

The effect of cold atmospheric plasma and linalool nanoemulsions against *Escherichia coli* O157:H7 and *Salmonella* on ready-to-eat chicken meat.

Cid R. González-González^a, Olaoniye Labo-Popoola^b, Gonzalo Delgado-Pando^c, Katerina Theodoridou^d, Olena Doran^b, Alexandros Ch. Stratakos^{b*}

^a Tecnológico Nacional de México campus Acayucan, Department of Biochemistry Engineering, Costera del Golfo Km 216.4, Acayucan, CP 96100, México

^b University of the West of England, Faculty of Health and Applied Sciences, Centre for Research in Biosciences, Coldharbour Lane, Bristol, BS16 1QY, UK

^c Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), José Antonio Novais 10, 28040 Madrid, Spain.

^d Institute for Global Food Security, Queen's University Belfast, Belfast, BT9 5AG, UK

* Corresponding author: alexandros.stratakos@uwe.ac.uk

Abstract

In this study, the antimicrobial efficiency of a novel low-voltage piezoelectric direct discharge generated cold plasma (CAP) and linalool-loaded nanoemulsion washing (LW) was assessed against *Salmonella* and *Escherichia coli* O157:H7 on ready-to-eat (RTE) chicken meat. The antimicrobial activity of the nanoemulsified linalool and its effect on cell membrane permeability were also determined *in vitro*. Nanoemulsified linalool showed similar antimicrobial activity against *Salmonella* and *E. coli* O157:H7. The antimicrobial activity of CAP (0-5 min) and LW (0-25 min) treatments were determined individually and in combination in different sequence of application on RTE chicken. Individual treatments of CAP (5 min) and LW (25 min) achieved similar reduction levels for *E. coli* O157:H7 and *Salmonella* (1.4–1.8 log CFU/g). The combination of CAP (5 min) followed by LW (25 min) showed the highest reductions, 2.76 and >3.24 log CFU/g for *E. coli* O157:H7 and *Salmonella*, respectively, without significantly affecting the lipid oxidation levels of RTE chicken meat. This is the first report on the antibacterial effect of a piezoelectric direct discharge generated cold plasma to enhance food safety. Cold plasma, alone or in combination with linalool nanoemulsions, can be adopted by the food industry for decontamination of RTE chicken meat.

ABREVIATIONS:

Atmospheric pressure plasma jet (APPJ); Cold atmospheric plasma (CAP); Corona discharge (CD); Dielectric barrier discharge (DBD); Essential oils (EO); Linalool-loaded nanoemulsion washing (LW); Reactive oxygen and nitrogen species (RONS); Ready-to-eat (RTE).

Keywords

Cold plasma; linalool nanoemulsion; Ready-to-eat chicken meat; *Salmonella*; *E. coli* O157:H7

1 Introduction

Ready-to-eat (RTE) products have been gaining increasing popularity around the world during the last few years due to their convenience. RTE chicken breast is found precooked and generally already packaged in

the market. It does not require further preparation before consumption, unless preheating is chosen by the consumer for enhancing palatability. At the same time, although RTE foods offer many advantages, they have been linked to foodborne disease outbreaks (Stratakos & Koidis, 2015). Surface cross-contamination of RTE chicken with foodborne pathogenic bacteria may occur during the packaging stage or post-treatment manipulation due to contact with contaminated surfaces or water (Carrasco, Morales-Rueda, & García-Gimeno, 2012). *Salmonella* and *Escherichia coli* O157:H7 are amongst the most important and common pathogenic Gram-negative bacteria responsible for foodborne diseases worldwide in meat (Koutsoumanis et al., 2019; Rouger, Tresse, & Zagorec, 2017). Several outbreaks involving these two pathogens have been reported due to the consumption of poultry meat and poultry containing products (EFSA & ECDC, 2018; Koutsoumanis et al., 2019; Niemira, Boyd, & Sites, 2018). According to the Center for Disease Control and Prevention (CDC, 2021) numerous outbreaks in the USA for the period 2011-2018 have been linked to products that contain RTE chicken meat (e.g. chicken rolls, chicken salad, and grilled chicken). Among them, *E. coli* was the cause of 7 outbreaks, 146 illnesses, and 26 hospitalisations whereas *Salmonella* has been the cause of 9 outbreaks, 698 illnesses, and 112 hospitalizations. Contamination of chicken meat in RTE products can be a result of cross-contamination from the fresh-cut vegetables that are contained with the meat, during the packaging stage or post-treatment manipulation due to contact with contaminated food contact surfaces, water or food handlers (Carrasco et al., 2012). To increase microbiological safety, food processors utilise a wide range of food treatments, including the use of organic acids (e.g., acetic, lactic, citric acid) as well as chlorine, chlorine dioxide, trisodium phosphate and acidified sodium chlorite (Keener, Bashor, Curtis, Sheldon, & Kathariou, 2004). However, the increasing consumers' concern about the safety of chemical treatments of food has forced processors to look for alternative decontamination strategies.

The use of essential oils (EO) as a decontamination method against yeast, moulds, and bacteria has been reported (Quesada, Sendra, Navarro, & Sayas-Barberá, 2016; Zhang, Liu, Wang, Jiang, & Quek, 2016). Linalool is a monoterpenoid with a potent antimicrobial activity which has been found in EO produced from basil, rosewood, and citrus (Gao et al., 2019; Yang, Khan, & Kang, 2015). It is generally recognised

as safe by the Food and Drug Administration (FDA) (Tripathi & Mishra, 2016), has shown low cytotoxicity (Politano et al., 2008) and is an approved substance under Regulation (EC) No 1907/2006 - REACH. These characteristics make linalool very promising ingredient for the food industry.

Cold atmospheric plasma (CAP) is an emerging processing technology extensively investigated for its surface decontamination capacity. CAP has shown promising antimicrobial activity against bacteria on various fruits, vegetables (Niemira et al., 2018), and meat products (Stratakos & Grant, 2018). Plasma is considered the fourth state of matter, is produced by the complete or partial ionisation of a gas by application of electric discharges. CAP is composed of electrons, ions, radicals, and UV photons (Pasquali et al., 2016), and is commonly produced with the use of the helium, argon, and oxygen (Gök, Aktop, Özkan, & Tomar, 2019). CAP can be generated by using microwaves, radio frequencies, but also using various set-ups with high voltage input such as i) dielectric barrier discharge (DBD) consisting of an electric discharge occurring between two electrodes separated by an insulating dielectric barrier; ii) atmospheric pressure plasma jet (APPJ) consisting in a pulsed electric arc generated by a high voltage discharge and a compressed gas flowing through the plasma section and then ejected through a jet head; and iii) corona discharge (CD) consisting in the ionisation of a neutral fluid, generally air, by a current flowing from an electrode with a high potential generating a region of plasma around (Tappi et al., 2016). On the other hand, the piezoelectric direct discharge (PDD), consist of a piezoelectric transformer generator that converts electric energy of low voltage AC into mechanical oscillations to produce high voltage AC. This end acts as an electrode to generate electric discharges ionising the air to produce a plasma state. This technology has the advantage of being compact, low cost operation, and has been recently proven to effectively kill bacteria on different surfaces (Gonzalez-gonzalez, Hindle, Saad, & Stratakos, 2021; Timmermann et al., 2021).

Studies have also shown that combination of decontamination treatments (Hurdle Technology), including either a combination of physical interventions or a treatment consisting of physical, natural, and biological interventions can be a more effective tool in eliminating foodborne pathogens when compared to a single intervention (Chen et al., 2012). Stratakos, Delgado-Pando, Linton, Patterson, & Koidis (2015)

demonstrated the synergistic antimicrobial effect of high-pressure processing and essential oil-based active packaging against *Listeria monocytogenes* on RTE chicken breast. CAP technology has strong application potential in the food industry. Thus, to accelerate its adoption the application of this non-thermal technology alone or in the combination with other interventions should be explored in depth as a means of ensuring food safety and maintain quality.

The aim of the study was to explore for the first time the efficacy of a low-voltage piezoelectric direct discharge-generated cold atmospheric plasma, and nanoemulsions loaded with linalool either alone or combined, to control the health risk from *Salmonella* and *E. coli* O157:H7 in RTE chicken breast. The two methods were also applied in different order to identify the most efficient combination. Finally, the effect of these methods on lipid oxidation was also explored in order to study the potential effects on meat quality.

