Food
Programme

Teagasc Technology Updates

A collection of technology updates highlighting the findings and key technologies from food research projects within Teagasc

Teagasc
Agriculture and Food Development Authority

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Compiled by Jane Kavanagh and Ciarán Arshad
For more Technology Updates see www.teagasc.ie/publications

Professor Gerry Boyle
Director, Teagasc

Foreword

As a leading organisation in the fields of agriculture and food research in Ireland, Teagasc seeks to achieve industry impact from its research.

We are committed to transferring the latest discoveries from our portfolio of over 350 research projects to end users to ensure that our research has an impact and delivers a benefit to our stakeholders and the Irish economy. As part of this effort, we publish Technology Updates in respect of all completed research projects. These reports are designed to ensure transfer of new research information to the advisory and training services and to the end-user, and to provide an easily accessible record of the main research findings. I am delighted at the publication of this collection of Technology Updates from our Food Programme. They present many exciting results that can assist the food industry to innovate their processes and products to deliver greater profitability, new high value products and enhanced safety and traceability along the food chain.

The research leading to these results was generally conducted in collaboration with other research institutes, mainly Irish universities and Institutes of Technology, and we acknowledge and hugely value those collaborations. Many other external bodies or food companies were instrumental in conducting this research and achieving these results, and we again greatly value those contributions.

Research requires investment and Teagasc uses external funding to supplement its Grant-in-aid, which provides the majority of funding for its research. In particular, funding from the FIRM programme run by the Department of Agriculture, Food and the Marine, EU programmes such as the various Framework Programmes, the Marine Institute, Science Foundation Ireland and Enterprise Ireland was vital for the conduct of these research projects.

Teagasc is extremely fortunate to have a dedicated workforce, including the research, technical, administrative and general support staff, who carried out these research projects. In particular, I would like to highlight the contribution of funded contact staff (research, technical and post-doctoral fellows) and Walsh Fellows. They are essential to our research programme, and their presence brings a vitality and dynamism to our research centres that is invaluable.

I would also like to thank the many staff who assisted in the writing, review and collation of this collection of Technology Updates, as well as those staff who provided the design, layout, format and inspiration for an easy to read report on research project outcomes.

Professor Gerry Boyle
Director, Teagasc

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Teagasc Food Programme Mission

To foster science-based innovation for the Agri-Food sector in a way that will lead to economic development and profitability through careful and targeted planning with a clear focus on the needs of the food industry.

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Teagasc Vision for Food

Stimulating and catalyzing innovation in the Irish Food Sector through research, development and impact

The Teagasc Food Programme provides a scientific platform to support food innovation in large, medium and small enterprises. It comprises of a world class competitive research programme that is carried out in state of the art laboratories and pilot plant facilities by our highly experienced food scientists and technologists. In total it delivers a unique technology and business development centre across the country. We work with many strategic partners in Ireland and abroad ensuring that our programme is relevant, has high impact and has scientific excellence. The programme is sufficiently targeted and applied to generate new opportunities for the Irish food industry based on a combined culture of innovation and excellence in food research.

Importantly, this programme comprises research areas which must deliver an economic and social dividend to the food industry in both the short and long term. The Food Programme therefore focuses on areas such as food processing, food for health and food sustainability across our major food sectors such as meat, dairy, seafood, prepared consumer foods and food ingredients. In addition the programme supports the novel technologies for the production of safe and nutritious food by indigenous food companies.

We operate from two main campuses in Dublin (Ashtown) and Cork (Moorepark Fermoy). Both sites have state of the art food pilot plant and incubation facilities. We engage with major multi-national companies, SMEs and high potential start-ups in many different ways. These 'Gateways' range from research agreements, contract research services, rental of facilities and the Enterprise Ireland research and development vouchers.

These new Technology Updates from our Food Programme aim to stimulate dialogue and action between food companies (and stakeholders) and our key food researchers at our centres which will lead to increasing innovation in our important food sector. The key contact details are provided in each up-date. Do not hesitate to contact our researchers (or myself) to discuss your innovation needs, our research findings and opportunities to innovate and collaborate together.

Food Biosciences

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Introduction

The core objective of the Food Biosciences Department is to engage in advanced research and technology development in support of the Irish Agri-Food industry sector. Activities are organised into three research areas: an extensive Food for Health sub-programme; a Cheese Microbiology and Biochemistry sub-programme and a Milk and Product Quality sub-programme.

The Food Biosciences Department is a partner in the Alimentary Pharmabiotic Research Centre (www.ucc.ie/research/apc), Food for Health Ireland (www.fhi.ie), NutraMara (www.nutramara.ie) Eldermet (http://eldermet.ucc.ie), and the Irish Phytochemical Food Network (www.ipfn.ie).

The objectives are:

- To extract and/or modify food components and provide bio-functional molecules, as food solutions to address key societal diet related health concerns including gut health, cardiovascular disease, obesity, diabetes and infant nutrition.
- To exploit micro-organisms, microbial metabolites and bacteriophage as agents to control deleterious or pathogenic organisms in food systems or the gastrointestinal tract.
- To focus on the application of micro-organisms and their enzymes to impact on the sensory, textural, techno-functional properties and health benefits of a range of foods, with particular emphasis on cheese.

Food for Health: Research on the key societal diet related health concerns focuses on:

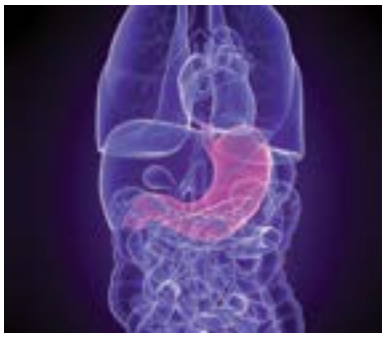
- Extraction and isolation of bioactive components from a range of food grade biological sources (milk, meat and marine origin).
- Extraction of phytochemicals from fruits, vegetables and cereals.
- Application of high throughput bioassays to screen for putative bioactive compounds.
- Identification and structural characterisation of bioactives.
- Elucidation of the physiological mechanism(s) of action of bioactive molecules.
- Assessment of bioavailability.
- Understanding the relationship between gut microflora and health status, and the potential of food to programme a healthy gut microflora.
- Biocontrol agents (bacteriocins and bacteriophage) for spoilage/pathogen control.

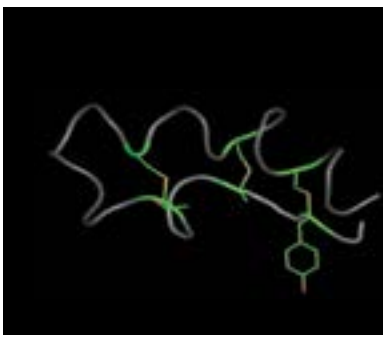
Cultures and flavour analysis: Research focuses on the:

- Application of micro-organisms and enzymes to the manufacture and ripening of cheese.
- Selection, identification and production of novel starter cultures of commercial interest.
- Investigating approaches to control and modulate the key sensory properties of cheese.
- Demonstration of the natural health benefits of cheese and application of novel approaches to enhance such benefits.

Milk Quality: While milk produced in Ireland is highly regarded there is an ongoing need to ensure that quality standards are maintained. Milk quality research is a cross departmental activity in the Food Programme and performed in association with AGRIP and focuses on:

- The microbial quality of raw milk.
- The growth of spore forming bacteria in milk processing streams.





Food Chemistry and Technology

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Introduction

The Food Chemistry & Technology Department is located at both the Teagasc Food Research Centres, with dairy science carried out primarily in Teagasc Moorepark and meat and cereals research at Teagasc Ashtown.

Dairy research focuses on cheese, infant formula and dairy-based ingredients. Meat research focuses on quality, whole chain management and recovering value from meat processing streams. Cereals research is focused on product quality and innovation in the bakery industry.

The objectives are:

- To understand proteins, minerals and other ingredient interactions in food systems.
- To understand the impact of ingredient composition, processing and storage conditions on micro- and macro-structural properties, and how this impacts the quality of dairy, meat and cereal products.
- To facilitate product modification and process optimisation through intelligent process design.
- To extract value from meat processing waste streams and by-products.
- To add value to an expanding milk pool.

Ingredient development and Infant formula/ nutritional beverage research focuses on:

- The design of 'SMART' protein based ingredients in dehydrated form suitable for export *via* understanding of protein chemistry and the effects of thermal processing.
- Investigating the control of protein aggregation, mainly by understanding self aggregation of whey proteins and interaction with casein.
- Studying the interactions of proteins, carbohydrates and minerals in concentrated colloidal systems through optimisation of formulation and processing conditions.

Cheese research focuses on:

- Developing non-cheddar table cheeses and cheeses/cheese ingredient products for food service.
- Investigation of structure/function relationships and physical properties (e.g. macrostructure, rheology, functionality, opacity) by controlling protein-protein interactions, protein-mineral interactions, and product structure *via* alteration of milk composition, milk treatments, process modifications, type/level of added ingredients, and/or storage conditions.
- The development of 'SMART' processes for cheesemaking.
- Cheesemaking efficiency/component recovery and influence of stage of lactation and manufacturing process.

Meat research focuses on:

- Meat quality from a whole-chain meat management perspective.
- The application of genomic, proteomic, metabolomic and imaging technologies to elucidate underlying post-mortem molecular mechanisms and structural characteristics involved in regulating meat quality and the identification of biological markers of quality.
- Investigating food matrix properties, for the generation of targeted food systems which deliver consistency in product quality.
- Identifying physico-chemical parameters which influence sensory performance and technological functionality of processed meats.
- Strategies for recovery of value through generation of higher value functional products from the meat processing chain.

Cereals research focuses on:

- The development and transfer of enabling science and technology to support the bakery industry's needs and requirements for product quality and innovation.
- Fundamental and applied research on the links between food/ingredient structure and functionality.

Food Safety

Introduction

The safety and the integrity of food is fundamental to the sustainability and continued development of the Irish Agri-food sector and a 'risk based total chain approach' to food safety management is essential to reduce level of food borne illness.

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The objective of the Food Safety programme is to provide the science to underpin a total chain risk based approach to food safety focusing on microbial and chemical contaminants in the "farm to fork" food chain.

The objectives are:

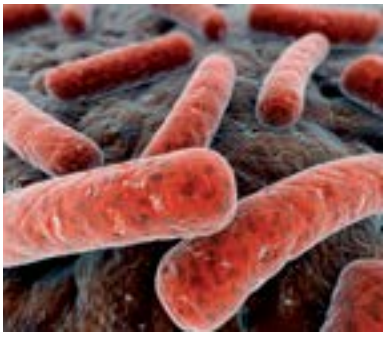
- To understand the transmission, behaviour, virulence potential of key and emergent microbial pathogens and to a lesser extent, spoilage organisms in Irish food.
- To develop predictive microbial models and quantitative risk models which can be used to assess and manage microbial risk.
- To develop novel controls for microbial pathogens and spoilage organisms.
- To develop state of art methods to detect and monitor for chemical contaminants including residues from veterinary drugs, environmental contaminants and natural toxins in the food supply.
- To develop quantitative risk models and exposure models for key chemical contaminants in the Irish food supply.

The Microbiology Programme addresses key food bacterial pathogens and specific food spoilage issues along the complete "farm to fork" chain with the main focus on zoonotic pathogens. The research areas addressed include:

- Pathogen transmission and tracking using molecular epidemiological tools.
- Pathogen behaviour and survival in the food chain including adaptation to stresses and resistance to antibiotics and biocides.
- Pathogenicity, virulence and its molecular basis.
- Development of predictive modelling and quantitative risk assessment models.
- Development of novel interventions, strategies, particularly biocontrols.
- Specific issues related to microbial spoilage (packaged meat) and quality (milk) that are of concern to the Irish food industry.
- Some of the main issues being addressed are verocytoxigenic *E. coli* in meat and dairy sectors; *Salmonella* in pigs and pork, *Listeria* monocytogenes in dairy and ready to eat foods, *Campylobacter* in poultry, and pathogen resistance to antibiotics and biocides.

Chemical contaminants: The research on chemical contaminants focuses in particular on veterinary drug residues, and includes research on environmental contaminants and natural toxins in the food supply. The research includes:

- Development of state of art methods to detect chemical contaminants using mass spectroscopy and biosensor technologies.
- Investigation of the fate of residues during processing.
- Application of the technologies as part of the national residue monitoring plan.
- The data and technologies underpin a quantitative risk based approach to the control and management of chemical residues in the food.



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Food Industry Development

Introduction

Irish food businesses face constant challenges to be competitive, sustain existing and new markets and comply with a demanding regulatory environment. Teagasc, working in conjunction with other national development agencies, provides a comprehensive support service for the food processing industry with a particular focus on supporting Small and Medium sized Enterprises (SME) and start up food businesses.

The objectives are:

- To provide technology development and problem solving supports for the food SME sector, food start up businesses and related stakeholders, through specialist technical training courses and seminars, company specific consultancy, product development and testing, and a technical information service.
- To support research knowledge and technology transfer to industry and other stakeholders, through specific research dissemination activities, and interactions with industry in training, consultancy, product development and technical information.

Technical Training Courses and Seminars

- Specialist technical training courses and seminars are provided in key areas of emerging technologies, legislation, and outputs from the food research programmes. Topics encompass food safety, quality management systems, food processing, ingredient and packaging innovations.

Product Development Supports

- This includes technical advice, access to modern food processing plant and product testing in microbiological, chemical, physical and sensory analysis.

Consultancy, Food Assurance Standards

- Provides support, usually in-company, in the development, implementation and maintenance of industry and regulatory food assurance standards.

Pilot Plant facilities

- A wide range of modern food processing facilities and equipment are available to food businesses at both Teagasc Ashtown and Teagasc Moorepark. An ultra modern pilot plant, Moorepark Technology Ltd., containing the most up-to-date and versatile pilot scale processing equipment exists on the Moorepark campus. MTL has a wide range of capabilities in general food and food ingredient development and is arranged in a modular structure of self-contained processing areas which guarantees single client access and total confidentiality.
- At Teagasc Ashtown the Meat Industry Development Unit encompasses a pilot scale meat facility incorporating a licensed abattoir, production units for processing and packaging of meat under controlled refrigeration systems and a cooked meats facility.

Technical Information Service

- This service is provided for food businesses relating to problem solving or requests for information on new product development, commercial or regulatory requirements for food production.

Food Market Research Unit

- This unit located on the Teagasc Ashtown campus is part of the Teagasc Rural Economy and Development Programme. In addition to undertaking strategic research to understand key drivers of consumer behaviour, market developments and innovation processes, it underpins the activities of the Food Programme. Specifically, it collaborates on many projects within the Food Programme to ensure that technical outputs are market-oriented and developed based on an understanding of consumer requirements.

Food Technology and Knowledge Transfer

Introduction

Innovation is one of the keys to accelerating economic recovery. The Teagasc Technology and Knowledge Transfer Strategy underpins the implementation of a systematic, effective and flexible technology transfer process which promotes science based innovation in the Agri-Food Sector.

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A 'culture' of technology transfer and an environment in which it can flourish exists within the Food Research Programme. Teagasc food researchers demonstrate leadership, flexibility, entrepreneurial and other skills in order to deliver an effective technology transfer function in their area. The newly established Teagasc Technology Transfer Office assists researchers and their teams to build collaborations with industry.

Technology and Knowledge Transfer mechanisms take many forms within the Food Programme and include industry collaborations, contract research, services to large public/private contractors, agreements or licensing, acquisition of technologies, in addition to commercial service arrangements (incorporating training courses, consultancy provision and other services).

Central to these mechanisms are mutual trust, credibility, confidentiality, clear business objectives and high quality customer relationship management. Teagasc values its extensive industry interactions and has stringent polices regarding IP management, contract agreements and confidentiality.

The objectives are:

- To develop best practice in technology transfer.
- To develop a Technology Marketing Portfolio which ensures our technologies, capabilities and expertise are widely accessible (at www.teagasc.ie) and describes:
  - *Technology Offers* that can be marketed for exploitation by potential users.
  - *Technology Updates* which summarise the main findings of Teagasc research projects and describe technologies which can be further developed in partnership with external clients.
  - *Specialised Services* which are offered from the Ashtown and Moorepark sites.
  - *Technology Expertise* which gives an overview of our advanced technical equipment and pilot plant facilities.
  - *Experts* which profiles our key contact research and development staff.
- To foster key collaborations and strategic partnerships with industry and state agencies by proactively engaging with external stakeholders to align our technologies and capabilities to solution focused programmes and projects.

Our TTO staff are ready to assist in facilitating, enhancing and supporting the transfer of IP resources and information to the food industry and other stakeholders.

For further information view our Food Portfolio at http://www.teagasc.ie/research/collaboration/

Food Bioscience

Year	Author	Title	RMIS No.	Page
2014	Cotter, P	Controlling obesity-associated gut microbes	5971	12
2014	Rai, D.K	Anti-oxidant and anti-microbial compounds from dandelion root, fenugreek and bitter melon	6038	15
2014	Rai, D.K	Potato peels: a rich source of pharmaceuticals and bioactives	5961	18
2013	Beresford, T	Mining for milk based bio-actives using microbial fermentations	5939	21
2013	Beresford, T	Novel Strategies for Optimization of Cheddar Cheese Manufacturing Process	5952	24
2013	Giblin, L	Food Solutions for Weight Management	5942	28
2013	Kilcawley, K	Natural Ingredient Cheese Solutions	5938	31
2013	Rea, M	Culture Collections in Teagasc Food Research Centre Moorepark	6312	34
2013	Ross, R.P	Probiotic lactobacilli survival and impact in the animal gut	5972	36
2013	Ross, R.P	Alimentary Pharmabiotic Centre: Microbe/microbe interactions in the gastrointestinal tract	5271	39
2013	Stanton, C	APC: production of microbial metabolites by gut bacteria	5274	43
2011	Stanton, C	Impact of exogenous factors in the development of allergy in infants: (EFRAIM)	5858	47
2010	Stanton, C	Health promoting bioactives from cider yeast	5932	51
2008	Ross, R.P	The milk proteome: a tool for understanding milk quality and functionality	5550	54
2007	Ross, R.P	Genetic Tools for Improvement of Food Cultures	5027	56
2006	Kilcawley, K	Development of a highly functional cheese sauce	5115	59

Food Chemistry and Technology

Year	Author	Title	RMIS No.	Page
2014	Fenelon, M	Re-engineering process technology for the manufacture of infant formula	5949	64
2014	Fenelon, M	In-situ starch modification in food formulations using protein	5950	67
2014	Hamill, R	A food matrix approach to meat product development	5957	70
2013	Allen, P	Accelerated meat curing using Ultrasound and Pulsed Electric Fields	5962	74
2013	Brodkorb, A	Bioactive dairy protein complexes – *in vitro* and *in vivo* digestion	5947	77
2013	Fenelon, M	Bio-sensitives advanced stabilisation	5953	80
2013	Guinee, T.P	Design and development of Realistic food Models with well-characterized micro- and macro-structure and composition (DREAM)	5983	83
2013	Kelly, P.M	Water activity control and texture stabilisation of high protein snack bars	5951	88
2013	Kelly, P.M	Pre-commercial scale-up of biologically active milk protein hydrolysates (FHI Project WP3)	5940	92
2012	Kelly, P.M	Improved whey permeate drying using high pressure gas/liquid dosing during spray atomisation	5986	96
2011	Allen, P	Predicting beef eating quality	5718	99
2011	Guinee, T.P	Technology for healthier pork products	5979	103
2010	Auty, M.A.E	Updating Cheesemaking Efficiency	5607	107
2008	Allen, P	Properties of nano-fibrillar whey proteins	5418	111
2008	Kelly, P.M	Technological advances in spray drying of functional ingredients for automated beverage vending	5435	114

Food Safety

Year	Author	Title	RMIS No.	Page
2014	Danaher, M	Detection of Endocrine Disrupting Agents in Milk	6141	120
2013	Danaher, M	Safe and Healthy Foods	5856	123
2013	Duffy, G	BASELINE: Risk targets in milk and dairy products	5994	127
2012	Burgess, K	Biocide tolerance in foodborne pathogens	5954	130
2012	Duffy, G	*ProSafeBeef*: Assessment of microbiological and chemical safety of beef	5705	134
2012	Duffy, G	Genomics of gram negative food poisoning bacteria of animal origin	5854	138
2012	Duffy, G	Risk Assessment Network of Ireland	5855	141
2011	Jordan, K	Improved bio-traceability of unintended micro-organisms and their substances in food and feed chains	5691	144
2009	Jordan, K	Detection and surveillance of *Enterobacter sakazakii* (*Cronobacter* spp.) along the infant formula food chain	5561	148

Technology Updates

Food Bioscience

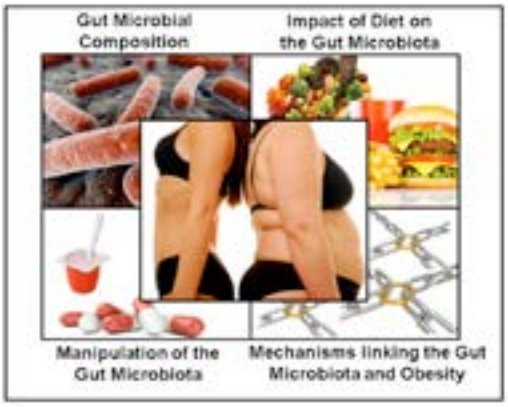
Project number:
5971

Date:
June, 2014

Funding source:
Teagasc

Project dates:
March 2009-Apr 2014

Controlling obesity-associated gut microbes



Collaborating Institutions:
University College Cork
Alimentary Pharmabiotic Centre

Teagasc project team:
Dr. Paul Cotter (PI)
Prof. Paul Ross

External collaborators:
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Prof. Fergus Shanahan, University College Cork, Alimentary Pharmabiotic Centre
Dr. Eileen Murphy, University College Cork, Alimentary Pharmabiotic Centre

Compiled by:
Paul Cotter

Key external stakeholders:

Irish food industry, Irish consumers.

Practical implications for stakeholders:

The microbes in our gut (gut microbes) can contribute to weight gain. There is an opportunity to develop new weight management strategies by altering these microbial populations in a beneficial way. Through the course of this research we have provided evidence that antimicrobial-producing probiotics, whey protein and exercise have the potential to bring about such beneficial changes.

Main results:

Through a variety of animal and human studies, we have investigated the obesity associated gut microbiota and have employed a variety of approaches with a view to changing this population in a beneficial way.

- We have established that a bacteriocin (antimicrobial) producing probiotic (*Lactobacillus salivarius* UCC118) more considerably alters the gut microbiota than a non-bacteriocin producing equivalent and that the changes induced bring about a short-term reduction in weight gain.
- We have revealed a desirable, high microbial diversity in the gut of elite athletes and have revealed a correlation between this high diversity with exercise and protein consumption, respectively.

Opportunity/Benefit:

As a consequence of our studies, the potential benefits of employing bacteriocin-producing probiotics, protein and exercise to modulate the gut microbiota in a beneficial way have been highlighted. Further research will focus on optimizing the use of these intervention strategies (individually and in combination) to control obesity associated gut microbes.

1. Project background:

There are now several emerging lines of evidence to suggest that the gut microbiota has a role in energy and metabolic homeostasis. Recent evidence from animal and human studies indicates that the composition of the gut microbiota may be involved in the development of obesity. Other studies highlight the role of the gut microbiota in the regulation of energy homeostasis, insulin resistance, non-alcoholic fatty liver disease and energy, lipid and amino acid metabolism. These findings highlight the opportunity for new research to examine the ability of selected bioactives and interventions to modulate the composition of the gut microbiota in a manner that may contribute to the prevention of obesity and obesity-related conditions.

2. Questions addressed by the project:

To what extent can (bacteriocin producing) probiotics, diet and exercise alter the obesity-associated gut microbiota in a beneficial way?

3. The experimental studies:

Study 1: Increased efficiency of energy harvest, due to alterations in the gut microbiota (increased Firmicutes and decreased Bacteroidetes), has been implicated in obesity in mice and humans. However, a causal relationship is unproven and contributory variables include diet, genetics and age. Therefore, we explored both diet-induced obesity (DIO) and genetically-determined obesity (ob/ob) for changes in microbiota and energy harvesting capacity over time. Methods: Seven-week old male ob/ob mice were fed a low-fat diet and wild-type mice were fed either a low-fat diet or a high-fat diet (DIO) for 8 weeks (n=8/ group). They were assessed at 7, 11 and 15 weeks of age for: fat and lean body mass (NMR); fecal and cecal short chain fatty acids (SCFA, gas chromatography); fecal energy content (bomb calorimetry) and microbial composition (metagenomic pyrosequencing).

Study 2: The gut microbiota is an environmental regulator of fat storage and adiposity. Whether the microbiota represents a realistic therapeutic target for improving metabolic health is unclear. This study explored two antimicrobial strategies for their impact on metabolic abnormalities in murine diet-induced obesity: oral vancomycin and a bacteriocin-producing probiotic (*Lactobacillus salivarius* UCC118 Bac(+)). Male (7-week-old) C57BL/J6 mice (9–10/group) were fed a low-fat (lean) or a high-fat diet for 20 weeks with/without vancomycin by gavage at 2 mg/day, or with *L. salivarius* UCC118Bac(+) or the bacteriocin-negative derivative *L. salivarius* UCC118Bac(-) (each at a dose of 1×10(9) cfu/day by gavage). Compositional analysis of the microbiota was by 16S rDNA amplicon pyrosequencing.

Study 3: Since extremes of exercise often accompany extremes of diet, we assessed the impact of diet and exercise on the gut microbiota by studying professional athletes from an international rugby union squad. Two groups were included to control for physical size, age and gender. Compositional analysis of the microbiota was explored by 16S rRNA amplicon pyrosequencing. Each participant completed a detailed food frequency questionnaire.

4. Main results:

Study 1: A progressive increase in Firmicutes was confirmed in both DIO and ob/ob mice reaching statistical significance in the former, but this phylum was unchanged over time in the lean controls. Reductions in Bacteroidetes were also found in ob/ob mice. However, changes in the microbiota were dissociated from markers of energy harvest. Thus, although the fecal energy in the ob/ob was significantly decreased at 7 weeks, and cecal SCFA increased, these did not persist and fecal acetate diminished over time in both ob/ob and DIO mice, but not in lean controls. Furthermore, the proportion of Firmicutes and Bacteroidetes did not correlate with energy harvest markers. Conclusion: The relationship between the microbial composition and energy harvesting capacity is more complex than previously considered. While compositional changes in the fecal microbiota were confirmed, this was primarily a feature of diet-induced rather than genetically-induced obesity. In addition, changes in the proportions of Firmicutes and Bacteroidetes were unrelated to markers of energy harvest which changed over time. The possibility of microbial adaptation to diet and time should be considered in future studies.

Study 2: Analysis of the gut microbiota showed that vancomycin treatment led to significant reductions in the proportions of Firmicutes and Bacteroidetes and a dramatic increase in Proteobacteria, with no change in Actinobacteria. Vancomycin-treated high-fat-fed mice gained less weight over the intervention period despite similar caloric intake, and had lower fasting blood glucose, plasma TNFα and triglyceride levels compared with diet-induced obese controls. The bacteriocin-producing probiotic had no significant impact on the proportions of Firmicutes but resulted in a relative increase in Bacteroidetes and Proteobacteria and a decrease in Actinobacteria compared with the non-bacteriocin-

producing control. No improvement in metabolic profiles was observed in probiotic-fed diet-induced obese mice but a short-term reduction in weight gain was apparent.

Study 3: As expected, athletes and controls differed significantly with respect to plasma creatine kinase (CK, a marker of extreme exercise), inflammatory and metabolic markers. More importantly, athletes had a higher diversity of gut microorganisms, representing 22 distinct phyla, which in turn positively correlated with protein consumption and CK. The results provide evidence for a beneficial impact of exercise on the gut microbiota diversity but also indicate that the relationship is complex and related to accompanying dietary extremes.

5. Opportunity/Benefit:

The research team has developed expertise with respect to undertaking animal and clinical studies to assess the impact of a variety of gut microbiota targeting strategies to control weight gain. Other interventions, foods, supplements etc could be investigated in a similar way and the team is available for collaboration in this area.

The specific benefits of employing probiotics, exercise and protein will be the focus of further attention and, again, the team is open to collaboration in these areas.

6. Dissemination:

The results of this project have been transferred through presentations by the associated researchers to companies and the general public. In addition, a number of peer-reviewed and popular press publications have resulted with the study involving the Irish rugby team attracting a significant amount of attention in the national and international press.

Main publications:

Clarke S.F., Murphy E.F., O'Sullivan O., Lucey A.J., Humphreys M, Hogan A., Hayes P., O'Reilly M, Jeffery I.B., Wood-Martin R., Kerins D.M., Quigley E., Ross R.P., O'Toole P.W., Molloy M.G., Falvey E., Shanahan F and Cotter, P.D. (2014) 'Exercise and associated dietary extremes impact on gut microbial diversity' Gut 2014 Published online first 09/06/2014 doi: 10.1136/gutjnl-2013–306541.

Clarke S.F., Murphy E.F., O'Sullivan O., Ross R.P., O'Toole P.W., Shanahan F. and Cotter P.D. (2013)

'Targeting the microbiota to address diet-induced obesity: a time dependent challenge' PLoS One. 8(6):e65790.

Murphy E.F., Clarke S.F., Marques T.M., Hill C., Stanton C., Ross R.P., O'Doherty R.M., Shanahan F. and Cotter P.D.(2013) 'Antimicrobials: Strategies for targeting obesity and metabolic health?' Gut Microbes 4:48–53.

Murphy E.F., Cotter P.D., Hogan A., O'Sullivan O., Joyce A., Fouhy F., Clarke S.F., Marques T.M., O'Toole P.W., Stanton C., Quigley E.M., Daly C., Ross P.R., O'Doherty R.M. and Shanahan F. (2013). 'Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity.' Gut 62:220–6.

Clarke S.F., Murphy E.F., Nilaweera K., Ross P.R., Shanahan F., O'Toole P.W. and Cotter P.D. (2012) 'The gut microbiota and its relationship to diet and obesity: new insights' Gut Microbes 3:186–202.

Murphy EF, Cotter PD, Healy S, Marques TM, O'Sullivan O, Fouhy F, Clarke SF, O'Toole PW, Quigley EM, Stanton C, Ross PR, O'Doherty RM, Shanahan F. (2010) 'Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models' Gut 59:1635–42.

Popular publications:

http://issuu.com/spin35/docs/spin_61_all/24

Cotter P.D. (2013) 'Gut microbes and obesity' TResearch Autumn 2013 p. 28.

Anti-oxidant and anti-microbial compounds from dandelion root, fenugreek and bitter melon

Project number:
6038
Date:
October, 2014
Funding source:
Teagasc
Project dates:
Oct 2009 – Jan 2014

Collaborating Institutions:
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Teagasc project team:
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Dr. Dilip Rai
Dr. Thomas Smyth
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External collaborators:
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Compiled by:
Dilip Rai

Key external stakeholders:

Vegetable growers/processors, functional food manufacturers, government authorities/legislators, consumers, food research scientists.

Practical implications for stakeholders:

The bioactive constituents in these species, in particular *Taraxacum officinale* (dandelion) roots, offer promising leads as sources of natural alternatives to synthetic food additives/preservatives.

Main results:

- The ethyl acetate extracts (1 mg/ml) of *Trigonella foenum-graecum* (fenugreek) seeds had the highest antioxidant activity (DPPH IC_{50} = 212 µg/ml) but showed no anti-microbial activity.
- The ethyl acetate extract of *Momordica charantia* (bitter melon) exhibited antimicrobial activity against *S. aureus*, MRSA and *B. cereus* strains (MIC = 62.5 – 93.8 µg/ml) while the *n*-hexane extract and a methanol-hydrophilic dialysed extract of *M. charantia* fruit demonstrated the best antioxidant activity in comparison to all other extracts from this species (DPPH IC_{50} = 575 – 648 µg/ml).
- Dandelion roots (*T. officinale*) contains 1, 5-dicaffeoylquinic acid as a major antioxidant compound while its ethyl acetate extract demonstrated the strongest antimicrobial activity against *S. aureus*, MRSA and *B. cereus* strains (MIC = 250 – 500 µg/ml).
- A number of previously unreported compounds (4-Hydroxyphenylacetic acid derivatives of inositol) were isolated from dandelion root that could have useful biological properties not under investigation here.

Opportunity/Benefit:

Dandelions are under-utilised plants and often considered as weeds. However dandelion roots were shown to have substantial anti-oxidant and anti-microbial properties. The outcomes of the project demonstrated that these under-utilised plants can serve as excellent source of natural anti-oxidant and anti-microbial agents and therefore can be potentially exploited as natural food preservatives and for nutraceutical applications.

1. Project background:

Since ancient times plants have been exploited by mankind for their associated medicinal properties. As our basic understanding of medicinal plants continued to develop so too did our expertise in successfully isolating their active components. Whilst the use of medicinal remedies gradually began to decline in favour of synthetic alternatives, the use of plants has remained strong in developing countries as an alternative medicine. Today the biological efficacies of plant derived metabolites are again receiving interest as active components in functional foods for general health and also to delay the onset of many diseases such as cancer, diabetes and inflammatory disorders. The development and refinement of biological screening strategies and analytical technologies over time has greatly enhanced the continued identification of bioactive compounds from plant origin. However, the pharmacological benefits of many traditional medicinal plants and the underlying compounds responsible for their purported efficacy remain largely unexplored or poorly understood. To this end the present study aimed to investigate the biological efficacy of three medicinal plants (*Momordica charantia*, *Trigonella foenum-graecum* and *Taraxacum officinale*) with long histories of use to treat various disorders but with little information on the compounds responsible for these properties.

2. Questions addressed by the project:

The existing literature on the antioxidant and anti-microbial activities of these three species were inconclusive. The project therefore addresses the following questions:

- Do these three species have anti-microbial and anti-oxidant activities and what level of activity do they have?
- What is the identity and chemical structure of the compound(s) responsible for the bioactivity?
- How much of these bioactive compounds are present in the respective species?

3. The experimental studies:

Crude extracts of the samples were prepared using various solvents of varying polarity. Solvent-solvent partitioning and/or molecular weight cut off filtration were employed on the crude extract fractions. Further purification was carried out using normal and reverse phase flash and preparative chromatography. All the fractions were investigated for in-vitro antioxidant activities and anti-microbial activities against a wide range of food-borne pathogens. The fractions that showed the strongest antioxidant and antimicrobial activities were further enriched using a combination of chromatographic techniques. The constituent bioactive compounds were then identified and quantified with hyphenated spectroscopic methods. The LC-NMR facility at TFRCA was particularly useful in this regard.

4. Main results:

- The ethyl acetate extracts of *T. foenum-graecum* (fenugreek) seeds at 1 mg/mL demonstrated the strongest antioxidant activity (DPPH IC_{50} = 212 µg/ml) but showed no anti-microbial activity.
- The ethyl acetate extract of *M. charantia* (bitter melon) demonstrated strongest antimicrobial activity against *S. aureus*, MRSA and *B. cereus* strains with (MIC = 62.5 – 93.8 µg/ml while the *n*-hexane extract and a methanol-hydrophilic dialysed extract of *M. charantia* (bitter melon) fruit demonstrated the best antioxidant activity in comparison to all other extracts from this species (DPPH IC_{50} = 575 – 648 µg/ml).
- Dandelion roots (*T. officinale*) contains 1, 5-dicaffeoylquinic acid as a major antioxidant compound while its ethyl acetate extract demonstrated the strongest antimicrobial activity against *S. aureus*, MRSA and *B. cereus* strains (MIC = 250 – 500 µg/ml).

- A number of previously unreported compounds (4-Hydroxyphenylacetic acid derivatives of inositol) were isolated from dandelion root that could have useful biological properties not under investigation here.

In summary, the ethyl acetate extract of *T. officinale* root has demonstrated strong antioxidant and antimicrobial properties which may warrant further investigation in food matrices as a potential functional food ingredient.

5. Opportunity/Benefit:

Dandelions are under-utilised plants and often considered as weeds. However dandelion roots were shown to have substantial anti-oxidant and anti-microbial properties. Therefore outcomes of the project demonstrated that these under-utilised plants can serve as excellent source of natural anti-oxidant and anti-microbial agents and therefore can be potentially exploited as natural food preservatives and for nutraceutical applications.

6. Dissemination:

The technology has been transferred in a number of ways, primarily through scientific A1 publications and conferences as outlined below:

Main publications:

Kenny, O., Smyth, T.J., Hewage, C.M., & Brunton, N.P. (2014). Quantification of caffeoylquinic acid derivatives in bioactivity-guided fractions of an antioxidant ethyl acetate extract from dandelion (Taraxacum officinale) root. International Journal of Food Science and Technology, DOI: 10.1111/ijfs.12668.

Kenny, O., Smyth, T.J., Walsh, D., Kelleher, C.T., Hewage, C.M., & Brunton, N.P. (2014). Investigating the potential of under-utilised plants from the Asteraceae family as a source of natural antimicrobial and antioxidant extracts. Food Chemistry, 161, 79 – 86.

Kenny, O., Smyth, T.J., Hewage, C.M., & Brunton, N.P. (2014). Antioxidant properties and quantitative UPLC-MS/MS analysis of phenolic compounds in dandelion (Taraxacum officinale) root extracts. Free Radicals and Antioxidants, 4(1), 55 – 61.

Kenny, O., Smyth, T.J., Hewage, C.M., & Brunton, N.P., McLoughlin, P. (2014). 4-hydroxyphenylacetic acid derivatives of inositol from dandelion (Taraxacum officinale) root characterised using LC-SPE-NMR and LC-MS techniques. Phytochemistry, 98, 197 – 203.

Kenny, O., Smyth, T.J., Hewage, C.M., & Brunton, N.P. (2013). Antioxidant properties and quantitative UPLC-MS analysis of phenolic compounds from extracts of fenugreek (Trigonella foenum-graecum) seeds and bitter melon (Momordica charantia) fruit. Food Chemistry, 141(4), 4295 – 4302.

Kenny, O., Smyth, T.J., McLoughlin, P., Hewage, C.M., Brunton, N.P. & Rai, D.K. (2011). Isolation and Structural Characterisation of a Bioactive compound from Bitter Melon. *Recent Advances in Synthesis and Chemical Biology X Symposium*, University College Dublin, 9th December 2011.

Popular publications:

Kenny, O., Smyth, T.J., McLoughlin, P., Walsh, D., Brunton, N.P., Rai, D.K., & Hewage, C.M. (2012). Bioactivity Guided Fractionation, Isolation and Characterisation of Plant Secondary Metabolites from Bitter Melon (*Momordica charantia*) Fruit, Fenugreek (*Trigonella foenum-graecum*) Seeds and Dandelion (*Taraxacum officinale*) Roots. *Teagasc Walsh Fellowships Book of Abstracts*, RDS Hall, Ballsbridge, Dublin 4, 22 November 2012.

Kenny, O. (2014). Investigation and characterisation of antioxidant and antibacterial compounds from bitter melon (Momordica charantia) fruit, fenugreek (Trigonella foenum-graecum) seeds and edible Asteraceae species. *PhD Thesis*.

Potato peels: a rich source of pharmaceuticals and bioactives

Project number:
5961

Date:
October, 2014

Funding source:
DAFM

Project dates:
Dec 2008 – July 2014

Collaborating Institutions:
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Largo Foods, Ashbourne, Co. Meath
Wilson's Country, Craigavon,Co. Armagh.

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Dr. Stuart Collins, University College Cork

Compiled by:
Dilip Rai

Key external stakeholders:

Potato growers, Potato processors, pharmaceuticals, functional food manufacturers, government authorities/legislators, consumers, food research scientists.

Practical implications for stakeholders:

Large volumes of potato peels as by-products are generated as a result of processing of foods. This project highlighted the potential use of this waste as a source of bio-active compounds for bio-pharmaceutical and natural bio-control agents.

Main results:

- A set of optimised methods for the extraction, isolation, purification and characterisation of glycoalkaloids was developed. Optimised large scale solid liquid extraction of glycoalkaloids from potato peels yielded ~90% pure aglycone solanidine (500 mg) following acid hydrolysis and flash chromatography separation.
- The purified aglycone glycoalkaloid, solanidine, had a high potential to synthesize novel anticancer and apoptotic drugs. As many as 29 novel compounds structurally related to glycoalkaloids had been synthesized and tested against a number of cell-line assays.
- None of the 9 different cultivars exceeded the threshold of toxicity of glycoalkaloids content of 1 mg/g. As expected, room temperature storage influenced the greater production of glycoalkaloids in peels when compared to potatoes stored at chilled temperature.
- Glycoalkaloids and potato peel extracts enriched in glycoalkaloids did not possess anticancer potential nor did they induce apoptosis nor showed cardioprotective effects. However, they demonstrated anti-inflammatory and immuno-modulatory potentials. Whilst the potato peel peptides showed anti-inflammatory, anti-hypertensive and modest anti-oxidant activities.

- Pelleted potato peels rich in glycoalkaloids contorlled the level of nematode *Globodera pallida* in conjunction with crop rotation or nematicide and more importantly the light treated pelleted peels had significantly higher 'suicide hatch' rate of potato nematodes.

Opportunity/Benefit:

Outcomes of the project will especially be of use to the potato processors as the development of methodologies for the recovery of valuable compounds from their waste stream will allow them to exploit a potentially valuable resource. This strategy also seeks to harness new technologies for use in plant sciences, food innovation and bio-pharmaceutical applications. In addition, levels of toxic glycoalkaloids in the Irish fresh potato cultivars will be determined for the safety of the consumers and also investigate the effect of commercial storage conditions of potatoes used by the processing industries. The outcomes of the project will also indirectly address the call for sustainable agriculture development as it seeks to find an environmentally safe solution for the control of potato nematodes, a major pest of potato crops, which cause significant damage and losses.

1. Project background:

Processing of potatoes involves the generation of large amounts of waste especially in the form of peel. However, the potato peel is a rich source of a group of chemical compounds known as glycoalkaloids. These compounds are known toxins to humans at high levels (>200 mg/kg), however, some evidence indicates that they may possess anti-carcinogenic activities and other health benefits when present at lower levels indicating a possible use in the phyto-pharmaceutical industry. In addition glycoalkaloids can act as a hatch agent which may be explored to control nematode infestation in potato crops. This project aims to examine the potential of potato peel waste as a source of glycoalkaloids for the control of nematodes by developing methodologies and schemes for recovery of these potentially valuable compounds. In addition, an in-depth investigation of the bio-activity and toxicity of these agents will be undertaken. After removal of glycoalkaloids the remaining waste will be tested for presence of potential bioactive peptides.

2. Questions addressed by the project:

The project addresses the following specific questions:

- Can extraction methodologies be developed to recover glycoalkaloids in sufficient amount from a potato peel by-product for bio-pharmaceuticals and examine the efficacy of glycoalkaloids enriched peels as nematode control agents?
- What are the levels of glycoalkaloids in Irish whole potato cultivars and how does the commercial storage by the potato processing industry can affect their levels?
- Could the detoxified peels (after removal of toxic glycoalkaloids) serve as source of bio-active peptides?

3. The experimental studies:

A number of extraction technologies from conventional thermal (solid-liquid, pressurized liquid) and novel non-thermal (ultrasonication, pulsed electric field and pulsed light) extraction techniques were employed. Response surface methodology assisted the optimisation of extraction of crude glycoalkaloids in high yield. Further enrichment and isolation of glycoalkaloids were achieved using a combination of flash-chromatography and preparatory chromatography. Synthetic analogues of glycoalkaloids were prepared in Prof. Anita Maguire's lab. Ammonium sulphate was employed to precipitate peel proteins which where hydrolysed using trypsin. Biological assays such as immune-modulatory and against cancer cell-lines were investigated. Identification and quantification of the glycoalkaloids and peptides were carried out on an UPLC-MS/MS systems.

4. Main results:

- A set of optimised methods for the extraction, isolation, purification and characterisation of glycoalkaloids was developed. Optimised large scale solid liquid extraction of glycoalkaloids from potato peels yielded ~90% pure aglycone solanidine (500 mg) following acid hydrolysis and flash chromatography separation.
- The purified aglycone glycoalkaloid, solanidine, had a high potential to synthesize novel anticancer and apoptotic drugs. As many as 29 novel compounds structurally related to glycoalkaloids had been synthesized and tested against a number of cell-line assays.
- None of the 9 different cultivars exceeded the threshold of toxicity of glycoalkaloids content of 1 mg/g. As expected, room temperature storage influenced the greater production of glycoalkaloids in peels when compared to potatoes stored at chilled temperature.

- Glycoalkaloids and potato peel extracts enriched in glycoalkaloids did not possess anticancer potential nor did they induce apoptosis nor showed cardioprotective effects. However, they demonstrated anti-inflammatory and immuno-modulatory potentials. Whilst the potato peel peptides showed anti-inflammatory, anti-hypertensive and modest anti-oxidant activities.
- Pelleted potato peels rich in glycoalkaloids contorlled the level of nematode *Globodera pallida* in conjunction with crop rotation or nematicide and more importantly the light treated pelleted peels had significantly higher 'suicide hatch' rate of potato nematodes.

5. Opportunity/Benefit:

Development of anti-carcinogenic and anti-inflammatory drug from a cheap and easily accessible source will be useful for pharmaceutical industries. A number of compounds synthesised in this project had shown very good anti-cancer activity with excellent IC_{50} values and could have potential in the future as possible target candidates for the pharmaceutical industry. The light treated pelleted peels have potential for acceptance by the organic certification bodies, thus opening up a new market for PCN control products.

6. Dissemination:

The technology has been transferred in a number of ways, primarily through scientific A1 publications, conferences and an industry workshop as outlined below:

Main publications:

Sinead E. Milner, Nigel P. Brunton, Peter W. Jones, Nora M. O' Brien, Stuart G. Collins and Anita R. Maguire. (2011). Bioactivities of Glycoalkaloids and their Aglycones from Solanum species. *Journal of Agriculture and Food Chemistry*, 59 (8), 3454–3484.

Kenny, O.M., Brunton, N.P., Rai, D.K., Collins, S.G., Maguire, A.R., Jones, P.W. and O'Brien, N.M. (2013). Cytotoxic and apoptotic potential of potato glycoalkaloids in a number of cancer cell lines. *Journal of Agricultural Science and Applications,* 2(4): 184–192.

Kenny, O.M., Brunton, N.P., Hossain, M.B., Rai, D.K., Collins, S.G., Maguire, A.R., Jones, P.W. and O'Brien, N.M. (2013). Anti-inflammatory properties of potato glycoalkaloids in stimulated Jurkat and Raw 264.7 macrophages. *Life Sciences,* 92(13), 775–782.

Hossain, M. B., Tiwari, B. K., Gangopadhyay, N., O'Donnell, C., Brunton, N. P. and Rai, D. K. (2014). Ultrasonic extraction of steroidal alkaloids from potato peel waste. *Ultrasonics Sonochemistry*, 21(4), 1470–1476.

Hossain, M., Aguiló-Aguayo, I, Lyng J.L, Brunton, N.P. and Rai, D.K. (2014). Effect of pulsed electric field and pulsed light pre-treatment on the extraction of steroidal alkaloids from potato peel Innovative Food Science and Emerging Technologies. *Innovative Food Science and Emerging Technologies*, *In Press*. DOI: 10.1016/j.ifset.2014.10.014.

Conference Abstracts

O'Brien, NM, O'Callaghan YC, Foley DA, McCarthy FO, Maguire AR (2010). Cytotoxic and apoptotic effects of stigmasterol oxides in U937 cells. Proceeding of the 8th Euro Fed Lipid Congress, Munich, Germany. 21–24 November 2010.

Hossain M.B., Brunton N.P., Smyth T., Rai D.K. (2012). Response surface optimization of solid-liquid extraction condition for the extraction of glycoalkaloids from potato peel. International Conference on Food safety, Quality and Nutrition: Greening the Food Industry. Manchester Metropolitan University, UK. 11–13th April 2012. Page 15.

Hossain M.B., Brunton N.P., Smyth T., Rai D.K. (2012). Effect of drying methods on the glycoalkaloids content of potato. International Conference on Food safety, Quality and Nutrition: Greening the Food Industry. Manchester Metropolitan University, UK. 11–13th April 2012. Page 90.

Hossain M.B., Tiwari B.K., O'Donnell C., Rai D.K., Brunton N.P. (2012). Extraction of glycoalkaloids from potato peel waste. IFT Conference, Las Vegas, USA.June 25-June 28, 2012.

Hossain. M.B., Aguiló-Aguayo. A., Brunton. N.P., Rai. D.K. (2013). Optimization of pressurized liquid extraction of glycoalkaloids from potato peel using response surface methodology. EUROFOODCHEM XVII, Istanbul, Turkey, May 7–10, 2013.

Industry Workshop: Waste not Want not – Recovering value from Food Waste, 7th February 2014 – A book of abstracts.

Popular publications:

Hossain. M.B. and Rai, D.K. (2014). Storage conditions and potato glycoalkaloids. *TResearch,* Summer edition, pages 16–17.

Potatoes appeal to our health' in *The Irish Times,* July 16, 2009 under Science section.

Mining for milk based bio-actives using microbial fermentations

Key external stakeholders:

Irish Dairy Industry, dairy farmers, consumers.

Practical implications for stakeholders:

Added functionality of casein, whey and milk based powders with health benefits beyond those associated with nutrition, increased profitability to the Irish milk sector.

Improved health benefits to the consumer.

Main results:

The key results were.

- Dairy associated microbes with extracellular proteolytic activity were identified.
- Fermented casein, whey and skim milk based substrates and water soluble extracts from commercial cheeses, were made into freeze-dried powders, a number of which had bioactivity across a range of health indicator assays.
- Optimized fermentation and post-fermentation heat treatments were established that retained bioactivity.

Project number:
5939

Date:
November, 2014

Funding source:
Enterprise Ireland and Industry

Project dates:
Jan 2009 – Jun 2013

Collaborating Institutions:
Dublin City University
University College Dublin
Univercity College Cork
University of Limerick
Carbery
Dairygold
Glanbia
Kerry

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UCC (Ted Dinan)
UCD (James Lyng)
UCD (Doleres O'Riordan)
UCC (David kerins)
UCD (Mike Gibney)
UCD (Philip Newsholme)
UCD (Torres Sweeney)
DCU (Christine Loscher)
Teagasc-Moorepark Food Research Centre (Phil Kelly)
Teagasc-Moorepark Food Research Centre (Linda Giblin)

Compiled by:
Paul Simpson and Tom Beresford

Opportunity/Benefit:

The range of bioactivities associated with the microbial fermented milk products will increase the functionality of milk-based ingredients, adding market value and extending the applications for the dairy industry. The development of products containing the bioactive ingredients will directly benefit public health. This project was a component of FHI, the primary objective of which was to attempt to release peptides from milk proteins that demonstrate bioactivity in the areas of interest to FHI.

1. Project background:

In 2004 Enterprise Ireland (EI) with a number of Irish dairy industry representatives established a common functional foods research strategy, FHI; the center-piece of which was to "mine" for constituents in milk that could have an impact on defined health areas.

Bioactive peptides are described as "food-derived components (genuine or generated) that in addition to their nutritional value exert a physiological effect on the body". Such peptides can be encrypted within a protein and require proteolysis for their release and activation. Microbes, including lactic acid bacteria, have evolved extracellular proteolytic enzymes that enable them to breakdown the principal milk proteins, casein and whey into smaller peptides that can be transported into the cell for further metabolism. In some cases these peptides have been shown to have bioactivity and in the current study the aim was to identify additional bioactive components and extend the health benefits associated with them.

2. Questions addressed by the project:

- Could Teagasc identify proteolytic microbes, specifically LAB, capable of producing peptides from casein, whey and milk based substrates?
- Would these crude hydrolysates display bioactivity with a variety of health benefits relating to obesity, immunity, cardiovascular disease, glucose metabolism and infection?
- Can the production of bioactive products from bacterial fermentations be commercially optimized?

