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

13F444 - Innovative process technologies for the fresh produce industry

Final Report

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

SUMMARY

The main objectives of this project were to design, build and test two different cold plasma technologies for the fruit and vegetable sector. These consisted of a dry (plasma gas) and a wet (plasma water) design. These technologies were tested and optimised for antimicrobial efficacy for selected fresh produce. Based upon these findings the selected technologies were combined with Modified Atmosphere Packaging (MAP) to test the combined interventions to provide both product safety and shelf-life extension. Both technologies were effectively tested and scaled. The water design was found to be particularly effective in terms of efficacy, quality retention and ease of scaling. By combing this design with appropriate MAP the benefits of both interventions were realised. The impact of the work demonstrated that the plasma-water approach is a realistic alternative to chlorine or Peracetic acid (PA) active washing.

KEYWORDS

Pathogens, Shelf-life

ACRONYM	InnoFresh
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Section 1 - Research Approach & Results

Start Date

01 December 2013

End Date

31 August 2018

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 5: Technology validated in relevant environment

NRPE Priority area

Sustainable Food Production and Processing

Total DAFM Award

€471,794.00

Total Project Expenditure

€387,759.99

Rationale for undertaking the Research

The Fruit & Vegetable (F&V) industry forms a major component of our food supply and is an important contributor to the Irish economy with the total retail fresh produce market valued at €1.53bn in 2018 (Bord Bia, 2019). However, despite strong consumer demand and growth in the F&V industry, the sector is not without its challenges, in particular in relation to the short-shelf life of fresh F&V, the risk of foodborne pathogens (especially in light of the highly publicised 2011 Entero-Hemorrhagic Escherichia coli (EHEC) crisis), as well as the sustainability of the sector in relation to water and energy use. Ireland's competitiveness in the sector requires the latest technological innovation in processing and packaging solutions to ensure competitive supply chains. Consumer demands for convenience has increased the market share of fresh cut products which are more perishable in nature, due to higher respiration and transpiration rates. These characteristics at best decrease shelf-life, and at worst create a breeding ground for serious food poisoning problems. While fresh F&V consumption is linked to a plethora of health benefits, it can also be a source of foodborne illness. Globally, there has been an increase in the number of outbreaks of foodborne illness associated with fresh foodstuffs, and in particular ready-to-eat fruit and vegetables. In 2008 a World Health Organization (WHO) report on microbial safety in fresh produce recognized leafy green vegetables having the highest risk in the fresh produce category. It is therefore critical that effective decontamination steps are in place to ensure consumer protection and confidence in fresh produce. The F&V industry is heavily dependent on chlorine as a sanitiser to assure the safety of their produce. However, in light of concerns about its antimicrobial efficacy for produce, as well as about the environmental and health risks associated with the formation of carcinogenic halogenated disinfection by-products, there is increasing pressure on the industry to eliminate chlorine from the disinfection process and find a suitable alternative. Moreover, the use of chlorine for the disinfection of fresh produce is banned in some EU countries such as Germany and Switzerland. The F&V industry is a major consumer of water at different stages of fresh and processed F&V processing, especially during washing and disinfection. Dry preservation technologies would allow a considerable reduction in water usage as well as wastewater generation. A proposed solution to this problem is atmospheric cold plasma (ACP). For shelf-life extension Modified Atmosphere Packaging (MAP) is an established technology that can help to increase the post-harvest life of fresh cut F&V, but the Irish fresh-cut market showed little evidence of application of MAP to fresh cut F&V, showing significant opportunities for process optimisation and tailoring for specific fresh cut produce. In view of the above there is a clear need to provide producers and packers of fresh F&V, with an affordable, safe and effective method of offering microbiological safety assurance, while at the same time increasing shelf life and retention of nutritional quality.

