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

1

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.

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

26   the market. It does not require further preparation before consumption, unless preheating is chosen by the

27   consumer for enhancing palatability. At the same time, although RTE foods offer many advantages, they

28   have been linked to foodborne disease outbreaks (Stratakos & Koidis, 2015). Surface cross-contamination

29   of RTE chicken with foodborne pathogenic bacteria may occur during the packaging stage or post-treatment

30   manipulation due to contact with contaminated surfaces or water (Carrasco, Morales-Rueda, & García-

31   Gimeno, 2012). *Salmonella* and *Escherichia coli* O157:H7 are amongst the most important and common

32   pathogenic Gram-negative bacteria responsible for foodborne diseases worldwide in meat (Koutsoumanis

33   et al., 2019; Rouger, Tresse, & Zagorec, 2017). Several outbreaks involving these two pathogens have been

34   reported due to the consumption of poultry meat and poultry containing products (EFSA & ECDC, 2018;

35   Koutsoumanis et al., 2019; Niemira, Boyd, & Sites, 2018). According to the Center for Disease Control

36   and Prevention (CDC, 2021) numerous outbreaks in the USA for the period 2011-2018 have been linked

37   to products that contain RTE chicken meat (e.g. chicken rolls, chicken salad, and grilled chicken). Among

38   them, *E. coli* was the cause of 7 outbreaks, 146 illnesses, and 26 hospitalisations whereas *Salmonella* has

39   been the cause of 9 outbreaks, 698 illnesses, and 112 hospitalizations. Contamination of chicken meat in

40   RTE products can be a result of cross-contamination from the fresh-cut vegetables that are contained with

41   the meat, during the packaging stage or post-treatment manipulation due to contact with contaminated food

42   contact surfaces, water or food handlers (Carrasco et al., 2012). To increase microbiological safety, food

43   processors utilise a wide range of food treatments, including the use of organic acids (e.g., acetic, lactic,

44   citric acid) as well as chlorine, chlorine dioxide, trisodium phosphate and acidified sodium chlorite (Keener,

45   Bashor, Curtis, Sheldon, & Kathariou, 2004). However, the increasing consumers' concern about the safety

46   of chemical treatments of food has forced processors to look for alternative decontamination strategies.

47   The use of essential oils (EO) as a decontamination method against yeast, moulds, and bacteria has been

48   reported (Quesada, Sendra, Navarro, & Sayas-Barberá, 2016; Zhang, Liu, Wang, Jiang, & Quek, 2016).

49   Linalool is a monoterpenoid with a potent antimicrobial activity which has been found in EO produced

50   from basil, rosewood, and citrus (Gao et al., 2019; Yang, Khan, & Kang, 2015). It is generally recognised

51   as safe by the Food and Drug Administration (FDA) (Tripathi & Mishra, 2016), has shown low cytotoxicity

52   (Politano et al., 2008) and is an approved substance under Regulation (EC) No 1907/2006 - REACH. These

53   characteristics make linalool very promising ingredient for the food industry.

54   Cold atmospheric plasma (CAP) is an emerging processing technology extensively investigated for its

55   surface decontamination capacity. CAP has shown promising antimicrobial activity against bacteria on

56   various fruits, vegetables (Niemira et al., 2018), and meat products (Stratakos & Grant, 2018). Plasma is

57   considered the fourth state of matter, is produced by the complete or partial ionisation of a gas by application

58   of electric discharges. CAP is composed of electrons, ions, radicals, and UV photons (Pasquali et al., 2016),

59   and is commonly produced with the use of the helium, argon, and oxygen (Gök, Aktop, Özkan, & Tomar,

60   2019). CAP can be generated by using microwaves, radio frequencies, but also using various set-ups with

61   high voltage input such as i) dielectric barrier discharge (DBD) consisting of an electric discharge occurring

62   between two electrodes separated by an insulating dielectric barrier; ii) atmospheric pressure plasma jet

63   (APPJ) consisting in a pulsed electric arc generated by a high voltage discharge and a compressed gas

64   flowing through the plasma section and then ejected through a jet head; and iii) corona discharge (CD)

65   consisting in the ionisation of a neutral fluid, generally air, by a current flowing from an electrode with a

66   high potential generating a region of plasma around (Tappi et al., 2016). On the other hand, the piezoelectric

67   direct discharge (PDD), consist of a piezoelectric transformer generator that converts electric energy of low

68   voltage AC into mechanical oscillations to produce high voltage AC. This end acts as an electrode to

69   generate electric discharges ionising the air to produce a plasma state. This technology has the advantage

70   of being compact, low cost operation, and has been recently proven to effectively kill bacteria on different

71   surfaces (Gonzalez-gonzalez, Hindle, Saad, & Stratakos, 2021; Timmermann et al., 2021).

72   Studies have also shown that combination of decontamination treatments (Hurdle Technology), including

73   either a combination of physical interventions or a treatment consisting of physical, natural, and biological

74   interventions can be a more effective tool in eliminating foodborne pathogens when compared to a single

75   intervention (Chen et al., 2012). Stratakos, Delgado-Pando, Linton, Patterson, & Koidis (2015)

76 demonstrated the synergistic antimicrobial effect of high-pressure processing and essential oil-based active

77 packaging against *Listeria monocytogenes* on RTE chicken breast. CAP technology has strong application

78 potential in the food industry. Thus, to accelerate its adoption the application of this non-thermal technology

79 alone or in the combination with other interventions should be explored in depth as a means of ensuring

80 food safety and maintain quality.

81 The aim of the study was to explore for the first time the efficacy of a low-voltage piezoelectric direct

82 discharge-generated cold atmospheric plasma, and nanoemulsions loaded with linalool either alone or

83 combined, to control the health risk from *Salmonella* and *E. coli* O157:H7 in RTE chicken breast. The two

84 methods were also applied in different order to identify the most efficient combination. Finally, the effect

85 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.*

86 The antimicrobial activity of linalool (Sigma-Aldrich, UK) was evaluated against *S. enterica* serovar

87 Typhimurium DT104, *S. enterica* serovar Senftenberg, and *E. coli* NCTC 12900, which is a Shiga toxin

88 negative serotype O157:H7 strain. All three strains were activated in Tryptone Soya Agar plus 0.6% yeast

89 extract (TSAYE, Oxoid, UK) at 37 ℃ for 24 h and then maintained in slopes of the same medium at 4 ℃.

