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

13F497 - Reducing Mycotoxin levels in plant derived foods and beverages

Final Report

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

SUMMARY

Main objectives:

- To develop strategies to reduce mould and detoxify contaminated cereals using physical, chemical and biological methods.
- To evaluate the effectiveness of decontamination treatments on fungal and mycotoxin levels of grains/flour/bread/malt/beer.
- To analyse the impact of intervention strategies on the viability (malt) and sensory properties (bread and beer) of ingredients/foods/beverages.
- To disseminate research to Irish agri-food industry and the international scientific community.

Results:

A new analytical test was developed and validated for the analysis of >40 mycotoxins in cereals using a QuEChERS extraction procedure prior to detection by LC-MS/MS (liquid chromatography coupled to tandem mass spectrometry. This method was applied on the project to analyse research study samples from UCC, farm trials samples obtained from DAFM and Teagasc, and Irish cereal samples supplied by industry. Research has been carried out to assess the impact of different factors on mycotoxin occurrence including variety, organic/conventional, spring/winter crop, lodging trials, crop rotation, climatic factors and processing using Teagasc research samples and DAFM trials. This research will help industry to develop strategies to reduce mycotoxins in oats through improved agronomy practices.

Impact:

The methodology developed on the project was applied to generate knew knowledge on the incidence of mycotoxin residues in Irish Oats. We have been using the methodology to assess the impact of different factors on mycotoxin occurrence including variety, organic/conventional, spring/winter crop, lodging trials, crop rotation, climatic factors and processing. The samples include a survey collected from Irish cereal farms, Teagasc research trials and DAFM trials. This research is very relevant new guidance levels that have been proposed for the sum of T2 and HT-2 toxins in unprocessed and processed oats. The outputs from this research will help industry to develop strategies to reduce mycotoxins in oats through improved agronomy practices.

KEYWORDS

Mycotoxins; Reduction; Foods.

ACRONYM	MycoRed
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Section 1 - Research Approach & Results

Start Date

01 April 2014

End Date

31 March 2019

Research Programme

Food Institutional Research Measure

TRL Scale

3: Experimental Proof of Concept

NRPE Priority area

Processing technologies and novel materials

Total DAFM Award

€499,987.00

Total Project Expenditure

€438,704.62

Rationale for undertaking the Research

Fungal contamination is problematic in a wide range of food products and is the main cause of product and concomitant economic losses. Furthermore, fungal mycotoxin production can cause serious public health hazards in foods. In spite of scientific research, the introduction of good agricultural practices (GAP) and good manufacturing practices (GMP) during food production, storage and distribution, fungal mycotoxin contamination continues to be a problem. An estimated 25% of the world's food crops are contaminated by mycotoxins during growth and storage, which can result in serious food-borne illness. In future decades, temperatures in Western/Atlantic European countries will increase by 2.0–3.5°C and will be accompanied with more precipitation causing impaired crop drying and increased mycotoxins in stored products with subsequent amplified waste and health hazard issues. In addition to mycotoxin-related health issues, financial impacts on the farmer after EU rejection of their crops amounts to €7,300-11,000 in administrative costs as well as extensive losses of revenue due to crop re-classification as feed/waste. Additionally, due to global food security issues, predicted cereal shortages and the 40% increase forecast in global food demand over the next 30 years. This project brought together a multidisciplinary team of researchers from Teagasc, UCC and QUB with extensive expertise in food processing technologies, biotechnology and analytical chemistry to address the challenges.

Methodology

A previously developed method for the analysis of approximately 20 mycotoxins was extensively modified on the project to allow the analysis of 42 mycotoxins in cereal samples. The method was carefully optimised during the early stages of the project to ensure accurate and precise analysis. Once developed a standard operation procedure was wrote for the method, which was then validated to EU guidelines.

A total of 220 Lactic acid bacteria from UCC were screened using agar-based methods against five different relevant fungi using the mycotoxin method developed by Teagasc. A total of five lactic acid bacteria were identified and identified using PCR. Furthermore, modifications of the five LABs regarding the bacterial growth

medium and conditions were undertaken, in order to increase the yield of active antifungal compounds. The most efficient formulations (cells and supernatant) were tested in the three food model systems of grain storage, bread making and beer making.
Various physical decontamination strategies (high hydrostatic pressure, ultrasound, vacuum packaging and microwaves with and without sodium hypochlorite)) were applied to 5% fungal infected grains prior to storage. Important processing parameters, such as time, temperature and intensity, of each treatment were adjusted to achieve best results in terms of microbial decontamination and grain quality deterioration.
Various chemical sanitizers, such as quaternary ammonium compounds (QACs), cold plasma, various organic acids and their respective salts were tested. Results were compared sanitizers routinely used in the food industries. In addition, the combination of different chemical and physical treatments was evaluated.
Antifungal lactic acid bacteria were applied during the malting process, in order to increase the microbial stability and safety of the resulting wort. Thereby, the bacterial cell-free supernatant was used during malting instead of water.