2 Materials and Methods

2.1 Linalool antimicrobial activity against *E. coli* O157:H7 and *Salmonella*.

The antimicrobial activity of linalool (Sigma-Aldrich, UK) was evaluated against *S. enterica* serovar Typhimurium DT104, *S. enterica* serovar Senftenberg, and *E. coli* NCTC 12900, which is a Shiga toxin negative serotype O157:H7 strain. All three strains were activated in Tryptone Soya Agar plus 0.6% yeast extract (TSAYE, Oxoid, UK) at 37 °C for 24 h and then maintained in slopes of the same medium at 4 °C. The antimicrobial activity of linalool was evaluated by a disc diffusion assay according to Stratakos et al. (2018) by pouring one mL of inoculum of each pathogen individually containing approximately 10⁷ CFU/mL onto TSAYE agar plates and left to dry. Sterile filter paper discs (6 mm) were impregnated with linalool solution at 8% (v/v) in DMSO and placed on the inoculated agar and then incubated at 37 °C for 24 h. The diameter corresponding to the inhibition zone (DIZ) around the disc was measured. To determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of linalool, the two-fold tube dilution method was used as described by Zhu, Du, Fox, & Zhu (2016) following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2017). Linalool was diluted from 8% to 0.015625% (v/v) in Muller-Hinton broth (MHB) containing 0.15% (w/v) agar. After an overnight

incubation, the bacterial cultures in MHB were harvested by centrifugation, washed with phosphate buffer saline solution (PBS), and diluted at approximately 6 log CFU/mL in MHB with 0.15% agar. Afterwards, each tube containing linalool was inoculated with the respective bacterial suspension aiming a concentration of 5 log CFU/mL and then incubated at 37°C for 24 h. Non-inoculated tubes containing the same growth medium were used as a negative control. Inoculated tubes without linalool were used as positive control. The tubes with no visible growth were considered as the MIC. One hundred µL aliquots were taken from the tubes that showed no visible growth and were plated onto TSAYE agar. The lowest concentration of linalool that did not show microbial growth after plating out was considered as the MBC. All the experiments were conducted in triplicate.

2.2 Preparation and characterisation of nanoemulsions loaded with linalool

Stable nanoemulsions loaded with linalool were prepared using the spontaneous emulsification method using food grade reagents (Chang, McLandsborough, & McClements, 2013). Briefly, 4 g of linalool (Sigma-Aldrich, UK), were added to 6 g of medium chain triglyceride oil (Miglyol 812, IOI Oleo, Germany) and mixed for 5 min (600 rpm). Subsequently, 10 g Tween 80® (Sigma-Aldrich) was added to the oil mixture and mixed for another 5 min. The Tween 80/oil mixture (20 g) was titrated (2 mL/min) into 80 g of 5.0 mmol/L sodium citrate buffer (pH 6.8) and was mixed at 600 rpm for 15 min. Subsequently, the emulsion was sterilised by passing through a sterile 0.22 µm syringe filter and stored in sterile 50 mL tubes at 4 °C. Droplet size and polydispersity index (PDI) were measured using dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, UK).

2.3 Bacterial time-kill assay and cell membrane permeability for nano-emulsified linalool

A time-kill assay for nano-emulsified linalool was carried out as follows. Nano-emulsified linalool was added at a concentration of 8000 ppm in sterilised meat simulation medium consisting in 20 g of bacteriological peptone, 16 g of Lab Lemco, 8 g of yeast extract, 0.2 g of MgSO₄ · 7H₂O, 0.038 g of MnSO₄ · H₂O, 1 mL of Tween 80, 5 g of sterile lactic acid, and 40 g of NaCl per litre of water. Overnight cultures of *E. coli* O157:H7 and a cocktail (at 1:1 ratio) of *S. Seftenberg* and *S. Typhimurium* in MHB were washed

twice in PBS and inoculated at 5 log CFU/mL into the meat simulation medium with nano-emulsified linalool solutions, mixed and incubated at 37 °C. The samples were taken at 0, 2, 4, 8 and 24 h for cell enumeration by serial dilution and plated onto TSAYE agar. Cell membrane permeability for all three pathogens was indirectly measured using an electric conductivity assay to provide an insight into the antimicrobial mechanism nanoemulsified linalool according to Zhang et al. (2016). Specifically, membrane permeability was assessed by measuring changes in electric conductivity, using an electric conductivity meter (Jenway 4200, U.K.) after treatment for 6 hours. All experiments were performed in triplicate.

2.4 Preparation of RTE chicken samples

Commercially manufactured RTE chicken breast fillets were procured from a local retailer and transferred to the laboratory. The heat-treated (cooked) RTE samples were transferred to the lab on ice and kept under refrigeration. Whole pieces of cooked chicken (10 ± 0.2 g) were prepared and inoculated as described in section 2.5. The treatments were as follows:

- i) Cold atmospheric plasma treatment (CAP): RTE samples were treated with cold plasma for 0, 1, 2, 3, 4, and 5 min. These time points were chosen according to previous studies conducted on meat (Stratakos & Grant, 2018; Xiang et al., 2018). Moreover, a short time period, would make this technology more attractive to the food industry.
- ii) Nanoemulsion wash (LW): RTE samples were treated by immersion in a 1 L solution containing nano-emulsified linalool at 8000 ppm for 0, 5, 10, 15, 20, and 25 min.
- iii) Combination of cold plasma and nanoemulsion wash: RTE samples were treated with two different sequential combinations: CAP/LW and LW/CAP (CAP for 5 min and LW for 25 min for both sequences tested).

2.5 Bacterial strains used and inoculum preparation for RTE chicken breast

E. coli O157:H7 and a cocktail consisting of *Salmonella* (*S.*) *enterica* Typhimurium and *S. Senftenberg* (1:1 ratio) were used to artificially inoculate the RTE chicken breast. *E. coli* O157:H7 and the *Salmonella* cocktail were inoculated onto the cooked meat separately. For each bacterial strain individually, a loopful

of a fresh Tryptone soya agar plus 0.6% yeast extract (TSAYE, Oxoid Limited, Basingstoke, UK) slope culture was inoculated into 10 mL of brain heart infusion broth (BHI, Oxoid) and incubated at 37 °C for 24 h. Subsequently, 100 µL of a 10^{-4} dilution of this broth culture in the maximum recovery diluent (Oxoid), was inoculated into another 10 mL of BHI broth and incubated at 37 °C for 24 h, until the stationary phase of growth was reached. The final 10 mL cultures were harvested by centrifuging at $3600\times g$ for 30 min, washed thrice in phosphate-buffered saline (PBS), and the pellet was resuspended in a final volume of 10 mL PBS to achieve an approximately 8-9 log CFU/mL. To prepare the *Salmonella* cocktail, equal quantities of the 2-strain cell suspensions were mixed well in a plastic 50 mL centrifuge tube to produce the 2-strain cocktail. To emulate the surface contamination of RTE products, the suspension (100 µL) was inoculated on the surface of chicken samples (10 ± 0.2 g) (six samples per each time point), to achieve an initial inoculum level of approximately 5 log CFU/g.

2.6 Cold atmospheric plasma treatment of RTE chicken

The cold plasma treatment was performed using a handheld cold plasma generation device Piezobrush PZ2 (Relyon Plasma, Germany), which uses a Piezoelectric Direct Discharge (PDD®) Technology. This technology is based on the direct electrical discharge of a piezo-ceramic transformer into a working gas which is able to transform a low input voltage into a high output voltage as shown in Figure 1. The input voltage of DC power was 15 V and at a frequency of 50 kHz. The plasma was produced using atmospheric air, with the air feed being achieved by an internal axial fan. The treatments were performed in atmospheric conditions (at approximately 22 °C and 60% of Relative Humidity) by mounting the device on a stand in a vertical position and placing the chicken meat samples on a Petri dish at 10 mm of distance from the plasma source. Independent experiments were performed in triplicate.

2.7 RTE chicken treatment with nanoemulsions loaded with linalool

The prepared linalool nanoemulsions were diluted 5-fold to avoid a droplet size increase. After this dilution, the concentration of linalool in the nanoemulsions was 8000 ppm. The meat samples (6 samples per each

time point) were immersed individually in 1 L of antimicrobial nanoemulsion (8000 ppm) for up to 25 min and the pathogen levels were determined as described in section 2.8.

2.8 *E. coli* O157:H7 and *Salmonella* enumeration

A 10^{-1} dilution of chicken meat sample was prepared by adding 90 mL of the maximum recovery diluent (MRD) (Oxoid code CM733). The dilution was homogenised for 1 min in a Seward stomacher. Further 10-fold dilutions were prepared in 9 mL MRD, as necessary. An aliquot of each of the 10-fold dilutions was poured onto the plates using Sorbitol MacConkey agar with the addition of Cefixime-Tellurite (CT-SMACK) and enumerated after incubation at 37 °C for 24 h. Each sample was plated out in duplicate. The suspected *E. coli* O157:H7 colonies were confirmed with the *E. coli* O157 Latex test kit (DR0620). For enumeration of pathogenic *Salmonella*, a 100 µl aliquot of each of the appropriate 10-fold dilutions was spread plated on brilliant green agar plates (Oxoid, CM0329) and incubated at 37 °C for 24 h.