3. The experimental studies:

Some 300 strains from the Teagasc Moorepark Culture Collection, representing the genera *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Enterococcus*, *Brevibacterium*, *Corynebacterium*, *Lactobacillus*, *Bifidobacterium*, *Bacillus* and *Pediococcus*, were screened for potential proteolytic activity against casein and whey proteins. In addition, a further 120 strains from species with reported proteolytic activity in milk were also sourced and tested. From this analysis 110 strains were further selected for fermentation of either casein, whey of milk based substrates. Freeze-dried powders from 170 fermentations were tested in one or more of six health bioassays, from which 85 potential bioactive powders were identified. From these bioactive powders 25 were further selected to establish if bioactivity could be retained under optimized fermentation conditions and post-fermentation heat treatments, resulting in a further 140 samples for follow-on testing. Moreover, using technologies based on membrane separation, reverse-phase HPLC and iso-electric charge, crude hydrolysates were fractionated with a view to characterizing the bioactive component, and a further 500 samples were generated. Follow-on testing of samples in the various health bioassays have to-date confirmed a number of key findings:

- Bioactivity could be achieved under commercially acceptable fermentation parameters of 1% inoculation under aerobic conditions for 16 hours at 30°C in substrates that could be heat sterilized pre and post fermentation.
- A bioactive component associated with an anti-infective crude casein-based hydrolysate was identified, through mass-spectroscopy and subsequent synthetic peptide testing, as a nine amino-acid peptide cleaved from the end of the alpha-casein protein.
- A metalloprotease proteolytic enzyme was associated with a crude casein hydrolysate that enhanced the satiety and immune systems and regulated cardiovascular responses. For the immune response, bioactivity could be generated using a cell free enzymatic preparation.
- A non-peptide based metabolite was associated with a *Bacillus licheniformis* strain that enhanced satiety.

4. Main results:

- Extracellular proteolytic activity of Lactic Acid Bacteria was in general poor, resulting in no discernible hydrolysis of casein or whey proteins in dairy-based substrates for the majority of strains.
- Proteolytic bacteria were identified from non-LAB sources and represent a new category of technological advantageous dairy-related bacteria.
- Bacterial fermentations of casein, whey and skim milk-based substrates generated crude hydrolysates that harbored a range of bioactivities across health assays related to obesity, immunity, cardiovascular disease, infection, glucose regulation, and gut-flora modulation.
- Fermentation and bioactive powder production conditions were considered to be commercially viable and within existing dairy milk processing practices required to produce food-grade ingredients.

5. Opportunity/Benefit:

Dairy-based powders containing functional ingredients with targeted health effects will add market value to the Irish dairy ingredient sector and help improve public health.

6. Dissemination:

Main publications:

Marcone S, Haughton K, Simpson P, Belton O, Fitzgerald DJ (2014) Milk-derived bioactive peptides inhibit endothelial-dependent adhesive interactionswith monocytes via PPAR-_ dependent regulation of NF-kappa B. submitted.

McArdle M, Roche H. (2012) The Anti-Inflammatory Potential of Milk Derived Bio-actives. Nutrition Society Postgraduate Meeting Cork, Ireland.

Ben Larbi N., Canavan M. Simpson P, Loscher CE. (2011) Anti-inflammatory benefits of milk derived hydrolysates. Irish Society for Immummology Annual Meeting, Galway, Ireland.

O'Brien L, Schellekens H, Dinan T, Cryan J, Fitzgerald GF, Stanton C. (2011) Enhancement of calcium bioavailability via milk bioactives. Proceedings of the Annual Walsh Fellowship Conference, Ireland.

Phelan M, Kerins D (2011) Milk-derived bioactive peptides: selected cardiovascular effects. Food and Function Journal 2: 153–16.

Bruen C, O'Halloran F, Kett A, Chaurin V, Fenelon M, Nilaweera K, Kelly A, McGrath B, McSweeney P, Cashman K, Giblin L. (2010) Effects of dairy protein hydrolysates on satiety. International Congress on Obesity, Stockholm, Sweden.

Project number:
5952

Date:
November, 2014

Funding source:
DAFM

Project dates
Nov 2012 – Sep 2013

Collaborating Institutions:
University College Cork
University of Limerick

Teagasc project team:
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Compiled by:
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Novel Strategies for Optimization of Cheddar Cheese Manufacturing Process

Key external stakeholders:

Dairy Industry.

Practical implications for stakeholders:

Consistency in terms of quality and yield are vital in ensuring an economic return from the commercial production of Cheddar cheese. Seasonal variation in the lactose content of Irish milk and residual galactose accumulation in cheese arising for introduction of new starter systems have the potential to impact on Cheddar cheese quality.

Starter culture systems were developed that can greatly reduce residual lactose levels in ripening cheese and curd washing during manufacture was demonstrated as a means of controlling lactose levels in cheese and thus improving consistency in manufacture of quality cheese.

Main results:

Starter systems containing galactose metabolizing *St. thermophilus* and *Lb. paracasei* strains have the potential to remove residual galactose from ripening cheese and reduce some of the quality issues associated with galactose in cheese including off flavours, inconsistency in composition and browning on cooking.

Curd washing during manufacture was demonstrated as a means of reducing unfermented lactose in, and altering the sensory properties of, Cheddar cheese.

Opportunity/Benefit:

Starter systems investigated as part of this project demonstrated that levels of residual galactose that accumulate in cheese manufactured using *St. thermophilus* containing starter systems can be controlled.

The data generated clearly indicate how curd washing regimes may be applied for cheesemaking under different conditions (milk protein levels, pH at set and at whey drainage, different calcium levels) to control the level of lactose and lactic acid in the cheese from a quality perspective, and to differentiate sensory properties.

1. Project background:

The large scale commercial Cheddar cheese industry recognized early the importance of controlling compositional parameters that could be measured easily in industry (moisture-in-non-fat substances, pH, NaCl, fat-in-dry-matter) as the key to their strategy for producing consistently high quality cheese. While many of these parameters are monitored by Irish Cheddar producers, production of premium quality cheese still cannot be guaranteed. The key objective of this project is to investigate other parameters likely to cause variation in quality but which could also be determined easily in industry within a quality assurance programme with a view to developing further process and product-specific strategies for maximizing Cheddar quality. Such factors include levels of residual galactose and lactose-to-protein ratio in the cheese. On completion of this project, it is envisaged that a number of easily implementable approaches to monitoring and optimizing quality will be developed.

2. Questions addressed by the project:

- How does variation in milk lactose content influence residual lactose and lactic acid levels in Cheddar cheese?
- What is effect of varying residual lactic acid in Cheddar cheese?
- Does residual galactose impact Cheddar cheese quality and can strategies based on starter systems be developed to control it?
- Can we develop strategies to control the effects of lactose level to buffering on Cheddar cheese quality?

3. The experimental studies:

To assess the impact of residual lactose on cheese quality a bank of *Streptococcus thermophilus* were evaluated for lactose metabolism, production of galactose, salt sensitivity and acidification rate. Galactose-positive and galactose-negative strains were evaluated in cheese-making as adjuncts cultures. The interactive effect of pH at whey drainage (pH 6.15 and 6.45) or salting level (1.6 and 2.7%) and use of galactose positive/negative *St. thermophilus* strains on Cheddar cheese quality were evaluated in triplicate pilot scale cheese manufacturing trials followed by a full ripening study.

To assess the impact of lactose-in-moisture (L/M) content of the cheese, cheeses were manufactured at pilot scale where the lactose levels were altered either by addition of lactose to the cheese milk or through curd washing during manufacture. Using these approaches L/M was varied from 5.3 to 3.8.

4. Main results:

The results of cheese-making trials to assess the interactive effects of drain pH and starter culture system indicated that:

- Increasing the drain pH from pH6.15 to pH6.45 increased the cheese moisture content by ~2%; reduced fat, protein, salt and pH at day 14 of cheese ripening time; and also reduced the counts of *St. thermophilus* throughout ripening.
- The cheese manufacture time was ~30 minutes faster than the control cheese when using galactose positive *St. thermophilus*.
- By using the galactose negative *St. thermophilus,* there was an accumulation of residual galactose in cheese during ripening (~0.2%). However, at higher drain pH, the residual galactose in the cheese made with the galactose negative strain was much lower (~ 0.05%).
- Galactose-positive or galactose-negative strains had no significant effects on primary or secondary proteolysis during cheese ripening.
- Reducing the drain pH from 6.45 to 6.15 resulted in: a 1% increase in cheese protein. This in turn led to a decrease in the moisture and MNFS and significantly decreased hardness, fracture strain and fracture stress.
- With respect to sensory analysis, high drain pH control cheese had a fruity flavour, creamy texture and a fruity/savory odor. Cheese made with the galactose-negative *St. thermophilus* at high drain pH had a sweaty and rancid flavour. Cheese made with a galactose positive *St. thermophilus* strain at high drain pH tended to have a salty taste and sweaty odor. Low drain pH control cheese had a buttery flavour, caramel odor and sweet taste. Cheese made with galactose-negative or galactose-positive *St. thermophilus* at low drain pH had a pungent flavour and odor and had an acid taste.

The results of cheese-making trials to assess the interactive effects of salt level and starter culture system indicated that:

- Reducing the salt levels resulted in higher levels of total lactate in cheese hence lower pH, reduced levels of lactose, proteolysis, hardness and fracture properties.
- Cheeses made with adjunct cultures had higher levels of proteolysis, especially, secondary proteolysis as measured by PTA-SN and total free amino acids.
- By using the galactose negative *St. thermophilus*, there was an accumulation of residual galactose in cheese during ripening (~0.1%). However, at lower salt level, the residual galactose in the cheese made with the galactose negative strain was much lower (~ 0.02%).
- Inclusion of a galactose positive *Lactobacillus paracasei* strain in combination with the galactose negative *St. thermophilus* strain as the adjunct culture significantly reduced the residual galactose in cheese during ripening and lowering the cheese pH.
- Sensory analysis indicated that high salt cheeses had a more sweet, buttery, salt, and acid flavour attributes while the control cheese is caramel and bitter and the cheese made with galactose positive *St. thermophiles* strain is more sweaty, fruity and buttery, and the cheese made with galactose negative *St. thermophiles* strain is fruity and acid, and the cheese made with galactose negative *St. thermophiles* strain plus galactose positive *Lb. paracasei* strain is salty, pungent and rancid.
- Low salt cheeses had a bitter, pungent bunt and rancid flavour attributes in which the control cheese is bitter, caramel and buttery while the cheese made with galactose positive *St. thermophiles* strain is more waxy, sulphur and rancid, and the cheese made with galactose negative *St. thermophiles* strain is fruity and sweet, and the cheese made with galactose negative *St. thermophiles* strain plus galactose positive *Lb. paracasei* strain is caramel, buttery and acid.

The results of cheese-making trials to assess the impact of lactose-in-moisture (L/M) content of the cheese that:

- Reducing the L/M level, through increased curd washing, resulted in lower levels of residual lactose, total lactate and D-lactate and higher pH values, but had little effect on gross composition, galactose content, level of primary (pH 4.6 soluble N) or secondary (PTA or free amino acids) proteolysis, or on the levels of starter bacteria (~ 10^7–10^8 cfu/g on day 1) or non-starter lactic acid bacteria NSLAB (~ 10^7cfu/g at 180 d). Nevertheless, reducing the L/M content led to cheeses that were, overall, firmer and less brittle, that had lower levels of some volatile compounds. Sensory evaluation found that reducing the L/M content resulted in Cheddar cheeses being less acid, more buttery, sweeter, saltier and creamier than non-washed cheeses that had more 'sweaty', pungent and farmyard-like sensory notes. Residual lactose content in the 270-day old cheese varied from ~ 0.2% in the control non-washed cheese to ~ 0% in washed-curd cheese with an L/M of 3.9.
- The response of altering L/M content of cheeses, through curd washing, differed depending on the calcium content of the cheese. The mean pH of standard-calcium cheeses (SCa, 770 mg/100g) over the 270-d ripening period increased significantly with curd washing and ripening time, in contrast to the reduced-calcium cheese (RCa, 660 mg/100 g), for which pH was not affected by either of the latter parameters. The RCa washed-curd cheeses had a more buttery, caramel odor and flavour, and a more bitter, less sweet, and nutty taste than the SCa washed-curd cheeses, whereas the RCa non-washed cheeses had a more pungent and less fruity flavour, a less fruity odor, a saltier, more-bitter, and less acidic taste, and a more astringent mouthfeel than SCa non-washed cheeses. The level of unfermented lactose decreased in all cheeses during the 270-day maturation period, with levels in the RCa non-washed-curd cheese being significantly higher than that in all other cheese (washed and non-washed SCa cheeses, washed RCa cheese) at all ripening times.
- Increasing the milk protein from to 3.3 and 4.0% using UF and varying the L/M ratio in the cheese indicated that increasing the level of curd washing and concentrated milk protein reduced concentrations of lactose and total sugars-to-protein ratio in cheese, increased cheese pH, especially at advanced ripening times (not by protein), increased the protein levels and decrease the moisture and MNFS in cheese. High protein cheese tended to be harder, exhibit increased fracture stress and fracture strain. Sensory Analysis indicated high protein cheeses tended to have caramel, buttery and sweet/cheesy flavour, with a fruity and savoury odour, while low protein cheeses tended to have more savoury, onion, farmyard and pungent flavour and more acid taste. With increased curd washing, the cheeses tended to be fruitier, buttery, and sweet and had less 'farmyard' flavour.

5. Opportunity/Benefit:

The composition of culture systems used for Cheddar manufacture has changed in recent years, principally due to the common inclusion of *St. thermophilus*. Since *St. thermophilus* primarily metabolizes only the glucose moiety of lactose, galactose accumulates during manufacture leading potential problems including flavour defects, compositional inconsistency, browning on cooking. The starter systems developed in this project significantly reduced residual galactose in Cheddar cheese and these can be adapted for large scale commercial production.

The current study has shown that significant levels of unfermented lactose may remain in Cheddar cheese even after long maturation times of 270 days, with the magnitude being influenced by the lactose level in the milk, the extent of curd washing and calcium content of the cheese. Residual lactose may be undesirable to those consumers predisposed to lactose intolerance.

The current study has found curd washing to be a very effective means at reducing and controlling the levels of residual lactose in Cheddar cheeses varying in calcium content. This is particularly relevant to Irish manufacturers who process milk with large seasonal- and lactational- changes in lactose content into cheese. Apart from being a tool to reduce the level of residual lactose in cheese, curd washing also, via its effects on pH and calcium distribution between soluble and colloidal states, proved to be means by which the sensory properties of cheese can be differentiated, to an extent that varies with lactose level in milk, extent of curd washing and calcium content of the cheese.

6. Dissemination:

The research has been presented at a number of national and international conferences including the 8th Cheese Symposium, Cork, 39th and 41st Annual Research Conference, Food, Nutrition & Consumer Sciences. Cork, Teagasc Walsh Fellowship Seminar, Dublin and IDF Cheese Ripening & Technology Symposium, Madison, Wisconsin, USA.

Main publications:

Hou, J., Hannon, J.A., McSweeney, P.L.H., Beresford, T.P. and Guinee, T.P. (2012). Effect of curd washing on composition, lactose metabolism, pH, and the growth of non-starter lactic acid bacteria in full-fat Cheddar cheese. *International Dairy Journal* 25, 21–28.

Hou. J., Hannon. J.A., McSweeney. P.L.H., Beresford. T.P., Guinee. T.P. (2014). Effect of curd washing on cheese proteolysis, texture, volatile compounds, and sensory grading in full fat Cheddar cheese. *International Dairy Journal*. 34: 190–198.

Hou, J., McSweeney, P.L.H., Beresford, T.P. and Guinee, T.P. (2014). Effect of curd washing on the properties of reduced-calcium and standard-calcium Cheddar cheese. Journal of Dairy Science 97, 5983–5999.

Food Solutions for Weight Management

Project number:
5942

Date:
Oct, 2014

Funding source:
Enterprise Ireland

Project dates:
Nov 2009 – Oct 2013

Collaborating Institutions:
University College Cork
University College Dublin
University of Limerick
Dublin City University
under the FHI umbrella
www.fhi.ie.

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University College Cork
Prof. Ted Dinan, University
College Cork
Prof. Alan Kelly, University
College Cork
FHI academic and
industrial partners

Compiled by:
Linda Giblin

Key external stakeholders:

Dairy Industry, Food manufactures, Consumer.

Practical implications for stakeholders:

Teagasc, under the Food for Health Ireland (FHI) umbrella, is striving to deliver foods that enhance satiety.

- Ingestion of such foods may reduce portion size and/or frequency leading to a reduction in food intake over time.
- The aim is to identify ingredients that 'make you feel fuller for longer'

Main results:

- One thousand dairy fractions were screened in high throughout satiety assays *in vitro*.
- Of these, eleven lead functional compounds were identified; eight activating satiety receptors in the brain and three increasing satiety hormone secretion in the intestine.
- Three of these 'leads' have been proven to reduce food intake over time in animal trials.
- Successful scale up to 100 litres has occurred with at least one lead functional compound.

Opportunity/Benefit:

- We have identified eleven milk derived ingredients that enhance satiety.
- These ingredients can potentially be used by the food industry in weight management/slimming products.
- An economical viable scaled up satiety enhancing ingredient with strong scientific data benefits the consumer in the 'battle of the bulge'.
- A major achievement has been the establishment of high throughput bioassays for satiety and adiposity which are now available as a compound screening platform to industry.

1. Project background:

Obesity is a global health concern. In Europe alone, obesity and obesity related illness are responsible for over 1 million deaths each year. By 2015 almost 1.5 billion consumers worldwide will be overweight or obese. The diet and weight management market is, therefore, worth over €13.9 billion. An increase in portion size has been identified as a major contributing factor to the obesity epidemic. Several of the drug-based therapies currently on the market to treat obesity either lack efficacy or have adverse side-effects. Food-based solutions can be more easily adapted into daily life. The weight management/ slimming market primarily focuses on boosting satiety or heat generation. Satiety is the feeling of fullness. The presence of food in the gut causes secretion of several satiety hormones that control food intake.

Protein is generally regarded as the most potent stimulus of satiety. Bovine milk protein is of particular interest as several studies have correlated increased milk and dairy consumption with positive effects on body weight, metabolic control and glycaemia.

The aim of this project was to screen milk derived fractions for their ability to enhance satiety. Ingestion of foods containing such ingredients may reduce portion size and/or frequency leading to a reduction in food intake.

2. Questions addressed by the project:

- Can milk fractions increase satiety secretion from intestinal cells in vitro?
- If so, can these lead functional compounds reduce food intake in animal trials?
- What is the effective dose?
- Can these lead functional compounds survive gut transit?
- Can production of these lead functional compounds be scaled up and is this scale-up economical?
- What is the bioactive and what is it's mechanism of action?

3. The experimental studies:

We employed two different cell-based high-throughput assays to screen milk fractions for satiety and fat burning activities. These assays used the murine enteroendocrine cell line, STC-1 as the *in vitro* gut model and the differentiated 3T3-L1 cell line as the *in vitro* adipocyte model. The high throughput satiety assays were based on HTRF technology and measured cAMP and calcium flux. 'Hits' were then funneled into satiety-specific bioassays that measure levels of satiety signals (GLP-1, PYY and CCK) using qRT-PCR and MSD technology. Positives from here were labeled as lead functional compounds and were progressed into animal studies and scale-up.

An in-house scale-up team (led by Dr. Phil Kelly) focused on the economic potential of pre-commercial scale up of the lead functional compounds. Lead functional compounds were given to mice via oral gavage or intra-peritoneal injection and food intake levels recorded over time. Follow-up, and arguably more accurate, studies were then performed in pigs. A lead functional compound at different concentrations in a food matrix was fed to pigs. Blood samples were taken every 15 minutes over a 2 hour period and levels of satiety signals in the blood measured. Although researchers at Teagasc have focused on screening milk fractions, the screening assays developed are applicable to screen any food fraction for promoting satiety or fat burning.

4. Main results:

Dr. Linda Giblin's team screened > 1000 milk fractions in high throughput satiety assays. Of these, approximately 15% were funneled into specific satiety assays. We identified three 'hits', capable of dose dependently increasing GLP-1 secretion from the STC-1 intestinal cells. Two of these were progressed to rodent studies. With our collaborators at UCC, the team performed oral gavage experiments and simulated upper gastric digestions with one lead functional compound. This gave us some anecdotal evidence of bioactivity survival during gut transit.

Parallel work focused on modification of production protocols so that 100 litres scale-up was achieved economically. The optimized protocol (change in % solids, enzyme concentration, pH, freeze drying V spray drying survival) resulted in increased biofunctionality.

To determine bioactive peptides, the lead functional compound was fractionated and tested in cellular bioassays. 'Best guess' bioactive peptides were synthesized and tested.

With our front runner, we performed a post-prandial pig trial to look at effective dosage, food matrix and allow for blood sample collection post-ingestion. Satiety hormones in blood were measured every 15 mins for 4 hours post-ingestion, The team at Moorepark concluded that this lead functional compound needed protection from the harsh conditions of the gut to maintain bioactivity. The project has entered a very exciting phase with a human trial now underway.

During the lifetime of this project we developed a large number of bioassays (high medium and low throughput) to screen compounds (food or chemicals) for their ability to modify satiety and adiposity. As such a spin off from this project was a number of confidential industrial projects.

5. Opportunity/Benefit:

The satiety enhancing ingredients identified can potentially be incorporated into weight management/ slimming products by the food industry.

Discovering ingredients that target satiety are of public good as these ingredients will aid the consumer manage their weight.

A major achievement has been the establishment of high throughput bioassays for satiety and adiposity which are now available as a compound screening platform to industry.

6. Dissemination:

Technology transfer will be managed via FHI www.fhi.ie.

Main publications:

Book Chapters:

Giblin, L., McCarthy, T., Gil-Lozano, M., Gagnon, J.D.and Brubaker, P.L. (2014) 'Enteroendocrine Cell Models for Screening Food Bioactives..' *INFOGEST*, Springer (in press).

McCarthy, T., Green, B.D., Calderwood, D., Gillespie, A., Cryan, J.F. and Giblin, L. (2014) 'STC-1 cells.' *INFOGEST*, Springer (in press).

Journal Articles:

Bruen, C.M., O'Halloran, F., Cashman, K.D. and Giblin, L. (2012) 'The effects of food components on hormonal signalling in gastrointestinal enteroendocrine cells.' *Food and Function,* 3(11): 1131–43.

Bruen, C.M., Kett, A.P., O'Halloran, F., Chaurin, V., Fenelon, M.A., Cashman, K.D. and Giblin, L. (2012) 'Effect of gelatinisation of starch with casein proteins on incretin hormones and glucose transporters *in vitro*.' *British Journal of Nutrition* 107: 155–163.

Four scientific papers submitted to peer reviewed international journals.

Conference Presentations:

Diacetyl (2,3 butanedione), increases GPR120 production and cell surface expression *in vitro*. Triona McCarthy, T., Bruen, C., Schellekens, H., Cryan, J.F. and Giblin, L. (2013) *Young Life Scientists' Symposium 2013 – Cell Signalling*, Cork, Ireland 11th Sept.

Bruen, C.M., Schellekens, H., Simpson, P., Cryan, J.F., Dinan, T.G. and Giblin, L. (2013) 'Identification and characterisation of a milk protein hydrolysate that increases satiety signalling *in vitro* and reduces feed intake *in vivo*.' *European Congress on Obesity* 2013 Liverpool, U.K. 12–15 May.

'Dairy components and satiety – a taste of things to come?' McCarthy, T., Cryan., J.F. and Giblin , L. (2012) *UCC Doctoral Showcase*, Cork, Ireland March.

Bruen C., Hannon J., O'Halloran F., Cashman K. and Giblin L. (2011) 'Mechanistic investigations into the effect of volatile dairy compounds on in vitro satiety signaling'. 40th *Annual UCC Food Research Conference*, Cork, Ireland April.

Effects of dairy protein hydrolysates on satiety Bruen, C., O'Halloran, F., Kett, A., Chaurin, V., Fenelon, M., Cashman, K. and Giblin, L. (2010) *International Congress on Obesity*, Stockholm, Sweden11–15 July.

Bruen C., Hannon J., O'Halloran F., Cashman K. and Giblin L. (2011) 'The effects of aromatic compounds on taste transduction and gastrointestinal satiety signalling.' *Teagasc Walsh Fellowship Conference*. Dublin, Ireland Nov.

Bruen, C., O'Halloran, F., Kett, A., Fenelon, M., Cashman, K. and Giblin, L. (2009) 'Gut hormonal signaling in response to protein-carbohydrate food components.' *European Congress on Obesity* Amsterdam, The Netherlands, 5–8 May.

Popular publications Press Clippings:

'FHI program identifies new bioactive peptides in Milk Proteins.' (2010) *ADP1 American Dairy Products Institute*, May Release.

'FHI milk mining starts to strike gold.' (2010) *Nutriceutical Business and Technology*, June Release.

'FHI identifies new bioactive peptides.' (2010) Dairy Industries International, July Release.

'FHI programme identifies new bioactive peptides in milk proteins.' (2010) *Food ingredients*, Aug Release.

'Next satiety foods will contain cocktail of ingredients.' (2011) *Food navigator*, July Release.

'Milk protein could be sold as diet drink.' (2012) *Sunday Times*, 14 Oct.

'Food solutions for weight management – Satiety enhancing bioactives.' (2012) *T-Research*, 7(3) 16–17 ISSN1649–8917.

Natural Ingredient Cheese Solutions

Project number:
5938
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Funding source:
DAFM
Project dates:
Dec 2008 – July 2013

Collaborating Institutions:
University of Limerick
University College Cork

Teagasc project team:
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(University of Limerick)
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University College Cork
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Compiled by:
Kieran N Kilcawley

Key external stakeholders:

Dairy Industry, Food Manufacturers.

Practical implications for stakeholders:

Methods to augment, accelerate and diversify cheese flavour.

- A method to attenuate lactic acid bacteria for use as adjuncts to augment cheese flavour.
- Rapid methods to screen lactic acid bacteria for flavour potential.
- Database of key volatile cheese flavour compounds.
- Protocols for production of cheese concentrates, using attenuated lactic acid bacteria.
- Development of a yeast based encapsulation system to augment cheese flavour development.
- Use of camel chymosin to alter texture in low moisture part-skim Mozzarella.
- Protocols for accelerating and diversifying cheese flavour in Ingredient cheese applications.

The study has highlighted a number of approaches to control, augment, accelerate and diversify cheese flavour in a range of different applications.

Main results:

- Microfluidization is a useful technique to attenuate lactic bacteria and yeast to enhance their flavour development capability.
- Attenuated yeast can be used to entrap enzymes critical for cheese flavour development and to control their subsequent release into the cheese matrix during ripening to accelerate flavour development.
- Production of fast-ripened cheeses with diverse flavours for use in ingredient applications.
- Model system to rapidly screen lactic acid bacteria, enzymes and yeasts for cheese flavour development.

Opportunity/Benefit:

Researchers involved in this project have the experience and expertise to aid producers to alter existing cheese products or develop new cheese flavour concepts using natural lactic acid bacteria, yeasts, enzymes. Extensive knowledge and expertise exists in the flavour chemistry facility at Teagasc Food Research Centre Moorepark in the extraction, concentration and identification of cheese flavour volatiles. Consultancy and contract research opportunities are available to both national and international clients in the area of cheese flavour development.

1. Project background:

The use of cheese as an ingredient has grown steadily and is predicted to continue to do so as consumers demand more sophisticated high quality natural semi-processed and processed foods due to less available "quality time". The market now demands natural wholesome cost effective products and cheese meets this demand as it can provide a multitude of functions, with regard to visual, textural, flavour and aroma appeal. The rationale behind this project involves the development of natural cheese concentrates and a range of cost effective natural ingredient cheeses to meet this growing market. This project explores and exploits natural biochemical pathways in lactic acid bacteria and yeasts to create concentrated cheese flavours and ingredient cheeses with diverse flavours to meet current and future market demands for clean label dairy products.

2. Questions addressed by the project:

- Can cheese flavour development be controlled and accelerated in the production of cheese concentrates, cheese and cheeses for ingredient applications?
- What different mechanisms can be used to accelerate natural cheese ripening that can be practically applied at commercial level?
- Is microfluidization a feasible technique attenuated lactic acid bacteria and yeasts for use as adjunct cultures in cheese ripening?
- Can concentrated cheese flavours be developed using attenuated lactic acid bacteria?
- Can attenuated yeast be used to deliver key flavour producing enzymes into the cheese matrix to accelerate ripening?
- Can suitable model systems be developed to rapidly screen lactic acid bacteria and yeasts for their ability to produce key cheese flavour compounds?
- What is the impact of adding hydrolysed dairy substrates/concentrated cheese flavours into the cheese matrix during manufacture?
- What is the impact of different chymosin sources on the quality of low-moisture part skim Mozzarella cheese?
- What is the combined effect of applying different techniques to boost cheese flavour formation in semi-hard type cheeses?

3. The experimental studies:

- The results clearly highlighted that incorporation of adjunct cultures and increased ripening temperatures can be easily applied to control flavour formation in semi-hard cheese, but that other strategies, such as incorporation of attenuated yeast, entrapped cell free extract and added hydrolyzed dairy substrates can also be successfully applied.
- Microfluidization was proven to be a very simple, practical technique to control the degree of attenuation of lactic acid bacteria and yeast for use as adjuncts in cheese flavour development.
- Attenuated cultures can be used in combination with commercial enzymes to develop concentrated cheese flavours.
- Yeast attenuated by microfluidization was successfully used to entrap a cell free extract of lactic acid bacteria and subsequently used to augment, control and accelerate flavour development in cheese for ingredient applications.

- Two separate model systems were successfully used to rapidly screen lactic acid bacteria for their cheese flavour potential.
- A spray dried cheese concentrate consisting primarily of hydrolysed sodium caseinate, cream and water was successfully added to a Cheddar type cheese process during dry salting. This provided more available substrate for metabolic reactions during the early stages of cheese ripening to augment, accelerate and diversify flavour formation.
- The use of camel chymosin did not affect composition or meltability of low-moisture part skim Mozzeralla cheese, but did increase proteolysis which subsequently impacted on cheese texture.
- The combined effects of the addition of adjunct cultures, increased ripening temperatures, inclusion of attenuated yeast, entrapped cell free extracts and inclusion of additional hydrolysed dairy substrates modified flavour development in semi-hard type cheeses.

4. Main results:

- Optimisation of attenuation by microfluidization of lactic acid bacteria and yeasts for use as adjuncts in the development of concentrate cheese flavours, cheeses or ingredient type cheeses.
- Successful entrapment of enzymes in attenuated yeast and subsequent use in semi-hard type cheeses.
- Development of model systems to rapidly screen lactic acid bacteria for cheese flavour potential.
- Production of concentrated cheese flavours using enzymes and attenuated lactic acid bacteria.
- Incorporation of spray dried cheese concentrates into cheese curd during production to augment flavour development.
- Comparison of the impact of camel and calf chymosin in the production of low-moisture part skim Mozzeralla.
- Development of a data base of key volatile compounds that are involved in cheese flavour.
- Assessment of the impact of a range of combined approaches (adjunct cultures, raised ripening temperatures, attenuated yeast, entrapped enzymes & added cheese concentrates) to augment, control, accelerate or diversify flavour in cheeses for ingredient applications.

5. Opportunity/Benefit:

Consultancy and contract research opportunities are available to both national and international clients in the cheese flavour area.

6. Dissemination:

The information generated as part of this study was disseminated over the project lifespan on 34 occasions (workshops, conferences, symposia and meetings).

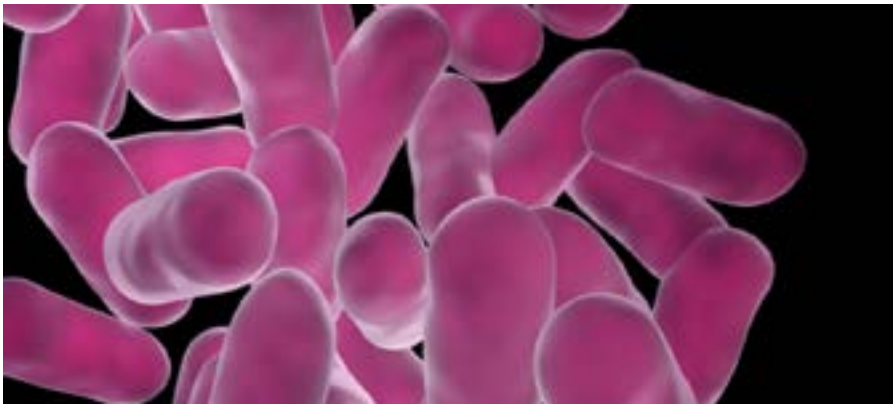
Main publications:

Yarlagadda, A.B., Wilkinson, M.G., Ryan, S.P., Doolan, I.A., O'Sullivan, M.G & Kilcawley, K.N (2014), Utilisation of a cell-free extract of lactic acid bacteria entrapped in yeast to enhance flavour development in Cheddar cheese. *International Journal of Dairy Technology*, 67, (1) 21–30.

Yarlagadda, A.B., Wilkinson, M.G., O'Sullivan, M.G & Kilcawley, K.N (2014). Utilisation of microfluidization to enhance enzymatic and metabolic potential of lactococcal strains as adjuncts in Gouda type cheese. *International Dairy Journal*, 38, (2), 124–132.

Moynihan, AC., Govindasamy-Lucey, S., Jaeggi, JJ., Johnson, M.E, Lucey, J.A & McSweeney, P.L.H (2014), Effect of camel chymosin on the texture, functionality, and sensory properties of low-moisture, part-skim Mozzarella cheese, *Journal of Dairy Science*, 97, (1), 85–96.

Culture Collections in Teagasc Food Research Centre Moorepark



Project number:
6042 and 6312

Date:
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Funding source:
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Project dates:
2008 – 2013

Collaborating Institutions:
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Prof. Catherine Stanton

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Compiled by:
Mary Rea and
Olivia McAuliffe

Key external stakeholders:

Dairy Industry, food manufacturers, pharm industry, research community.

Practical implications for stakeholders:

The culture collections in the Teagasc Food Research Centre Moorepark provide banks of bacterial cultures with potential for exploitation as dairy starters, adjunct cultures and probiotics for the Food and Pharma industries and the research community.

Main points

The main functions of the DPC and APC culture collections are:

- To provide a central repository for safe housing and cataloguing of DPC and APC Biobanks.
- To provide researchers within Teagasc and APC and interested stakeholders with accurate data regarding the potential applications, safety and quality of strains within the collections.
- To provide unambiguous traceability for IP protection and accountability.

Main results:

DPC and APC culture collections contain 7000 and 62,000 strains respectively. The DPC culture collection predominately consists of strains of lactic acid bacteria of the genera *Lactococcus*, *Lactobacillus* and *Streptococcus*. These bacteria have been isolated over many years from a variety of dairy-associated sources. In addition, this collection also houses bacteria and yeasts isolated from surface ripened cheese, many food, animal and human Class 2 pathogens and also bacteriophages isolated from both dairy and environmental sources. More recently the biobank associated with the APC contains strains isolated from human intestinal samples which have potential for exploitation as probiotics for the treatment of anti-inflammatory diseases such as IBD and IBS, anti-*Clostridium difficile* probiotics and antimicrobials in addition to strains producing bioactive metabolites such as conjugated linoleic acid and exopolysaccharides.

Opportunity/Benefit:

The DPC and APC culture collections are available to researchers in Teagasc Food Research Centre, researchers in the APC and companies for exploitation in the Food or Pharma or Veterinary arena.

1. Project background:

The culture collection held at Teagasc Food Research Centre Moorepark, called the DPC Culture Collection, currently holds in excess of 7,000 strains of bacteria and yeast. This collection was begun over 40 years ago and is constantly growing based on the evolution of the Food Research Programme. These bacteria were isolated from a wide variety of foods and from environmental, human, veterinary and clinical samples. In addition the collection houses a large number of strains that have been purchased from culture collections worldwide and are used as reference strains.

Historically the strains deposited in the collection were isolated predominantly from fermented dairy foods such as cheese, yoghurts and fermented beverages. However, more recently, due to the increasing interest in the health promoting role of the bacteria that make up the gut microbiome, this collection has expanded to include a wide range of potential probiotic microorganisms isolated from the animal and human gastrointestinal tract. In addition and situated on the Moorepark Campus are three additional culture banks associated with (i) the Alimentary Pharmabiotic Centre (~63,000 strains) which studies the role of gut microbiota in health and disease (ii) the Eldermet project (http://eldermet.ucc.ie) (6,000 strains) and (iii) the Infantmet project (4,000 strains) which study the gut microbiota of people at the extremes of life i.e. the elderly and the infants respectively.

2. Questions addressed by the project:

How best can the cultures in the biobanks be stored and characterised to make the strains more accessible for commercialisation?

3. The experimental studies:

All the strains are stored in duplicate stocks at -80°C and catalogued. In so far as is possible all information pertaining to the strains is captured in order to build up background information for future commercial exploitation of the strains.

Molecular methods such as 16S rDNA gene sequencing (for species identification) and genome finger printing methods such as pulsed field gel electrophoresis, to determine the genetic relationship of strains of the same species, were used for classification of the strains.

Many strains have been assessed as potential starter cultures in food fermentation, screened for their probiotic potential and for their ability to produce antimicrobial compounds and bioactive molecules.

4. Main results:

The DPC and APC culture collections house strains with potential for the following applications:

- Lactic acid bacteria and certain non-dairy bacteria suitable for use as starter/adjunct cultures in fermented foods.
- Lactic acid bacteria and yeasts suitable for flavour modification in foods.
- Smear cheese bacteria with applications for surface ripened cheese or with capabilities for flavour modification of fermented dairy products.
- Lactic acid bacteria and *Bifidobacterium* species with potential as probiotics producing both antimicrobial compounds and bioactive metabolites.
- A range of bacterial species in addition to lactic acid bacteria with antimicrobial producing ability or the capacity to produce bioactive metabolites or flavour compounds.
- A range of foodborne pathogens for research purposes.

5. Opportunity/Benefit:

All strains in the collections housed in TFRC Moorepark, unless restricted (i.e. prior license to another party) are available for exploitation to the Dairy, Food and Pharma sectors subject to discussions with the Technology Transfer Offices in Teagasc and University College Cork.

6. Dissemination:

Main publications:

Rea, M.C. D. Alemayehu, R. P. Ross C. Hill, (2013) Gut Solutions to a gut problem: bacteriocins, probiotics and bacteriophage for the control of *Clostridium difficile* infection. J. Med Microbiol. 62: 1369–1378.

Alemayehu, D., J. A. Hannon, O. McAuliffe, R.P. Ross. (2014). Characterization of plant-derived lactococci on the basis of their volatile compounds profile when grown in milk. Int. J. Food Microbiol. 172: 57–61.

Popular publications:

Pharmabiotics – health benefits beyond the gut T Research Vol 8 No 3 Autumn 2013.

Probiotic lactobacilli survival and impact in the animal gut

Project number:
5972

Date:
November 2014

Funding source:
Science Foundation Ireland

Project dates:
Sept 2008 – Aug 2013

Collaborating Institutions:
University College Cork

Teagasc project team:
Prof. Paul Ross
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External collaborators:
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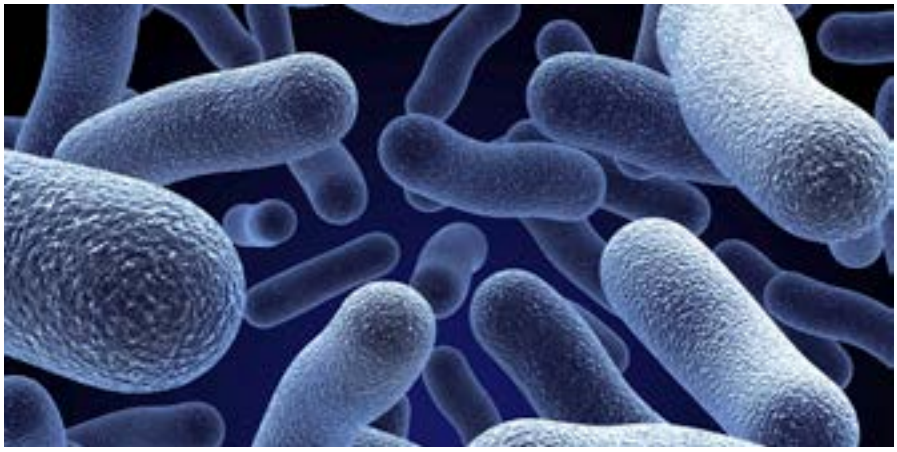
Compiled by:
Michelle O' Donnell



Key external stakeholders:

Animal feed manufacturers; thoroughbred racehorse industry, veterinary health professionals.

Practical implications for stakeholders:

- This project provides first time information on the microbial ecology of the equine, and other mammalian species gut.
- This project also provides information on commensal lactobacilli found in the gut microbiota of humans and animals.

Main results:

- The project provided definitive genome-based evidence to support the fermentation patterns of sixteen strains of *Lactobacillus ruminis*, and has identified prebiotic carbohydrates with the potential to promote *L. ruminis* growth *in vivo*.
- This project identified the core faecal microbiota of ruminants, hindgut fermenters and mono-gastric animals co-localised to a single farm in Ireland.
- The project provided details for the first time, on the faecal microbiota of thoroughbred racehorses, both active and at rest.
- Analysis of the thoroughbred horse microbiota has revealed *Lactobacillus equi* to be a predominant *Lactobacillus* species in the hindgut. Genome analysis identified genes and enzymes highlighting *L. equi* adaptations to the herbivorous gastrointestinal tract of the horse, including fructan hydrolases.
- Having sequenced the genome of *Lactobacillus equi*, will help to further understand the microbial ecology of the equine hindgut and the influence lactobacilli have on it.

Opportunity/Benefit:

The outcomes of this project is of relevance for the basic understanding of commensals/probiotics, potential mammalian applications, and potential alternatives to in-feed antibiotics for the animal production industry and generation of information of direct relevance for human probiotic consumption.

1. Project background:

Lactobacilli constitute a diverse genus of the Gram Positive bacteria found ubiquitously in nature and also within the gastrointestinal tract of humans and animals. At least twenty species appear to be the dominant Lactobacillus species in human faeces or the GI tract. They represent a wealth of potentially useful organisms and products for exploitation. Lactobacilli are often the target of dietary treatments like prebiotics as they are considered beneficial, commensal bacteria. Therefore, identification of the pathways involved in prebiotic utilization in an autochthonous member of the mammalian microbiota (*L. ruminis*) would greatly benefit the functional food sector.

The microbiota of both humans and animals in conjunction with dietary intake can have an effect on the health of the host. Many diseases including IBD, IBS and laminitis are thought to be caused by microbial dysbiosis within the gut. Elucidation of the herbivore microbiota would therefore be of benefit for animal husbandry and the economy.

2. Questions addressed by the project:

- What are the key properties of lactobacilli that promote survival in the gut, and what is the effect of introducing Lactobacillus on the gut microbiota?
- Can a combination of genome sequencing, comparative and functional genomics, and animal feeding trials identify gut survival and host interaction mechanisms?

3. The experimental studies:

In order to identify the factors involved in motility, prebiotic utilization and growth within *L. ruminis* we sequenced the genome of six strains from a variety of human and animal sources. The genomes of each were analysed and metabolic pathway maps were developed. A number of genes and operons were identified as being key to the utlisation of prebiotics (fructoligosaccharides, galactooligosaccharides and soybean-oligosaccharides).

This project was also interested in the effect that digestion type had on the microbiota of domesticated herbivores. In order to research this, fecal samples were collected from a commercial mini-farm which housed 10 different animal species. These 10 animal species could be divided into three digestion types – ruminant, hindgut fermenter or mono-gastric fermenter. Bacterial DNA was extracted from each sample and sent for pyrosequencing. Following bioinformatics analysis the resulting taxa were compared, in order to identify a core microbiota present in domesticated herbivores, regardless of digestion type.

4. Main results:

- Identification of the genetic basis for motility and carbohydrate utilization in *L. ruminis*.
- The first study to investigate the microbiota of the Irish Thoroughbred racehorse using next generation sequencing techniques.
- We determined the genome sequences of six strains of a commensal lactobacillus spp. *L. ruminis*.
- Analysis of the microbiota of Thoroughbred racehorses, miniature ponies, deer, donkeys, Kune-kune pigs, pygmy goats, llamas, alpaca, rabbits and chinchillas.

5. Opportunity/Benefit:

This project has highlighted the need for further research on the effect of diet on performance animals. It also forms a knowledge base for future studies into the microbiota of domesticated herbivores and its influence on health status.

6. Dissemination:

Main publications:

O'Donnell MM, Harris HMB, O'Toole PW, Ross RP. (2014) The Genome of the Predominant Equine Lactobacillus Species, *Lactobacillus equi*, Is Reflective of Its Lifestyle Adaptations to an Herbivorous Host. Genome Announc. 2014 Jan 16;2(1).

O'Donnell MM, Harris HMB, Jeffreys IB, Claesson MJ, Younge B, O'Toole PW, Ross RP. (2013) Hindgut microbiome of the Irish Thoroughbred racehorses. Letters in Applied Microbiology, 57, 6, 2013, 492–501.

O'Donnell MM, O'Toole PW, Ross RP. (2013) Catabolic flexibility of mammalian-associated lactobacilli. Microb Cell Fact. 2013 May 16;12:48.

Riboulet-Bisson E, Sturme MH, Jeffery IB, O'Donnell MM, Neville BA, Forde BM, Claesson MJ, Harris H, Gardiner GE, Casey PG, Lawlor PG, O'Toole PW, Ross RP. (2012) Effect of Lactobacillus salivarius bacteriocin Abp118 on the mouse and pig intestinal microbiota. PLoS One. 2012;7(2).

Neville, B. A., Forde, B. M., Claesson, M. J., Darby, T., Coghlan, A., Nally, K., Ross, R. P. & O' Toole, P. W. (2012). Characterization of pro-inflammatory flagellin proteins produced by Lactobacillus ruminis and related motile lactobacilli. PLOS One 7, e40592.

Forde BM, Neville BA, O'Donnell MM, Riboulet-Bisson E, Claesson MJ, Coghlan A, Ross RP, O'Toole PW. (2011) Genome sequences and comparative genomics of two Lactobacillus ruminis strains from the bovine and human intestinal tracts. Microb Cell Fact. 2011 Aug 30;10 Suppl 1:S13.

PhD awarded to Michelle O'Donnell.

Popular publications:

Betting on Bacteria, Bernard Dixon, Microbes Magazine Animalcules section, March 2014 https://www.microbemagazine.org/images/New%20Folder/Mar2014/znw00314000090.pdf.

Alimentary Pharmabiotic Centre: Microbe/ microbe interactions in the gastrointestinal tract

Alimentary Pharmabiotic Centre
Interfacing Food & Medicine

Project number:
5271
Date:
November 2014
Funding source:
Science Foundation Ireland
Project dates:
Feb 2004 – Sept 2013

Key external stakeholders:

Food manufacturers, pharmaceutical industry, gastroenterologists, wider research community.

Practical implications for stakeholders:

It is widely recognised that the gut microbiota plays an important role in human health and this is currently one of the most dynamic, complex and exciting areas of research in both the food and pharmaceutical arenas. The mining of the gastrointestinal tract (GIT) has revealed that the gut microbiota represents a repository of potential therapeutic molecules for food and pharmaceutical applications.

Main results:

- The antimicrobial Thuricin CD has been patented and is licensed to Alimentary Health for the treatment or prevention of *Clostridium difficile* infection.
- Bacteriophages MR299–2 and NH-4 can eliminate *Pseudomonas aeruginosa* in a murine model of Cystic Fibrosis and this combined with other results has led to the establishment of Phageworks™ – a one-stop development and IP licensing company bringing phage based products to market for customers.
- The novel antimicrobial Bactofencin LS1 is effective for the control of both *Listeria monocytogenes* and *Staphylococcus aureus*. Bactofencin LS1 has been patented and is currently undergoing a programme of investigation with the Irish SME Sigmoid Pharma.

Opportunity/Benefit:

A bank of novel antimicrobials produced by GI microbiota is available for development.

Collaborating Institutions:
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Teagasc project team:
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Dr. Paul Cotter
Dr. Debebe Alemayehu
Dr. Eileen O'Shea
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Dr. Daniel Burke
Dr. Alan Marsh

External collaborators:
Prof. Fergus Shanahan
Prof Colin Hill and the APC team
Prof John Vederas

Compiled by:
Sheila Morgan and Mary Rea

1. Project background:

This project aimed to understand the role of antimicrobials produced by the gut microbiota, and the conditions governing their activity in the gastro-intestinal tract (GIT). Characterisation of a range of novel isolates was undertaken and their activity investigated both *in vitro* and *in vivo* in order to improve our understanding of the role of antimicrobials produced by the gut microbiota, and the conditions governing their activity in the GIT. To gain regulatory approval for gut microbes for use in food or pharma industries, applications have to be underpinned by high quality scientific data. Therefore, studies were undertaken to gain a thorough understanding of the safety, structure/function relationships and mode of action of isolates and the bioactive compounds they produce. In addition literature reports have suggested that probiotic drinks demonstrate a protective effect against CDI (*Clostridium difficile* infection) in patients undergoing antibiotic treatment. *C. difficile* is now the most common cause of hospital acquired diarrhea and the incidence of hypervirulent strains is increasing worldwide. Therefore reducing *C. difficile* carriage and outgrowth by novel probiotics was a primary target of research.

2. Questions addressed by the project:

- Can we attempt to understand the role of microbe-microbe interactions in determining the overall composition and flux of the human intestinal microflora?
- Can we control *Clostridium difficile* overgrowth using novel antimicrobials?
- Can we isolate novel antimicrobials and apply these to control undesirable microbes?
- Can we confirm a role for bacteriocins as colonising and anti-infective mechanisms in probiotics?
- Can we isolate a probiotic strain with the capability to reduce *C. difficile* carriage?

3. The experimental studies:

We performed substantial high throughput screening of the microbiota and isolated and characterized a variety of antimicrobial producing strains.

We undertook a number of studies in parallel relating to the prevalence and carriage of *Clostridium difficile* in a range of population demographics and found that various increased rates of carriage is associated with poor gut health – with healthy subjects having a carriage rate of 1–2% compared to IBS (4–5%), IBD (11%), institutional aged (20%) and cystic fibrosis (50%). A major outcome was the isolation and characterisation of Thuricin CD, a post-translationally modified bacteriocin with activity against *Clostridium difficile*. In two consecutive publications in the Proceedings of the National Academy of Science this bacteriocin was shown to have a very narrow spectrum of activity such that it killed *C. difficile* without the associated collateral damage of gut microbiota associated with the antibiotics normally used to treat CDI namely vancomycin and metronidazole. In further experiments rectal delivery of Thuricin CD resulted in a 100 fold reduction of Clostridium *difficile* carriage *in vivo* in mice. As a result of these data, Thuricin CD is licensed to Alimentary Health for treatment or prevention of *Clostridium difficile* infection.

After intensive screening of the DPC and APC culture collections three lactobacillus strains with potential to reduce *C. difficile* carriage were identified. These strains are currently being evaluated for their IP/licencing potential.

A further specific goal was to characterise novel antimicrobials produced by gut microbiota. Within this goal we had significant success at mining the gut for bacteriophage with the ability to kill *Pseudomonas aeruginosa*, an opportunistic superbug that infects the lungs of patients suffering from Cystic Fibrosis. In collaboration with visiting Professor James G. Martin of McGill University, we demonstrated that bacteriophages MR299–2 and NH-4 can eliminate *Pseudomonas aeruginosa* in a murine model. This finding, combined with other bacteriophage research performed within the APC, has led to the establishment of Phageworks™ – a one-stop development and IP licensing company bringing phage based products to market for customers.

