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FINAL REPORT

Plasma Activated Water: Investigating opportunities to
reduce food waste in the poultry industry

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Panel Review Statement

End Food Waste CRC recognises the value of knowledge exchange and the importance of objective peer review. It is committed to encouraging and supporting its research teams in this regard. The author(s) confirm(s) that this document has been reviewed and approved by the Project Leader and Industry Partner.

This project has also been evaluated by the End Food Waste CRC publication review panel. These reviewers evaluated its:

- Methodology articulated clearly
- Positioning of findings within the current literature
- Acknowledged compliance with food safety standards
- Conclusions against results
- Relevant human and/or animal ethic approvals obtained

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Industry Partner Foreword

Feather&PECK is a pasture-raised egg producer committed to regenerative farming and reducing chemical inputs across our operations. Through this project, we have had the opportunity to explore the application of plasma activated water (PAW) as an alternative cleaning and sanitising solution for egg processing.

The project has demonstrated the potential for PAW to reduce reliance on traditional chemical sanitisers while maintaining food safety outcomes. This aligns strongly with our commitment to reducing chemical inputs and continuously improving the sustainability of our production systems.

The findings provide a promising pathway for future implementation of PAW technology within our business. Beyond the potential cost savings associated with reduced chemical use, the technology offers an opportunity to further strengthen our environmental credentials and support the values that are important to our customers. We appreciate the collaborative approach taken throughout the project and look forward to exploring the next steps in bringing these outcomes into practice.

John Byrne

Director

Feather&PECK eggs

Executive Summary

Food waste arising from bacterial spoilage remains a significant challenge for the Australian poultry industry, contributing to reduced product shelf life, increased costs, and avoidable environmental impacts. Eggs and chicken meat are particularly vulnerable to bacterial contamination by spoilage organisms and foodborne pathogens, prompting widespread use of chemical sanitisers throughout poultry production and processing. While chlorine-based sanitisers are effective and widely adopted, industry interest is growing in alternative technologies that can maintain food safety, reduce chemical use, and support more sustainable production systems.

This project investigated the potential of plasma activated water (PAW) as an alternative sanitising technology for poultry products. PAW is generated using water, air, and electricity, producing a solution that has reactive oxygen and nitrogen species with strong antimicrobial properties. In partnership with Feather&PECK, the project evaluated the effectiveness of PAW for egg washing and chicken meat sanitisation, comparing its performance directly against conventional chlorine-based sanitising treatments. The project objective was to determine whether PAW could reduce bacterial contamination and thereby contribute to extended storage, reduced food waste, improved profitability, and lower reliance on conventional chemical sanitisers.

Results demonstrated that PAW is an effective antimicrobial treatment for both eggs and chicken meat. For eggs, short exposure times significantly reduced total viable bacterial counts on eggshell surfaces. Importantly, a 10-second PAW treatment (similar to current industry practice) provided substantial bacterial reduction while causing minimal impact on eggshell cuticle integrity. Comparative testing showed that PAW performed similarly to the industry-standard chlorine sanitiser Circhlor in reducing bacterial contamination.

An eight-week egg storage study further demonstrated that eggs treated with PAW maintained bacterial loads comparable to those treated with Circhlor across storage temperatures of 5°C, 15°C, and 25°C. Egg quality (freshness) was not compromised by the PAW treatment. These findings indicate that PAW can provide equivalent sanitation performance to chlorine-based treatments without compromising egg quality.

For chicken meat, PAW demonstrated antimicrobial efficacy. For a range of commercially relevant cuts, including skin-on and skin-off breast and thigh portions and wings, PAW consistently reduced bacterial loads during refrigerated storage. In several cases, PAW outperformed chlorine treatments by maintaining significantly lower bacterial counts over time. These results suggest that PAW may provide enhanced control of spoilage microorganisms and support longer storage periods for poultry products.

Overall, the project achieved its primary objective of demonstrating that PAW is a viable alternative to conventional chlorine-based sanitisers for poultry applications. For egg washing, PAW delivered sanitation performance equivalent to the current industry standard while maintaining egg quality and cuticle integrity. For chicken meat, PAW performed as well as or better than chlorine treatments in reducing bacterial contamination during refrigerated storage, highlighting its potential to contribute to storage.

Beyond its antimicrobial performance, PAW offers several potential sustainability benefits. It can be generated on-site using water, air, and electricity, leaving no chlorine residues and reducing dependence on transported chemical sanitisers. The technology also aligns with industry efforts to improve environmental performance, reduce chemical inputs, and support the transition towards more sustainable food production systems.

The findings from this project provide a strong foundation for further technology development and commercial implementation. Subsequent work is already underway through Australian Economic Accelerator funding to advance the technology readiness level and evaluate PAW within integrated poultry processing systems. Future research should focus on regulatory pathways, food safety validation, commercial-scale implementation, and quantification of shelf-life extension benefits under real-world operating conditions.

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Introduction

Bacterial contamination is a major challenge for the poultry industry and contributes to food loss, reduced shelf life, and food safety risks [1,2]. Bacterial contamination contributes to Australia's estimated \$36 billion in annual food losses [3]. On both eggs and chicken meat, spoilage bacteria are of significant economic concern because they reduce product quality and shelf life, while pathogenic and zoonotic bacteria present additional food safety and public health risks [1,2].

On eggs, microbial contamination of the eggshell surface can similarly lead to spoilage and increase the risk of foodborne disease. For chicken meat, spoilage is commonly associated with psychrotrophic bacteria capable of proliferating under refrigeration conditions [4]. *Pseudomonas*, *Serratia*, *Acinetobacter*, and *Morganella* species are among the most common spoilage organisms associated with poultry meat deterioration [5]. Consequently, bacterial decontamination processes are widely used throughout both the egg and poultry meat industries to improve food safety and extend product shelf life.

Chlorine-based sanitisers are among the most commonly used antimicrobial agents in poultry processing. In commercial egg production, eggs are routinely washed to reduce microbial contamination on the eggshell surface, typically using chlorine-based sanitisers and quaternary ammonium compounds [6]. However, studies have suggested that some chemical sanitisers used during egg washing may alter the eggshell surface and damage the protective cuticle layer [7]. Egg washing practices also vary considerably across the Australian egg industry [8]. In addition, chlorine-based sanitisers may leave chemical residues and are unsuitable for certified organic egg production systems.

Similarly, poultry meat processing plants employ several antimicrobial interventions to reduce bacterial contamination on broiler carcasses, including inside/outside washing, chilling to 4°C, and immersion chilling in chlorinated water [9]. Although chlorine-based sanitisers are effective, concerns regarding occupational health and safety, chemical residues, and negative consumer sensory perceptions have prompted investigation into alternative sanitising technologies. Other chemical alternatives, including peroxyacetic acid (PAA), acetic acid (AA), and acidified sodium chlorite (ASC), have also been investigated for the Australian chicken meat industry [10-12], although these treatments may present cost, handling, or safety limitations.

As a result, there is growing industry interest in studying new chemical-free and environmentally sustainable washing or sanitising technologies capable of maintaining food safety while potentially reducing spoilage and food waste. This study investigated plasma activated water (PAW) as an alternative to conventional chlorine-based washes for both eggs and chicken meat. PAW possesses highly bactericidal physicochemical properties, including low pH and elevated concentrations of reactive oxygen and nitrogen species (RONS), which contribute to broad-spectrum antimicrobial activity.