90 The antimicrobial activity of linalool was evaluated by a disc diffusion assay according to Stratakos et al.

91 (2018) by pouring one mL of inoculum of each pathogen individually containing approximately 10^7

92 CFU/mL onto TSAYE agar plates and left to dry. Sterile filter paper discs (6 mm) were impregnated with

93 linalool solution at 8% (v/v) in DSMO and placed on the inoculated agar and then incubated at 37 ℃ for

94 24 h. The diameter corresponding to the inhibition zone (DIZ) around the disc was measured. To determine

95 the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of

96 linalool, the two-fold tube dilution method was used as described by Zhu, Du, Fox, & Zhu (2016) following

97 the Clinical and Laboratory Standards Institute guidelines (CLSI, 2017). Linalool was diluted from 8% to

98 0.015625% (v/v) in Muller-Hinton broth (MHB) containing 0.15% (w/v) agar. After an overnight

99   incubation, the bacterial cultures in MHB were harvested by centrifugation, washed with phosphate buffer

100 saline solution (PBS), and diluted at approximately 6 log CFU/mL in MHB with 0.15% agar. Afterwards,

101 each tube containing linalool was inoculated with the respective bacterial suspension aiming a concentration

102 of 5 log CFU/mL and then incubated at 37ºC for 24 h. Non-inoculated tubes containing the same growth

103 medium were used as a negative control. Inoculated tubes without linalool were used as positive control.

104 The tubes with no visible growth were considered as the MIC. One hundred µL aliquots were taken from

105 the tubes that showed no visible growth and were plated onto TSAYE agar. The lowest concentration of

106 linalool that did not show microbial growth after plating out was considered as the MBC. All the

107 experiments were conducted in triplicate.

108

2.2   Preparation and characterisation of nanoemulsions loaded with linalool

109 Stable nanoemulsions loaded with linalool were prepared using the spontaneous emulsification method

110 using food grade reagents (Chang, McLandsborough, & McClements, 2013). Briefly, 4 g of linalool

111 (Sigma-Aldrich, UK), were added to 6 g of medium chain triglyceride oil (Miglyol 812, IOI Oleo, Germany)

112 and mixed for 5 min (600 rpm). Subsequently, 10 g Tween 80® (Sigma-Aldrich) was added to the oil

113 mixture and mixed for another 5 min. The Tween 80/oil mixture (20 g) was titrated (2 mL/min) into 80 g

114 of 5.0 mmol/L sodium citrate buffer (pH 6.8) and was mixed at 600 rpm for 15 min. Subsequently, the

115 emulsion was sterilised by passing through a sterile 0.22 µm syringe filter and stored in sterile 50 mL tubes

116 at 4 °C. Droplet size and polydispersity index (PDI) were measured using dynamic light scattering

117 (Zetasizer Nano ZS, Malvern Instruments, UK).

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

118 A time-kill assay for nano-emulsified linalool was carried out as follows. Nano-emulsified linalool was

119 added at a concentration of 8000 ppm in sterilised meat simulation medium consisting in 20 g of

120 bacteriological peptone, 16 g of Lab Lemco, 8 g of yeast extract, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.038 g of $MnSO_4$

121 $\cdot H_2O$, 1 mL of Tween 80, 5 g of sterile lactic acid, and 40 g of NaCl per litre of water. Overnight cultures

122 of *E. coli* O157:H7 and a cocktail (at 1:1 ratio) of *S.* Seftenberg and *S.* Typhimurium in MHB were washed

123 twice in PBS and inoculated at 5 log CFU/mL into the meat simulation medium with nano-emulsified

124 linalool solutions, mixed and incubated at 37 ℃. The samples were taken at 0, 2, 4, 8 and 24 h for cell

125 enumeration by serial dilution and plated onto TSAYE agar. Cell membrane permeability for all three

126 pathogens was indirectly measured using an electric conductivity assay to provide an insight into the

127 antimicrobial mechanism nanoemulsified linalool according to Zhang et al. (2016). Specifically, membrane

128 permeability was assessed by measuring changes in electric conductivity, using an electric conductivity

129 meter (Jenway 4200, U.K.) after treatment for 6 hours. All experiments were performed in triplicate.

2.4   Preparation of RTE chicken samples

130 Commercially manufactured RTE chicken breast fillets were procured from a local retailer and transferred

131 to the laboratory. The heat-treated (cooked) RTE samples were transferred to the lab on ice and kept under

132 refrigeration. Whole pieces of cooked chicken (10 ± 0.2 g) were prepared and inoculated as described in

133 section 2.5. The treatments were as follows:

134   i)   Cold atmospheric plasma treatment (CAP): RTE samples were treated with cold plasma for 0,

135   1, 2, 3, 4, and 5 min. These time points were chosen according to previous studies conducted

136   on meat (Stratakos & Grant, 2018; Xiang et al., 2018). Moreover, a short time period, would

137   make this technology more attractive to the food industry.

138   ii)   Nanoemulsion wash (LW): RTE samples were treated by immersion in a 1 L solution

139   containing nano-emulsified linalool at 8000 ppm for 0, 5, 10, 15, 20, and 25 min.

140   iii) Combination of cold plasma and nanoemulsion wash: RTE samples were treated with two

141   different sequential combinations: CAP/LW and LW/CAP (CAP for 5 min and LW for 25 min

142   for both sequences tested).

143

2.5   Bacterial strains used and inoculum preparation for RTE chicken breast

144 *E. coli* O157:H7 and a cocktail consisting of *Salmonella (S.) enterica* Typhimurium and *S.* Senftenberg (1:1

145 ratio) were used to artificially inoculate the RTE chicken breast. *E. coli* O157:H7 and the *Salmonella*

146 cocktail were inoculated onto the cooked meat separately. For each bacterial strain individually, a loopful

147   of a fresh Tryptone soya agar plus 0.6% yeast extract (TSAYE, Oxoid Limited, Basingstoke, UK) slope

148   culture was inoculated into 10 mL of brain heart infusion broth (BHI, Oxoid) and incubated at 37 °C for 24

149   h. Subsequently, 100 μL of a 10^{-4} dilution of this broth culture in the maximum recovery diluent (Oxoid),

150   was inoculated into another 10 mL of BHI broth and incubated at 37 °C for 24 h, until the stationary phase

151   of growth was reached. The final 10 mL cultures were harvested by centrifuging at 3600×g for 30 min,

152   washed thrice in phosphate-buffered saline (PBS), and the pellet was resuspended in a final volume of 10

153   mL PBS to achieve an approximately 8-9 log CFU/mL. To prepare the *Salmonella* cocktail, equal quantities

154   of the 2-strain cell suspensions were mixed well in a plastic 50 mL centrifuge tube to produce the 2-strain

155   cocktail. To emulate the surface contamination of RTE products, the suspension (100 μL) was inoculated

156   on the surface of chicken samples (10 ± 0.2 g) (six samples per each time point), to achieve an initial

157   inoculum level of approximately 5 log CFU/g.

