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**Cold Atmospheric Gas Plasma Disinfection of Chicken Meat and Chicken Skin
contaminated with *Listeria innocua***

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ABSTRACT

Gas plasmas generated at atmospheric pressure and ambient temperatures offer a possible decontamination method for poultry products. The efficacy of cold atmospheric gas plasmas for decontaminating chicken skin and muscle inoculated with *Listeria innocua* was examined. Optimization of operating conditions for maximal bacterial inactivation was first achieved using membrane filters on which *L. innocua* had been deposited. Higher values of AC voltage, excitation frequency and the presence of oxygen in the carrier gas resulted in the greatest inactivation efficiency, and this was confirmed with further studies on chicken muscle and skin. Under optimal conditions, a 10 s treatment gave > 3 log reductions of *L. innocua* on membrane filters, an 8 min. treatment gave 1 log reduction on skin, and a 4 min. treatment gave > 3 log reductions on muscle. These results show that the efficacy of gas plasma treatment is greatly affected by surface topography. Scanning electron microscopy (SEM) images of chicken muscle and skin revealed surface features wherein bacteria could effectively be protected from the chemical species generated within the gas plasma. The developments in gas plasma technology necessary for its commercial application to foods are discussed.

Keywords: cold atmospheric gas plasma, decontamination, chicken skin, chicken muscle, *Listeria innocua*,

1. Introduction

Poultry carcasses are commonly contaminated with enteric pathogens such as *Salmonella*, *Campylobacter* and *Listeria monocytogenes* (Jacobsreitsma et al., 1994; Murphy et al., 2004), the possibility of cross-contamination of poultry carcasses post slaughter is high. Decontamination of poultry carcasses is therefore desirable. Various decontamination technologies have been proposed including the use of various chemical agents such as alkali (Rodriguez De Ledesma et al., 1996), physical methods such as steam treatment (James et al., 2007) and biological control with bacteriophages (Carvalho et al., 2010), but only treatment with water supplemented with chlorine or a chlorinating agent is used commercially. The effects of such decontaminating treatments are limited (Oyarzabal, 2005; Russel and Axtell, 2005). There is, therefore, need for an effective technology that can be operated within commercial constraints.

The role of gas plasmas in microbial inactivation has been studied since the mid 1990s, but their potential for food decontamination purposes has only recently been recognised. Gas plasmas are usually generated by means of an external electric field; when the voltage applied to a gas exceeds a certain threshold value the gas will become ionized. Gas plasmas comprise mixtures of electrons, ions, atomic species, free radicals and UV photons, all of which have the capability of inactivating micro-organisms (Perni *et al.*, 2007). In order to decontaminate foods without bringing about undesired changes, gas plasmas should ideally be operated at or near room temperature. Until quite recently this could only be achieved under vacuum, which is inconvenient and expensive. Recent advances in plasma source technology allow the generation of plasmas having both spatial and temporal stability at ambient temperatures and atmospheric pressure using relatively simple and inexpensive equipment (Kogelschatz, 2002). Cold atmospheric plasmas have been reported to be very effective against a wide range of microorganisms, including biofilm-formers and bacterial spores (Montie *et al.*, 2000; Deng *et*

68 *al.*, 2005; Vleugels *et al.*, 2005). Although most of the previous work on bacterial inactivation
69 has been conducted with bacteria deposited on the surface of abiotic materials such as
70 membrane filters and glass slides, data on the disinfection of foodstuffs is steadily
71 accumulating. To give examples, Perni *et al.*, (2008b) examined the inactivation of a number
72 of micro-organisms on mango and melon, whilst Kim *et al.*, (2011) reported on the
73 disinfection of bacon.

74 In the present work, the effectiveness of cold atmospheric plasmas for inactivating *Listeria*
75 *innocua*, a non- pathogenic surrogate for *Listeria monocytogenes*, (Perni *et al.*, 2006) on the
76 surface of both membrane filters and chicken breast (skin and muscle) was examined. A range
77 of plasma operating conditions were first investigated in order to arrive at those that were
78 optimum for the inactivation of *L. innocua* deposited on the surface of membrane filters.
79 Those proving to be the most effective conditions were then applied to chicken skin and then
80 further modified for maximum efficacy. Following this, chicken muscle was treated under
81 these optimised conditions.