The intensive screening for antimicrobials also uncovered a completely novel bacteriocin produced by *Lactobacillus salivarius* DPC 6502. We identified Bactofensin LS1, a new type of cationic bacteriocin as part of the specific goal devoted to novel antimicrobials produced by the gut. Bactofensin LS1, produced by a porcine intestinal isolate, is highly positively charged which is very unusual in a prokaryotic peptide. Indeed in this respect it demonstrates closer similarities to eukaryotic defensins and plant derived antimicrobial peptides, than those of bacterial origin. It has been demonstrated to be effective for the control of both *Listeria monocytogenes* and *Staphylococcus aureus*. Bactofensin LS1 has been patented and is currently undergoing a programme of research with the Irish SME Sigmoid Pharma.

4. Main results:

- Thuricin CD, an antimicrobial with activity against *Clostridium difficile* was isolated, identified and characterized at the molecular, structural and mechanism of action level. This antimicrobial has applications in the food and pharma sectors.
- Bacteriophage, isolated from the gastrointestinal tract, were found to be effective for the elimination of *Pseudomonas aeruginosa* in an animal model, indicating its potential as a treatment for individuals suffering from Cystic Fibrosis. These bacteriophages have potential for applications in the pharma sector.
- The antimicrobial, Bactofensin LS1, a gut isolate has been demonstrated to have the ability to control *Listeria monocytogenes* and *Staphylococcus aureus*, two food pathogens. This antimicrobial has applications in the food industry.

5. Opportunity/Benefit:

Outputs from this research are of significance in a number of areas including food, medical devices, pharmaceutical and veterinary industries. Microbial isolates and metabolites mined from the gut environment have potential applications for the improvement of food safety and human health.

6. Dissemination:

Main publications:

Rea, M.E., E. Clayton, P. O'Connor, F. Shanahan, B. Kiely, R.P. Ross and C. Hill. 2007. Antimicrobial activity of lacticin 3147 against clinical *Clostridium difficile* strains. J. Med. Microbiol. 56:940–946.

Gardiner, G.E., MC. Rea, B. O'Riordan, PM. O'Connor, S. Morgan, PG. Lawlor, PB. Lynch, MC. Cronin, RP. Ross and C. Hill 2007. Fate of the two component lantibiotic lacticin 3147 in the gastrointestinal tract. Applied and Environmental Microbiology. 73: 7103–7109.

Clayton, E.M., M.C. Rea, F. Shanahan, E.M. Quigley, B.E. Kiely, C. Hill and R.P. Ross. 2009. The vexed relationship between *Clostridium difficile* and Inflammatory Bowel Disease – an assessment of carriage in an outpatient setting among patients in remission. American Journal of Gastroenterology 104:1162–9.

O'Shea, E.F., G.E. Gardiner, P.M. O'Connor, S. Mills, R.P. Ross and C. Hill. 2009. Characterization of enterocin and salivaricin producing lactic acid bacteria from the mammalian gastrointestinal tract. FEMS Microbiology Letters 2009 29:24–34.

Rea, M., C.S. Sit, E. Clayton, P.M. O'Connor, R.M. Whittal, J. Zheng, J.C. Vederas, R.P. Ross and C. Hill. 2010. Thuricin CD, a post-translationally modified bacteriocin with a narrow spectrum of activity against *Clostridium difficile*. PNAS (Proc Natl Acad Sci USA) 107:9352–7.

O'Shea, E.F., P.M. O'Connor, P.D. Cotter, R.P. Ross and C. Hill. 2010. Synthesis of Trypsin-Resistant Variants of the Listeria-Active Bacteriocin Salivaricin P. Applied and Environmental Microbiology 76:5356–5362.

Marsh, A.J., O. Sullivan, R.P. Ross, P.D. Cotter and C. Hill. 2010. In silico analysis highlights the frequency and diversity of type 1 lantibiotic gene clusters in genome sequenced bacteria. BMC Genomics 11:679.

Rea, M.C., A. Dobson, O. O'Sullivan, F. Crispie, F. Fouhy, P.D. Cotter, F. Shanahan, B. Kiely, C. Hill and R.P. Ross. 2011. Microbes and Health Sackler Colloquium: Effect of broad- and narrow-spectrum antimicrobials on *Clostridium difficile* and microbial diversity in a model of the distal colon. Proc. Natl Acad Sci USA 108 Suppl 1:4639–44.

Clayton, E.M., C. Hill, P.D. Cotter and R.P. Ross. 2011. Real-time PCR assay to differentiate Listeriolysin S-positive and –negative strains of *Listeria monocytogenes*. Applied and Environmental Microbiology 77:163–71.

O'Shea, E.F., P.M. O'Connor, E.J. Raftis, P.W. O'Toole, C. Stanton, P.D. Cotter, R.P. Ross and C. Hill. 2011. Production of multiple bacteriocins from a single locus by gastrointestinal strains of *Lactobacillus salivarius*. J. Bacteriology 193:6973–82.

Clayton, E.M., M.C. Rea, F. Shanahan, E.M.M. Quigley, B. Kiely, R.P. Ross and C. Hill. 2012. Carriage of *Clostridium difficile* in outpatients with irritable bowel syndrome. Journal of Medical Microbiology 61, 1290–1294.

Alemayehu, D., P.G. Casey, O. McAuliffe, C.M. Guinane, J.G. Martin, F. Shanahan, A. Coffey, R.P. Ross and C. Hill. 2012. Bacteriophages MR299–2 and NH-4 can eliminate *Pseudomonas aeruginosa* in the murine lung and on cystic fibrosis lung airway cells. MBio. 2012 Mar 6;3(2);e00029–12.

Rea, M.C., O. O'Sullivan, F. Shanahan, P.W. O'Toole, C. Stanton, R.P. Ross and C. Hill. 2012. *Clostridium difficile* carriage in elderly subjects and associated changes in the intestinal microbiota. J. Clinical Microbiology 867–875.

O'Shea, E.F., P.D. Cotter, C. Stanton, R.P. Ross and C. Hill. 2012. Production of bioactive substances by intestinal bacteria as a basis for explaining probiotic mechanisms: Bacteriocins and conjugated linoleic acid. International Journal of Food Microbiology 152:189–205.

Rea, M.C., D. Alemayehu, R.P. Ross and C. Hill. 2013. Gut Solutions to a Gut Problem: bacteriocins, probiotics and bacteriophage for control of *Clostridium difficile* infection. J Medical Microbiology 62:1369–78.

Marsh, A.J., O. O'Sullivan, C. Hill, R.P. Ross and P.D. Cotter. 2013. Sequencing-based analysis of the bacterial and fungal composition of kefir grains and milks from multiple sources. PLos One19;8(7):e69371.

Marsh, A.J., O. O'Sullivan, C. Hill, R.P. Ross and P.D. Cotter. 2013. Sequence-based analysis of the microbial composition of water kefir from multiple sources. FEMS Microbiology Letters:348:79–85.

O'Shea, E.F., P.D. Cotter, R.P. Ross and C. Hill. 2013. Strategies to improve the bacteriocin protection provided by lactic acid bacteria. Current Opinion in Biotechnology 24: 130–134.

O'Shea, EF, PM O'Connor, O. O'Sullivan, PD Cotter, R.P. Ross and C. Hill. 2013. Bactofencin a, a new type of cationic bacteriocin with unusual immunity. MBio. 2013 Oct 29;4(6). Doi:pii:e00498–12.

Rea MC, D. Alemayehu, P.G. Casey, P.M. O'Connor, P.G. Lawlor, M. Walsh, F. Shanahan, B. Kiely, R.P. Ross and C. Hill. 2014. Bioavailability of the anti-Clostridial Bacteriocin Thuricin CD in Gastrointestinal Tract. Microbiology 160:439–45.

Marsh, A.J., O. O'Sullivan, C. Hill, R.P. Ross and P.D. Cotter. 2014. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungue) samples. Food Microbiology. 2014 38:171–8.

Patents:

Ross, R.P., Stanton, C., Hill, C. and Fitzgerald, G.F. (2009) Antimicrobial Peptides and Bacterial Strains that Produce them. Publication No.: US2009214498.

Hill, C., Rea, M. and Ross, R.P. (2010) Thuricin CD an Antimicrobial for specifically targeting *Clostridium difficile*. Publication No: MX2010005908

Ross, R.P., et al (2010) *Pseudomonas aeruginose* Bacteriophage(s) and uses thereof. Patent filed December 2010. EU Patent Application No: 10195995.

APC: production of microbial metabolites by gut bacteria

Key external stakeholders:

Food manufacturers, dairy industry, pharmaceutical companies, research communities; public health agencies and health professionals; policymakers.

Practical implications for stakeholders:

Availability of probiotic bacteria and functional foods enriched in probiotics and beneficial microbial metabolites with potential beneficial effects on gut health, metabolic health and in reducing allergy onset.

Main results:

- This research led to the identification and exploitation of human gut bacteria for production of a wide range of bioactives such as conjugated linoleic acid (CLA), short chain fatty acids (SCFA), exoploysaccharides and gamma-amino butyric acid (GABA).
- A range of potential probiotic bacteria shown to exhibit health benefits to gut, metabolic and cognitive health, based on cell-based bioassays, and in vivo studies are available as a result of this work.

Opportunity/Benefit:

The development of Functional Foods enriched in probiotic bacteria and beneficial microbial metabolites for health promotion and extension of the range of added value specialized functional foods and dietary ingredients with health claims.

Project number:
5274

Date:
Nov 2014

Funding source:
Science Foundation Ireland

Project dates:
Jan 2008 – Dec 2013

Collaborating Institutions:
University College Cork

Teagasc project team:
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Prof. Tony Ryan
Gene Dempsey
Brendan Murphy
Prof. Eamonn Quigley
Prof. Ted Dinan
Prof. John Cryan.

Compiled by:
Catherine Stanton

1. Project background:

The aims of this research were to identify and exploit human gut bacteria for production of a wide range of bioactives such as conjugated linoleic acid (CLA), short chain fatty acids (SCFA) and gamma-amino butyric acid (GABA). The genomes of the CLA-producing *Bif. breve* DPC 6330, and CLA, EPS and bacteriocin producing *Bif. longum* DPC 6315 strains were sequenced. The *Bif. longum* DPC 6315 was used for development of functional foods (yoghurt) enriched in an exopolysaccharide (EPS), with enhanced yoghurt texture, compared with control. Animal studies have investigated the impact of administration of acid CLA producing strains on host fatty acid composition, with an emphasis on adipose tissue and brain, and implications for gut microbial composition. We found that different CLA-producing members of the same species-B. *breve* strains influenced host fatty acid metabolism and gut microbiota composition in different ways suggesting that CLA-production by these strains is not solely responsible for their ability to modify host fatty acid composition following dietary supplementation. Strain-strain differences are also important factors with respect to modulation of the gut microbial community by ingested microorganisms. Furthermore, we have investigated the influence that different dietary fatty acids have on mechanisms underlying the development of obesity, diabetes and other risk factors described as 'The Metabolic Syndrome' and effects on the composition of the gut microbiota.

2. Questions addressed by the project:

- To investigate the microbiota for production of bioactive fatty acid metabolites.
- To determine the fate of microbial fatty acids in murine and porcine models.
- To mine the microbiota for production of neuroactive metabolites.

3. The experimental studies:

- Assessing the ability of a panel of human gut derived microbes to produce bioactives, including CLA, GABA, EPS and ability modulate the immune system via TLR-4.
- Molecular characterisation of the microbes of human gastrointestinal origin.
- Assessment of the probiotic potential of human infant gut derived bifidobacteria and lactobacilli.

4. Main results:

The main findings of the project were as follows:

Mining isolates from the human gastrointestinal tract:

infant gut

Twenty faecal samples were obtained from 10 preterm infants; faecal samples and gastric juice were taken on weeks 2 and 4 of age. DNA samples have been extracted from the faecal samples and 16s ribosomal compositional analysis was performed. The pH of the gastric juice was recorded and cytokine analysis of the gastric juice was performed. Isolates were screened for ability to produce conjugated fatty acids, EPS and neuroactive metabolites.

ileostomy samples

Six ileostomy samples were obtained from a 6 week old infant; samples were taken on a weekly basis on weeks 6 to 12. The ileostomy was reversed and intestinal continuity restored. Three faecal samples were collected from the infant at 16, 18 and 32 weeks of age. DNA was extracted from the ileal fluid and faecal samples. In order to investigate colonization at an early stage of life, a 16s ribosomal compositional study on the ileostomy samples was carried out using pyrosequencing. The ileostomy samples were plated on bifidobacteria and lactobacilli selective media to enumerate the culturable bacteria from these 2 genera from the samples and isolates were screened for ability to produce conjugated fatty acids, EPS and neuroactive metabolites.

colostomy samples

Eight colostomy samples were obtained from a 4 week old infant; samples were taken on weeks 4, 5, 6, 9, 10, 16, 21 and 28 of age. The infants had received a vaccination on week 9 and had changed from breast feeding to formula and solid food between weeks 21 and 28. DNA was extracted from the colon fluid and a 16s ribosomal compositional study on the colostomy samples was carried out using pyrosequencing. The colostomy samples were also plated on bifidobacteria and lactobacilli selective media to enumerate the culturable bacteria from these 2 genera from the samples and isolates were screened for ability to produce conjugated fatty acids, EPS and neuroactive metabolites.

human appendix

Seven appendix (incl. healthy, inflamed, child and adult samples) samples were collected and analysed for the microbial content by culturing-dependant

and culturing-independent sequencing techniques. Microbial culturing revealed the presence of a range of organisms within and adhering to the organ including *Lactobacillus salivarius*, *Lactobacillus plantarum*, *Bifidobacterium longum*, *Streptococcus mutans*, *Streptococcus anginosus*. Bacteriocin screening identified a *Lactobacillus salivarius* strain from within the appendix producing an active abp118 bacteriocin previously associated with gut isolates. 16S compositional analysis has been completed on the appendix samples 1–5 and on one 1 comparative stool sample. Bioinformatic analysis indicated considerable differences at Phylum (Firmicutes (31–74%), Actinobacteria (1–14%)) family and genus level between appendix samples.

Genome sequencing of CLA-producing strains

Bif. breve DPC 6330: The genome sequence of the CLA-producing *Bif. breve* DPC 6330 has been completed in draft. The draft genome includes 2,385,951 bases with a G+C content of 58.6%. A total of 1,881 protein-coding genes were predicted in the genome. Order of the assembled contigs was determined by mapping against the *B. breve* UCC2003 genome. The two genomes are similar in size, GC content and gene synteny. Variation in certain functional categories include regulatory protein content, putative mobile elements present (prophage-like elements) and in cell defence (restriction-modification and CRISPR) systems.

Bif. longum DPC 6315: The genome of the CLA-producing *Bif. longum* DPC 6315 was fully mined for potential probiotic traits. A *B. longum* lantibiotc operon was identified as was a putative EPS operon of ~20kb with a predicted priming glycosyltransferase (*wblE* gene). Screening for the presence of the *wblE* gene in a number of CLA producing *Bif longum* isolates revealed the presence of the gene in 5 of the 9 tested strains, two of which did not produce an evident EPS. An EPS was produced in 7% lactose broth by 5 of the 9 tested strains. Molecular characterisation of the EPS operon in *Bif. longum* DPC 6315 was undertaken. The *wblE* gene was cloned and expressed in an expression plasmid (pNZ44) under the control of constitutive promoter and was successfully expressed in a *Lb. paracasei* 338 host (non-EPS background) but did not produce an apparent EPS suggesting the importance of other genes in the operon. Disruption of the *wblE* gene in the *Bif. longum* DPC 6315 background is ongoing. The inability to make the strain competent to date has to be overcome to successfully knockout the gene in this strain.

Characterisation of EPS produced by *Bif. longum* DPC 6315

The EPS was soluble in water, was characterised using Gas Chromatography Mass Spectrophotometry and found to be a glucose-galactose type polysaccharide consisting of 60% glucose, 30% galactose and 5–10% each of rhamnose and mannose. It also contained glucosamine and traces of mannosamine. It contained inferred linkages of terminal glucose, terminal galactose, 3-linked glucose and 6-linked glucose in a ratio of 4:2:3:2 respectively. The EPS does not contain much branching and is similar to other bifidobacterial EPS that have been published.

EPS producing *Bif. longum* DPC 6315 and development of functional foods (yoghurt) enriched in an exopolysaccharide (EPS)

Yoghurt trials were performed with the *Bif. longum* DPC 6315 strain. 14% RSM + 1% yeast extract was fermented using *Bif. longum* DPC 6315 and CH1 starter cultures from CSK to produce a test yoghurt. *Bif. longum* DPC 6315 was inoculated at $1x10^9$ CFU/ml at the start of fermentation. The control yoghurt was made using CH1 cultures only. The syneresis of the test yoghurt was favourable being significantly lower than the control over 28 days. *Bif. longum* DPC 6315 had not reduced in numbers at the end of fermentation but decreased by ~2 log CFU/ml over 4 weeks at 4°C. There were no significant differences in lactic acid concentration or pH values for both the test and control yoghurts over the 4 weeks. Confocal laser scanning microscopy revealed that the EPS produced by *Bif. longum* DPC 6315 was located at the edges of the pores of the protein aggregates in the yoghurt.

Contrasting effects of *Bifidobacterium breve* NCIMB 702258 and *Bifidobacterium breve* DPC 6330 on the composition of brain fatty acids and gut microbiota

A study comparing the impact of CLA-producing *B. breve* (*B. breve* NCIMB 702258 *and B. breve* DPC 6330) on fat distribution and composition in mice, and the effect of these *B. breve* strains on the composition of the microbiota was conducted. It was demonstrated that the response of fatty acid metabolism to administration of bifidobacteria is strain-dependent, and further that strain-strain differences are important factors with respect to modulation of the intestinal microbial community by ingested microorganisms.

Comparison of the effects of different dietary fatty acids on development of the Metabolic Syndrome and implications for gut microbiota composition

A feeding trial (16 weeks) to assess the effects of dietary fatty acids from different high fat sources (palmitic acid, oleic acid, linoleic acid, linolenic acid) and a low fat, high sucrose diet on the development of The Metabolic Syndrome; particularly obesity, type 2 diabetes and non-alcoholic fatty liver disease has been completed. Significant increases in fat as a percentage of body composition from diets enriched with palmitic acid were found, and higher fasting glucose levels in this group which may indicate development of hyperglycemia. The cecum weight was significantly reduced in the diet enriched with palmitic acid compared with all other diets. Pyrosequencing was performed on cecal content which confirmed an effect of the HF diets on the composition of the microbiota.

5. Opportunity/Benefit:

The opportunity is the potential for the future generation of probiotic strains which may enhance human health. New scientific knowledge was generated on microbial bioactives with modulation of host fat composition, and host health, and new functional food developments. These achievements will further enhance the reputation of the APC in probiotic/functional food research. Highly-competent PhD researchers were trained, thereby contributing to the development of Fourth Level education in Ireland. Patents on CLA and GABA producing strains were filed for future commercial exploitation by Irish Industry.

6. Dissemination:

Main publications:

Mills, S., C. Stanton and R.P. Ross. 2009. Microbial production of Bioactives: from Fermented Functional Foods to Probiotic Mechanisms. Australian Journal of Dairy Technology Vol 64, No. 1 Feb 2009 41–49.

Coakley, M., S. Banni, M.C. Johnson, S. Mills, S., R. Devery, G.F. Fitzgerald, R.P. Ross and C. Stanton. 2009. Inhibitory effect of conjugated a-linolenic acid (CALA) from bifidobacteria of intestinal origin on SW480 cancer cells. Lipids: Volume 44, Issue 3:249–256.

Wall, R., R.P. Ross, C.A. Ryan, S. Hussey, B. Murphy, G.F. Fitzgerald and C. Stanton. 2009. Role of Gut Microbiota in Early Infant Development. Clinical Medicine – Pediatrics 2009:3 45–54.

Hennessy, A., R.P. Ross, R. Devery and C. Stanton. 2009. Optimization of a reconstituted skim milk based medium for enhanced CLA production by bifidobacteria. Journal of Applied Microbiology 106:1315–1327.

Wall, R., R.P. Ross, F. Shanahan, L. O'Mahony, C. O'Mahony, M. Coakley, O. Hart, P. Lawlor, E.M. Quigley, B. Kiely, G.F. Fitzgerald and C. Stanton. 2009. Metabolic activity of the enteric microbiota influences the fatty acid composition of murine and porcine liver and adipose tissues. American Journal of Clinical Nutrition 2009;89(5):1393–401.

Kearney, N., X.C. Meng, C. Stanton, J. Kelly, G.F. Fitzgerald, and R.P. Ross. 2009. Development of a Spray Dried Probiotic Yoghurt containing Lactobacillus paracasei NFBC 338. International Dairy Journal 19:684–689.

Dinan, T., L. Siggins, P. Scully, S. O'Brien, R.P. Ross and C. Stanton. 2009. Investigating the inflammatory phenotype of major depression: focus on cytokines and polyunsaturated fatty acids. Journal of Psychiatric Research 43(4):471–6.

Shanahan, F., C. Stanton and R.P. Ross 2009. Pharmabiotics: Bioactives from Mining Hose-Microbe-Dietary Interactions. Functional Foods Review June 2009 Vol.1 No.1 p.20–25.

Clarke, G., S.M. O'Mahony, A.A. Hennessy, R.P. Ross, C. Stanton, J.F. Cryan and T.G. Dinan. 2009. Chain reactions: early-life stress alters the metabolic profile of plasma polyunsaturated fatty acids in adulthood. Behav. Brain, Res. 14;205(1):319–21.

Marques, M.T., R. Wall, R.P. Ross, G.F. Fitzgerald, C.A. Ryan and C. Stanton, C. 2010. Programming Infant Gut Microbiota –influence of dietary and environmental factors. Current Opinion in Biotechnology 21:149–156.

Impact of exogenous factors in the development of allergy in infants: (EFRAIM)

Key external stakeholders:

Food manufacturers, dairy industry, pharmaceutical companies, research communities; public health agencies and health professionals; policymakers.

Practical implications for stakeholders:

- Consumption of raw or farm milk has recently been shown to have an inverse association with the development of allergies in children, when consumed in the first year of life. The incidence of allergy in children and adults is increasing consistently and has detrimental effects on the lives on affected individuals.
- Milk is pasteurized to extend shelf-life and to reduce the risk of milk borne pathogens, but pasteurization may affect some labile milk components currently unknown that have health benefits in terms of allergy prevention.
- Ingestion of probiotic bacteria may have beneficial effects on reducing allergy onset.

The aim of this study was to identify allergy protective components in raw milk and bacteria. Foods supplemented with allergy reducing components may in future contribute to reduced incidence of allergy. This project compared the effects of exposure of raw and pasteurised milk to different human cells, assessed a panel of gut bacteria for their potential for allergy prevention and assessed the bacterial populations of Irish milk using new technologies.

Project number:
5858

Date:
Nov 2012

Funding source:
EU Framework 7

Project dates:
Jan 2008 – Dec 2011

Collaborating Institutions:
University College Cork.

EFRAIM Cohort – "Mechanisms of early protective exposures on allergy development"

Teagasc project team:
Prof. Catherine Stanton (PI)
Prof. Paul Ross
Mr. Robert McCarthy
Dr. Susan Mills

External collaborators:
Prof. Ger Fitzgerald UCC
Prof. Johnathan Hourihane, CUH

Compiled by:
Catherine Stanton

Main results:

- A differential genetic response of human intestinal cells to raw and pasteurised milk was confirmed using microarray technology and cell based bioassays developed in EFRAIM.
- The casein fraction made from raw milk was found to harbour immunomodulating activity relative to casein from pasteurised milk, using cell based bioassays developed in EFRAIM.
- The microbiota composition of raw and pasteurised milk from several producers around Ireland were identified using new sequencing technologies.
- A range of bacteria of human intestinal origin were shown to exhibit immune altering properties using cell-based bioassays, which may contribute to our understanding of how the infant gut microbiota contributes to the healthy development of the immune system, thus reducing the potential for allergy development later in life.

Opportunity/Benefit:

From a scientific perspective, the identification of milk based bioactives for allergy prevention would represent a major breakthrough, while from an economic perspective the identification of dairy-based bioactives related to allergy prevention would lead to the development of added value specialized functional ingredients with health claims. The opportunity exists to further investigate the potential of milk components and bacteria to reduce the incidence of allergy in children and adults. This could lead to future nutritional solutions for allergy prevention.

1. Project background:

Atopic diseases linked to immune dysfunction are increasing at a rapid rate, with many blaming such increases in incidence on the "hygiene theory" and in so doing link it to (over)-food processing and in some cases heat processes such as pasteurization. Recent studies have demonstrated that children raised in environments where they are exposed to a large variety of microbial sources such as the farming environment are less likely to develop atopic allergy (Riedler et al., 2001; Rey et al., 2009). Many potential sources in the farming environment have been correlated with eliciting protective effects such as animal contact/sheds, dust in hay lofts and consuming raw unpasteurised cows milk in the first year of life (Lima et al., 2010; Rey et al., 2009; Riedler et al., 2001). While the exact nature of these protective components in raw milk are unknown, it is entirely logical that mothers milk contains such programming substances to direct and programme biological processes in the infant at a very early stage of life. Such "inbuilt intelligence" would be of particular relevance to education of the developing immune system where antigen exposure may create protection against disease in later life. The objectives of this project were to probe the scientific basis for the health attributes of raw versus pasteurized milk in relation to this health aspect and to uncover heat labile constituents present in raw milk that have a direct effect on the immune system and more specifically which are related to asthma development later in life. Therefore, the immune altering properties of raw milk were compared with pasteurized milk, using cell-based bioassays, with a view to identifying labile allergy protective components in raw milk.

2. Questions addressed by the project:

- What are the differential genetic responses of human intestinal cells to raw versus pasteurised milk?
- Does exposure to raw milk affect previously identified genes associated with the "allergy protective effect" of drinking raw milk *in-vitro*?
- Which milk/dairy fractions harbour immunomodulating activity?
- Does the immune altering nature of milk and milk fractions change with heat treatment?
- How does the microbiota composition of raw milk compare with pasteurised milk?
- Do *Lactobacillus* strains interact with the innate immune component Toll like receptor 4 (TLR4)?
- What are the immune altering properties of bifidobacteria isolated from infant gastrointestinal tract of infants?

3. The experimental studies:

- *In-Vitro* characterisation of the immuno-modulatory effects of Raw/Farm milk versus pasteurised milk exposures.
- Assessing the ability of a panel of lactobacilli to modulate the immune system via TLR-4.
- Global genetic comparison of human intestinal cells exposed to raw or pasteurised cows milk.
- Molecular characterisation of the microbial composition of raw and pasteurised cow's milk.
- Assessment of the immunogenic potential of human infant gut derived Bifidobacteria.
- Generation of a transfection based immune assay to assess milk fractions.

4. Main results:

The main findings of the project were as follows:

- Raw milk was seen to significantly ($p<0.05$) down regulate CD14 (previously associated with the allergy protective effect of raw milk consumption) compared to pasteurised milk and the controls used. Pasteurised milk was seen to upregulate ($p<0.05$) CD14 when compared to PBS controls. Raw milk stimulation resulted in a significant increase ($p<0.001$) in the cytokines IL-2 and IL-12p70 production with no significant difference observed in IL-10 production between milk exposures. A significant shift in the immunmodulatory potential of milk due to pasteurisation was demonstrated in this bioassay in particular following the down regulation of CD14 in PBMC's exposed to the raw milk samples and upregulation following exposure to the pasteurised milk samples. The significant increase in the Th1 associated cytokines IL-2 and IL-12p70 due to treatment with raw milk being observed in this experiment may support the theory of farm milk consumption eliciting an allergy protective effect.
- All lactobacilli tested for TLR 4 interacting capabilities were shown to induce inflammatory status in unstimulated cell models. A number of bacteria tested showed the ability to improve Lipopolysaccharide induced inflammation. A TLR-4 blocker was unable to alter the immuno-modulating effects elicited by the lactobacilli tested suggesting that there was no significant TLR-4 interaction occurring in this model. While this is not an exhaustive investigation on the ability of all *Lactobacillus* species to signal via TLR-4, the inclusion of commercial probiotics in this study would suggest that the main areas of bacteria host interaction by which probiotic lactobacilli infer their health promoting effects, lies outside TLR-4 interaction.
- The milk microbiota from Irish milk producers was determined using a number of culture-independent techniques, including flow cytometry, real-time qPCR and high throughput sequencing. The combination of the techniques employed reveals the presence of a previously unrecognised and diverse bacterial population in unpasteurised cow's milk. Most notably, the use of high throughput sequencing resulted in a number of bacterial genera being identified in milk samples for the first time. These included *Bacteroides, Faecalibacterium, Prevotella* and *Catenibacterium*. Our culture-independent analyses also indicate that the bacterial population of pasteurised milk is more diverse than previously appreciated but that non-thermoduric bacteria within these populations are likely to be in a damaged, non-culturable form. It is thus apparent that the application of state-of-the-art approaches can provide a detailed insight into the bacterial composition of milk and could potentially be employed in the future to investigate the factors that influence the composition of these populations. .
- Bifidobacteria strains isolated from infant fecal material, able to alter significantly gene expression of gene involved in the immune system in human intestine/immune system co-culture model were identified. A *Bifidobacterium breve* identified as a consistant coloniser of the infant gut showed significant proinflammatory potential in an immune cell only exposure model and was able to significantly induce the expression of both IL-6 and TNF alpha cytokines after 2 and 24 hour incubation. As a strain of bacteria that was shown to be persistent in colonising the infant gut from birth to 3 months of age, it is interesting that *B. breve* was shown to be so inflammatory. This may suggest that colonisation of the new born by immune stimulating bacteria is involved in the development of the local immune system and gut health.
- Milk fractions were generated for use in the *in vitro* transfection based bioassay. A differential immune effect was observed in response to raw or pasteurised whole casein in the bioassay. Whole casein fractions were further broken down and separated by size exclusion methods. Homogenised casein fractions were also generated in order to investigate the effect of homogenisation on activity previously described. The immune altering effect that was shown as a result of whole casein fractions was not reproduced for any one of the fractions tested. This maybe due to the loss of a synergistic effect of a number of components separated during fractionation process.

5. Opportunity/Benefit:

The opportunity is the potential for the future generation of probiotic strains which may reduce the incidence of allergy development.

As such this project would develop systems to reintroduce heat labile components back into milk and in so doing preserve much of the original health-associated attributes of milk while maintaining safety for the consumer. Importantly, the foods which result will be classed as Functional

Foods and as such will hold a premium in the marketplace. Given the growth which is occurring in this food sector and the projections for the future we expect that ingredients which carry a health claim such as reduction in risk of asthma development will contribute to our European Dairy Industry in having a competitive advantage in the future. At Teagasc Research Centre Moorepark, there is long standing expertise in innovative ingredient development from a technology perspective. This expertise will be brought to bare in the development of fractions of raw milk which retain an immunoreactivity. Once characterized in detail the active molecule(s) from these fraction(s) will be characterized at a molecular level and then developed in an ingredient format to be reintroduced into processed milk and dairy products.

6. Dissemination:

Main publications:

M.van Boekel, Vincenzo Fogliano, Nicoletta Pellegrini, Catherine Stanton, Gabriele Scholz, Sam Lalljie, Veronika Somoza, Dietrich Knorr, Pratima Rao Jasti and Gerhard Eisenbrand (2009). A REVIEW ON THE BENEFICIAL ASPECTS OF FOOD PROCESSING. Mol Nutr Food Res. Sep;54(9):1215–47.

Marques, T.M., Wall, R., Ross, R.P., Fitzgerald, G.F., Ryan, T. and Stanton, C. (2010). Programming infant gut microbiota: influence of dietary and environmental factors. Curr Opinion Biotech. Apr;21(2):149–56.

Mills S, Ross RP, Stanton C (2011). Milk Intelligence: Mining Milk for Bioactive Substances Associated with Human Health International Dairy Journal. 2011;21(6):377–401.

Quigley, L., O. O'Sullivan, C. Stanton, T.P. Beresford, R.P. Ross, G.F. Fitzgerald and P.D. Cotter. (2013). The complex microbiota of raw milk. FEMS Microbiol Rev 2013 37(5):664–98.

McCarthy R, Mills S, Ross RP, Fitzgerald GF and Stanton C (2014). Bioactive Peptides from Casein and Whey Proteins. (Book chapter) Milk and Dairy Products as Functional Foods (Ed. A. Kanekanian), First Edition, Published by John Wiley & Sons, Ltd.

Quigley, L., R. McCarthy, O. O'Sullivan, T.P. Beresford, G.F. Fitzgerald, R.P. Ross, C. Stanton and P.D. Cotter. (2013). The microbial content of raw and pasteurized cow's milk as determined by molecular approaches. J. Dairy Sci. 2013 96(8):4928–37.

R.J. McCarthy, R.P Ross, G.F. Fitzgerald, and C. Stanton (2015). The immunological consequences of pasteurisation: comparison of the response of human intestinally-derived cells to raw versus pasteurised milk. Int. Dairy Journal, 40: 1–6.

McCarthy R. (2013). PHD THESIS: The immunological consequences of pasteurization, Microbiology Department, University College Cork.

Health promoting bioactives from cider yeast

Project number:
5932
Date:
March, 2012
Funding source:
Enterprise Ireland
Project dates:
Nov 2008 – Oct 2010

Collaborating Institutions:
Cybercolors

Teagasc project team:
Prof. Catherine Stanton (PI)

External collaborators:
Mr. Noel Sexton
Cybercolors

Compiled by:
Catherine Stanton

Key external stakeholders:

Food manufacturers, dairy industry, pharmaceutical companies, research communities; public health agencies and health professionals; policymakers.

Practical implications for stakeholders:

Beta glucan is a bioactive polysaccharide which has FDA approval for the reduction of cardiovascular risk, the leading cause of death and morbidity in the EU. A cardioprotective diet enriched in dietary fiber, and in particular beta glucan is recommended to protect against the development of cardiovascular disease. Furthermore, food-derived ACE (Angiotensin-I-converting enzyme)-inhibitory peptides have been shown to reduce peripheral blood pressure and exert an antihypertensive effect *in vivo* following ingestion. In this project, bioactive components (ACE inhibitory/antihypertensive peptides and beta glucan) were isolated and characterised from Natural Yeast, which was a by-product of the cider production process.

Main results:

- Laboratory scale trials, involving autolysis and hydrolysis of spent cider yeast, were optimised for production of yeast extracts, enriched in free amino acids, flavour-enhancing components and bioactive ACE-inhibitory peptides.
- Pilot scale trials were performed but further technical trials are required.
- Economic and financial analysis of the prototype products developed in this project were undertaken, and results indicated that the process for their production (involving spray drying at 20%) was not commercially viable, with further technical trials required to overcome this difficulty.

Opportunity/Benefit:

The opportunity exists to further investigate the potential waste stream of Cider production in collaboration with industrial personnel. The research group benefited from improved links with industry (Cybercolors).

1. Project background:

A previous Innovation Partnership project had shown that bioactive ACE inhibiting peptides can be generated from Cider Yeast by enzymatic hydrolysis. Bioactive peptides are defined as 'food-derived components that in addition to their nutritional value, exert a physiological effect in the body'. Biological activities associated with bioactive peptides include immunostimulatory, antibacterial, antihypertensive and opioid-like properties. The Cell wall fraction produced as a result of autolysis of Cider Yeast Cells is also a source of naturally occurring beta glucan – a bioactive polysaccharide with cholesterol-lowering properties.

2. Questions addressed by the project:

- Could yeast extracts containing bioactive peptides be produced through hydrolysis with commercially available proteolytic enzymes?
- Could anti-ACE peptides be identified using *in-vitro* assays for anti-hypertension?
- Could the ACE-inhibitory peptides and yeast beta glucan be characterized using Reverse-Phase High Performance Liquid Chromatography (RP-HPLC), Molecular Size profiling, Mass Spectrometry and Sequencing?
- Could the products be evaluated for stability during processing?
- Could naturally occurring beta glucan and other galactomannans in Natural Apple Yeast be isolated and measured?
- Could the process be scaled up to pilot production of fractions with promising bioactive properties, for production of pre-commercial product?

3. The experimental studies:

- Method development for the release of yeast bioactive peptides through hydrolysis with commercially available proteolytic enzymes.
- ACE-inhibition assays to identify anti-ACE peptides for anti-hypertension from cider yeast.
- Reverse-Phase High Performance Liquid Chromatography (RP-HPLC), Molecular Size profiling, Mass Spectrometry and Sequencing for characterization of yeast-derived bioactive peptides.
- Method development for the isolation and measurement of naturally occurring beta glucan and other galactomannans in Natural Apple Cider Yeast.
- Pilot scale production of fractions exhibiting bioactive properties.

4. Main results:

This project involved the isolation and characterisation of bioactive components (ACE-inhibitory (antihypertensive) peptides and beta glucan from Natural Yeast) recovered as a by-product of the cider production process. The bioactive nature of ACE-inhibitory peptides was stimulated as a result of release via hydrolysis of the yeast substrate using proteolytic enzymes. It was then proposed to produce bioactive fractions exhibiting bioactive properties at pilot scale and their incorporation into functional foods and beverages. In addition, the concentrated cell wall by-product fraction of the bioactive peptide extraction process was investigated as a source of beta glucan, a bioactive polysaccharide which has FDA approval for the reduction of cardiovascular risk. During the course of this project, a protocol for autolysis/hydrolysis of spent cider yeast was developed and optimised at laboratory scale. Hydrolysis was performed with two commercial proteolytic enzymes in order to generate yeast extracts rich in free amino acids, flavour-enhancing components and bioactive peptides to pilot-scale.

- Laboratory scale trials involving autolysis and hydrolysis of spent cider yeast were optimised for production of yeast extracts, enriched in free amino acids, flavour-enhancing components and bioactive ACE-inhibitory peptides.
- Lab-scale trials were undertaken, in which yeast substrate (natural apple yeast) was autolysed and hydrolysed using a range of proteolytic enzymes for release of cell wall, and cell-free supernatant fractions, which were stabilised by freeze drying.
- Beta glucan yields were quantified in cell wall, and cell-free dried yeast powders and detected at high concentrations in the cell wall fraction, with similar concentrations as present in the commercially available reference beta-glucan sample.

- Mass balance for beta-glucan isolation completed, and process design for recovery from Natural Apple Yeast.
- Scale up and optimisation of process technologies for the large scale production of beta glucan rich fractions from autolysed/ hydrolysed cider yeast based on decanting centrifugation at pilot-scale.
- Pilot scale trials were performed using decanting technology to fractionate the cell wall and various yeast extract supernatants (arising from the different proteolytic enzyme combinations).
- Spray drying of the beta glucan rich cell wall retentates yielded inferior powders, due to the high water binding capacity of the cell wall material, thus leading to a highly viscous liquid, which could not be further concentrated by evaporation.
- Further lab scale trials were undertaken, in which various commercial enzymes were used in attempts to reduce viscosity, with reduction in viscosity obtained using carbohydrate-degrading enzymes Biocellulose and Depol 667P.
- Prototype cell wall powders were produced and assessed for economic and financial feasibility and assessed for market acceptability, with encouraging results and commitment of customers to taking 500–1000 kg of product, following testing and approval.
- Economic and financial analysis of the prototype products developed in this project were undertaken, and results indicated that the process for their production (involving spray drying at 20%) was not commercially viable, with further technical trials required to overcome this difficulty.

5. Opportunity/Benefit:

The opportunity is the availability of bioactive components which have positive impacts on human health, such as anti-ACE peptides and beta glucan from the waste stream of Cider production. The exploitation of these bioactive substances as functional food ingredients, using optimized fermentation technology, initially at lab scale, followed by optimized large scale production were the ultimate goals of the technical phase of this project. Protocols were developed for optimising yields of beta glucan yields in cell wall, and cell-free dried yeast powders (with similar concentrations recovered, as present in the commercially available reference b-glucan products). The economic prospects were promising for the beta glucan rich fractions arising from this project. Prototype cell wall powders produced within this project were assessed for economic and financial feasibility and market acceptability, with encouraging results and commitment of customers to taking 500–1000 kg of product, following testing and approval.

6. Dissemination:

The research findings have been made available to the Industrial partner and to stakeholders, particularly the Irish food industry for applications.

Project number:
5550
Date:
November 2014
Funding source:
DAFM
Project dates:
Mar 2005 – Sept 2008

Collaborating Institutions:
University College Cork

Teagasc project team:
Prof. Paul Ross
Ms. Paula O'Connor

External collaborators:
Prof. Alan Kelly

Compiled by:
Sheila Morgan

The milk proteome: a tool for understanding milk quality and functionality

Key external stakeholders:

Cheese manufacturers.

Practical implications for stakeholders:

This study has a very high relevance for the Irish cheese industry, and its need to supply high quality products over the whole year. As milk composition changes over the lactation cycle, milk at late lactation stage is less suitable for cheese manufacturing due to the changing plasmin levels.

Main results:

- We clearly demonstrated differences in proteolysis in cheeses made from milk taken over different stages of the lactation cycle.
- From this study, it could be seen that there are significant changes in the profile over the lactation cycle and, while similar studies have been done on this topic, the application of proteomic tools gives another a deeper insight into the specific changes occurring due to proteolysis.
- Proteomics is a very helpful tool to characterize the differences between cheese samples during ripening and also over lactation.

Opportunity/Benefit:

This project has developed significant additional research capacity in a very new field (proteomic analysis of food systems) which offers new advanced analytical capability of interest in the context of a range of new research project areas, including analysis by food companies. In addition, the project involved applying these tools to applied research questions of direct scientific and industrially-relevant interest (e.g., impact of seasonality and somatic cell count on dairy

product quality). Additional knowledge on milk quality issues is of indirect economic impact by providing additional knowledge for dairy companies in Ireland.

1. Project background:

The objective of this project was to use proteomic tools to explore aspects of the milk protein and enzyme system of scientific and industrial significance. The term 'proteomics' refers to a family or toolkit of techniques relatively recently developed, to separate and identify complex mixtures of proteins. While this approach has been adopted in many fields of biological research, it has not been extensively used for analysis of dairy proteins (which can be very complex in composition and interactions in dairy products), and this project was designed to exploit its advantages for this purpose.

Throughout the project, the proteomic tools developed have been shown to be an excellent and precise approach for analysing milk quality, in terms of proteins and enzymes present. Milk contains a complex protein and enzyme system, and the huge increase in resolving power, and potential for characterisation, offered by proteomic tools presents a much more vivid and rounded picture of the milk protein system, and in particular, changes induced either on the farm or due to processing.

2. Questions addressed by the project:

- Can we use proteomic tools to explore compositional changes in milk over a complete lactation?
- Can we use proteomic tools to investigate changes in cheese protein/peptides during ripening?

3. The experimental studies:

During this project, milk over a whole lactation cycle was obtained from the experimental herd in Moorepark at approximately monthly intervals, and Cheddar cheese was manufactured on a one liter scale. The composition was analysed after one day and the cheese was ripened for 6 months; the ripening process was studied by Urea-PAGE and 2-DE, as well as plasmin activity. It could clearly be seen that there are differences in proteolysis of the different cheeses made over the lactation cycle which, despite the small scale of the study, was the first such demonstration. This study thus has a very high relevance for the Irish cheese industry, and its need to supply high quality products over the whole year. The milk composition changes over the lactation cycle and milk at late lactation stage is less suitable for cheese manufacturing. From this study, it could be seen that there are significant changes in the profile over the lactation cycle and, while similar studies have been done on this topic, the application of proteomic tools gives another insight into this topic. 2-DE is a very helpful tool to characterise the differences between cheese samples during ripening and also over lactation.

4. Main results:

- There were clearly differences in the protein/peptide composition of milk samples obtained over lactation stage.
- The major compositional difference between cheeses manufactured at different stages of lactation was moisture, which correlated positively with advancing lactation.
- One- and 2-D gel electrophoresis showed proteolysis during 6 months ripening was more developed in later lactation stages.
- The proteomic patterns of Cheddar cheeses produced at different lactation stages suggests that the variability in hydrolysis of caseins and derived low molecular mass products in cheese is mainly due to plasmin.

5. Opportunity/Benefit:

This project has developed significant additional research capacity in a very new field (proteomic analysis of food systems) which offers new advanced analytical capability of interest in the context of a range of new research project areas, including analysis by food companies. The findings of this research may be of interest to the cheese manufacturers, as the seasonality of milk supply in some countries cannot guarantee the production of high quality products in certain months.

6. Dissemination:

Main publications:

Hinz K, O'Connor PM, O'Brien B, Huppertz T, Ross RP, Kelly AL. (2012). Proteomic study of proteolysis during ripening of Cheddar cheese made from milk over a lactation cycle. J Dairy Res. 2012 May;79(2):176–84.

Hinz K, O'Connor PM, Huppertz T, Ross RP, Kelly AL. (2012) Comparison of the principal proteins in bovine, caprine, buffalo, equine and camel milk. J Dairy Res. 2012 May;79(2):185–91.

Genetic Tools for Improvement of Food Cultures

Project number:
5027

Date:
November 2014

Funding source:
Teagasc

Project dates:
Mar 2001 – Dec 2007

Collaborating Institutions:
University College Cork
Cork Institute of Technology

Teagasc project team:
Prof. Paul Ross
Dr. Olivia McAuliffe
Dr. Susan Mills
Dr. Debebe Alemayehu

External collaborators:
Prof. Gerald Fitzgerald
Dr. Aidan Coffey

Compiled by:
Sheila Morgan

Key external stakeholders:

Starter culture suppliers, fermented dairy food producers, dairy research community.

Practical implications for stakeholders:

- A food-grade cloning system for application in food safety and food quality arena.
- A system where genes for key metabolic and industrial traits can be under-/over-expressed.
- Characterisation of the phage resistance determinants for food-grade improvement of starter cultures.
- Identification of the PyrR gene as a target for the development of bacteriophage resistance strategies in starter cultures.

Main results:

- We over-expressed the plasmid-borne Mg^{2+}/Co^{2+} transporter and investigated it's potential as a marker gene for direct insertional inactivation in lactococci.
- We identified the genetic determinants involved in phage resistance in the conjugative lactococcal plasmid pMRC01.
- We identified the *pyrR* gene as a potential target for improvement of phage resistance properties in starter cultures.

Opportunity/Benefit:

The use of recombinant food cultures requires a food-grade approach to the design of systems for their genetic manipulation. The Genetic Tools for Improvement of Food Cultures program focused on the development of safe and sustainable genetic manipulation systems for various food-grade fermentative and probiotic bacteria. Given that the ultimate aim of research on the biotechnology of food cultures has been to genetically improve strains for food use, much of the focus in recent years has been on the development of self-cloning systems, which rely on genetic elements naturally occurring in the genus. However, the presence of inherent background resistances associated with many of these food-grade markers has placed limitations on their use, something we address in this project. Additionally the wealth of genomic data and information available can be viewed as a positive resource and can be mined for gene-finding strategies.

1. Project background:

Food-grade cloning systems totally exclude the use of foreign DNA in molecular manipulation by using only host DNA or the DNA from closely related food bacteria. This food grade cloning approach is essential in the improvement of bacteria as cell factories (producing a range of valuable metabolites impacting on food flavour, human nutrition and health) for food use, especially since the resulting strains may not be considered as "GMOs" as described in EU Directives 219 and 220.

2. Questions addressed by the project:

- Can we target selectable marker systems for food-grade DNA vectors?
- Can we apply these systems in a range of starter bacteria?
- Can we exploit genomics to find genes encoding key metabolic and industrial traits?

3. The experimental studies:

In developing food-grade genetic tools for lactococcal cultures, we have identified and characterised the Mg2+/Co2+ transport system, CorA, from the lactococcal plasmid pAH90. CorA is the principal Mg^{2+} transport system among the eubacteria. It is also involved in the uptake of Co^{2+}, and hence its presence can be lethal where environmental Co^{2+} concentrations are above a certain threshold. Genetic determinants for CorA activity, comprising the genes **orf18** and **corA**, are found on the lactococcal plasmid, pAH90. Insertionally inactivating the pAH90 **orf18/corA** determinants in the presence of Co^{2+} allows cells to grow in concentrations that are otherwise toxic to the cell. We report on the potential of using the lactococcal Mg^{2+} and Co^{2+} transport determinants as a tool for genetic manipulation of lactococci to eliminate background resistances when they are used in conjunction with another marker. In the absence of *corA*, L. lactis NZ9800 is capable of growth in $CoCl_2$ to concentrations of 3–4 mM. In the presence of the *corA* gene and the orf preceding *corA*, *orf18*, these concentrations of $CoCl_2$ are inhibitory. It was found that *corA* is inactive in the absence of *orf18*, a gene encoding a protein of unknown function. Based on the sensitivity phenotype observed, the use of *corA* as a tool for screening clones in *Lactococcus* through insertional inactivation was investigated. Insertional inactivation of the *corA* gene in the presence of $CoCl_2$ allows cells to grow at a concentration of cobalt which would otherwise be lethal to the cell. The lacticin immunity gene was selected for cloning in the *corA/orf18* construct, due to our ability to monitor the phenotype of this gene in addition to the insertional inactivation. We observed that only cells with *ltnI* cloned into the *corA* gene were capable of growth on $CoCl_2$ at a concentration of 2.5 mM. Furthermore, *ltnI* was functional in this construct. This system has the potential to provide an added advantage to current food-grade selectable markers often associated with background resistance by eliminating these resistances and increasing the rate of identification of recombinant clones and the potential of this system has been successfully demonstrated in certain starter lactococci.

An abortive infection mechanism has been previously associated with the fully sequenced, 60.2 kb, conjugative plasmid, pMRC01 (Coakley et al., 1997; Ryan et al., 1996). The mechanism has been shown to target the phage-lytic cycle at a point after phage DNA replication. Plaques formed by the large, prolate-headed phage c2 on L. lactis MG1363 cells harbouring pMRC01 are approximately six-fold smaller than the plaques formed on the MG1363 host itself. The exact location of this Abi mechanism remained unknown. We have identified four genes involved in the Abi mechanism of pMRC01 by a sequential gene-knockout approach that exploits the conjugative nature of the plasmid. In total, four orfs (49, 50, 51, and 52) were linked to the phage resistance phenotype, two of which are homologous to regulatory proteins while the other two encode putative transmembrane proteins.

To exploit the wealth of genomic information generated from starter bacteria (most notably L. lactis IL1403) whole genome microarrays have been used as tools in 'gene-finding' strategies. We exploited DNA microarrays to mine the lactococcal IL1403 genome for genes responsive to infection with the large prolate-headed lactococcal phage c2. This allowed us to identify and characterise potential targets for new anti-bacteriophage strategies. Using these arrays, the global gene expression profile of Lactococcus in response to infection by c2-type and 936-type phages has been evaluated. This approach was used to mine the IL1403 genome for genes that are positively regulated upon infection with phage c2.

4. Main results:

- Insertional inactivation of the plasmid-encoded determinants for Mg^{2+} and Co^{2+} transport, *orf18/corA*, provides a tool for screening recombinant clones in *Lactococcus*, based on the observation that overexpression of *orf18/corA* results in cell growth inhibition on certain concentrations of $CoCl_2$.

- We identified the potential of using the lactococcal Mg^{2+}/Co^{2+} transport determinants as a tool for insertional inactivation in lactococci, by eliminating background resistances when used in conjunction with another food-grade marker.
- We overexpressed the plasmid-borne Mg^{2+}/Co^{2+} transporter and investigated its potential as a marker gene for direct insertional inactivation in lactococci and can make the following overall conclusions.
- (i) CorA requires the preceding Orf18 for Co^{2+} transport activity, and they function as a gene pair, which occurs in at least three other lactococcal strains.
- (ii) Strains overexpressing the **orf18/corA** determinants cannot grow on $CoCl_2$ concentrations of >2.0 mM, whereas strains in which **orf18** has been replaced with **ltnI** and **corA** has been truncated can grow in concentrations greater than this value.
- (iii) Since the spontaneous resistance of lactococci expressing **orf18/corA** on solid media after transformation (in the absence of nisin) is less than 1 in 10^9 CFU, the system could be used to identify clones in which the gene has been disrupted.
- (iv) The system should be applicable to the vast majority of dairy lactococci given the low incidence of plasmid-borne **corA**.
- The potent Abi mechanism associated with the conjugative plasmid pMRC01 involves a four-gene operon, two of which are homologous to regulatory proteins while the other two encode putative transmembrane proteins.
- Induction of this Abi operon resulted in a slower growth rate for the culture.
- Mining the *Lactococcus lactis* IL1403 genome array showed that a number of genes differentially regulated upon phage c2 infection were directly involved in the *de novo* synthesis of pyrimidine nucleotides; *pyrE, pyrF, carB, pyrR, pyrP* and *pyrB*, demonstrating that infecting phage enhance host enzymes involved in nucleotide biosynthesis to generate precursors for phage DNA synthesis.
- It appears that PyrR, the regulator of pyrimidine synthesis in *Lactococcus*, is a limiting factor for phage replication, by limiting the production of pyrimidines. The *pyrR* gene is a potential target for improvement of the phage resistance of food cultures.
- Overexpression of PyrR allows starter acidification rates to be controlled.