2.6   Cold atmospheric plasma treatment of RTE chicken

158 The cold plasma treatment was performed using a handheld cold plasma generation device Piezobrush PZ2

159 (Relyon Plasma, Germany), which uses a Piezoelectric Direct Discharge (PDD®) Technology. This

160 technology is based on the direct electrical discharge of a piezo-ceramic transformer into a working gas

161 which is able to transform a low input voltage into a high output voltage as shown in Figure 1. The input

162 voltage of DC power was 15 V and at a frequency of 50 kHz. The plasma was produced using atmospheric

163 air, with the air feed being achieved by an internal axial fan. The treatments were performed in atmospheric

164 conditions (at approximately 22 ºC and 60% of Relative Humidity) by mounting the device on a stand in a

165 vertical position and placing the chicken meat samples on a Petri dish at 10 mm of distance from the plasma

166 source. Independent experiments were performed in triplicate.

2.7   RTE chicken treatment with nanoemulsions loaded with linalool

167 The prepared linalool nanoemulsions were diluted 5-fold to avoid a droplet size increase. After this dilution,

168 the concentration of linalool in the nanoemulsions was 8000 ppm. The meat samples (6 samples per each

169   time point) were immersed individually in 1 L of antimicrobial nanoemulsion (8000 ppm) for up to 25 min

170   and the pathogen levels were determined as described in section 2.8.

171

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

172   A 10^{-1} dilution of chicken meat sample was prepared by adding 90 mL of the maximum recovery diluent

173   (MRD) (Oxoid code CM733). The dilution was homogenised for 1 min in a Seward stomacher. Further 10-

174   fold dilutions were prepared in 9 mL MRD, as necessary. An aliquot of each of the 10-fold dilutions was

175   poured onto the plates using Sorbitol MacConkey agar with the addition of Cefixime-Tellurite (CT-

176   SMACK) and enumerated after incubation at 37 °C for 24 h. Each sample was plated out in duplicate. The

177   suspected *E. coli* O157:H7 colonies were confirmed with the *E. coli* O157 Latex test kit (DR0620). For

178   enumeration of pathogenic *Salmonella,* a 100 μl aliquot of each of the appropriate 10-fold dilutions was

179   spread plated on brilliant green agar plates (Oxoid, CM0329) and incubated at 37 °C for 24 h.

2.9   Lipid oxidation analysis

180   Lipid oxidation was measured by thiobarbituric acid reactive substances value (TBARS) as described by

181   Delgado-Pando et al., (2011). TBARS were expressed as mg of malondialdehyde (MDA) kg^{-1} meat. The

182   values were the means of duplicate measurements of 4 different samples from the same treatment. Briefly,

183   5 g samples were weighed and 35 mL of trichloroacetic acid (TCA) (7.5%) was added. Afterwards, the

184   samples were homogenised for 30 s, centrifuged at 3000×g for 2 min and then filtered. Five millilitres of

185   filtrate were transferred to plastic tubes, and 5 mL of 0.02 mol L^{-1} thiobarbituric acid (TBA) (1:1, TBA:

186   filtrate) were added. The samples were vortexed and left to stand for 20 h at 20 °C. Subsequently, the

187   samples were vortexed again, and an aliquot was used to measure the absorbance at 532 nm, equivalent to

188   malonaldehyde (MDA) concentration with the use of 98-well plates using a microplate reader FLUOstar

189   Omega (BMG Labtec, UK). The blank was prepared by adding 5 mL of TCA and 5 mL of TBA. Untreated

190   meat and chicken meat washed with distilled water only were used as negative control. The calibration

191   curve was prepared using 1,1,3,3-tetramethoxypropane.

2.10   Statistical analysis

192   A two-way ANOVA was performed to test the levels of bacteria cell enumeration using time and strains as

193   fixed factors when testing linalool nanoemulsion or CAP or a combination of both on chicken breast. Tukey

194   post-hoc analysis was carried out at a significance level of $P < 0.05$ to compare the mean values among the

195   groups. All experiments were carried out in three independent trials with at least two samples for each

196   observation. Analyses were carried out using RStudio version 1.2.1335 working with R version 3.6.1 for

197   Mac (R-Core-Team, 2019).

3   Results and Discussion

3.1   Antimicrobial activity of linalool

198   In this study, we evaluated the antimicrobial activity of linalool against *E. coli* O157:H7 and *S.*

199   Typhimurium and *S.* Senftenberg. The results (Table 1) for linalool, in terms of DIZ values, showed an

200   antibacterial effect against all strains tested. *E. coli* O157:H7 was found to have higher MIC and MBC

201   values, thus exhibiting a lower susceptibility when compared to the two *Salmonella* strains, which showed

202   the same MIC and MBC values. Since the *Salmonella* strains showed the tolerance to linalool, a cocktail of

203   both *Salmonella* strains (1:1 ratio) was prepared to perform subsequent experiments.

204   Previous studies have also shown similar linalool antimicrobial activity. DIZ values for linalool of

205   21.0 ± 1.0 mm and 12.5 ± 1.3 mm have been reported for *S.* Typhimurium and *S.* Senftenberg (Kisluk,

206   Kalily, & Yaron, 2013). Zengin & Baysal (2014) reported MIC values of 0.7% & 0.6 % against *S.*

207   Typhimurium and *E. coli* O157:H7, respectively, using linalool diluted in nutrient broth. Also, Prakash,

208   Vadivel, Rubini, & Nithyanand (2019) reported MBC and MIC values of 1.25% for *S.* Typhimurium. Any

209   differences observed between studies could be due to methodological variations (e.g. different growth

210   media) and different bacterial strains used (Rivas et al., 2010).

