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

Food Institutional Research Measure

Final Report

'Development of high protein bars as vehicles for functional ingredient delivery (PROBar)

DAFM Project Reference No: 13/F/513

Start date: 01/05/2014

End Date: 30/04/2015

Principal Coordinator and Institution:

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Collaborating Research Institutions and Researchers: None

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	5	6 X	7

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

Priority Area (s)	**H Food for Health**

Key words: *Health promotion, high value nutrition, functional foods, wellness products*

1. Rationale for Undertaking the Research

The need for the research executed during this project was described in the report of the Research Prioritisation Steering Group (2011), which emphasised a need for the '*development and delivery of functional foods*' and that '*major focus on nutrition, probiotics, novel bioactives/functional ingredients*' is required as part of the national strategy for science, technology and innovation. Food Harvest 2020 stated that '*R&D should be targeted to new product development, with a greater focus on product areas with added-value potential, such as nutraceuticals and functional foods*'.

This FIRMplus 'PROBar' study extended the outcomes of its fore-running FIRM-funded project 'Water activity control and texture stabilisation of high protein snack bars' in a practical way. The latter project highlighted the complexity underlying ingredient interactions in the highly concentrated environment and, particularly, how they impact on textural change (hardening) during storage. The purposes of PROBar was to adopt optimal formulations and appropriate protein combinations from its parent project as a platform with which to improve the carbohydrate and lipid profiles of high-protein bars and to determine the capacity of bars to carry and maintain probiotic stability throughout product shelf-life. The project seeks to address the technical challenges in maintaining the textural stability of re-formulated (healthier) high-protein bar matrices and the effects of composition, processing and storage on the viability of probiotic bacteria included therein.

2. Research Approach

A three-stranded approach was undertaken during the course of this work. This included:

1. The development of physically stable, protein-based matrices, capable of protecting functional ingredients (probiotic bacteria and/or prebiotics) during manufacture and storage. Optimisation of formulations was carried out with emphasis on health. These included comparison of whey and soya protein isolates, the effects of whey protein hydrolysis and addition oil blends with fatty acid compositions (melting profiles) that enhance the functional requirements of texturally-stable bars.

2. The viability of probiotic strains was determined over a range of water activities (a_w). The effects of bacterial addition to individual ingredient classes (solid or liquid phases), pre-conditioning of probiotics in selected growth media and encapsulation/coating of probiotics to minimise moisture uptake and thereby enhance viability.

3. The stability of prebiotics as a function of non-enzymatic browning reactions and the synergistic effects of prebiotic-probiotic inclusion were determined in high-protein bar systems. Work on encapsulation of prebiotics was not carried out as per original proposal. Work on the development of effective barriers to moisture uptake by probiotic bacteria was deemed more important and resources were directed that that effect.

3. Research Achievements/Results

A range of whey protein isolate (WPI) and soy protein isolate (SPI) -based model formulations, differing in protein concentration, were compared for their suitability as ingredients for the manufacture of texturally stable, high-protein bars. Non-protein ingredients included glucose-fructose syrup (1:1 w/w) and glycerol. Bars were stored at 37°C and examined intermittently for a number of physical indicators

of textural stability. Water activity, increased during storage and was higher in WPI systems compared to SPI. Water activity values remained below levels associated with microbial degradation. Colour change during storage, associated primarily with Maillard reactions, was broadly similar in WPI and SPI systems. Hardening during storage represents the most important deteriorative physical change in high-protein bars and was dependent on both protein concentration and type. Bars containing WPI did not harden during storage to the same extent as those containing SPI. Although the physical stability of SPI bars was enhanced by inclusion of lecithin, WPI-based bars were deemed to be more acceptable from a sensory perspective. The maximum acceptable level of protein inclusion into protein bars with was established at 35% (w/w). Bars containing WPI at protein concentrations below 30% (w/w) were physically unstable and prone to protein-sugar phase separation.

The effect of oil type on the development of hardening in WPI-based bars was examined by inclusion of coconut oil (CO) and sunflower oil (SO). These oils differed in the proportion of saturated fatty acids and had melting points of 35° and -35 °C, respectively. Bars containing SO had higher water activity values during storage but were less susceptible to hardening. It is likely that inclusion of liquid fat provided a more effective barrier to moisture diffusion from the liquid (glucose-fructose syrup) to the dry, protein powder phase. The transfer of moisture from high to low water activity constituents has been established as the driving force for bar hardening. The order of addition of ingredients also affected hardening. Mixing of WPI with glucose-fructose syrup prior to addition of SO enhanced long-term physical stability.