5. Opportunity/Benefit:

We have demonstrated the potential of using the lactococcal Mg2+/Co2+ transport genetic determinants as a tool for insertional inactivation in lactococci, by eliminating background resistances when used in conjunction with another food-grade marker. This system provides an added advantage to current food-grade selectable markers often associated with background resistance, by eliminating these resistances and increasing the rate of identification of recombinant clones and the potential of this system has been successfully demonstrated in certain starter lactococci.

The potent Abi mechanism encoded on pMRC01 can be transferred in a food-grade manner into a variety of starter cultures, leading to the concomitant improvement in their phage resistance.

6. Dissemination

Main publications:

Mills S, Coffey A, Hill C, Fitzgerald GF, McAuliffe O, Ross RP. 2005 Insertional inactivation of determinants for Mg2+ and Co2+ transport as a tool for screening recombinant Lactococcus species clones. Appl Environ Microbiol. 2005 Aug;71(8):4897–901.

Mills S, Coffey A, McAuliffe OE, Meijer WC, Hafkamp B, Ross RP. 2007 Efficient method for generation of bacteriophage insensitive mutants of Streptococcus thermophilus yoghurt and mozzarella strains.

Mills S, McAuliffe OE, Coffey A, Fitzgerald GF, Ross RP. 2006 Plasmids of lactococci – genetic accessories or genetic necessities? FEMS Microbiol Rev. 2006 Mar;30(2):243–73.

Trotter, M., McAuliffe, O., Callanan, M., Edwards, R., Fitzgerald, G. F., Coffey, A. Ross, R. P. 2006. Genome analysis of the obligately lytic phage 4268 of Lactococcus lactis provides insight into its adaptable nature. Gene. 366 (1): 189–199.

McAuliffe, O., Ross, R. P. and Fitzgerald, G. F. 2006. The new phage biology: from genomics to applications. In Bacteriophage: Genetics and Molecular Biology, Horizon Scientific Press.

Development of a highly functional cheese sauce

Project number:
5115
Date:
May, 2011
Funding source:
DAFM
Project dates:
July 2003 – June 2006

Key external stakeholders:

Dairy Industry, Food Manufacturers.

Practical implications for stakeholders:

- Ultra High Temperature (UHT) and *sous vide* cheese sauces were developed.
- A new process to create concentrated cheese flavours was developed. This new process allows a diverse range of concentrated cheese flavours to be developed from base dairy substrates.
- In addition a spray dried concentrated cheese flavour was also in production and information as to minimize losses of volatile key flavour compounds was highlighted.

Main results:

- A method for producing retort cheese sauces.
- Novel method for the production of concentrated cheese flavours.
- A greater understanding of the flavour potential and use of dairy cheese lactic acid starter bacteria in the production of concentrated cheese flavours.
- How manipulation of pH can impact on losses of key volatile cheese flavour compounds during spray drying.
- How manipulation of pH can impact on sensory perception.

Opportunity/Benefit:

Consultancy and contract research opportunities are available to both national and international clients in the area of enzyme-modified cheese and concentrated cheese flavours.

A HPLC method to quantify short chain volatile free fatty acids was developed and is now available as a technical service to industry.

A detailed one-two day course on all aspects of enzyme-modified cheese has been developed and is available to industry on request.

Collaborating Institutions:
None

Teagasc project team:
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Dr. Diarmuid Sheehan
Mr Jim Kelly
Dr. Brendan O'Kennedy

External collaborators:
None

Compiled by:
Kieran N Kilcawley

1. Project background:

Cheese sauce is widely used in the convenience food sector in pre-prepared and prepared meals. In addition to providing flavour, cheese sauces are often required to provide functional and visual roles, i.e. mouth-feel, texture and colour. Cheese sauce is mainly comprised of cheese powder (dehydrated cheese), emulsifying salts and fillers such as starch, whey, flour, buttermilk, maltodextrins or skim milk. Cheese powder is the most expensive ingredient in the cheese sauce formulation and it is envisaged that this cost could be reduced by replacing a proportion of the cheese powder with natural concentrated cheese flavours, such as enzyme-modified cheese. Concentrated natural cheese flavours are typically produced by enzymatic hydrolysis of cheese curd under controlled conditions. The main advantages of natural concentrated cheese flavours over natural cheese, nature identical flavours and synthesized chemical cheese flavours are as follows: 100% natural, produced cost-effectively, have long shelf lives, consistent flavour and texture; much more intense flavours than natural cheese. The aim of this project was to produce high quality natural concentrated cheese flavours or enzyme-modified cheeses, minimize losses of volatile cheese flavour compounds during drying and to incorporate these natural flavours into cheese sauce formulations.

2. Questions addressed by the project:

- Develop concentrated cheese flavours from base dairy ingredients and utilize lactic acid bacteria and exogenous enzymes.
- Development of process for spray drying enzyme-modified cheese with minimum loss of important volatile flavour compounds.
- Formulation of cheese sauce containing enzyme-modified cheese as a replacement for a part of the cheese powder.
- Evaluation of novel cheese sauce functionality & sensory characteristics.

3. The experimental studies:

Sodium caseinate and skim milk powder were used to create a 10 % protein base substrate. This was pre-hydrolysed with commercial enzymes (proteinase and peptidase) to a specific level of proteolysis. Five commercial starter cultures used as adjunct cultures in Natural Cheddar cheese production were selected on the basis of their low acidification rates at the reaction temperatures of the process chosen to enhance the background “cheesy” notes from this hydrolysed protein base. Dose rates were optimized to give a specific level of secondary proteolysis after incubation under controlled conditions. These products were subsequently evaporated to higher solids and spray dried with sodium caseinate using an anhydrous pilot scale drier to create dried natural cheese concentrates. Another fat based product was created by blending sterile water and anhydrous butterfat and hydrolysing with a commercial lipase under controlled conditions to a specific level of free butyric acid. The hydrolysed fat formed two separate phases on cooling, a clear liquid phase with high concentrations of water soluble free fatty acids and a solid phase with high concentrations of water and fat soluble fatty acids, mono-, di- and tri-acylglycerides. This solid phase was homogenized with skim milk powder and spray dried using an anhydrous pilot scale dryer resulting in a final product with a fat in dry matter of > 50%. The liquid phase was pre-blended with maltodextrin and skim milk powder and spray dried. Losses of key volatile free fatty acids during drying were minimized by increasing the pH prior to drying to convert them into insoluble sodium salts. This simple pH adjustment significantly minimized losses of butyric acid. By blending different ratio’s of these dried protein and fat products, a range of different concentrated cheeses flavours were produced. Selected cheese flavours were then incorporated into a specific formula to create different types of cheese sauces, also consisting of sodium caseinate, anhydrous butterfat, water, sodium chloride and emulsifying salts. The impact of varying pH on the sensory perception of this formula was evaluated. Ranked preference analysis identified a preference for products at lower pH and that statistically differences existed between the products at different pH’s. A sous vide cheese sauce which can be stored at refrigeration temperatures for 10 days and a ultra high temperature (UHT) product with a shelf life of 3 months at room temperature were created using a Barriquand Steriflow Cooker. The sensory rheological characteristics were evaluated after production over storage.

4. Main results:

- A method for producing retort cheese sauces.
- Novel method for the production of concentrated cheese flavours.
- A greater understanding of the flavour potential and use of dairy cheese lactic acid starter bacteria in the production of concentrated cheese flavours.
- How manipulation of pH can impact on losses of key volatile cheese flavour compounds during spray drying.
- How manipulation of pH can impact on sensory perception.

5. Opportunity/Benefit:

Technical Service:

A HPLC method to quantify short chain volatile free fatty acids was developed and is now available as a service to industry.

Training Course:

A detailed one-two day course on all aspects of enzyme-modified cheese has been developed and is available to industry on request.

Consultancy/contract research:

Consultancy and contract research opportunities are available to both national and international clients in the area of enzyme-modified cheese and concentrated cheese flavours.

6. Dissemination:

Open Day/WorkShops:

Relay Workshop 29 Moorepark: Cheese Ingredients in Consumer Foods.

New Generation EMCs K. Kilcawley.

Minimising Flavour Loss D. Sheehan.

NIZO Food Research Ede, Netherlands:

New Developments in cheese science and technology – K. Kilcawley.

Main publications:

Kilcawley, K.N., Wilkinson, M.G & Fox, P.F. (2006) 'A novel two-stage process for the production of enzyme-modified cheese' *Food Research International*, 39, 619–627.

Lee, B.H., Kilcawley, K.N., Hannon, J.A., Park, S.Y., Wilkinson, M.G & Beresford, T.P (2007) 'The use of viable and heat-shocked Lactobacillus helveticus DPC 4571 in Enzyme-Modified Cheese Production' *Food Biotechnology*, 21, 149–143.

Technology Updates

Food Chemistry and Technology

Project number:
5949
Date:
November, 2014
Funding source:
DAFM
Project dates:
Oct 2008 – Feb 2014

Re-engineering process technology for the manufacture of infant formula

Collaborating Institutions:
University College Cork

Teagasc project team:
Dr. Mark Fenelon
Eoin Murphy
Dr. John Tobin
Dr. Mark Auty
Dr. Sean Hogan

External collaborators:
Prof Yrjo Roos, University College Cork.

Compiled by:
Mark A. Fenelon

Key external stakeholders:

- Dairy Ingredients and Infant Formula Sector.
- Dairy Processing Equipment Manufactures.
- Academic and Research Institutions.

Practical implications for stakeholders:

The study aimed to re-engineer process technology for the manufacture of infant milk formula (IMF) by modification of formulation dynamics and use of steam shockwave injector (Maklad-Fluid GmbH) technology:

- A greater understanding of the impact of macronutrient interaction (upon heating) on viscosity during IMF manufacture has been achieved and can be utilised for new formulation development.
- High solids infant formulations can be processed using a shockwave steam injector.
- IMF concentrate manufactured with a selectivity hydrolysed whey protein ingredient has application in high dry matter processes for reduced energy costs and more sustainable processing.

Main results:

The study demonstrated that heat-induced changes in infant formula associated with whey protein (denaturation, viscosity) are not only a function of concentration but are also dependent on interactions between macronutrients. Selectively hydrolysed proteins were shown to be an effective way of reducing viscosity, while maintaining good emulsification capacity, in heat-treated high solids concentrates of 1st age (0 – 6 months) infant formula. A new energy efficient high solids process for manufacture of infant formula with lower viscosity was developed using a shockwave steam injector.

Opportunity/Benefit:

The research provided a platform for understanding the heat-induced changes associated with macro-nutrient interactions in IMF for development of new formulations. In addition, technology has been developed for processing formulations at high solids using novel energy efficient approaches based on new ingredients and processing techniques. The new knowledge / process can be exploited by end users i.e., ingredient manufactures and infant, adult and medical nutritional beverage sectors.

1. Project background:

Infant formula (IMF) manufacture plays a significant role in the Irish dairy industry as a large consumer of milk and high quality dairy ingredients. The scale of operation is such that multinational IMF companies involved provide a vital channel to market across large geographical regions for Irish dairy processors and their ingredients. The current project provides latest research in ingredient and process innovation to support and build research capability for both the ingredient and infant formula sector.

2. Questions addressed by the project:

The project aims to address the need to manufacture ingredients / infant formula in a more sustainable and energy efficient way. The project provides new knowledge and mapping techniques on how protein ingredients interact with other nutrients within an infant formula process. The new process is based on a high solids approach whereby manufacturers bypass processing steps such as the evaporator to reduce operation costs.

3. The experimental studies:

The project consisted of three distinct experimental phases:

(i) Studies to understand the consequences of the interactive effects of macro-nutrients (protein, fat and carbohydrate) on hydration of ingredients, lactose solubility, and the subsequent colloidal stability during processing and spray drying

(ii) Investigation of new technological approaches (high shear shockwave injector) for manufacture of infant formula at high total solids content (> 55% solids)

(iii) Development of innovative intermediate protein ingredients to support the process re-engineering objectives of (i) and (ii) above

4. Main results:

- Thermal behaviour of macronutrients (casein, whey, lactose, fat) was studied, in isolation and combination, over a range of concentrations. Addition of phosphocasein to whey protein solutions elevated the denaturation temperature of β-lactoglobulin. Secondary structural changes in whey proteins occurred at higher temperatures in dispersions containing phosphocasein, however, the final extent of viscosity increase was similar to that of whey protein alone. Addition of lactose to whey protein solutions delayed secondary structural changes, increased denaturation and reduced viscosity post-heat treatment.
- A new energy efficient high solids process for manufacture of infant formula was developed using a shockwave steam injector. This study evaluated the use of an inline rotor-stator mixer (Ytron™) followed by direct steam injection to disperse and heat-treat (110°C, 3 s) high-solids (60% w/w) formulations, for the production of powdered infant milk formula. Formulations subjected to the steam injection process had significantly ($P<0.05$) lower viscosity compared to control formulations, made using indirect heat treatment, at equivalent solids contents (55% w/w); this was partly attributed to lower levels of whey protein denaturation. Prior to spray drying, volume mean particle size of high-solids steam injection processes was not significantly different ($P>0.05$), 2.04±0.22 than the control, 1.82±0.04 µm. Powders produced using the new process had statistically similar surface free fat content, wettability and dispersibility to the control powder. The study showed that it is possible to produce quality model infant milk formula powders from a high-solids concentrates while considerably reducing process complexity.
- Selectively hydrolysed proteins were shown to be an effective way of reducing viscosity during concentration / heating, while maintaining good emulsification capacity, in heat-treated high solids wet-mixes of a 1st age infant formulations. Heat-treated and homogenised wet-mixes containing hydrolysed ingredients had significantly ($P < 0.05$) lower viscosities than formulations containing non-hydrolysed ingredients. Ingredients in which b-Lactoglobulin was selectivity hydrolysed resulted in wet-mixes with lowest viscosity. The reconstitution properties of powders made from selectively hydrolysed wet-mixes were comparable or better than those powders made using intact, non-hydrolysed wet-mixes. Production of low viscosity wet-mixes, using selectively hydrolysed whey proteins, provides a potential mechanism for drying IMF at high dry matter content while maintaining good concentrate atomisation and ensuing powder functionality (flowability, wettability).
- Process mapping techniques have been developed to monitor the behaviour of dairy ingredients in different product formulations. These techniques have been used for troubleshooting in commercial applications.

5. Opportunity/Benefit:

The project provides new understanding and scientific methodologies related to the re-engineering of processes for manufacture of infant formula and also the incorporation of novel protein ingredients into infant formulations. The new knowledge should help ingredients and infant formula companies to continue to develop research capabilities, placing them at the forefront of international research in these areas. A parallel objective is to support development of functional ingredients by Irish ingredient manufacturers for use in infant and adult formulated beverages. Opportunities for the ingredients and infant formula sector include a new process for high solids processing and detailed information on incorporation of new protein ingredients into nutritional beverages. The new process developed in this project is highly exploitable as it can provide companies with the ability to manufacture food/beverage formulations in an energy efficient and sustainable way. In addition, the project has generated many research tools such as predicative models and methodologies for ingredient suppliers to incorporate ingredients into nutritional beverage formulations.

6. Dissemination:

Presentations

Scientific workshop 19th April 2011: Opportunities to grow the infant milk formula sector, the technical capability within Teagasc and UCC: Title of presentation: Re-Engineering Process Technology for the Manufacture of Infant Formula. Presented by M. A. Fenelon.

The effect of high velocity steam injection on the colloidal stability of concentrated emulsions for the manufacture of infant formulations. Abstract at 11th International Congress on Engineering and Food, May 22–26 2011, Athens – Greece. Presented by Eoin G. Murphy.

Invited speaker at International Conference on Functional Dairy Foods (ICFDF 2011). 'New High Solids Process for Infant Formula Manufacture'. Held in Karnal, India 16th to 19th November 2011. Presented by M. A. Fenelon.

Teagasc Walsh Fellow Seminar, Re-Engineering Infant Formula Manufacture. 2011. Presented by E. G. Murphy.

Invited speaker at 5th IDF/INRA International Symposium on SDDP. Title of presentation "High solids processing of infant formula using a shockwave steam injector". Venue: St. Malo, France 19th to 21st June 2012. Presented by M. A. Fenelon.

Lactose solubility in concentrated protein systems measured by refractometry. Poster at Sustainable Dairy Technology Conference, UCC, September 2012. Presented by Eoin G. Murphy.

Murphy, E.G., and M.A. Fenelon. Relay Research UpDate: New process technology aimed at nutritional beverage manufacturers, project code FQ013.

Main publications:

1. Murphy, E.G., J.T. Tobin, Y.H. Roos and M.A. Fenelon. 2011. The effect of high velocity steam injection on the colloidal stability of concentrated emulsions for the manufacture of infant formulations. Procedia Food Science 1; 1309–1315

2. Murphy, E.G., J.T. Tobin, Y.H. Roos and M.A. Fenelon. 2013. A high-solids steam injection process for the manufacture of powdered infant milk formula. Dairy Science & Technology Vol 93; Issue 4–5 pp 463–475

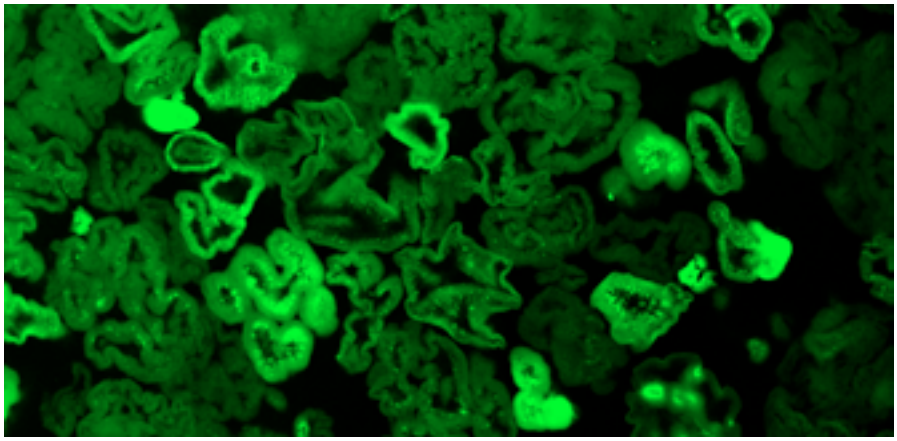
3. Murphy, E.G., M.A. Fenelon, Y.H. Roos and S. A. Hogan. 2014. Decoupling Macronutrient Interactions during Heating of Model Infant Milk Formulas. Journal Agricultural & Food Chemistry 62, 10585–10593

4. Murphy, E.G., Y. H. Roos, S. A. Hogan, P. G. Maher, C. G. Flynn, and M. A. Fenelon. 2015. Physical stability of infant milk formula made with selectively hydrolysed whey proteins. International Dairy Journal 40; 39–46

Popular publications:

T-Research Article – The infant formula sector in Ireland. M Fenelon and Phil Kelly. Volume 5: Number 4. Winter 2010

In-situ starch modification in food formulations using protein



Project number:
5950
Date:
Nov, 2014
Funding source:
DAFM
Project dates:
Nov 2008 – Feb 2014

Collaborating Institutions:
University College Cork

Teagasc project team:
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Brid Treacy
Dr. Valérie Chaurin
Dr. Joseph Kehoe

External collaborators:
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Prof Alan Kelly
Dr. Seamus O Mahony

Compiled by:
Mark Fenelon

Key external stakeholders:

- Dairy ingredients and Starch Industry.
- Prepared foods and Nutritional beverage manufacturers.
- Academic and Research Institutions.

Practical implications for stakeholders:

The objective was to study the behaviour of mixed protein-starch systems with a view to understanding protein starch interactions as a possible mechanism for in-situ alternation to starch functionality.

- Structure of the starch pastes can be altered by the presence of the proteins (intact or hydrolysed).
- Gelatinisation temperature of starch and denaturation temperature of proteins can be synergistically used to create new food structures.
- A novel rheological reactor cell can be used for simultaneous measurement of viscosity and in-vitro digestion of protein-starch mixtures.

Main results:

- The gelatinisation temperature of potato starch is lower than the temperature for whey protein denaturation / aggregation; thus in mixtures of potato starch and whey proteins, starch granules swell before denaturation / aggregation of the protein occurs, resulting in a reduction in viscosity and change in functionality.
- Hydrolysed whey protein resulted in a reduction in potato starch granule swelling during heating.
- Different blends of dairy proteins were evaluated in the presence of pre-gelatinised starch for changes in viscosity during in-vitro digestion using a newly designed rheological reactor cell. The study found that a blend of casein and α-lactalbumin may provide viscosity increase and release of peptides / amino acids for use in commercial applications, e.g., anti-reflux infant formula.

Opportunity/Benefit:

New knowledge on the effect of intact and hydrolysed dairy proteins on the pasting properties of waxy maize and potato starch can be utilised for development of structure in beverage and prepared food applications. The methodologies developed in this study can be used to evaluate ingredients under simulated (in-vitro) gastrointestinal digestion for use in development of functional, medical or therapeutic beverages.

1. Project background:

Experimental investigations at Teagasc (FIRM project 06RDTMFRC445) have shown that the individual caseins (b-casein and a-casein) have the ability to alter pasting behavior of waxy maize starch, however, the exact mechanism for this altered behavior has not been fully elucidated. While research has been carried out independently on both protein and starch the effect of protein and in particular, high value added protein ingredients on the physicochemical properties of starch have not been extensively studied. This project describes findings from model systems to investigate dairy protein – starch interactions for use in the development of new ingredients with application in formulated foods, imitation cheese and nutritional beverages.

2. Questions addressed by the project:

In many food applications starch is gelatinised in the presence of proteins and other non-starch carbohydrates. Consequently, there is a need for mechanistic studies on starch – dairy protein interaction to support the extensive use of these ingredients in food products. The project addresses this gap in knowledge and has resulted in the establishment of new rheological, thermal and microscopic methodologies for the investigation of protein-starch mixtures including a novel rheological reactor cell developed during the FIRM funded project for simultaneous measurement of viscosity and digestion.

3. The experimental studies:

The project comprised these key tasks:

- Investigation of the effect of dairy proteins on the gelatinisation behavior of starch (waxy maize and potato).
- Evaluation of the microscopic, rheological, thermal and dynamic behavior of selected protein/starch mixtures with the aim of understanding the effect that dairy proteins have on starch functionality.
- The effect of various conditions of pH, salt and protein concentration on starch functionality was investigated.
- In-Vitro characterisation of viscosity development and digestion kinetics of protein-starch mixtures using a novel rheological reactor cell simulating the effect of viscosity on digestion kinetics.
- Effect of hydrolysed whey proteins on starch pasting properties.
- Microscopic investigations using imaging techniques that involve shear and stationary measurements.

4. Main results:

The gelatinisation temperature of potato starch is lower than the temperature for whey protein denaturation / aggregation. As a result, the starch can swell before the whey protein has denatured / aggregated; the net effect is a reduction in peak viscosity and change in functionality. The aggregation of the intact whey proteins during the starch gelatinisation process has a significant effect on the viscosity of the pastes formed. However, when the starch is gelatinised before the whey protein aggregation occurs, as is the case with potato starch, the pasting curve is significantly modified when compared to waxy maize where its gelatinization temperature is similar to the denaturation temperature of the whey proteins. The study demonstrates a mechanism whereby the functionality of food systems can be altered by selection of starches with gelatinsation temperatures differing from the denaturation temperature of the protein system used.

The addition of casein increased the onset temperature of starch gelatinsation; this was observed in rheological and differential scanning callorimetry studies. The addition of whey protein isolate to potato starch reduced peak viscosity. The peak viscosity of the waxy maize starch increased when the casein was added. The final viscosity of the waxy maize starch pastes was not altered by the presence of the hydrolysates but whey protein isolate and pre-aggregated WPI increased the final viscosity significantly.

The functionality of starch was studied using a prototype rheological reactor cell. This cell was attached to a controlled-stress rheometer to measure rheological properties of model infant formula emulsions containing pre-gelatinised starch under simulated gastro-intestinal digestion in-vitro.

The cell can evaluate viscosity changes in complex multi-phase food systems under different physiological conditions (i.e., ionic strength, shear, pH and hydrolysis by peptic or amylolytic enzymes). It was demonstrated that, while starch was a critical component, protein type also affected viscosity development during acidification. The highest viscosity was achieved with a phosphocasein / starch mixture, followed by b-lactoglobulin / starch and then a-lactalbumin / starch mixture. Of the protein mixtures analysed, a combination of casein and α-lactalbumin provided viscosity increase and digestion kinetics, i.e., release of peptides / amino acids that may have application in special purpose beverages, e.g., anti-reflux infant formulae. The study also highlighted the importance of pH and protein buffering during digestion, as it influences the activity of pepsin and thus breakdown of the casein matrix, with subsequently influences the ability of starch to contribute to viscosity development.

5. Opportunity/Benefit:

New techniques / methodologies have been developed at Teagasc and UCC, including rheological, microstructural and thermal, for elucidating the role of protein in modulation of starch paste functionality. These techniques can be applied to any combination of starch and protein for development of prototype food structures for industry. The findings from the study demonstrate the role of protein in modulating viscosity in the presence of starch on acidification during simulated digestion for application in nutritional, medical and therapeutic foods.

6. Dissemination:

Presentations

Abstract / Poster presented at International Symposium on Food Rheology and Structure – ISFRS 2009. Latest research on microstructure of hydrocolloids and starch. Influence of a- and b-casein on the gelatinisation and subsequent amylolytic digestion of waxy maize starch. Valérie Chaurin & Mark A. Fenelon.

Abstract / poster presented at XVIII INTERNATIONAL STARCH CONVENTION CRACOW-MOSCOW, Cracow, Poland June 21–25, 2010; Measurement of viscosity of an anti-reflux infant formula in-vitro using a rheological reactor cell, Brid Treacy, Anthony P. Kett, Valérie Chaurin, Alan L. Kelly, Mark A. Fenelon.

Presentation at the 4th working group / conference: Improving health properties of food by sharing our knowledge on the digestive process (INFOGEST) 2014. Presentation Title: 'A mass spectrometry study of the peptides produced during the in vivo digestion of α-Lactalbumin' – presented in conjunction with project FIRM 08/RD/TMFRC/650 BioA-Lac, finished 28 February 2013. The work involved the study of peptides produced from whey proteins (as produced in the current study) during digestion. Joseph Kehoe.

Relay UpDate: RU –FI014–1-DT: Modifying starch functionality in food formulations using proteins.

Relay UpDate: RU –FI014–2-DT: Starch / protein interactions influence digestion in the stomach.

Main publications:

Influence of milk proteins on the pasting behavior and microstructural characteristics of waxy maize starch. 2013. Anthony P. Kett, Valérie Chaurin, Sinead M. Fitzsimons, Edwin R. Morris, James A. O'Mahony, Mark A. Fenelon. Food Hydrocolloids 30: 661–67.

MSc Thesis (Brid Treacy) entitled: Starch and Dairy Protein Interactions in Anti-Reflux Infant Formula.

A food matrix approach to meat product development

Project number:
5957

Date:
May, 2014

Funding source:
DAFM

Project dates:
Dec 2008 – May 2014

Collaborating Institutions:
University College Cork

Teagasc project team:
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Compiled by:
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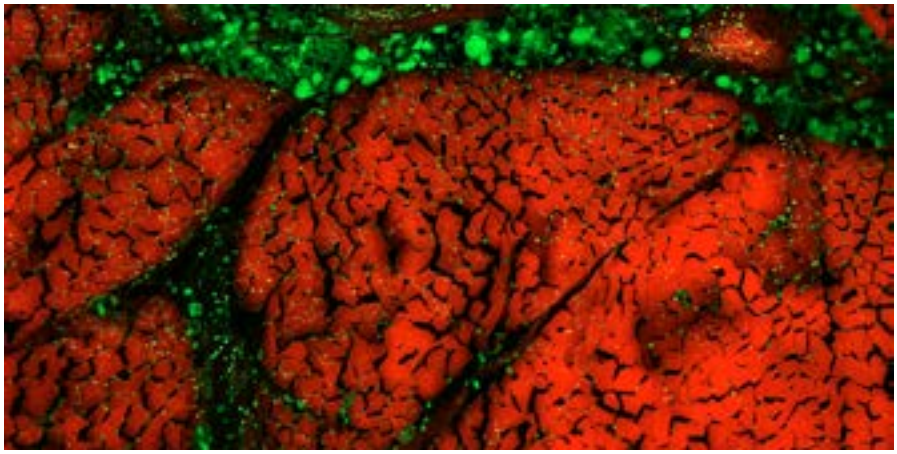

Key external stakeholders:

Primary and secondary meat processors; Ingredients companies; retailers; Regulatory agencies: DAFM.

Practical implications for stakeholders:

- Increasing consumer awareness of health issues associated with high dietary intake is driving the need for change in the products available to them. Therefore, the meat industry is examining the possibilities for meat products with reduced fat, salt and additives and assessing the potential for meat-based functional foods.
- Processed meat products represent complex systems that can be considered as a 'matrix' of interacting components. Processes and forces operating at the micro-scale impact on the macro-scale properties of the product.
- By improving our understanding of the impact of interactions between the food matrix and novel ingredients on technological and sensory performance, strategies were developed to optimise healthier versions of traditional meat products such as reduced fat and salt products and products including bioactive compounds and prebiotic fibres.

Main results:

- Comminuted product formulations (burgers, breakfast sausages, and frankfurters) with a wide range of salt and fat levels both above and below industry norms were assessed using consumer sensory panels and instrumental measurements. Several of the most preferred formulations represented a significant decrease in salt and fat content compared to typical retail counterparts (controls).
- Using response surface methodology, both comminuted and whole muscle products formulations containing functional ingredients, such as fibre, prebiotics, omega-3 fish oils and antioxidants were optimized and the most promising products were assessed by consumers.

- Detailed physico-chemical and ultra-structural analyses better elucidated the underlying forces governing overall product quality, the knowledge of which can be used in a more systematic scientific approach to new product development.
- Reducing fat and salt is possible without affecting consumers' perception of quality, while enhancing products with functional ingredients can also be achieved to further enhance their health profile.

Opportunity/Benefit:

Information and databases available to industry that can be used in future to predict the effects of alteration of various parameters on microstructure, molecular interactions and their relationship with product quality.

1. Project background:

Most of the existing processes applied in the development of meat products, have been developed empirically by testing the effect of a limited number of ingredients/inclusion levels/processing conditions on product quality and yield, which is pragmatic but does little to develop an understanding of the physic-chemical processes that govern the final product. Implementation of a systematic scientific approach in the controlled and efficient development of future meat products requires a basic application of chemical, biochemical, physical and biological principles and consideration of the meat system as a matrix of interacting components. The overall objectives of this project were to:

- enhance understanding of the effect of the complex interactions between the components of commercially relevant meat matrices with each other and with added ingredients on the technological and sensory properties of meat products.
- to further assess the interaction of ingredients with processing at the molecular and food matrix level.
- to generate information on the applicability of the comminuted meat matrix as a delivery system for bioactives.

2. Questions addressed by the project:

- What is the effect of modifying salt and fat level and their interactions on consumer acceptance and technological performance of commercially relevant meat products?
- Can model systems provide insight on the physic-chemical and technological properties of intrinsic components of meat, such as myofibrillar and sarcoplasmic proteins?
- Is it possible to develop a healthier meat product without compromise on sensory or technological performance?
- Are meat products suitable as delivery vehicles for healthy and bioactive ingredients?
- Can ultra-structural visualisation techniques, nuclear magnetic resonance and differential scanning calorimetry help us gain understanding on the interactions among food components?
- What is the consumer view of nutraceutical-containing meat products?

3. The experimental studies:

- Interaction between 1) purified intact myofibrils and 2) sarcoplasmic protein extracts from beef muscle with thermal processing and salt were studied to investigate gelation properties.
- Model systems representing a wide range of salt and fat levels for important comminuted products were established and assessed through technological andconsumer sensory analysis to identify where it was possible to reduce these ingredients below levels currently utilised in retail products.
- Once levels of salt and fat were optimised, putative bioactive ingredients were included in optimised formulations and assessed for sensory and technological performance and bioactive persistence through cooking was assessed.
- Functional and bioactive ingredients including rice starch and fructose were assessed in cooked ham products, both whole muscle and reformed.
- The freezing properties of brined muscle cuts were investigated and the potential of ultrasound to improve freezing time was assessed.
- Consumer surveys were carried out to identify consumer attitudes towards typical ingredients in meat products and nutraceutical-containing meat products.

4. Main results:

- Myosin and actin in extracted myofibrils differed in their thermal properties. For actin, there is a significant interaction effect between salt and thermal processing, whereas for myosin, the effects of salt and temperature are independent.
- Salt reduction in beef patties improved consumer sensory performance. The most consumer acceptable beef patty was that containing 40% fat with a salt level of 1%.

- For breakfast sausages, products containing 1.4% and 1.0% salt were significantly (P<0.01) more acceptable to consumers than higher salt levels. Producing a product within the limits of salt levels recommended by the FSAI is thus not only achievable but also can produce a superior product when rated by consumers.
- For frankfurters, lower fat levels (10% and 15%) with higher salt levels (2.5–3%) were significantly the most acceptable variants to consumers. Fat levels can be potentially reduced without significantly affecting overall acceptability, with a greater challenge for salt reduction.
- Optimisation of the sensory analysis of sausages containing a prebiotic dietary fibre (inulin) suggested that further reductions in fat can be achieved (>50% reduction) without affecting eating quality.
- Optimisation of burger formulation with a lower added-fat level (reduction of 7.8% compared to the control) substituted with commercial encapsulated fish oil was achieved with an acceptable desirability level in terms of technological performance. However, sensory analysis carried out on this optimised formulation showed that some flavour modification did occur.
- For whole muscle products, the application of power ultrasound had a positive effect on freezing properties (i.e. decreased the freezing time) of non-brined and brined round beef pieces compared to controls.
- Partial or total substitution of phosphates and dextrose with the inclusion of rice starch and/or fructo-oligosacharides is feasible in the manufacture of whole muscle hams; while it leads to a certain reduction in yield, a product of satisfactory quality (measured instrumentally and chemically) is obtained which may represent a healthier product compared with those currently available at retail.
- Consumer studies carried out on the whole muscle hams have indicated consumer preferences towards the removal of additives and support inclusions like dietary fibre as they could be further beneficial to their health.
- Retention of CoQ10 in fortified sausages and burgers was high after cooking (74–75%). Furthermore, results from *in vitro* digestibility assay showed a high rate of digestibility with up to 95% (sausages) remaining intact & reaching the absorption site in small intestine, indicating its potential as a functional ingredient.
- Reduced fat and salt, and CoQ10 fortified patties were more accepted by consumers compared to commercially available products and scored significantly higher for appearance. Reduced fat and salt, as well as the CoQ10 fortified, sausages were found to compare quite well to their commercial counterparts for overall acceptability, whereas commercial frankfurters were more favoured in comparison to reduced fat and CoQ10 fortified versions.
- Many of the respondents were willing to consume meat based functional food products but were not willing to pay more for them.
- Time domain NMR analysis gave a more detailed account of the binding properties between water and mixture components in sausages than empirically derived responses of cook loss and total expressible fluid. Three distinct peaks were identified that can be associated with water that is trapped, bound and free. Of these, trapped and free water were the most strongly correlated with moisture loss. Models for trapped and free water were significant and indicated samples with increasing inulin had higher trapped water and lower free water populations.
- The application of ultra-structural techniques was highly relevant and permitted visualisation of the physico-chemical changes underpinning the macro-scale technological and sensory parameters. For example, the reduction in fat in inulin enriched sausages was visualised by differential staining and confocal microscopy while the crystalline structure of the inulin molecule was visible using cryo-scanning electron microscopy.

5. Opportunity/Benefit:

A series of large datasets have been generated from more than ten experimental trials on a variety of model systems, and prototype case study products of comminuted, whole muscle and reformed products have been explored in detail. A large number of novel additives, many technologically functional, the majority of which are clean label, several of which are potentially bioactive, have been included in prototype products and comprehensively investigated so that their impact on techno-functional and sensory parameters of the selected products has been established. Furthermore, a number of processing interventions have been explored and novel processing interventions have been tested. Main findings have been published in peer-review journals. However, the data held in our databases is a further source of information of practical utility for the Irish meat processing

industry. Datasets from Teagasc and UCC have now been compiled and are stored in a single database in digital format at Teagasc. For example, companies may wish to use these datasets to explore in greater detail the attributes of a specific prototype product and its interaction with ingredients and processing. Finally, we have clearly demonstrated the benefits of optimisation through response surface methodology and this can be applied in future research partnerships with industry e.g. to investigate a new ingredient/ functional additive.

6. Dissemination:

This project was showcased at several Teagasc Gateways events and highlighted in the national press. Several peer-reviewed publications have emerged to date and aspects of the work were highlighted at international conferences such as the International Congress of Meat Science.

Main publications:

Keenan, D. F., Auty, M. A. E., Doran, L., Kerry, J.P., Hamill, R. M. (2014). Investigating the influence of inulin as a fat substitute in comminuted products using rheology, calorimetric and microscopy techniques. Food Structure, 01: 2014.

Keenan, D. F., V. C. Resconi, J. P. Kerry and R. M. Hamill (2014). Modelling the influence of inulin as a fat substitute in comminuted meat products on their physico-chemical characteristics and eating quality using a mixture design approach. Meat Science 96(3): 1384–1394.

Tobin, B. D., M. G. O'Sullivan, R. Hamill and J. P. Kerry (2014). Effect of cooking and in vitro digestion on the stability of co-enzyme Q10 in processed meat products. Food Chemistry 150: 187–192.

Tobin, B. D., M. G. O'Sullivan, R. Hamill and J. P. Kerry (2014). European consumer attitudes on the associated health benefits of neutraceutical-containing processed meats using Co-enzyme Q10 as a sample functional ingredient. Meat Science 97(2): 207–213.

Resconi, V. C., Keenan, D. F., Gough, S., Doran, L., Allen, P., Kerry, J. P., and Hamill, R. M. (2013). Starch and fibre in whole-muscle cooked ham: yield microstructure and sensory discrimination. Proceedings: 59th International Congress of Meat Science and Technology (ICoMST) 0–37, Izmir, Turkey, August 2013

Tobin, B.D., O'Sullivan, M. G., Hamill, R.M. and J. P. Kerry (2013). The impact of salt and fat level variation on the physiochemical properties and sensory quality of pork breakfast sausages. Meat Science, 93, 2, February 2013, 145–152.

Tobin, B.D., O'Sullivan, M. G., Hamill, R.M. and J. P. Kerry (2012). Effect of varying salt and fat levels on the sensory and physiochemical quality of frankfurters. Meat Science, 92, 4, pp. 659–666

Tobin, B.D., O'Sullivan, MG, Hamill, R.M., Kerry JP (2012). Effect of varying salt and fat levels on the sensory quality of beef patties. Meat Science, 91, 4, pp. 460–465.

McArdle, R, Hamill, RM and Kerry, JP (2011). Utilisation of hydrocolloids in processed meat systems. In: Processed meats: improving safety, nutrition and quality, p. 243–269. Edited by JP Kerry and JF Kerry, Woodhead Publishing.

Project number:
5962
Date:
November, 2012
Funding source:
EU Framework 7
Project dates:
Oct 2008 – Nov 2013

Collaborating Institutions
University College Dublin

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Compiled by:
Paul Allen

Accelerated meat curing using Ultrasound and Pulsed Electric Fields

Key external stakeholders:

- Meat processors.
- Food retailers.
- Consumers.

Practical implications for stakeholders:

Meat curing is one of the oldest meat preservation methods and it is still widely used today to produce a range of meat products with desirable characteristics. However, brine penetration into meat is a slow process so most processors use multi-needle injectors to produce bacon and ham in a few days rather than a few weeks. However, this produces products of lower quality. We have shown that the rate of brine penetration can be speeded up by applying high intensity ultrasound (US) to the meat while it is immersed in brine. Processors could use US to shorten processing times without adversely affecting the quality. Pulsed electric fields (PEF) is another novel technology with potential, but we have found that it is not as effective as US.

Main results:

- In lab-scale studies a range of US treatments (10, 25 or 40 min at US intensities of 4.2, 11 or 19 W cm^{-2}) increased the salt content of pork at a set time.
- Diffusion studies confirmed that the rate of salt uptake was increased (46%) by US treatment.
- In pilot scale studies (pork pieces of 300g approx.) three US treatments (2 h; 10.7, 17.1 or 25.4 W cm^{-2}) halved the time to reach a salt content of 2.2%
- US treatment did not affect any quality attributes.
- PEF treatment of pork prior to curing increased the salt uptake but only by about 17%
- US is easier to apply to meat pieces than PEF and it showed greater potential for reducing curing times.

Opportunity/Benefit:

Ultrasound is a technology that is already in use in the food industry. Commercial systems could be adopted to reduce curing times for high quality products. PEF systems are also available to the food industry, mainly for liquids. Although PEF showed some potential for accelerating the curing of pork it needs a considerable amount of development to optimise it for this purpose.

1. Project background:

Curing of pork to produce ham and bacon products that are safe and attractive to the consumer requires that the salt and other curing agents are evenly distributed throughout the meat. Penetration of salt into pork is a slow process, so long processing times are required with traditional processing. The industry uses multi-needle injectors to accelerate the process but this produces a lower quality product. Ultrasound (US) and Pulsed Electric Fields (PEF) are 2 novel technologies that have the potential to accelerate the rate of brine diffusion. By different mechanisms they both cause physical disruption of tissues which may allow faster ingress of salt into meat. These were both investigated to assess their potential for accelerating the curing of pork.

2. Questions addressed by the project:

- Can US accelerate the uptake of brine by pork?
- What is the optimum combination of frequency and treatment time?
- Does US treatment affect the sensory and technological properties of the ham?
- Can PEF accelerate the uptake of brine by pork?
- What is the optimum combination of field strength, frequency, pulse number and treatment time?
- Does PEF treatment affect the sensory and technological properties of the ham?

3. The experimental studies:

Pork cylinders were placed in a jacketed vessel with a coolant flowing through the outer jacket to remove heat. Brine was place above the meat and ultrasound was applied to the brine by a probe. Optimum treatment time and US frequency was determined by measuring the salt content of the pork after treatment. The rate on brine uptake was calculated by Fick's law.

Water compartmentalization in pork was assessed by NMR spectroscopy. Protein denaturation was measured by Differential Scanning Calorimetry.

The studies on US curing were scaled up using samples of 300g in sealed bags containing brine. US was applied in a US bath with two US probes inserted in the water in the bath.

The effect of PEF parameters on brine uptake was assessed on small (6 x 2 x 2 cm) samples in a batch PEF equipment. Samples were immersed in brine for a fixed time after PEF treatment when the salt content was assayed to determine the effectiveness of the PEF treatment.

4. Main results:

- The rate of salt uptake increased with increasing US intensity.
- There was no effect of US on water binding capacity (WBC) or cook loss.
- The only texture attributes affected by US were a reduction in gumminess and cohesiveness.
- The rate of diffusion (independent of temperature changes) was successfully modeled.
- US also caused protein extraction at the surface which would aid in ham processing.
- In a pilot scale study a 50% reduction in curing time was achieved by subjecting brines meat pieces sealed in a bag and immersed in a US bath with 2 US probes.
- PEF increased the uptake of salt by up to 13%
- PEF was most effective at low frequency (100Hz) and high pulse rate (300 pulses), though this combination gave the highest cook looses.

5. Opportunity/Benefit:

Ultrasound is a technology that is already in use in the food industry. Commercial systems could be adopted to reduce curing times for high quality products. PEF systems are also available to the food industry, mainly for liquids. Although PEF showed some potential for accelerating the curing of pork it needs a considerable amount of development to optimise it for this purpose.

6. Dissemination:

Main publications:

C. K. McDonnell, P. Allen, E. Duggan, J. M. Arimi, E Casey, G Dunne and J. G. Lyng (2013). The effect of salt and fibre direction on water dynamics, distribution and mobility in pork muscle: a low filed NMR study. Meat Science 95: 51–58.

C. K. McDonnell, P. Allen, C. Morin and J. G. Lyng (2013). The effect of ultrasonic curing on meat protein and water-protein interaction in meat. Food Chemistry, 147:245–251.

C. K. McDonnell, J. G. Lyng and P. Allen, (2014). The use of power ultrasound for accelerating the curing of pork. Meat Science, 98(2):142–149.

C. K. McDonnell, P. Allen, C., F. Chardonnereau, J. M. Arimi and J. G. Lyng (2014).

The use of pulsed electric fields for accelerating the curing of pork. LWT – Food Science and Technology 59(2) part 1:1054–1060.

Popular publications:

C. K. McDonnell, P. Allen, C. Morin and J. G. Lyng (2013). The effect of ultrasonic curing on water-protein interactions in meat. *Proceeding for the Institute of Food Technologists Annual Meeting*, 11–16th June 2013, Chicago, USA.

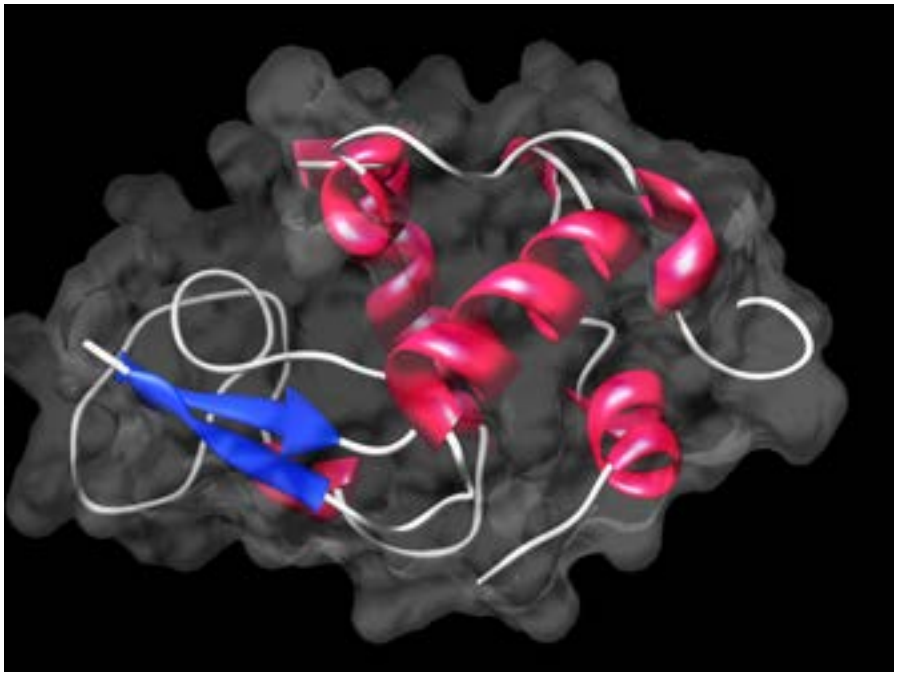
C. K. McDonnell, P. Allen, F. S. Chardonnereau, J. M. Arimi and J. G. Lyng (2013). The use of pulsed electric fields for accelerating the curing of pork. *Proceedings of the 42nd Annual Food Research Conference*, 27–28th June 2013, Dublin, Ireland, p. 13.

C. K. McDonnell, P. Allen, G. Duane, C. Morin, E. Casey, and J. G. Lyng. (2013). The influence of power ultrasound on NaCl diffusion in pork. *Proceedings of the 42nd Annual Food Research Conference*, 27–28th June 2013, Dublin, Ireland, p. 41.

C. K. McDonnell, P. Allen, J. M. Arimi and J. G. Lyng (2013). Optimisation of pilot-scale production of ultrasound-cured hams. *Proceedings of the 59th International Congress of Meat Science and Technology*, 18–23rd August 2013, Izmir, Turkey, p. 105.

C. K. McDonnell, P. Allen, F. S. Chardonnereau, J. M. Arimi and J. G. Lyng (2013). The use of pulsed electric fields for accelerating the curing of pork. *Proceedings of the 59th International Congress of Meat Science and Technology*, 18–23rd August 2013, Izmir, Turkey, p.16.

Bioactive dairy protein complexes – *in vitro* and *in vivo* digestion



Key external stakeholders:

Food, feed and pharmaceutical industry.

Practical implications for stakeholders:

- Whey proteins can act as delivery vehicles of small molecules such as fatty acids, thereby changing their biological activity.
- *In vitro* and *in vivo* tools are available within Teagasc to assess digestibility, bioaccessibility and bioavailability of food compounds.

Main results:

The key results were:

- α-lactalbumin (α-la) and β-lactoglobulin (β-lg), both whey proteins, can bind small hydrophobic molecules and act as delivery vehicles to cells.
- α-la and β-lg can alter the solubility of fatty acids, thereby affecting their biological activity e.g. increasing or decreasing their anti-tumour activity or delay the uptake of fatty acids.
- *In vivo* gastric digestion of α-lactalbumin in adults (n=10) provided valuable and novel insight into the mechanism and kinetics of protein breakdown.

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Collaborators in COST action INFOGEST FA1005

Compiled by:
André Brodkorb

Opportunity/Benefit:

The research team in Teagasc Moorepark has developed *in vitro* and *in vivo* tools to assess the digestive mechanism of food components. Assays such as bioaccessibility and bioavailability are now available to interested end users.

1. Project background:

The past decade has seen a large increase in interest from consumers and industry in functional foods and ingredients; these ingredients can provide the consumer with other health benefits beyond nutrition.

α-lactalbumin (α-la) is the main whey protein in human milk and is also present in bovine milk. It has been known that a cytotoxic complex can be formed when α-la associates with a major fatty acid, oleic acid. Studies on this complex have, until recently, primarily focused on its cytotoxic mode of action and the type of mammalian cells against which it was active. While it has been suggested that α-la/oleic acid complexes could be formed during the digestion of dairy products, no studies have been carried-out to verify this.

2. Questions addressed by the project:

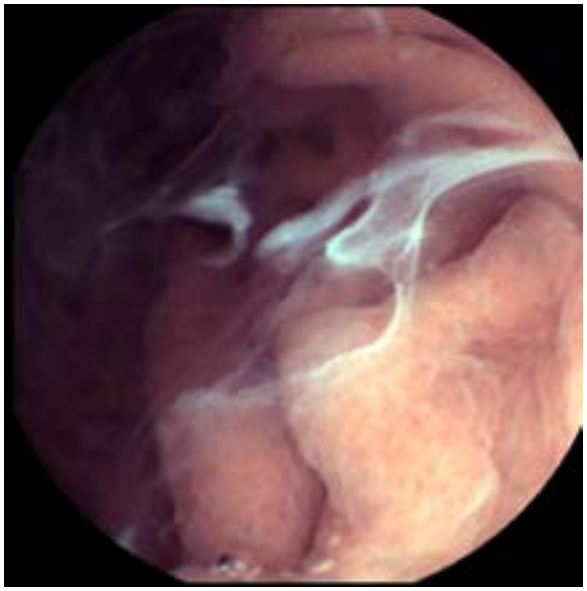
The main questions prior to 2008 were whether α-la/oleic acid-like complexes have any relevance for food and more specifically whether they could be formed *in vivo* during gastro-intestinal digestion. In addition, there was little or no information of the role of the individual components, protein vs. fatty acid, and whether other proteins and fatty acids can induce similar effects.