In collaboration with industry partner Feather&PECK, this project developed PAW formulations for cleaning eggs. Furthermore, PAW formulations were applied to chicken meat pieces. Sanitisation and food quality results are presented in comparison to conventional chemical washes. The goal of this project was to evaluate the efficacy of PAW in reducing bacterial contamination, therefore extending product shelf life, with the broader goals of reducing food waste, improving industry profitability, lowering chemical residues, and providing cleaner and safer food products to consumers.

Previous Research & Literature

Psychrotrophic bacteria are a major cause of spoilage in both eggs and chicken meat because they can replicate under refrigeration conditions [4,13]. Fresh chicken is highly perishable, with a shelf life of approximately 8–12 days depending on processing, handling, and storage conditions [14]. In contrast, whole eggs typically have a refrigerated shelf life of approximately six weeks. Common bacterial species associated with egg spoilage include *Proteus*, *Bacillus*, *Pseudomonas*, and *Staphylococcus* species [13].

In some commercial egg facilities, as is the case with industry Feather&PECK, washing, including the use of sanitisers, is an important part of supplying clean eggs that meet the market preference of consumers [personal communication, John Byrne Feather&PECK]. Rapid washing procedures (lasting approximately 30 seconds) generally includes pre-washing with warm water, surfactant washing, sanitisation with antimicrobial agents (typically chlorine-based at approximately 100–200 ppm), and blow-drying [15]. However, the porous nature of eggshells allows microbial contaminants and chemical sanitisers such as chlorine to penetrate the shell, raising concerns regarding food safety, chemical residues, and public health. The persistence of biofilms and the increasing threat of antimicrobial resistance (AMR) further highlight the need for safer and more effective sanitisation technologies.

Plasma activated water (PAW) has emerged as a promising non-thermal sanitisation and decontamination technology, either as a stand-alone treatment or in combination with conventional food-washing systems. PAW is produced when cold atmospheric plasma (CAP) interacts with water, generating a solution enriched with reactive oxygen and nitrogen species (RONS). These include short-lived species such as hydroxyl radicals, atomic oxygen, superoxide anions, and peroxynitrite, as well as longer-lived species including hydrogen peroxide, ozone, nitrite, and nitrate [16]. Plasma activation also alters other physicochemical properties, including reduced pH and increased conductivity and oxidation–reduction potential (ORP), all of which contribute to antimicrobial activity.

The complex composition of PAW underpins its antimicrobial properties, enabling rapid inactivation of bacteria through oxidative and nitrosative damage to cellular membranes, proteins, enzymes, and nucleic acids [17,18].

Multiple studies have demonstrated the efficacy of PAW in reducing microbial loads on fresh produce, meat, poultry, and food-contact surfaces, including activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus* [19–24]. However, PAW's use in controlling eggshell-associated bacteria remains poorly understood. Compared with conventional sanitisers such as chlorine-based agents, quaternary ammonium compounds, peracetic acid, and hydrogen peroxide [21,25,26], PAW offers several advantages. It is generated using only gas (e.g. air), water, and electricity; produces no harmful chemical residues; and its physicochemical properties can be tailored by adjusting plasma parameters such as voltage, gas flow, and treatment mode (e.g. dipping, spraying, or rinsing). These characteristics position PAW as a promising sanitising technology capable of improving food safety and reducing chemical use in egg and broader food processing industries.

1.1.1 Background Research on Plasma Activated Water (PAW)

Plasma is considered the fourth state of matter, alongside solids, liquids, and gases. Just as boiling converts a liquid into a gas, the addition of sufficient energy to a gas transforms it into plasma. Lightning is among the most familiar natural examples of a thermal (hot) plasma. Non-thermal or cold plasma technologies are emerging as chemical-free industrial disinfection methods for controlling pathogens in water [27] and food [28].

Plasma treatment of water generates a range of reactive oxygen and nitrogen species (RONS), including hydroxyl radicals (OH), hydrogen peroxide (H₂O₂), ozone (O₃), superoxide (O₂⁻), nitrite (NO₂⁻), nitrate (NO₃⁻), nitric oxide (NO), and peroxynitrite (ONOO⁻). The accumulation of ions and RONS decreases pH while increasing electrical conductivity and oxidation–reduction potential (ORP) [29].

The physicochemical properties of plasma activated water, and the concentration of individual RONS, depend on several factors including the plasma device used, carrier gas composition (e.g. air, helium, argon, or helium with oxygen), power settings, electromagnetic frequency, plasma exposure time, and environmental conditions during generation [30]. Recent work by Drs McWhorter and Richter demonstrated that higher RONS concentrations are positively associated with increased bactericidal activity in plasma water preparations [24].

Reactive oxygen species such as atomic oxygen, hydroxyl radicals, ozone, and hydrogen peroxide are known to damage bacterial DNA, proteins, membrane structures, and metabolic pathways [31]. Reactive nitrogen species similarly disrupt bacterial metabolism [32,33]. The synergistic interaction of RONS under low-pH conditions is believed to drive bacterial inactivation while minimising the potential for sublethal effects. Consequently, plasma activated water has attracted increasing interest within the food industry as an experimental alternative to chlorine-based and quaternary ammonium sanitisers.

1.2 Gaps in Current Knowledge

There has been limited investigation into the use of plasma activated water (PAW) for egg washing and chicken meat sanitation, with most existing studies focusing primarily on pathogen removal rather than potential improvements in storage, or food quality outcomes. Furthermore, relatively few studies have directly compared the efficacy of PAW with chlorine-based sanitisers, which remain the industry standard.

Additional knowledge gaps include the effects of chemical sanitisers on egg quality, freshness, and eggshell cuticle integrity, as well as comparative studies examining PAW applications in chicken meat processing. The aims and associated milestones of this project addressed these research gaps.

Methodology

Plasma Activated Water

PAW was generated using a modified plasma unit from Plasmacreat (Plasmacreat GmbH, Germany). Briefly, 1.5 L of autoclave-sterilised deionised water was treated for 45 min in a 2 L glass Schott bottle. The plasma generation was set to a fixed power supply with variable voltage to ensure target power levels were reached for the dielectric barrier discharge at 80 watts (W) and 300 W for the gliding arc. Air was the input gas source at a flow rate of 8 and 10 litres per min (L/min), respectively (Figure 1).