83 **2. Materials and Methods**

84 *2.1 Microorganisms and Cultivation*

85 *Listeria innocua* (ATCC 33090) was stored on cryobeads at -20 °C. Cells were activated by
86 transferring one of these beads into 100 mL Tryptone Soy Broth (TSB; Oxoid Ltd.,
87 Basingstoke, Hants, UK) in 500 mL Erlenmeyer flasks and incubating overnight at 30 °C and
88 140 rpm. This culture was streaked onto Tryptic Soya Agar plates (TSA; Oxoid Ltd.,)
89 incubated for 24 h at 30 °C. Working cultures were kept at 4 °C on TSA slopes, prepared
90 from single colonies of the TSA plates, and subcultured every two weeks.

Inocula for deposition on the surfaces of membrane filters and chicken tissue were prepared by transferring single colonies from TSA slopes to 500 mL Erlenmeyer flasks, each containing 100 mL of TSB. After 24 h incubation at 30 °C and 140 rpm, a loopful of cells from these cultures was used to inoculate 100 mL of fresh TSB in 500 mL Erlenmeyer flasks, incubated as before. Cultivation under these conditions yielded cell populations at stationary phase of growth with a concentration of approximately 5.0×10^9 CFU mL⁻¹. This second subculture, diluted to 5.0×10^7 CFU mL⁻¹ was used to surface inoculate membrane filters, chicken skin and muscle. Additionally, the same culture diluted approximately to 5.0×10^8 CFU mL⁻¹, was used to load sterile filter papers of 20 mm diameter (No. 1, Whatman, Fisher Scientific, Loughborough, UK) by immersion in the dilute culture for 15 min. These bacteria-laden filters were used to inoculate samples of chicken muscle as described below.

2.2 Preparation of membrane filters

For ease of manipulation, 0.20 µm pore size Whatman polycarbonate membrane filters of 25 mm diameter (Fisher Scientific, Loughborough, UK) were placed on agar plates (Technical Agar No. 3, Oxoid Ltd.,). Bacterial cell suspension (100 µL) was then carefully deposited onto the centre of the sterile membrane filters, and allowed to dry for 60 min. at room temperature in a laminar flow cabinet. All experiments were conducted in triplicate with three independent cultures.

2.3 Preparation of chicken skin and muscle samples

Fresh, raw, boneless chicken breasts with the skin attached were purchased at a local supermarket and transported immediately to the laboratory, where they were kept in a refrigerator overnight at 4°C before use. The entire skin was removed in one piece from the underlying muscle, using a sterile scalpel and forceps, and placed in Petri dishes. Skin tissue was of two types; rough, relatively thick skin and smoother thinner skin. A slice of

approximately 2 mm thickness was cut from the subcutaneous muscle and stored in Petri dishes. Discs of tissue were punched from both muscle and skin pieces using a sterile cork borer of 20 mm diameter. The resulting discs were placed singly in Petri dishes.

Bacterial suspension (100 μ L) was deposited onto the centres of the discs of skin and muscle, taking care not to allow the suspension to overflow the edges. After inoculation the tissue samples were kept at room temperature in a laminar flow cabinet for 60 min. to allow attachment. Additionally, bacteria-laden membrane filters (prepared as described above) were pressed lightly onto a dry, sterile filter paper for a few seconds to remove the excess liquid, placed onto prepared chicken samples for 10 min., then carefully removed with forceps. All experiments were conducted in triplicate with three independent cultures.

2.4 Cold atmospheric plasma apparatus

The cold atmospheric plasma system comprised a 1.5 mm inner diameter ceramic tube confining a helium-oxygen flow; a concentric, ring-shaped, 1 cm wide copper electrode wrapped around the ceramic tube and connected to a high-voltage power supply; and a disc electrode (ground electrode) placed 0.5 to 1.5 cm downstream of the nozzle of the ceramic tube (Figure 1). The powered electrode was energized with a purpose-built, high-voltage, AC power supply with a peak voltage of 6.5 to 16 kV, and a variable excitation frequency between 23 and 38.5 kHz. Helium (99.99 % purity) at a fixed flow rate of 5 L min.⁻¹ and oxygen at a flow rate of up to 100 mL min.⁻¹ were fed through the hollow ceramic tube for ionization. The ionized gas was flushed out of the electrode unit and into ambient air toward a point 0.5 to 1.5 cm downstream where the samples were placed connected to the ground electrode. In ambient air, the gas plasma appeared as a pale purple plume, termed a cold atmospheric plasma pen (CAP-Pen).

