



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

15F690 - Research supporting the unpasteurised milk and associated cheesemaking industry from a food safety perspective

Final Report

This project was funded under the Department of
Agriculture, Food and the Marine Competitive Funding
Programme.

SUMMARY

Main Objectives

- To develop the most suitable methods of pathogen testing for unpasteurised milk and associated cheese and to establish a suitable sampling plan.
- To assess levels of pathogenic bacteria in unpasteurised milk and associated cheese including.
- To assess the level of residues and contaminants in unpasteurised milk and in the associated cheese.
- To assess the toxicological risks in unpasteurised milk cheese including mycotoxins and Staphylococcal enterotoxins.
- To report and disseminate the results to the unpasteurised milk cheese producers with implantations and recommendations for improved working practices.

Results

No *L. monocytogenes*, *Salmonella* spp. were detected in any of the samples. No *L. monocytogenes* were detected in any of the swab samples. Other bacterial numbers were within any specifications set. *E. coli* numbers were >1 log cfu/g in laboratory-made and industry-made cheese ripened for about 60 days, irrespective of the starting numbers. In task 2, a method was established for the analysis of anthelmintic drug residues and applied to samples collected on the project, showing that samples did not contain any detectable residue. A multi-residue method that was previously developed for the analysis of 45 mycotoxins in cereals was adapted to the analysis of dairy samples and validated as a screening method. The application of the mycotoxin method to cheese samples showed that samples did not contain any detectable residues.

Impact

This research has demonstrated that all samples collected on the project complied with microbiological regulatory requirements. No residues of anthelmintic drugs were found in cheese samples, which indicated that farmers were complying product withdrawal times and weren't using products in any unlicensed applications. No residues of mycotoxins were detected in samples. This may be because animals were largely on a grass-fed, pasture-based diet. Mycotoxins contamination such as aflatoxin M1 in milk is mainly associated with cereal-based feeds.

KEYWORDS

Food safety; Cheese; Dairy

ACRONYM

RawMilkCheese

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PUBLICATION DATE

November 2020

Section 1 - Research Approach & Results

Start Date

01 November 2016

End Date

31 December 2018

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 6: Technology demonstrated in relevant environment

NRPE Priority area

Sustainable Food Production and Processing

Total DAFM Award

€149,627.00

Total Project Expenditure

€140,777.05

Rationale for undertaking the Research

The quality and safety of unpasteurised milk for the production of unpasteurised milk cheese is essential as this type of cheese is produced from smaller, if not single, herd sizes. Therefore, the risk of potential contaminants in their products is higher than if milk from a larger number of herds is pooled. Identifying microbiological, toxicological, contaminant and residue issues in the Irish unpasteurised milk cheesemaking industry will address these aims. The production of cheese using unpasteurised milk needs to be examined closely in order to minimise the risk to the consumer. An EFSA publication (EFSA scientific opinion on raw milk, 2015) recently identified several microbiological hazards transmissible through unpasteurised milk. However, the risks associated with unpasteurised milk cheese were not studied. Also highlighted was the need for improved risk communication to consumers, particularly susceptible/high risk populations, regarding the hazards and control methods associated with the consumption of raw milk. The presence of residues and contaminants from raw milk also needs to be assessed so that confidence in the end product can be assured in all aspects.

EU regulations require food producers to produce safe food, to identify food safety hazards in the food they produce, to identify measures to control these hazards and to verify that these controls are working. Due to the changing export market, Irish cheesemakers will need to adhere to more than Irish and E.U. systems. Therefore, support from the scientific community is needed in order to ensure food producers produce safe foods.

Methodology

The research was focused on three main themes which address the call text and the objectives:

1. Microbiological
2. Toxicological
3. Contaminants and residues

The research approach focussed on risk analysis of pathogens, residues and toxins from unpasteurised milk to unpasteurised milk cheese. The research focus was on the process of cheesemaking from the primary product (milk) to the cheese at dispatch. Our aim was to establish best working practices before, during and after cheesemaking within the cheesemaking facility. The results were disseminated to Irish unpasteurised milk cheesemakers, CAIS and FSAI. Participant reports and publications will lead to increased knowledge of the risks of unpasteurised milk cheese.