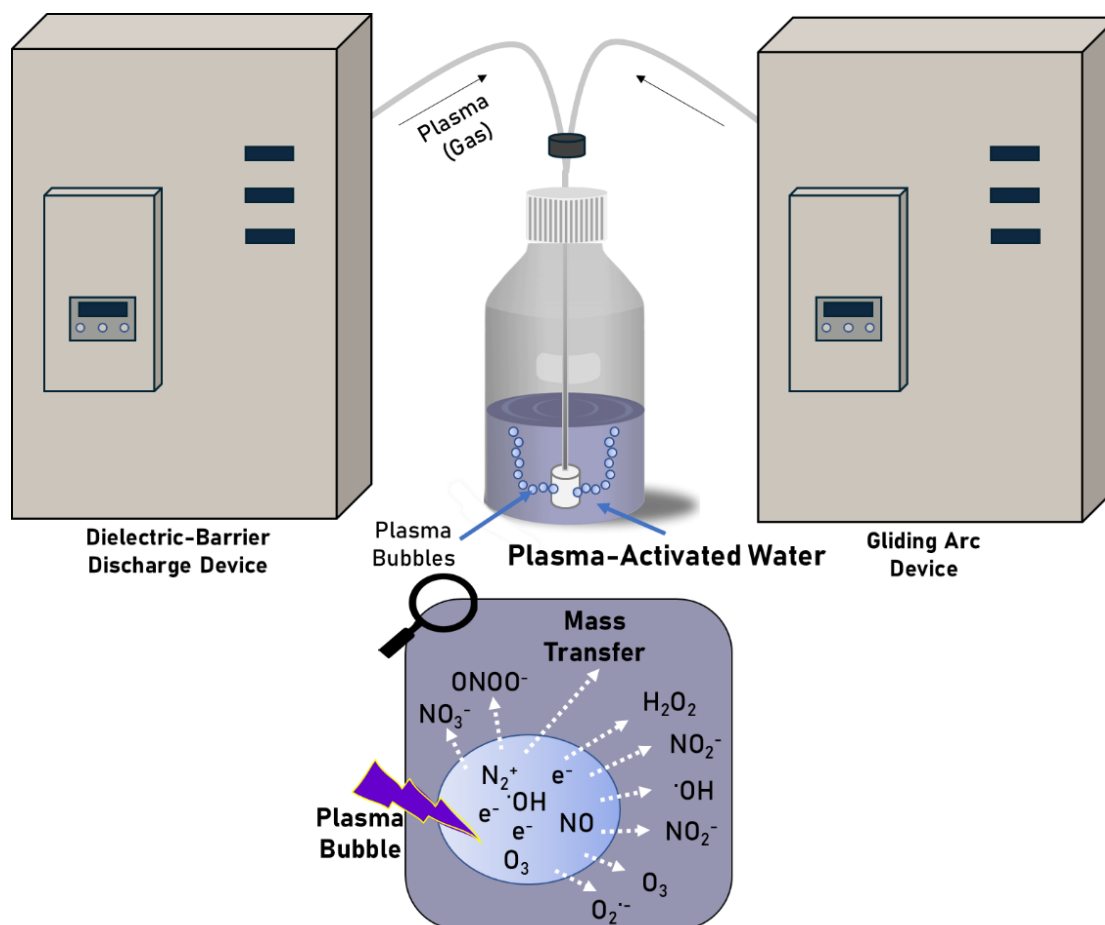


Figure 1. Schematic representation of the setup used in this study to generate PAW for the *in vitro* treatment of biofilms. 1.5 L of PAW was produced for 45 min using a dual-system plasma configuration comprising a dielectric barrier discharge device (80 W, air supplied at 8 L/min) and a gliding arc device (300 W, air supplied at 10 L/min). During PAW production, plasma bubbles rich in reactive species form within the water, enabling the mass transfer of these species from the gas phase (bubbles) to the liquid phase (water). Figure from authors' publication [17].

Materials and methods

Eggs used in this study were generously supplied by Feather&PECK. Eggs were obtained fresh and experiments were set up on the day they were collected. Chicken meat was obtained from commercial suppliers immediately post processing.

Circhlor was sourced from BASF/Chemetall. It was used at 1.5% v/v.

Cuticle Integrity

To assess treatment effects, the lower half of each egg was exposed to EDTA, plasma-activated water (PAW), or chlorine, while the upper half remained untreated as an internal control. Following a 10 s exposure, eggs were rinsed with water and air dried. Cuticle staining was performed using malachite green (5 min), followed by rinsing and drying. Eggshell colour (L^* , a^* , b^*) was measured using a handheld spectrophotometer (Konica Minolta CM-700d), and colour differences (ΔE) were calculated relative to untreated regions. Based on the L^* , a^* , and b^* values the ΔE was calculated using the following equation:

$$\Delta E = \sqrt{((L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2)}$$

In this calculation, the difference between the control untreated shell was evaluated against the treated eggshell cuticle.

Application of PAW to chicken eggs

The efficacy of PAW at reducing the load of microbes (total viable counts; TVC) on the eggshell surface was evaluated at 10s, 30s, 60s, 120s, and 300s exposure time.

Egg Storage Study

Bacterial counts

Bacteria were enumerated (TVC) weekly over the course of 8 weeks on plasma treated and control eggs. In this study, separate trials were conducted for eggs stored at 5 °C, 15 °C, or 25 °C.

Egg quality

Egg quality was measured using Haugh Units. A Haugh Unit is a measurement of the egg internal quality and freshness by calculating the height of the thick albumen (egg white) relative to its weight. Higher scores indicate better quality and fresher eggs. Egg quality was measured weekly for 8 weeks. Separate experiments were conducted for different storage temperatures: 5 °C, 15 °C, or 25 °C.

Culturing of Bacterial Organisms from Chicken Egg Shells.

Individual eggs were placed separately into sterile polyethylene bags containing 10 mL of sterile buffered peptone water (BPW). Each egg was gently agitated in the BPW for 1 min at ambient temperature to recover eggshell-associated bacteria, after which the egg was aseptically removed and the rinsate retained for microbiological analysis. Specifically, 1 mL aliquots of each rinsate were plated in duplicate onto selective agar media as follows: Violet Red Bile Glucose agar (Thermo Scientific) and Brilliance™ *Salmonella* agar (Oxoid) for isolation of *Enterobacteriaceae*; Brilliance™ CRE agar (Thermo Scientific) or CHROMagar™ *Acinetobacter* (CHROMagar) for *Acinetobacter* spp.; and Pseudomonas Isolation Agar (Thermo Scientific™ Remel™) supplemented with glycerol for *Pseudomonas* spp. Plates were incubated aerobically at 37 °C for 18–24 h.

From each positive sample, two presumptive colonies were randomly selected and purified by re-streaking on the corresponding selective medium. Presumptive identification was based on characteristic colony morphology: *Enterobacteriaceae* produced dark red–purple colonies with red–purple haloes (with *Serratia* spp. appearing blue on Brilliance™ *Salmonella* agar); *Acinetobacter* spp. appeared white or naturally pigmented on Brilliance™ CRE agar or red on CHROMagar™ *Acinetobacter*; and *Pseudomonas* spp. produced fluorescent green colonies with pyocyanin pigmentation. Purified isolates were streaked onto Heart Infusion (HI) agar and incubated overnight at 37 °C. Following incubation, isolates were preserved in glycerol stocks at –80 °C for long-term storage, while parallel agar-struck isolates were maintained at 4 °C for short-term storage prior to DNA extraction and preparation for subsequent sequencing.

Bacterial Isolate DNA Extraction & Sequencing

Genomic DNA was extracted from bacterial isolates recovered from eggshell surfaces using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, USA) according to the manufacturer's instructions. Briefly, 50 purified isolates were randomly selected to represent the diversity of bacteria recovered from the eggs. Each isolate was cultured overnight in 10 mL HI broth at 37 °C with shaking at 160 rpm. Cultures were centrifuged at 7,000 × g for 10 min, and cell pellets were resuspended in ultrapure water and BashingBead™ buffer. Mechanical lysis was performed using a TissueLyser II (Qiagen) at 30 Hz for 5 min, repeated twice to ensure complete cell disruption. Following bead beating, samples were centrifuged at 10,000 × g for 1 min, and the supernatants were transferred to clean tubes, mixed with Genomic Lysis Buffer, and centrifuged again at 10,000 × g for 1 min. DNA purification was completed using the kit's DNA Pre-Wash and Wash Buffers, and genomic DNA was eluted into clean collection tubes. DNA concentration and purity were assessed using a NanoDrop One spectrophotometer (Thermo Fisher Scientific), with yields (ng/μL) and A260/A280 ratios confirmed.