Intermediate moisture foods are generally regarded as microbially stable due to their ability to control water activity (a_w). Hence from the outset, it was anticipated that the physico-chemical environment of a high protein bar matrix was likely to be inimical to all microbial growth including that of probiotics. Very quickly it became apparent as the study got underway that this, indeed, is the case. Various avenues were explored such as preconditioning the probiotic by growth in different media, and inoculation into different phases of the bar formulation e.g. liquid (carbohydrates/oils) or solid (protein). These model studies indicated that inoculation of probiotics into the oil phase supported culture viability. As a result, chocolate was selected from a number of hydrophobic materials as a lipid-continuous system and used as a carrier medium into which probiotics could be injected. It was also established that, for optimum performance, the freeze-dried probiotic should be prepared from a live cell culture and suspended in a skim milk/prebiotic mixture before freeze-drying and subsequent dispersal in molten chocolate. Large chunks (ca. 10 mm^3) of cooled, solidified probiotic-containing chocolate was mixed and distributed throughout the bar matrix.

A series of complementary studies examining the physicochemical characteristics of high-protein bars used in probiotic inoculation and viability studies were carried out. *L. casei* was inoculated into bars via lipid or carbohydrate phases in bars containing WPI, hydrolysed whey protein (WPH), glucose-fructose syrup, glycerol and either coconut or rapeseed oil (RO). The extent of hardening during storage was greater in bars containing WPI (intact protein) compared to WPH. Formulations containing a 1:1 mixture of WPI and WPH were least susceptible to hardening. It has been previously reported that mixed protein systems impact beneficially on textural stability. The susceptibility to hardening of bars containing WPI and WPH was further enhanced through the inclusion of CO compared to RO (the influence of lipid melting point was opposite to that observed in single protein systems (and would appear to be indicative of interactive effects of ingredient addition). Non-enzymatic browning reactions were greater in bars containing WPH but did not correlate with hardening.
Previous work at Moorepark demonstrated that textural stability can be enhanced through minimising the osmotic potential between ingredients and further work was carried out to explore this phenomenon. A series of bars, were produced differing in initial water activities. Bars containing a glycerol/water ratio of 7:3 (w/w) were less susceptible to hardening compared to equivalent ones prepared at 9:1 (w/w) despite higher water activity and moisture contents. It is likely that changes to solvent quality affected the hydration and re-conformation behaviour of whey proteins and peptides.

Single strain pure freeze-dried *L. casei* 431(*Lactobacillus paracasei subsp.paracasei*) and Bifidobacterium BB-12® (Chr Hansen) were used in this study. The probiotics selected were clinically effective at seven log colony-forming units (CFU) per 100 g unit, and this was used as target concentration in the bars. The initial probiotic count of the freeze-dried ingredient was 11 log CFU/g. Initial studies of these probiotics with and without added ingredients showed long-term stability in the absence of water. The presence of water (added directly to probiotics or from diffusion processes within the bar) resulted in rapid decreases in bacterial counts. Such losses were further exacerbated during elevated temperature storage (37 ^{o}C).

Twelve bar formulations were manufactured and inoculated with probiotics (*L. case).* Probiotic counts were carried out following manufacture and at intervals during storage at three different temperatures (37°C, 20°C and 5°C) over three months. Two approaches to probiotic incorporation were used during bar manufacture i.e. dispersal in oil or sugar-based phases. Oil-based delivery of probiotics proved more stable with respect to viability compared to sugar-based carrier systems. Storage testing at 37°C and 20°C for one week and one month, respectively, indicated that probiotic stability in the bar formulations fell significantly short of the six-month target set for ambient temperature storage. Decreasing a_w by means of altering glycerol concentrations in bar formulations improved probiotic stability but remained below the storage target set. Vacuum packing of the bars was of no benefit with respect to probiotic survival.