Sampling

The samples, raw milk intended for raw milk cheese production, milk filters (obtained after milking), raw milk cheese curd and raw milk cheese after different ripening times, were obtained from nine raw milk artisan cheese producers in the south of Ireland (7 producing cow's milk cheese and 2 producing goat's milk cheese) over an 18-month period. For the residue and mycotoxin testing, overall, 147 samples were tested. The processing environment samples were taken by trained laboratory staff. The detection and enumeration of *L. monocytogenes* was performed according to ISO 11290:2017 parts 1 and 2, respectively. Samples were tested for the presence of *Salmonella* spp. using ISO 6579-1:2017 (by enrichment). The enumeration of beta-glucuronidase-positive *Escherichia coli*, herein after referred to as *E. coli*, was performed according to ISO 16649-2:2001. The enumeration of *Staphylococcus aureus* (coagulase-positive staphylococci) was done according to ISO 6888-2:1999/Amd.1:2003. The samples were tested for *Bacillus cereus* following the FDA Bacteriological Analytical Manual: Chapter 14 (FDA, 2012), with slight modifications. Anthelmintic and mycotoxin residues were detected using in-house previously published LC-MS/MS methods that were adapted to cheese samples.

Project Results

As a part of this project, raw milk, milk filter and raw milk cheese samples were collected from 9 raw milk cheese producers, 7 producing raw cow's milk cheese and 2 producing goat's milk cheese. Additionally, 6 of the cheesemaking facilities were swabbed for *L. monocytogenes* on 2 occasions each, 20 swab samples being taken on each occasion. Finally, the survival of *E. coli* was studied in a generic laboratory-made cheese and in cheese from one of the manufacturers. The milk, filter and cheese samples were tested microbiologically for *L. monocytogenes*, *Salmonella* spp., *S. aureus*, *B. cereus*, *E. coli* and for drugs. Standard methods were used for all milk, filter, cheese and swab samples. The survival of *E. coli* was tested at two different inoculation levels in the laboratory-made cheese for about 50 days and in the industry-manufactured cheese, it was tested over approximately 60 days. One hundred and forty-seven samples were tested for

anthelmintic drug residues and mycotoxins. No residues or mycotoxins were detected above the reporting levels. No *L. monocytogenes* was detected in any of the cheese or swab samples. No *Salmonella* spp. were detected and no drug residues were detected. *S. aureus*, *B. cereus* and *E. coli* were detected at varying levels in the samples, although for the majority of samples the numbers were below the detection limit. In both laboratory-made and industry-made cheeses, *E. coli* numbers decreased at varying rates in most cases, although they increased on occasion. In all cases, the numbers were >1 log cfu/g after about 60 days of ripening. The results obtained point to a good microbiological and residue quality of raw milk used for raw milk cheesemaking and of raw milk cheese in Ireland.

Section 2 - Research Outputs

Summary of Project Findings

Task 1 showed the absence of *L. monocytogenes* (in milk, cheese and swabs), and *Salmonella* spp. shows the high microbiological quality of raw milk that is used for raw milk cheese manufacture, from cow's and goat's milk and of the processing facilities. In Task 2, a method that was developed and validated for the analysis of >40 anthelmintics drugs in milk and the method was adapted to solid dairy samples (cheese and curds). The method was applied to the analysis of samples collected and all residue levels of anthelmintic drugs were found to be compliant with EU legislation. The results show good quality of milk and cheese in the raw milk cheese industry. The results of the analysis showed that no mycotoxin residues were detectable in the samples analysed.

The results will be of value to the cheesemakers in giving them confidence of the quality of their milk and cheese, with regard to residues and mycotoxins. In designing sampling plans for residue analyses, the results should be of help to policymakers. The results will be of relevance to regulatory authorities in that they show compliance of milk and raw milk cheese samples. It has to be highlighted that the samples analysed on this project are just a snapshot of batches of samples produced on the island. Contaminants like mycotoxins can vary depending on quality of the feed consumed by the animals, which can vary greatly between years. So, although this research has showed that the samples analysed were safe, industry needs to continue to adopt good practice and to continue the monitoring of product safety to satisfy regulatory and customer requirements.

Knowledge Transfer Activities

Research Output	Male	Female	Total Number
Post Doctorates	1	0	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	1	Lourenco, A., Fraga, M., De Colli, L., Moloney, M., Danaher, M., Jordan, K. (2020) Determination of the presence of pathogens and anthelmintic drugs in raw milk and raw milk cheeses from small scale producers in Ireland. <i>LWT</i> 130, August 2020, 109347. https://doi.org/10.1016/j.lwt.2020.109347 .
Publications in Peer Reviewed Scientific Journals	1	Jordan, K., Hunt, K., Lourenco, A., Pennone, V. (2018) <i>Listeria monocytogenes</i> in the Food Processing Environment. <i>Current Clinical Microbiology Reports</i> 5, 106–119.
Peer Reviewed Conference Papers	1	Lourenco, A, Jordan, K. In the proceedings of IAFP European Symposium on Food Safety. The Quality of Raw Milk (and Cheese Manufactured from It) in Ireland (Poster P2-27). 25–27 APRIL 2018 Stockholm, Sweden.