The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTACGACTT-3') with MyTaq™ Red Mix (Bioline). PCR amplification comprised an initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 64.3 °C for 15 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min. A second PCR was performed using the first-round PCR product under identical cycling conditions. Nuclease- free water was included as a negative control, and genomic DNA from *Salmonella enterica* serovar Typhimurium ATCC 14028 was used as a positive control in each PCR run. PCR amplicons were excised from agarose gels and purified using the PureLink™ Quick Gel Extraction Kit (Invitrogen). Purified products were quantified using a NanoDrop™ One spectrophotometer and submitted to the Australian Genome Research Facility for Sanger sequencing. Resulting sequences were analysed using BLASTn (NCBI) and species-level identification was assigned based on BLAST scores and ≥99% sequence identity.

Chicken Meat Storage Study

Chicken meat storage experiments were conducted using multiple cuts (skin-on and skin-off thigh, breast, and wings) to evaluate reductions in total aerobic bacterial counts following treatment with plasma-activated water (PAW). Fresh samples were collected from a commercial processing plant, where whole chicken carcasses were treated with chlorine in the spin chiller during processing. However, bacterial loads on meat pieces can increase following carcass cutting and further processing due to cross-contamination and increased handling. Therefore, PAW was evaluated as a post-cutting dip treatment to determine its potential to reduce bacterial contamination on chicken meat portions following processing.

For these experiments, a *Campylobacter jejuni* strain previously isolated from chicken meat was used. To generate an inoculum for the experiments, *C. jejuni* was cultured in nutrient broth No.2 (ThermoFisher) under microaerophilic conditions at 37 °C with shaking until an OD600 of 0.2 was achieved. The culture was then diluted in 1 L of 0.9% saline to achieve a final concentration of 10⁶ CFU/mL. Different chicken meat type pieces including wings, thigh with skin, thigh without skin, breast with skin, and breast

without skin were immersed in the *C. jejuni* suspension for 5 minutes and air-dried. The meat pieces were vacuum sealed and stored at 4°C until been treated with PAW or chlorinated water.

The air-dried meat pieces were weighed and subjected to one of the following treatments: unwashed, washed in 4 L of PAW, or in 4 L of 50 ppm chlorine solution for 30 seconds (10 pieces per treatment, total $n = 30$) at the day of inoculations, and at day 3, 6, 9, and 12 (depending on cut type) after inoculations. After treatment, the chicken meat pieces were washed in 100 mL buffered peptone water (BPW; ThermoFisher Scientific) for 2 minutes. The BPW was then collected for test total viable bacteria and total *Campylobacter* counts. Control groups which have inoculated but untreated meat pieces ($n = 10$) were also washed in BPW. For total viable bacterial counts, serial 10-fold dilutions were prepared in 0.9% saline, and 100 μ L of dilution was spread-plated onto nutrient agar and incubated overnight at 30 °C. For *Campylobacter* counts, a 10-fold dilution of the BPW was spread on *Campylobacter* Blood-Free Selective Agar plates (200 μ L per plate), which were incubated under microaerophilic conditions at 37 °C using the Oxoid *CampyGen* Sachet.

Bacterial Biofilm Formation

The following method is reproduced from the authors' publication [17]. From the 50 eggshell-associated bacterial isolates identified, a representative subset of four Gram-positive (*Staphylococcus arlettae*, *Lysinibacillus fusiformis*, *Priestia megaterium*, and *Peribacillus frigiditolerans*) and four Gram-negative (*Acinetobacter lwoffii*, *Escherichia coli*, *Pseudomonas fulva*, and *Pseudomonas stutzeri*) species were selected for further investigation. These species were selected to represent a combination of well-recognised foodborne or opportunistic pathogens and lesser-studied environmental bacteria commonly associated with soil, water, and poultry environments, for which biofilm formation remains poorly characterised. Single-species biofilms were developed in sterile, flat-bottom 96-well microtiter plates over 24, 48, and 72 h to assess formation dynamics and determine the optimal growth period. Following Vyas et al.[17] wells were inoculated with 150 μ L of diluted overnight bacterial cell cultures adjusted to approximately 1×10^6 colony forming units (CFU)/mL. Gram-positive isolates were grown in HI broth, and Gram-negative isolates in Luria–Bertani (LB) broth. Plates were incubated at 37 °C with gentle agitation (50 rpm), with the medium carefully removed via gentle pipetting and replaced with fresh sterile medium every 24 h.

Scanning Electron Microscopy

The following method is reproduced from the authors' publication [17]. Scanning electron microscopy (SEM) was utilised to examine the morphological alterations in biofilms and residing cells of *L. fusiformis*, *P. megaterium*, *E. coli*, and *P. stutzeri* following 1 min treatment with fresh PAW, relative to water control. Briefly, biofilms were grown for 48 h on 13 mm plastic Nunc Thermanox coverslips (Proscitech, USA) in a 12-well polystyrene plate, then treated with PAW or water control. Biofilms were prepared for SEM imaging using methods adapted from Vyas et al. [18] with the following modifications. Biofilms were pre-fixed for 30 min at 4 °C, followed by fixation for 1 h at 4 °C. After fixation, samples were washed and dehydrated through a graded ethanol series (30 %, 50 %, 70 %, and 3×100 %). Dehydrated biofilms were then treated with a 1:1 mixture of 100 % ethanol and hexamethyldisilazane (HMDS), followed by two successive changes of 100 % HMDS. After 30 min, the HMDS was removed and samples were allowed to air-dry. Dried biofilms were sputter-coated with a 10 nm platinum layer using a high-resolution sputter coater (Cressington Scientific Instruments, UK; 208HR) and imaged using a SU8600 cold field-emission ultra-high-resolution scanning electron microscope (Hitachi High-Tech, Japan). Images were acquired at random positions across each sample by a microscope technician blinded to the experimental conditions to minimise bias.

Statistical analyses

Data were analysed using GraphPad Prism 10.0 (GraphPad Software, Boston, MA, USA). For egg experiments, data are presented as the mean $\pm$ standard deviation (SD) or standard error of the mean (SE) as indicated. Log₁₀ reduction of bacteria following PAW exposure of different treatment times and different PAW ages were compared by mixed-effects model with Šídák's multiple comparisons test between all treatment groups, with a family-wise α threshold of 0.05. Residuals plots and QQ plots were used to assess assumptions of homoscedasticity and normality of the data, respectively. Intracellular ROS accumulation in PAW- and water control-treated biofilms were analysed by one-way ANOVA with Tukey's post hoc test. For chicken meat experiments, CFU counts from chicken meat cuts experiment were tested for normality using the Shapiro-Wilk normality test and are represented by the mean $\pm$ standard error of the mean (SEM). Total aerobic bacteria count and total viable *Campylobacter* data was analysed using a repeated measures one-way analysis of variance (one-way ANOVA) with Tukey's multiple comparisons test. Data were log₁₀ transformed. P values less than 0.05 were considered statistically significantly different.

Results

Eggs

Cuticle integrity

The eggshell cuticle is a critical protective barrier that limits microbial penetration through shell pores and contributes to egg shelf life. It is therefore essential that any egg-washing intervention does not adversely affect cuticle integrity. Notably, cuticle deposition at oviposition is inherently variable between eggs. Both PAW and chlorine treatments exhibited minimal impact on cuticle integrity under these conditions (Figure 2).

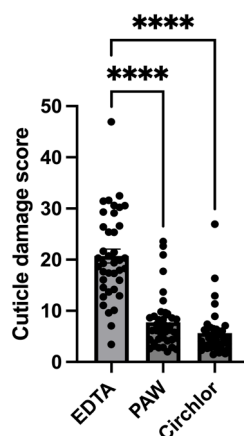


Figure 2. Effect of PAW on egg cuticle. ** p < 0.0001 n = 30 eggs per treatment**

While there were significant differences of either treatment (PAW or Circhlor) to the control, there was no significant difference between PAW and Circhlor after the 10 s treatment time.