3. The experimental studies:

A semi-dynamic *in vitro* gastric digestion model was established using computer controlled titration to mimic the pH gradient in an infant's stomach during digestion. The α-la/oleic acid complexes were digested, analysed for structural changes and tested with cell lines such as HL60, U937, PC12 and Caco2.

A human *in vivo* study was carried-out in the Mercy University Hospital in collaboration with the Alimentary Pharmabiotic Centre (APC) to follow the fate of α-la during digestion in the stomach of healthy adults.

A variety of novel complexes were formed from the proteins α-la, β-lactoglobulin (β-lg) and bovine serum albumin combined with a range of fatty



acids. The critical reaction parameters were identified. One detailed study was carried out on the bioavailability of β-lg – linoleic acid complexes. Bioaccessibility, delivery and uptake of linoleic acid was tested in enterocyte-like Caco-2 monolayers. Changes in the production or secretion of the enteroendocrine satiety hormone, cholecystokinin, were investigated.

International links were established with the leading groups working on food digestion as part of an EU-funded COST action INFOGEST. The PI André Brodkorb led a group to harmonise existing food digestion methods.

4. Main results:

- α-la/oleic acid complexes could be formed during *in vitro* gastric digestion.
- *In vivo* gastric digestion of α-la, using naso-gastric tube aspiration revealed a great insight into structural changes and the kinetics of protein hydrolysis. Capsule endoscopy provided real time images of gastric digestion of α-la, see image.
- Extensive physico-chemical characterization of a variety of α-la/oleic acid complexes was performed and substantial structural heterogeneities were discovered.
- Complexes of α-la and β-lg with a number of fatty acids revealed a clear trend: the amount of fatty acid is correlated to the cytotoxicity of the complex, i.e. the protein is the "mule", the fatty acid is the "drug"
- β-lg can significantly delay the uptake of linoleic acid in intestinal cells.

- In collaboration with the COST action INFOGEST, an international consensus was established for a harmonised static *in vitro* digestion method suitable for food, coordinated by the PI André Brodkorb.

5. Opportunity/Benefit:

Teagasc can provide expertise and/or service (assays and models) for testing food and food ingredients for their *in vitro* and/or *in vivo* digestibility, bioaccessibility and bioavailability.

6. Dissemination:

Main peer-reviewed publications:

*Le Maux, S., Giblin, L., Croguennec, T., Bouhallab, S., & Brodkorb, A. (**2012**). β-lactoglobulin as a molecular carrier of linoleate: characterisation and effects on intestinal epithelial cells in vitro. Journal of Agricultural and Food Chemistry, 60(37), 9476–9483. doi: 10.1021/jf3028396

*Brinkmann, C. R., Brodkorb, A., Thiel, S., & Kehoe, J. J. (**2013**). The cytotoxicity of fatty acid/α-lactalbumin complexes depends on the amount and type of fatty acid. European Journal of Lipid Science and Technology, 115(6), 591–600. doi: 10.1002/ejlt.201200165

*Le Maux, S., Bouhallab, S., Giblin, L., Brodkorb, A., & Croguennec, T. (**2013**). Complexes between linoleate and native or aggregated β-lactoglobulin: Interaction parameters and in vitro cytotoxic effect. Food Chemistry, 141(3), 2305–2313. doi: 10.1016/j.foodchem.2013.05.031

*Le Maux, S., Brodkorb, A., Croguennec, T., Hennessy, A. A., Bouhallab, S., & Giblin, L. (**2013**). β-Lactoglobulin-linoleate complexes: In vitro digestion and the role of protein in fatty acids uptake. Journal of Dairy Science, 96(7), 4258–4268. doi: 10.3168/jds.2013–6682

*Sullivan, L., Mok, K. H., & Brodkorb, A. (**2013**). The Formation of an Anti-cancer Complex Under Simulated Gastric Conditions. Food Digestion, 4(1), 7–18. doi: 10.1007/s13228–012–0030–0

De Azambuja, K., P. Barman, J. Toyama, D. Elashoff, G. W. Lawson, L. K. Williams, K. Chua, D. Lee, J. J. Kehoe, A. Brodkorb, R. Schwiebert, S. Kitchen, A. Bhimani and D. J. Wile (**2014**). Validation of an HPV16-mediated Carcinogenesis Mouse Model. In Vivo, 28(5), 761–767.

*Kehoe, J. J., & Brodkorb, A. (**2014**). Interactions between sodium oleate and α-lactalbumin: the effect of temperature and concentration on complex formation. Food Hydrocolloids, 34, 217–226. doi: 10.1016/j.foodhyd.2012.09.009

*Kehoe, J. J., Lišková, K., Mok, K. H., O'Brien, N., Kelly, A. L., & Brodkorb, A. (**2014**). Formation of cytotoxic α-lactalbumin / sodium oleate complexes: Concentration and temperature effects. International Dairy Journal, 38(1), 65–73. doi: http://dx.doi.org/10.1016/j.idairyj.2014.04.005

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Popular publications:

Reactivating HAMLET, Science Spin – Issue 56; Page 22–23 – January 2013, http://issuu.com/spin35/docs/supplement_2013/10.

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Bio-sensitives advanced stabilisation

Project number:
5953

Date:
November, 2014

Funding source:
DAFM

Project dates:
Oct 2008 – Mar 2013

Collaborating Institutions:
University College Cork

Teagasc project team:
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Patrick Maher
Dr. Mark Auty

External collaborators:
Prof Yrjo Roos, University College Cork.

Compiled by:
Mark A. Fenelon

Key external stakeholders:

Dairy Ingredients and Nutritional Beverage Manufacturers.

Academic and Research Institutions.

Practical implications for stakeholders:

The research investigates processes, such as dehydration, as a way of stabilising sensitive and bioactive food components in structure-forming food matrices.

- Stabilisation of high-value ingredients requires a thorough understanding of ingredient interactions during formulation, processing, storage and distribution. The research demonstrates the effects of altering the composition of the continuous phase of emulsions on microstructure and physical properties of resultant powders such as glass transition temperature, sugar crystallisation, and lipid oxidation.
- The production of nanoemulsions, using microfluidisation for spray drying, with carbohydrate glass-formers has potential as a technique for increased retention of active components and uniformity of powder particle structure.

Main results:

The project utilised microfluidisation equipment for the production of nanoemulsions (fat globule size ~ 150 nm), which may be used for encapsulation of lipid soluble bioactives by spray drying to produce powdered ingredients. Spray drying produced a solid, glassy matrix with sensitive components as part of the glassy material or entrapped in the structure-forming matrix (solid-oil dispersion). The research showed the impact of reducing the fat globule size on the physical properties of emulsions and powders. Spray dried nanoemulsions had altered microstructure compared to the control powders, with reduced levels of lipid oxidation but increased rates of lactose crystallisation. Partial replacement of lactose with sucrose, reduced glass transition temperature (T_g),

delayed lactose crystallization and reduced the extent of lipid oxidation in powders – a possible beneficial effect for long term storage of powders.

Opportunity/Benefit:

This research provides a comprehensive account of the fundamental properties of nanoemulsions in liquid and dried forms. The techniques described can be translated into improved product quality and stability with demonstrable benefits to the Irish industry as producers of high quality ingredients and foods for the international markets.

1. Project background:

The ability to stabilise bio-sensitive ingredients is an essential prerequisite to maximise the market potential of Irish dairy products. Stabilisation of high-value ingredients requires a thorough understanding of ingredient interactions during formulation, processing, storage and distribution. A wide range of factors, including state transition behavior, particle size distribution, microstructure and concentration, influence the stability of bulk dairy components and biologically sensitive materials alike. UCC and Teagasc (Moorepark) have the analytical capacity and expertise to characterise the physico-chemical behavior of a wide range of food ingredients and to provide insight into stabilisation strategies for bio-sensitive ingredients.

2. Questions addressed by the project:

The project investigates the extent to which formulation dynamics / processing technology, including liquid and emulsion formulation, can be used for stabilisation of sensitive food components using freezing (work carried out in UCC) and dehydration (described in this Report). The scope of the work extends to characterisation of those food materials used for stabilisation through understanding physicochemical properties, microstructure, interactions with water, and phase separation behavior.

3. The experimental studies:

The project consisted of 3 experimental phases:

1. To study the effect of varying viscosity and Tg temperature of nanoemulsions (sunflower oil, 11.5%) stabilised with different carbohydrate/ protein (Lactose or a 70:30 mixture of lactose: sucrose, 23.9%, sodium caseinate, 5.1%) ratios on subsequent emulsion stability.
2. Comparison of the physicochemical properties of spray dried nano- and control emulsions with varying water and sugar contents,
3. Characterisation of lactose crystallisation and microstructure of nanoemulsion powders, and measurement of powders with respect to lipid oxidation.

4. Main results:

- Spray drying experiments were carried out to investigate the effects of emulsion type (nanoemulsion vs. conventional emulsion) on encapsulation efficiency of both water- and fat-soluble biosensitive components in dehydrated systems. Nanoemulsions were less viscous and more stable in liquid form than conventional emulsions. Nanoemulsions produced by microfluidisation (particle size ~ 150 nm) had higher stability and lower viscosity compared to control emulsions (particle size ~ 1.2 mm), made using a homogeniser, and exhibited lower solvent extractable free fat in the resulting powder. Increasing the dryer outlet temperature from 80 to 90°C resulted in lower moisture, water activity, particle size, tapped bulk density and increased onset temperatures for T_g and crystallization (T_{cr}) of lactose in powders (measured by differential scanning calorimetry; DSC). Reducing the fat globule size by microfluidisation lowered T_{cr} of lactose, possibly due to lower levels of protein in the continuous phase resulting from increased fat surface area in nanoemulsions. Partial replacement of lactose with sucrose decreased T_g and delayed lactose crystallisation, as measured by dynamic vapour sorption (DVS). The effect of emulsion type on the stability of vitamins was also studied. Ascorbic acid (vitamin C) was more stable in powders produced from nanoemulsions than in powders produced using equivalent control emulsions. β-carotene content decreased slightly over time in powders in which sugars crystallised.

- The effect of emulsion type on powder structure was observed using a range of microstructural techniques, i.e., polarised light, confocal laser scanning and scanning electron microscopy. Nanoemulsion powders had a more uniform distribution of smaller sized oil droplets compared to larger, more scattered oil droplets in powders made from conventional emulsions. Using polarised light microscopy, differences in crystallisation of emulsions with lactose compared to emulsions with lactose/sucrose were observed. After lactose crystallisation, powder surfaces were uneven and ruptured, with crystals comprising a mixture of 5:3 molar ratio α- and β-lactose.
- A gas chromatographic headspace solid-phase microextraction (HS-SPME) method was used to quantify the volatile compounds pentanal and hexanal (indicators of lipid oxidation) in powders made from nano- and control emulsions, stored over 24 months. Levels of pentanal and hexanal were significantly ($P < 0.05$) lower in powders made from nanoemulsions compared to those from control emulsions, which was attributed, in part, to their altered structure and lower porosity (as determined by pycnometry). The study also showed that partial replacement of lactose with sucrose significantly ($P < 0.05$) reduced lipid oxidation.

5. Opportunity/Benefit:

The results of the present study advance possibilities for the Irish food industry to protect sensitive components through process development and product formulation. The methodologies developed in this study can be used by industry to better understand the structure of dehydrated materials and identify changes occurring during processing and storage of powders containing sensitive nutrients. This knowledge can be used for improvements in manufacturing of dairy powders and nutritional beverages.

6. Dissemination:

Main publications:

Maher, P.G., Fenelon, M.A., Zhou, Y., Haque, Md. K., Roos, Y.H. (2011). Optimization of β-casein Stabilized Nanoemulsions using Experimental Mixture Design. Journal of Food Science 76(8), 1108–1117

Maher P. G., Y. H. Roos and M. A. Fenelon. 2014. Physicochemical properties of spray dried nanoemulsions with varying final water and sugar contents. Journal of Food Engineering. Volume 126, Pages 113–119

Maher P. G., M. A Auty, Y. H. Roos, L.M. Zychowski and M. A. Fenelon. 2014. Microstructure and lactose crystallization properties in spray dried nanoemulsions. Food Structure. In Press.

Popular publications:

Fenelon, M. (2013). Critical parameters affecting emulsion stability pre-spray drying. Workshop on Biosensitives Advance Stabilisation, University College Cork, 7 March 2013.

Maher, P. (2013). Spray Dried Nano-emulsions for Biosensitives Stabilization. Workshop on Biosensitives Advance Stabilisation, University College Cork, 7 March 2013.

Auty, M. (2013). Imaging of Micro- and Nanostructures in Food Materials. Workshop on Biosensitives Advance Stabilisation, University College Cork, 7 March 2013.

Maher, P.G., Zhou, Y., Haque, K., Roos, Y.H., Fenelon, M.A. (2009). Optimisation of glass transition and rheological properties in nanoemulsions using a statistical mixture design. 39th UCC Annual Conference, Cork, Ireland.

Maher, P.G., Zhou, Y., Haque, M.K., Roos, Y.H., Fenelon, M.A. (2010). Physicochemical properties of b-casein stabilised nanoemulsions. Institute of Food Technologists (IFT) Annual Meeting in Chicago, IL. 17–20 July 2010.

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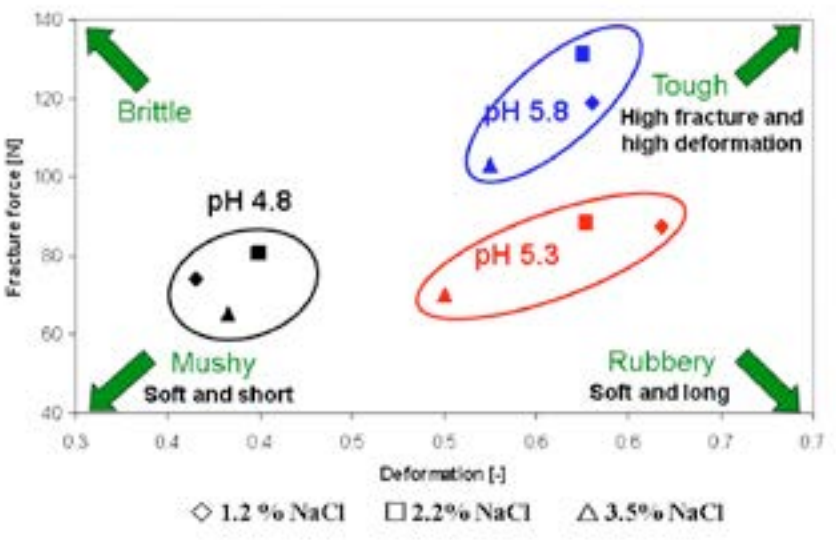
Maher, P.G., Roos, Y.H., Fenelon, M.A. (2012). Physico-chemical properties of dairy powders prepared from nanoemulsions. 5th IDF/INRA International Symposium of Spray Dried Dairy Products, June 19–21, 2012, St. Malo, France.

Maher, P.G., Roos, Y.H., Fenelon, M.A. (2012). Comparison of spray-dried emulsion and nanoemulsion dairy powders. Society of Dairy Technology Autumn Conference, September 5–7, 2012, Cork, Ireland.

Design and development of Realistic food Models with well-characterized micro- and macro-structure and composition (DREAM)



Key external stakeholders:

Cheese and Dairy Industry.

Practical implications for stakeholders:

A procedure for the preparation of a semi-hard rennet-curd model cheese in which composition is precisely controlled and which can be used to validate the growth/survival of microorganisms under different conditions.

A database on the effects of varying salt concentration and pH in model cheese on the survival of probiotic bacterial strains and on the chemical and rheological properties.

A data base on the survival of probiotic bacterial strains in full-salt and reduced-salt Cheddar cheeses during maturation.

Project number:
5983
Date:
November, 2014
Funding source:
EU Framework 7
Project dates:
Jun 2009 – May 2013

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Dr. Brian Byrne
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Collaborating Institutions:
INRA
ADRI Development
Campden BRI
Consiglio Nazionale delle Ricerche, Instituto di Scienze delle Produzioni
Actalia- Produits Laitiers
KOKI, Központi Élelmiszertudományi Kutatóintézet
INRA Transfert
Campden, BRI;CNRS, Centre National de la Recherché Scientifique
IRTA, Institut de Recerca I Tecnologia Agroalimentàries Alimentari
IFR, Institute of Food Research
TI Food and Nutrition, Stitching Top Institute Food and Nutrition
United Biscuits (UK) Limited
VTT, Valtion Teknillinen Tutkimuskeskus
Soredab, Soredab SAS
University of Ljubljana
Wageningen University

Compiled by:
T.P. Guinee

Main results:

1. A model cheese-making system was designed for the manufacture of semi-hard rennet-curd cheese (~47% dry matter, 18% protein, protein-to-fat ratio ~1) in which salt (1.2, 2.2 or 3.5%) and pH (4.8, 5.3 or 5.8) could be systematically controlled. The model cheeses were prepared from concentrated dispersions of micellar casein in an aqueous-based solvent comprised of water, fat and lactose.
2. The values for the coefficient of variation for salt, moisture, salt-in-moisture and pH in the model cheeses were ≤ 5%, indicating a high degree of reproducibility.
3. The survival of probiotic bacteria *Bifidobacteria* BB12 (BB12), incorporated into the model cheese at a level of ~ 10^8 cfu/g, was independent of variations in NaCl and pH at ripening times up to 96 d. However, after 150 d storage, the mean count of BB12 had decreased significantly (to ~ $10^{5.6}$ cfu/g) in the high-salt (3.5%) high-pH (5.8) cheese but not in the other cheeses.
4. The survival of probiotoc strain *Lactobacillus casei* LC-01 (LC-01), incorporated into the model cheese at ~ 10^8 cfu/g, was independent of pH variation in the high salt cheese (3.5%) at ripening times up to 47 day but decreased significantly to < 10^6 cfu/g in the low pH cheese (pH 4.8) after longer ripening times (96–150 day). However, the survival of LC-O1 was not affected by pH in the cheese with lower salt levels (1.2 and 2.2%).
5. A study on the survival of probiotic strains (BB12 and LC-01) in Cheddar cheese showed that both strains grew in the cheese during ripening, from ~ 10^8 cfu/g at 1 day ~ 10^{10} cfu/g at 60 day, and were unaffected by salt content of the cheese (1.4 %, 1.8 %).

Opportunity/Benefit:

The research makes available to the dairy industry a database on the effects of salt and pH on:

- the survival of two probiotic bacterial strains in a semi-hard model cheese (47% dry matter) and Cheddar cheese.
- the chemical, rheological and viscoelastic properties of model cheese.

1. Project background:

Cheese is a very diverse product with greater than 500 different varieties. Consequently, cheese differs tremendously in composition, structure, rheology and suitability as a matrix to host different bacteria, depending on variety. Hence, there is a requirement for generic realistic cheese models that can simulate this complexity and, thereby, facilitate evaluation of the impact of changing composition or processing conditions on the characteristics of cheese. Cheeses made using conventional technology can be quite variable in composition, texture and sensory properties. Such variation is in turn associated with variations in milk composition (e.g., lactose and casein contents), starter culture activity, manufacturing conditions (e.g., rennet-to-casein ratio), and partition of milk components (e.g. calcium, sodium chloride) between the curd and whey (which accounts for ~ 90% of the milk volume). Such inadvertent variation in cheese composition can make it difficult to establish the precise effects of parameters such as salt and pH on cheese properties.

The project aimed to develop a model cheese in which the effects of salt and pH, which vary widely across the large array of different cheese varieties, could be studied without the confounding effects of variations in other parameters such as weight ratio of cheese serum-to-cheese matrix, moisture content and calcium level. The principal focus was on the effect of salt and pH on the survival of probiotic bacterial strains and on the physico-chemical and rheological properties of the cheese.

2. Questions addressed by the project:

- Do variations in salt content and pH as found across the spectrum of different cheese varieties affect the survival of commonly used probiotic strains in cheese?
- How do variations in salt and pH affect the biochemical and physical properties of cheese in situations where the ratio of solvent (water and dissolved solutes)-to-solid cheese matrix (protein, fat and colloidal ash) is maintained constant as salt and pH are changed?

3. The experimental studies:

Development of a model cheese system for variation of salt and pH

A model cheese system was developed in which the solvent quality (salt content and pH) was changed while retaining a fixed weight ratio of solvent (water, soluble calcium and phosphate, lactose, lactic acid and salt) to matrix (fat, casein and colloidal minerals) were kept constant. The model cheese (~47% dry matter, protein-to-fat ratio ~1) was developed by dispersing micellar casein in an aqueous-based solvent comprised of water, fat and lactose. The resultant dispersion (pre-cheese), which was a liquid pre-cheese, was pasteurised at 80°C, and cooled to ~ 35°C, inoculated with coagulant (chymosin), acidifying agent (glucono-d-lactone) and salt. The treated pre-cheese was filled into the final package/mould and incubated at 32°C to allow in-situ gelation and transformation into the final cheese. The reproducibility of the model system in the manufacture of cheeses with different pH salt and pH values was evaluated by undertaking three separate trials on different occasions.

Evaluation of the effect of salt and pH on the survival of probiotic bacteria in model cheeses

Model cheeses with varying levels of salt (1.2, 2.2 or 3.5%) and pH (4.8, 5.3 or 5.8) were made in triplicate by varying the level of salt and glucono-d-lactone added to the model cheese system. Probiotic bacteria strains *Bifidobacteria BB12* (BB12) and *Lactobacillus casei* LC-01 (LC-01) (Chr. Hansen Ireland Limited, Little Island, Cork) were inoculated into the model pre-cheeses at a level of ~ 10^8 cfu/g prior to coagulation. The population of the probiotic strains cheeses was monitored over a 150-day storage period at 4°C. BB12 were enumerated on *Bifidobacteria* Selective Media with added BSM antimicrobial supplement (BSM: Fluka Analytical, Sigma Aldrich) incubated an-aerobically (Anaerocult® A; Merck KGaA, Germany) at 37°C for 3 days. LC-01 was enumerated on LBS agar (Becton Dickinson Co, Cockeysville, USA) incubated at 37°C for 4 days.

Survival of probiotic bacteria in full-salt and reduced-salt Cheddar cheeses

Full salt (1.8%) and reduced salt (1.4%) Cheddar cheeses were made in triplicate using the following treatments (Control, PB BB12, PB LC-01):

(a) Control full-salt (FSC) and reduced-salt (RSC) Cheddar cheeses, made with a commercial mesophilic lactic acid starter culture (R-704);

(b) PB BB12 full-salt (FSC) and reduced-salt (RSC) Cheddar cheeses, made with R-704 plus probiotic bacteria *Bifidobacteria* BB12 (BB12), inoculated into the cheesemilk at a level of $10^{7.8}$ cfu/ml;

(c) PB LC-01 full-salt (FSC) and reduced-salt (RSC) Cheddar cheeses, made with R-704 plus probiotic bacteria *Lactobacillus casei* LC-01 (LC-01), inoculated into the cheesemilk at a level of $10^{7.6}$ cfu/g.

The population of starter culture and probiotic bacteria in the cheese were enumerated over a 180-day ripening period. The cheeses were also evaluated for composition at 14 d, rheology characteristics, and grading scores at 6 month.

Effect of salt and pH on the chemical and rheological properties of model cheese

Model cheeses with varying salt content (1.2, 2.2 or 3.5%, w/w) and pH (4.8, 5.3 or 5.8) were manufactured in triplicate, as described above. The cheeses were stored for 5 days at 4°C and analysed for:

- Gross composition (fat, protein, moisture, salt, pH, Ca);
- Protein hydration, as determined from the level of expressible serum obtained on centrifugation of grated cheese at 12500 x g at 20°C.
- Texture profile analysis, using a two-bite compression test to 30% of samples original height at a rate of 1 mm/s, which enabled the calculation of fracture force (force required to fracture, bite 1), hardness (force at maximum deformation, bite 1), and chewiness (product of hardness x cohesiveness x springiness, using analysis of bites 1 and 2).
- Viscoelastic changes on heating from 25 to 85°C at a rate of 3K/min, using low amplitude strain oscillation, to obtain elastic modulus (G'), phase angle (d) – an index of fluidity of heated cheese.

4. Main results:

Survival of probiotic bacteria in model cheeses with different salt and fat levels

- The model cheese had the following composition: solvent (moisture, GDL and NaCl), 60%; protein, 18%; fat, 16.5%. The model cheese-making system showed a high degree of reproducibility (coefficient of variation < 5%) for levels of salt, pH and moisture for cheeses with a wide range of salt (1.2 – 3.5%, w/w) and pH (4.8 – 5.8) as found in different cheese varieties.

- *Bifidobacteria* BB12-inoculated cheeses maintained their target pH values at all salt levels and BB12 survival was independent of variations in salt content and pH, with counts between 10^7 and 10^8 cfu/g in all cheeses at 96 d. However, the count ($10^{5.6}$ cfu/g) of BB12 in the high-salt (3.5%) high-pH (5.8) cheese after 150 d storage at 4°C was lower than that (≥ 10^6 cfu/g) associated with probiotic foods. However, this reduction was not observed in the high-salt cheeses with lower pH values (4.8 and 5.3) which had mean counts of $10^{6.5}$ – $10^{8.5}$ cfu/g after 150 d storage.
- The pH of the LC-01 inoculated model cheeses with target pH values of 5.8 and 5.3 and containing 1.2% salt decreased to 4.7 – 4.8 after 1 day, while the pH of the corresponding cheeses with 2.2% NaCl had decreased to 4.7 – 4.8 after 96 day. The decrease in pH of the latter cheeses suggests that LC-01 grew during storage at 4°C and metabolized lactose to lactic acid. In contrast, there was no change in the pH of LC-01 cheeses with 3.5% NaCl. The levels of *L. casei* LC-01 recovered from all LC-01 inoculated cheeses (that maintained their target pH) remained at levels of ≥10^6 cfu/g up to 150 d storage.

Survival of probiotic bacteria in full- and reduced-salt Cheddar cheeses

- The addition of probiotic bacteria significantly increased the levels of moisture, moisture-in-non-fat substances, and fat-in-dry matter, and reduced levels of salt and salt-in-moisture of both the full-salt cheese (FSC) and reduced-salt cheese (RSC). The pH of the PB LC-01 FSC was significantly lower than that of the control FSC or the PB BB12 FSC, while pH of the PB LC-01 RSC and PB BB12 RSC were significantly lower than the corresponding control RSC. The latter trend may reflect the high levels of moisture and MNFS, and lower S/M content of the probiotic cheeses, especially the PB BB12 RSC. Reducing the salt content significantly increased the contents of moisture and fat, while significantly reducing salt-in-moisture content and pH. The lower pH of the reduced-salt cheeses reflects their higher moisture content which would lead to a higher level of lactic acid.
- The counts of probiotic-bacteria in the PB BB12 FS, PB BB12 RS, PB LC-01 FS and PB LC-01 RS cheeses increased from ~ 10^8 cfu/g in the cheese milk after inoculation to 10^9 – 10^{10} cfu/g in the cheese at day 1. Over the 180 day ripening period, the probiotic strains decreased by ~ 1 to 2 log units, reaching mean levels of ~ $10^{7.5}$ at day 180. As counts were > 10^6 cfu/g, the 180-day old ripened cheese can be referred to as probiotic cheese. Salt content did not significantly influence initial counts (on day 1) or the survival BB12 or LC-01 in Cheddar cheese. These results concur with those from the model system which found that salt levels in the range 1.2 – 2.2% (inclusive of the range in FS and RS cheddar cheeses: 1.4 – 1.8%) at pH values in the range 4.8 – 5.3 (inclusive of the range in FS and RS cheddar cheeses: 5.06– 5.21) had little effect on the survival of BB12 or LC-01.
- Despite the differences in composition, the probiotic culture did not significantly affect cheese hardness, fracture stress or fracture strain over a 6 month ripening period, apart from at 60 day when the fracture strain of probiotic cheeses was lower than that of the control cheeses.
- Relative to control FSC cheese, the BB12 FSC was considered very acceptable, and had a sweetish flavour note, balanced flavour, and creamy–smooth texture. Conversely, the PB LC-01 FSC cheese had a strong savoury, slightly rancid flavour more typical of very mature aged Cheddar (compared to the control cheese). Reducing the salt content resulted in all cheeses being described as being sharper, more acid, slightly bitter, and less balanced.

Effect of salt and pH on the chemical and rheological properties of model cheese

- For all salt levels, increasing pH from 4.8 to 5.3 significantly reduced cheese hardness, while increasing pH from 5.3 to 5.8 had little effect. Altering the salt concentration in final cheese did not significantly affect the hardness.
- Chewiness was highest at pH 5.3, and decreased with increasing salt concentration at all pH values.
- A plot of fracture force versus deformation at fracture point yielded a texture map indicating how the pH and salt level separated cheeses (Fig. 1). Model cheeses with low pH had low fracture force and deformation (fracture strain), indicating a tendency to be relatively soft and crumbly (short consistency). Conversely, the high pH cheeses had higher fracture force and deformation, suggesting an increasing tendency to tough, hard, rubbery texture as pH was increased. Increasing the pH from 4.8 to 5.3, which is typical of that of many hard cheese varieties, increased the deformation required at fracture, signifying an increase in rubberyness but not in hardness. Compared to pH, the effect of increasing salt content was relatively small, though statistically significant.

- Salt and also pH significantly altered the value of G' at 25°C. Lowest values were found at pH 5.3 at all salt concentrations and highest values at pH 4.8.
- Increasing pH increased the maximum phase angle (index of fluidity of the melted cheese) on heating the cheese from 25 to 82°C. Increasing the salt concentration led to an increase in the temperature at which phase angle attained a maximum value. In practical terms, the results indicated that increasing pH increased the fluidity of the melted cheese, while increasing salt from 1.2 to 3.5% increased the temperature required to obtain the maximum fluidity of the melted cheese.
- Protein hydration decreased significantly as pH was increased in the region 5.3 to 5.8, and as salt was reduced from 3.5 to 1.2%.

5. Opportunity/Benefit:

The research provides an extensive database on the effects of varying salt level in the range 1.2 to 3.5% and pH in the range 4.8 to 5.8 on the survival of common probiotic bacterial strains in cheese.

6. Dissemination:

Dream, Project Book of Results: Design and Development of Realistic Food Models with well-characterized micro-and macro-structure and composition (2013). University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, Slovenia.

Byrne, B., Jordan, K. and Guinee, T. P. (2012). Effect pH and NaCl on the survival of probiotic cultures in a model cheese system. In proceedings of 8th Cheese Symposium.

Piska, I., Byrne, B. and Guinee, T. P. (2012). Effect of sodium chloride on the properties of a model cheese system. In proceedings of 8th Cheese Symposium.

Water activity control and texture stabilisation of high protein snack bars

Project number:
5951
Date:
Nov 2014
Funding source:
FIRM
Project dates:
Apr 2009 – Mar 2013

Collaborating Institutions:
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Compiled by:
Phil Kelly

Key external stakeholders:

Dairy industry manufacturers of milk powders and dairy ingredients; formulators of nutritional snack products.

Practical implications for stakeholders:

High protein snacks are particularly susceptible to hardening during storage. As a critically important market outlet for functional milk protein ingredients, it is imperative to have an improved understanding of the ingredient interactions that may be contributing to this product defect.

- The outcomes of this research study now reveal a complex science occurring in a matrix of highly concentrated ingredients dispersed in an intermediate moisture environment.
- A database of information resulting from this research enables better choices of ingredients to be made in order to ensure improved shelf-life, and which can be utilised by ingredient manufacturers' support teams during engagement with their respective food formulator clients.
- Use of specialised analytical tools provide insights into changes at the macro- and micro-structural level.

Main results:

- The relative susceptibility to hardening of bar formulations featuring a range of dairy proteins was established under standardised conditions.
- Minimising water activity differences between liquid and solid components provides a means of controlling or delaying textural change.
- When appropriately directed, moderate levels of whey protein hydrolysis was highly effective in retarding bar hardening with time. Though not always the case e.g. hydrolysed casein, it appears that proteolytic effects at molecular or mesostructural levels may not be sufficient to significantly affect macrostructure especially when molecular jamming has yielded a highly confined, protein continuum.

Opportunity/Benefit:

A platform for testing ingredients, particularly proteins, in model protein bars is available. Expert knowledge and specialised analytical services are available to characterise performance and diagnose changes during the course of shelf life tests.

1. Project background:

The combined market for bars (sports/nutrition/snacks) in the USA is valued at $2 billion plus, having increased by a total of 169% since 1999, and is continuing to grow at the rate of 1–2% on an annual basis (Reed, 2007). With the advent of protein fortification, these healthy bars appeal to consumers engaged in sport, dieting and as meal replacements. It is estimated that over 10,000 tonnes protein ingredients are used in bar formulations to supply the US market alone, most of it supplied as milk protein by dairy companies.

A relatively simple process is involved in bar production. However, in the relatively low moisture environment of bars, the proteins present attract moisture and may exert considerable influence over bar texture. The result is that over time the bars may become progressively harder with increasing shelf life. Furthermore, marketing personnel emphasise that a high degree of innovation is required to help sustain future annual growth rates in bar consumption, and also to step up to even healthier standards through compositional improvements.

2. Questions addressed by the project:

The project was singularly focussed on addressing a key scientific issue e.g. what are the factors underlying textural changes in high protein bars during storage that ultimately result in hardness. Hence, the experimental studies were aimed at generating an improved understanding of the intermolecular interactions taking place in the concentrated intermediate moisture environment that constitutes the matrix of such high protein bars.

3. The experimental studies:

Methodologies were developed for the preparation of high protein bars, and protocols for accelerated storage trials were adopted in order to assess the effects of ingredient mixing, moulding and extrusion on the development of hardness in bars.

In depth analysis of formulated bars was undertaken using the analytical capability e.g. DVS analyser and NFIC equipment for food structure imaging. Ingredient composition and powder property manipulation was accomplished using Moorepark's scale-up facilities available i.e. BFE and MTL to prepare experimentally-dried ingredients for incorporation into bars.

The study commenced with an appraisal of a range of commercial high protein bars sourced in the USA according to their gross composition and ingredient classification. Analytical test protocols established to determine physico-chemical changes in bars with time included determination of moisture sorption isotherms (Teagasc and UCC), time-dependent changes in bar hardness by texture analyser (Teagasc and UCC), water activity (UCC), confocal laser scanning microscopy (CLSM) (Teagasc), differential scanning calorimetry (UCC) and changes in protein by reversed-phase HPLC (Teagasc). Time-dependent, protein-induced hardening was assessed as a function of composition during the course of elevated temperature storage.

The relationships between length scales and macrostructure in model, protein bars were investigated through in situ proteolysis of whey and caseinate proteins. Texture analysis, small angle oscillatory rheology, electrophoresis, gel permeation chromatography and infra-red spectroscopy were used to examine structural hierarchies via enzymatic deconstruction.

4. Main results:

The relative susceptibility to hardening of bar formulations featuring a range of dairy proteins was established under standardised conditions. However, it is cautioned that such comparisons are highly system dependent. Co-solvents were examined in both simple, binary systems and in model bar systems in order to elucidate this system dependency. When comparing the effects of a wide range of milk protein powders on textural change in model, high protein bars, solidification behaviour of powders was dependent on concentration (or volume) in a type-dependent manner and that protein powders have different windows of concentrations over which jamming and subsequent hardening occurs.

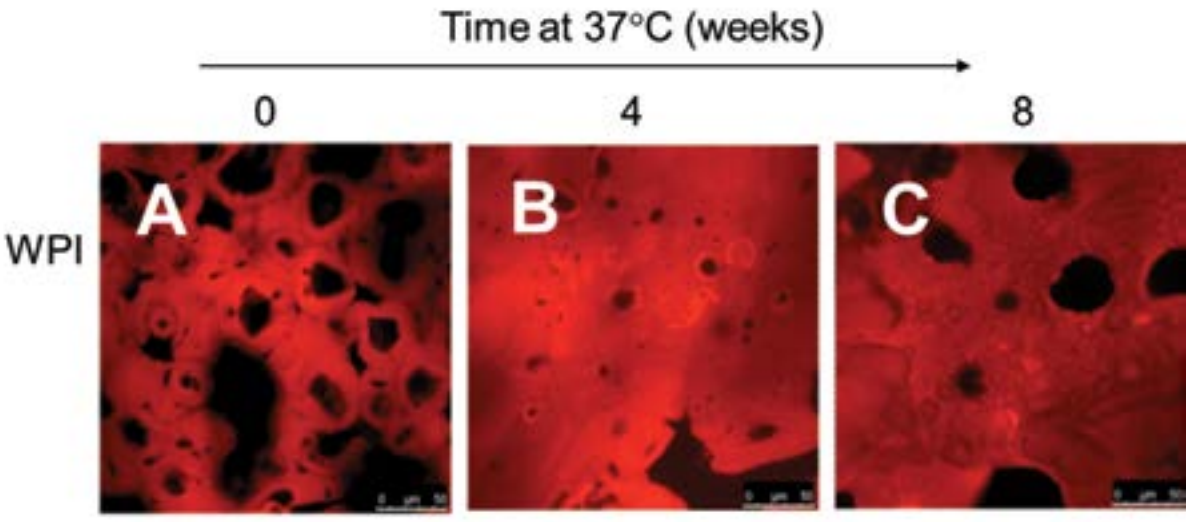

Figure 1. Microstructural changes occurring during accelerated storage testing at 37°C of bar formulations containing whey protein isolate (WPI)

- A wide range of protein powder physicochemical characteristics were correlated against textural changes in bars over time.
- Minimising water activity differences between liquid and solid components provides a means of controlling or delaying textural change.
- FT-IR measurements showed that water or solvent-induced plasticisation of protein powders in bar matrices was sufficient for protein-ingredient interactions to occur at a molecular level.
- Hardening in mixed protein bars resulted in a broadly linear response to ratio inclusion.
- Confocal scanning laser microscopy (CSLM) techniques were developed that allowed good quality imaging of physical changes in protein bars during storage. This led to a significant improvement on the fundamental understanding of the behaviour of dairy proteins and their interactions in highly-concentrated systems.
- Functionality differences in protein bars incorporating co-dried (CD) and dry-blended (DB) protein powders reflected structural and surface area effects attributable to the co-drying process. Physical interactions occurring between protein types during spray drying also impact on the functional characteristics of the resultant powders.

Effect of protein hydrolysis on hardening

The extent and effects of enzymatic hydrolysis differed between protein types. Only limited proteolysis was required to slow the extent of hardening in whey protein containing bars. In contrast, more extensive cleavage of calcium caseinate proteins caused greater hardening. It is possible that cleavage of high molecular weight calcium caseinate proteins produced altered functionalities, similar, in effect, to sodium caseinate, which contributes to extensive bar hardening. The findings suggest that proteolytic effects at molecular or mesostructural levels may not be sufficient to significantly affect macrostructure, particularly where the extent of jamming has yielded a highly confined, protein continuum.

Packing behavior of powder particles

Four whey powders, including three hydrolysates, were evaluated to determine the effects of particle interactions, moisture uptake, glass-transition temperature and deformation on structure formation, in particular the nature of the liquid-solid transition and the development of 'solidity'. Intact whey protein powder dispersions behaved as normally distributed, non-interacting spheres. By contrast, hydrolysate-based systems behaved as weakly, attractive colloidal particles, with a greater propensity for self-organisation. Frequency dependent approaches to the liquid-solid transition also differed as a function of hydrolysis. The study provides insight, from a fundamental rheological perspective, on the physical causes for lower susceptibility to hardening observed in hydrolysed powders. Such findings have potentially useful implications for the analysis of structure in particulate food systems.

Long term ambient storage trials

Long term ambient temperature storage studies of model whey protein bars, including whey protein hydrolysates, was carried out over a period of 18 months. Bars, produced at a relatively low volume fraction, did not demonstrate physical destabilisation, such as phase separation and remained stable with respect to hardening. Colour changes, visual appearance and overall sensory quality appeared acceptable. Equivalent samples, prepared at higher volume fraction underwent more extensive hardening but remained within estimated consumer acceptability limits. This work reinforced the need to establish the critical concentration dependence of protein powders in viscous continuous food matrices.

5. Opportunity/Benefit:

The resulting database of information enables better choices of ingredients to be made in order to ensure improved shelf-life, and can be utilised by Irish dairy company technical support teams during engagement with their respective clients. Background expertise in applying food microstructure imaging and other specialised analytical tools for this type of application can be made available.

6. Dissemination:

Informal communication and verbal updates were provided during the course of engagement with technical representatives of the dairy companies with specific ingredient manufacturing interest for this application.

A presentation entitled 'Optimising the shelf-life of high protein snack bars' was given by Sean Hogan Teagasc, Moorepark at RELAY Workshop 'Commercial opportunities for aggregate cereals and snack companies', 16th November 2010, UCC.

An in-house presentation was made to research staff and students at TRFC, Moorepark, by Sean Hogan entitled 'Influence of Co-dried Protein Powders on Textural Change in High-protein Bars', 14th March, 2012.

An in-house presentation was made to research staff and students at TRFC, Moorepark, by Sean Hogan entitled 'Soft matter characterisation of whey-based protein bars', 16th April, 2013.

Main publications:

Hogan, S.A., Chaurin, V., O'Kennedy, B.T. and Kelly, P.M. (2012). 'Influence of dairy proteins on textural change in high-protein bars'. *International Dairy Journal*, 26, 58 - 65.

Popular publications:

Kelly, P.M & Hogan, S. A. (2012) Technological adaptation of functional ingredients to improve texture of dairy-based energy bars. Oral presentation given at the IDF World Dairy Summit – Dairy Science & Technology Conference, 8th Nov 2012. Cape Town, South Africa.

Hogan, S.A., O'Kennedy, B.T., Huppertz, T. and Kelly, P.M Influences of co-dried, milk proteins on structural stability of high-protein bars. 5th International Symposium on Spray Dried Dairy Products, 19-21st June 2012, St. Malo. (Oral presentation)

Hogan, S., O'Kennedy, B., and Kelly, P. Influence of dairy proteins on textural change in high-protein snack bars. Flavour and Texture: Innovations in Dairy, 7th NIZO Dairy Conference, Papendal, The Netherlands. 21-23 September, 2011. (Oral presentation).

Kelly, P.M., O'Kennedy, B.T., Hogan, S.A., Chaurin V. and Roos, Y.H. 'Development of model systems to study select microstructure and chemical changes in high protein snack bars'. IDF Symposium on Microstructure of Dairy Products, 9-11th June, 2010, Tromso, Norway. (Poster and Oral presentation).

Hogan, S.A., Chaurin, V., O'Kennedy, B.T. and Kelly, P.M. 'Influence of milk proteins on hardness development in high-protein, solid food matrices'. European Federation of Food Science & Technology (EFFoST), 10-12th November, 2010. Dublin, Ireland. (Poster presentation).

Project number:
5940

Date:
October, 2014

Funding source:
Enterprise Ireland

Project dates:
June 2008 – May 2013

Collaborating Institutions:
University of Limerick
University College Dublin
University College Cork
Dublin City University

Teagasc project team:
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Prof. Torres Sweeney
Dr. Ashling Robinson and Dr. James Lyng, University College Dublin

Compiled by:
Phil Kelly

Pre-commercial scale-up of biologically active milk protein hydrolysates (FHI Project WP3)

Key external stakeholders:

This Industry-led, EI-funded Food for Health Ireland (FHI) project was co-funded by 4 major Irish dairy manufacturers Glanbia, Kerry, Carbery and Dairygold. The FHI project was governed by a consortium agreement drawn-up in conjunction with all participants which set out protocols for the uptake of results.

Practical implications for stakeholders:

Successful precommercial scale-up work at Moorepark retained bioactivity of FHI lead functional compounds (LFCs) i.e. enzymatically-produced milk protein hydrolysates and their sub-fractions in line with their original laboratory-based protocols, and also satisfied the microbiological specification necessary for formulation of the active ingredients in human clinical trial diets (undertaken by UCD).

- Pre-commercial scale-up contributed substantively towards the compilation of technological data which will be incorporated in scientific dossiers setting out health claims for individual LFCs to be submitted to the European Food Safety Authority (EFSA).
- In addition to the protocols and LFC's assigned by FHI, the pre-commercial scale-up team generated a novel casein-based hydrolysate and sub-fractions which was biologically active against multiple physiological functions (anti-inflammatory; endothelial and satiety-ghrelin)
- Technological developments employed to enrich biological activity during scale-up included advances in membrane separation technology e.g. charged- and electro-membrane based processes.

Main results:

The following is a list of outputs accomplished by the FHI pre-commercial scale-up team:

- No. protocols validated (laboratory): 150
- No. plant scale-up trials: 50 (small) and 35 (large)
- **LFC's** (Lead Functional Compounds)**:** 6 based on the MF025 hydrolysate series.
- **ACR** (Available Centre Result): 1 (Hypoallergenic Infant Dessert)
- **NPD** (Novel Product Development): 3 (Family Milk & HA Infant Dessert)
- Complementary research highlighted the benefits of protein aggregation-enhanced enzymatic hydrolysis.

Opportunity/Benefit:

Ground rules laid down in the FHI consortium agreement set out conditions for priority right of access by its Industry Partners to project outputs with commercial potential. Otherwise, expressions of interest in the scale-up and characterisation of FHI milk protein hydrolysates and their fractions will be entertained by the technology transfer officer. An FHI 'available centre result' (ACR) based on the novel formulation of a hypoallergenic infant food (desert-format) is currently licensed out for evaluation.

1. Project background:

The Food for Health Ireland (FHI) project represents an alliance of academic and industry partners with the single goal of creating a critical scientific mass to test scientific hypotheses surrounding the preparation of bioactive peptides from milk, investigate them *in vitro*, assess the mechanism by which their effects are exerted and undertake clinical studies using animals and humans to confirm their efficacy.

FHI featured an intelligent milk mining programme and an extensive bioassay analytical platform to screen over 1000 peptides in order to identify those with biological activity. Out of this, a total of 75 lead functional compounds (LFCs) or bioactives with a potential health benefit were qualified. A select number of these LFCs were scaled-up up in Moorepark's pilot plant facilities under good manufacturing practice (GMP) in order to generate key ingredients for evaluation in clinical trials (UCD) in the course of completing scientific dossiers in accordance with the European Food Safety Authority's (EFSA) procedures for establishment of health claims.

FHI industry partners were actively engaged in the research throughout by providing guidance and ingredient substrates for mining and scale-up studies. The industry partners through their own market interactions were able to set out mission critical information concerning end-product specifications and techno-economic constraints to guide the technology transfer process.

2. Questions by the project:

Pre-commercial scale-up (WP3) was required to take charge of upscaling laboratory protocols for the preparation of milk protein hydrolysates (and where relevant, their sub-fractions) generated by FHI's milk mining laboratories. The principle obligation on the WP3 precommercial scale-up team was to transfer protocols faithfully in accordance with their original biological characterization while at the same time adapting technology to accomplish more sustainable processes without loss of activity. In terms of the overall project, the team's contribution in preparing LFC ingredients for clinical trial created another critical knowledge input in the chain of event that complete the framing of individual health claims.

3. The experimental studies:

Replication of laboratory protocols at Moorepark required different scales of reactors for the initial hydrolysis step in order to confirm hydrolysis conditions and rates in collaboration with the bioassay testing platform. HPLC analyses provided a profile of the hydrolysate peptide mixture. Fractionation of hydrolysates was undertaken by means of membrane filtration technologies with membranes ranging in molecular weight cut-off (MWCO) from 50 kDa down to 1 kDa. These membrane separation steps were also scalable from laboratory to large pilot-plant.

Selective heat-treatments of enriched fractions of individual whey proteins and whey protein isolate(s) (WPI) were undertaken to evaluate their effects on aggregation behaviour and subsequent susceptibility to hydrolysis. This was examined at both a sub-molecular and macro-molecular level in line with varying degrees of hydrolysis (DH).

Selected hydrolysis processes of heat denatured/ aggregate proteins were successfully scaled-up in the pilot plant, and incorporated successive membrane filtration steps (cascade membrane fractionation) to produce a range of spray dried hydrolysate fractions with altered molecular weight (Mw) distributions and bio-functional characteristics. This enabled partition of both the

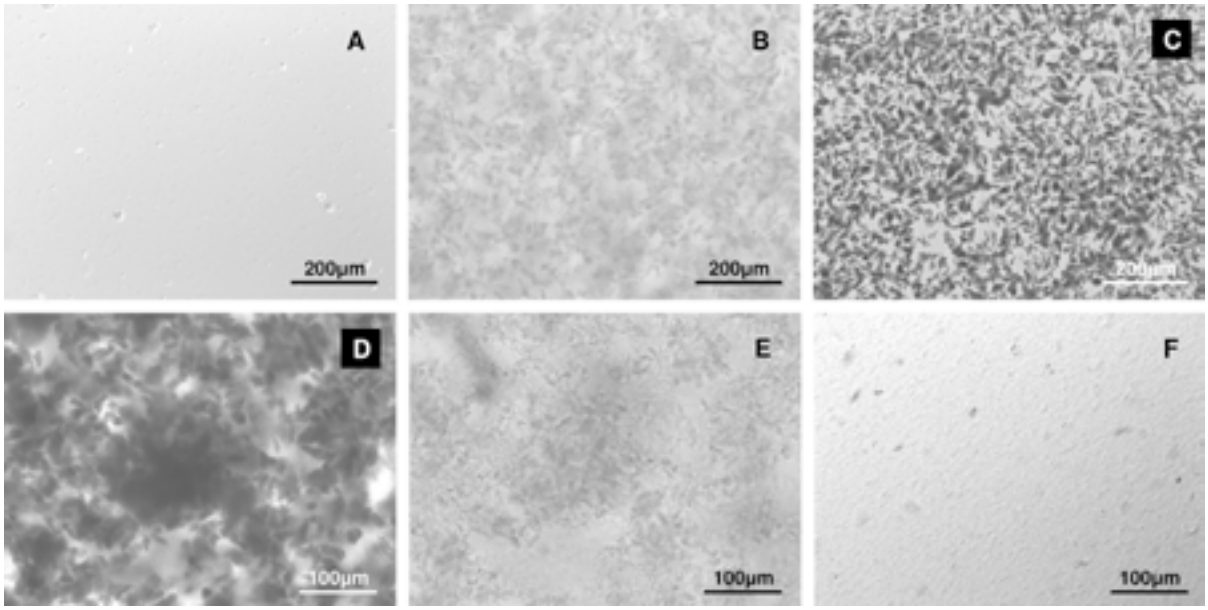

Figure. 1: Confocal laser micrographs of heat-induced aggregation of whey protein isolate with increasing intensity (frames A, B, C) and aggregate disintegration during the course of enzymatic hydrolysis (frames D, E, F) with accompanying reduction in mean particle size. Source: National Food Imaging Centre (Dr. Mark Auty)

iron chelating and the angiotensin-I-converting enzyme (ACE) inhibitory properties in both control and heat-treated systems.

4. Main results:

Of 150 validation tests undertaken at laboratory level on protocols received, 50 were progressed to small pilot scale processing (<5 kg dried hydrolysate ingredient output) from which 35 were taken to large pilot scale preparation and spray drying (<100 kg).

Developments in separation technologies e.g. use of electrofocussing, and charged membranes were key to the characterisation of bioactive peptides at approx. 1,000 Da. FTIR (Fourier transformed infra-red) technology provided some insights into the conformation changes taking place in whey protein structure. Aggregation-enhanced hydrolysis (Figure 1) not alone improved kinetics but also shaped final outcomes e.g. hydrolysis of denatured WPI favoured the generation of higher levels of free essential amino acids; lysine, phenylalanine and arginine compared to the unheated substrate. Distinct peptides release from the heat-treated system were mapped to parent molecules and theoretically attributed to certain endo-protease activities. The heat pre-treated substrates, which exhibited increased viscosity and surface hydrophobicity, demonstrated significantly increased ($P < 0.001$) hydrolysis rates with the enzymatic preparation Corolase® PP. The proteinaceous components were hydrolysed in the order: CMP > β-Lg A > β-Lg B > α-La. Hydrolysates (5 %DH) revealed an increase in soluble molecular weight (Mw) material greater than 30 kDa which was discerned to be material derived mainly from 1Leu-Arg40, 70Lys-Phe82 and 140Leu-Met145 regions of β-Lg and the 1Met-Ile20 region of CMP.