2.5 Microbial inactivation procedure and cell recovery

The CAP-Pen diameter of 3 mm was much smaller than the bacterial deposition area (approx. 1 cm in diameter), therefore plasma treatment was effected by keeping the plasma nozzle fixed and moving the sample beneath it in a zig-zag path by hand so that the entire surface was exposed to the plume. The duration of CAP-Pen treatment varied from 10 s to 8 min. These total treatment times were about 10 fold greater than the time that any part of the treated area was exposed to the plasma. Therefore it should be noted the D values quoted are in reality *apparent* D values. Following CAP-Pen treatment, cells were recovered from membrane filters by transferring them to sterile Universal bottles containing 10 mL of Ringers solution (Oxoid Ltd.) and agitating for 30 s with a vortex mixer. Chicken samples were transferred to stomacher bags containing 10 mL of sterile Ringers solution and surviving cells were recovered by treating for 30 s in the Stomacher 400 (Seward Ltd., Thetford, Norfolk, UK) at intermediate speed. Aliquots (100 μ L) of cell suspension or serial dilutions in Ringers solution were spread onto agar plates. Total bacterial counts were determined on TSA plates incubated at 30 °C for 24 h. *L. innocua* was enumerated on PALCAM *Listeria* selective agar base (Oxoid Ltd), supplemented with PALCAM *Listeria* selective supplement (Oxoid Ltd.). The plates were incubated at 30 °C for 48 h. All counts were performed in triplicate, and all the results reported here as CFU per square centimetre are mean values.

2.6 Mathematical Modelling of Bacterial Inactivation

The Baranyi inactivation model as modified by Xiong et al., (1989) was applied to all of the data presented here. This was done according to the form of the inactivation curve, and either applied to the entire data (in experiments I, II, V-VII) or, where more than one phase existed, to individual phases (in experiments III and IV). D values, the times required for one log-reduction of the microbial population, were calculated from the corresponding inactivation rate constants (k) i.e. $D = 2.303/k$.

2.7 Scanning electron microscopy (SEM)

Skin and muscle samples were prepared for SEM by chemically fixing them in 0.025 M phosphate buffer (pH 6.8) containing 3 % (v v⁻¹) glutaraldehyde for 1 h. Samples were then washed twice for 15 min. in the same phosphate buffer prior to dehydration in a series of increasing ethanol concentrations (20, 40, 60, 80 and 100 % v v⁻¹ ethanol in water; 15 min. in each solution). Then, samples were transferred from 100 % ethanol to 100 % acetone in a similar way (30, 50, 70, 90 and 100 % v v⁻¹ acetone in ethanol; 10 min. in each solution) and dried by critical point-drying with liquid CO₂ (Balzers CPD 030, BAL-TEC AG, Fürstentum, Liechtenstein). Membrane filters were also subjected to chemical fixation, but they were first dehydrated by incubation at 60 °C overnight. Following this, all the samples were mounted on aluminium stubs with double-sided carbon sticky-tape, sputtered with gold in a vacuum evaporator (Balzers SCD 004, BAL-TEC AG, Fürstentum, Liechtenstein), and visualized using a JEOL-6100 SEM microscope (JEOL Ltd., Tokyo, Japan).

2.8 Statistical analysis

Comparisons of experimental data, either in the form of log concentrations or log reductions, were evaluated by means of analysis of variance (ANOVA) tests. The multiple Range Test was used to distinguish means which differed significantly from each other. Standardized skewness and standardized kurtosis were used to assess if data sets were normally distributed. These analyses were performed using STATGRAPHICS PLUS for Windows 3.0[®] Package (Statistical Graphics, Washington, USA). Test statistics were regarded as significant when p was < 0.05 .

