

Project number: 5552
Funding source: DAFF (05/R&D/TD/370)

Date: March, 2012
Project dates: Oct 2008 – Mar 2009

Characterisation and enrichment of “buttermilk” fat globule membrane (MFGM) composition using novel technologies



Key external stakeholders:

Dairy processors, butter manufacturers, ingredient innovators, food and health researchers

Practical implications for stakeholders:

This project has demonstrated that the milk fat globule membrane (MFGM) residue contained within buttermilk possesses biological activity offers potential for greater commercial exploitation and adding value. A key implication for dairy producers and processors is a realisation that buttermilk as a by-product of buttermaking is presently under-utilised through processing into a relatively low-value commodity buttermilk powder.

- Expertise and analytical capability were developed, in relation to bioscience aspects and technological features of MFGM, which is key to understanding the fate of MFGM proteins and phospholipids during processing.
- Specific analytical capabilities developed during the project were made available to interested dairy processors thereafter in order to enable them characterise the composition of buttermilk and MFGM fractions generated by their processes. This, in turn, led to international food and nutritional company reaction e.g. expressions of interest on the part of infant milk formula manufacturers.

Main results:

- Analytical techniques were established which enabled for the first time the fate of MFGM proteins and phospholipids to be tracked during processing simulations performed on freshly-produced milk.
- MFGM proteins are partitioned mainly into buttermilk during cream churning, some of these proteins were also detected in the resulting butter. All major MFGM phospholipids, i.e. PE (phosphatidylethanolamine), PI (phosphatidylinositol), PC (phosphatidylcholine), PS (phosphatidylserine), SM (sphingomyelin), as well as high quantities of LC (lactosylceramide) were detected in the various sample streams irrespective of mechanical action and/or heat treatment of cream prior to processing.
- Significant anti-cancer effects were detected in the various buttermilk fractions produced experimentally.

Opportunity / Benefit:

Follow-on research is necessary to elaborate our scientific understanding of MFGM and document further biological evidence to support health benefit claims but the expertise developed from this project would be key to such commercially focused research and possible links with industry.

Collaborating Institutions:

Dublin City University

Teagasc project team: Dr. Phil Kelly (PI)
Dr. Brian Murray,
Dr. Catherine Stanton

External collaborators: Dr. Rosaleen Devery, DCU as Walsh Fellowship (A. Kuchta) co-supervisor

1. Project background:

The project aimed to explore a commercially, undeveloped component of milk, viz. the naturally occurring emulsifying layer around milk fat i.e. milk fat globule membrane (MFGM), known to be prevalent in a traditional milk beverage, *sour buttermilk*, that no longer enjoys popular appeal with consumers. MFGM is increasingly recognized to possess important biological activities. The composition and structure of MFGM is believed to take part in the body's defense mechanism when milk is ingested by the young of both bovine and human. In fact, for the most part, modern dairy processing destroys the MFGM in liquid milks through use of homogenization for stabilization of the creaming effect. While satisfactory from a nutritional point of view, the ramifications of such disruption in biofunctional terms is not at all understood. Furthermore, many of the protein constituents of MFGM (especially butyrophilin, xanthine oxidase) are extremely heat sensitive and are readily destroyed by relatively mild heat treatment.

2. Questions addressed by the project:

1. Examine the composition and characteristics of milk fat globule membrane (MFGM) contained in traditional buttermilk beverage by tracking the influences of different processes and its partitioning into various products streams (buttermilk; residual lipid content of skimmed milk and whey; fractions extracted from anhydrous milk fat production, and butter)
2. Does lactic fermentation (e.g. traditional sour cream buttermaking) influence buttermilk MFGM composition?
3. Is MFGM derived from the above sources bioavailable according to *in vitro* bioassays? especially to see if differences arise between raw and heated milk sources, and any other technological changes induced by routine dairy processes?
4. Can membrane-based separation technologies be used to enrich MFGM under both neutral and acidic conditions?
5. Is it possible to reformulate buttermilk-based beverages (acidic and neutral formats) in order to enhance their appeal to suit modern tastes?

3. The experimental studies:

The experimental design incorporated elements of artisanal practice in the collection of milk, gravity-based separation of cream and subsequent butter churning. Since no heat treatments were involved in any of all these traditionally-practiced steps, it was opportune to examine the impact of such 'mild' processing on changes to MFGM components and functionality, and go some way to addressing the question i.e. did our forbears who were reputed for their high consumption of traditional buttermilk actually enjoy both nutritional and functional benefits of this artisanally-produced dairy product? Thus, the following experimental conditions were studied (i) gravity cream separation versus centrifuge acceleration of creaming, (ii) heat treatments simulating forewarming for centrifugal separation and cream pasteurization, (iii) fermentation of milks, creams and buttermilk under controlled (using lactic acid bacteria) and 'wild' conditions, and (iv) use of cream washing to determine the extent to which MFGM components may be removed as well as processing-induced attachment of other milk components to MFGM

Experimentally-produced buttermilk samples and their fractions produced above were also subjected to *in vitro* anticancer screening against SW480 human colon cancer cells

Since traditional buttermilk-based beverage consumption gradually became less appealing in sensory terms over the years as consumers tastes changed, the project also addressed new formulation approaches using modern beverage formats which were adapted to incorporate the positive attributes of buttermilk MFGM.

4. Main results:

Analytical techniques were established which enabled for the first time the fate of MFGM proteins and phospholipids to be tracked during processing simulations performed on freshly-produced milk. It was observed that the migration of serum casein and whey proteins into cream during separation was independent of the separation method used (gravity at ambient temperature v. centrifugal at 50°C) and

resulted in little difference in their respective contents in butters and buttermilks subsequently produced. Washing of the cream prior to butter production removed some but not all of the casein and whey proteins present. In addition, heat treatment of milk prior to cream separation and/or cream also increased the incorporation of casein and whey in cream, buttermilk and/or butter.