Strong iron-binding peptides were also identified which were found to be *in vitro* gastric stable. A positive correlation ($P < 0.01$) was established between the average Mw of fractions and ferrous (Fe2+) chelating capability. Upon solid phase extraction these fractions possessed high total concentrations of the basic amino acids and possessed ferrous chelation equivalent to 84.4 µM EDTA. The strongest ACE inhibitory fractions were the 1 kDa permeates of both control and prior heat-treated WPI process streams (activity as IC50 = 0.17 g L-1). Isoelectric focussing (IEF) of the hydrolysate fraction further increased ACE-inhibition in fractions collected within the pH range 6.1 – 6.6.

5. Opportunity/Benefit:

A hydrolysate preparation (MF 025) developed by the Moorepark WP3 team generated multiple biological hits i.e. anti-inflammatory, endothelial function and satiety / ghrelin activation. Six LFC's were produced in the course of sub-fractionating the parent hydrolysate – further study is underway to underpin

IP claims (Teagasc/UCC collaboration) which are in the course of being established for the fraction responsible for ghrelin activation. The process for enrichment of iron-binding peptides may be a possible contender for commercial evaluation. The IP offer of this know-how would need to be firstly made to the FHI industry partner before being put on general release.

6. Dissemination:

A commercialisation protocol within the FHI consortium agreement sets out rules whereby the industry partners have first opportunity to express interest in evaluating project outputs in conjunction with their business interests for a limited time period.

Main publications:

Gaudel C, Nongonierma AB, Maher S, Flynn S, Krause M, Murray BA, Kelly PM, Baird AW, FitzGerald RJ, Newsholme P. (2013) A whey protein hydrolysate promotes insulinotropic activity in a clonal pancreatic β-cell line and enhances glycemic function in ob/ob mice. *J Nutr.*143:1109–14. doi: 10.3945/jn.113.174912

O'Loughlin, I.B., Murray, B.A., Kelly, P.M., FitzGerald, R.J. and Brodkorb, A. (2012). Enzymatic hydrolysis of heat-induced aggregates of whey protein isolate. *Journal of Agricultural and Food Chemistry* 60: 4895 – 4904.

O'Loughlin, I.B., Murray, B.A., Brodkorb, A, FitzGerald, R.J. and Kelly, P.M. (2014a). Pilot-scale production of hydrolysates with altered bio-functionalities. *International Dairy Journal* 34:146 – 152.

O'Loughlin, I.B., Murray, B.A., FitzGerald, R.J., A. Brodkorb, Robinson, A.A, Holton, T.A. and Kelly, P.M. (2013). Whey protein isolate polydispersity affects enzymatic hydrolysis outcomes. *Food Chemistry* 141: 2334 – 2342.

O'Loughlin, I.B., Murray, B.A., Brodkorb, A., FitzGerald, R.J. & Kelly, P.M. (2014b). Production of whey protein isolate hydrolysate fractions with enriched ACE-inhibitory activity. *International Dairy Journal* 38, 101 – 103.

O'Loughlin, I.B., (2014) Enzymatic hydrolysis of heat-denatured whey proteins. PhD thesis. University of Limerick.

Popular publications:

Ian O'Loughlin, PhD Walsh Fellow, attached to the project was the recipient of the following awards during the period 2010–13 for his research achievements:

2010: Young Researcher winner of ***Travel Grant for attendance at the European PhD Conference*** on Food Science & Technology, Berlin.

2011: Annual Food Science & Technology Conference, UCC – Best Student Presentation.

2012: University of Limerick, Department of Life Sciences Annual Student Conference – Best Student Presentation.

2012: Winner of the Eamonn P. McCormick medal for Best Student Poster presented at the **Society of Dairy Technology Conference** UCC, September 2013

2013: Shortlisted for Young Scientist Award at the 8th NIZO Dairy Conference.

Project number:
5986
Date:
Nov, 2014
Funding source:
Dairy Levy
Project dates:
Sept 2009 – Dec 2012

Collaborating Institutions:
None

Teagasc project team:
Dr. Phil Kelly (PI)
Mr. Jim Kelly

Compiled by:
Phil Kelly

Improved whey permeate drying using high pressure gas/liquid dosing during spray atomisation

Key external stakeholders:

Irish dairy processors and whey ingredient manufacturers on behalf of their dairy farmer members.

Practical implications for stakeholders:

- Modification of the feed dosage systems using high pressure gas dosing into the concentrate line to nozzle atomisers of spray driers looks promising as a means of improving permeate drying without undue deposit formation.
- Such a high pressure gas/liquid dosing is uniquely installed on Moorepark's MTL Tall-form drier and may be availed of by stakeholders and clients to pursue more detailed R&D investigations.
- Complementary on-site specialised analytical services such as microscopy (National Food Imaging Centre), rheology and particle size monitoring serve enable a comprehensive development programme to be pursued.

Main results:

High pressure CO_2 dosing in the concentrate feed line to the spray atomiser would appear to potentially benefit whey permeate drying. It would appear that the beneficial effects may be attributable more to changes in powder physical properties rather than alteration of the glass transition states. It is recommended to that careful control of the gas dosing is exercised in order not to impact negatively on the wettability behavior of the powders.

Opportunity/Benefit:

Processing conditions established during the course of the study may be used by dairy company R&D personnel in order to accomplish improved spray drying of whey permeates using novel technologies installed on the pilot plant drying facilities are Moorepark Technology Ltd. The results of such investigations would be readily scalable to industrial manufacturing scenarios.

1. Project background:

Global Whey production is currently expanding at an annual rate of +2%. While whey powder represents the high level of production at 2.38 Mt, its annual growth rate of +1% pales in comparison with permeate powder which is enjoying 10–20% annual expansion, albeit from a much smaller base (0.76 Mt). Permeate powder is increasingly sought for product formulation and standardisation purposes. However, it is a challenging product to dry using standard spray drier configurations because of stickiness behaviour associated with lactose transition states.

Both whey and permeate drying use broadly similar technologies. However, differences emerge at a physico-chemical level: absence of true protein in permeate makes drying more difficult, viscosity increases in concentrates (> 40%TS), while the thixotropic nature of permeate concentrates means that viscosity decreases with increasing shear. In addition, economic factors predominate since permeate is a low value by-product stream with lower TS than whey, hence the impetus is towards energy efficient processes that require as much removal of water as possible by evaporation before drying.

2. Questions addressed by the project:

- The objective of the project was to investigate whether adaptation of the feed conditions to the nozzle atomiser of a spray drier could be used to favourably influence resulting permeate powder particle formation so as to allow improved flow without undue deposit formation in the drying chamber and ancillary components e.g. cyclone, rotary valve and fluidised beds.
- The question was whether a novel high pressure gas/liquid dosing system installed on Moorepark's MTL Tall-form drier for modification of food ingredient functionality would benefit the drying of permeate feedstocks by minimising stickiness and particle adhesion within the drier chamber.

3. The experimental studies:

Spray drying of permeate concentrate containing 40% TS at 65°C was undertaken with 3 levels (0.13; 0.25; 0.50%) of high pressure CO_2 injection. The Tall-form spry drier was configured to operate with 3 spray nozzle lances with drying inlet and outlet temperatures of 185°C and 85°C, respectively. The external fluidized beds VF1 and VF2 were 65°C and 30°C, respectively. Spray drying was also conducted in both regular and agglomerated mode. By this is meant that 'regular' powder generally has poorer dissolution behavior. Agglomeration, on the other hand, exploits the recycling of fine powder particles into the spray atomization zone during drying in order to promote clustering of small semi-dry particles with large ones – the resulting 'agglomerates' facilitate improve wettability and dispersibility characteristics during subsequent powder contact with water.

Key powder properties monitored throughout the individual treatments includes Bulk Density, Particle Density and Wettability.

4. Main results:

High pressure CO_2 dosing during permeate spray drying resulted in powders which were more porous (contributing to more rapid drying of powder particles) and reduced deposit formation. However, excess dosage of high pressure CO_2 tended to make powders more bulky. For this reason, judicious use of gas dosing is required in order to maintain drying with minimal deposit formation over time at the expense of small compromises in powder bulkiness.

Effects of high pressure CO_2 dosing into the concentrate feed line

- There was no visual evidence of chamber deposits/stickiness occurred during drying of permeate
  - Only slight differences in the material state of the powders as expressed by the T-Tg values (glass transition behaviour) were observed.
- Flowability improvement
  - High pressure gas-injected powders were more porous, and contributed to a more rapid drying powder particles.
  - Incremental changes in particle structure as a result of gas injected appeared to be more significant that particle surface characteristics.
- Bulk Density
  - Excess gas injection has a potential downside as it tends to make powders more bulky, hence it is necessary to strike a balance between stickiness control and powder bulk density distortion.

Table 1. Lower levels of CO_2 dosing at the lower level favours retention of desired Bulk Density and better Wettability properties.

Mode	% CO_2 dosing	Bulk density (g/mL)	Wettability (sec)	
Regular powder*	0.13	0.53	17.5	optimum CO_2 dosing conditions for regular powder
	0.25	0.33	> 60	
	0.5	0.2	> 60	
Agglomerated powder	0.13	0.34	24	optimum CO_2 dosing conditions for agglomerated powder
	0.25	0.32	9	
	0.50	0.2	> 60	

* Typical bulk density of regular powder (without gas dosing): 0.76

5. Opportunity/Benefit:

Modification of the feed dosage systems to the nozzle atomisers of spray driers looks promising as a means of improving permeate drying without undue deposit formation. It appears that the more rapid drying occurring as a consequence of better particle porosity may be pivotal in influencing stickiness behaviour. The process however, requires validation at industrial scale where higher concentrate solids and different lactose crystallization conditions prevail. As a first step, it is recommended that permeate and whey concentrates prepared on industrial scale evaporators should be tested on the pilot scale Tall-form drier which uniquely incorporates the above described high pressure gas dosing system at Moorepark Technology Ltd.

6. Dissemination:

J. Kelly, P.M. Kelly, D.J. O'Callaghan & S.A. Hogan (2009) *Spray drying whey permeate*, presentation given at the 4th International Symposium on Spray Dried Dairy Products Symposium, 15–17th April, Melbourne, Australia.

Popular publications:

Dairy Levy Funding Research Report 2013

Predicting beef eating quality

Key external stakeholders:

Beef processors, retailers.

Practical implications for stakeholders:

Beef processors could use the Meat Standards Australia (MSA) grading system to sort individual cuts into eating quality classes priced accordingly. Such a guarantee of expected eating quality could increase the share of the market particularly at the premium end. For optimum eating quality boning should not be carried out on the day after slaughter. Processors and retailers need to consider the negative effects of MAP on eating quality.

Main results:

- The MSA palatability grading scheme uses a predictive model to assess the eating quality of individual cuts from each carcass and assigns them to a quality class.
- Although the model was developed in Australia using Australian consumers our research showed that it worked equally well for Irish beef and Irish consumers.
- The model was tested over a wide range of carcass types and for three cooking methods (grill, roast and thin slice) with over 1600 consumers tasting over 1100 samples.
- Factors of particular importance to the Irish beef industry (breed, sex, electrical stimulation, aitch-bone hanging, prolonged ageing) were accounted for by the model.
- Boning at 24 v 48 hours post mortem had a small negative effect on eating quality and this was not accounted for by the model.

Project number:
5418
Date:
October, 2013
Funding source:
DAFM
Project dates:
April 2005 – June 2008

Collaborating Institutions:
University College Cork
University College Dublin

Teagasc project team:
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Dr. Karen Brandon
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Dr. Maeve Henchion and
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External collaborators:
Prof. DaWen Sun and
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College Cork

Compiled by:
Paul Allen

- PiVac, a novel method of avoiding cold shortening of hot boned beef (Tenderbound) produced meat of equal quality to cold boning.
- High resolution imaging using hyperspectral imaging can predict eating quality attributes with a high degree of accuracy.
- High oxygen MAP promotes lipid oxidation leading to off-flavours and protein oxidation leading to less tender meat.
- Irish consumers preferred meat from MAP packs with 50% oxygen despite a high level of lipid oxidation.

Opportunity/Benefit:

Irish beef processors could use the MSA system to sort beef into quality classes and supply the market with beef of guaranteed quality.

1. Project background:

Previous research has shown that the eating quality of Irish beef is variable. This agreed with major studies carried out in Australia and USA. In those two countries there is some attempt at assessing likely eating quality as part of the carcass grading, but the EUROP carcass grading scheme is not an attempt related to palatability. Meat and Livestock Australia addressed the problem of declining beef sales due to inconsistent palatability by devising a grading system that predicts the eating quality of the main beef cuts from individual carcasses cooked by a number of different methods. The inputs for the model are all the live animal and post slaughter factors that are known to affect palatability. The effects of these factors were assessed by around 65,000 consumers who scored samples for tenderness, juiciness, flavour and overall liking. These were combined into a Meat Quality Score (MQS) using appropriate weightings, and cut off points have been determined for four quality categories, denoted by stars. The system has proven to be successful in Australia, as evidenced by the number of carcasses graded by the MSA system increasing each year and consumers being willing to pay a premium for graded cuts. Adoption of the MSA system or something similar by the Irish beef industry could improve consumer confidence in the eating quality of Irish beef and increase exports. The model can also be used as a management tool to optimise eating quality and as a training tool to demonstrate to producers how to improve eating quality.

Packaging method is a factor known to affect eating quality that is not included in the model. High oxygen modified atmosphere packaging (MAP) which is now widely used to preserve the attractive bright red colour retail cuts of beef has been shown to adversely affect the flavour and tenderness. In collaboration with UCC studies were carried out to determine the optimum MAP packaging conditions for Irish beef.

An alternative to the palatability at critical control points (PACCP) approach to predicting eating quality that was adopted by MSA is to take non-invasive measurements that are correlated with eating quality. Hyperspectral Imaging (HI) is a technology that shows potential in this regard. A study was carried out in collaboration with UCD to determine whether HI could predict eating quality traits and intramuscular fat.

2. Questions addressed by the project:

The MSA model for predicting palatability is based on a large dataset of Australian beef samples tasted by Australian consumers. It allows the Australian beef industry to market beef cuts with an expected eating quality grade – 3-star, 4-star and 5-star. If this model were suitable for application by the Irish beef industry it would enable then to market beef of more consistent quality. The main question addressed by the project is whether the MSA model would be as accurate at predicting the eating quality assessments made by Irish consumers for Irish beef. A secondary question is whether the main processing factors commonly used in Irish factories are accurately accounted for by the model. If the latter were not the case then a modified model could be considered for Ireland.

In relation to MAP packaging the issues addressed were firstly the optimum percentage of oxygen to maximise eating quality as assessed by Irish consumers and secondly the effect of meat to head space ratio on the eating quality and shelf life.

In relation to HI the issues addressed were how accurately could HI predict eating quality traits and intramuscular fat and what was the best approach to building the predictive models.

3. The experimental studies:

Firstly, a trial was carried out in collaboration with MSA to determine the performance of the model on Irish beef and Irish consumers compared to Australian beef and Australian consumers. Samples from 18 carcasses were taken from a relatively homogenous group of heifers and a similar set of samples were selected in Australia and shipped to Teagasc Ashtown. Duplicates of the latter set were scored by Australian consumers. Irish consumers scored both the Irish and Australian samples. Samples were grilled, roasted or cooked by Yakiniku method (thin slice). The resulting MQS scores were compared

with the model predictions and the Irish and Australian scores were compared with each other.

Secondly, samples were collected from a more variable group of carcasses to test the performance of the model over a wider range. These samples were cooked and presented to Irish consumers.

Finally, a series of experiments were conducted to test the model for a number of factors known to be important for the Irish industry. These included breed, sex, high and low voltage stimulation, aitch bone hanging, boning time and ageing time (maturation). These samples were also presented to Irish consumers and their scores were compared with predictions from the model.

4. Main results:

The overall conclusion from the comparative trial was that the model fits Irish beef and Irish consumers at least as well as it fits the Australian beef and consumers for which it was developed. There were some small differences in the relative importance of tenderness and flavour to Irish and Australian consumers.

The model also worked quite well for the variable sample and accounted for the factors of low voltage electrical stimulation (LVES) and ageing time (14 v 28 days) with good accuracy.

How well the model accounted the effects of high voltage electrical stimulation (HVES), breed and sex was tested with cattle from the Teagasc herd at Johnstown Castle. Overall the mean deviations from the model were small for both sexes, both breeds and for HVES and non-stimulated carcases. The only significant sex effect on the eating quality was a higher satisfaction rating for the eye of round sample from heifers compared to steers while the only significant breed effect was a higher score for Charolais eye of round samples than for Aberdeen Angus samples. HVES samples tended to score higher than non-stimulated samples though these differences were significant only for the striploin.

The effect of carcass suspension technique in conjunction with LVES was tested in another experiment. Overall the model was a good fit for both hanging methods with and without stimulation with small mean deviations from the model for all groups. Accuracy was significantly better for aitch bone hung samples, with Achilles tendon hung samples having mean deviations greater than 10 units. Aitch bone hung samples had lower (more tender) average Warner Bratzler Shear Force (WBSF) 14 day values, indicating the tenderising effect of this hanging method on some muscles.

The effect of boning time on eating quality and how this was accounted for by the model was studied. The MSA model assumes that samples are boned out after rigor has been fully resolved which may not be the case on the day after slaughter when boning is often done. The model significantly overestimated the eating quality of 24-hour boned samples suggesting that rigor resolution was had not been completed at this time. Hot (1 hour) and warm (4 hour) boning were also studied. Both hot and warm boning resulted in the mean actual scores for grilled striploin and roasted topside samples being lower than predicted by the model. This is not surprising as the model was developed for cold boning and it is known that removing cuts prior to rigor results in shortening and consequent toughening. These results suggest that the model is not appropriate for hot-boned and warm-boned samples.

Tightly wrapping hot-boned muscles using the PiVac machine has been shown to avoid the negative effect of hot-boning on tenderness. How well the model worked for such samples was tested. The model fitted PiVac samples as well as it did control samples. Mean deviations were small for both groups and both cuts (striploin and topside) indicating no biases. The absolute differences were smaller for PiVac striploin samples suggesting a better fit to the model. This may have arisen because the PiVac system has been shown to reduce variability in tenderness.

Much beef is now sold in high oxygen modified atmosphere packaging (MAP). Recent studies have shown that this may decrease tenderness due to oxidation of the proteases and adversely affect the flavour due to lipid oxidation. The effect of varying the oxygen content of MAP packs on the eating quality of beef steaks was studied in collaboration with UCC. Sensory panels preferred steaks stored in 50% oxygen (0 to 80% studied). This result was more or less repeated with a larger consumer test, which showed a preference for steaks stored in 40% oxygen. TBARS tests confirmed that lipid oxidation had occurred in these steaks suggesting that Irish consumers prefer some degree of rancidity. Protein oxidation and consequent reduced tenderness was also confirmed in these samples suggesting that the flavour effect overrides the tenderness effect.

In collaboration with UCD a novel image analysis system for predicting beef palatability was developed. Using a range of data and image analysis approaches, models to predict the eating quality attributes and WBSF values with a high degree of accuracy have been developed. This could be used alone or to augment the MSA model predictions.

5. Opportunity/Benefit:

Irish beef processors could use the MSA grading system or a version of it optimised for Irish beef to sort cuts according to their expected eating quality. Markets could then be supplied with beef of more consistent eating quality which could be sold to consumers with an eating quality guarantee. This could help the industry to maintain or even grow their share of the premium markets for beef.

6. Dissemination:

Main publications:

Zakrys, P. I., Hogan, S. A., O'Sullivan, M. G., Allen, P., Kerry, J. P. (2008). Effects of oxygen concentration on sensory evaluation and quality indictors of beef muscle packed under modified atmosphere. *Meat Science*, 79, 648–655.

Zakrys, P. I., O'Sullivan, M. G., Allen, P., Kerry, J. P. (2009). Consumer acceptability and physiochemical characteristics of modified atmosphere packed beef steaks. *Meat Science*, 81, 720–725.

Zakrys, P. I., O'Sullivan, M. G., Allen, P., O'Neill, E. E. and Kerry, J. P. (2010). Investigation of the effects of commercial carcass suspension (24 and 48 hours) on meat quality in modified atmosphere packed beef steaks during chill storage. Food Research International, 43.1: 277–284.

Zakrys-Waliwander, P I; O'Sullivan, M G; Walsh, H; Allen, P; Kerry, J P. (2011). Sensory comparison of commercial low and high oxygen modified atmosphere packed sirloin beef steaks. *Meat Science,* 88.1: 198–202.

Zakrys-Waliwander, P I; O'Sullivan, M G; O'Neill, E E; Kerry, J P. (2012). The effects of high oxygen modified atmosphere packaging on protein oxidation of bovine M. longissimus dorsi muscle during chilled storage. *Food Chemistry*, 131.2: 527–532.

Jackman, P., Sun, D.-W., Allen, P. and Downey, G. (2008). Prediction of beef eating quality from colour, marbling and wavelet texture features. *Meat Science,* **80(4)**, 1273–1281.

Jackman, P., Sun, D. W. and Allen, P. (2009). Comparison of the predictive power of beef surface wavelet texture features at high and low magnification. *Meat Science*, **82(3)**, 353–356.

Jackman, P., Sun, D-W and Allen, P. (2009). Comparison of various wavelet texture features to predict beef palatability. *Meat Science,* **83(1),** 82–87.

ElMasry, G; Da-Wen, Sun; Allen, P. (2012). Near-infrared hyperspectral imaging for predicting colour, pH and tenderness of fresh beef. *Journal of Food Engineering,* 110.1: 127–140.

Jackman, P; Da-Wen, Sun; Allen, P. (2011). Recent advances in the use of computer vision technology in the quality assessment of fresh meats. *Trends in Food Science & Technology*, 22.4: 185–197.

ElMasry, G; Sun, D-W and **Allen, P.** (2011). Non-destructive determination of water-holding capacity in fresh beef by using NIR hyperspectral imaging. Food Research International 44. 9: 2624–2633.

Jackman, P; Sun, DaWen; **Allen, P**. (2010). Prediction of beef palatability from colour, marbling and surface texture features of *longissimus dorsi*. Journal of Food Engineering 96. 1: 151–165.

Jackman, P., Sun, D.-W., Allen, P., Brandon, K. and White, A. (2010). Correlation of consumer assessment of longissimus dorsi beef palatability with image colour, marbling and surface texture features. Meat Science 84, 3: 564–568.

Jackman, P., Sun, D. W. and Allen, P. (2009). Comparison of the predictive power of beef surface wavelet texture features at high and low magnification. Meat Science vol. 82, no. 3, p. 353–356.

Jackman, P., Sun, D.-W. and Allen, P. (2009). Automatic segmentation of beef longissimus dorsi muscle and marbling by an adaptable algorithm. (2009d). Meat Science 83(2): 187–194.

Jackman, P., Sun, D.-W. and Allen, P. (2009). Comparison of various wavelet texture features to predict beef palatability. (2009c). Meat Science 83(1): 82–87.

Jackman, P., Sun, D.-W., Du, C.-J. and Allen, P. (2009). Prediction of beef eating quality from colour, marbling and wavelet texture features using homogeneous carcass treatment. *Pattern Recognition,* 42(5): 751–763.

Technology for healthier pork products

Key external stakeholders:

- Meat processors.
- Ingredients companies.
- Food Retailers.
- Consumers.

Practical implications for stakeholders

The information generated by this project will assist meat processing companies to develop healthier meat products that are as appealing and satisfying to eat as standard versions of traditional products such as sausages and luncheon roll. Healthier means containing less salt and/or fat and using natural ingredients such as plant derived antioxidants and prebiotic fibres.

Main results:

- The salt content of pork sausages was reduced from 2.5% to 1.4% without a noticeable change in sensory attributes, composition, emulsion stability, lipid oxidation or shelf life. High pressure processing at 200 MPA may assist in producing reduced salt sausages with the same functional properties as controls.
- An acceptable breakfast sausage with 39% less calories enriched with 2.5% prebiotic fibre was produced with equivalent sensory and quality attributes to standard sausages.
- A phytosterol ester was incorporated into a reduced salt pork breakfast sausage with organoleptic properties which were favoured by the trained sensory panel.
- Grape seed extract (GS) and Rosemary-Pomegranate (RP) extract had no effect on sausage appearance, overall liking, tenderness, flavour or juiciness liking.
- Half the nitrite in a pork luncheon roll was replaced with tomato powder without negatively affecting sensory attributes.

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Opportunity/Benefit:

There is growing consumer and hence retailer interest in "clean" label foods and functional foods. This research has shown that healthier versions of traditional meat products such as sausages and pork luncheon roll can be produced that are just as acceptable to consumers as standard versions of the same products. Meat processors wishing to exploit this potential can use these results to guide the development new products or collaborate with the team at Teagasc Research Centre Ashtown.

1. Project background:

Traditional meat products such as sausages and cooked ham are often high in fat, salt and contain additives to prolong shelf life, improve colour and prevent oxidation. Consumers are aware of the possible health risks of these products but enjoy eating them. Producing healthier versions of these traditional meat products would therefore find favour with many consumers. In this project, which was part of a large EU project aimed at improving the entire pork production chain from farm to fork, we aimed to develop the knowledge to produce healthier versions of traditional meat products that would be just as acceptable to consumers as standard versions.

2. Questions addressed by the project:

- Are low salt sausages as acceptable to consumers as higher salt versions?
- Can high pressure processing play a role in reducing the salt content?
- Do low fat sausages containing a prebiotic fibre taste as good as standard sausages?
- Do sausages containing a phytosterol ester taste as good as standard sausages?
- Can natural antioxidants such as rosemary-pomegranate extract and grape seed extract be used in sausages without affecting flavour or appearance?
- Can tomato powder be used to replace some of the nitrite in pork luncheon roll?

3. The experimental studies:

The combined effect of high pressure processing (HPP) and salt level on the sensory and technological properties of pork breakfast sausages was examined over a 21 day storage period. Four pressure treatments (0.1 (control = atmospheric pressure), 200, 300 and 400 MPa) were applied to lean and fat before sausage fabrication. Sausages were manufactured to contain a salt level of 2.5% or 1.4% and refrigerated for 21 days at 4°C. Shelf life, composition, texture and sensory analysis assessments were carried out.

Pork breakfast sausages were prepared at two fat levels (22% (standard) and 13% (reduced fat)) containing a prebiotic fibre (inulin) at four levels (0, 2.5, 5 and 7.5%). The influence of fat and prebiotic fibre addition on the organoleptic and textural properties of the pork sausages was determined.

Pork sausage were made with three levels of the phytosterol *Vegapure*: control with no added phytosterol (VG0), with 1% phytosterol added (VG1) and with 2% phytosterol added (VG2). The sausages were stored for 0, 10, 15, and 20 days when quality and technological parameters analysed.

Grape Seed extract (GS) was added at 100 (GS100) and 200 (GS200) µg/g and Rosemary-Pomegranate (RP) extract was added at 250 (RP250) and 500 (RP500) µg/g to breakfast pork sausages. An analysis of the technological and sensory attributes of these sausages was then carried out.

Nine treatments of pork luncheon rolls were produced with three sodium nitrite levels (0, 0.05 and 0.1%), three tomato pulp powder levels (0, 1.5 and 3 %) at three storage times (2, 7 and 14 days). The effects of cooked pork luncheon roll enriched with tomato powder on composition (protein, fat, moisture and ash), pH, colour, nitrosomyoglobin content, lipid oxidation, residual nitrate content, shelf life, texture and sensory analysis were investigated.

4. Main results

The salt level can be reduced to 1.4% without a noticeable change in sensory attributes, composition, emulsion stability, lipid oxidation and shelf life (TVC). High pressure treatment may have a role in producing reduced fat sausages as pressurised samples had higher pH, lower cook losses and slightly altered colour and texture attributes compared to non-pressurised controls. HPP in the range 200 to 400 MPa did not promote lipid oxidation and reduced TVC levels. A moderate pressure level of 200 MPa resulted in a sausage product with similar cook loss, colour, water holding capacity, texture and sensory attributes to control sausages at both salt levels, suggesting HPP improved protein extraction. However, HPP at 300–400 MPa would not be recommended as higher pressure levels altered binding properties and therefore texture and sensory attributes of the pork breakfast sausages.

The incorporation of inulin at levels of 5 and 7.5% significantly reduced pH and emulsion stability of the raw breakfast sausages. An average calorie reduction between standard and reduced fat breakfast sausages of 23% was achieved. It is possible to manufacture a standard pork breakfast sausage with up to 5% inulin in the final product without significant detrimental changes in the overall product quality. It is also feasible to produce an acceptable breakfast sausage with 39% less calories enriched with 2.5% prebiotic fibre without sacrificing overall sensory and quality attributes.

The incorporation of a phytosterol ester, *Vegapure,* to reduced salt pork breakfast sausages did not have any detrimental effects on lipid stability or sausage composition and organoleptic properties were favoured by the trained sensory panel. The addition of *Vegapure* had no effect on the redness or yellowness values of the raw pork sausages while a small but significant difference in lightness values on days 2 and 10 was observed. The addition of *Vegapure* had no effect on the pH, cook loss, or emulsion stability of the sausages and did not affect lipid oxidation during storage. For texture analysis, the addition of *Vegapure* reduced hardness and springiness, while no obvious trends were observed for chewiness, cohesion force and gumminess. No significant difference was observed for any of the sensory descriptors, indicating a positive result that *Vegapure* has the potential to be incorporated into the sausage better without negatively affecting the organoleptic properties or product quality of the sausage. This resulted in an acceptable functional meat product.

Addition of Grape seed extract (GS) and Rosemary-Pomegranate (RP) extract at both concentrations had no effect on pH, WHC, emulsion stability, colour, composition or texture profile of pork breakfast sausages. RP significantly reduced the cook loss relative to the control. GS100, GS200 and RP500 had reduced lipid oxidation. GS and RP at both levels had no effect on sausage appearance, overall liking, tenderness, flavour or juiciness. These results demonstrate the potential of natural flavonoid containing extracts to the meat industry in the development of novel healthy functional meat products.

Increasing the level of nitrite affected the colour, increased the pH of the cooked product, the nitrosomyoglobin value and the lipid oxidation but decreased the residual nitrite content. The reduction in nitrites did not significantly affect the composition and texture of the pork luncheon rolls. Tomato powder reduced the pH and increased redness and yellowness values of both the raw and the cooked product. Tomato powder affected the texture by decreasing the hardness, gumminess and chewiness and increasing the cohesiveness particularly at a level of 3%. TVCs for all treatments and storage days were below the safe limit for this type of cooked product. A sensory evaluation was performed using a trained panel on day 2 of storage. The pork luncheon roll formulated with 50 mg nitrite and 1.5% tomato had similar or enhanced sensory attributes compared to the luncheon roll containing no tomato and the high level of nitrite, resulting in a reduced nitrite enhanced pork luncheon roll product.

5. Opportunity/Benefit:

There is growing consumer and hence retailer interest in “clean” label foods and functional foods. The meat products sector is behind other sectors such as the dairy sector in supplying these needs. Furthermore meat products are perceived by many to be unhealthy due to their high fat, salt and artificial ingredients. This research has shown that healthier versions of traditional meat products such as sausages and pork luncheon roll can be produced that are just as acceptable to consumers as standard versions of the same products. There are opportunities for the meat industry to reduce the salt content of sausages to about 1.4% without affecting functionality or flavour. Natural antioxidants such as grape seed extract or rosemary-pomegranate extract can be used in place of artificial antioxidants. Fat can be partially replaced by a prebiotic fibre such as inulin without affecting sensory attributes and a phytosterol ester which has positive associations with health can be incorporated. Finally, nitrite which also has a negative image but is essential for colour development can be partially replaced by tomato powder.

6. Dissemination:

Main publications:

Daly, T., Ryan, E., Aherne, S. A., O’Grady, M. N., Hayes, J., Allen, P., Kerry, J. P. and O’Brien, N. (2010). Bioactivity of ellagic acid-, lutein- or sesamol-enriched meat patties assessed using an in vitro digestion and Caco-2 cell model system. Food Research International, 43(3): 753–760.

Hayes, J.E., Stepanyan, V., O’Grady, M.N., Allen, P. and Kerry, J.P. (2010). Evaluation of the effects of selected phytochemicals on quality indices and sensorial properties of raw and cooked pork stored in different packaging systems. Meat Science, 2010, 85(2): 289–296,

Hayes, J. E., Stepanyan, V., O'Grady, M. N., Allen, P. and Kerry, J. P. (2010). Effect of lutein, sesamol, ellagic acid and olive leaf extract on the quality and shelf-life stability of packaged raw minced beef patties. Meat Science, 84(4): 613–620.

Hayes, J.E., Stepanyan, V., Allen, P., O'Grady, M.N. and Kerry, J.P. (2010). Evaluation of the effects of selected plant-derived nutraceuticals on the quality and shelf-life stability of raw and cooked pork sausages. LWT – Food Science and Technology, 2010, 44(1): 164–172,

Hayes, J. E., Allen, P., Brunton, N., O'Grady, M. N. and Kerry, J. P. (2011). Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: Olive leaf extract (Olea europaea L.), lutein, sesamol and ellagic acid. Food Chemistry, 2011, vol. 126, no. 3, p. 948–955.

Hayes, J E; Canonico, I; Allen, P. Effects of organic tomato pulp powder and nitrite level on the physicochemical, textural and sensory properties of pork luncheon roll. (2013). Meat Science, 95(3): 755–762.

Popular publications:

J.E. Hayes, P. Allen (2011). The effect of inulin as a prebiotic fibre on organoleptic and technological properties of standard and low fat pork breakfast sausages. Proceedings: 57th International Congress of Meat Science and Technology, Copenhagen, Denmark. (Oral Presentation).

J.E. Hayes, P. Allen (2011). The development of functional pork breakfast sausages containing flavonoid rich extracts: Sensory and technological impact. Proceedings: 57th International Congress of Meat Science and Technology, Copenhagen, Denmark. (Poster presentation).

J.E. Hayes, P. Allen (2011). Monitoring the effects of high pressure processing, salt levels and refrigerated storage on sensory and technological properties of pork sausages. Proceedings: 57th International Congress of Meat Science and Technology, Copenhagen, Denmark. (Poster presentation).

Updating Cheesemaking Efficiency

Key external stakeholders:

Irish Cheese and Dairy Industry.

Practical implications for stakeholders:

Manufacturing efficiency is a key aspect of cheese manufacture which influences cheese composition, milk component recoveries and plant profitability.

A major outcome of the project is the provision of new information on the comparative effects of bovine chymosin and camel chymosin on Cheddar cheese making efficiency, and the effects of high heat treatment of milk at different pH on its rennet gelation and curd forming characteristics. It also provides an extensive compendium on the effects of milk quality and cheese manufacturing conditions on cheese making efficiency and quality in the form of 2 monographs (Moorepark Monographs 1 and 2) published in 2010.

Main results:

1. The use of chymosin of camel origin (*Camelus dromedarius*) or *Rhizormucor miehei* rennet in place of bovine chymosin (*Bos taurus*) as coagulant in the experimental manufacture of Cheddar cheese had significant effects on recovery of fat from milk to cheese, cheese yield, and age-related changes in primary proteolysis and texture. These effects depended on the level of coagulant (number of milk clotting activity units added) and firmness of the milk gel at cutting.
2. The effects of increasing pH from 6.6 to 7.5 during high heat treatment of milk (80°C for 5 min) resulted in depletion in the content of k-casein on the casein micelle and an increase in the level in the milk serum to an extent depending on pH. Desk-top cheesemaking studies indicated that increasing the milk pH during heating accentuated the adverse effects of high heat treatment on the rennet coagulability of the milk at pH 6.55 and its cheesemaking characteristics.

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Collaborating Institutions:
None

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3. Two monographs (Moorepark Monograph 1. Cheese manufacture: Quality Characteristics of the milk; Moorepark Monograph 2. Cheese Manufacture: Control and prediction of quality characteristics), on the effects of milk quality and cheese, manufacturing conditions on cheese making efficiency and quality were prepared and distributed to Irish Dairy industry in 2010.

Opportunity/Benefit:

The research makes available to the dairy industry a database of information on the effects of key cheesemaking parameters on manufacturing efficiency and cheese quality. The comparative study on different coagulants provides statistically validated, practically-applicable information on the impacts of the bovine chymosin, camel chymosin and *Rhizormucor miehei* coagulants on cheesemaking efficiency and changes in the proteolysis and texture of Cheddar cheese during maturation. The cheese manufacture monographs provide a user-friendly reference source of practical information directly applicable to optimization of cheese manufacturing efficiency and quality.

1. Project background:

Irish cheese production '000 tonnes has undergone a marked increase in the last decade from ~ 115,000 tonnes in 2002 to ~ 180, 000 tonnes in 2011, and now utilizes ~ 31% of total domestic milk production. Similarly, global production has grown dramatically in the same period and is now estimated at ~ 18 million tones annually. Such growth reflects an increasing global population, higher living standards and the adaptability of cheese to modern food service practices. This has resulted in a large expansion of international cheese trade leading to greater distances between the producer and the consumer. Coinciding with this, there has been an increase in demand for more consistent quality and innovative cheese products, differentiated with respect to sensory properties, usage characteristics, and nutrient profiling. Such demand is driven by higher consumer expectations, health agencies, legislators, suppliers and retailers in pursuit of greater market share. This in turn has necessitated a more rigorous approach to engineering cheese quality / characteristics, compliance with international standards (e.g. *Codex Alimentarius*), quality consistency and cost-effective manufacture.

Cheese manufacture *per se* essentially involves gelation of cheesemilk, dehydration of the gel to form a curd which is treated and ripened according to the variety of cheese. Curd manufacture and maturation are highly complex processes that involve controlled fermentation of lactose to lactic acid, protein aggregation and syneresis, enzymatic-induced hydrolysis of proteins and fat, and other biochemical/microbiological events that are variety specific. Cheese composition and quality are effected by the interactive effects of many factors including milk composition and quality, range and magnitude of control variables applied to the milk and curd during manufacture, and ripening conditions.

The project examines the effects of milk quality and manufacturing conditions on cheesemaking efficiency, and on the composition and quality of cheese, with emphasis on Cheddar cheese – the principal variety produced in Ireland.

2. Questions addressed by the project:

- How do different commercially-available coagulants, and especially the recently-introduced Camel chymosin, affect cheesemaking efficiency and key changes in the cheese during maturation?
- Can the cheesemaking potential of high-heat treated milk be improved by alteration of pH during heating?
- Is there a ready-available user-friendly information source of information on the factors affecting milk quality and on cheesemaking efficiency and quality (based on results from Moorepark and published literature) available to professions in the Irish Dairy and Cheese Industries?

3. The experimental studies:

Three different coagulants were evaluated in pilot-scale Cheddar cheese manufacture: Bovine chymosin (BC), camel chymosin (CC) and *Rhizormucor miehei proteinase* (Hannilase; Han). The MCAs of the rennets at pH 6.55 and 35°C was monitored using the Foss Lattodinamograffo coagulometer. The resultant 5 treatments were undertaken based on added milk clotting activity units (MCA) and gel firmness (GF) at cut, as measured using low amplitude strain oscillation rheometry on the rennet-treated cheesemilks: BC (MCA, 11.6; GF 30 Pa), Han (MCA, 11.6; GF 30 Pa) CC1 (MCA, 11.6; GF 30 Pa), CC2 (MCA, 8.7; GF 20 Pa), CC3 (MCA, 8.7; GF 30 Pa). The treatments were undertaken in quadruplicate in March/April 2011. Component recoveries (fat and protein) and yield were measured using a mass balance on the composition and weights of inputs (cheesemilk, rennet, salt) and outputs (cheese, whey streams). Cheeses were stored at 8°C and monitored for composition (14 days), proteolysis (30, 90, 180 days) and texture/rheology at 75 % compression (180 days).

Milk samples were pH adjusted to 6.5, 7.0 and 7.5, heated to 80°C for 5 min, cooled to 20°C, re-adjusted to pH 6.55, and evaluated for rennet coagulation properties using low strain oscillation rheometry. Following heat treatment and re-adjustment to pH 6.55, the milks were ultracentrifuged at 100, 000 g. The unheated milks, heated milks, and ultracentrifuged supernatants were analyzed for protein composition using reversed phase HPLC and particle size using ZetaMaster. Simultaneously, curds were manufactured at bench level from these milks (heated at pH 7.5, 7.0 and 6.5) and evaluated composition and meltability.

Published literature and Moorepark data (previously published and unpublished) was reviewed for impacts of variations in milk composition and quality, and manufacturing conditions, on cheesemaking efficiency and quality. Monographs for distribution to the Irish Dairy Industry were compiled using this review.

4. Main results:

1. The use of camel chymosin as coagulant, under the conditions specified for treatment CC2, significantly increased fat recovery and cheese yield relative to the corresponding values for bovine chymosin (treatment BC). Camel chymosin treatments (CC1 and CC3) did not significantly affect the latter parameters. Conversely, *Rhizormucor miehei* (Han) reduced fact recovery and cheese yield, compared to BC.
2. Camel chymosin treatments (CE1, CE2, CE3) significantly reduced the level of primary proteolysis during maturation but did not significantly affect the texture of the cheese. In contrast, *Rhizormucor miehei* (Han) did not significantly affect the level of primary proteolysis, but significantly reduced the firmness, fracture stress or fracture strain of 180 day-old cheese.
3. Increasing pH of milk at high heat treatment from 6.5 to 7.0 – 7.5 increased in the concentration of all caseins in the serum, as monitored by reverse phase HPLC, especially _-casein which increased from ~ 30% of total k-casein at pH 6.5 to ~ 90% at 7.5. Moreover, it also led to an increase in the levels of whey proteins remaining in the serum following heat treatment, from ~ 41% of total at pH 6.5 to 100% at 7.5. These changes coincided with the development of two particle types in the heated milk, namely a k-casein-depleted casein micelles (typically ~ 200 nm) and k-casein-denatured whey protein particles (kCnWPPs; typically 40 – 50 nm diameter). Increasing the pH at heat treatment reduced the rennet gelation time (RGT), but significantly reduced gel firming rate and gel firmness. The reduction in curd firmness, which impaired the suitability of the heated milk for coagulation, was more pronounced at pH 7.0 than at 7.5. The poorer rennet coagulation properties at the higher pH values of heating caused a marked deterioration in curd quality, with the curd becoming wetter, softer and more difficult to recover, especially at pH 7.0. The adverse effect on results suggest that hydrolysis of the caseino-macropeptide region of the k-casein from the kCnWPPs leads to their destabilisation in the presence of k-casein-depleted micelles, and these aggregates sterically impede the fusion/aggregation of casein micelles into a gel.
4. Moorepark Monograph 1 discusses the effect of following on milk quality for cheese manufacture: composition, state of the components (ratio of globular: free fat; degree of hydrolysis of casein or fat), the levels of indigenous and contaminating enzyme activity (from bacteria, somatic cells), and levels of contaminants and chemical residues. It concludes that milk quality concept is a dynamic entity, and a continuous quality improvement approach is required to meet the requirements of different stakeholders including the cheese manufacturer and the consumer. Moorepark Monograph 2 considers the effects of variations in various cheesemaking parameters including milk pre-treatments, gelation conditions, curd-whey treatments in cheese vat, and curd treatments ex-vat. It concludes that owing to this complexity, it is essential that the raw material, the unit operations and ripening conditions are strictly controlled to ensure that the desired properties are consistently achieved. To this end, Monograph 2 proposes a basic approach to quality assurance to reduce variation in cheese composition and quality; the approach advises the implementation of a Quality Assurance scheme that focuses on more objectively-defined standard operating procedures (SOPs), process validation and continuous quality improvement.

5. Opportunity/Benefit:

The research provides an extensive database on the effects of different factors (milk quality, cheesemaking conditions including coagulants) on cheesemaking efficiency and cheese quality and proposes a QA scheme for same. It also shows how the interact effects of heat treatment and pH on milk vis-à-vis its cheesemaking properties and provide insights into the development of novel dairy ingredients (k-casein depleted micelles, k-casein-whey protein aggregates) which may find potential use in areas such as specialized nutritional beverages or smart ingredients for food formulation or milk protein standardization.

6. Dissemination:

The results of this project have been transferred in large part to the Irish Dairy manufacturers by way of circulation of Moorepark Monographs 1 and 2, group discussions with companies, and interactions with individual companies.

Main publications:

Guinee, T.P. and O'Brien, B. (2010) 'The Quality of Milk for Cheese Manufacture' In Technology of Cheesemaking (eds. B.A. Law and A.Y. Tamime), 2nd edition, pp. 1–67. John Wiley & Sons Ltd, Chichester, West Sussex, UK. ISBN: 978-1-4051-8298-0.

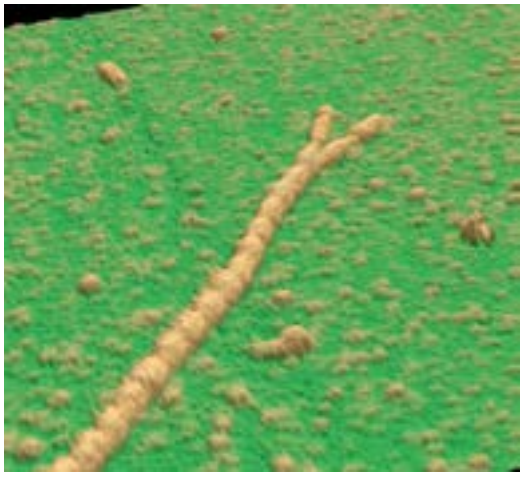
Guinee T.P. and O'Callaghan, D.J. (2010) 'Control and Prediction of Quality Characteristics in the Manufacture and Ripening of Cheese' In Technology of Cheesemaking (eds. B.A. Law and A.Y. Tamime), 2nd edition, pp. 260–329. John Wiley & Sons Ltd, Chichester, West Sussex, UK. ISBN: 978-1-4051-8298-0.

Popular publications:

Guinee T.P. and O'Brien B. (2010) 'Moorepark Monograph 1, Cheese Manufacture: Quality characteristics of milk', Teagasc Food Research Centre: Moorepark, Fermoy, Ireland. ISBN 1-84170-559-4.

Guinee T.P., & O'Callaghan D.J. (2010) 'Moorepark Monograph 2, Cheese Manufacture: Control and prediction of quality characteristics', Teagasc Food Research Centre: Moorepark, Fermoy, Ireland. ISBN 1-84170-561-6.

Properties of nano-fibrillar whey proteins



Project number:
5607
Date:
September, 2013
Funding source:
DAFM
Project dates:
Oct 2006 – Mar 2010

Key external stakeholders:

- Dairy Industry.
- Food and Ingredient Manufacturers.
- Biotechnology companies.
- Academic Institutions.

Practical implications for stakeholders:

The main objective was to produce fibrillar whey proteins at the nano-scale and assess their potential as functional ingredients. Main outcomes included:

- Optimised conditions for producing stable nanofibrillar whey proteins.
- Nanotechnology expertise in characterising the structure and formation mechanism of fibrillar proteins.
- Shown that nanofibrils can be used to create low salt gels, foams and biofilms.
- Development of nano-fibrils into a spray dried ingredient.
- Established a research platform of expertise in food nanotechnology.

Main results:

- Mechanism for forming nanofibrillar whey proteins has been established.
- Functionality of the nanofibrils has been assessed.
- Spray dried nanofibrils have been produced.
- New atomic force microscopy expertise has been gained.

Opportunity/Benefit:

This has established Ireland's first food nanotechnology platform based on nano-engineering food structures. Whey-based nanofibrils have unique functionality, in particular they are excellent foaming agents that can be used to replace more expensive ingredients such as egg-white. In addition, nanofibrils can be used as texturing agents in food products, for example to produce low-salt gels.

Collaborating Institutions:
Materials and Surface Science Institute
University of Limerick
Institute of Food Research
Norwich
Wageningen University

Teagasc project team:
Dr. Mark Auty (PI)
Dr. Lizhe Wang
Ms Daniela Oboroceanu
Dr. Andre Broskorb

External collaborators:
Prof Edmond Magner, MSSI, University of Limerick
Prof Vic Morris, IFR,Norwich
Dr. Paul Venema, Food Biophysics, University of Wageningen

Compiled by:
Mark A.E. Auty

1. Project background:

Recent research has demonstrated that many globular food proteins, including bovine whey proteins such as b-lactoglobulin, can self-assemble into fibrils ("nano-fibrils") at high temperature and low pH. Nano-fibrils typically have a thickness of 2 – 5nm and are highly poly-disperse with lengths up to 15 mm. Whey, a major by-product of cheese processing, represents an indigenous source of these novel food structuring agents. The potential of these nano-fibrillar assemblies as functional ingredients in foods has yet to be exploited.

2. Questions addressed by the project:

The objectives of this project are to optimize conditions for nano-fibril assembly and to investigate their stability and potential as food ingredients. This research will also establish a new food nanotechnology platform, enabling development of new scientific techniques, novel food structures and functional ingredients at the nano-scale.

3. The experimental studies:

The project comprised these key tasks:

- Elucidate mechanism of formation and determine nano-fibril stability.
- Characterization of nano-fibril microstructure and length distribution.
- Preparation and characterization of nano-fibrillar hydrogels.
- Functional properties (foaming, gelling, emulsification) of nano-fibrils as potential food ingredients.

4. Main results:

- The optimal conditions for nano-fibrillar production from b-lactoglobulin or WPI and potential scale-up are now established: pH 2.0, 80 °C/60min.
- b-lactoglobulin forms much longer fibrils (> 15 mm long, 3 nm thick) compared to other food proteins.
- Whey protein isolate (WPI) or concentrate (WPC) can equally be used to generate nano-fibrils: purified BLG is not necessary.
- Unique atomic force microscopy expertise is now established in Teagasc.
- Technology transfer – Nano-fibril length characterization by flow birefringence (Wageningen University).
- Effect of high pressure processing on nano-fibril length characterised.
- Weak gels can be formed at lower protein concentrations (< 5 %) when using fibrillar whey proteins compared to non-fibrillar counterparts.
- Both freeze- and spray dried nano-fibrillar powders were produced; rehydration confirmed the presence of intact nano-fibrils.
- Nano-fibrils are effective foaming agents and produce more stable foams than non-fibrillar protein or egg white (ovalbumen).
- Microfluidization of nano-fibrils enhances foaming capacity significantly.
- Multi-lamellar films comprising sodium alginate, sodium caseinate and calcium chloride were prepared and overlaid with WPI nanofibrillar films.

5. Opportunity/Benefit:

Whey protein in the form of nano-fibrils < 5 nm thick, have unique functional properties. In particular nano-fibrils can be used to 1) form low-salt food gels and 2) produce food foams.

6. Dissemination:

Presentations

Auty, M.A.E., Wang, L., Oboroceanu, D. and Brodkorb, A. (Invited presentation). Characterization of Nanofibrillar Whey Proteins by Atomic Force Microscopy. Asylum Research UK Forum. St. Johns College, Oxford, 16 April 2007.

Wang, L., Oboroceanu, D. and Auty, M.A.E. 2008. Characterisation of nanofibrillar assembly of beta-lactoglobulin. 14th World Congress of Food Science & Technology, Shanghai, China October 19 – 23.

Wang, L., Oboroceanu, D.,Nijboer, A., Brodkorb, A., Magner, E., Venema, P. & Auty, M.A.E. 2009. Nano-fibrillar milk protein assemblies as precursors to novel food structures,IDF World Summit, Berlin 20 – 24 September 2009.

Auty, M.A.E. (Invited Presentation). Food Structure Research, 5th Council Meeting of Chinese Association of Animal Product Processing Research. Nanjing, China, 24 October 2008.

Auty, M.A.E. (Invited Presentation). Food Microstructure Research. Key Laboratory for Dairy Science, Harbin, China, 27 October 2008.

Auty, M.A.E. (Invited Presentation). Agro-Food Structure Research. Institute of Quality Standards & Testing Technology for Agro-Products, Beijing. 31 October 2008.