3. Results and Discussion

3.1 Inactivation on membrane filters

Plasma inactivation of *L. innocua* deposited on membrane filters at a range of experimental conditions is shown in Figure 2 and Table 1. The temperature of samples undergoing treatment never exceeded 30° C. Increasing the peak voltage, whilst maintaining the gap distance at 1.5 cm, had relatively little effect. However, decreasing the gap distance to 1.0 cm resulted in a biphasic inactivation curve in contrast to the monophasic curves obtained for experiments I and II. A substantial reduction in D value for the first phase of inactivation was followed by a second phase that had a D value almost one order of magnitude greater. This pattern of inactivation was maintained as the voltage was further increased to 7.0 kV with a reduction in D value for both phases of the inactivation curve. Further increasing the voltage to 8.0 kV resulted in a return to monophasic inactivation kinetics, and a reduction in the D value to 4.5 s. Increasing the voltage still further necessitated a decrease in frequency to 23.0 kHz in order to maintain stable plasma operation. under these conditions oxygen was supplied to the CAP. This yielded a D value of 3.4 s. at a peak voltage of 9.0 kV and 3.1 s. at 11.0 kV. Table 2 shows that *L. innocua* was inactivated to below the detection limit (1×10^3 CFU cm⁻²) after only 10s treatment in experiments V, VI and VII. Inactivation curves displaying biphasic (or even multiphasic) behaviour have previously been interpreted as being indicative of the occurrence of inactivation by different plasma species (Montie *et al.*, 2000; Moisan *et al.*, 2001; Kong *et al.*, 2009; Kong *et al.*, 2005).

Perni *et al.* (2008b) reported that 1.5 s of plasma exposure were sufficient to inactivate *L. monocytogenes* on membrane filters ($\approx 4 \times 10^6$ CFU/cm²) to below the detection limit, although their treatment conditions (30 kHz, 8 kV and 25 mL oxygen min.⁻¹) were not identical to those employed here. Plasma exposure time to achieve no detectable cell counts was of the same order for both species, although the slightly higher value obtained here

indicates that *L. innocua* may be marginally more resistant to plasma treatment than is *L. monocytogenes*.

When oxygen was added to the helium flow (experiments VI and VII), peak voltage and excitation frequency had to be re-set to achieve stable plasma operation. Molecular gases have higher breakdown voltages than atomic gases, and therefore the applied voltage had to be increased when oxygen was mixed with helium. For the CAP-pen used here it was necessary to increase the voltage to 9 kV at an excitation frequency of 38.5 kHz. However, this was found to result in considerable overheating in, and subsequent failure of, the transformer used in the power supply. To prevent overheating the excitation frequency was reduced to 23 kHz and this resulted in stable operation. The key parameter for comparing the lethality of plasmas is the number densities of the relevant biocidal plasma species. These were taken to be oxygen atoms since they had previously been identified as a key biocidal species in atmospheric He-O₂ plasmas (Deng et al., 2007a). The optical emission intensities at 777 nm and 845 nm (not shown here) were taken as an indirect indicator of atomic oxygen concentrations. Using this criterion, conditions in experiment IV and V were found to be similar to those in VI and VII, respectively. Moreover, previous studies have shown that plasma characteristics do not change in any significant way in the frequency range of 20 to 50kHz (Deng and Kong, 2004).

Although the D values were of the same order of magnitude, log reductions at 10 s in experiments VI and VII were significantly different to the inactivation achieved in the absence of oxygen (experiment V). Therefore, carrier gas composition played a decisive role in plasma inactivation as would be expected (Deng *et al.*, 2007b; Perni *et al.*, 2007).

3.2 Inactivation on skin from chicken breast

234
235 Viable counts recovered from chicken skin before inoculation were of the order of 4.0×10^5
236 CFU cm², but did not include listeriae. Viable counts after CAP-Pen decontamination of
237 inoculated skin under various experimental conditions are shown in Table 2. The plasma
238 operating parameters that were most effective with membrane filters were applied to the
239 treatment of chicken skin. However, the dielectric properties of membranes and chicken skin
240 are different, and this necessitated adjustment of the operating conditions in order to achieve
241 stable operation. In particular, different peak voltages, excitation frequencies, carrier gas and
242 plume length (experiments IV to VIII) were tested within the plasma stability range, in
243 addition to studying the effect of cell attachment (experiment IX) as well as skin type
244 (experiment III).