Egg Storage Experiments Results

Effect of PAW on Reducing Bacterial Load

The efficacy of PAW at reducing the load of microbes (TVC) on the eggshell surface was evaluated at multiple time points. The greatest log reduction was observed after 120 second exposure time (Figure 3).

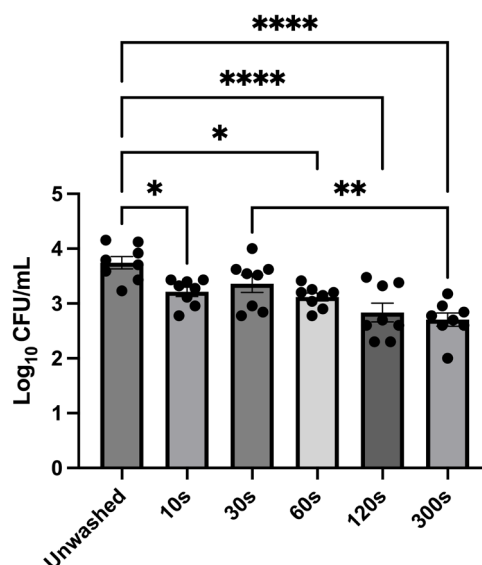


Figure 3. TVC on eggs following PAW treatment at different time points. * $p < 0.05$, ** $p < 0.01$, ** $p < 0.0001$, $n = 8$ per treatment group.**

An exposure as short as 10 s provided a significant knock-down of TVC of bacteria. Increasing the treatment time to 60 s did not provide any further benefit, as TVC at 10, 30 and 60 s were not statistically different. The greatest decrease in TVC was measured at the 120 s time point. However, after exposure, there was clear evidence of damage to the egg cuticle as it was found peeling off following the wash. Therefore, the optimal wash time was determined to be 10 seconds, which aligns with current practices for Circhlor washing.

Effect of PAW treatments and egg storage temperature on TVC

The efficacy of PAW in reducing bacterial contamination on eggs over time was evaluated by measuring the TVC. Counts are shown for 8 weeks storage at three temperatures (Figure 4).

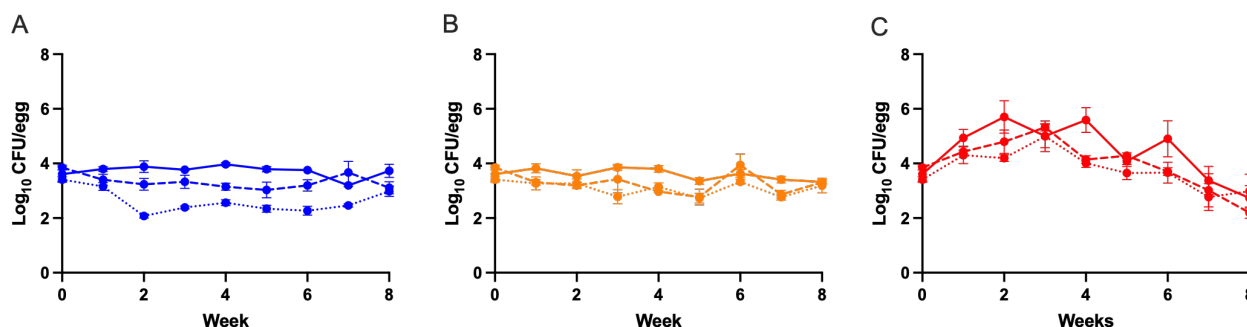


Figure 4. Effect of PAW on TVC at three different temperatures. Eggs were stored at 5 °C (A), 15 °C (B), or 25 °C (C). Unwashed eggs were the control (solid lines) and treatments were Circhlor (hashed lines) and PAW (dotted lines). TVC was evaluated over an 8-week period.

As shown in the figure, TVC measured after Circhlor treatment or PAW treatments were essentially the same, meaning that the PAW performed as well as the industry standard. As for trends, TVC remained essentially constant for the 5 °C and 15 °C storage trials. This means that the total bacterial load did not increase during storage at these conditions and was controlled by either sanitiser for all three temperature studies. At 25 °C, the TVC increased during the first 4 weeks of storage and then decreased between weeks 4-8; additionally, trends were the same for PAW, Circhlor and unwashed eggs.

Effect of PAW and egg storage temperature on quality

Egg quality was measured using Haugh Units. Higher scores indicate better quality and fresher eggs. Haugh Units are graphed for Circhlor and PAW treatments for eggs stored over 8 weeks at different temperatures (Figure 5).

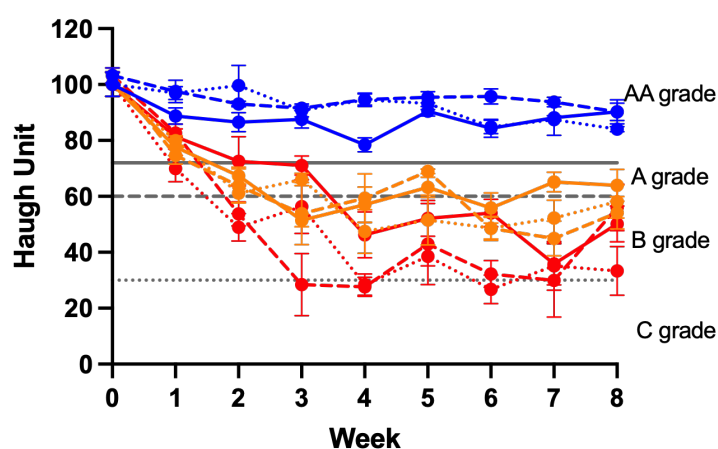


Figure 5. Effect of wash treatment on egg quality. Unwashed eggs were the control (solid lines) and treatments were Circhlor (hashed lines) and PAW (dotted lines). Eggs were stored at 5 °C (blue), 15 °C (orange), or 25 °C (red). Egg grades (AA, A, B, C) corresponding to Haugh units are shown.

Outcomes of this experiment demonstrate that temperature is a dominant driver in maintaining egg quality. Regardless of wash treatment, eggs stored at 5°C retained consistent quality over the 8 week experiment and remained as AA grade eggs. Eggs stored at 15 and 25°C exhibited the fastest decrease in quality (Figure 5).

Bacteria cultured and identified from chicken eggs

The identity of bacteria present on unwashed eggs is rather poorly characterised but could be important to understand ways of improving egg shelf life. A study was conducted to culture bacteria from eggshells and characterise these at the species taxonomic level (Table 1).

Table 1. Identification of various Gram positive and negative bacteria cultured from eggs in this study

Staining	Bacteria genus & species
Gram +	<i>Staphylococcus arlettae</i>

Gram + *Staphylococcus saprophyticus*

Gram + *Staphylococcus cohnii*

Gram + *Staphylococcus warneri*

Gram + *Staphylococcus succinus*

Gram + *Bacillus mycoides*

Gram + *Lysinibacillus fusiformis*

Gram + *Priestia megaterium*

Gram + *Peribacillus frigoritolerans*

Gram - *Acinetobacter lwoffii*

Gram - *Pseudomonas fulva*

Gram - *Pseudomonas stutzeri*

Gram - *Escherichia coli*

Nine Gram-positive and four Gram-negative bacterial species were identified. According to available information, some of these species are associated with spoilage (*Bacillus mycoides*, *Pseudomonas fulva*, *Pseudomonas stutzeri*) [34,35]. Characterisation of bacterial species commonly found on eggs will be important in future work when understanding how to best apply PAW to control the bacterial species responsible for spoilage.