Auty, M.A.E. (Invited keynote speaker) A review of the latest imaging techniques for characterizing food structure in "Emerging Imaging Techniques" session at IDF Dairy Microstructure Symposium, Tromso, Norway, 11 June 2010.

Main publications:

Oboroceanu, D., Wang, L., Brodkorb, A., Magner, E., Auty, M.A.E. 2010. Characterization of β-lactoglobulin fibrillar assembly using atomic force microscopy, polyacrylamide gel electrophoresis and in situ Fourier transform infrared spectroscopy. *Journal of Agricultural and Food Chemistry*, **58:** 3667–3673.

Oboroceanu, D., Wang, L., Kroes-Nijboer, A., Brodkorb, A., Venema, P., Magner, E. & Auty, M.A.E. 2011. The effect of high pressure microfluidization on the structure and length distribution of whey protein fibrils. *International Dairy Journal*, 21: 823 – 830.

Oboroceanu, D., Wang, L., Magner, E. & Auty, M.A.E. 2014. Fibrillization of whey proteins improves foaming capacity and foam stability at low protein concentrations. *Journal of Food Engineering*. 121: 102–111.

Technological advances in spray drying of functional ingredients for automated beverage vending

Project number:
5435

Date:
Nov, 2012

Funding source:
DAFM

Project dates:
Jan 2005-Sept 2008

Collaborating Institutions:
None

Teagasc project team:
Dr. Phil Kelly (PI)
Mr. Jim Kelly

External collaborators:
None

Compiled by:
Phil Kelly

Key external stakeholders:

Manufacturers of milk powders and dairy ingredients.

Practical implications for stakeholders:

Technologies were developed to produce functional powders suitable for reconstitution/dispensing as either hot or cold beverages.

- Installing an in-line high pressure gas/liquid injection system on the concentrate feed to the spray atomiser of a milk drier facilitated the production of dried ingredients with extensive foaming properties suitable for use in cappuccino-based beverage formulations.
- *Development of foaming powder for hot beverage formulation and vending* – a knowledge-base was established on the performance of different injection gases used and their interactions with concentrate formulation and process variables on powder characteristics.
- *Development of cold mixed smoothie-style beverages from textured dairy-fruit dry blends* – 'smoothie' style powders containing fruit/dairy ingredient blends with desired physical characteristics e.g. texture, viscosity and phase stability were successfully developed for dispensing in prototype vending machines.

Main results:

The immediate effect of using either nitrogen gas or liquid CO_2 injection during atomisation was improved powder agglomeration and associated decline in bulk densities (from 0.56g/cc to 0.12g/cc) as well reduced moisture contents. This was also reflected in changes to the particle size distribution and particle density – the latter reduced from 1.2334g/cc to 0.599g/cc).

Interrelationships were established between drying parameters and powder properties (bulk density, particle size distribution, occluded air, interstitial air, particle density, wettability, foam height using a coffee dispenser at t=0 min, foam height after 5 min, and moisture content) specific to cappuccino beverages. Significant relationships, in particular, were established between powder bulk density and cappuccino foam stability using CO_2 (foam stability = 5.556-(5.532*Bulk Density) and N_2 (foam stability = 5.017–4.573*Bulk Density) dosing.

Opportunity/Benefit:

Adding functionality and value to spray dried ingredients

This technology may be incorporated with some adaptation by ingredient drying manufacturers to prepare fat-filled base or fully formulated powders for supply to branded food companies with channel dominance in food service markets. Relevant pilot scale technologies at Moorepark may be availed of to support technology transfer initiatives.

1. Project background:

The concept of gas injection during spray atomisation was first explored during the 1960s by a team of researchers at the US Department of Agriculture. At the time these workers were motivated by the prospect of producing reconstituted milk with flavour profiles resembling fresh milk – an attribute that is absent in conventional spray dried milk powders. Although the innovation attracted little commercial attention at the time, a peripheral observation was an improvement in powder reconstitution properties and increased foaming tendency due to the effects of the injected gas. Thus, it was opportune to revisit this technology since foaming is now a desired property in functional ingredients that are designated for use in automated vending systems, particularly those which mix and dispense cappuccino-based drinks.

2. Questions addressed by the project:

An overall aim was to enhance spray drier functionality for the development of innovative ingredients for beverage applications. The project's specific objective was to:

- Modify concentrate feed atomization conditions in order to influence physical properties of resulting powders especially those affecting reconstitution behavior e.g. tendency to foam.
- Update the technical specification of a prototype gas injection system by engineering a microprocessor-controlled precision dosing system.
- Apply modern analytical techniques and instrumentation to observe the effects of gas injection on resulting powder properties.

3. The experimental studies:

A high pressure in-line gas / liquid injection system was configured and installed on the concentrate feed line to the spray atomiser on the MTL Tall-form spray drier. This microprocessor-controlled unit was designed to meter precise low dose amounts of gaseous N_2, air, or liquid CO_2 under high pressure during spray drying. Initial technological hurdles faced included limitations e.g. injection nozzle sizing posed by operating at pilot rather than industrial scale. Secondly, a means of overcoming variability in gas dosing arising from declining pressures in supply storage cylinders had to be resolved.

An experimental matrix was designed to establish the interrelationships between coffee whitener formulation and process parameters during high pressure spray atomization and drying on physical properties, including foaming, of the resulting powders. An empirical foam test was set up by adapting a commercial automated cappuccino-dispensing machine in order to provide realistic foam generation volumes.

Hot beverage formulations centered around cappuccinos and latte's were formulated in order to simulate the diversity of conditions by which such products may be vended and retailed e.g. vending using a single-sachet where all ingredients are pre-blended before mixing, or using dual sachets (separate coffee and creamer units) which are combined only at the point of mixing. Another scenario simulated a consumer application where retail packs of coffee/creamer pre-blends are prepared using manual (spoon) stirring.

The extensive foaming characteristics of powders achieved using the high pressure gas/liquid injection technology was also applied to the development of novel smoothie-style beverage concepts which could be reconstituted from functionalised blends of dried milk protein and fruits ingredients. Alternative ingredient texturisation approaches based on protein-hydrocolloid interactions were also explored in order to impart the desired sensory and textural properties in smoothie-style beverages.

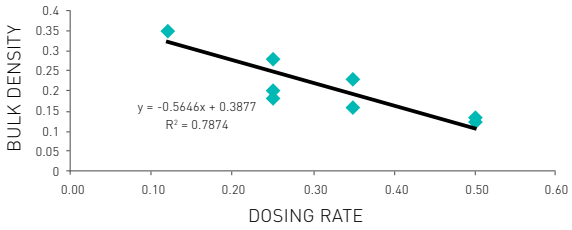
4. Main results:

High pressure gas injection into the concentrate feed during spray is highly effective in promoting foamability of subsequent powders for use in hot beverages such as cappuccino.

The immediate effect of using either nitrogen gas (0.12 – 0.5% of feed) or liquid CO_2 dosage during atomisation was evident from the improved powder agglomeration and concomitant decline in bulk densities (from 0.56g/cc to 0.12g/cc) as well reduced moisture contents. This was also reflected in changes to the particle size distribution and particle density (reduced from 1.2334g/cc to 0.599g/cc).

By creating too low a bulk density, there was a risk of generating foams that were too voluminous so that powder dispersibility and sinkability were restricted during reconstitution.

Formulation effects



Targeted bulk densities were achieved more readily using WPC- instead of SMP-based based formulations e.g. 0.19 g/cc and 0.35 g/cc for WPC and SMP, respectively at 0.12% N_2 dosage.

Powder agglomeration effects with/with gas injection

Since high pressure gas injection contributed to the formation of powder agglomerates, it was necessary to isolate its additional contribution from the powder agglomeration process settings already configured on the spray drier. With the drier configured in regular (control) drying mode i.e. without gas injection, powder bulk density was 0.68 g/cc compared to typical values of 0.5 g/cc for agglomerated control powders. With gas injection during the regular (conventional non-agglomerated) mode of drying, bulk density was 0.2g/cc compared to 0.15 g/cc for agglomerated powders. Particle density for the control was 1.194g/cc compared to ~ 0.4 g/cc for the gas-injected agglomerated powder. On the otherhand, gas injecting without the agglomerated mode function could only yield a particle density of 0.651 g/cc. However, irrespective of the evident differences in bulk densities and particle densities, foaming capacity and stability from a cappuccino perspective were similar to those values obtained in earlier trials.

Development of powder foaming test

A commercial automated powder-based cappuccino dispensing machine (Flavia) was successfully adapted as a standardised method of generating data on foaming tendency that would be more realistic for testing the performance of the experimentally produced powders (Figure 1). The instrument generated a pressurised jet of hot water at 70°C to create turbulence in the cupholder containing the test powder. The cupholder was also mounted on a rotating platform.

Interrelationships between process parameters and powder foaming

Correlation coefficients were established for drying parameters and powder properties (Bulk density, Particle size distribution, Occluded air, Interstitial air, Particle density, Wettability, Foam height using the adapted FlaviaÔ coffee dispenser at t=0 min, Foam height after 5 min, moisture content) specific to cappuccino beverages using Sigma Plot analysis. For example, significant relationships between bulk density and cappuccino foam stability using CO_2 (foam stability = 5.556-(5.532*Bulk Density) and N_2 (foam stability = 5.017–4.573*Bulk Density) dosing were statistically established.

Development of an automated smoothie-based beverage dispensing system

Smoothie powders containing fruit/dairy ingredient blends (88/12 and 76/24, respectively) suitable for vending dispensers were successfully developed having regard to key physical characteristics e.g. texture, viscosity and phase stability. The role of pectin supplementation (fruit-based hydrocolloid) proved particularly effective as a texture enhancer in selected formulations e.g. strawberry rather than apple. During this study, an experimentally-developed whey protein-based ingredient was superior to other forms of added dairy ingredients

and represented a significant innovative achievement in terms of developing a tailored functional ingredient formulation for smoothie dispensing.

5. Opportunity/Benefit:

Growing food service opportunities are the result of how well the food industry is able to respond with novel concepts to consumer needs for high quality and healthy beverages 'on the go' e.g. market growth in smoothie-based beverages. The individualised and personalised nature of smoothie and juice bar beverage service has been primarily responsible for its market growth. However, such a service is self-limiting by virtue of the dependency on location (usually shopping malls, central stations etc), labour intensity, and availability during the 'working day'. In this regard, the proposed automated dispensing system provides consumers not only a quality beverage but a refreshing drink experience that is superior to that encountered with retailed bottled smoothies. It is with this context in mind that these ideas have been promoted to interested food companies.

This research project generated knowledge and expertise at three levels which are continuing to be exploited.

(i) Elaboration of research infrastructure at large pilot scale spray drying with 'bolt-on' innovative gas injection technology to extend the functionality of spray dried ingredients – these facilities are now accessed by national and international food companies.

(ii) Development of competence and skills in powder technology and increased scientific understanding of its effects on powder properties – a knowledge base that is attracting active engagement by national and international food companies.

(iii) Functional ingredient concepts such as foaming powders for hot beverage use (cappuccino), as well as texture enhancement of cold beverages (smoothies) reconstituted during powder dispensing at vending points.

6. Dissemination:

Non-disclosure agreements were signed with a number of companies in the course of discussing the notion of an automated smoothie-beverage dispenser. An SME engineering company constructed a basic (manually operated) prototype dispenser (tentatively called 'Smoothex') to demonstrate the concept.

The success of the gas injection technology has been brought to the attention of the dairy and ingredient manufacturers, and in a number of instances the technology has been exploited for other ingredient functionality purposes.

Main publications:

J.T. Tobin, S.M. Fitzsimons, A.L. Kelly, P.M. Kelly & M.A. Fenelon *Microparticulation of mixtures of whey protein and inulin*. Intl. J. Dairy Technol. 63, 32–40.

Popular publications:

Invited paper on *Effects of using high pressure gas/liquid injection during spray atomisation on powder properties*. J. Kelly, & P.M. Kelly presented to 3rd International Conference on Spray Drying of Milk Products – San Francisco, 27–28th February 2007.

Invited keynote address: *Whey permeate drying: a review*. J. Kelly, P.M. Kelly, D.O'Callaghan & S.A. Hogan presented to 4th International Conference on Spray Drying of Milk Products, Melbourne, Australia.

High pressure N2 gas or Liquid CO2 injection during spray atomisation – powder functionality and ingredient innovation, J. Kelly in RELAY Workshop on *An Insight into Current Milk Powder Research and Innovation*, Moorepark, 18th April 2007.

New ideas for dairy-based beverages, P.M. Kelly in RELAY Workshop on *Commercial opportunities for developing new beverage concepts*, UCC 16th September 2009.

Whey Permeate Drying: A Review, J. Kelly, in RELAY Workshop No. 62 – *Latest research to Improve Spray Drying of Milk and Ingredients*, Moorepark, 21st October 2009.

Technology Updates

Food Safety

Detection of Endocrine Disrupting Agents in Milk

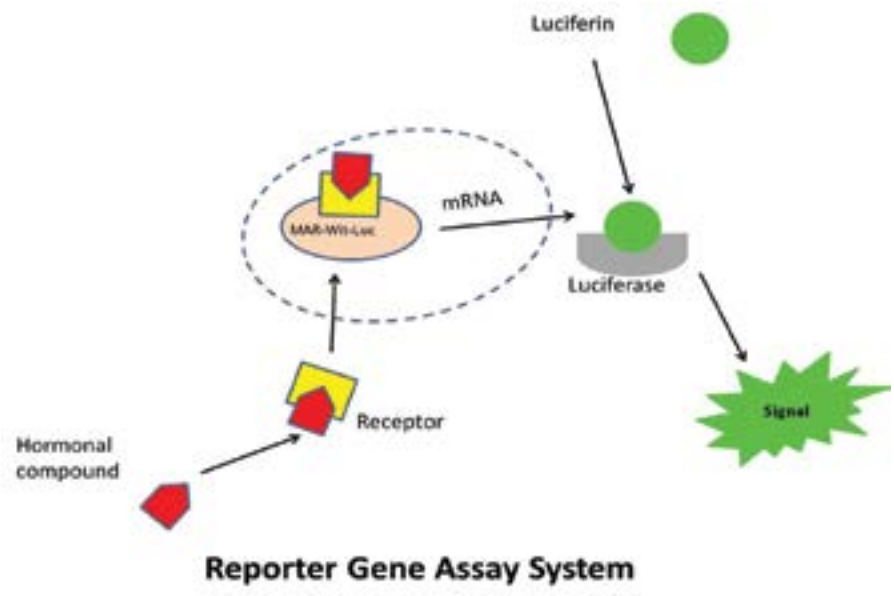
Project number:
6141
Date:
November, 2014
Funding source:
Teagasc
Project dates:
Oct 2010 – Sep 2014

Collaborating Institutions:
Queen's University Belfast

Teagasc project team:
Dr. Martin Danaher (PI)

External collaborators:
Dr. Lisa Connolly
Queens University Belfast

Compiled by:
Martin Danaher



Reporter Gene Assay System

Key external stakeholders:

Dairy industry, Dairy farmers, Agri-businesses, Policy makers.

Practical implications for stakeholders:

Endocrine disruptor agents (EDAs) comprise of both naturally occurring and synthetic chemicals. Some of these chemicals can transfer into milk due to environmental contamination, feed contamination, leaching from milking machine components, cleaning agents or processing. This research has shown that endocrine disruptors can be successfully detected in milk using receptor assays. However, chemical analysis using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is required to accurately measure and identify each compound. Unfortunately, a wider range of EDAs could not be detected because there are more amenable to GC-MS analysis, which was not available at the time.

Using the technology developed on this project low levels of EDAs were found in milk samples but further investigations should be carried out to identify the source of residues. More extensive methodology is required to properly investigate a wider range of phthalates, which have been detected in dairy products in other EU countries.

Main points

- The technology developed on the above project provides two validated solutions for detecting EDAs in milk.
- End-users can use the technology to screen for endocrine disrupting chemicals in milk and be confident that dairy is safe for consumption.

Main results:

- Two new methods were developed to analyse endocrine disrupting agents in milk using an estrogenic reporter gene assay and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).
- The technologies were applied to a range of different types of milk and infant formula.
- A range of endocrine disruptors were detected in samples including the natural hormone progesterone and low levels antimicrobials, phytoestrogens and benzyl butyl phthalate.

Opportunity/Benefit:

This technology is now available as a tool to monitor the safety of milk.

1. Project background:

Endocrine disruptors are chemicals which mimic natural hormonal compounds or that can otherwise interfere with normal endocrinal function and have significant implications for human health, being implicated in cancers, malformations, infertility and obesity. A large number of chemicals can be classed as endocrine-disrupting. Some of these are natural substances, such as endogenous hormones and phytoestrogens; others are artificial in origin, such as pesticides, bisphenol A, phthalates, alkylphenols and persistent organic pollutants.

In the research project, two different approaches were employed to detect and measure endocrine disrupting chemicals in milk. The first was a targeted test based on liquid chromatography coupled to tandem mass spectrometry, which is suitable for accurately quantifying known endocrine disruptors. The other approach was based on cell based assay, which can detect the overall endocrine disruptor activity and is suitable for unknown agents.

2. Questions addressed by the project:

- Can methodology be developed to detect endocrine disrupting agents in milk?
- What endocrine disruptors are present in milk and at what levels?

3. The experimental studies:

This research project set out to assess biological activity of different contaminants, which gain entry to the food chain as well as analyze a variety of milk samples for their total estrogenic hormonal load and chemical composition.

The assessment of environmental contaminants was performed employing an estrogenic reporter gene assay (RGA). Milk samples were analyzed using two assays, namely, a screening reporter gene assay and a quantitative LC-MS/MS method which were validated according to 2002/657/EC guidelines.

4. Main results:

A number of contaminants were evaluated in the reporter gene assay and showed activity including UV filters, parabens, phthalates, pyrethroid pesticides and their metabolites, stressing a possible risk for consumers if exposed to mixtures of these compounds.

The development and validation of both screening and confirmatory methods yielded two fast and highly reliable assays with suitable sensitivity for screening for estrogenic activity above the levels resulting from the presence of endogenous hormones as well as chemical confirmation of nineteen EDAs in milk at sub parts per billion (ppb) levels.

Results of the screening of a range of milk samples revealed the presence of low level mixtures of EDAs of natural origin, such as myco- and phytoestrogens, but also man-made chemicals such as antimicrobials. Nevertheless, the chemical contamination did not translate to enhanced estrogenic hormonal load in majority of samples. Only 3% of those tested showed increased estrogenic load, which origins have not been confirmed.

Employment of fractionation with subsequent concomitant biological and untargeted chemical analysis was investigated as an additional tool which could provide invaluable insight into the composition and possible origin of the biological activity detected in milk. Additionally, analysis of masked EDAs present in milk have been performed by samples reassessment with the inclusion of an enzymatic deconjuation step and revealed higher concentrations of contaminants resulting in an increased estrogenic load.

The research highlights the importance of ongoing screening of food commodities for EDA contamination and highlights the advantages of employing combined biological and chemical assays to facilitate accurate risk assessment.

5. Opportunity/Benefit:

- Two new tests have been developed on this project and can be used by the dairy industry to measure endocrine disrupting compounds in milk.
- These tests can be used for public good by the dairy industry to improve the quality and safety of milk and dairy products produced on the island.

6. Dissemination:

PhD Thesis

Wielogorska E., Developing assays to detect and quantify endocrine disrupting compounds in milk. (November 2014) PhD Thesis. Queens University Belfast.

Main publications:

Wielogorska E., Elliott, C.T., Danaher, M and Connolly, L. (2014) 'Validation and application of a reporter gene assay for the determination of estrogenic endocrine disruptor activity in milk' *Food and Chemical Toxicology* 69:260–266.

Wielogorska E., Elliott, C.T., Danaher, M., Chevalier, O and Connolly, L. (2015) 'Validation of an ultra high performance liquid chromatography – tandem mass spectrometry method for detection and quantitation of 19 endocrine disruptors in milk' *Food Control* 48:48–55.

Wielogorska E., Elliott, C.T., Danaher, M and Connolly, L. (2014) 'Endocrine disruptor activity of multiple environmental food chain contaminants' *Toxicology in Vitro* (in press).

Popular publications:

Wielogorska E., Elliott, C.T., Danaher, M., Chevalier, O and Connolly, L. 'Validation of an ultra high performance liquid chromatography – tandem mass spectrometry method for detection and quantitation of 19 endocrine disruptors in milk.' ASSET Food Integrity and Traceability Conference, 8th – 10th April 2013, Belfast, UK.

Wielogorska E., Elliott, C.T., Danaher, M., Chevalier, O. and Connolly, L. 'Endocrine disruptor activity of multiple environmental food chain contaminants' ASSET Food Integrity and Traceability Conference, 8th – 10th April 2013, Belfast, UK.

Safe and Healthy Foods

Key external stakeholders:

Aquaculture, pork, poultry, beef, egg and honey producers; regulatory agencies, retailers, importers, animal health companies, food safety laboratories and consumers.

Practical implications for stakeholders:

Safe & Healthy Foods programme set out to improve the safety of food consumed or produced on the island of Ireland through the development of new analytical methods and food databases.

A suite of new residue test methods were developed that cover nearly 150 different analytes. The range of compounds covered included veterinary drugs, feed additives, hormonal agents and pyrrolizidine alkaloids in different foods. The application of these tests showed that food consumed On the island is generally of high purity. Residues were detected in a very small proportion of samples rendering them non-compliant. However, >99.6% of samples were residue free. A range of food safety databases were developed or updated on the project including the National Food Residue Database, Veterinary Drug and Feed Additives Databases (VetFAD) and the Central Microbial Database. A new comprehensive food ingredient database (INFID), which has been used to estimate the intake of four sweeteners (aspartame, saccharin, acesulfame K, sucralose) were within the Acceptable Daily Intake levels for preschool children. The Irish Food Compositional Database was updated with current data on nutrients and bioactive components for a range of different foods.

Main points

- The newly developed databases and technologies will allow stakeholders to significantly improve the safety and quality of food products produced on the island.
- The newly developed tools will allow the stakeholders to more effectively target resources and give better value for money.

Project number:
5856
Date:
November, 2014
Funding source:
DAFM
Project dates:
Dec 2007–Dec 2013

Teagasc project team:
Dr. Martin Danaher (Project Coordinator/PI)
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External collaborators:
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Prof. Albert Flynn, University College Cork
Prof. Francis Butler, University College Dublin
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Dr. John Egan, Department of Agriculture Food & the Marine
Dr. Montserrat Gutierrez, Department of Agriculture Food & the Marine

Compiled by:
Martin Danaher and Kieran Jordan

Main results:

- New multi-residue test methods developed for nearly 150 contaminant residues in food.
- New databases were developed covering the area of food safety and food consumption.
- Food surveys and exposure assessments were completed showing that the food we eat is very safe.

Opportunity/Benefit:

During the project, new knowledge and technologies have been developed that can be used to improve the quality and safety of food products consumed or produced on the island.

1. Project background:

Safe and Healthy Foods was a large multidisciplinary project that focused on the integration of food safety and public health research on the island of Ireland. The project encompassed three main work areas, namely, chemical contaminants, nutrition & health and biological contaminants. The chemical contaminant research focused on the development of new improved multi-residue tests and their application to generate new exposure data for risk assessments. The development of new food databases was a major focus on the project, which acknowledged both the need to update current databases and develop new databases. Database development, including work on The National Food Residue Database (NFRD), the National Food Ingredients Database (INFID), the Irish Supplemental Food Composition Database, the Central Microbial Database and the Veterinary Medicinal Products & Feed Additives Database, was necessary.

2. Questions addressed by the project:

Can new multi-residue tests be developed to improve the safety of food consumed and produced on the island of Ireland?

Can the research team develop new databases that can be used for food safety and public health applications?

How safe is the food that we consume or produce on the island of Ireland?

3. The experimental studies:

Researchers at Teagasc and AFBI developed new multi-residue LC-MS/MS tests to detect veterinary drug and feed additive residues in food. This work included the development of new extensive multi-residue tests for the detection of antibiotic, anticoccidial and dye residues. The project team also set out to develop test methods for aminoglycoside antibiotics, which are very polar drugs that are extremely difficult to reliably analyze in food. CIT researchers, in collaboration with Teagasc, developed the first Irish tests for detecting pyrrolizidine alkaloid residues in food and herbal products. Advanced screening tests based on receptor assays were developed by QUB for the detection of sex hormonal contaminants in dietary supplements. All methodologies were validated to 2002/657/EC guidelines. Comprehensive surveys were carried out by the project team to determine the incidence of residues in different food products using existing and newly developed methods. Exposure assessments were carried out by UCD researchers, using data generated during the project, and these datasets were populated into the NFRD. VetFAD and the Central Microbial Database were developed in collaboration between researchers and regulatory agencies as new tools to support food safety activities on the island of Ireland. Extensive food consumption surveys were carried out by UCC and UCD to update the National Food Ingredients Database (INFID) and the Irish Supplemental Food Composition Database.

4. Main results:

Chemical residues

- A range of new analytical methods were developed and validated by the project team:
- A new multiplex screening method, which was applied to detect of four nitrofuran residues in honey.
- A multi-residue test was developed to measure 23 anticoccidial residues in eggs, milk and animal tissue using LC-MS/MS.
- AFBI developed a test for 13 triarylmethane and phenothiazine dyes in fish and poultry tissues.
- LC-MS/MS and multiplex immunoassays were developed to detect 18 aminoglycoside residues in honey. These are now the most comprehensive methods for the determination of aminoglycosides and significant improvements over existing methodologies.
- CIT developed a method to detect 14 pyrrolizidine alkaloids in honey, milk and herbal products.

- Multi-class methods were developed on the project to detect >60 antibiotic residues in aquaculture tissues.
- QUB developed sex hormonal receptor assays to detect known and unknown hormonal agents in dietary supplements.

Surveys were carried out by the project team during the project using food products purchased in retail surveys and collected in collaboration with the Irish food industry. Samples covered both domestic and imported foods. In general, results of the surveys showed that >99.6% of the samples were residues free of pharmaceutical agents. In agreement with previous research, pyrrolizidine alkaloids (PAs) were detected in a number of honey and herbal tea samples collected on the project. High levels of PAs were detected in honey samples from Australia and New Zealand compared to Europe. Exposure and risk assessments are currently being run on these samples to determine the risk to the consumer.

Microbial databases

A fully functional national microbial database was successfully developed, which incorporates data on the major pathogens in Irish food generated by the major regulatory and research institutions in Ireland. This is the first time such a database has been created and it is now fully operational with three years of data in the system. All partners have been issued with login details and can access the database online. The database is a repository of valuable information that can be used to underpin quantitative risk assessments for a large number of product / pathogen combinations. The data can be used to generate risk assessments of direct relevance to the industry as required. The microbial database represents a major resource to the regulatory and research institutions in Ireland working in food safety. This database includes capacity to sub-typed isolates (by PFGE to PulseNet standard and serotyping) of three relevant food poisoning organisms (Listeria monocytogenes, Salmonella and VTEC). This database represents an important resource for food safety management in Ireland. The database gives the ability to epidemiologically track sources of isolates, and isolate similarities and the ability to link food-poisoning cases and identify outbreaks. This capability allows food safety regulatory agencies manage food borne illness outbreaks and allows the potential identification of the food responsible for the outbreak.

Food ingredient databases

A comprehensive food ingredient database has been developed where detailed information on the ingredients of foods consumed by adults and children is recorded and has been quality controlled. Furthermore, a report on patterns of food additives in Irish foods has been supplied to the Food Safety Authority of Ireland and exposure assessments to artificial sweeteners (beyond the scope of this task) have been completed. Intakes of the four sweeteners (aspartame, saccharin, acesulfame K, sucralose) were shown to be within the Acceptable Daily Intake levels for preschool children.

The updated Irish Supplemental Food Composition Database is a supplement to the UK Tables of Food Composition that has been compiled by UCD from food consumption records collected in the Irish national food consumption surveys (the National Children's Food Survey, the National Teens' Food Survey, the National Adult Nutrition Survey and the National Pre-school Nutrition Survey; www.iuna.net), which together cover food consumption from age 1 – 90 years. Data are included for a total of 1533 new or updated food codes. The database facilitates ongoing monitoring (with FSAI) of intakes of nutrients, bioactive constituents and botanical and herbal products in Ireland and underpins Ireland's contribution to the EU level data collection required by EFSA.

5. Opportunity/Benefit:

A range of new technologies were developed on the project and will be available from the individual partners following the completion of the project. Some tests have been accredited to ISO17025 standard and are provided as a service for the food industry. Several databases were developed on the project, which can be used by industry for food safety purposes and the development of healthier food products.

6. Dissemination:

Dissemination outputs from the project team included 19 peer reviewed papers/book chapters, four PhD theses, one MSc thesis, 54 scientific presentations, two national reports, seven popular non-scientific publications, four YouTube videos and nine workshops.

Main publications:

Kinsella, B., O'Mahony, J., Malone, E., Moloney, M., Cantwell, H., Furey, A., Danaher, M. (2009). Current trends in sample preparation for growth promoter and veterinary drug residue analysis. Journal of Chromatography A, 1216:46, 7977–8015.

Edward M. Fox, Niall deLappe, Patricia Garvey, Paul McKeown, Martin Cormican, Nola Leonard, and Kieran Jordan. 2012. Pulsed-field gel electrophoresis (PFGE) analysis of *Listeria monocytogenes* isolates of clinical, animal, food, and environmental origin from Ireland. Journal of Medical Microbiology 61, 540–547.

O'Mahony J., Moloney, M., McConnell, R.I., Benchikh, E.O., Lowry, P., Furey, A., Danaher, M. (2011). Simultaneous detection of four nitrofuran metabolites in honey using a multiplexing biochip screening assay. Biosensors and Bioelectronics 26 4076–4081.

Radovnikovic, A., Moloney, M., Byrne, P., Danaher, M. UPLC-MS/MS method for detection of nitrofuran residues in plasma. (2011). Journal of Chromatography B 879 159–166.

Cronly, M., Behan, P., Foley, B., Danaher, M., Malone, E., Regan, L. (2011). Survey of 11 nitroimidazole residues in hen and duck eggs from the Irish market. Food Additives & Contaminants: Part B 4:2 79–87.

Moloney, M., Clarke, L., O'Mahony, J., Gadaj, A., O'Kennedy, R, Danaher, M., (2012) Determination of 20 coccidiostats in egg and avian muscle tissue using ultra highperformance liquid chromatography–tandem mass spectrometry. Journal of Chromatography A 1253 94– 104.

O'Mahony, J., Clarke, L., Whelan M., O'Kennedy, R., Lehotay, S.J., Danaher M. (2013). The use of ultra-high pressure liquid chromatography with tandem mass spectrometric detection in the analysis of agrochemical residues and mycotoxins in food – challenges and applications. Journal of Chromatography A 1292 83–95.

Griffin, C.T., Danaher, M., Elliott, C.T., Kennedy, D.G. (2013) Detection of Pyrrolizidine Alkaloids in Commercial Honey using Liquid Chromatography-Ion Trap Mass Spectrometry. Food Chemistry 136 1577–1583.

O'Mahony, J., Moloney, M., McCormack, M., Nicholls, I.A., Mizaikoff, B., Danaher, M. (2013). Design and Implementation of an Imprinted Material for the Extraction of Bisphenol A from Milk. Journal of Chromatography B 931 164–169.

Radovnikovic, A., Conroy, E-R, Gibney., M., O'Mahony, J., Danaher, M. (2013) Residues analyses and exposure assessment of the Irish population to nitrofurans metabolites from different food commodities in 2009–2010. Food Additives & Contaminants: Part A 30:11 1858–1869.

Clarke, L., Moloney, M., O'Mahony, J., O'Kennedy R., Danaher M. (2013). Determination of 20 coccidiostats in milk, duck muscle and non-avian muscle tissue using UHPLC-MS/MS. Food Additives & Contaminants Part A. 30:6 958–969.

Clarke, L., Fodey, T.L., Crooks, S.R.H., Moloney, M., Delahaut, P., O'Kennedy, R., Danaher, M. (2014). A review of coccidiostats and the analysis of their residues in meat and other food. Meat Science. 97 358–374.

Griffin, C.T., O'Mahony, J., Danaher, M., Furey, A. (2014). Liquid Chromatography Tandem Mass Spectrometry Detection of Targeted Pyrrolizidine Alkaloids in Honeys Purchased within Ireland. Food Analytical Methods (in press).

Gadaj, A., di Lullo, V., Cantwell, H., McCormack, M., Furey, A., Danaher, M. (2014) Determination of nitroimidazole residues in aquaculture tissue using ultra high performance liquid chromatography coupled to tandem mass spectrometry. Journal of Chromatography B 960 105–115.

BASELINE: Risk targets in milk and dairy products

Key external stakeholders:

Dairy industry, European Food Safety Authority (EFSA)

Practical implications for stakeholders:

The study focused on the risk posed by *Listeria monocytogenes* in raw and pasteurised milk cheese. The study showed that *L. monocytogenes* grew at a faster rate on pasteurised milk cheese compared to raw milk cheese during the storage period following ripening. A quantitative risk assessment model predicting the growth and survival of *Listeria monocytogenes* in raw and pasteurised milk cheese, from farm to fork showed that the mean level of exposure to *L. monocytogenes* in contaminated cheese was higher for raw milk cheese (2.22 $\log_{10}$ cfu g^{-1}) compared to pasteurised milk cheese (<1 $\log_{10}$ cfu g^{-1}). This model can support food processors to optimise conditions to reduce *L. monocytogenes* growth in cheese and to comply with EC2073/2005.

This research was carried out as part of a multi-national EU Framework project, *BASELINE* which focused on research to provide harmonised and validated sampling strategies, supporting European policies in food safety and suitable for food producers to collect comparable data, to improve quantitative risk analysis of selected biological and chemical agents.

Research by Teagasc in this project focused on the growth kinetics of *L. monocytogenes* in semisoft rind washed cheese prepared from raw and pasteurised milk, in the storage period following ripening. Additionally work focused on predicting the risk posed by the *Listeria monocytogenes* contamination arising from the farm environment as well as cross-contamination at processing and retail level, and subsequent human exposure, using a quantitative risk assessment modeling approach.

Project number:
5994

Date:
Jan 2014

Funding source:
EU Framework 7

Project dates:
June 2008 – Nov 2013

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Dr. Kieran Jordon
Dr. Uma Tiwari
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Agence Francaise De Securite Sanitaire Des Aliments, France
Hungarian Food Safety Office, Budapest

Compiled by:
Geraldine Duffy and Uma Tiwari

Main results:

- *L. monocytogenes* grew at a slower rate on the raw milk cheese compared to the pasteurised milk cheese at all the storage temperatures investigated.
- The simulated quantitative risk assessment model showed that the mean level of exposure to *L. monocytogenes* in contaminated cheese was higher for raw milk cheese (2.22 $\log_{10}$ cfu g^{-1}) compared to pasteurised milk cheese (<1 $\log_{10}$ cfu g^{-1}).
- A model sensitivity analysis highlighted the critical factors for exposure to *L. monocytogenes* from both cheeses were the serving size of the cheese, storage days and temperature at distribution stage.
- The model showed that when the Performance Objective (PO) for *L. monocytogenes* in raw milk cheese was set at ≤ 2 log cfu g^{-1} at retail level, nearly 10.34 % of product was predicted to exceed this PO limit, whereas the model predicted 100% of pasteurised milk cheese met the PO target.

Opportunity/Benefit:

The study showed that growth kinetic models can facilitate prediction of *L. monocytogenes* growth during shelf-life and will help to demonstrate compliance with food safety criteria (EC 2073/2005). Further, the quantitative risk assessment conducted based on a farm-to-fork approach also showed possible cross-contamination of raw milk at farm level and retail level. Such model predictions, will allow food processors and policy makers to identify the possible routes of contamination in cheese processing and to reduce the risk posed to human health.

1. Project background:

The BASELINE project objective was to develop and deliver harmonised and validated sampling protocols and innovative analytical methods to detect and quantify relevant biological and chemical food risks. The project also focussed on supporting advances in food safety risk assessment to provide the food industry with new insights on pathogens and chemicals in their products and processes. The aim was to generate new knowledge on sampling schemes for risk assessment by using a mathematical approach for different groups of food products including seafood, eggs and egg products, fresh meats, milk and dairy products and plant products. Teagasc main focus in the BASELINE project was on the selection and optimisation of sampling plans for the different risks in milk and dairy products. Researchers focused on developing mathematical models to assess the growth behaviour of *L. monocytogenes* on ripened raw and pasteurised milk stored at retail level. An additional aim was to develop a quantitative risk assessment, which included a prediction of contamination arising from the farm environment as well as cross-contamination at processing and retail level, and subsequent human exposure, using a quantitative risk assessment modeling approach.

2. Questions addressed by the project:

- What is the difference in growth behaviour of *L. monocytogenes* on raw and pasteurised milk cheese?
- What are the interaction effects of water activity and pH on the growth of *L. monocytogenes*?
- What are the risk factors for transmission of *L. monocytogenes* in the cheese chain, farm to fork level?
- What is the probability of human exposure to *L. monocytogenes* following consumption of contaminated raw or pasteurised milk cheese?

3. The experimental studies:

- The growth of *L. monocytogenes* in semi-soft rind washed cheese made from raw and pasteurised milk was investigated at three different storage temperatures (4, 10 and 15°C) over a 28 day period, simulating storage following ripening. Changes in water activity (a_w) and pH in cheeses were also monitored during storage. Response surface models were used to model the interaction of storage temperature and time on a_w, pH and *L. monocytogenes* population. Growth curves were fitted using Baranyi, modified Gompertz and Logistic models at all storage temperatures for both cheeses, and model parameters were statistically analysed.
- The growth kinetics of *L. monocytogenes* in raw and pasteurised milk cheese was modelled from farm to fork, using a Bayesian inference approach combined with a quantitative risk assessment. The modelling approach included a prediction of contamination arising from the farm environment as well as cross-contamination at processing and retail level, and subsequent human exposure.

4. Main results:

- *L. monocytogenes* grew at a slower rate on the raw milk cheese compared to the pasteurised milk cheese at all the storage temperatures investigated. A higher specific growth rate was observed for *L. monocytogenes* in pasteurised milk cheese (0.18 – 0.85 Day^{-1}) compared to raw milk cheese (0.05 – 0.37 Day^{-1}) at all storage temperatures.
- The interaction of pH, a_w and *L. monocytogenes* showed that the population increased with rise in pH however a decreasing trend in a_w for both cheese types was observed.
- The simulated quantitative risk assessment model showed that the mean level of exposure to *L. monocytogenes* in contaminated cheese was higher for raw milk cheese (2.22 $\log_{10}$ cfu g^{-1}) compared to pasteurised milk cheese (<1 $\log_{10}$ cfu g^{-1}).
- A model sensitivity analysis highlighted the critical factors for exposure to *L. monocytogenes* from both cheeses, were the serving size of the cheese, storage days and temperature at distribution stage.
- The model showed that when the Performance Objective (PO) for *L. monocytogenes* in raw milk cheese was set at ≤ 2 log cfu g^{-1} at retail level, nearly 10.34 % of product was predicted to exceed this PO limit, whereas the model predicted 100% of pasteurised milk cheese met the PO target.
- Simulated exposure level following the consumption of contaminated raw milk cheese showed nearly 97% of product was above the Food Safety Objective (FSO) of ≤ 1 $\log_{10}$ cfu g^{-1}. All pasteurised products meet this FSO. Thus indicating better safety criteria for pasteurised milk cheese compared to raw milk cheese.

5. Opportunity/Benefit:

The study showed that growth kinetic models can facilitate prediction of *L. monocytogenes* growth during shelf-life and will help to demonstrate compliance with food safety criteria (EC 2073/2005). Further, the quantitative risk assessment conducted based on a farm-to-fork approach also showed possible cross-contamination of raw milk at farm level and retail level. Such model predictions, will allow food processors and policy makers to identify the possible routes of contamination along the cheese processing and to reduce the risk posed to human health.

6. Dissemination:

Main publications:

Rivas, L. and Duffy, G. (2010). The European Project BASELINE: Selection and Improving of fit-for-purpose Sampling Procedures for Specific Foods and Risks. International Association of Food Protection (IAFP). European Symposium. 9–11th June 2010. P2–22.

Tiwari, U., Walsh, D., and Duffy, G. (2012). Effect of storage conditions on growth of *Listeria monocytogenes* in pasteurized cheese. International Conference, Global Food Safety: Solutions for Today and Tomorrow. Crowne Plaza Hotel, Blanchardstown, Dublin, October 23 to 25th 2012. Pg. 116. ISBN 84170–591–8.

Tiwari, U., Walsh, D., Duffy, G. (2014) Modelling the interaction of storage temperature, pH, and water activity on the growth behaviour of *Listeria monocytogenes* in raw and pasteurised semi-soft rind washed milk cheese during storage following ripening. *Food Control* (*submitted*).

Tiwari, U., Cummins, E., Valero, A., Walsh, D., Dalmasso, D., Jordon, K., Duffy, G. (2014) Quantitative risk assessment of *Listeria monocytogenes* contamination in raw and pasteurised milk cheese from farm to fork. *Risk Analysis* (*submitted*).

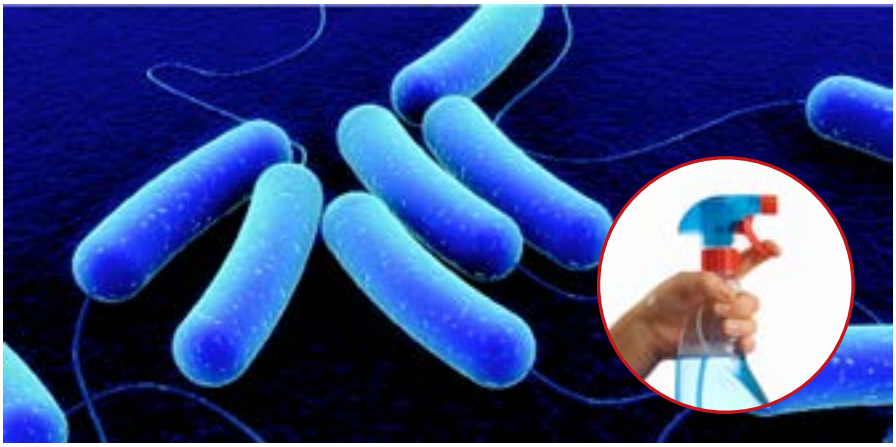
Biocide tolerance in foodborne pathogens

Project number:
5954

Date:
November 2012

Funding source:
DAFM

Project dates:
Dec 2008-May 2012



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Compiled by:
Kaye Burgess

Key external stakeholders:

Food industry, biocide producers, regulatory authorities.

Practical implications for stakeholders:

The outcome of this project is a greater understanding of how foodborne pathogens including *E. coli* O157 and *Salmonella* spp. respond to the presence of biocidal agents, with a particular emphasis on triclosan.

- A panel of verocytotoxigenic *E.coli* (VTEC) and *Salmonella* isolates were found to have minimum inhibitory concentrations (MIC) less than the recommended working concentrations of a number of commercial biocide formulations, although some possessed an MIC of greater than 50% of the working concentration of some agents. This highlights the importance of strict adherence to manufacturer guidelines and appropriate training of personnel.
- Mutants with an enhanced tolerance to triclosan were readily obtained for both *Salmonella* and VTEC. In the case of *Salmonella* corresponding alterations to the strains' antibiotic profiles were observed, illustrating an additional public health risk.
- A spectroscopic method was developed for the detection of quaternary ammonium compounds on stainless steel surfaces, allowing for the detection of residue build up which may constitute a risk for pathogen exposure to sub lethal concentrations of such agents. This would increase the likelihood of resistance developing.

Main results:

A bank of foodborne pathogen isolates were tested against commercial biocide formulations. Although all isolates had an MIC below the recommended working concentration for all the biocide formulations tested a concern is that for some isolate-biocide combinations the MIC was 50% of the working concentration. Such a concentration may easily occur in real world situations, either due to over dilution, handler error or high organic load. Through this study the transcriptomic and proteomic response of triclosan tolerant *E. coli* O157 and

Salmonella mutants in comparison with their reference strains were characterised in detail, identifying key responses for each pathogen. Subsequent phenotypic studies showed key changes which may contribute to enhanced pathogen persistence. A spectroscopic method was developed for measuring the potential buildup of biocidal agents on industrial surfaces.

Opportunity/Benefit:

The findings of this project provide a detailed analysis of the response of two key foodborne pathogens to sub lethal exposure to biocides commonly used in the farm to fork chain and how these responses may contribute to pathogen persistence in the food chain. The project findings underline the key importance of utilising biocidal agents as directed. Furthermore, the spectroscopic method developed and validated as part of this project is readily transferable to industry for the measurement of the buildup of biocide residues on industrial surfaces.

1. Project background:

Biocides are deployed at all stages of the farm-to-fork continuum to eliminate or reduce pathogenic microorganisms and thus decrease the likelihood of human or animal exposure to such infectious agents. However, the emergence of biocide resistant bacteria has been reported. In contrast to their increased usage there has not been a corresponding increase in the understanding of the microbial response to biocides. It is vital that this is investigated as it is unknown if the development of resistance has an impact on bacterial response to stresses such as those encountered in food processing. It may enhance virulence of strains or may contribute to subsequent resistance to antibiotics. Such events may thus contribute to the enhanced persistence and dissemination of pathogenic strains in the food chain and pose an increased risk to consumer health. In an effort to fill these knowledge gaps the objectives of this project were (i) to establish the biocide resistance in a panel of key Gram-negative pathogens, namely *Salmonella* and verocytotoxigenic *E. coli*, (ii) to compare the response of biocide resistant strains with that of parent strains to stresses such as those encountered in food processing, (iii) to provide an understanding of biocide resistance using genomic, proteomic and metabolomic technologies and (iv) to determine if biocide resistance impacts on virulence characteristics and gene transfer. Such information will be crucial so that guidance can be provided to the food industry regarding appropriate biocide usage so as to minimise the development of biocide resistance.

2. Questions addressed by the project:

- Do a bank of verocytotoxigenic *E. coli* and *Salmonella* isolates have a minimum inhibitory concentration greater than the working concentration of eight biocide formulations used in the Irish food industry?
- Is there a correlation between biocide and antibiotic resistance?
- What are the genomic, proteomic and phenotypic differences between biocide susceptible and biocide tolerant foodborne pathogens?
- Does biocide tolerance impact on other stress responses in foodborne pathogens?
- In dry cleaning processes can biocide residues build up on surfaces?

3. The experimental studies:

A multidisciplinary approach was required to address the research questions posed. A high throughput microtitre plate based screening strategy was developed to determine the MIC and minimum bacteriocidal concentration (MBC) of a large panel of VTEC and *Salmonella* strains for eight commercial biocide formulations used by the Irish food industry. Concurrently, antibiotic resistance profiles were also generated for all strains. Whole genome microarray studies were used to determine the transcriptomic changes in mutants with increased tolerance to triclosan, with the studies validated using real time quantitative PCR. This work was correlated with a proteomic study done using two dimensional fluorescence difference gel electrophoresis. Phenotype microarrays were utilised to identify metabolomics changes in triclosan tolerant mutants. Attachment and invasion studies were undertaken using cell culture assays and biofilm studies were undertaken using agar based and microtitre plate assays. Finally, a spectroscopic assay was developed for the rapid measurement of quaternary ammonium compounds on stainless steel surfaces.

4. Main results:

The results obtained in this project are described in detail in the publications listed below and further manuscripts currently in review and therefore a brief overview is provided here. The first task of this project focused on the testing of a large panel of VTEC and *Salmonella* isolates for potential resistance to eight commercial biocide formulations which were selected in consultation with Irish food industry representatives. In all cases the MICs were found to be less than the recommended working

concentration. Of concern is that in some cases the MIC was over 50% of the recommended working concentration. Initially mutants were selected with increased tolerance to commercial biocides but the resistance phenotype was not stable. Therefore a subsequent panel of stable mutants with increased tolerance to triclosan, benzylkonium chloride or chlorhexidine was selected. For all mutants the MIC for commercial biocide formulations was not altered. For the *E. coli* isolates the antibiotic resistance profiles were also not altered but for the some of the *Salmonella* mutants an increase in antibiotic resistance was observed.

The biocide tolerant mutants were compared to their reference strains in a number of different ways. They were compared for their tolerance to common food processing stresses, namely heat and low pH, but in general no significant differences were observed. Transcriptomic studies using whole genome microarrays were undertaken to compare the gene expression of the strains upon sublethal exposure to the biocide. A wealth of information was obtained in these studies, one example being the upregulation of the flagellar assembly pathway in the triclosan tolerant *E. coli* O157 isolate with 33 of 38 genes being highly upregulated. Transmission electron microscopy indicated the presence of extended flagella in this strain. Flagella are connected with biofilm formation and motility which may contribute to strain persistence and virulence. A proteomic study was undertaken using fluorescent 2D DIGE which corroborated the results obtained in the transcriptomic study. It also showed the upregulation of general metabolism proteins and outer membrane proteins in the biocide tolerant mutants. Efflux pump inhibitor studies also showed the clear involvement of efflux pumps in tolerance to the biocides tested. Triclosan targets FabI, a protein involved in fatty acid biosynthesis but the work undertaken in this project clearly indicates that high level triclosan resistance involves a number of different cellular mechanisms.

In a parallel study a spectroscopic method was developed and validated for the measurement of quaternary ammonium compound residues on stainless steel surfaces. This is particularly relevant in dry cleaning regimes. The study showed that these residues could remain stable on stainless steel surfaces for at least six days.

5. Opportunity/Benefit:

This project has provided a much greater insight into foodborne pathogen response to sub lethal exposure to biocidal agents commonly used in commercial formulations. It provides an in depth understanding of the key pathways affected by exposure to low levels of biocide and therefore how this may impact on a number of factors including persistence, antibiotic resistance and resistance to other food processing stresses. Such knowledge is of benefit to food industry managers, regulators and policymakers considering the utilisation of such agents.

The spectroscopic method developed as part of this project would be useful to the quality control sector of food processing factories. The instrument required for residues monitoring, a spectrophotometer, is widely available in industrial plants which makes this technique beneficial to the food industry.

6. Dissemination:

Dissemination of the results of this project was achieved through a number of different routes. A stakeholder focused conference highlighting the results of the project was held on November 10th 2012 at the Teagasc Food Research Centre Ashtown. Discussions regarding the results of this project have taken place with industry collaborators and with the relevant sections of the Department of Agriculture, Food and the Marine and the Food Safety Authority of Ireland. Furthermore, a number of peer reviewed papers have been published as a result of this project and are listed below. A number of other manuscripts are currently in review prior to publication. Both oral (14) and poster (10) presentations have been given regarding various aspects of the project at national and international conferences and workshops throughout the lifetime of the project. Two PhD theses have been submitted and an MSc is currently being finalised.

Main publications:

Sheridan Á, Lenahan M., Condell O., Bonilla-Santiago R., Sergeant K., Renaut J., Duffy G., Fanning S., Nally J. E. and C.M. Burgess. (2013) Proteomic and phenotypic analysis of verocytotoxigenic *Escherichia coli* (VTEC) with tolerance to triclosan. *Journal of Proteomics* 80: 78–90

Sheridan À., M. Lenahan, G. Duffy, S. Fanning and C.M. Burgess (2012). The potential of biocide tolerance in *Escherichia coli* and its impact on the response to food processing stresses. *Food Control*, 26:98–106.

Condell O., C. Iversen, S. Cooney, K. Power, C. Walsh, CM Burgess and S. Fanning (2012). Efficacy of biocides used in the modern food industry to control *Salmonella* – links between biocide tolerance and resistance to clinically relevant antimicrobial compounds. *Applied and Environmental Microbiology* 78(9):3087–97

Condell O, Sheridan Á, Power KA, Bonilla-Santiago R, Sergeant K, Renaut J, Burgess C, Fanning S, Nally JE. (2012) Comparative proteomic analysis of *Salmonella* tolerance to the biocide active agent triclosan. *Journal of Proteomics* 75(14):4505–19.