2.9 Lipid oxidation analysis

Lipid oxidation was measured by thiobarbituric acid reactive substances value (TBARS) as described by Delgado-Pando et al., (2011). TBARS were expressed as mg of malondialdehyde (MDA) kg⁻¹ meat. The values were the means of duplicate measurements of 4 different samples from the same treatment. Briefly, 5 g samples were weighed and 35 mL of trichloroacetic acid (TCA) (7.5%) was added. Afterwards, the samples were homogenised for 30 s, centrifuged at 3000×g for 2 min and then filtered. Five millilitres of filtrate were transferred to plastic tubes, and 5 mL of 0.02 mol L⁻¹ thiobarbituric acid (TBA) (1:1, TBA: filtrate) were added. The samples were vortexed and left to stand for 20 h at 20 °C. Subsequently, the samples were vortexed again, and an aliquot was used to measure the absorbance at 532 nm, equivalent to malonaldehyde (MDA) concentration with the use of 96-well plates using a microplate reader FLUOstar Omega (BMG Labtec, UK). The blank was prepared by adding 5 mL of TCA and 5 mL of TBA. Untreated meat and chicken meat washed with distilled water only were used as negative control. The calibration curve was prepared using 1,1,3,3-tetramethoxypropane.

2.10 Statistical analysis

A two-way ANOVA was performed to test the levels of bacteria cell enumeration using time and strains as fixed factors when testing linalool nanoemulsion or CAP or a combination of both on chicken breast. Tukey post-hoc analysis was carried out at a significance level of $P < 0.05$ to compare the mean values among the groups. All experiments were carried out in three independent trials with at least two samples for each observation. Analyses were carried out using RStudio version 1.2.1335 working with R version 3.6.1 for Mac (R-Core-Team, 2019).

3 Results and Discussion

3.1 Antimicrobial activity of linalool

In this study, we evaluated the antimicrobial activity of linalool against *E. coli* O157:H7 and *S. Typhimurium* and *S. Senftenberg*. The results (Table 1) for linalool, in terms of DIZ values, showed an antibacterial effect against all strains tested. *E. coli* O157:H7 was found to have higher MIC and MBC values, thus exhibiting a lower susceptibility when compared to the two *Salmonella* strains, which showed the same MIC and MBC values. Since the *Salmonella* strains showed the tolerance to linalool, a cocktail of both *Salmonella* strains (1:1 ratio) was prepared to perform subsequent experiments.

Previous studies have also shown similar linalool antimicrobial activity. DIZ values for linalool of 21.0 ± 1.0 mm and 12.5 ± 1.3 mm have been reported for *S. Typhimurium* and *S. Senftenberg* (Kisluk, Kalily, & Yaron, 2013). Zengin & Baysal (2014) reported MIC values of 0.7% & 0.6 % against *S. Typhimurium* and *E. coli* O157:H7, respectively, using linalool diluted in nutrient broth. Also, Prakash, Vadivel, Rubini, & Nithyanand (2019) reported MBC and MIC values of 1.25% for *S. Typhimurium*. Any differences observed between studies could be due to methodological variations (e.g. different growth media) and different bacterial strains used (Rivas et al., 2010).

Studies on the antimicrobial mechanism of monoterpenes, such as linalool, have shown their ability to damage and penetrate the cell membrane and, consequently, to interfere with cellular functions (Cristani et al., 2007). Linalool increases the membrane permeability leading to the leakage of functional cell material

in *Salmonella* (Kalily, Hollander, Korin, Cymerman, & Yaron, 2016). Also, microscopy imaging data for *L. monocytogenes* and *E. coli* O157:H7 have also shown the lytic effect of linalool on the cell wall and cytoplasmic membrane the osmotic balance and causing the loss of ions and functional molecules (Gao et al., 2019; Zengin & Baysal, 2014). Despite advantages linked to linalool, such as its plant origin and hypo-allergenicity, it is a volatile compound with a relatively short half-life (Pereira, Severino, Santos, Silva, & Souto, 2018). Moreover, its lipophilic nature causes difficulties to its application in food systems with high water content. The encapsulation of linalool in nanoemulsions, provides different benefits: increased water solubility, longer stability, attenuated organoleptic effects, and an increased contact surface area with the bacterial cell (Pereira et al., 2018). To enhance the antimicrobial capability of linalool and circumvent possible application limitations, we employed a spontaneous emulsification process to encapsulate linalool. Dynamic light scattering analysis showed that the linalool nanoemulsions produced had an average particle size of 103.24 ± 3.31 nm and with a PDI of 0.244 ± 0.0085 .

The time-kill assay results in Figure 2 show the antimicrobial activity of the nanoemulsified linalool for both pathogens. More specifically, exposure to linalool nanoemulsions reduced *Salmonella* and *E. coli* O157 counts below the detection limit after 4 h of exposure (i.e., > 4 log CFU/mL) with *Salmonella* showing a higher susceptibility to linalool nanoemulsion than *E. coli* ($P = 0.022$). The effect of the nanoemulsion components (Tween 80 and medium chain triglyceride oil) on all three bacterial strains was also determined by disc diffusion assay to examine if the antimicrobial effect observed could also be attributed to these components. The results exhibited no inhibition zones for all strains and concentrations used (6% and 10% for the medium chain fatty acids and Tween 80, respectively), showing these compounds do not contribute to the antimicrobial activity under the experimental conditions used. However, previous studies have suggested that the main effect of these compounds is to enhance the mass transport of EO molecules to the interstitial spaces and across the cell membrane increasing their antimicrobial effect (Yegin, Perez-Lewis, Zhang, Akbulut, & Taylor, 2015).

238 Additionally, to get an insight into the antimicrobial mechanism, a cell membrane permeability assay was
239 performed by determining the changes in electrical conductivity of bacterial cultures treated with the
240 nanoemulsified linalool. As showed in Figure A1, linalool nanoemulsions caused a pronounced increase in
241 the electric conductivity after 6 h of treatment revealing that there was leakage of ions (e.g., Na^+ , H^+ , K^+)
242 compared to the untreated control linalool ($P < 0.001$). All three strains studied exhibited a similar trend
243 with control samples showing no significant changes. These results suggest that increased membrane
244 permeability is a determinant factor in the antimicrobial activity mechanism of the nanoemulsions.

245 Thus, the high antimicrobial effect of linalool nanoemulsions could be attributed to the following factors:
246 i) the lower hydrophobicity of the nanoemulsified linalool and its increased solubility and easier contact
247 with the bacterial cell, ii) nanoemulsions enhance the diffusion of essential oil particles thus facilitating
248 penetration within the membrane causing damage (Moghimi, Ghaderi, Rafati, Aliahmadi, & McClements,
249 2016), iii) the fusion of the emulsifier itself with the cell membrane allowing the essential oil subcellular
250 size particles to reach their target in the cytosol (Yegin et al., 2015), iv) a sustained and gradual release of
251 bioactive molecules from the emulsified nanodrops, and v) the electrostatic interaction of positively charged
252 nanodroplets attracted to the negatively charged microbial cells (Donsì & Ferrari, 2016). Therefore,
253 nanoemulsified linalool is a promising technology for disinfection in the food industry.

255 **3.2 Antimicrobial treatments on RTE chicken meat**

256 **3.2.1 Linalool nanoemulsion washing**

257 The results from linalool nanoemulsion washing (LW) treatment on RTE chicken meat by immersion are
258 shown in Figure 3A. Results showed a similar antimicrobial effect on both pathogens with no significant
259 difference between them ($P > 0.05$). A 5 min wash reduced the pathogen counts by 0.88 and 0.76 log CFU/g
260 for *Salmonella* and *E. coli* O157:H7, respectively. Moreover, a gradual decrease in the levels of the
pathogens was observed as the washing time increased, which resulted in a 1.83 and 1.67 log CFU/g

reduction for *Salmonella* and *E. coli* O157:H7, respectively. No significant difference was found between the results obtained during 15 and 25 min of exposure for both strains ($P>0.05$).

Washing with water as a first step is a common practice in the food industry for lowering microbial load. Previous studies reported that water washing led to a small reduction of *E. coli* levels (0.26 - 0.40 log CFU/mL) in beef (Stratakos & Grant, 2018). Our results demonstrate that LW applied to RTE chicken meat lowers the microbial load reducing the risk from foodborne pathogens. Similar studies have been reported of EO nanoemulsions applied on different types of meat. Stratakos and Grant (2018) reported a reduction of *E. coli* cells on beef after 5 min wash with carvacrol (1.13 log) and thyme EO (1.09 log) nanoemulsions. Moraes-Lovison et al. (2017) reported a reduction of approximately 1.5 log CFU/g for *E. coli* after 3 days of storage when oregano (*Origanum vulgare*) EO nanoemulsion (5g essential oil 100/g nanoemulsion) was incorporated into chicken pâté. To the best of our knowledge, this is the first report demonstrating the potential of linalool nanoemulsion wash on chicken RTE chicken meat for its antimicrobial capabilities.