Methodology

The proposal's research approach was to design and test two different cold plasma technologies for the F&V sector. These consisted of a dry (plasma gas) and a wet (plasma water) design. These technologies were tested and optimised for antimicrobial efficacy for selected fresh produce. Based upon these findings he selected technologies were combined with MAP to test the combined interventions aimed at providing both product safety and shelf-life extension. The key research methodologies were:

Prototype design and optimisation:

The project integrates novel dry and wet processing technologies based upon cold plasma discharge to deliver pasteurisation-like capabilities with a quality by design approach for modified atmosphere packaging to assure safety and maximise shelf life. The initial work at TU Dublin focused on the design and manufacture of these prototype dry and wet plasma technologies for product decontamination. Designs were produced for each approach followed by testing of suitable construction material options for each design. Lab scale prototypes for the plasma jet (dry) and plasma Jacuzzi (wet) were constructed and used for testing prior to scale-up tasks. The performance of various cold plasma approaches in terms of antimicrobial efficacy was tested for typical microbes of concern. Initial studies on the quality and nutritional parameters of plasma treated produce were carried out to support process optimisation.

Microbiology testing:

In parallel with process development and optimisation the designs were evaluated for their anti-microbial efficacy. The target bacterial included both pathogens and spoilage microflora along with testing against the challenging conditions of biofilm formations. With respect to microbiological safety, the testing focused on the key pathogens of concern; E. coli O157:H7, Salmonella spp. and Listeria monocytogenes. The research also studied any adaptive or stress response by the microorganisms to the plasma treatments. Resistance studies were performed to evaluate any potential for induced resistance to plasma compared to untreated controls.

Combining with MAP:

The optimised plasma technologies developed by TU Dublin were sent to UCC to test the benefits of combining the interventions with MAP for shelf-life extension. The combination of cold plasma and MAP for cherry tomatoes was investigated throughout controlled storage at optimal (10˚C) and abused (20˚C) conditions. The effects of plasma treatment were studied for these tests on the key quality parameters including weight loss, brix, colour and pH.

Project Results

Prototype reactor developed:

Two different cold plasma technologies were designed and built. These consisted of a gas-based tunnel where a multi-jet plasma design was employed for treatment of fresh product and a plasma-water reactor for washing of fruit and vegetables. The tunnel design consisted of the team designing unique plasma jets which included testing the dielectric materials of construction. Based upon this optimisation work a jet design was selected and 12 identical systems built. These were incorporated into a ring design with a common gas feed and power source. The power source was modified to account for the challenge of driving the 12 jets simultaneously. A tunnel was constructed to prevent the loss of the plasma species to the open environment. The tunnel was found to increase the species intensity by over two-fold.

For the plasma-water reactor two different designs were constructed, these included a porous membrane design at the base of a water bath and a multi-element porous ceramic diffuser which inserts into the water bath from above. Both designs were tested for plasma species generation, stability and process efficacy. Based upon the results both designs were found to work well as plasma wash water reactors, each offering different advantages for integration and scale-up to fresh produce washing designs.

Both systems where characterised in terms of electrical and optical diagnostics with a view to offering an approach for process control. A simple optical emission spectroscopy approach was found to offer an easy and non-invasive technique for control of the designs.

Efficacy demonstration:

Both plasma technologies were found to be effective for inactivation of the target microorganisms. The work demonstrated that the efficacy of the 'dry' plasma technology and the 'wet' plasma wash water could decontaminate microorganisms on fresh produce. The plasma wash water was selected as the most suitable technology for fresh produce that can be washed. Plasma water achieved control comparable to chlorine treatment of L. innocua, and eradicated P. fluorescens populations below the detection limit, offering potential for delayed spoilage and shelf-life improvement. In addition, micro-bubbling of the plasma effluent and high-speed agitation helped distribute the generated reactive species, providing greater microbial reactive species interactions.

The treatments were also shown as promising technology for the reduction and eradication of principle indicator viable bacterial populations present in a mature biofilm formed in lettuce broth or on lettuce under different conditions (mono or mixed biofilms). All the result presented highlight the complexity of the mono/mixed biofilm, the influence of interspecies interaction between pathogenic and spoilage bacteria in biofilm, culture and biofilms setup conditions, temperature, substrate surface and composition of substrate. Further, efficacy of water-based plasma treatment was evaluated against bacterial population from fresh produce and in the wash, water generated from fresh produce washing.

