Food Institutional Research Measure

Final Report

Targeting the Glycome of the Milk Fat Globule Membrane for anti-infective properties (GLYCOMFGM)

DAFM Project Reference No: 10RDTMFRC708

Start date: 1/3/12

End Date: 30/6/16

Principal Coordinator and Institution: Dr. Rita Hickey, Teagasc Food Research Centre
Email: rita.hickey@teagasc.ie

Collaborating Research Institutions and Researchers: Prof. Lokesh Joshi, National University of Ireland, Galway

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

<table>
<thead>
<tr>
<th>Basic/Fundamental</th>
<th>Applied</th>
<th>Pre Commercial</th>
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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 and M. Processing Technologies and Novel Materials

Key words: Milk fat Globule membrane, Anti-infective, Glycoprotein, E coli
1. **Rationale for Undertaking the Research**

The alarming increase in antibiotic-resistant bacteria makes the search for novel means of fighting infections imperative. For that reason, instead of searching for factors that kill bacteria, research should focus on compounds that can prevent the onset of infection. It is well documented that adhesion of enteric bacteria is required for colonization and subsequent development of disease. Therefore, one attractive possibility is the use of agents which interfere with bacterial adhesion to the host. Some of the most efficient anti-adhesion agents identified to date are present in foodstuffs - as best exemplified by human milk glycans which protect newborns against infections. Such glycans can display structural homology to host cell receptors or pathogenic lectins, thus functioning as receptor decoys. MFGM from buttermilk is a rich source of glycosylated proteins and an ideal source to mine for glycosylated bioactives. Therefore, this project aimed to explore a commercially undeveloped component of milk i.e the milk fat globule membrane (MFGM). Many biological functions have been attributed to MFGM e.g. prebiotic activity, antiadhesion effects, anti-inflammatory properties, an involvement in brain development, and other uncharacterised effects. However, there are very few commercial products on the market which capitalise on these functions. Identification of the components within MFGM which confer these effects may aid in highlighting its commercial potential. Following the initial investigative phase, the project subsequently exploited modern separation technologies for generating various MFGM streams. In vitro tests were performed to validate the bioactivity of the MFGM based powders generated.

2. **Research Approach**

Different processing streams for MFGM were exploited to generate glycoprotein enriched fractions e.g. (a) Raw whole milk, (b) Separated cream (with/without washing) (c) Heat treated milk and cream (with/without washing) (d) Buttermilks produced from churning (buttermaking) creams (b) and (c), (e) Butteroil serum resulting from conversion of creams (b) and (c) to anhydrous milk fat (AMF), (f) Fermented buttermilks from (d), (g) Residual skim milk lipid fraction, (h) Residual cheese whey lipid fraction (i) Membrane filtered fractions (retentates/permeates) of (d), (e), (f) and (g), (j) Spray dried buttermilks (d) and enriched fractions from (i), (k) Polar lipid-depletion of the more biologically active materials from (d) - (h) as a means of selectively enriching glycoprotein moieties of MFGM. Bioactive fractions underwent gastric digestion using a simulated model. Undigested samples were retained as controls. Lectin microarray analysis of MFGM provided detail on how glycosylation of the proteins change over lactation. Genomic microarrays were employed to identify any processes in intestinal cells which were influenced by exposure to buttermilk. Lectin and neoglycoconjugate microarrays were employed to investigate and distinguish surface glycosylation patterns and binding preferences of various E. coli strains. Validation that glycoprotein fractions which bind the bacteria can in fact prevent adherence to human cells was performed using well established cell line models of infection. Finally, prototype beverages were formulated to incorporate anti-infective glycoprotein ingredients.
Research Achievements/Results

Defatted bovine milk fat globule membrane (dairy stream K) was found to inhibit the association of enterohaemorrhagic *Escherichia coli* O157:H7 with human HT-29 cells. This work was published: Ross S. A., Lane J. A., Kilcoyne M., Joshi L. and Hickey R. M.* (2016) Defatted bovine milk fat globule membrane inhibits association of enterohaemorrhagic *Escherichia coli* O157:H7 with human HT-29 cells. International Dairy Journal. 59:36-43.

This MFGM fraction was observed to reduce the association of several *E. coli* O157:H7 strains with the HT-29 cells. This activity was strain-specific and concentration dependent. The parameters of the experimental assays were varied to identify the potential mechanisms by which the MFGM fraction exerted its activity. The anti-infective activity was shown to occur as a result of the MFGM interacting with the *E. coli* rather than the HT-29 cells. This study identified the potential use of defatted bovine MFGM to prevent the onset of *E. coli* infections in humans.