3.2.2 Cold atmospheric plasma treatment

In the current study, a piezoelectric direct discharge generator was utilised to produce cold non-equilibrium plasma using ambient air. The effect of cold atmospheric plasma at different short exposure times (0 - 5 min) against *E. coli* O157:H7 and *Salmonella* on the surface of RTE chicken meat is presented in Figure 3B. The antimicrobial activity exhibited by CAP on RTE chicken breast was time dependent showing similar reduction in both pathogens. Approximately 1-log CFU/g reductions were achieved after 1 min treatment and the highest reductions were 1.8 log and 1.47 CFU/g for *Salmonella* and *E. coli* O157 respectively, after 5 min of treatment. The time dependent antimicrobial effect observed can be attributed to an accumulation of reactive oxygen and nitrogen species (RONS) (Tappi et al., 2016). No significant difference was found between the counts of the two pathogens at 2 min or longer treatments ($P > 0.05$). According to Timmerman et al. (2021), the air-fed cold plasma system utilised in this study has been found to produce O_3 , NO_2 , and NO with the temperature of the produced plasma reaching around 30 °C. When these different reactive species in cold plasma come in contact with the bacteria present on food, it leads to

breaking of structural chemical bonds (e.g. C–O, C–C) in the cell membrane causing damage to it. These reactive species can also enter the bacterial cell through the compromised cell membrane and further interact with intracellular components thus leading to cell death (Pasquali et al. 2016).

Previous studies have shown the antimicrobial potential of CAP on raw chicken meat. For instance, DBD plasma treatment for 3 minutes showed a reduction of up to 2.54 log of *S. enterica* inoculated on skinless chicken breast, whereas reductions of 1.31 log were achieved when treating pieces without the chicken skin removed (Dirks et al., 2012). A recent study on RTE chicken meat, showed that a 39 kV DBD in package cold plasma treatment applied for 3.5 min on RTE chicken cubes resulted in a 3.9 log CFU/cube and 3.7 log CFU/cube for *E. coli* O157:H7 and *Salmonella*, respectively (Roh, Oh, Lee, Kang, & Min, 2020). Given that the RTE chicken meat is already cooked, changes in the meat structure occur under high temperature during the cooking process thus forming a new surface topography (Bertram, Engelsen, Busk, Karlsson, & Andersen, 2004). It has been reported that bacterial cell deposition into the fissures existent in chicken meat tissue may protect bacteria against reactive species thus affecting the antimicrobial effectiveness of CAP (Noriega, Shama, Laca, Díaz, & Kong, 2011). Therefore differences in the inactivation levels achieved between studies could at least partially be attributed to different surface topographies, however, other factors such as the type and configuration of the CAP system can also influence inactivation efficiency. To the best of our knowledge, this is the first report of a low-voltage CAP system tested for meat surface decontamination achieving microbial reductions comparable to those achieved by high-voltage systems.

Based on the current results, both cold plasma and linalool nanoemulsion washing were shown to be effective against both pathogens. To facilitate adoption of cold plasma decontamination in the food industry, it is important to investigate the effect of cold plasma in combination with other interventions. Such an approach would assist with improving food safety without compromising food quality. Therefore, in this study we also investigated the efficiency of a combination of cold plasma and linalool nanoemulsion wash.

3.2.3. Combination of Cold plasma and linalool nanoemulsion washing

Figure 4 presents the effect of the combination of cold plasma and nanoemulsion washing on the inactivation of *E. coli* O157:H7 and *Salmonella* in RTE chicken meat. Cold plasma (for 5 min) and nanoemulsion washing (for 25 min) treatments were applied one after the other at two different sequences. The combined treatments significantly reduced the levels of both pathogens ($P < 0.001$) compared to the control. Specifically, for *E. coli* O157:H7, when the nanoemulsion wash was followed by CAP (LW/CAP), it resulted in a 2.38 log CFU/g reduction whereas when the CAP treatment was applied first (CAP/LW) the reduction was 2.76 log CFU/g. In addition, the LW/CAP treatment resulted in a 2.96 log CFU/g of *Salmonella*. When CAP/LW was applied, the pathogen levels dropped below the detection limit (>3.24 log CFU/g reduction) resulting in a more pronounced reduction compared to the reverse sequence (Fig. 4B).

Considering that washing with water may reduce the bacterial population by 0.26 – 0.40 log (Stratakos & Grant, 2018), cell reductions were calculated by taking the control wash value as baseline (washing with buffer only). The resulting reduction after the combination of both methods is comparable to the sum of the reduction of both techniques applied individually. This indicates that the two methods have an additive antimicrobial effect, with inactivation levels significantly higher than when the hurdles were applied individually.

We hypothesised that the more pronounced antimicrobial effect observed for both pathogens when CAP was applied first could be attributed to the mechanical etching effect on the cell membrane resulting in pore formation (i.e. electroporation) (Huang et al., 2020), or sub-lethal bacterial injuries (Govaert et al., 2019), making them more susceptible to linalool penetration thus increasing the killing efficiency. Future research should take into account any possible overestimation of the treatments' efficacy due to sub-lethal injuries. Previous studies have shown that the combination of different methods with CAP may achieve increased bacterial reduction. Cui, Wu, Li, & Lin (2017) reported a synergistic effect, achieving a reduction of 2.8 log in *L. monocytogenes* levels when combining lemongrass essential oil (5 mg/mL, 30 min) with cold nitrogen plasma treatment (2 min) in pork loin, whereas applying these treatments individually resulted in

a 0.58 log and 0.96 log reduction, respectively. Also, Lis et al., (2018) applied CAP to RTE sliced ham fillets, achieving reductions of 1.14 log in *S. Typhimurium* and 1.02 log in *L. monocytogenes* after 20 min of treatment. Higher reductions were observed after combining CAP with cold storage at 8 °C packed in a controlled atmosphere (1.84 log for *Salmonella* and 2.55 log for *L. monocytogenes*). The results of this study demonstrate that the combination of CAP followed by LW can significantly increase food safety resulting in a very promising strategy that could be adopted by the meat industry.

3.3 Lipid oxidation

Application of CAP on food surfaces could result in increased lipid oxidation leading to negative effects of organoleptic properties and a reduced shelf life (Dirks et al., 2012). Lipid oxidation products confer unpleasant odours and rancid taste to meat. Malondialdehyde (MDA) is a polyunsaturated fatty acid oxidation product and is considered to be a major marker of lipid oxidation (Moutiq, Misra, Mendonça, & Keener, 2020). The RONS generated by CAP may initiate lipid oxidation on the meat surface, such as pork, beef, chicken and seafood (Gavahian, Chu, Mousavi Khaneghah, Barba, & Misra, 2018). Thus, the effect of the combined treatments on lipid oxidation, as levels of MDA in RTE chicken, was determined. The results in Figure 5 showed that although the treated samples had a slightly higher lipid oxidation level (0.36 and 0.34 mgMDA/kg for LW/CAP and CAP/LW, respectively) compared to the control samples (0.32 and 0.31 mgMDA/kg for control and control-wash, respectively), the differences were not significant ($P > 0.05$). The high standard deviation observed can be attributed to the high variation within meat samples. It is evident from the results that for both treatments the mean lipid oxidation values were maintained at very low levels. The mean lipid oxidation values were much lower compared to the values where oxidation starts impacting the sensory properties on meat products, which is generally 2 - 2.5 mg MDA/kg (Domínguez et al., 2019). This is in agreement with previous studies showing that chicken breast is more stable to plasma-induced oxidation than red meats due to its lower content of fat, ferric heme pigment, and myoglobin (Gavahian et al., 2018; Lee et al., 2016). Further studies are required to evaluate the organoleptic effects, including any potential effects on flavour, that CAP alone or combined with LW might cause to the RTE cooked chicken meat. Although this work has focused on Gram-negative bacteria, other relevant pathogens

for the RTE-food industry, such as *L. monocytogenes*, will also need to be investigated in future experiments.

Conclusions

This study demonstrated that cold plasma and linalool nanoemulsion wash are effective against *Salmonella* and *E. coli* O157:H7. Also, combining the two methods can significantly enhance the decontamination effect against these pathogens on RTE cooked chicken meat without affecting lipid oxidation, an important meat quality indicator. These methods can be applied before the meat is packaged and sealed to increase the safety margin of RTE meat. Although the cost associated with the application of the two methods was beyond the scope of the study, the nanoemulsion production, and the running of the cold plasma device (low-voltage) using atmospheric air, costs are expected to be low. The use of linalool could potentially increase the cost, however since it is utilised in small quantities the cost is not anticipated to increase substantially. These results provide a strong platform for facilitating the commercial adoption of cold plasma by the food industry.

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Conflict of interest:

The authors declare that there is no conflict of interest.