Project Results

Main Results:
Researchers at Teagasc and QUB developed a new analytical test for analysing 42 mycotoxins in cereals using LC-MS/MS detection (liquid chromatography coupled to tandem mass spectrometry. The method is capable of measuring regulated, emerging and masked mycotoxins in cereal samples. This method was applied as a tool on the project to analyse research study samples from UCC, farm trials samples obtained from DAFM, Teagasc, and Irish farm samples supplied by industry. Research was carried out to assess the impact of different factors on mycotoxin occurrence including variety, organic/conventional, spring/winter crop, lodging trials, crop rotation, climatic factors and processing using Teagasc research samples and DAFM trials.
Researchers at UCC optimised lactic acid bacteria-based (LAB) decontamination strategies to produce highly efficient antifungal solutions when applied in vitro. In situ application of these solutions was partially successful in prolonging bread shelf life and reducing fungal proliferation in malted grains. Physical and chemical decontamination strategies were evaluated and optimised to improve sanitation efficiency and prevent product quality deterioration. The outcome from this research showed that no single treatment can produce ideal results.
Novel decontamination strategies optimised in Tasks 3 and 4 were evaluated for potential of industrial applications in Task 5. It became evident that the suitability of the decontamination strategies strictly depends on the type of food products investigated. Vacuum packaging, high hydrostatic pressure, microwave, sorbate and propionate treatments, were found to be the most effective physicochemical treatments which fully inhibited fungal development and mycotoxin production. Quality deterioration was found due to the microwave treatment as a result of the internal heating. Among the different biological strategies to reduce contamination of cereals, the antifungal QPS (qualified presumption of safety)-LAB were able to ensure both an effective biological preservative strategy and enhancing the quality characteristics of the end-products. In addition to microbial stability, the antifungal method implemented in this study resulted in a higher extract yield due to the extra modified malt grains obtained. The unique antifungal characteristics of L. reuteri R29 supernatant should be further tested in pilot scale (bakery/brewery plant) to demonstrate/validate their efficiency at the industry level.

Conclusions
The new multi-mycotoxin test provides efficient analysis of samples using LC-MS/MS and can be used as a tool to improve food safety. The test was applied on the project to analyse the effectiveness of decontamination strategies optimised by researchers at UCC. The new test was used to investigate the occurrence of mycotoxins in different cereal production systems showing a strong influence of crop rotation. Antifungal LABs and their fermentation products were found to be very promising candidates to serve as bio-preservatives. Successful application of antifungal LAB and their respective fermentation products into the food processing chain as bio-

preservatives, could help to satisfy the consumer desire for less chemical preservatives and high product quality and safety.

Among the novel intervention strategies evaluated, vacuum packaging, high hydrostatic pressure, sorbate or propionate and QPS-LAB were found to be most effective.

Section 2 - Research Outputs

Summary of Project Findings

Methodology for the analysis of mycotoxins is a significant over existing tests available nationally. The method almost doubles the number of toxins included in the scope of analysis and the throughput of the method has been enhanced through the application of sample/data handling approaches developed by Teagasc. The method has been applied on the project to generate an extensive dataset of results on the occurrence of mycotoxins in raw Irish oats produced under organic, conventional and crop rotated systems. This data has been used to support the setting more favourable EU Maximum limits for mycotoxins.

Chemical and biological strategies to reduce contamination of cereals, brewing and bakery products were investigated and optimised in terms of efficiency and end-product quality characteristics. It became evident that the suitability of the decontamination strategies strictly depends on the type of food products investigated. Among the different strategies to reduce contamination of cereals, the application of antifungal QPS-LAB were able to ensure both an effective biological preservative strategies and enhancing the quality characteristics of the end-products. The project team have published several papers including reviews that provide a roadmap for successful implementation mycotoxin reduction strategies.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD Students	2	0	2

Summary of Academic Outputs

Research Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	8	1. Schmidt, M., Zannini, E., Arendt, E.K., Screening of post-harvest decontamination methods for cereal grains and their impact on grain quality and technological performance. European Food Research and Technology (2019) 245:1061–1074. Schmidt, M., Zannini, E., Lynch, K.M. and Arendt, E.K. (2018). Novel approaches for Chemical and Microbiological shelf-life extension of cereal crops. Critical Reviews in Food Science and Nutrition. 59(21):3395. 2. Schmidt, M., Zannini, E. and Arendt, E.K. (2018). Recent Advances in Physical Post-Harvest Treatments for Shelf-Life Extension of Cereal Crops. Foods 7 (4), 45. 3. Schmidt, M., Lynch, K.M., Zannini, E. and Arendt, E.K. (2018). Fundamental study on the improvement of the antifungal activity of Lactobacillus reuteri R29 through increased production of phenyllactic acid and reuterin. Food Control 88, 139. 4. Schmidt, M., Zannini, E. and Arendt, E.K. (2017). Impact of post-harvest degradation of wheat gluten proteins by Fusarium culmorum on the resulting bread quality. European Food Research and Technology 243(9), 1609. 5. Schmidt, M., Horstmann, S., De Colli, L., Danaher, M., Speer, K., Zannini, E. and Arendt, E.K. (2016). Impact of fungal contamination of wheat on grain quality criteria. Journal of Cereal Science 69, 95.