211   Studies on the antimicrobial mechanism of monoterpenes, such as linalool, have shown their ability to

212   damage and penetrate the cell membrane and, consequently, to interfere with cellular functions (Cristani et

213   al., 2007). Linalool increases the membrane permeability leading to the leakage of functional cell material

214   in *Salmonella* (Kalily, Hollander, Korin, Cymerman, & Yaron, 2016). Also, microscopy imaging data for

215   *L. monocytogenes* and *E. coli* O157:H7 have also shown the lytic effect of linalool on the cell wall and

216   cytoplasmic membrane the osmotic balance and causing the loss of ions and functional molecules (Gao et

217   al., 2019; Zengin & Baysal, 2014). Despite advantages linked to linalool, such as its plant origin and hypo-

218   allergenicity, it is a volatile compound with a relatively short half-life (Pereira, Severino, Santos, Silva, &

219   Souto, 2018). Moreover, its lipophilic nature causes difficulties to its application in food systems with high

220   water content. The encapsulation of linalool in nanoemulsions, provides different benefits: increased water

221   solubility, longer stability, attenuated organoleptic effects, and an increased contact surface area with the

222   bacterial cell (Pereira et al., 2018). To enhance the antimicrobial capability of linalool and circumvent

223   possible application limitations, we employed a spontaneous emulsification process to encapsulate linalool.

224   Dynamic light scattering analysis showed that the linalool nanoemulsions produced had an average particle

225   size of 103.24 ± 3.31 nm and with a PDI of 0.244 ± 0.0085.

226   The time-kill assay results in Figure 2 show the antimicrobial activity of the nanoemulsified linalool for

227   both pathogens. More specifically, exposure to linalool nanoemulsions reduced *Salmonella* and *E. coli*

228   O157 counts below the detection limit after 4 h of exposure (i.e., > 4 log CFU/mL) with *Salmonella* showing

229   a higher susceptibility to linalool nanoemulsion than *E. coli* ($P = 0.022$). The effect of the nanoemulsion

230   components (Tween 80 and medium chain triglyceride oil) on all three bacterial strains was also determined

231   by disc diffusion assay to examine if the antimicrobial effect observed could also be attributed to these

232   components. The results exhibited no inhibition zones for all strains and concentrations used (6% and 10%

233   for the medium chain fatty acids and Tween 80, respectively), showing these compounds do not contribute

234   to the antimicrobial activity under the experimental conditions used. However, previous studies have

235   suggested that the main effect of these compounds is to enhance the mass transport of EO molecules to the

236   interstitial spaces and across the cell membrane increasing their antimicrobial effect (Yegin, Perez-Lewis,

237   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.

254

255 ## 3.2   Antimicrobial treatments on RTE chicken meat

3.2.1   Linalool nanoemulsion washing

256 The results from linalool nanoemulsion washing (LW) treatment on RTE chicken meat by immersion are

257 shown in Figure 3A. Results showed a similar antimicrobial effect on both pathogens with no significant

258 difference between them (P > 0.05). A 5 min wash reduced the pathogen counts by 0.88 and 0.76 log CFU/g

259 for Salmonella and *E. coli* O157:H7, respectively. Moreover, a gradual decrease in the levels of the

260 pathogens was observed as the washing time increased, which resulted in a 1.83 and 1.67 log CFU/g

261   reduction for *Salmonella* and *E. coli* O157:H7, respectively. No significant difference was found between

262   the results obtain during 15 and 25 min of exposure for both strains (P>0.05).

263   Washing with water as a first step is a common practice in the food industry for lowering microbial load.

264   Previous studies reported that water washing led to a small reduction of *E. coli* levels (0.26 - 0.40 log

265   CFU/mL) in beef (Stratakos & Grant, 2018). Our results demonstrate that LW applied to RTE chicken meat

266   lowers the microbial load reducing the risk from foodborne pathogens. Similar studies have been reported

267   of EO nanoemulsions applied on different types of meat. Stratakos and Grant (2018) reported a reduction

268   of *E. coli* cells on beef after 5 min wash with carvacrol (1.13 log) and thyme EO (1.09 log) nanoemulsions.

269   Moraes-Lovison et al. (2017) reported a reduction of approximately 1.5 log CFU/g for *E. coli* after 3 days

270   of storage when oregano (*Origanum vulgare*) EO nanoemulsion (5g essential oil 100/g nanoemulsion) was

271   incorporated into chicken pâté. To the best of our knowledge, this is the first report demonstrating the

272   potential of linalool nanoemulsion wash on chicken RTE chicken meat for its antimicrobial capabilities.

273   **3.2.2 Cold atmospheric plasma treatment**

274   In the current study, a piezoelectric direct discharge generator was utilised to produce cold non-equilibrium

275   plasma using ambient air. The effect of cold atmospheric plasma at different short exposure times (0 - 5

276   min) against *E. coli* O157:H7 and *Salmonella* on the surface of RTE chicken meat is presented in Figure

277   3B, The antimicrobial activity exhibited by CAP on RTE chicken breast was time dependent showing

278   similar reduction in both pathogens. Approximately 1-log CFU/g reductions were achieved after 1 min

279   treatment and the highest reductions were 1.8 log and 1.47 CFU/g for *Salmonella* and *E. coli* O157

280   respectively, after 5 min of treatment. The time dependent antmicrobial effect observed can be attributed to

281   an accumulation of reactive oxygen and nitrogen species (RONS) (Tappi et al., 2016). No significant

282   difference was found between the counts of the two pathogens at 2 min or longer treatments (P > 0.05).

283   According to Timmerman et al. (2021), the air-fed cold plasma system utilised in this study has been found

284   to produce O_3, NO_2, and NO with the temperature of the produced plasma reaching around 30 °C. When

285   these different reactive species in cold plasma come in contact with the bacteria present on food, it leads to

286 breaking of structural chemical bonds (e.g. C–O, C–C) in the cell membrane causing damage to it. These

287 reactive species can also enter the bacterial cell through the compromised cell membrane and further

288 interact with intracellular components thus leading to cell death (Pasquali et al. 2016).

289 Previous studies have shown the antimicrobial potential of CAP on raw chicken meat. For instance, DBD

290 plasma treatment for 3 minutes showed a reduction of up to 2.54 log of *S. enterica* inoculated on skinless

291 chicken breast, whereas reductions of 1.31 log were achieved when treating pieces without the chicken skin

292 removed (Dirks et al., 2012). A recent study on RTE chicken meat, showed that a 39 kV DBD in package

293 cold plasma treatment applied for 3.5 min on RTE chicken cubes resulted in a 3.9 log CFU/cube and 3.7

294 log CFU/cube for *E. coli* O157:H7 and *Salmonella*, respectively (Roh, Oh, Lee, Kang, & Min, 2020). Given

295 that the RTE chicken meat is already cooked, changes in the meat structure occur under high temperature

296 during the cooking process thus forming a new surface topography (Bertram, Engelsen, Busk, Karlsson, &

297 Andersen, 2004). It has been reported that bacterial cell deposition into the fissures existent in chicken meat

298 tissue may protect bacteria against reactive species thus affecting the antimicrobial effectiveness of CAP

299 (Noriega, Shama, Laca, Díaz, & Kong, 2011). Therefore differences in the inactivation levels achieved

300 between studies could at least partialy be attributed to different surface topographies, however, other factors

301 such as the type and configuration of the CAP system can also influence inactivation efficiency. To the best

302 of our knowledge, this is the first report of a low-voltage CAP system tested for meat surface

303 decontamination achieving microbial reductions comparable to those achieved by high-voltage systems.