Chicken Meat Storage Study

Bacterial counts were enumerated on different cuts of chicken meat cuts during storage to compare the efficacy of PAW with chlorine in a controlling bacterial contamination over time (Figure 6).

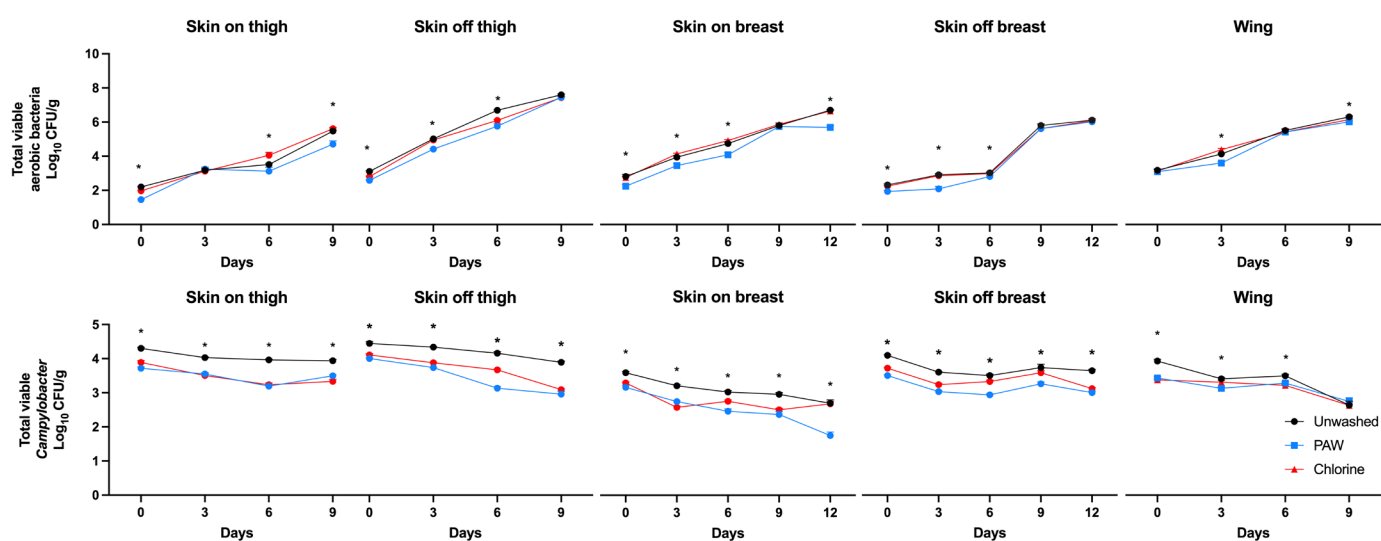


Figure 6. Changes in total aerobic bacterial (upper panel) and *Campylobacter* (lower panel) counts on different chicken meat cuts which treated with PAW or chlorine over time. Samples included skin on thigh, skin on breast, and wings, skin off thigh, and skin off breast. Treated samples were stored at 4 °C and surface bacteria were recovered at days 0, 3, 6, 9, and 12. Data represent mean log CFU/g. An asterisk (*) denotes that PAW treated samples had significantly less bacteria loads than untreated meat cuts ($p < 0.05$).

Total aerobic bacterial counts increased progressively in untreated controls, consistent with slow bacterial growth during refrigerated storage. Both PAW and chlorine treatments delayed bacterial growth; however, PAW consistently demonstrated greater antimicrobial efficacy. Across all cuts, PAW-treated samples maintained lower TVC throughout storage, indicating sustained antimicrobial activity. Chlorine treatment provided moderate inhibition but was less effective than PAW. Skin-off breast and wing samples exhibited lower overall microbial loads, suggesting that surface structure influences bacterial attachment and treatment efficacy. PAW treatment resulted in significantly lower *Campylobacter* counts compared with the untreated control, demonstrating its effectiveness in reducing this foodborne pathogen on chicken meat cuts (Figure 6).

Microscopic Evaluation of Antibacterial Properties of PAW

To better understand the antimicrobial properties of PAW, an *in vitro* study evaluated the microstructures of bacteria cultured from eggshells before and after PAW treatment.

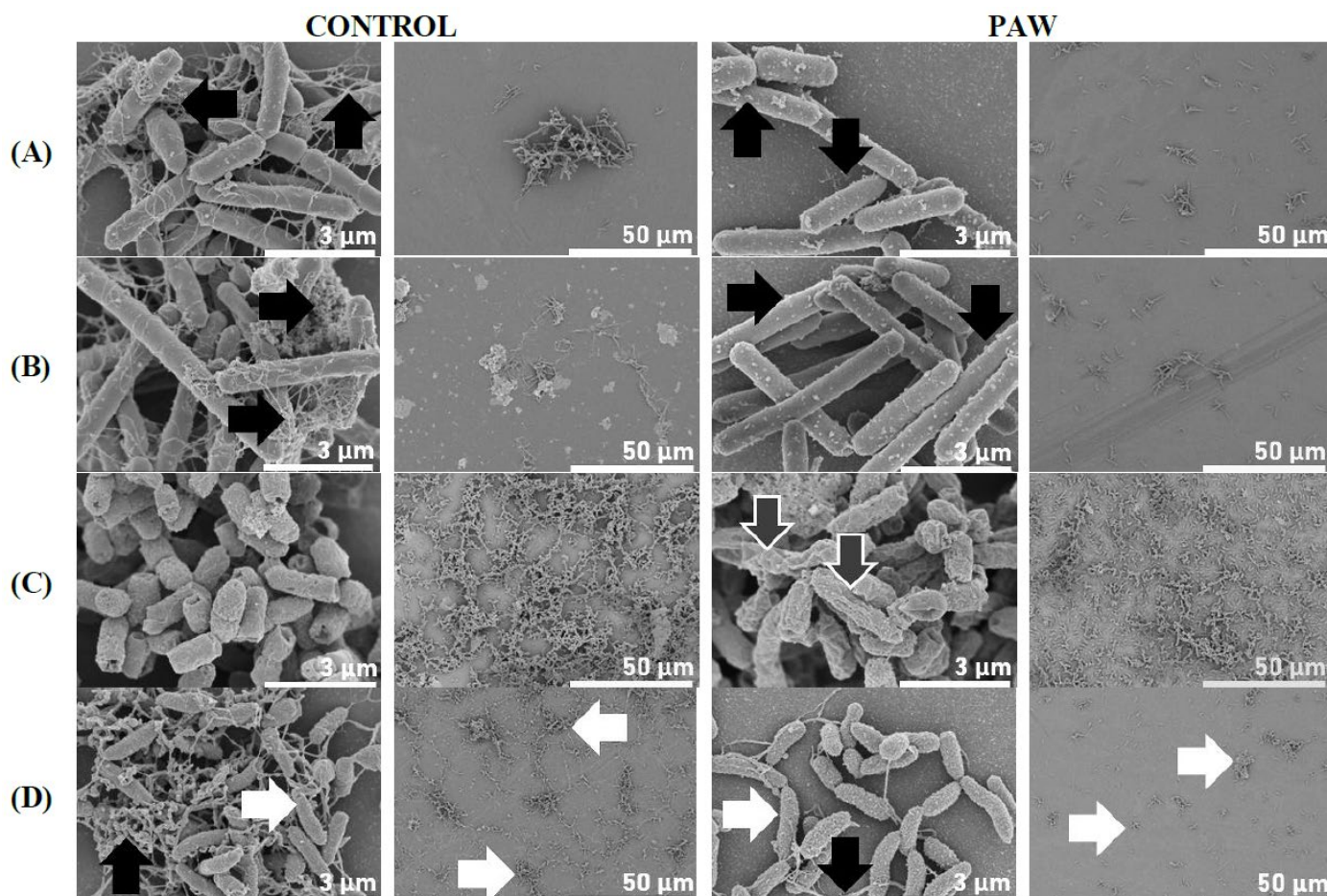


Figure 7. SEM reveals distinct morphological and structural alterations in bacterial biofilms and their cells following PAW treatment. 48 h biofilms of (A) *L. fusiformis*, (B) *P. megaterium*, (C) *E. coli*, and (D) *P. stutzeri* were exposed to freshly generated PAW or water control for 1 min. Observed morphological changes included extracellular polymeric substance reduction (black arrows), cell surface shrinkage and deformation (grey arrows), and localised removal of biofilm cells (white arrows). Figure reproduced from reference [17]