While MFGM proteins are partitioned mainly into buttermilk during cream churning, some of these proteins were also detected in the resulting butter. All major phospholipids, i.e. PE (phosphatidylethanolamine), PI (phosphatidylinositol), PC (phosphatidylcholine), PS (phosphatidylserine), SM (sphingomyelin), as well as high quantities of LC (lactosylceramide) were detected in all samples irrespective of mechanical action and/or heat treatment of cream prior to processing.

At the outset, buttermilk and fermentation would have been seen as synonymous since buttermilk would have evolved over the years as a beverage from milk/cream that might have soured naturally during prolonged storage. However, during monitoring of antimicrobial activity, natural raw buttermilk possessed activity against gram + *E. coli* and this increased upon heat treatment of buttermilk. Prior cream washing, however, decreased antimicrobial activity of buttermilk against *E. coli* which suggested that some bioactive components may have been lost in the process. A most surprising finding was that in all cases lactic acid fermentation decreased the antimicrobial activity of buttermilks.

Using microfiltration (MF) as a means of enriching MFGM concentration, it was found that the antimicrobial activity of natural and washed buttermilks was retained and concentrated in the retentate fraction during processing using either 0.8 μm or 1.4 μm MF membranes. Some antimicrobial activity was also present in the permeate fraction following processing of natural cream buttermilks in contrast to washed cream buttermilks which did not.

Significant anti-cancer effects were found in the various buttermilk fractions produced experimentally. Sphingolipids in a pure form: ceramide, sphingosine, lactosylceramide and sphingomyelin showed high anticancer activity against SW480 human colon cancer cells which suggest that these individual MFGM phospholipids are most likely responsible for the bioactivity. Experimentally-prepared sweet (unfermented) buttermilks as well as their MF retentates and permeates inhibited growth of SW480 human colon cancer cells while possessing no toxic effect on FHC normal human colon cells. Permeate fractions from commercial cream buttermilk (washed and unwashed) had lower cytotoxic activity than feed and retentate fractions. Washed samples, resulting in greater MFGM concentration showed higher anti-cancer activity than unwashed samples (feed samples). Cream pasteurisation didn't influence cytotoxic activity of resulting buttermilk. Decreased anticancer activity was also observed in the buttermilks after fermentation with different strains - the exception being 2 lactic cultures which diminished bioactivity to a lesser degree.

Freeze - and spray - drying had a significant influence on anticancer activity of buttermilk – the biological effect was effectively lost as a result of spray drying.

5. Opportunity/Benefit:

During the course of the project, the analytical capabilities developed have been made available to interested dairy processors in order to enable them characterise the composition of buttermilk and MFGM fractions generated by their processes. This would not have been possible prior to this project as no such capability was in place nationally. We have explored with others the potential for recovery and analytical characterisation of residual MFGM material in whey arising from cheesemaking.

Based on the projects findings, the recommendation to industry is to avoid fermentation as an optional processing route when pursuing different beverage formats based on buttermilk or MFGM-enriched extracts.

The most significant industry-impact in the short-term was the incorporation of the project's findings into the workings of the industry-led, Enterprise Ireland-funded Food for Health Ireland (FHI) project – Work Package (WP) 5 – Early infant nutrition.

A consumer focus of the project was to explore beverage formats using either traditional buttermilk or buttermilk-derived MFGM-enriched ingredients as a basis for supplementation of conventional beverages. Taking on board the apparent reluctance on the part of today's consumers to drink buttermilk in its natural form, we have been able formulate fruit-flavoured variants of buttermilk which are quite appealing and have a lighter consistency than, for example, a yoghurt-based fermented milk drink. In the short term, the aim to

generate bioactive MFGM-rich ingredients for use as ingredients in modern beverage formats was affected for some unknown reason by loss of bioactivity encountered during spray drying

Ms. Anna Kuchta (Walsh Fellowship, PhD student) was the recipient of the Dublin City University Orla Benson 2009 Award. This award enables young researchers to visit research laboratories to learn new techniques. Anna Kuchta chose to travel to Prof. G. Caderni's cancer research laboratory at University of Florence, Italy in order to become acquainted with test facilities and use of small animal model for anti-cancer evaluation.

6. Dissemination:

Dissemination was implemented using all regular channels of research communication e.g. peer-reviewed and popular, technical publications, participation in symposia and seminars, RELAY-organised workshops and technical newsbrief issued to industry, and individual dairy company engagement.

Main publications:

Kuchta, A.M., Kelly, P.M., Stanton, C. & Devery, R. A. (2012) 'Milk fat globule membrane – a source of polar lipids for colon health? A review' *International Journal of Dairy Technology* DOI: 10.1111/j.1471-0307.2011.00759.x

Kuchta, A.M. (2010) PhD thesis 'Investigation of the health benefits of Buttermilk fat globule membrane lipid components', *submitted to Dublin City University*, November 2010.

Kuchta, A. M., Devery, R., Murray, B., Stanton, C, Kelly, P. M. (2010) 'Analysis of Possible Pathways Involved in Buttermilk's Antiproliferative Activity in *in vitro* Studies. Effect of sweet and fermented buttermilk samples on growth of SW480 human colon cancer cells and FHC human normal colon cells'. *Nutrition and Cancer (submitted)*

Popular publications:

Kelly, P.M., & Murray, B.M. (2009) 'Buttermilk – a 'health blast' from the past!' *TResearch* 4, 14-16.

7. Compiled by: Dr Phil Kelly and Dr. Brian Murray