		6. De Colli, L., K. De Ruyck, M. F. Abdallah, J. Finnan, E. Mullins, S. Kildea, J. Spink, C. Elliott and M. Danaher (2021). Natural Co-Occurrence of Multiple Mycotoxins in Unprocessed Oats Grown in Ireland with Various Production Systems. Toxins 13(188) 1. 7. De Colli, L., C. Elliott, J. Finnan, J. Grant, E. K. Arendt, S. P. McCormick and M. Danaher (2020). Determination of 42 mycotoxins in oats using a mechanically assisted QuEChERS sample preparation and UHPLC-MS/MS detection. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 1150, 122187.
PhD Theses	2	1. Schmidt, M. 2018. Fundamental study on perspectives for post-harvest bioprotection of cereal crops. PhD Thesis, University College Cork. 2. De Colli, L. 2021. Development of multi-mycotoxin UHPLC-MS/MS methods and application to oat and beer samples.

Intellectual Property

- Individual datasets have been kept under the stewardship of researchers at Teagasc and UCC.
- The SOP for the new method for mycotoxin analysis has been wrote in ISO17025 format and has been stored in the Quality Management System at Teagasc.

Summary of other Project Outputs

Project Outputs	Details	Total No.
New Technology	New method developed for the analysis of 42 mycotoxins in cereals.	1

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Industry	New datasets developed reporting on the occurrence of mycotoxins in Irish oat samples collected from different production systems and different seasons.
Industry	New knowledge generated on the impact of different chemical, biological and physical treatments on the reduction of fungal contamination of grains and cereal products.

Dissemination Activities

Activity	Details
Other	1. Schmidt, M., Zannini, E. and Arendt, E.K. Impact of fungal contamination on grain and bread quality. 4. Fruehjahrstagung des Weihenstephaner Instituts fuer Getreideforschung (WIG), WIG, Freising, Germany, 21-22nd April 2015 (Oral Presentation). 2. Schmidt, M., Zannini, E. and Arendt, E.K. Optimization of antifungal activity of Lactobacillus reuteri R29 and potential application in food production. IUFoST, 18th World Congress of Food Science and Technology, Dublin, Ireland, 21 - 25th August 2016. (Oral Presentation). 3. Schmidt, M. and Arendt, E.K. Optimization of antifungal activity of Lactobacillus reuteri R29 and potential application in food production. VII International Symposium on Sourdough; Sourdough for Health, Cork, Ireland, 06 - 08th June 2018 (Poster Presentation). 4. Schmidt, M. and Arendt, E.K. Optimization of antifungal activity of Lactobacillus reuteri R29 and potential application in food production. LAB12, 12th International Symposium on Lactic Acid Bacteria, Egmond Aan Zee, The Netherlands, 27 – 31st August 2017 (Poster Presentation). 5. De Colli L., Elliott C., Danaher M., Development of a multi-residue method for the analysis of mycotoxins, including masked mycotoxins, in cereal-based food by UHPLC-MS/MS. 43rd Annual Food Research Conference, 10-11th December 2014, University College Dublin (UCD), Dublin, Ireland (awarded as the best poster presentation in the “Food Safety” section). 6. De Colli L., Elliott C., Danaher M., Development of a multi-residue method for the analysis of mycotoxins, including masked mycotoxins, in cereal-based food by UHPLC-MS/MS. 7th International Symposium on Recent Advances in Food Analysis (RAFA), 3-6th November 2015, Prague, Czech Republic (poster presentation). 7. De Colli L., Elliott C., Danaher M., Development and validation of a multi-residue method for the analysis of mycotoxins in cereal-based food by UHPLC-MS/MS. 44th Annual Food Research Conference, 14th December 2015, Teagasc Moorepark, Cork, Ireland (poster presentation). 8. De Colli L., Elliott C., Danaher M., Development and validation of a multi-residue method for the analysis of mycotoxins in cereal-based food by UHPLC-MS/MS. 18th World Conference of Food Science and Technology (IUFoST), 21-25th August 2016, RDS, Dublin, Ireland (poster presentation). 9. De Colli L., Elliott C., Danaher M., Simultaneous determination of 40 mycotoxins in Irish oats: a high throughput UHPLC-MS/MS analytical method. 46th Annual Food Science and Technology Conference, 67th December 2017, Teagasc Ashtown, Dublin (Ireland) (poster presentation). 10. De Colli L., Elliott C., Danaher M., Simultaneous determination of 42 mycotoxins in Irish oats: a high throughput UHPLC-MS/MS analytical method. 10th Forum of the World Mycotoxin Conference, 12-14th March 2018, Amsterdam (The Netherlands) (poster presentation).