A range of different hydrophobic materials (oils/fats/wax/chocolate) were tested as coating agents for probiotics. Results confirmed that the protective effect increased with the hardness (high melting points) of fat/oils, i.e. paraffin wax >chocolate>coconut oil/palm oil>liquid oils. The effects of mixing probiotic cultures with molten chocolate as a primary protection system before subsequent incorporation into bar formulations was pursued using different sizes of chocolate pieces, followed by storage at ambient and elevated temperatures. It was concluded that chocolate pieces of ca. 10mm^3 offered better protection to probiotics compared to smaller pieces (with greater surface area).

It is well-established that prebiotics act as an energy source for probiotics and stimulate growth of beneficial bacteria in the digestive tract. A range of freeze-dried, prebiotic (galacto- (GOS) and fructo-oligosaccharides (FOS)) and probiotic containing powders were prepared, mixed into molten chocolate added to model bars. Freeze dried cultures, prepared in the presence of prebiotics and skim-milk were most viable over time. Enrobing of model bars with an outer layer of chocolate was also investigated in order to maximise the longevity of the probiotics.

Following 28 days storage at 20°C, no significant decrease was observed in the numbers of viable probiotic bacteria. Storage at 37°C, however, was associated with a drastic decline in bacterial numbers with no live bacteria present at day 14.

A stable delivery system for probiotic bacteria in a high-protein snack bar formulation was developed using chocolate as the primary protectant of probiotics. This hydrophobic coating was necessary to prevent uptake of moisture by bacteria as a consequence of osmotic equilibration. Involved in this was the optimization of robust probiotic bacteria, which were stabilized by means of freeze drying in the presence of prebiotics and skim milk. Probiotics in the resultant formulations were able to withstand manufacture and ambient temperature storage for one month with no significant decline.

4. Impact of the Research

Two major stake-holders can benefit from the outcomes of this research: (i) dairy ingredient innovators and suppliers in business to business relationships with formulators of protein bars, and (ii) SME companies interested in producing healthy, high protein snack-based foods to local markets. Dairy ingredient manufacturers typically are engaged in the export of large volumes of ingredients to bar formulators whom they also support with considerable technical support along with introduction of innovative ideas. The knowledge gained and the approaches taken during the course of this this project represent a significant source of material of use to those seeking practical information on technical aspects of bar formulation and functional ingredient stabilisation.

The PROBar concept is of immediate appeal to consumers increasingly preoccupied with overweight, obesity and dietary aids that support post-exercise recovery. The Interim report released in 2015 by the WHO's Commission on Ending Childhood Obesity (ECHO) highlighted that in 2013 42m infants and young children were overweight or obese, by 2025,70m young children will be overweight or obese if current trends continue, and that the rate of increase is 30% higher in low- and middle-income countries than that of developed countries.

A number of Irish food companies have significant interests in nutrition products, probiotics and prebiotics. These include Glanbia, Kerry Group, Dairygold, Carbery, Alimentary Health Ltd., Atlantia Food CRO and Biovea. Efforts to develop protein applications is reflected in interest in all three product groups. Major European and global companies such as Danisco, Winclove b.v., Valio, Metagenics, Micropharma, Pepsico and Pfizer Nutrition have all expressed similar interest. Along with these, companies such as Danone, Nestle, Abbott, all of whom include GOS into their infant formulas, are known extending their product range.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research:

Technical discussions were undertaken with Professor Y.H. Roos of UCC.

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

A number of new protein bar formulations and processes for introduction and stabilisation of functional food ingredients were developed.

Results can be used to provide formulation technology and food micro-structural control that allow production of 'high-tech' protein nutritional products for the health-conscious consumer. Such products will appeal, in particular, to those involved in sports, exercise regimes and weight loss programmes and meet the demands of a discerning and knowledgeable public.

Inclusion of specific probiotics and prebiotics into protein bars, provides an alternative means for their delivery and consumption. The effects of addition of hydrolysed whey proteins were determined and work demonstrated that high quality, digestible protein can be used to promote textural stabilisation of bars.

It was demonstrated that properly formulated bar matrices provide the structural medium for stabilisation and delivery of sensitive, bioactive ingredients. Thermodynamic incompatibility of

hydrophobic (oils/fats) and hydrophilic (proteins, carbohydrates) phases creates a segregative, internal structure that can be exploited for targeted insertion and separation of functional ingredients.