304 Based on the current results, both cold plasma and linalool nanoemulsion washing were shown to be

305 effective against both pathogens. To facilitate adoption of cold plasma decontamination in the food

306 industry, it is important to investigate the effect of cold plasma in combination with other interventions.

307 Such an approach would assist with improving food safety without compromising food quality. Therefore,

308 in this study we also investigated the efficiency of a combination of cold plasma and linalool nanoemulsion

309 wash.

310

3.2.3. Combination of Cold plasma and linalool nanoemulsion washing

311   Figure 4 presents the effect of the combination of cold plasma and nanoemulsion washing on the

312   inactivation of *E. coli* O157:H7 and *Salmonella* in RTE chicken meat. Cold plasma (for 5 min) and

313   nanoemulsion washing (for 25 min) treatments were applied one after the other at two different sequences.

314   The combined treatments significantly reduced the levels of both pathogens (P < 0.001) compared to the

315   control. Specifically, for *E. coli* O157:H7, when the nanoemulsion wash was followed by CAP (LW/CAP),

316   it resulted in a 2.38 log CFU/g reduction whereas when the CAP treatment was applied first (CAP/LW) the

317   reduction was 2.76 log CFU/g. In addition, the LW/CAP treatment resulted in a 2.96 log CFU/g of

318   *Salmonella*. When CAP/LW was applied, the pathogen levels dropped below the detection limit (>3.24 log

319   CFU/g reduction) resulting in a more pronounced reduction compared to the reverse sequence (Fig. 4B).

320   Considering that washing with water may reduce the bacterial population by 0.26 – 0.40 log (Stratakos &

321   Grant, 2018), cell reductions were calculated by taking the control wash value as baseline (washing with

322   buffer only). The resulting reduction after the combination of both methods is comparable to the sum of the

323   reduction of both techniques applied individually. This indicates that the two methods have an additive

324   antimicrobial effect, with inactivation levels significantly higher than when the hurdles were applied

325   individually.

326   We hypothesised that the more pronounced antimicrobial effect observed for both pathogens when CAP

327   was applied first could be attributed to the mechanical etching effect on the cell membrane resulting in pore

328   formation (i.e. electroporation) (Huang et al., 2020), or sub-lethal bacterial injuries (Govaert et al., 2019),

329   making them more susceptible to linalool penetration thus increasing the killing efficiency. Future research

330   should take into account any possible overestimation of the treatments' efficacy due to sub-lethal injuries.

331   Previous studies have shown that the combination of different methods with CAP may achieve increased

332   bacterial reduction. Cui, Wu, Li, & Lin (2017) reported a synergistic effect, achieving a reduction of 2.8

333   log in *L. monocytogenes* levels when combining lemongrass essential oil (5 mg/mL, 30 min) with cold

334   nitrogen plasma treatment (2 min) in pork loin, whereas applying these treatments individually resulted in

335 a 0.58 log and 0.96 log reduction, respectively. Also, Lis et al., (2018) applied CAP to RTE sliced ham

336 fillets, achieving reductions of 1.14 log in *S.* Typhimurium and 1.02 log in *L. monocytogenes* after 20 min

337 of treatment. Higher reductions were observed after combining CAP with cold storage at 8 ℃ packed in a

338 controlled atmosphere (1.84 log for *Salmonella* and 2.55 log for *L. monocytogenes*). The results of this

339 study demonstrate that the combination of CAP followed by LW can significantly increase food safety

340 resulting in a very promising strategy that could be adopted by the meat industry.

3.3   Lipid oxidation

341 Application of CAP on food surfaces could result in increased lipid oxidation leading to negative effects of

342 organoleptic properties and a reduced shelf life (Dirks et al., 2012). Lipid oxidation products confer

343 unpleasant odours and rancid taste to meat. Malondialdehyde (MDA) is a polyunsaturated fatty acid

344 oxidation product and is considered to be a major marker of lipid oxidation (Moutiq, Misra, Mendonça, &

345 Keener, 2020). The RONS generated by CAP may initiate lipid oxidation on the meat surface, such as pork,

346 beef, chicken and seafood (Gavahian, Chu, Mousavi Khaneghah, Barba, & Misra, 2018). Thus, the effect

347 of the combined treatments on lipid oxidation, as levels of MDA in RTE chicken, was determined. The

348 results in Figure 5 showed that although the treated samples had a slightly higher lipid oxidation level (0.36

349 and 0.34 mgMDA/kg for LW/CAP and CAP/LW, respectively) compared to the control samples (0.32 and

350 0.31 mgMDA/kg for control and control-wash, respectively), the differences were not significant (P > 0.05).

351 The high standard deviation observed can be attributed to the high variation within meat samples. It is

352 evident from the results that for both treatments the mean lipid oxidation values were maintained at very

353 low levels. The mean lipid oxidation values were much lower compared to the values where oxidation starts

354 impacting the sensory properties on meat products, which is generally 2 - 2.5 mg MDA/kg (Domínguez et

355 al., 2019). This is in agreement with previous studies showing that chicken breast is more stable to plasma-

356 induce oxidation than red meats due to its lower content of fat, ferric heme pigment, and myoglobin

357 (Gavahian et al., 2018; Lee et al., 2016). Further studies are required to evaluate the organoleptic effects,

358 including any potential effects on flavour, that CAP alone or combined with LW might cause to the RTE

359 cooked chicken meat. Although this work has focused on Gram-negative bacteria, other relevant pathogens

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

361   experiments.

362

363   **Conclusions**

364   This study demonstrated that cold plasma and linalool nanoemulsion wash are effective against *Salmonella*

365   and *E. coli* O157:H7. Also, combining the two methods can significantly enhance the decontamination

366   effect against these pathogens on RTE cooked chicken meat without affecting lipid oxidation, an important

367   meat quality indicator. These methods can be applied before the meat is packaged and sealed to increase

368   the safety margin of RTE meat. Although the cost associated with the application of the two methods was

369   beyond the scope of the study, the nanoemulsion production, and the running of the cold plasma device

370   (low-voltage) using atmospheric air, costs are expected to be low. The use of linalool could potentially

371   increase the cost, however since it is utilised in small quantities the cost is not anticipated to increase

372   substantially. These results provide a strong platform for facilitating the commercial adoption of cold

373   plasma by the food industry.