11. De Colli L., Elliott C., Danaher M., Mycotoxins in Irish oats - a three year national survey. Food Asset 2018, 28-31st May 2018, Queen's University Belfast (QUB), Belfast (Northern Ireland) (poster presentation).

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	New knowledge has been generated on the occurrence of mycotoxins in Irish oats, which has been published in peer reviewed literature. Analytical reports have been provided to industry stakeholders that provided the samples
Identify any knowledge transfer activities executed within the project.	Analytical reports have been provided to industry stakeholders that provided the samples. The output of this research has been disseminated more broadly through the Oats Ireland Forum in January 2021.
List any impacts resulting from the knowledge transferred during the project.	Research from the project has helped the processors develop more effective strategies for the sampling and analysis of mycotoxins in cereal samples. The results from the research the Irish Industry agree more favourable maximum residue limits forT2 and HT2 toxins in oats.

Section 3 – Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
EU R&I programmes	€194,198.00	Teagasc have been funded on the EU interreg project (Agritox.eu), which has been funded to mid-2022 to carry out research on mycotoxins in cereals in the Atlantic area.
Exchequer National Funding	€132,139.00	Teagasc have also funded by EI and the Irish dairy industry to carry out research on contaminant residues in dairy products on FHI (Food for Health Ireland). As part of the this project we are planning to investigate different residues (including mycotoxins) in processed dairy products.
Exchequer National Funding	€262,470.00	2021R460 Mycotoxin-I (2022-2026) Mycotoxins in Cereals (funded by DAFM). Total funding 1,661,552
Exchequer National Funding	€10,633.00	MycoSafe Mycotoxins in Cereals (Funded by DAFM). Funded by Safefood. Total funding €114,080

Future Strategies

The research from this project will be built on through ongoing projects that have been funded in collaboration with UCD, NUI Maynooth and QUB. As part of this work, we are closely collaborating with Crop Research Scientists at Teagasc Oakpark. The bulk of this work will be carried out on the DAFM project Mycotoxin-I. A major focus of this project is to develop a standardised method of the analysis of mycotoxins using methodology developed by Austrian researchers. This methodology will be used to build more extensive datasets on mycotoxin occurrence in Irish cereals. Researchers at Teagasc Oakpark and UCD will carry out research to help identify the cause of mycotoxins i.e., the pathogenic fungi involved. While researchers at NUI Maynooth will used machine-based learners' tools to interrogate project data.

Project Publications

1. Schmidt, M., Horstmann, S., De Colli, L., Danaher, M., Speer, K., Zannini, E. and Arendt, E.K. (2016). Impact of fungal contamination ofwheat on grain quality criteria. Journal of Cereal Science 69, 95.
2. Schmidt, M., Zannini, E. and Arendt, E.K. (2017). Impact of post-harvest degradation of wheat gluten proteins by Fusarium culmorum on the resulting bread quality. European Food Research and Technology 243(9), 1609.
3. Schmidt, M., Zannini, E., Lynch, K.M. and Arendt, E.K. (2018). Novel approaches for Chemical and Microbiological shelf-life extension of cereal crops. Critical Reviews in Food Science and Nutrition. 59(21):3395.
4. Schmidt, M., Zannini, E. and Arendt, E.K. (2018). Recent Advances in Physical Post-Harvest Treatments for Shelf-Life Extension of Cereal Crops. Foods 7 (4), 45.
5. Schmidt, M., Lynch, K.M., Zannini, E. and Arendt, E.K. (2018). Fundamental study on the improvement of the antifungal activity of Lactobacillus reuteri R29 through increased production of phenyllactic acid and reuterin. Food Control 88, 139.
6. De Colli, L., C. Elliott, J. Finnan, J. Grant, E. K. Arendt, S. P. McCormick and M. Danaher (2020). Determination of 42 mycotoxins in oats using a mechanically assisted QuEChERS sample preparation and UHPLC-MS/MS detection. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 1150, 122187.

7. De Colli, L., K. De Ruyck, M. F. Abdallah, J. Finnan, E. Mullins, S. Kildea, J. Spink, C. Elliott and M. Danaher (2021). Natural Co-Occurrence of Multiple Mycotoxins in Unprocessed Oats Grown in Ireland with Various Production Systems. Toxins 13(188) 1.