References

Bertram, H. C., Engelsens, S. B., Busk, H., Karlsson, A. H., & Andersen, H. J. (2004). Water properties

during cooking of pork studied by low-field NMR relaxation: Effects of curing and the RN--gene.
Meat Science. [https://doi.org/10.1016/S0309-1740\(03\)00132-3](https://doi.org/10.1016/S0309-1740(03)00132-3)

Carrasco, E., Morales-Rueda, A., & García-Gimeno, R. M. (2012). Cross-contamination and
recontamination by Salmonella in foods: A review. *Food Research International*, 45(2), 545–556.
<https://doi.org/10.1016/j.foodres.2011.11.004>

CDC. (2021). Center for Disease Control and Prevention (CDC) National outbreak reporting system (2021).
Retrieved April 17, 2021, from <https://wwwn.cdc.gov/norsdashboard/>

Chang, Y., McLandsborough, L., & McClements, D. J. (2013). Physicochemical properties and
antimicrobial efficacy of carvacrol nanoemulsions formed by spontaneous emulsification. *Journal of*
Agricultural and Food Chemistry, 61(37), 8906–8913. <https://doi.org/10.1021/jf402147p>

Chen, J. H., Ren, Y., Seow, J., Liu, T., Bang, W. S., & Yuk, H. G. (2012). Intervention Technologies for
Ensuring Microbiological Safety of Meat: Current and Future Trends. *Comprehensive Reviews in*
Food Science and Food Safety, 11(2), 119–132. <https://doi.org/10.1111/j.1541-4337.2011.00177.x>

CLSI. (2017). *Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement*
M100. Wayne, PA: Clinical and Laboratory Standards Institute. *Performance standards for*
antimicrobial susceptibility testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and
Laboratory Standards Institute. <https://doi.org/10.1039/C4DT01694G>

Cristani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M. G., Micieli, D., ... Trombetta, D.
(2007). Interaction of four monoterpenes contained in essential oils with model membranes:
Implications for their antibacterial activity. *Journal of Agricultural and Food Chemistry*, 55(15),
6300–6308. <https://doi.org/10.1021/jf070094x>

Cui, H., Wu, J., Li, C., & Lin, L. (2017). Promoting anti-listeria activity of lemongrass oil on pork loin by
cold nitrogen plasma assist. *Journal of Food Safety*, 37(2), 1–10. <https://doi.org/10.1111/jfs.12316>

406 Delgado-Pando, G., Cofrades, S., Ruiz-Capillas, C., Solas, M. T., Triki, M., & Jiménez-Colmenero, F.
 407 (2011). Low-fat frankfurters formulated with a healthier lipid combination as functional ingredient:
 408 Microstructure, lipid oxidation, nitrite content, microbiological changes and biogenic amine
 409 formation. *Meat Science*, 89(1), 65–71. <https://doi.org/10.1016/j.meatsci.2011.03.022>

410 Dirks, B. P., Dobrynin, D., Fridman, G., Mukhin, Y., Fridman, A., & Quinlan, J. J. (2012). Treatment of
 411 raw poultry with nonthermal dielectric barrier discharge plasma to reduce campylobacter jejuni and
 412 salmonella enterica. *Journal of Food Protection*, 75(1), 22–28. [https://doi.org/10.4315/0362-](https://doi.org/10.4315/0362-028X.JFP-11-153)
 413 028X.JFP-11-153

414 Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A
 415 comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*, 8(10), 1–31.
 416 <https://doi.org/10.3390/antiox8100429>

417 Donsì, F., & Ferrari, G. (2016). Essential oil nanoemulsions as antimicrobial agents in food. *Journal of*
 418 *Biotechnology*, 233, 106–120. <https://doi.org/10.1016/j.jbiotec.2016.07.005>

419 EFSA, E. F. S. A., & ECDC, E. C. for D. P. and C. (2018). Multi-country outbreak of Salmonella Agona
 420 infections possibly linked to ready-to-eat food. *EFSA Supporting Publications*, 15(7), 1465E.
 421 <https://doi.org/10.2903/sp.efsa.2018.EN-1465>

422 Gao, Z., Nostrand, J. D. Van, Zhou, J., Zhong, W., Van Nostrand, J. D., Zhou, J., ... Guo, J. (2019). Anti-
 423 listeria Activities of Linalool and Its Mechanism Revealed by Comparative Transcriptome Analysis.
 424 *Frontiers in Microbiology*, 10(December), 1–13. <https://doi.org/10.3389/fmicb.2019.02947>

425 Gavahian, M., Chu, Y. H., Mousavi Khaneghah, A., Barba, F. J., & Misra, N. N. (2018). A critical analysis
 426 of the cold plasma induced lipid oxidation in foods. *Trends in Food Science and Technology*,
 427 77(April), 32–41. <https://doi.org/10.1016/j.tifs.2018.04.009>

428 Gök, V., Aktop, S., Özkan, M., & Tomar, O. (2019). The effects of atmospheric cold plasma on inactivation

429 of *Listeria monocytogenes* and *Staphylococcus aureus* and some quality characteristics of pastirma—
 430 A dry-cured beef product. *Innovative Food Science and Emerging Technologies*, 56(November 2018),
 431 102188. <https://doi.org/10.1016/j.ifset.2019.102188>

432 Gonzalez-gonzalez, C. R., Hindle, B. J., Saad, S., & Stratakos, A. C. (2021). Inactivation of *Listeria*
 433 *monocytogenes* and *Salmonella* on stainless steel by a piezoelectric cold atmospheric plasma
 434 generator. *Applied Sciences (Switzerland)*, 11(8). <https://doi.org/10.3390/app11083567>

435 Govaert, M., Smet, C., Vergauwen, L., Ećimović, B., Walsh, J. L., Baka, M., & Van Impe, J. (2019).
 436 Influence of plasma characteristics on the efficacy of Cold Atmospheric Plasma (CAP) for
 437 inactivation of *Listeria monocytogenes* and *Salmonella Typhimurium* biofilms. *Innovative Food*
 438 *Science and Emerging Technologies*, 52(January), 376–386.
 439 <https://doi.org/10.1016/j.ifset.2019.01.013>

440 Huang, M., Zhuang, H., Zhao, J., Wang, J., Yan, W., & Zhang, J. (2020). Differences in cellular damages
 441 induced by dielectric barrier discharge plasma between *Salmonella typhimurium* and *Staphylococcus*
 442 *aureus*. *Bioelectrochemistry*, 132, 107445. <https://doi.org/10.1016/j.bioelechem.2019.107445>

443 Kalily, E., Hollander, A., Korin, B., Cymerman, I., & Yaron, S. (2016). Mechanisms of resistance to linalool
 444 in *Salmonella* Senftenberg and their role in survival on basil. *Environmental Microbiology*, 18(11),
 445 3673–3688. <https://doi.org/10.1111/1462-2920.13268>

446 Keener, K. M., Bashor, M. P., Curtis, P. A., Sheldon, B. W., & Kathariou, S. (2004). Comprehensive
 447 Review of *Campylobacter* and Poultry Processing. *Comprehensive Reviews in Food Science and Food*
 448 *Safety*. <https://doi.org/10.1111/j.1541-4337.2004.tb00060.x>

449 Kisluk, G., Kalily, E., & Yaron, S. (2013). Resistance to essential oils affects survival of *Salmonella*
 450 *enterica* serovars in growing and harvested basil. *Environmental Microbiology*.
 451 <https://doi.org/10.1111/1462-2920.12139>

452 Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., ... Davies,
 453 R. (2019). Salmonella control in poultry flocks and its public health impact. *EFSA Journal*, 17(2),
 454 e05596. Retrieved from <https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5596>

455 Lee, H., Yong, H. I., Kim, H. J., Choe, W., Yoo, S. J., Jang, E. J., & Jo, C. (2016). Evaluation of the
 456 microbiological safety, quality changes, and genotoxicity of chicken breast treated with flexible thin-
 457 layer dielectric barrier discharge plasma. *Food Science and Biotechnology*, 25(4), 1189–1195.
 458 <https://doi.org/10.1007/s10068-016-0189-1>

459 Lis, K. A., Boulaaba, A., Binder, S., Li, Y., Kehrenberg, C., Zimmermann, J. L., ... Ahlfeld, B. (2018).
 460 Inactivation of Salmonella Typhimurium and Listeria monocytogenes on ham with nonthermal
 461 atmospheric pressure plasma. *PLoS ONE*, 13(5), 1–21. <https://doi.org/10.1371/journal.pone.0197773>

462 Moghimi, R., Ghaderi, L., Rafati, H., Aliahmadi, A., & McClements, D. J. (2016). Superior antibacterial
 463 activity of nanoemulsion of Thymus daenensis essential oil against E. coli. *Food Chemistry*, 194, 410–
 464 415. <https://doi.org/10.1016/j.foodchem.2015.07.139>

465 Moraes-Lovison, M., Marostegan, L. F. P., Peres, M. S., Menezes, I. F., Ghiraldi, M., Rodrigues, R. A. F.,
 466 ... Pinho, S. C. (2017). Nanoemulsions encapsulating oregano essential oil: Production, stability,
 467 antibacterial activity and incorporation in chicken pâté. *LWT - Food Science and Technology*, 77,
 468 233–240. <https://doi.org/10.1016/j.lwt.2016.11.061>