374

375   **Acknowledgements**

377

378   **Conflict of interest:**

379   The authors declare that there is no conflict of interest.

380

381   **References**

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

383   during cooking of pork studied by low-field NMR relaxation: Effects of curing and the RN--gene.

384   *Meat Science*. https://doi.org/10.1016/S0309-1740(03)00132-3

385   Carrasco, E., Morales-Rueda, A., & García-Gimeno, R. M. (2012). Cross-contamination and

386   recontamination by Salmonella in foods: A review. *Food Research International*, *45*(2), 545–556.

387   https://doi.org/10.1016/j.foodres.2011.11.004

388   CDC. (2021). Center for Disease Control and Prevention (CDC) National outbreak reporting system (2021).

389   Retrieved April 17, 2021, from https://wwwn.cdc.gov/norsdashboard/

390   Chang, Y., McLandsborough, L., & McClements, D. J. (2013). Physicochemical properties and

391   antimicrobial efficacy of carvacrol nanoemulsions formed by spontaneous emulsification. *Journal of*

392   *Agricultural and Food Chemistry*, *61*(37), 8906–8913. https://doi.org/10.1021/jf402147p

393   Chen, J. H., Ren, Y., Seow, J., Liu, T., Bang, W. S., & Yuk, H. G. (2012). Intervention Technologies for

394   Ensuring Microbiological Safety of Meat: Current and Future Trends. *Comprehensive Reviews in*

395   *Food Science and Food Safety*, *11*(2), 119–132. https://doi.org/10.1111/j.1541-4337.2011.00177.x

396   CLSI. (2017). *Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement*

397   *M100. Wayne, PA: Clinical and Laboratory Standards Institute*. *Performance standards for*

398   *antimicrobial susceptibility testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and*

399   *Laboratory Standards Institute*. https://doi.org/10.1039/C4DT01694G

400   Cristani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M. G., Micieli, D., … Trombetta, D.

401   (2007). Interaction of four monoterpenes contained in essential oils with model membranes:

402   Implications for their antibacterial activity. *Journal of Agricultural and Food Chemistry*, *55*(15),

403   6300–6308. https://doi.org/10.1021/jf070094x

404   Cui, H., Wu, J., Li, C., & Lin, L. (2017). Promoting anti-listeria activity of lemongrass oil on pork loin by

405   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-

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 pastırma—

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., Stratakos, 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   Stratakos, 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   Stratakos, 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   Stratakos, 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.

521   https://doi.org/10.1088/1361-6463/abb900

522   Tripathi, A. K., & Mishra, S. (2016). Chapter 16 - Plant Monoterpenoids (Prospective Pesticides). In B. T.-
523   E. P. M. for F. S. Omkar (Ed.) (pp. 507–524). San Diego: Academic Press.
524   https://doi.org/https://doi.org/10.1016/B978-0-12-803265-7.00016-6

525   Xiang, Q., Liu, X., Li, J., Ding, T., Zhang, H., Zhang, X., & Bai, Y. (2018). Influences of cold atmospheric
526   plasma on microbial safety, physicochemical and sensorial qualities of meat products. *Journal of Food*
527   *Science and Technology*. Springer India. https://doi.org/10.1007/s13197-017-3020-y

528   Yang, X. N., Khan, I., & Kang, S. C. (2015). Chemical composition, mechanism of antibacterial action and
529   antioxidant activity of leaf essential oil of Forsythia koreana deciduous shrub. *Asian Pacific Journal*
530   *of Tropical Medicine*. https://doi.org/10.1016/j.apjtm.2015.07.031

531   Yegin, Y., Perez-Lewis, K. L., Zhang, M., Akbulut, M., & Taylor, T. M. (2015). Development and
532   characterization of geraniol-loaded polymeric nanoparticles with antimicrobial activity against
533   foodborne bacterial pathogens. *Journal of Food Engineering*, *170*, 64–71.
534   https://doi.org/10.1016/j.jfoodeng.2015.09.017

535   Zengin, H., & Baysal, A. H. (2014). Antibacterial and antioxidant activity of essential oil terpenes against
536   pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM
537   microscopy. *Molecules*, *19*(11), 17773–17798. https://doi.org/10.3390/molecules191117773

538   Zhang, Y., Liu, X., Wang, Y., Jiang, P., & Quek, S. Y. (2016). Antibacterial activity and mechanism of
539   cinnamon essential oil against Escherichia coli and Staphylococcus aureus. *Food Control*.
540   https://doi.org/10.1016/j.foodcont.2015.05.032

541   Zhu, H., Du, M., Fox, L., & Zhu, M. J. (2016). Bactericidal effects of Cinnamon cassia oil against bovine
542   mastitis bacterial pathogens. *Food Control*, *66*, 291–299.
543   https://doi.org/10.1016/j.foodcont.2016.02.013