SEM was used to qualitatively assess the effects of PAW treatment on bacterial biofilm cells and overall biofilm architecture (Figure 7). PAW appears to have several species-dependent mechanisms of action on bacterial cells and their associated biofilms. These include removal of extracellular polymeric substance, shrinking and deforming cells, and removal of adhered biofilm cells. All these associated changes are related to putative mechanisms of action for PAW acting on extracellular polymeric substances and cellular components.

Discussion

Bacterial contamination on foods by spoilage organisms leads to undesirable food loss and waste [36,37]. Furthermore, contamination by human pathogenic bacteria on poultry products is associated with illness and sometimes death [38]. For these

reasons, food industries seek ways to control unwanted bacterial contamination of foods by using sanitisation methods. In the poultry industry, chemical sanitisers are frequently used [10,34].

Feather&PECK currently uses an egg washing machine to clean eggs prior to further processing, storage and shipping. This machine consists of a conveyor belt that moves eggs through a series of brushes, under a stream of sanitiser. At Feather&PECK, the currently used practice uses the chlorine based chemical Circhlor (1.5%) and a conveyor speed that exposes eggs to washing for approximately 10 s.

Industries like Feather&PECK and poultry processors are interested in investigating alternatives to chlorine-based sanitisers. Chlorine sanitisers have a mild bleaching effect which controls microorganism growth, but can leave chemical residues and an associated smell, particularly for chicken meat [39,40].

An alternative sanitiser is plasma activated water (PAW). PAW is simply water that has had an electrical discharge passed through it. A carrier gas, such as air, when bubbled through the water in this discharge dissociates its component gasses (nitrogen and oxygen) into various reactive species which also includes reactions with dissociated water molecules. These reactive oxygen and nitrogen species have antibacterial properties that do not rely on chlorine and could be considered a chemical-free, antibacterial sanitising solution.

The main goal of this project was to evaluate PAW competitively against industry-standard chlorine sanitisers for controlling bacterial contamination on chicken eggs and meat.

Chicken Eggs

It can be concluded that PAW's antibacterial performance is equal to Circhlor in egg washing trials. In an 8-week storage study, the total viable counts remaining on chicken eggs were similar after Circhlor and PAW treatments. The PAW application time of 10 s (currently used in industry) reduced the TVC on chicken eggs and had minimal impact on cuticle integrity (comparable to Circhlor). On egg freshness, PAW and Circhlor again performed similarly. PAW and Circhlor both had an effect on the egg cuticle after a 10s exposure, giving a cuticle damage score ranging from 5-8; for comparison, these values were significantly lower than the positive control, EDTA (damage score around 20).

The effectiveness of PAW as an antibacterial agent was demonstrated by its ability to significantly impair bacteria integrity and adhesion to surfaces, as seen by shrinkage and deformation of bacterial cells and removal of secreted extrapolymeric substances on surfaces. This antibacterial mechanism of action is consistent with mechanisms that link changes to bacterial cell walls and biofilms to oxidative damage from reactive oxygen and nitrogen species [41].

A secondary finding of this research project discovered that a key determinant for maintaining egg freshness and shelf life is to store eggs at 5 °C.

Chicken Meat

It can be concluded that for chicken meat washing, PAW is an acceptable alternative to chlorine sanitisers, with PAW performing at least as well as chlorine washes in reducing TVC at day 9 compared to unwashed. For thigh (skin on and skin off), breast (skin on), wings (skin on) PAW outperformed chlorine washes in reducing TVC on days ranging from 6 to 12. The presence of chicken skin on the cuts seems to influence the effectiveness of washes for an undetermined reason. Potential explanations may be related to the surface texture or the presence of tissues within the skin that could potentially influence bacterial association on surfaces and how effective sanitisers are at removing bacteria on these tissues.

It is worth further investigating the implications of this finding in the context of shelf life extension. Future studies with more replicates could be carried out to understand if the better sanitising power of PAW (compared to chlorine) would allow bacterial

counts to be controlled to the extent that chicken pieces could remain within acceptable limits of bacterial growth for longer storage times.

Considerations for Implementation

While PAW is a “chemical free” free sanitiser, in the sense that its only material component is water, it nevertheless contains long-lived reactive oxygen and nitrogen species. The consumer safety of using PAW will need to be studied within industry guidelines for sanitiser use which is regulated by Food Standards Australia and New Zealand (FSANZ). It is recommended that a desktop study be conducted to research how chemical sanitisers are regulated and approved by FSANZ. It is suggested that since industry sanitisers such as chlorine and quaternary ammonium formulations have an antibacterial mechanism of action that relies on the presence of long-lived reactive oxygen species, that approval for PAW formulations could follow this pathway, and be regulated when compliant with the regulations pertinent to these approved chemicals.

Implementation of PAW systems for washing poultry products

Questions to consider for poultry industries looking to implement PAW as an alternative to chlorine sanitisers.

- 1) How can PAW be generated in sufficient quantity to meet the needs of chicken and egg washing facilities?
- 2) What is the cost for implementing PAW?
- 3) Who manufactures PAW and how is it supplied and stored?
- 4) Can PAW be implemented using existing equipment currently used for washing using conventional chlorine sanitisers (spray systems, dip systems, compatibility with egg and meat processing lines)?
- 5) Can PAW washing solutions be safely disposed in a manner that complies with environmental protection?

The answers to these questions can be determined by further research and extension activities and cost/benefit analysis for implementing new systems

Translational outcomes from this research project

During this project, the technology readiness was assessed at level 4 (**Lab Testing/Validation of Alpha Prototype Component** /**Process:** Design, development and lab testing of components/processes. Results provide evidence that performance targets may be attainable based on projected or modelled systems.)

An outcome from this project was a successful grant application to A/Prof Katharina Richter and the team from the Australian Economic Accelerator scheme and its plan to further advance the technology readiness level from 4 to 5. This Australian Economic Accelerator (AEA) project is currently exploring the answers to the types of questions asked above and investigating the market opportunity and commercialisation pathway to adoption.

Conclusions & Recommendations

The purpose of this research project was to evaluate plasma activated water as an alternative wash/sanitiser to ones currently used for chicken egg and meat washing..

For egg washing, when competitively compared to Circhlor sanitiser, PAW had performance equal to this industrial chemical (controlling bacterial growth and preserving freshness).

For chicken meat washing, when competitively compared to a chlorine sanitiser, PAW had performance equal to or better than industrial chlorine-based sanitisers.

Based on these results, work is now being undertaken to advance PAW's technology readiness to achieve TRL 6 to evaluate PAW in an integrated/semi-integrated egg washing system in collaboration with Feather&PECK.

As PAW washing has demonstrated comparative effectiveness equal to or better than current industry sanitation practices, it is further recommended to evaluate the food safety aspects of using PAW and regulatory testing needed to evaluate its approved use in future food sanitation applications.