469 Moutiq, R., Misra, N. N., Mendonça, A., & Keener, K. (2020). In-package decontamination of chicken
 470 breast using cold plasma technology: Microbial, quality and storage studies. *Meat Science*,
 471 159(September 2019), 107942. <https://doi.org/10.1016/j.meatsci.2019.107942>

472 Niemira, B. A., Boyd, G., & Sites, J. (2018). Cold Plasma Inactivation of Escherichia coli O157:H7
 473 Biofilms. *Frontiers in Sustainable Food Systems*, 2(August), 1–7.
 474 <https://doi.org/10.3389/fsufs.2018.00047>

475 Noriega, E., Shama, G., Laca, A., Díaz, M., & Kong, M. G. (2011). Cold atmospheric gas plasma
 476 disinfection of chicken meat and chicken skin contaminated with *Listeria innocua*. *Food*
 477 *Microbiology*, 28(7), 1293–1300. <https://doi.org/10.1016/j.fm.2011.05.007>

478 Pasquali, F., Stratakis, A. C., Koidis, A., Berardinelli, A., Cevoli, C., Ragni, L., ... Trevisani, M. (2016).
 479 Atmospheric cold plasma process for vegetable leaf decontamination: A feasibility study on radicchio
 480 (red chicory, *Cichorium intybus* L.). *Food Control*, 60, 552–559.
 481 <https://doi.org/10.1016/j.foodcont.2015.08.043>

482 Pereira, I., Severino, P., Santos, A. C., Silva, A. M., & Souto, E. B. (2018). Linalool bioactive properties
 483 and potential applicability in drug delivery systems. *Colloids and Surfaces B: Biointerfaces*,
 484 171(August), 566–578. <https://doi.org/10.1016/j.colsurfb.2018.08.001>

485 Politano, V. T., Lewis, E. M., Hoberman, A. M., Christian, M. S., Diener, R. M., & Api, A. M. (2008).
 486 Evaluation of the developmental toxicity of linalool in rats. *International Journal of Toxicology*,
 487 27(2), 183–188. <https://doi.org/10.1080/10915810801977948>

488 Prakash, A., Vadivel, V., Rubini, D., & Nithyanand, P. (2019). Antibacterial and antibiofilm activities of
 489 linalool nanoemulsions against *Salmonella Typhimurium*. *Food Bioscience*, 28(January), 57–65.
 490 <https://doi.org/10.1016/j.fbio.2019.01.018>

491 Quesada, J., Sendra, E., Navarro, C., & Sayas-Barberá, E. (2016). Antimicrobial Active Packaging
 492 including Chitosan Films with *Thymus vulgaris* L. Essential Oil for Ready-to-Eat Meat. *Foods*.
 493 <https://doi.org/10.3390/foods5030057>

494 R-Core-Team. (2019). R: A Language and Environment for Statistical Computing. Vienna, Austria: R
 495 Foundation for Statistical Computing. Retrieved from <https://www.r-project.org>

496 Rivas, L., McDonnell, M. J., Burgess, C. M., O'Brien, M., Navarro-Villa, A., Fanning, S., & Duffy, G.
 497 (2010). Inhibition of verocytotoxigenic *Escherichia coli* in model broth and rumen systems by

498 carvacrol and thymol. *International Journal of Food Microbiology*.
 499 <https://doi.org/10.1016/j.ijfoodmicro.2010.01.029>

500 Roh, S. H., Oh, Y. J., Lee, S. Y., Kang, J. H., & Min, S. C. (2020). Inactivation of Escherichia coli O157:H7,
 501 Salmonella, Listeria monocytogenes, and Tulane virus in processed chicken breast via atmospheric
 502 in-package cold plasma treatment. *Lwt*, 127(March), 1–7. <https://doi.org/10.1016/j.lwt.2020.109429>

503 Rouger, A., Tresse, O., & Zagorec, M. (2017). Bacterial Contaminants of Poultry Meat: Sources, Species,
 504 and Dynamics. *Microorganisms*, 5(3), 50. <https://doi.org/10.3390/microorganisms5030050>

505 Stratakis, A. C., Delgado-Pando, G., Linton, M., Patterson, M. F., & Koidis, A. (2015). Synergism between
 506 high-pressure processing and active packaging against Listeria monocytogenes in ready-to-eat
 507 chicken breast. *Innovative Food Science and Emerging Technologies*, 27, 41–47.
 508 <https://doi.org/10.1016/j.ifset.2014.11.005>

509 Stratakis, A. C., & Grant, I. R. (2018). Evaluation of the efficacy of multiple physical, biological and
 510 natural antimicrobial interventions for control of pathogenic Escherichia coli on beef. *Food*
 511 *Microbiology*, 76(January), 209–218. <https://doi.org/10.1016/j.fm.2018.05.011>

512 Stratakis, A. C., & Koidis, A. (2015). Suitability, efficiency and microbiological safety of novel physical
 513 technologies for the processing of ready-to-eat meals, meats and pumpable products. *International*
 514 *Journal of Food Science and Technology*. <https://doi.org/10.1111/ijfs.12781>

515 Tappi, S., Gozzi, G., Vannini, L., Berardinelli, A., Romani, S., Ragni, L., & Rocculi, P. (2016). Cold plasma
 516 treatment for fresh-cut melon stabilization. *Innovative Food Science and Emerging Technologies*.
 517 <https://doi.org/10.1016/j.ifset.2015.12.022>

518 Timmermann, E., Bansemer, R., Gerling, T., Hahn, V., Weltmann, K. D., Nettesheim, S., & Puff, M.
 519 (2021). Piezoelectric-driven plasma pen with multiple nozzles used as a medical device: Risk
 520 estimation and antimicrobial efficacy. *Journal of Physics D: Applied Physics*, 54(2), 025201.

<https://doi.org/10.1088/1361-6463/abb900>

- Tripathi, A. K., & Mishra, S. (2016). Chapter 16 - Plant Monoterpenoids (Prospective Pesticides). In B. T.-E. P. M. for F. S. Omkar (Ed.) (pp. 507–524). San Diego: Academic Press.
<https://doi.org/10.1016/B978-0-12-803265-7.00016-6>
- Xiang, Q., Liu, X., Li, J., Ding, T., Zhang, H., Zhang, X., & Bai, Y. (2018). Influences of cold atmospheric plasma on microbial safety, physicochemical and sensorial qualities of meat products. *Journal of Food Science and Technology*. Springer India. <https://doi.org/10.1007/s13197-017-3020-y>
- Yang, X. N., Khan, I., & Kang, S. C. (2015). Chemical composition, mechanism of antibacterial action and antioxidant activity of leaf essential oil of Forsythia koreana deciduous shrub. *Asian Pacific Journal of Tropical Medicine*. <https://doi.org/10.1016/j.apjtm.2015.07.031>
- Yegin, Y., Perez-Lewis, K. L., Zhang, M., Akbulut, M., & Taylor, T. M. (2015). Development and characterization of geraniol-loaded polymeric nanoparticles with antimicrobial activity against foodborne bacterial pathogens. *Journal of Food Engineering*, 170, 64–71.
<https://doi.org/10.1016/j.jfoodeng.2015.09.017>
- Zengin, H., & Baysal, A. H. (2014). Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules*, 19(11), 17773–17798. <https://doi.org/10.3390/molecules191117773>
- Zhang, Y., Liu, X., Wang, Y., Jiang, P., & Quek, S. Y. (2016). Antibacterial activity and mechanism of cinnamon essential oil against Escherichia coli and Staphylococcus aureus. *Food Control*.
<https://doi.org/10.1016/j.foodcont.2015.05.032>
- Zhu, H., Du, M., Fox, L., & Zhu, M. J. (2016). Bactericidal effects of Cinnamon cassia oil against bovine mastitis bacterial pathogens. *Food Control*, 66, 291–299.
<https://doi.org/10.1016/j.foodcont.2016.02.013>

544

545 **Figure Legends**

546 **Figure 1.** Schematic representation of the CAP system Piezobrush ® PZ2 utilising piezoelectric direct
547 discharge (PDD®) technology with a standard nozzle. Adapted from Timmermann et al. (2021).

548 **Figure 2.** Killing assay comparison of the antimicrobial activity of linalool nanoemulsion against
549 *Salmonella* and *E. coli* O157:H7. Meat simulation medium without linalool nanoemulsion was used as
550 control. The detection limit was 1 log CFU g⁻¹. Each point represents the mean ± standard deviation (n=3).

551 **Figure 3.** A) Effect of linalool nanoemulsion washing on RTE chicken meat after 0 (control), 5, 10, 15,
552 20, 25 min of treatment, and B) effect of cold atmospheric plasma on RTE chicken meat after 0 (control),
553 1, 2, 3, 4, 5 min of treatment. The detection limit was 2 log CFU/g. Each point represents the
554 mean ± standard deviation (n=3).

