collaboration

It was the aim of the project to create intellectual property and the project team has succeeded in improving the existing micro-bead formulation. Hence during the project there was no direct industry collaboration except for some communication regarding their needs (2 companies).

The developed technology is currently being offered to one company for valuation for a