Popular publications:

Burgess C. (2009). Biocide resistance: a food safety challenge? The Ashtown Food Innovator, issue 6.

Lenahan M., Á. Sheridan, G. Duffy, S. Fanning, O. Condell, C.M. Burgess, (2011). Microbial tolerance to biocides. TResearch, Volume 6: Number 2, 22–23.

ProSafeBeef: Assessment of microbiological and chemical safety of beef

Project number:
5705
Date:
September, 2013
Funding source:
EU Framework
Project dates:
Mar 2007 – Dec 2012

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Dr. Martin Danaher
Dr. Declan Bolton
Dr. Kaye Burgess

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University College Dublin;
Ghent University;
University of Bristol;
Agricultural University of Poznan;
University of Veterinary Medicine Austria;
Aristotle University of Thessaloniki;
Aarhus School of Business;
Danish Meat Research Institute;
University College Cork;
RIKILT, Institute of Food Safety;
Queen's University Belfast;
International Atomic Energy Agency;
British Nutrition Foundation;
University of Novi Sad;
Institute of Agro-Food Research and Technology, Spain;
Universidad Federale de Sao Paulo;
Universidade de Sao Paulo;
USDA, Western Regional Research Centre;
University of Guelph;
Institute of Environmental Science and Research, New Zealand.

Compiled by:
Geraldine Duffy

Key external stakeholders:

Beef sector, Regulators, FSAI.

Practical implications for stakeholders:

The study indicated that the risk posed by the microbial pathogens and chemical residues examined in beef in this study was generally Iow. Nonetheless the study showed that the hide was an important vehicle of microbial pathogen contamination into the abattoir and would thus be a key target for risk reduction measures. A new technology for anthelmintic drug residues was developed and is now in use by the Irish national reference laboratory where it is used for the control and monitoring of food of animal origin for such residues according to EU legislation ensuring beef and food safety.

This research was carried out as part of a multi-national EU Framework project, *ProSafeBeef* which focused on research and innovation to improve beef safety and quality. Research on beef safety at Teagasc focused on the risk posed by microbial pathogens and chemical residues in beef.

Main results:

- Overall, the occurrence of the four pathogens examined (verocytotoxigenic *E.coli, Listeria monocytogenes, Campylobacter* and *Salmonella*) in the beef chain was low, though many of the isolates that were recovered had traits similar to those seen in human illness causing strains highlighting the need for continued vigilance in risk management of such pathogens along the beef chain (farm to fork).
- Verocytotoxigenic *E.coli* are a human health concern with new serotypes of these pathogens being linked to human illness in recent years. In this study *E. coli* 0157, the most common type of VTEC inhuman illness, was also the most commonly recovered VTEC from beef. Emergent serogroups were recovered at a lower prevalence, and the majority of these isolates did not have the combination of virulence genes typically seen in human disease causing strains.

- During slaughter, it was shown by genetic finger-printing that the source of pathogens on a carcass could be from an animal's own hide or from hide of another animal being slaughtered on the same day, highlighting that the hide is a key target in the chain for interventions.
- A new state-of-the art Mass Spectroscopy (UHPLC-MS/ MS) method was developed for the detection of 38 anthelmintic drug residues. The method was validated according to Commission Decision 2002/657/EC and accredited to ISO 17025 standard. The method was then applied to assess occurrence of anthelmintic residues in 1061 retail beef samples from across Europe over a two year period. Results showed that the risk of exposure to EU consumers from anti-parasitic drug residues in beef was negligible.

Opportunity/Benefit:

The study showed that the hide was an important vehicle of microbial pathogen contamination into the abattoir and would thus be a key target for risk reduction measures. A new technology for anthelmintic drug residues was developed and has been transferred to a number of EU laboratories, thus harmonising the approach of residue control for beef consumed by EU consumers. This research underpins the safe image of EU beef, ensuring consumer confidence and safeguarding international investment in the sector.

1. Project background:

It is well recognised that food production animals including bovine animals shed a diverse range of micro-organisms in their faeces, some of which may be pathogenic including, verocytotoxigenic *E. coli* (VTEC), *Listeria monocytogenes, Salmonella* and *Campylobacter.* Such pathogens can persist and circulate in the farm environment posing a risk for contamination of the food and water chain while during beef slaughter and dressing, these pathogens can potentially be transferred from contaminated bovine hide or the gastrointestinal contents onto the beef carcass. While it is known that food pathogens are circulating in the beef chain, few studies have comprehensively tracked or quantified these pathogens in the beef chain or assessed the human clinical significance of strains transmitted by this vehicle. Research at Teagasc aimed to address this gap in knowledge at key stages of the beef chain, farm, beef slaughter and retail.

Additional research from a chemical beef safety perspective focused on anti-parasitic drugs which are important for the control and treatment of helminths such as roundworm, tapeworm and fluke infections in beef-producing animals. Many of these products are licensed for use and are safe for use if product labels are adhered to. However, undesirable levels of residues may be detected in beef if the incorrect dosage is administered; if the drug is not licensed for use in that species; or if withdrawal periods are not adhered. To address this concern, the aim of research at Teagasc was to develop a method for the determination of anti-parasitic drug residues and to then apply the methods to assess levels of these residues in retail beef.

2. Questions addressed by the project:

- What is the occurrence and transmission of *Salmonella*, *Campylobacter* and VTEC, including *E. coli* O157, at key stages in the beef chain (farm, slaughter and retail)?
- What is the human virulence potential of pathogen recovered from beef?
- What is the risk posed by anti-parasitic drug residues in beef?

3. The experimental studies:

- Field studies were performed on 10 Irish beef farms to examine the incidence and spread of *Salmonella*, *Campylobacter*, VTEC and *E. coli* O157. Bovine faecal samples were examined for the pathogens and all isolates were assessed for human virulence potential.
- Samples of bovine hides, and carcass (pre chill) of the same tracked animal were collected from beef abattoirs over a 3 year period. Ground beef samples were collected during the same time period from retail outlets. All samples were examined for prevalence and concentration of four pathogens (verocytotoxigenic *E. coli, Listeria monocytogenes, Campylobacter* and *Salmonella*). Isolates recovered were characterised, genetically finger printed and assessed for human virulence potential.
- A new state-of-the art UHPLC-MS/ MS method was applied to the detection of 38 anthelmintic drug residues. The method was validated according to Commission Decision 2002/657/EC and accredited to ISO 17025 standard and then applied to assess the occurrence of these residues in a two year study of 1061 retail beef samples from across Europe.

4. Main results:

- In farm faecal samples, 2% and 3% of samples were positive for *Salmonella*, and *Campylobacter*, respectively. All of the *Salmonella* detected were S. Typhimurium. *Campylobacter* species included *C. jejuni* (4 farms). This particular serotype of *Salmonella* and species of *Campylobacter* are the most common types seen in human illness.
- Verocytotoxigenic *E. coli* isolates was recovered on all farms examined with a wide diversity of serotypes recovered including *E. coli* O157 and emergent serogroups. Apart from the *E. coli* O157 isolates few of the emergent serotypes had the combination of virulence genes typically seen in human disease-causing strains.
- VTEC were also recovered from beef at slaughter, on hides (17%) and on carcass (1%). *E. coli* 0157 was the most common serogroup recovered. It was noted that as for farm isolates, only a small proportion of the non O157 VTEC had the combination of virulence genes typically seen in human disease-causing strains.
- The prevalence of *Listeria monocytogenes* ranged from 14% on carcass to 29% on ground beef, highlighting that cross contamination and growth of this pathogen can occur in the beef chain. The most common serotypes recovered were 1/2a followed by 4b (the most common serotype in human illness) and the majority of isolates contained a key virulence gene (*lmo*2821) seen in human illness strains.
- The prevalence of *Campylobacter* on hides was high (51%) but was very low on ground beef samples (1%) indicating that the pathogen did not survive the environmental conditions experienced in the beef chain (chilling, drying etc.). *Campylobacter jejuni* (the most common species in human illness) was also the most common species recovered from beef.
- The prevalence of *Salmonella* was low with a diversity of serogroups recovered.
- Genetic finger-printing showed the source of pathogens on a carcass could be its own hide or the hide of another animal slaughtered on the same day, highlighting that the hide is a key target for risk reduction measures.
- A mass spectroscopy method (UHPLC-MS/ MS) was developed for the detection of 38 different anthelmintic drug residues. The method was validated according to Commission Decision 2002/657/EC and the technology was transferred to the Irish national reference laboratory for anti-parasitic drugs where it is used for the control and monitoring of food of animal origin according to EU legislation ensuring food safety.
- Research showed that 26 of the 1061 beef samples analysed contained residues of the anti-parasitic drugs, and of these 26, only one was at a non-compliant level. The non-compliant sample contained low levels of ivermectin, however, as there was no maximum residue limit (MRL) for ivermectin in muscle, any presence of the drug is deemed non-compliant. These results indicate that the risk of exposure to EU consumers from anti-parasitic drug residues in beef is negligible.

5. Opportunity/Benefit:

The study showed that the hide was an important vehicle of microbial pathogen contamination into the abattoir and would thus be a key target for risk reduction measures. A new technology for anthelmintic drug residues was developed and has been transferred to a number of EU laboratories, thus harmonising the approach of residue control for beef consumed by EU consumers. This research underpins the safe image of EU beef, ensuring consumer confidence and safeguarding international investment in the sector.

6. Dissemination:

Main publications:

Bolton DJ, Ennis C, McDowell D. (2013)Occurrence, Virulence Genes and Antibiotic Resistance of Enteropathogenic *Escherichia coli* (EPEC) from Twelve Bovine Farms in the North-East of Ireland. 10.1111/zph.12058. [Epub ahead of print]

Ennis C., McDowell D, Bolton DJ. (2012). The prevalence, distribution and characterisation of Shiga toxin – producing *Escherichia coli* (STEC) serotypes and virulotypes from a cluster of bovine farms. *J Appl Microbiol.* 113(5):1238–1248.

Rhoades, J.R., Duffy, G., and Koutsoumanis, K. (2009) Prevalence and concentration of verocytotoxigenic *Escherichia coli, Salmonella enterica* and *Listeria monocytogenes* in the beef production chain: A review. *Food Microbiology, Volume* 26, I 4, 357–376

Thomas, K.M., McCann, M., Collery, M.M., Logan, A., Whyte, P., McDowell, D.A., and Duffy, G. (2012). Tracking Verocytotoxigenic *Escherichia coli* 0157, 026,0111, 0103 and 0145 in Irish Cattle. *Int J. Food Micro* 15(153):288–296

Cooper, K. M., Kennedy, D. G., & Danaher, M. (2012a). ProSafeBeef and anthelmintic drug residues – A case study in collaborative application of multi-analyte mass spectrometry to enhance consumer safety. Analytical and Bioanalytical Chemistry, 404(6–7), 1623–1630.

Cooper, K. M., Whelan, M., Kennedy, D. G., Trigueros, G., Cannavan, A., Boon, P. E., Wapperom, D. and Danaher, M. (2012) Anthelmintic drug residues in beef: UPLC-MS/MS method validation, European retail beef survey, and associated exposure and risk assessments. Food Additives & Contaminants: Part A, 29, 746–760.

Cooper, K. M., Whyte, M., Danaher, M., & Kennedy, D. G. (2012). Emergency slaughter of casualty cattle increases the prevalence of anthelmintic drug residues in muscle. Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 29(8), 1263–1271.

Cooper, K.M., Whelan, M., Danaher, M., Kennedy, D.G. Stability during cooking of anthelmintic veterinary drug residues in beef (2011) Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 28 (2), pp. 155–165.

Whelan, M., Kinsella, B., Furey, A., Moloney, M., Cantwell, H., Lehotay, S.J., Danaher, M. Determination of anthelmintic drug residues in milk using ultra high performance liquid chromatography-tandem mass spectrometry with rapid polarity switching (2010) Journal of Chromatography A, 1217 (27), pp. 4612–4622.

Kinsella, B., Whelan, M., Cantwell, H., McCormack, M., Furey, A., Lehotay, S.J., Danaher, M. A dual validation approach to detect anthelmintic residues in bovine liver over an extended concentration range (2010) Talanta, 83 (1), pp. 14–24.

Popular publications:

Tracking key pathogens in the beef chain. Prosafebeef TechKnowledge Stakeholder Digest No. 7. www.prosfebeef.eu.

Methods for the Detection of Anti-Parasitic Drug Residues in Beef. Prosafebeef TechKnowledge Stakeholder Digest No. 10. www.prosfebeef.eu.

Genomics of gram negative food poisoning bacteria of animal origin

Project number:
5854
Date:
September, 2013
Funding source:
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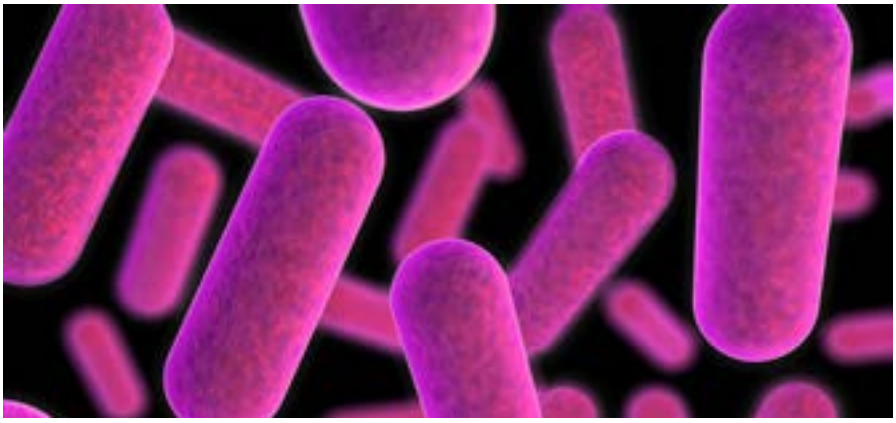
Project dates:
Nov 2007 – Dec 2012



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Teagasc project team:
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Compiled by:
Geraldine Duffy
Kaye Burgess
Kieran Jordan
Declan Bolton

Key external stakeholders:

Food and in particular the pork sector,
Regulators, FSAI Practical

Practical implications for stakeholders:

Approximately 40% of Salmonella *Typhimurium* isolates examined readily attached to food contact surfaces and formed biofilms (bacterial populations on surfaces). In biofilms, *Salmonella* can persist for long periods of time, and pose a risk of contamination in food production. Research showed that particular genes and proteins needed to be expressed to allow formation of biofilms by *Salmonella*, and these could be targeted in development of new biocides.

Main results:

This project investigated the responses of food-borne bacteria to various stresses at the genomic and proteomic level. Research at Teagasc focused on the ability of *Salmonella* Typhimurium isolates to attach and persist as biofilms (bacterial populations attached to surfaces) in food production.

- Of the *Salmonella* Typhimurium isolates (n= 172) examined, which were recovered from the pork chain in Ireland or of human clinical origin, about 40% had the ability to form biofilms on stainless steel and plastic surfaces. Among clinical isolates 73% attached to PVC plastic compared to 53.3% of pork isolates. This indicates that the ability to persist on surfaces may be enhancing the transmission of *Salmonella* through the food chain to the consumer.

- *Salmonella* in biofilms formed at pH 5 showed increased expression of virulence genes *hilA* and *invA* compared to those from biofilms formed at neutral pH 7 indicating that acidic environments in food production plants may enhance the ability of *Salmonella* to cause food borne illness.
- In acidic environments, genes related to *Salmonella* motility i.e. flagella structures (*Flagellin*) were down regulated in cells from biofilms as compared to non surface attached (planktonic) cells. Genes related to cell-to-cell signaling and transport of exopolysaccharides across the outer membrane, were upregulated and needed for successful biofilm formation. Proteomic analysis also revealed that the switch from planktonic to biofilm status required up regulation of proteins associated with glycolysis, cell-to-cell signaling and protein transport.
- Therefore design of biocidal agents that specifically interfere with glycolysis and cell-to-cell signaling and that enhance flagella formation could help inhibit biofilm formation by S. Typhimurium in food processing facilities.

Opportunity/Benefit:

The data generated in this project gives a fundamental understanding on the persistence and biofilm formation by *Salmonella* on contact surfaces used in food production and could support industry in control of this pathogen and the development of novel targeted biocidal agents.

1. Project background

Salmonella Typhimurium is a food-borne pathogen of importance to public health in Ireland and is a leading cause of human bacterial gastroenteritis. S. Typhimurium DT104 is frequently isolated from the pig/pork chain and this type of *Salmonella* is believed to have enhanced ability to attach to surfaces and to form biofilms (surface attached bacterial communities). When attached to surfaces and in biofilms, bacteria are believed to be better protected from anti-microbial controls and to persist for longer periods in the food environment. Cells existing in biofilms may exhibit different gene and protein expression when compared to non surface attached (planktonic) bacterial cells. The aim of the research was to gain a fundamental understanding, at a phenotypic, genomic and proteomic level, as to how *Salmonella* Typhimurium isolates recovered from the Irish pork chain attach to and persist as biofilms on food production surfaces.

2. Questions addressed by the project:

- How well do *Salmonella* Typhimurium DT104 isolates recovered from the Irish pork chain and from human illness, attach to food contact surfaces (PVC, stainless steel) and form biofilms?
- How is attachment and biofilm formation by *Salmonella* Typhimurium DT104 to surfaces impacted by acidic environments in food production environments?
- How are gene and protein expression in *Salmonella* Typhimurium DT104 impacted in biofilm formed at acidic pH?

3. The experimental studies:

- *Salmonella* Typhimurium isolates (n= 172) from the pork chain and of human clinical origin, were examined for their ability to attach to and form biofilms on stainless steel and plastic surfaces.
- Isolates were examined for presence of *Salmonella* genomic island 1 (SGI1), a 43 kb chromosomal genomic island that contains an antibiotic resistance gene cluster which confers multi-drug resistance to epidemic S. Typhimurium DT104. It has been proposed that the presence of SGI1 may also increase biofilm formation. *Salmonella* strains were examined for sections of SGI1 by PCR.
- *Salmonella* Typhimurium DT104 employs an acid tolerance response allowing it to adapt to acidic environments. The risk that these acid adapted cells pose to food safety could be enhanced if they also produce biofilms under acidic conditions. *Salmonella* were exposed to lactic acid and their ability to form biofilms on stainless steel was examined by looking at expression of selected genes related to regulation and virulence.
- A proteomics approach was used to examine differential protein expression in S. Typhimurium biofilm formed under acid conditions. Expression of selected proteins was examined by 2-D gel electrophoresis.

4. Main results:

Research focused on the ability of *Salmonella* Typhimurium isolates from the pork chain to attach and persist as biofilms (bacterial populations attached to surfaces) in food production.

- Of the *Salmonella* Typhimurium isolates (n= 172) examined of human clinical origin and the pork chain, 40% had the ability to form biofilms on stainless steel and plastic surfaces. Among clinical isolates 73% attached to PVC plastic compared to 53% of pork isolates. This indicates that the ability to persist on surfaces may be enhancing the transmission of *Salmonella* through the food chain to the consumer.
- *Salmonella* genomic island 1 (SGI1) was present in isolates which were weak and strong biofilm formers indicating a weak association of this chromosomal genomic island with biofilm production.
- *Salmonella* from biofilms formed at pH 5 showed increased expression of virulence genes *hilA* and *invA* compared to those from biofilms formed at neutral pH 7 indicated that acidic environments in food production plants are enhancing the ability of *Salmonella* to cause food borne illness.
- In acidic environments, genes related to *Salmonella* motility, (*Flagellin* which is involved in production of flagella structures) were down regulated in biofilms compared to non surface attached (planktonic) cells. Genes related to cell-to-cell signaling and transport of exopolysaccharides across the outer membrane, were up regulated and needed for successful biofilm formation. Proteomic analysis also revealed that the switch from planktonic to biofilm attached status required up-regulation of proteins associated with glycolysis, cell-to-cell signaling and protein transport.
- Therefore design of biocidal agents that specifically interfere with glycolysis and cell-to-cell signaling or that enhance flagella formation (motility) could help inhibit biofilm formation by S. Typmiurium in food processing facilities.

5. Opportunity/Benefit:

The data generated in this project gives a fundamental understanding on the persistence and biofilm formation by *Salmonella* on contact surfaces used in the food sector and could support industry in control of this pathogen and in the development of targeted biocidal agents.

6. Dissemination:

Main publications:

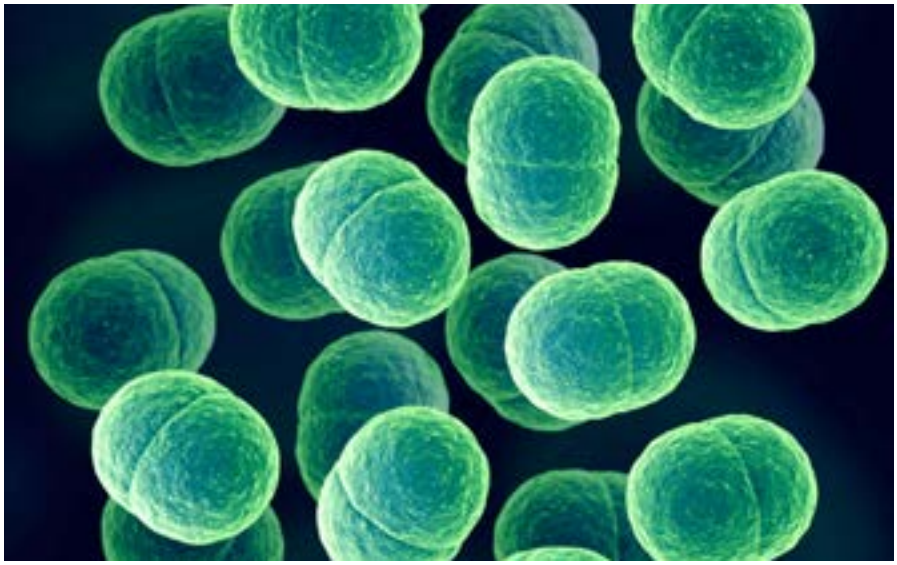
O'Leary, Denis, M. Mc Cabe, E., McCusker, M.P, Martins, M., Fanning, S. and Duffy, G. (2013) Microbiological study of biofilm formation in isolates of *Salmonella enterica* Typhimurium DT104 & DT104b cultured from the modern pork chain *International Journal of Food Microbiology* 5;161(1):36–43.

Martins. M., McCusker, M., McCabe, E., O'Leary, D., Duffy, G. and Fanning, S (2013). Study of the phenome(s) of Salmonella Typhimurium DT104 cultured from selected points across the pork production food chain – evidence of metabolic switching and implications for food safety. *Applied and Environ Micro.* 79(18):5437.

Popular publications:

O'Leary, D., McCabe, E., McCusker, M., Martins, M., Fanning, S. and Duffy, G., (2012). Effect of lactic acid treatment on biofilm production and gene expression in the food-borne pathogen *Salmonella enterica* Typhimurium DT104. International Conference, Global Food Safety: Solutions for Today and Tomorrow. Crowne Plaza Hotel, Blanchardstown, Dublin, October 23 to 25th 2012. Pg. 94, ISBN 84170–591–8.

Risk Assessment Network of Ireland



Project number:
5855
Date:
June, 2014
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Project dates:
Nov 2007 – Nov 2012

Collaborating Institutions:
University College Dublin

Teagasc project team:
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Dr. Geraldine Duffy
Ms. Karen (Triona) Hunt

External collaborators:
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University College Dublin

Compiled by:
Kieran Jordan and
Geraldine Duffy

Industry Impact

The study assessed the impact of two food pathogens on the safety of raw milk cheese for the benefit of raw milk cheesemakers and the public in general. The study showed that risks associated with *Staphylococcus aureus* are low, while those associated with *Listeria monocytogenes* are more significant.

Key external stakeholders:

Raw milk cheese industry; Policymakers, Food researchers.

Practical implications for stakeholders:

The study assessed the risk posed by two food pathogens (*Staphylococcus aureus* and *Listeria monocytogenes*) in raw milk cheesemaking. A range of samples (n=117), including milk, curds, whey and cheese, from 5 raw milk suppliers, and 4 raw milk cheesemakers were analysed for coagulase positive *S. aureus*. Of the isolates obtained, 17% had toxin producing ability and produced only Staphylococcal Enterotoxin C (SEC) which is generally animal rather than food associated. The other classical enterotoxins SEA, SEB or SED (food poisoning associated) were not produced. No toxin was produced in raw or pasteurised milk or in sterile reconstituted skim milk stored below 14°C for 24 h and no SEC was produced during cheesemaking. *L monocytogenes* was found at a level of 300 colony forming units/ml in the milk of one cow with sub-clinical infection. While the numbers of naturally occurring *L. monocytogenes* increased in milk and during cheesemaking, this increase did not appear to be due to growth.

This research was carried out as part of a large national network project, *Risk Assessment Network of Ireland* which focused on the application of microbial quantitative risk assessment as a tool to underpin risk management actions. Research by Teagasc in this project focused on the risk posed by two food pathogens on the safety of raw milk cheese.

Main results:

- None of the *S. aureus* isolates recovered from raw milk or cheese produced the endotoxins SEA, SEB or SED, nor did they harbour the enterotoxin encoding genes *sea, seb, sed* or *see*.
- 17% of *S. aureus* isolates produced Staphylococcal enterotoxin C (SEC)
- Cheesemaking inhibited staphylococcal toxin production as did storage temperatures below 14°C.
- Optimum conditions for toxin production in reconstituted skim milk were 37°C at pH 6.5
- *Listeria monocytogenes* was found in raw milk from one cow at a level of 300 cfu/ml, though there was with no evidence of infection in the animal.
- Although numbers of naturally occurring *L. monocytogenes* increased in milk and during cheese making, this increase did not appear to be due to growth.

Opportunity/Benefit:

The opportunity was to assess the impact of *S. aureus* and *L. monocytogenes* on the safety of raw milk cheese for the benefit of raw milk cheesemakers and the public in general. The study showed that there were different risks associated with each pathogen.

1. Project background:

Coagulase positive *Staphylococcus aureus* is a Gram-positive, facultative anaerobe that is ubiquitous in nature. Some, but not all strains produce staphylococcal enterotoxins (SEs) which are potent emetic agents causing staphylococcal food poisoning (SFP). SFP has recently been reported to have a low hospitalisation rate of 6.4%; however, due to the nature of the toxin the symptoms are rarely severe, leading to high levels of under-reporting. *S. aureus* strains produce a wide range of toxins (presently 121 SEs) of which toxins, SEA, SED and SEE are the most frequently associated with food, and are therefore most clinically relevant, while SEC is associated with animal origin. *S. aureus* is one of the major causes of bovine mastitis, and in cases of sub-clinical mastitis can easily contaminate raw milk. With no heat treatment prior to manufacture of raw milk cheese, and milk being an ideal growth medium for bacteria, numbers of *S. aureus* could potentially increase. And if subsequent growth opportunities occur during cheesemaking, it is possible that numbers could be relatively high in cheese. Approximately 10% of cheese in Europe is made from raw milk, presenting a considerable potential risk to public health.

Listeria monocytogenes is a pathogenic bacterium that can cause Listeriosis in humans and various animal species. In humans, foodborne *L. monocytogenes* causes large outbreaks of Listeriosis, with a mortality rate of 9% to 44%. In raw milk and the dairy environment, the source of *L. monocytogenes* contamination is mainly from poor silage and bedding. *L. monocytogenes* can cause bovine mastitis, and in cases of sub-clinical mastitis in cows, can go undetected if the milk remains visually unchanged, and with no clinical symptoms the contamination can normally persist even after treatment. Raw milk can thus be contaminated by direct excretion into the milk. The purpose of this study was to assess the risks associated with *S. aureus* and *L. monocytogenes* in raw milk.

2. Questions addressed by the project:

Do *S. aureus* and *L. monocytogenes* pose a threat to public health in raw milk cheese?

To address this issue, the following questions were asked:

- What is the occurrence of toxin producing *S. aureus* in raw milk?
- What are the factors that affect toxin production in milk and cheese?
- Can naturally occurring *L. monocytogenes* grow during cheese making?

3. The experimental studies:

- One hundred and seventeen samples, including milk, curds, whey and cheese, from 5 raw milk suppliers, to 4 raw milk cheesemakers in the South of Ireland, were analysed for coagulase positive *S. aureus*.
- The effect of different combinations of temperature and pH on production of SEC by *S. aureus* was investigated in batch cultures of 10% sterile reconstituted skim milk for up to 72 h. A full factorial experiment of 4 temperatures (25, 30, 37 and 40°C) and 4 pH values (5.5, 6.0, 6.5 and 7.0) was undertaken.
- Toxin production was also studied in pasteurised and unpasteurised milk at temperatures of 25, 30, 37 and 40°C with uncontrolled pH.
- During routine sampling of bulk raw milk on a dairy farm, the pathogenic bacteria *Listeria monocytogenes* was found to be a contaminant, at numbers < 100 cfu/ml. Milk samples were collected from the individual cows and analysed for *L. monocytogenes*.

4. Main results:

- The 151 isolates characterised represented up to 2 isolates from each of the 81 positive samples. The results showed 83.2% of the isolates did not contain the staphylococcal enterotoxin genes or the toxin producing capability tested for. From only one supplier, 26 isolates contained the *sec* genes and produced SEC. Within these 26 isolates there were only 2 PFGE types. None of the isolates from any of the 5 suppliers produced SEA, SEB or SED toxin nor did they harbour the *sea*, *seb*, *sed* or *see* genes. One SEC producing isolate showed no toxin production at 96 and 74 h in sterile 10% reconstituted skim milk at 10°C and 12°C, respectively. Some SEC was produced at 14°C and 16°C after 74 and 55 h, respectively.
- The results indicate that milk used for raw milk cheese production in Ireland poses a limited risk to public health.
- The optimum controlled conditions for SEC production were 37°C/pH6.5. At temperatures of 25, 30, 37 and 40°C with uncontrolled pH, a lower concentration of SEC was produced compared to the controlled pH conditions. In pasteurised and unpasteurised milk, no SEC production occurred at temperatures up to 30°C after 72 h.
- *L. monocytogenes* excretion (at 280 cfu/ml) from one of the 4 mammary quarters of one dairy cow out of 180 was identified.
- A strain with an indistinguishable pulsed-field gel electrophoresis pattern was isolated from the bulk milk. Environmental swabs taken at the dairy environment were negative for the presence of *L. monocytogenes*. The results indicated a possible case of excretion of the *L. monocytogenes* directly into the milk.
- Although numbers of *L. monocytogenes* increased in milk, there was no evidence that growth had occurred.

5. Opportunity/Benefit:

The opportunity was to assess the risk posed by two food pathogens (*S. aureus*, *L. monocytogenes*) on the safety of raw milk cheese. The study showed that there were different risks associated with each pathogen.

6. Dissemination:

The information generated from this study was disseminated by publication of the work in relevant peer reviewed journals and at meetings and workshops with farmhouse cheese makers.

Main publications:

Hunt, K., Schelin, J., Rådström, P., Butler, F., and Jordan, K. (2012). Classical enterotoxins of coagulase-positive *Staphylococcus aureus* isolates from Raw Milk and products for Raw Milk Cheese Production in Ireland. *Dairy Science and Technology*, 92, 487–499.

Hunt, K., Drummond, N., Murphy, M., Butler, F., Buckley, J., and Jordan, K. (2012). A case of subclinical mastitis resulting in bovine raw milk contamination with *Listeria monocytogenes*. *Irish Veterinary Journal*, 65: 13–18.

Hunt, K., Butler, F., and Jordan, K. (2014). Factors affecting Staphylococcal Enterotoxin C $_{\text{bovine}}$ production in milk. *International Dairy Journal*, 39:41–46.

Improved bio-traceability of unintended micro-organisms and their substances in food and feed chains

Project number:
5691

Date:
July 2013

Funding source:
EU Framework 7

Project dates:
Jan 2007-Dec 2011

Key external stakeholders:

- EU – the funding agency.
- Researchers.
- Irish Farmhouse Cheesemakers Association.
- Food Safety Authority of Ireland.

Practical implications for stakeholders:

This research has had an impact as follows:

- *Listeria monocytogenes* occurrence on farms was about 19%, indicating that cross contamination could occur on farms.
- Using predictive modelling for determination of growth of *L. monocytogenes* in food is not always accurate.
- Similar strains of *L. monocytogenes* can be isolated from multiple food chains.
- Of 109 raw milk samples tested, 6% contained *L. monocytogenes*.
- The results suggest that the farm environment external to the processing environment may in some cases be the source of processing environment contamination with *L. monocytogenes*.
- The data obtained contributes to a better understanding of the potential risk that *L. monocytogenes* presents to cheese producers (growth on the product, if it is contaminated) and constitutes a very useful set of data for further modelling studies in food.
- Persistent strains of *L. monocytogenes*, that are more difficult to control, were identified in some processing environments.

Collaborating Institutions:
Danish Technical University
Copenhagen University of Veterinary Medicine, Vienna.

Teagasc project team:
Dr. Kieran Jordan
Dr. Edward Fox
Sol Schvartzman

External collaborators:
Danish Technical University
Copenhagen University of Veterinary Medicine, Vienna.
There were 45 other participants in the project

Compiled by:
Kieran Jordan

Main results:

- Sixteen cheesemaking facilities were sampled during the production season at monthly intervals over a one-year period. Thirteen facilities were found to have samples positive for *L. monocytogenes* on at least one occasion.
- 19% of samples at farm level were positive for *L. monocytogenes*.
- This study demonstrates the prevalence of *L. monocytogenes* in the dairy farm and processing environments and the need for good hygiene practices to prevent its entry into the food chain.
- Predictive modeling is not always applicable to food.

Opportunity/Benefit:

- Contamination of food processing facilities (not food) was shown. There is an opportunity to use this pre-emptive knowledge to improve hygiene at processing facilities and prevent future issues with food contamination.
- Predictive modeling is not always applicable to food – challenge studies are necessary.
- A database of pulsed field gel electrophoresis (PFGE) profiles of *L. monocytogenes* isolates from Ireland was generated.

1. Project background:

L. monocytogenes is a foodborne bacteria responsible for the disease listeriosis. Although listeriosis is uncommon, the mortality associated with it is 20–30%. *L. monocytogenes* occurs widely in the environment so elimination of it is an unrealistic aim. Awareness of its occurrence and routes of transmission are essential in its control, particularly with respect to strains that persist in processing environments. PFGE is a valuable tool in tracing routes of contamination and in comparing isolates obtained from different sources. Regulations in the EU allow up to 100 cfu/ml (g) in foods that cannot support growth of the organism. Therefore, knowledge on the ability of foods to support growth of *L. monocytogenes* and the factors affecting such growth is essential.

2. Questions addressed by the project:

- What is the occurrence of *L. monocytogenes* in cheese processing facilities and on farms?
- What are the sources and putative transfer routes of *L. monocytogenes*?
- Is there a difference in growth of *L. monocytogenes* in raw and pasteurised milk?
- Can models of growth that are generated in laboratory media be used to describe growth in food?
- Are there similar strains of *L. monocytogenes* isolated from different sources?

3. The experimental studies:

- Farms and cheese processing facilities were sampled for *L. monocytogenes*.
- Mathematical models to describe the growth of *L. monocytogenes* on the surface of cheese was used.
- The difference between growth of *L. monocytogenes* in pasteurised and unpasteurised milk cheese was studied.
- PFGE profiles from *L. monocytogenes* isolates from different sources in Ireland were determined establishing a national database of *L. monocytogenes* profiles.

4. Main results:

L. monocytogenes in the farm and processing environments

This study aimed to determine the occurrence of *L. monocytogenes* in 1) the Irish dairy farm environment and 2) the cheese processing environment.

1) Two hundred ninety-eight environmental samples were collected from 16 farms in the southern region of Ireland. A number of farms within the group supply raw milk to the unpasteurized milk cheese industry. The samples taken included cow faeces, milk, silage, soil, water, etc. Samples were enriched in *Listeria* enrichment broth and incubated for 48 h, followed by plating on chromogenic agar Listeria Ottavani & Agosti and further incubation of the plates for 24 to 48 h. Presumptive *L. monocytogenes* isolates were purified and confirmed by PCR targeting the *hly* gene. And 51 isolates were compared using PFGE. Overall, 19% of the samples (57 of 298) were positive for *L. monocytogenes*. These were serotyped using conventional and PCR methods; serotypes 1/2a, 1/2b, and 4b made up 78% of the typeable isolates. A correlation was found between the level of hygiene standards on the farm and the occurrence of *L. monocytogenes*. There was little difference in the occurrence of *L. monocytogenes* between farms supplying milk to the unpasteurized milk cheese industry and those supplying milk for processing. From the 51 isolates examined by PFGE, there were 40 individual PFGE types. Four of the PFGE types were common to multiple farms, and five farms had isolates with indistinguishable PFGE types in multiple locations on the farm. Indistinguishable PFGE types were common to multiple farms in different geographical locations up

to 200km apart. The results indicate multiple niches for the organism in the dairy farm environment. The presence of *L. monocytogenes* in samples related to animals other than cattle indicates that there are multiple possible vectors of contamination. The farm environment harbors a diverse collection of *L. monocytogenes* isolates that must be considered as possible agents of food contamination.

2) Sixteen cheesemaking facilities were sampled during the production season at monthly intervals over a one year period. Samples were divided into 4 categories; cheese, raw milk, processing environment and external to the processing environment (samples from the farm such as silage, bedding, and pooled water). In order to attempt to identify the source, persistence and putative transfer routes of contamination with the *L. monocytogenes* isolates, they were differentiated using PFGE and serotyping. Thirteen facilities were found to have samples positive for *L. monocytogenes*. Of the 250 isolates, there were 52 different pulsotypes. No pulsotype was found at more than one facility. Two facilities had persistent pulsotypes that were isolated on sampling occasions at least 6 months apart. Of the samples tested, 6.3% of milk, 13.1% of processing environment and 12.3% of samples external to the processing environment, respectively, were positive for *L. monocytogenes*. Pulsotypes found in raw milk were also found in the processing environment and on only one occasion one of the pulsotypes from raw milk was also found in cheese. One of the pulsotypes isolated from the environment external to the processing facility was found on the surface of cheese, however, a number of them were found in the processing environment. The results suggest that the farm environment external to the processing environment may in some cases be the source of processing environment contamination with *L. monocytogenes*.

This study demonstrates the prevalence of *L. monocytogenes* in the dairy farm and processing environments and the need for good hygiene practices to prevent its entry into the food chain.

Strain comparisons

A retrospective analysis of isolates of 222 *L. monocytogenes* strains from human and non-human sources in Ireland was undertaken by PFGE. Human clinical isolates from other countries were also examined. Eight small clusters of human and non-human isolates (mostly serotype 4b) that were indistinguishable from one another were detected, suggesting potential sources for human infection. For non-human isolates, some PFGE types appeared to be exclusively associated with a single source, whereas other PFGE-types appeared to be more widely disseminated. Indistinguishable, or highly related clusters of isolates of Irish and non-Irish origin suggest that some PFGE patterns may be globally distributed.

Modelling growth of *L. monocytogenes*

The dynamics of the physicochemical characteristics of foods help to determine if growth of pathogens will occur in the food. The aim of this work was to determine if growth initiation varied between a cheesemaking, milk and tryptic soy broth (TSB). Growth of a two-strain mix of *L. monocytogenes* at combinations of four initial pH values and five water activity (a_w) values was determined in cheese, milk and TSB. Each condition was repeated six times, and growth initiation probability was modelled with logistic regression models. Growth initiation boundaries were obtained for each matrix type. The results showed that the growth limits were matrix dependent. In the three matrix types, a_w was the most important factor affecting the probability of growth initiation. The growth interface wideness and position in cheese, milk and TSB were dissimilar, indicating that the use of models evaluated in TSB or milk could not be used to predict the behaviour of *L. monocytogenes* under cheesemaking conditions. Predictive models generated in liquid media are not necessarily adaptable to solid food, and the generation of real food models is necessary.

No growth of *L. monocytogenes* occurred during raw milk cheesemaking, whereas growth did occur in pasteurised milk. During ripening, growth occurred in raw milk cheese, but inactivation occurred in pasteurised milk cheese. The behaviour observed for *L. monocytogenes* was modelled using a logistic primary model coupled with a secondary cardinal model, taking into account the effect of physicochemical conditions (temperature, pH, water activity and lactate). This complex model had an acceptable quality of fit on the experimental data. The estimated optimum growth rates can be used to predict the fate of *L. monocytogenes* during cheese manufacture in raw or pasteurized milk in different physicochemical conditions. The data obtained contributes to a better understanding of the potential risk that *L. monocytogenes* presents to cheese producers.

5. Opportunity/Benefit:

- Contamination of food processing facilities (not food) was shown. There is an opportunity to use this pre-emptive knowledge to improve hygiene at processing facilities and prevent future issues with food contamination.

6. Dissemination:

Technology transfer to various audiences included:

Scientific community: the project resulted in 9 papers in peer reviewed journals, 7 book chapters, 2 PhD theses, 14 conference presentations and 1 article in T-Research.

Conference: a Conference on Listeria for science and industry was held 2009, 2010, 2011 and 2012. The data generated from this project was disseminated at these conferences.

Main publications:

Edward M. Fox, Niall deLappe, Patricia Garvey, Paul McKeown, Martin Cormican, Nola Leonard, and Kieran Jordan. 2012. Pulsed-field gel electrophoresis (PFGE) analysis of *Listeria monocytogenes* isolates of clinical, animal, food, and environmental origin from Ireland. Journal of Medical Microbiology, in Press.

Edward M. Fox, Nola Leonard and Kieran Jordan. 2011. Physiological and transcriptional characterisation of persistent and non-persistent *Listeria monocytogenes* isolates. Applied and Environmental Microbiology, 77, 6559–6569.

M.S. Schvartzman, A. Maffre, F. Tenenhaus-Aziza, M. Sanaa, F. Butler, K. Jordan. 2011. Modelling the fate of *Listeria monocytogenes* during manufacture and ripening of smeared cheese made with pasteurised or raw milk. International Journal of Food Microbiology, 145, S31–S38.

Popular publications:

Troubleshooting the environmental source of contamination in a small food manufacturing plant. Kieran Jordan, Karen Hunt and Edward Fox. 2012. Case studies in food safety and authenticity. J. Hoorfar. Published by Woodhead Publishing.

Listeria monocytogenes in milk, cheese and the dairy environment. Anthony D. Hitchins, Kieran Jordan, Martin Wagner and Moez Sanaa. 2011. In: Rapid detection, characterization and enumeration of food-borne pathogens, Chapter 18, pages 257–284. Editor: J. Hoorfar, ASM Press.

Jordan, K.N. and Burgess, K. (2011). How safe is our food? In: Oral Presentation at What's for Lunch? Conference, Brussels, 20-Sep-2011.

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Detection and surveillance of *Enterobacter sakazakii* (*Cronobacter* spp.) along the infant formula food chain

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Key external stakeholders:

Infant milk formula industry, Food Safety Authority of Ireland.

Practical implications for stakeholders:

Cronobacter spp. is a key food safety issue for the infant formula sector. Apart from an obligation to meet the regulatory microbiological criteria for this pathogen, the sector would be severely damaged by any food safety scare affecting infants consuming these products. This study has focused on transmission sources and survival characteristics of *Cronobacter* spp. The study highlighted that *Cronobacter* can occur widely in the environment and are particularly associated and adapted to survive in dry environs.

Main results:

- *Cronobacter* spp. are not 'ubiquitous' in the environment and would be best described as 'widespread but infrequent' as it appears they have found a particular niche in dry environments.
- Dry ingredients added to milk powder may have a role in transmission of *Cronobacter* spp.
- *Cronobacter* spp. are resilient, surviving the time/temperature profile experienced during spray-drying, in soil, in rumen fluid, in inulin and lecithin (ingredients in infant formula manufacture)
- An adaptive tolerance response to sub-lethal heat that confers increased heat resistance can be induced. However, the increased heat tolerance was not transferred to increased survival potential in a dry environment. Changes in the ratio of saturated to unsaturated fatty acids in the cell membrane appear to be responsible for this adaptation.

Opportunity/Benefit:

This project has generated knowledge about the transmission and survival of *Cronobacter* in the farm to fork chain which will underpin risk management of this pathogen.

1. Project background:

Enterobacter sakazakii (renamed *Cronobacter*) has emerged as a rare cause of life-threatening illness resulting in meningitis, septicaemia and enterocolitis, particularly in neonates, premature infants, low birth weight infants and immunocompromised infants. The presence of this organism in dry infant milk formula products and the potential for improper handling of formulas has been implicated in several clinical cases. The overall objective of this study was to reduce the microbiological risks related to powdered infant milk formula, contaminated with *E. sakazakii*.

2. Questions addressed by the project:

- Are the microbiological media in current (2006) use suitable for isolation of *Cronobacter* spp. under all conditions?
- Are *Cronobacter* spp. ubiquitous in the environment?
- Will *Cronobacter* spp. survive under different conditions and in different matrices?
- Can *Cronobacter* spp. exhibit an adaptive response to heat ? If so, what is the mechanism of this adaptation and will the increased survival potential be transferred?
- Is rpoS important in survival of *Cronobacter* spp.

3. The experimental studies:

Studies were conducted on:

Sampling for occurrence of Cronobacter

Samples from pilot processing plants, households, milk powder processing environment, farm environment, and bovine faeces, animal feed, and foods sold at retail processing environment, were examined for *Cronbacter.*

Survival of Cronobacter spp. under different conditions

- During spray drying: Four strains of *Cronobacter* spp. were inoculated into 35% reconstituted skim milk at 10^7 and 10^2 cfu/g dry wt.
- In faeces at different temperatures: Faecal samples were inoculated with *Cronobacter* and survival on the soil and in containers stored outdoors was examined over time.
- In inulin and lecithin: inulin and lecithin were inoculated with isolates of *Cronobacter sakazakii*. Samples were stored and examined for *Cronobacter sakazakii*..
- D-values: The thermo-tolerance of the five strains was investigated in reconstituted Infant Milk Formula at 55, 60 and 65°C.
- In rumen fluid and simulated gastric juice: models of the bovine abomasum and rumen were inoculated with *Cronobacter* strains and survival was examined over time in these environs using an adapted ISO /DTS 22964 culture protocol.

Adaptation of Cronobacter

The impact of adaption on survival of *Cronobacter* spp. was examined by adapting cells to a sub-lethal heat treatment of 46°C for 30 min prior to lethal stress at 52°C. The mechanism of adaptation was further investigated using flow cytometry. Unlike the traditional indirect plate count method, flow cytometry can provide direct information on the metabolic and physiological status of bacterial cells and can be used to compare adapted and unadapted cells.

Stress response

RpoS is a protein that is involved in the stress response of some bacteria. Strain 823, whose genome has been sequenced, was found to possess an amber mutation (a termination code) at amino acid position 201, resulting in the strain expressing a truncated form of RpoS. The other strains in this study did not contain this amber mutation (i.e. strains 532, 784 and 796). Apart from this one nucleotide difference, both strains possess identical *rpoS* sequences. This study aimed to determine the effect of RpoS on stress tolerance of *Cronobacter* spp., and also to determine if the truncated RpoS produced by strain 823 still retained a function in stress tolerance of the strain.

4. Main Results

Sampling for occurrence of Cronobacter

Of all the diverse samples examined only 2 (0.57%) were positive. However, of the 43 samples from a milk powder processing environment, 12 (28%) were positive. The results showed that occurrence was high in a powder processing plant and in dry matrices but occurrence was relatively low in other environments. The results indicate that *Cronobacter* spp. have an ecological niche in dry environments. However, the term 'ubiquitous' does not accurately describe their occurrence as this implies that they are *omni-present*. The term '**widespread but infrequent**' better describes their occurrence. Furthermore, it was shown that the FDA and ISO methods in use in 2006 did not recover *att* strains, especially in conditions where there were a large number of competing microflora. Since 2006, the FDA and ISO methods have been improved with use of modified media.

Survival of Cronobacter spp. under different conditions

- During spray drying all strains survived the process and were detected in the powders with a low inoculum and enumerated in all the powders with the high inoculum for at least 12 weeks.
- In faeces, *Cronobacter* survived 105 days in sealed containers and was detectable after 112 days in soil.
- In inulin and lecithin three of four strains were still detectable in both matrices after 338 days storage. Higher numbers of the environmental strains were recoverable after 338 days than the clinical strains.
- The thermo-tolerance of a clinically derived type strain, NCTC 11467^{T} and a mutant strain were shown to be significantly more thermo-tolerant than other strains in reconstituted Infant Milk Formula at 55, 60 and 65°C..
- In rumen fluid and simulated gastric juice there was no significant changes in the number of *Cronobacter* in rumen fluid over a 24 h period but it was undetectable after 30 min incubation in the model abomasum.

Adaptation of Cronobacter

The results showed that survival of *Cronobacter* spp. at 52°C was greater in milk-grown cells than in broth-grown cells. They also showed that the survival potential of heat stressed cells was increased if cells were adapted to a sub-lethal heat treatment of 46°C for 30 min prior to lethal stress at 52°C. The acquired survival potential following adaptation was not transferred to survival in a dry environment or to survival during reconstitution of artificially contaminated milk powder by conventional or microwave heat. The ratio of membrane unsaturated to saturated fatty acids decreased, possibly resulting in a more rigid membrane in adapted cells. The alterations in the ratio of fatty acids in the membrane may explain the adaptation.

The mechanism of adaptation was further investigated using flow cytometry. Unlike the traditional indirect plate count method, flow cytometry can provide direct information on the metabolic and physiological status of bacterial cells and can be used to compare adapted and unadapted cells. The flow cytometry studies showed that indicators of metabolic activity, such as Fluorescein diacetate (FDA), Carboxy-fluorescein diacetate succinimidyl 3,3'-dihexylocarbocyanine iodide [CFDAse, DiOC6(3)] and Hydroethidide (HE), showed increased intensity in adapted cells (46°C for 30 min) compared to unadapted cells. In addition, Reactive Oxygen Species, an indicator of cell death, were increased in un-adapted cells.

Stress response

Strain 532 was found to have a greater tolerance to acid at low pH (pH 3, pH 3.5) than strain 823, indicating that the truncated RpoS had lost some of its function. To investigate this further, the *rpoS* gene of strain 823 was completely disrupted (by gene knock-out, by means of homologous recombination, involving double cross-over of DNA flanking the gene of interest) resulting in a mutant strain 823Δ*rpoS*. The acid tolerance of wild-type strain 823 was compared with that of the strain 823Δ*rpoS*, at pH 3.5 over 4.5 hours, using stationary phase cells. There was an approximate 2 log-cycles difference in survival after 3 h, the wild-type strain surviving better. These results indicate that *rpoS* plays a role in acid tolerance of *Cronobacter* spp., and that the truncated *rpoS* strain 823 still appears to retain some function in stress tolerance.

5. Opportunities/Benefits

Knowledge about the transmission and survival of *Cronobacter* in the farm to fork chain will underpin risk management of this pathogen.

6. Dissemination

The scientific knowledge generated in the project was disseminated via peer publications, popular publication, conferences and workshops.

Main publications:

Arku, B., Fanning, S. and Jordan, K (2011). Heat Adaptation and Survival of *Cronobacter* spp.

(Formerly *Enterobacter sakazakii*). *Foodborne Pathogens and Disease*, 8(9): 975–981.

Walsh, D., Molloy, C., Carroll, J., Cagney, C., O'Brien, S., Fanning S., Iversen, C. and Duffy, G. (2011). Survival characteristics of environmental and clinically derived strains of *Cronobacter sakazakii* in infant milk formula (IMF) and ingredients. *J Appl Microbiol*. 110(3):697–703.

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Popular

Arku, B. Fox, E., Mullane, N., Fanning, S. and Jordan, K.N. (2007). Survival of *Cronobacter spp.* during spray-drying. Proceedings of the IDF World Dairy Symposium in Dublin, Oct. 2007.

Jordan. K, and Duffy, G (2007). *Enterobacter sakazakii, an emerging pathogen.* T-Research.

1st International Conference on *Cronobacter* spp. held at UCD in January 2009.

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