Combined Plasma-MAP technologies and process validation:

The plasma wash technology was Cold Plasma was investigated as an intervention technology to reduce microbiological contamination without compromising key quality parameters. Equilibrium modified atmosphere packaging (EMAP) technology offers the possibility to control the respiration rates of produce. To test the potential of combining the technologies, cherry tomatoes were investigated under controlled temperatures throughout storage for both microbial reduction and shelf-life extension. Cherry tomatoes were packed in trays with an oriented polypropylene film single perforated (270 µm) stored at optimal (10˚C) and abused (20˚C) storage conditions. Importantly, the plasma treatment did not show significant negative influences on weight loss, brix, colour and pH of the produce. The packaging was found to be an important factor in preventing weight loss and changes in total soluble solid content. A higher microbiological growth was observed within the packed produce, while the washing step with cold plasma before packaging-controlled microorganism growth. Temperature as expected had a great impact on the shelf life, as cherry tomatoes kept at 10˚C could be stored for 35 days while at 20˚C they lost their integrity after 14 days. The combination of cold plasma and EMAP design was demonstrated as an effective approach to ensure the quality of cherry tomatoes, which suggests that they have potential to be used as hurdle technology and could be explored to reduce food waste by extending the shelf life of fresh produce.

Section 2 - Research Outputs

Two prototype atmospheric plasma reactors were designed and built. These protypes included the optimisation of the power source, the reactors and process monitoring system. Follow-one designs are being tested with a view to scale-up and industrial adoption of the technology.

The research points to potential applications for the technology for numerous food applications. In particular, the use of the approach for fresh produce washing is established. Produce could include both fruit and vegetables. A major application would be in the use of leafy greens such as lettuce where the industry is seeking alternatives to chlorine and peracetic acid. However, the design could be applicable to other foods which are washed such as chicken. Discussions and follow-on testing of the concepts are ongoing with One Harvest (Australia) for lettuce washing and Pilgrim's Pride Corporation (USA) for chicken washing.

Food Harvest 2020 suggests, "Irish companies must seek new markets, develop new product streams that meet changing consumer demand, as well as finding new ways to assert Ireland's environmentally friendly credentials to target the premium end of the market with high-value products". The replacement of traditional disinfection chemicals such as chlorine would support the assertion that Irish produce is environmentally friendly. The approach may also facilitate opening new markets through such green credentials but also via enhanced shelf-life extensions. Given the high losses of produce associated with the horticultural sector, any technologies which can help reduce waste is supported under these policies. Finally, the outputs of INNOFRESH may also address the identified industrial need stemming from regulatory requirements for process effluent treatment and public demand for clean water treated in an environmentally sustainable manner.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
Post Doctorates	0	1	1
PhD Students	0	1	1
MSc Students	1	0	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	4	The peer review papers published between 2017 and 2019 are listed in the publication section of this report.
PhD Theses	1	Apurva Patange, March 2018. Thesis title: 'Microbiological interaction of cold plasma technology'.
Peer Reviewed Conference Papers	15	The full list of the Popular non-scientific publications and abstracts including those presented at conferences by members of this project are listed in the publication section of this report.

Intellectual Property

The plasma reactors developed during this project were shown to be very effective, particularly the plasma wash water reactor and should have real potential in industry. The potential for IP capture was discussed however it was difficult to define a clear inventive step for a technology patent and it is established on the literature, meaning that a process patent was not possible.

Summary of other Project Outputs

Project Outputs	Details	Total No.
New Technology	Two prototype atmospheric plasma reactors were designed and built. These protypes included the optimisation of the power source, the reactors and process monitoring system. Follow-one designs are being tested with a view to scale-up and industrial adoption of the technology.	2