544

Figure Legends

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

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

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

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

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

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

566

567   Figure 1

Axial fan

Driver
Electronics

Piezoelectric transformer

Low voltage input

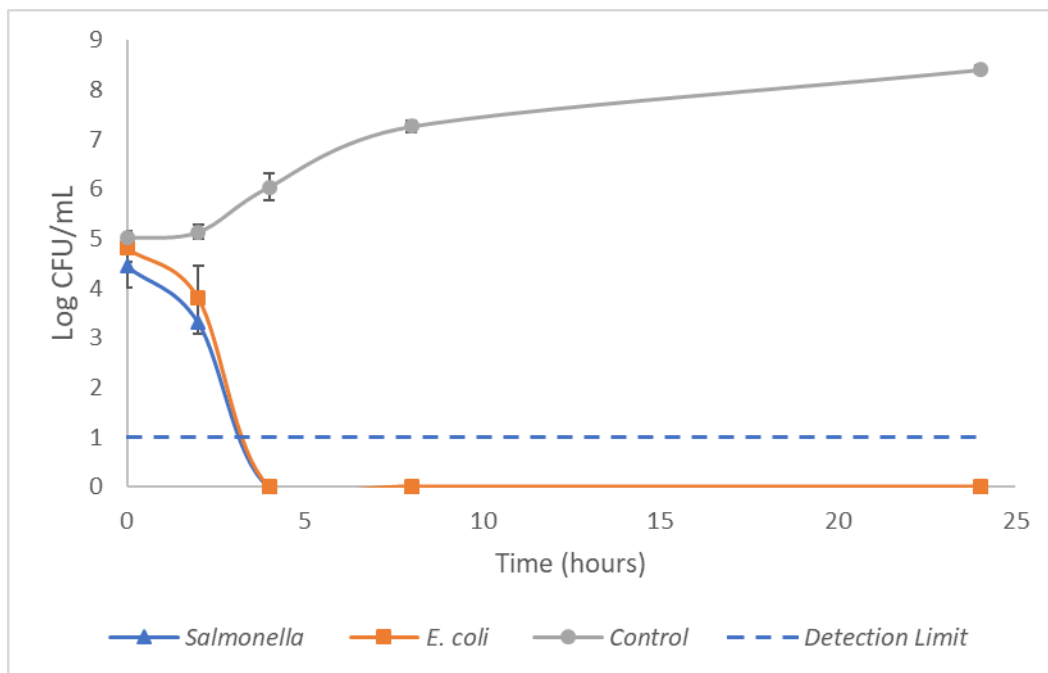Cold plasma
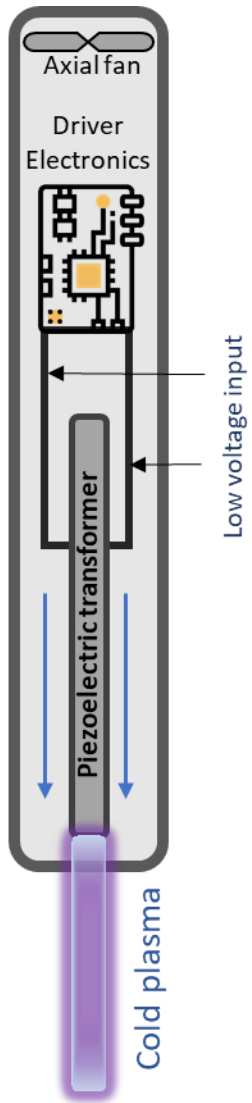
568

569

570

571

572

573

574   Figure 2

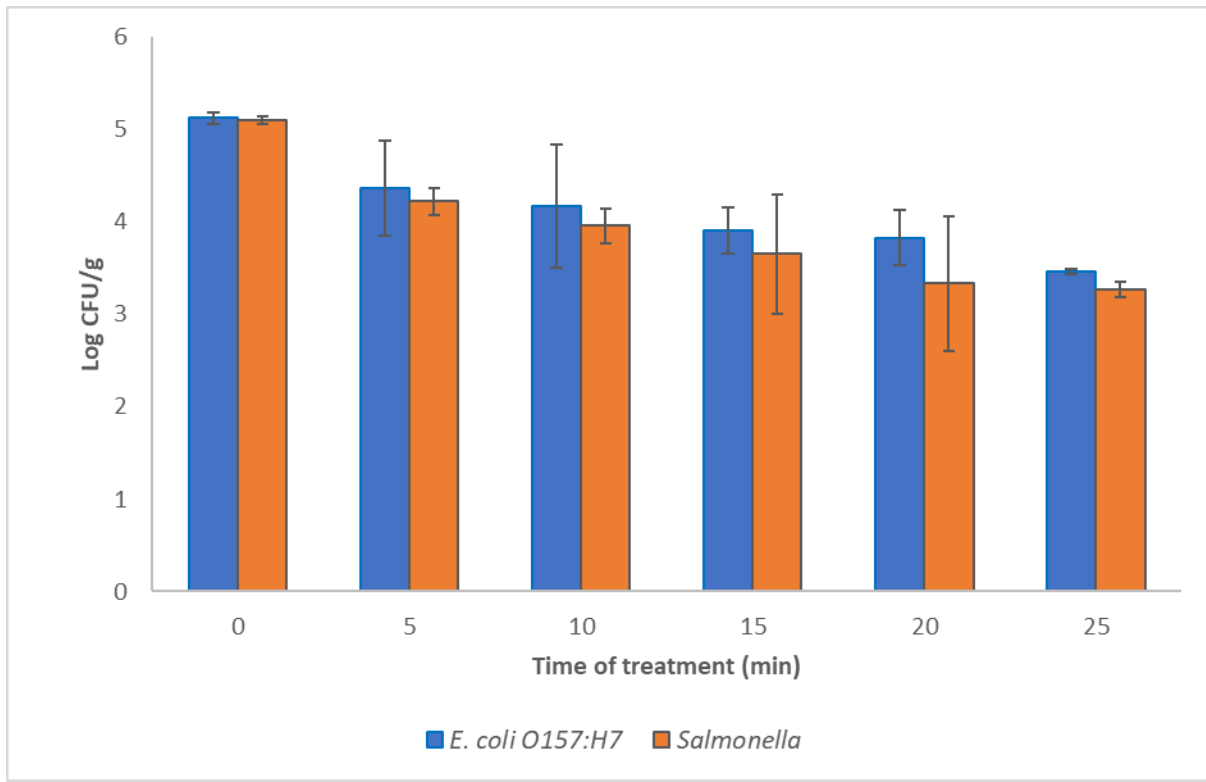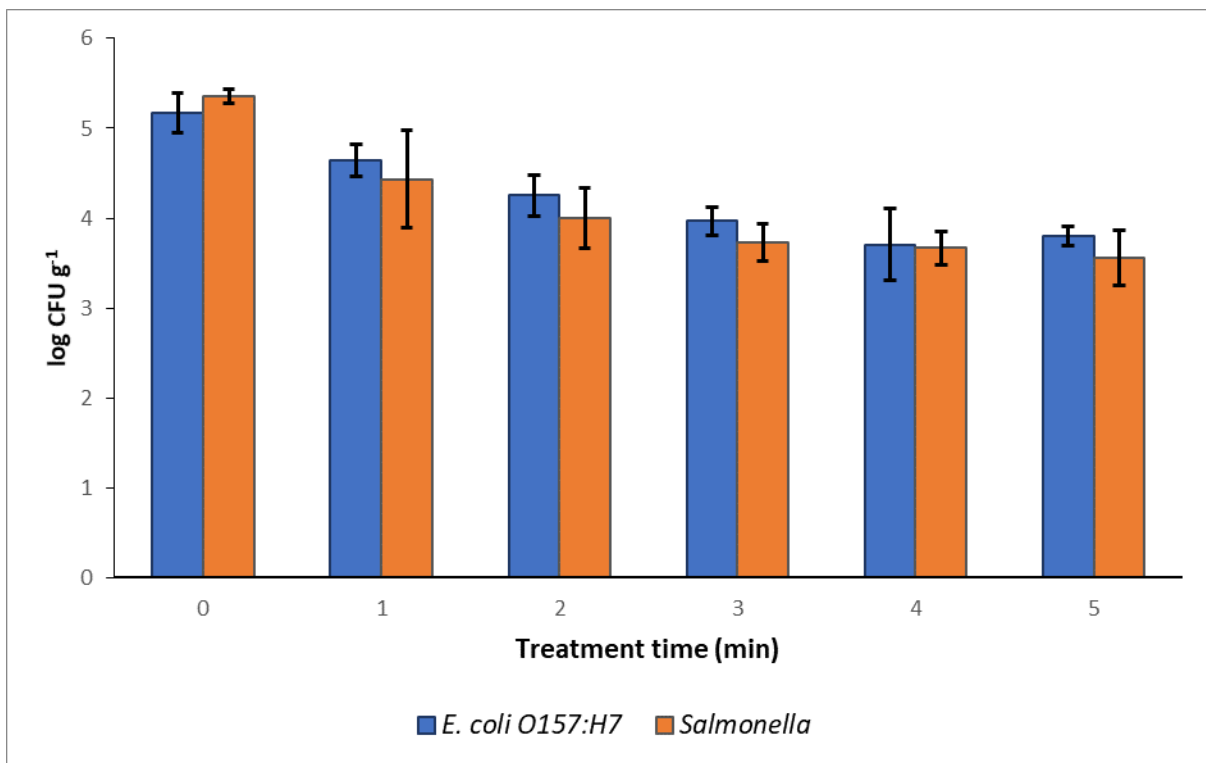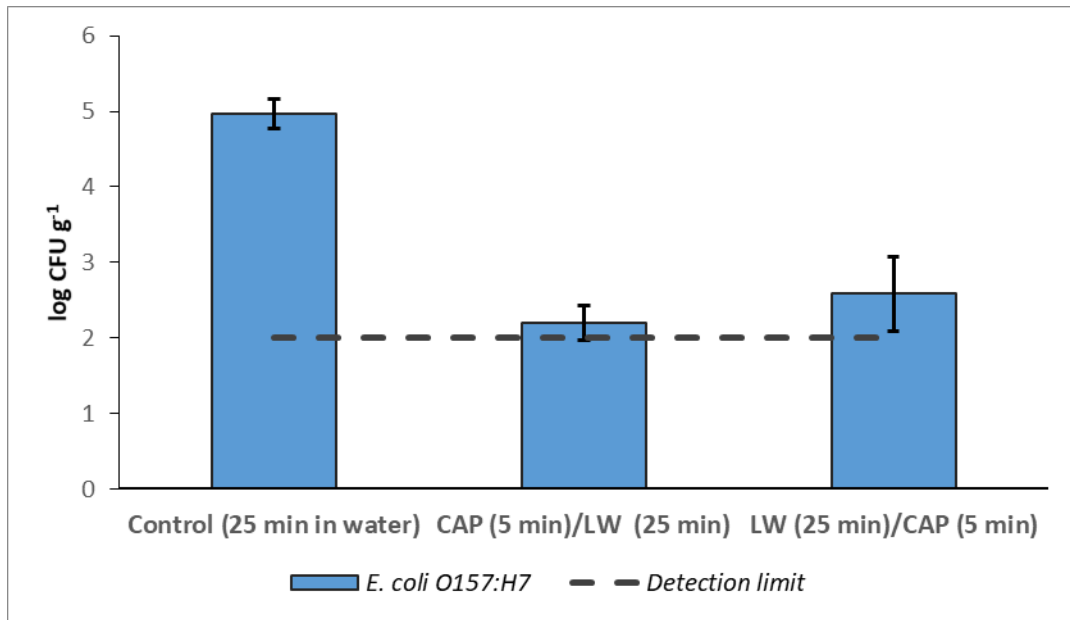575