245 Reductions in viable counts were considerably below those achieved using membranes, and
246 the D values were correspondingly higher. In experiments I to III, skin samples were treated
247 for both 4 and 8 min., but no significant reductions in bacterial numbers with treatment times
248 of up to 4 min. were achieved. Therefore in subsequent experiments treatment was for 8 min.
249 only.

250 Experiments I and II were conducted under conditions previously identified as representing
251 the best conditions for inactivation on membrane filters (V and VII, respectively). After 4
252 min., the conditions in experiment II resulted in significantly increased log reductions of the
253 total microflora, but not of *L. innocua*. However, after 8 min. of exposure, there were
254 significant decreases in numbers of both the total microflora and *L. innocua*. Curiously,
255 higher levels of inactivation were achieved in the absence of oxygen in the carrier gas
256 (experiment I), which contrasts with the results obtained using membrane filters. When
257 experiments I and II are compared to experiments conducted with membrane filters under the

same conditions (V and VII, respectively), significantly higher ($p < 0.05$) D values (approx. 200 and 600 fold higher, respectively) and log reductions (4- and 15- fold higher, respectively) were achieved with membrane filters with much shorter contact times. Thus chicken skin exerts a protective effect on microorganisms present at the surface.

The effect of skin type i.e. thick rough skin versus thin and relatively smooth skin, on plasma effectiveness was revealed as negligible from experiments II and III, since no significant differences ($p > 0.05$) were found in log reductions at any of the treatment times.

High excitation frequencies have been reported to expand the plasma stability range and enable more abundant reactive plasma species, such as oxygen atoms and hydroxyl radicals to be generated, and this has been correlated with increased anti-microbial efficiency of plasmas (Walsh and Kong, 2006). The importance of this parameter, at constant peak voltage, is evident by comparing experiments II and IV, since significantly higher reductions ($p < 0.05$) were achieved at the highest than at the lower frequencies for both total and listerial populations.

Increasing the oxygen flow rate whilst maintaining all other parameters constant resulted in a significant reduction in D values, as is evident by comparing the results for experiments V and VI. The D value for the latter experiment was not significantly different to that obtained in experiment I. However, when the oxygen flow was set at 100 mL min^{-1} (experiment VII), more effective inactivation was achieved than in experiment VI. To try to improve plasma effectiveness, the distance between the plasma source and the chicken skin (the 'gap' in Table 2) was decreased to 0.5 cm (experiment VIII). Significantly lower log reductions ($p < 0.05$) were achieved for *L. innocua* and total microflora as compared to experiment VII, although reductions were comparable to those in experiments I, and IV to VI. However, D values for total counts and *L. innocua* in experiment VIII were not significantly different ($p > 0.05$) from

the corresponding values in experiment VII. Cell migration into skin tissue during attachment may have occurred and this could well have affected plasma effectiveness, since cells could be transported beyond the reach of plasma species as Perni *et al.* (2008b) demonstrated for the plasma treatment of fresh cut fruit surfaces. To confirm this supposition, samples were treated immediately after deposition onto chicken skin (experiment IX) under the same conditions as in experiment VII. D values for total and listerial counts were not significantly different ($p > 0.05$) between experiments VII and IX. For these same experiments, no significant differences were found between log reductions of *L. innocua*, whereas values for the total microflora were significantly lower ($p < 0.05$), although they were of the same order as for experiments I, IV to VI and VIII.

Plasma conditions in experiment VII resulted in the most effective inactivation of *L. innocua* on chicken breast skin with a reduction of just under 1 log and a D value of about 9 min.

3.3 Inactivation on chicken breast muscle

Chicken skin acts as a physical barrier to microbial migration into the inner tissues, but severed blood vessels or skin cut during skinning, boning and portioning may contaminate the underlying tissue (Avens *et al.*, 2002). Microbiological analysis of uninoculated muscle revealed the absence of listeriae and total counts of only 2.5×10^3 CFU cm⁻². Previous experiments conducted with plasma membranes and chicken skin allowed identification of plasma operating conditions that are more effective for inactivation. These were applied to the treatment of chicken muscle (Table 3).