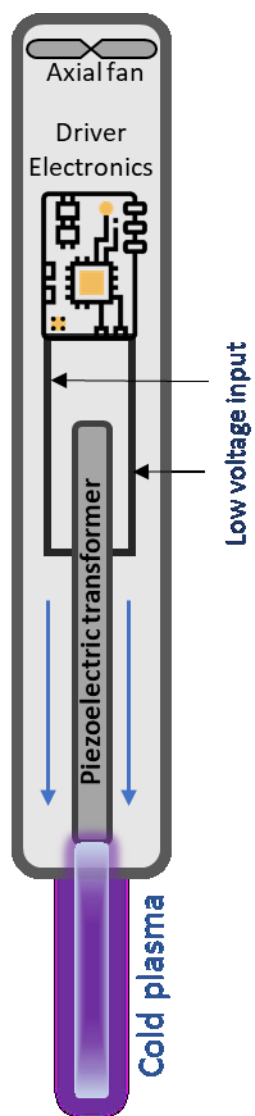
555 **Figure 4.** Comparison of combined antimicrobial treatments: cold atmospheric plasma (CAP) for 5 min
556 followed by linalool emulsion washing (LW) for 25 min or vice versa for A) *E. coli* O157:H7 and B)
557 *Salmonella*. * Counts for CAP/LW in *Salmonella* were below the detection limit represented by the dashed
558 line. The bars represent the mean ± standard deviation (n=3).

559 **Figure 5.** Lipid oxidation value expressed as mg of malondialdehyde (MDH)/kg on RTE chicken meat after
560 cold atmospheric plasma (CAP; 5 min) treatment followed by washing in linalool nanoemulsion (LW; 25
561 min) or vice versa (LW/CAP). Control = no wash, no CAP. Control = wash with only buffer and no CAP.
562 The bars represent the mean ± standard deviation (n=4).

563 **Figure A1.** Cell permeability assay conducted by measuring relative electric conductivity in cells of
564 *Salmonella* and *E. coli* O157:H7 exposed to control (buffer) and nanoemulsified linalool. The bars represent
565 the mean ± standard deviation (n=3).

566

567 Figure 1



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569

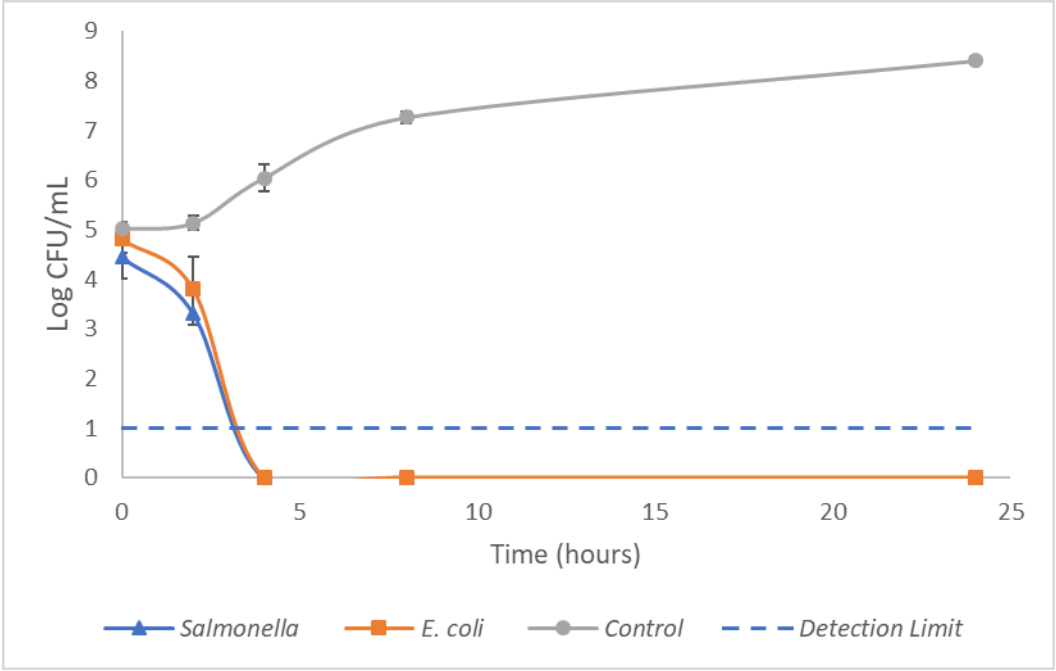
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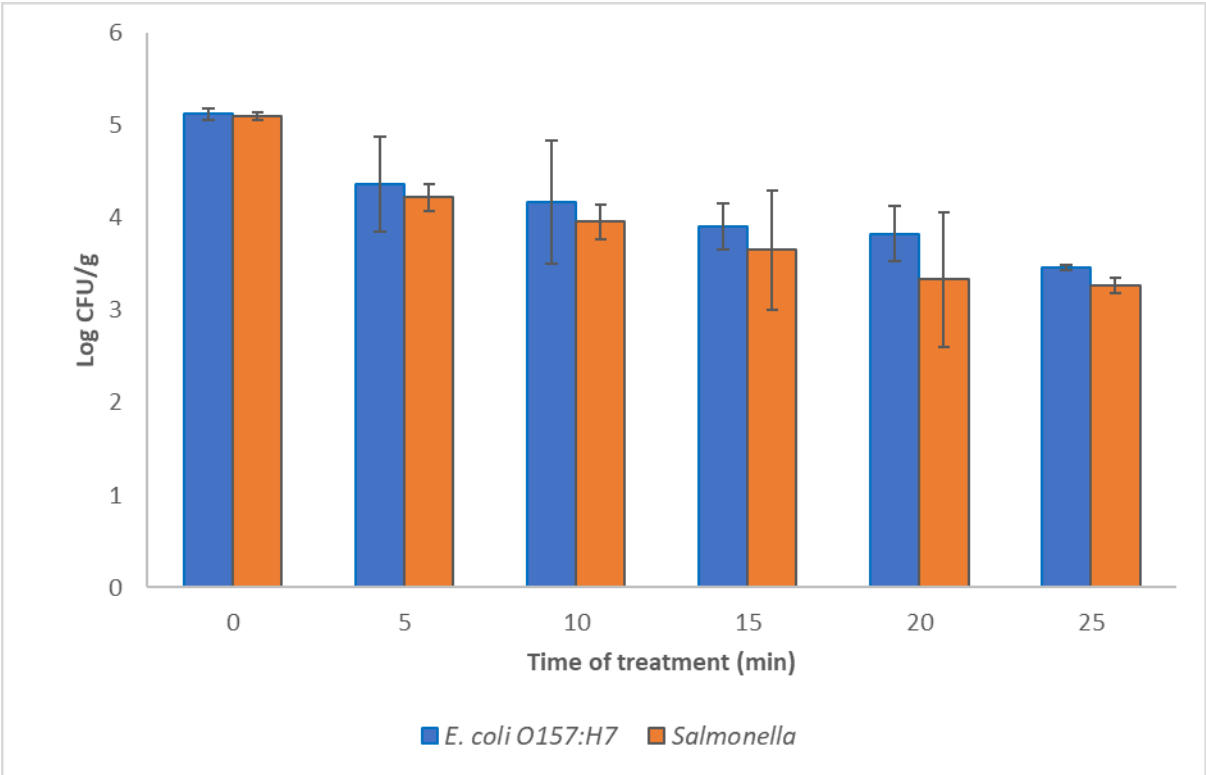
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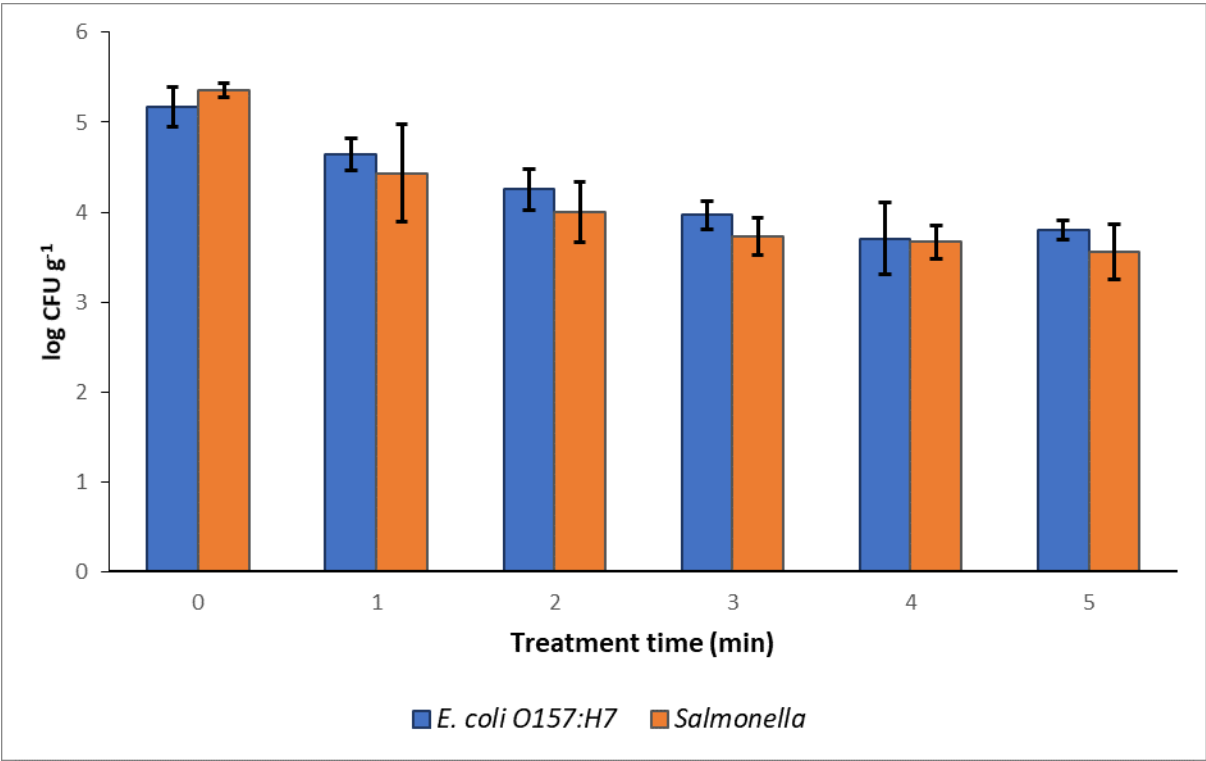
Figure 2