Impact and Ongoing Monitoring

Before project commencement, the following impacts were predicted:

Reduction of Food Waste. The sanitising performance of PAW was comparable to chlorine in the case of eggs. While it seems unlikely that shelf life could be extended beyond what is already possible with chlorine, the finding that bacteria could be controlled with a new chemical free sanitiser could have other economic and environmental impacts for the industry as noted below. For chicken meat, the project showed some promising initial results that PAW might control bacterial growth better than chlorine for some applications. CI McWhorter has funding from AgriFutures to continue investigating the potential to reduce food waste by product shelf life extension.

Increased Profitability. Chlorine sanitation on food is associated with negative consumer perception. Marketing poultry products as "chlorine free" could potentially unlock increased industry profitability for manufacturers who use PAW instead of chlorine sanitisers. There also might be cost benefits for PAW utilisation as opposed to chlorine; however, these are harder to predict as PAW is a new technology and a niche process, and chlorine is already a low-cost sanitiser.

Greenhouse gas emission savings. Chlorine bleach is a product of the chlor-alkali industry which uses energy intensive electrolytic processes. Furthermore, concentrated chlorine and chlorine gas byproducts are hazardous chemicals and require regulation for transport, which increases the supply-chain costs. On the other hand, in the future, PAW could be generated on-site for poultry industries with the major cost (electricity consumption) reduced by using green energy. Again, it is difficult to assess the greenhouse gas savings for switching from chlorine to PAW because of the size and scale of manufacturing. As with profitability, estimating GGE savings will require a technoeconomic assessment.

Circular economy jobs created. It is reasonable to predict that if the technology was adopted, that 8 jobs could be created by 2033, and 8 by 2048. These jobs may be engineers for installing and maintaining PAW systems, and technicians for running the units.

Future leaders graduated. CIs Richter and Coad co-supervise PhD student Bjoern Kolbe, currently in his last year. While Bjoern's PhD research was not sponsored by CRC funding, he benefited from this project and his broader focus of studying PAW's physicochemical properties and applications to other food and agriculture applications.

Industry people trained. If the technology was widely adopted by the poultry industry in Australia, the number of people predicted to be employed per year would be around 50. Training would be needed for producing PAW, change over of sanitation equipment, and quality control.

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APPENDIX A – Project Summary

Project Summary

'Plasma Activated Water: Investigating opportunities to reduce food waste in the poultry industry' Project

KEY POINTS

- Plasma activated water (PAW) performed as well as conventional chlorine sanitisers for cleaning eggs and reducing bacterial contamination.
- PAW reduced bacterial growth on chicken meat and, in several cases, outperformed chlorine-based treatments during refrigerated storage.
- Egg quality and freshness were maintained following PAW treatment, with no greater impact on eggshell integrity than current industry practices.
- PAW is generated using only water, air and electricity, offering a promising alternative to conventional chemical sanitisers.
- The technology has potential to extend poultry product shelf life, helping reduce food waste.

THE CHALLENGE

Eggs and chicken meat can become contaminated by spoilage organisms during production and processing, reducing shelf life and increasing the likelihood of products being discarded before consumption. While chlorine-based sanitisers are widely used to control bacterial contamination, there is growing demand for alternatives that can maintain food safety while reducing chemical use, residues and environmental impacts.

THE OPPORTUNITY

Plasma activated water (PAW) is an emerging sanitisation technology that combines water, air and electricity to generate naturally occurring antimicrobial compounds. Unlike conventional chlorine-based sanitisers, PAW leaves no chlorine residues and can potentially be produced on-site using renewable energy. If proven effective at commercial scale, PAW could help poultry, improve product quality, lower environmental impacts and meet increasing consumer demand for sustainable food production practices.

OUR RESEARCH

Evaluating a New Sanitising Technology

Researchers from Adelaide University worked with industry partner Feather&PECK to evaluate PAW as an alternative to conventional chlorine-based sanitisers used in chicken egg cleaning.

Egg Washing and Storage Studies

The project examined how effectively PAW reduced bacterial contamination on eggshells and whether it affected egg quality or the protective eggshell cuticle. Eggs treated with PAW were monitored over eight weeks under different storage conditions and compared with eggs treated using industry-standard sanitising practices.

Chicken Meat Storage Studies

Chicken meat cuts including breast, thigh and wings were treated with PAW and compared with conventional chlorine treatments. Researchers measured bacterial growth during refrigerated storage to determine whether PAW could help extend control microbial load.

Understanding How PAW Works

The team also identified bacterial species commonly found on eggshells and investigated how PAW damages bacterial cells and biofilms, providing insight into its antimicrobial mode of action

OUTCOMES

This project demonstrated that plasma activated water is an effective sanitising technology for poultry products.

For eggs, PAW reduced bacterial contamination and performed comparably to the industry-standard chlorine sanitiser. Egg quality and freshness were maintained throughout storage, and PAW had no greater impact on eggshell cuticle integrity than existing washing practices.

For chicken meat, PAW reduced bacterial growth during refrigerated storage and, in several cases, achieved better microbial control than chlorine-based treatments. These findings suggest PAW may offer opportunities to extend shelf life and reduce spoilage-related losses.

The project also expanded scientific understanding of the bacteria commonly found on eggshells and provided visual evidence of how PAW disrupts bacterial cells and biofilms.

Overall, the research establishes PAW as a promising alternative to conventional poultry sanitisers and provides a strong evidence base for further development and commercial evaluation.

IMPACT

This research supports the End Food Waste CRC's goal of researching evidence-based interventions with the potential to reduce food waste across Australia's food supply chain.

By improving control of spoilage bacteria, PAW has the potential to extend the shelf life of eggs and poultry products, reducing losses during storage, distribution and retail. The technology may also help improve future industry profitability by reducing product waste and increasing product quality.

Because PAW is generated using water, air and electricity, it offers a pathway to reduce reliance on conventional chemical sanitisers and their associated environmental impacts. The technology could also contribute to lower greenhouse gas emissions by reducing food waste and enabling more sustainable food processing practices.

More broadly, the project strengthens Australia's position in developing innovative food technologies that improve food safety, sustainability and resource efficiency.

NEXT STEPS

The next phase of research will focus on moving PAW from laboratory validation towards commercial implementation.

Researchers are currently investigating how PAW can be integrated into poultry processing systems at industrial scale, including evaluating production capacity, operational costs and compatibility with existing washing equipment. Additional work is also required to support regulatory approval pathways and confirm food safety requirements for commercial adoption. Many of the predicted impacts will only be realised with continued investment into further scientific research.

Future studies will further quantify shelf life extension benefits under commercial conditions and assess the economic and environmental impacts of large-scale implementation.

With promising results already achieved, PAW is now progressing towards pilot-scale testing and technology commercialisation, bringing the poultry industry closer to a sustainable, chemical-free sanitisation alternative that can help reduce food waste and improve food system resilience.

PROJECT TEAM

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PROJECT REPORTS/PUBLICATIONS

(Preprint) Vyas, H.K.N.; Abdo, A.I.; Kolbe, B.H.; Boahen, A.; Jia, S.; McWhorter, A.R.; Coad, B.R.; Richter, K. Plasma-Activated Water: An Emerging Technology for the Control of Eggshell-Associated Biofilms. *bioRxiv* **2026**, 2026.2001. 2012.699152.

PROJECT WEBPAGE

<https://endfoodwaste.com.au/plasma-activated-water/>

ENDFOODWASTE

COOPERATIVE RESEARCH CENTRE

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