Intellectual Property

Method for the detection of anthelmintic drug residues in cheese has been validated and a standard operating procedure is available in the laboratory. There are no plans to exploit this methodology.

The method for the analysis of mycotoxins in dairy will need further development work but potentially could be licensed as a tool to monitor mycotoxins in milk.

Summary of other Project Outputs

Project Outputs	Details	Total No.
New Technology	New method validated for the detected of anthelmintic drug residues in cheese.	1

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Other	As stated in earlier sections, the samples analysed on this project were compliant with legislation. There are no limits set for residues of veterinary drugs or mycotoxins in processed dairy samples. However, samples did not contain any detectable residues. It is possible, that more sensitive methods are needed to be developed to detect mycotoxins in cheese to be able to detect these toxic substances.

Dissemination Activities

Activity	Details
Workshops at which results were presented	<ol style="list-style-type: none">1. safefood knowledge network workshop. Tuesday 10 April Teagasc Moorepark, Cork.2. safefood knowledge network workshop. Tuesday 17 April Fernhill House Hotel, Clonakilty.3. safefood knowledge network workshop. Wednesday 18 April Brehon Hotel, Killarney.4. safefood knowledge network workshop. Tuesday 15 May Sligo Park Hotel, Sligo.5. safefood knowledge network workshop. Wednesday 16 May Raheen Woods Hotel, Athenry.6. safefood knowledge network workshop. Wednesday 06 June Mullingar Park Hotel, Mullingar.7. safefood knowledge network workshop. Thursday 07 June Brandon House Hotel, New Ross.8. safefood knowledge network workshop. Tuesday 04 September Everglades Hotel, Derry/Londonderry.9. safefood knowledge network workshop. Wednesday 05 September Mossley Mill, Belfast.10. safefood knowledge network workshop. Tuesday 25 September Crowne Plaza Blanchardstown, Dublin.
Other	<ol style="list-style-type: none">1. Report to the safefood knowledge networks on the microbiological and residue analysis of raw milk used for raw milk cheesemaking and on raw milk curd/cheese (FIRM project 15F690)2. Report to the FSAI on the microbiological and residue analysis of raw milk used for raw milk cheesemaking and on raw milk curd/cheese (FIRM project 15F690)

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	<ul style="list-style-type: none">• New knowledge has been generated on this project on the occurrence pathogens, anthelmintic drug residues and mycotoxins in process or final cheese samples.• One peer reviewed paper was published.• Two reports were also produced for the safefoods knowledge networks newsletter and the FSAI.
Identify any knowledge transfer activities executed within the project.	<ul style="list-style-type: none">• The results from this analysis have been reported back to producers for individual samples on one to one basis. Research from the project was disseminated through a peer viewed publication, international conferences and national workshops.• Research from the project was disseminated to stakeholders through a number of workshops completed in association with safefood's knowledge networks at different venues including Cork, Kerry, Sligo, Galway, Westmeath, Wexford, Derry, Belfast and Dublin.
List any impacts resulting from the knowledge transferred during the project.	<ul style="list-style-type: none">• The knowledge generated on this project has contributed to the development of regulatory advice concerning unpasteurised milk cheese with regard to microbiological, mycotoxin and residue issues. This research has shown based on the samples analysed that unpasteurised milk cheese was found to be safe but can be further improved using the tools developed and applied on this project.• The data gathered in the project has added to the scientific understanding of unpasteurised milk and cheese safety. The further use and development of methods to assess the safety of dairy foods in this project will also add to the scientific capability and will be of benefit in future projects. The profiles of Irish food safety research has been improved through the publication of two peer reviewed papers on the project.

Section 3 – Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
None	€0.00	Not applicable

Future Strategies

We plan to further research the area of mycotoxins in dairy through Food for Health Ireland. We have submitted a 3-year research fellow on Mycotoxins in Dairy through the Research Leaders 2020 programme. However, the applicant is unlikely to take up this position as he has just received a year research position at UGENT. We will probably resubmit this application with another candidate through the Marie Curie programme.

Project Publications

1. Lourenco, A., Fraga, M., De Colli, L., Moloney, M., Danaher, M., Jordan, K. (2020) Determination of the presence of pathogens and anthelmintic drugs in raw milk and raw milk cheeses from small scale producers in Ireland. *LWT* 130, August 2020, 109347. <https://doi.org/10.1016/j.lwt.2020.109347>.
2. Jordan, K., Hunt, K., Lourenco, A., Pennone, V. (2018) *Listeria monocytogenes* in the Food Processing Environment. *Current Clinica IMicrobiology Reports* 5, 106–119.