586 Figure 3

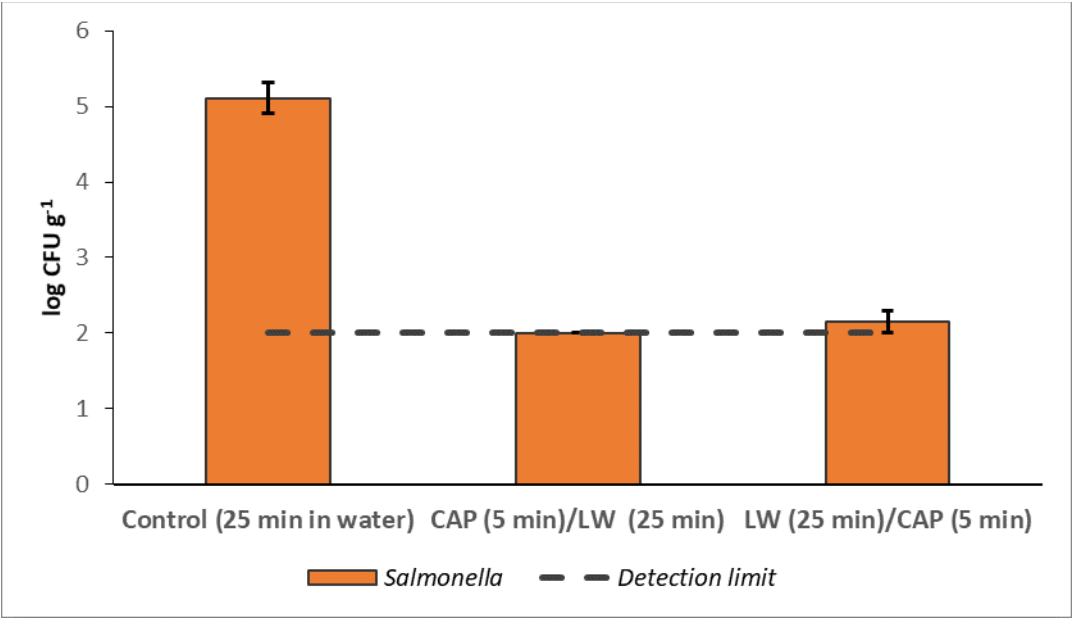
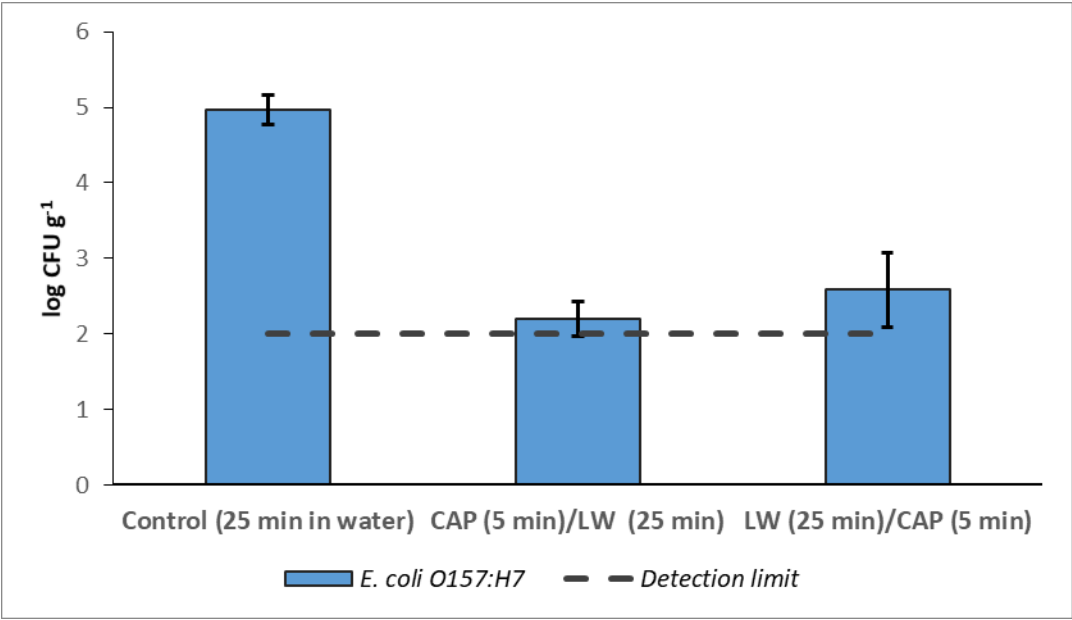


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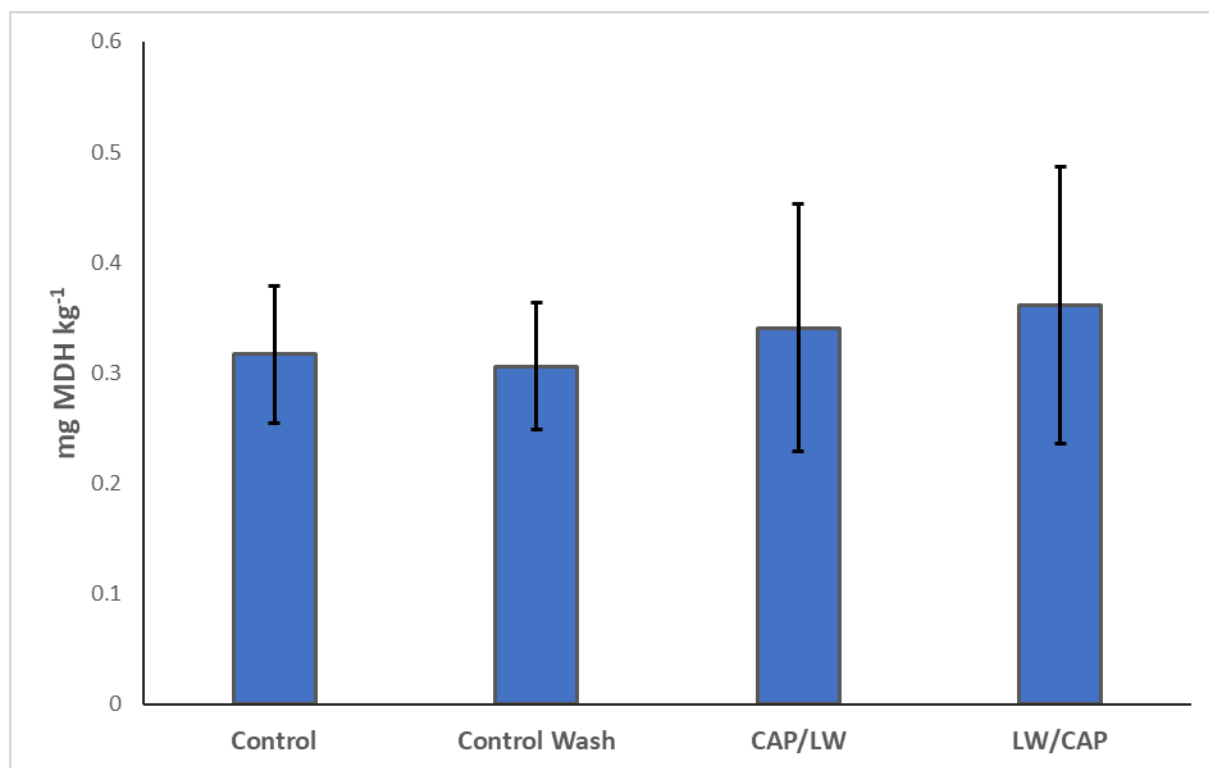


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589 Figure 4



595 Figure 5



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