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Industry	The research points to potential applications for the technology for numerous food applications. In particular, the use of the approach for fresh produce washing is established. Produce could include both fruit and vegetables. A major application would be in the use of leafy greens such as lettuce where the industry is seeking alternatives to chlorine and peracetic acid. However, the design could be applicable to other foods which are washed such as chicken. Discussions and follow-on testing of the concepts are ongoing with One Harvest (Australia) for lettuce washing and Pilgrim's Pride Corporation (USA) for chicken washing.
Other	Under Ireland' strategic plans, Food Harvest 2020 and Food Wise 2025, the Department of Agriculture, Food and Marine (DAFM), outline how Ireland will play a key role in meeting the population's demands for food, while overcoming the challenges of food security and climate change. Food Harvest 2020 suggests, "Irish companies must seek new markets, develop new product streams that meet changing consumer demand, as well as finding new ways to assert Ireland's environmentally friendly credentials to target the premium end of the market with high-value products". The replacement of traditional disinfection chemicals such as chlorine would support the assertion that Irish produce is environmentally friendly. The approach may also facilitate opening new markets through such green credentials but also via enhanced shelf-life extensions. Given the high losses of produce associated with the horticultural sector, any technologies which can help reduce waste is supported under these policies. Finally, the outputs of INNOFRESH may also address the identified industrial need stemming from regulatory requirements for process effluent treatment and public demand for clean water treated in an environmentally sustainable manner.
Other	A collaboration was established with the Leibniz Institute for Plasma Science and Technology, Greifswald, Germany (PI Dr Jörg Ehlbeck). The collaboration compared the technology developed in this project against a cold plasma wash water technology developed at Leibniz.

Dissemination Activities

Activity	Details
Workshops at which results were presented	Plasma Medicine Workshop June 10th/11th, 2015, Goettingen, Germany. Daniela Bohem attended a workshop for training and collaboration. Presented poster entitled: Biological applications of cold atmospheric plasma - methods, mechanisms and cellular responses. 10-11/06/2015

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	The major knowledge output of this work was the demonstration of the efficacy of plasma water as an active wash technology for fresh produce. The designs developed showed that the technology was more effective than chlorine and had minimal effects on the quality attributes of the tested produce. Knowledge on the technology design, control parameters and scale-up criteria were created.
Identify any knowledge transfer activities executed within the project.	A report on the technology developed, its performance and potential for possible commercialisation was sent to the industrial partner. A number of publications were produced which detailed the technology and its performance at bench scale.
List any impacts resulting from the knowledge transferred during the project.	Based upon this work, there is continued interest in commercialising this technology, particularly the plasma wash water. Dr Bourke is involved in a collaboration with a German group where a pilot-scale system has been developed. Their work is published in the recent paper: Plasma-Functionalized Water: from Bench to Prototype for Fresh-Cut Lettuce. Food Engineering Reviews (2020). Prof. Cullen is involved with scaling the plasma wash water with an Australian Fresh produce company (One Harvest). In the USA, a collaboration between Drexel University and SmartWash Solutions are also developing a plasma wash system.

Section 3 – Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding	Funding €	Summary
None	€0.00	n/a

Future Strategies

The potential of atmospheric plasma as a food treatment technology is now established. The project's PI (PJ Cullen) set up a start-up based in Ireland to develop plasma-based technology for environmental applications (PlasmaLeap Technologies). Through this company a prototype fresh produce bubble reactor (pilot scale) has been designed, although the design of the reactor is different than the approach taken in this project the efficacy of plasma for fresh produce treatment was established.

Project Publications

Peer-reviewed publications, International Journal/Book chapters:

1. High voltage atmospheric cold air plasma control of bacterial biofilms on fresh produce. 2019. A Patange, D Boehm, D Ziuzina, PJ Cullen, B Gilmore, P Bourke. International journal of food microbiology 293, 137-145.
2. Efficacy of cold plasma functionalised water for improving microbiological safety of fresh produce and wash water recycling. 2019. Apurva Patange, Peng Lu, Daniela Boehm, P.J. Cullen and Paula Bourke. Food Microbiology, Volume 84, 103226.
3. Sousa A.R., Oliveira J.C., *Sousa-Gallagher M.J. (2017) Determination of the respiration rate parameters of cherry tomatoes and their joint confidence regions using closed systems, J. Food Engineering, 206, 13-22.
4. Tumwesigye K.S., Sousa A.R., Oliveira J.C., *Sousa-Gallagher M.J. (2017). Evaluation of novel bitter cassava film for equilibrium modified atmosphere packaging of cherry tomatoes, Food Packaging & Shelf Life, 13, 1-4.