Such systems can be tailor made to include a wide variety of bioactive ingredients and micro-nutrients. Bar formulations can be readily modified to carry targeted nutrients for specific niche markets such as formulations for gender or age groups. Such product flexibility should offer considerable potential in terms of the Irish export market.

Findings from this project and its parent FIRM-funded project 'Water activity control and texture stabilisation of high protein snack bars' provided the technical platform for work presently being undertaken in Pillar 3 of the Dairy Processing Technology Centre (DPTC).

(iii) Outcomes with economic potential

N/A

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

The PROBar FIRMplus project was a sequel to a previous DAFF funded Project Reference No: 08/R&D/TMFRC/651 entitled *Water activity control and texture stabilisation of high protein snack bars*. This project made significant progress explaining the key parameters that drive textural changes during storage. The PROBar project built on to the outcomes of the previous project in so far as it adopted one of its outcomes i.e. an optimised bar formulation. PROBar went one step further though by cleaning up the commodity fats and carbohydrate ingredients used in the base formulation and substituting with healthier versions of the same ingredients. Combinations of glucose and fructose were also adopted in place of sugar (corn) syrups in order to avoid complexity associated with the high viscosity of the latter. Hence, a revised, better balanced (healthier) bar option was adopted for the complementary studies involving probiotics and prebiotics. Such developments contribute to national and social policies that seek to encourage a healthy approach to food consumption and an awareness of proper nutrition.

4 (b) Summary of Research Outputs

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

Two articles are currently in preparation for submission to peer-reviewed international scientific journals:

1. Working title: *Hunt, T., Potes, N., Rosberg-Cody, E. Hogan, S.A., Kelly, P.M. Shelf life stability of dried Lactobacillus casei 431 and Bifidobacterium animalis ssp. lactis (BB-12®) in a protein rich composite dairy bar*. *Target Journal: Food Microbiology*

2. Working title: *Potes, N., Rosberg-Cody, E., Hunt, T., Hogan, S., Kelly, P.M. Physico-chemical requirements of high-protein bars as vehicles for delivery of functional ingredients. Target Journal: International Dairy Journal*

(ii) Popular non-scientific publications and abstracts including those presented at conferences: *Potes, N., Rosberg-Cody, E., Hunt, T., Hogan, S., Kelly, P. and Roos, Y. Effects of interactions of food components on viability of Lactobacillus casei in high protein systems (2015). 29th EFFoST International Conference, 10-12th November, Athens.*

(iii) National Report: None

(iv) Workshops/seminars at which results were presented:
Teagasc Food Research Centre Research Seminar (02/02/15)

(v) Intellectual Property applications/licences/patents: None

(vi) Dr. Sean Hogan has been invited to speak at the forthcoming IDF World Dairy Summit, October 16-20th, Rotterdam, where he will present findings from the present project. Abstract title: *Development of high-protein bars as vehicles for delivery of functional ingredients.*

5. Scientists trained by Project

Total Number of PhD theses: Not applicable

Total Number of Masters theses: Not applicable

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
	1	0.05
Total	**1**	**0.05**

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	3	1.9
PhD students		
Masters students		
Temporary researchers		
Other		
Total	**3**	**1.9**

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended
Dr Naritchaya Potes	Writing Successful Grant Applications 15 and 16 January 2015 Induction Training 27 February 2015

9. Project Expenditure

Total expenditure of the project:	€99,351
Total Award by DAFM:	€99,351
Other sources of funding including benefit in kind and/or cash contribution(specify):	€0

Breakdown of Total Expenditure

Category	Teagasc	Total
Contract staff	16,602	
Temporary staff		
Post doctorates	55,401	
Post graduates		
Consumables	4,420	
Travel and subsistence	0	
Sub total	76, 423.46	
Durable equipment		
Other		
Overheads	22,927.04	
Total	**99,350.50**	

10. Leveraging

n/a

11. Future Strategies

Despite significant advances in stabilising probiotics in the bar systems storage viability remained below the target time of six months at ambient temperature. Further work on prevention of moisture sorption by probiotic cultures through encapsulation and/or coating techniques is required. Demonstrable long-term storage viability is likely to have generated interest by potential industry end-users.

Staff turnover and the necessity for re-training and familiarisation with laboratory procedures impacted on the research. It should recognised, however, that a significant body of work was generated during the life-span of the project.