In experiment I, no significant differences ($p > 0.05$) were observed after 8 min. of exposure, when compared to the initial concentration, whereas significant reduction in numbers was achieved in experiment II. However, microbial response to plasma treatment was markedly

different in experiment III. The survival curve (not shown) displayed biphasic behaviour, with a turning point at 10 s of CAP-Pen exposure, although significant differences were not found between both D values in the overall comparison. Log reductions in experiments I, II and III at 8 min. of plasma exposure, were significantly different ($p < 0.05$), with a much higher inactivation having been achieved in experiment III than in I and II (55-fold and 8-fold higher, respectively). Such a markedly different cell response to plasma treatment may be a reflection of differences in the inoculation procedure (direct deposition in experiment I and II *versus* filter contact in III), since plasma effectiveness has been reported to depend on the cell exposure method (Kong et al., 2005; Kong et al., 2009).

Significantly lower log reductions and higher D values were achieved with chicken muscle in experiment I (in the absence of oxygen), when compared to the same conditions on membrane filters (experiment V, Table 1) and skin (experiment I, Table 2). However, when compared with experiment II and the corresponding treatment on chicken skin (experiment IV), no significant differences were found. Therefore, the presence of oxygen in the carrier gas played a more decisive role on muscle than on skin, since inactivation in experiment II was seven-fold higher than in experiment I, whereas similar inactivation was achieved between skin experiments I and IV. Finally, log reductions in muscle experiment III were significantly higher than the values obtained for the corresponding skin experiment (VII).

Although the level of inactivation of *L. innocua* achieved on chicken muscle was lower than that on membrane filters, the highest level achieved, approximately 3.3 log reductions, would undoubtedly be of commercial interest.

3.4 Plasma effectiveness and surface topography

Whereas all experiments were conducted under broadly similar conditions, the effectiveness of plasma treatment decreased in the transition from membrane filters to chicken muscle, and

from that to chicken skin. This is strongly suggestive that surface topography plays a significant role in inactivation using gas plasmas. An average of 5.2×10^6 cells were deposited on each membrane, and from theoretical considerations based on the dimensions of *L. innocua* cells (assumed to be cylinders with dimensions of $1 \mu\text{m} \times 0.5 \mu\text{m}$) and the membrane surface area (1 cm^2) stacking would start to occur only as the cells exceeded a concentration in excess of 2×10^7 cells. Therefore, we can be reasonably certain that all cells on the surface of the membrane were equally exposed to the gaseous plasma species.

An SEM image of skin removed from chicken breast is shown in Figure 3a, and reveals a highly irregular topography. It is significant that SEMs taken after inoculation of the skin with *L. innocua* did not reveal the presence of an abundance of bacteria at the surface. In Figure 3b for example, only a small cluster of cells is visible slightly to the right of the centre of the image. It is possible that bacteria which were deposited at the surface were drawn through capillary action into feather follicles and other surface irregularities. Kim *et al.* (1996) reported that bacteria could migrate from the surface to depths up to about $140 \mu\text{m}$. Microorganisms at these distances from the surface have previously been reported to be largely unaffected by either thermal or chemical treatments. Moreover, the highest levels of inactivation achieved by these methods was typically no more than 1 log-reduction (Thomas and McMeekin, 1980), the maximum value reported in this work.

The surface of the chicken muscle immediately following the two different inoculation procedures employed here is shown in Figures 4a and 4b, the former was obtained after direct deposition, and the latter following filter contact. Figure 4a clearly reveals the presence of fissures. As rigor develops, muscle fibres have been reported to undergo radial shrinkage, and pull away from surrounding connective tissue (Frank, 2001). The resulting fissures, which

could provide a route for bacterial penetration, may be enlarged in the presence of excess water. Direct deposition causes the tissue to take up water and swell and this could cause bacteria to be drawn into the tissues with the water and become entrapped between swollen fibres. It has been reported that bacteria may be carried to depths of up to 25 μm by this means (Thomas and McMeekin, 1987; Auty et al., 2005). However, after inoculation by filter contact, a dense fibre network covering the muscle surface was observed (Figure 4b), and this possibly prevented cell migration through these channels. Figures 4c and 4d reveal bacteria concentrated around irregularities at the surface of chicken muscle. Some of these cells would presumably still be vulnerable to plasma species, but others might be protected by cells situated above them.