Popular non-scientific publications and abstracts including those presented at conferences:

1. Ziuzina, D., Patil, S., Cullen, P.J., Boehm, D., Bourke, P. Dielectric Barrier Discharge Atmospheric Cold Plasma for Inactivation of Pseudomonas aeruginosa biofilms. Oral presentation at the 5th International Conference on Plasma Medicine in Nara, Japan, 18-23 May 2014.
2. Han, L., Patil, S., Cullen, P.J., Bourke, P. Inactivation Mechanism of Atmospheric Cold Plasma against Escherichia coli and Staphylococcusaureus in Liquid. Poster presented at the 5th International Conference on Plasma Medicine in Nara, Japan. 18-23 May 2014.
3. Ziuzina, D., Han, L., Cullen, P.J., Bourke, P. Effect of in-package high voltage atmospheric cold plasma on L. monocytogenes in model media, internalised and bacterial biofilms formed on lettuce at different storage conditions. Oral presentation at Safefood Knowledge Networks Conference. Blanchardstown, Ireland, 16th September 2014.
4. Han L., Ziuzina D., Cullen, P.J. and Bourke P. Atmospheric cold plasma inactivation of Listeria monocytogenes: Role of ROS in different media. Poster at Safefood Knowledge Networks Conference, Blanchardstown, Ireland, 16th September 2014.
5. Ziuzina, D., Han, L., Cullen, P.J., Bourke, P. Effect of different storage conditions on decontamination efficacy of high voltage atmosphericcold plasma against E. coli in model media, internalised and bacterial biofilms formed on lettuce. Poster at Safefood Knowledge Networks Conference. Blanchardstown, Ireland, 21st October 2014.
6. Han L., Heslin C., Boehm D., Cullen, P.J. and Bourke, P. Atmospheric cold plasma inactivation of Escherichia coli: Role of ROS in different media and gases. Poster at Safefood Knowledge Networks Conference. Blanchardstown, Ireland, 21st October 2014.
7. Boehm, D., Bourke, P., Ziuzina, D., Han, L., Misra, NN, Keener, K., Cullen, PJ, Nov. 2015, Freising, Germany. In-package cold plasma sterilization. Recent advances in surface sterilization and disinfection. Symposium on Recent Advances in Surface Sterilization and Disinfection, Nov. 19th/20th, 2015, Fraunhofer Institute Freising, Germany.
8. Tank A., Oliveira J.C., Sousa-Gallagher M.J. Performance of modified atmosphere packaging (MAP) systems using bio-based and non-biobased packaging films Innovations in Food Packaging, Shelf Life & Food Safety Conference. September 14-17th, Munich, Germany.
9. Tank A., Oliveira J.C., Sousa-Gallagher M.J. Effect of relative humidity (RH) on the effective permeability of bio-based and non-bio-based films used for modified atmosphere packaging. Innovations in Food Packaging, Shelf Life & Food Safety Conference. September 15-17th, Munich, Germany.
10. Tank A., Oliveira J.C., Sousa-Gallagher M.J. Evaluation of Effective permeability of bio-based and non-bio-based films used for equilibrium modified atmosphere packaging (EMAP). 44th Annual Food Research Conference, Moorepark, Ireland.
11. Lu Han, Daniela Boehm, PJ Cullen, Paula Bourke "Investigations of atmospheric cold plasma against E.Coli mutants. 6th Congress of European Microbiologists (FEMS 2015).
12. Apruva Patange, Daniela Boehm, PJ Cullen, Paula Bourke. Investigations of Brochothrix thermosphacta by atmospheric cold plasma 6th Congress of European Microbiologists (FEMS 2015).
13. Daniela Boehm, Lu Han, Caitlin Heslin, Apruva Patange, Dana Ziuzina, PJ Cullen, Paula Bourke. Susceptibility of food contaminating microbial communities to atmospheric cold plasma. 6th Congress of European Microbiologists (FEMS 2015).
14. Apurva Patange, Chaitanya Sarangapani, Daniela Boehm, P J Cullen, Paula Bourke. Atmospheric Cold Plasma treatment of microbiological and organic pollutants in wastewater. Oral presentation at 26th Environ 2016, 22-24 March 2016.
15. Apurva Patange, Daniela Boehm, P.J. Cullen, Paula Bourke. Atmospheric cold plasma treatment of fresh produce bacterial biofilms. Oral presentation at 30th EFFoST International Conference. 28-30 November 2016.