576

577
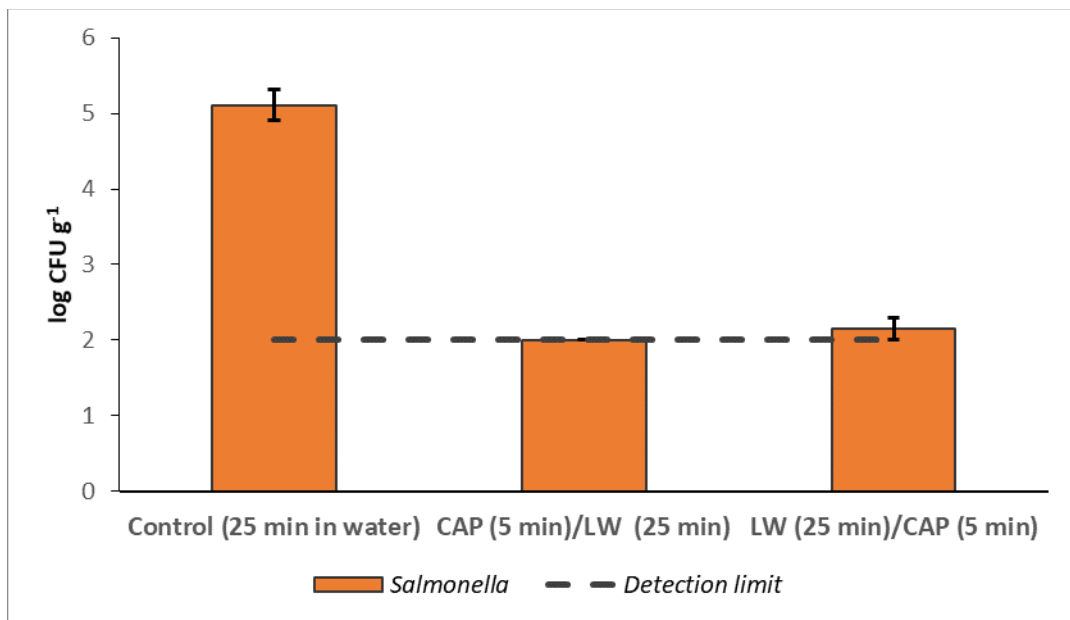
578

579

580

581

582

583

584

585

586   Figure 3

587

588

589   Figure 4

590

591

592

593

594

595   Figure 5

596