Since the glycan portion of milk glycoconjugates may be an important factor in their health-promoting activities, the changes occurring in buttermilk, a source of MFGM, were profiled for their altered glycosylation throughout the course of lactation. Buttermilk samples were generated at 13 time points during lactation for three multiparous animals and their glycosylation patterns were profiled using lectin microarrays and lectin blotting. The data suggested differences in glycosylation, including N-linked glycosylation, sialylation and fucosylation, between early and late time points. In addition, differences were evident between individual animals at various time points throughout the lactation cycle. The findings of this study may be invaluable in order to target the isolation of glycosylated ingredients for functional foods from particular lactation time points to maximise the abundance of particular glycan structures. This work was published: Ross, S.A Gerlach, J.Q. Gill, S.K. Lane, J.A. M. Kilcoyne, R.M. Hickey, L. Joshi. Temporal alterations in the bovine buttermilk glycome from parturition to milk maturation. Food Chemistry, 211, 329-338, 2016.

The most bioactive fraction identified from the MFGM samples, dairy stream K, was formulated into beverages. After undergoing, the various treatments, it was found that the heat treatments and storage conditions had very little effect on the structural characteristics of MFGM with the exception of sedimentation which was observed for all treatments after the 2 month storage period. Overall these studies indicate the potential of bovine MFGM fractions to promote human health by reducing the threat of infection and modulating immune response and inflammation. Moreover, the selection of bovine MFGM from certain lactation stages may be advantageous in order to increase the level of bioactivity by using the most appropriate glycosylation profile in the dairy fraction.
4. **Impact of the Research**

4(a) **Summary of Research Outcomes**

(i) Collaborative links developed during this research

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

It is too early to see impacts such as these yet.

(iii) Outcomes with economic potential

Buttermilk, the by-product of butter production, is essentially skim milk containing large amounts of MFGM. This currently under-exploited milk product is interesting as a crude source of anti-infective components. Defatted MFGM could translate as a valuable infant formula component to inhibit the colonisation of certain pathogens in the developing infant. The positive effect of MFGM on gut epithelial cells means that it may be a promising and valuable functional food component for promoting gut health.

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

Not applicable as it is too early to see impacts such as these yet.

4 (b) **Summary of Research Outputs**

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


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


(iii) National Report
(iv) Workshops/seminars at which results were presented


(v) Intellectual Property applications/licences/patents

None

(vi) Other

Both Prof. Joshi and Dr. Hickey were members of the Food for Health Ireland (FHI) 2 research consortium funded by Enterprise Ireland which includes research labs and industrial partners. Dr. Hickey has made regular reports to FHI2 which have included updates of other funded projects including this project. Relevant stakeholders who attend these meetings include the dairy and infant formula industries (Glanbia, Kerry Group, Ornua, Dairygold and Carbery), infant nutrition and dairy research communities (UCC, UCD, NUIM, UL and DCU) and the state agency Enterprise Ireland.


https://twitter.com/seankynetd/status/716967748534927360

Interviewed by TippFM (Jim Finn) for the Weekend show on the role of milk glycans in human health (March 2014)

5. Scientists trained by Project

Total Number of PhD theses: 1

Sarah Ross was awarded her PhD (September 30th 2016):
Ross, S. (2016). Targeting the glycome of the milk fat globule membrane for anti-infective properties. The National University of Ireland, Galway, the Degree of Doctor of Philosophy

Total Number of Masters theses: 0

6. Permanent Researchers

<table>
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<th>Institution Name</th>
<th>Number of Permanent staff</th>
<th>Total Time contribution (person)</th>
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<tr>
<th>Type of Researcher</th>
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<th>Total Time contribution (person years)</th>
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<td>PhD students</td>
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<td>Masters students</td>
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<tr>
<td>Temporary researchers</td>
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<tr>
<td>Other</td>
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<tr>
<td><strong>Total</strong></td>
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**7. Researchers Funded by DAFM**

**8. Involvement in Agri Food Graduate Development Programme**

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<tr>
<th>Name of Postgraduate / contract researcher</th>
<th>Names and Dates of modules attended</th>
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<tr>
<td>Sarah Ross*</td>
<td>&quot;Career Development for Food Researchers&quot; November, 2014</td>
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*Please note Sarah Ross had an exemption from NUIG regarding credits as she was based so far from the university

**9. Project Expenditure**

- Total expenditure of the project: €275,413.87
- Total Award by DAFM: €273,176.70
- Other sources of funding including benefit in kind and/or cash contribution (specify): €0

Breakdown of Total Expenditure
10. **Leveraging**

Not applicable to date.

11. **Future Strategies**

We are continuing the work on MFGM at Teagasc and are preparing 2 further manuscripts for submission to peer reviewed journals.