3.6 Prospects for employing gas plasmas for the decontamination of chicken tissue

This work has demonstrated that cold atmospheric gas plasmas have the potential to decontaminate chicken muscle. The results obtained with chicken skin are comparable to those obtained by other techniques that fall under the category of minimal processing and are testament to the difficulties in decontaminating this type of tissue owing to its topography. The plasma device employed was a laboratory model and treatment of chicken on a commercial scale would require the issue of scale-up to be addressed. Advances in this direction are already being made; Cao et al., (2010) have described the construction of a so-called '2-D plasma brush' that comprises multiple plasma plumes. Treatment times with such a device would be significantly reduced over those quoted above, and further scale-up would result in a concomitant decrease in the times necessary to bring about the requisite level of surface decontamination in industrial settings.

The chemical species formed during plasma treatment are highly reactive, and as a result, very short-lived. Therefore treatment with gas plasmas should not result in the formation of compounds that could be viewed as residues. However, it remains necessary to assess the effects of plasma treatment on the sensory and nutritional properties of the foods undergoing treatment in a systematic and exhaustive manner. Such studies were beyond the scope of the present work, although treatment as described above did not result in any obvious changes to the appearance of the chicken muscle or skin.

Lastly, it would be important to take into account consumer perception of any novel decontamination technology and before commercialisation of plasma treatment in the food industry could become a reality, this would need to be addressed.

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FIGURE CAPTIONS

Figure 1. Schematic of the cold atmospheric plasma pen (CAP-Pen) apparatus

Figure 2. Plasma inactivation of *Listeria innocua* deposited on the surface of membrane filters: ●-experiment I; ▲-experiment II; ▲-experiment III; ◆-experiment IV; ■-experiment V; ◆-experiment VI; ■-experiment VII (see experimental conditions in Table 1). Dashed lines correspond to concentrations below the detection limit. Figure 3. Scanning electron micrographs. a) non-inoculated skin sample (x1000 magnification), b) cluster of bacteria present on inoculated skin (x1000 magnification).

Figure 4. Scanning electron micrographs a) muscle sample newly inoculated by aliquot deposition (x2500 magnification), b) muscle sample newly inoculated by filter contact (x2000 magnification) and C) and D) muscle sample inoculated by filter contact after 24 h of incubation at 25 °C (x 2000 and x 6000 magnification, respectively).

FIGURE 1

FIGURE 2

TABLE 1. Inactivation of *Listeria innocua* deposited on membrane filters

Exp. ¹	CAP-Pen Treatment Conditions					Log-reductions ^{2,3} (log CFU/cm ²)					D-values ⁴ (s)
	Gap (cm)	Voltage (kV)	Frequency (kHz)	He (L/min)	O ₂ (mL/min)	10 s	30 s	1 min	2 min	3 min	
I _(a)	1.5	6.0	38.5	5.0	0.0	0.27 ± 0.07 ^a _A	0.86 ± 0.17 ^b _A	1.05 ± 0.03 ^b _A	1.96 ± 0.31 ^c _A	2.95 ± 0.40 ^d _A	60.7 ± 7.8 ^a
II _(a)	1.5	6.5	38.5	5.0	0.0	0.16 ± 0.14 ^a _A	0.49 ± 0.01 ^b _B	1.05 ± 0.15 ^c _A	2.48 ± 0.10 ^d _B	3.00 ± 0.06 ^e _A	56.0 ± 0.8 ^{a,b}
III _(b, c)	1.0	6.5	38.5	5.0	0.0	0.15 ± 0.04 ^a _A	0.63 ± 0.12 ^b _B	1.92 ± 0.23 ^c _B	3.15 ± 0.01 ^d _C	3.34 ± 0.01 ^d _A	37.0 ± 1.4 ^{b,c} *322.1 ± 35.1 ^d
IV _(b)	1.0	7.0	38.5	5.0	0.0	0.19 ± 0.01 ^a _A	1.01 ± 0.10 ^b _A	2.68 ± 0.01 ^c _C	3.37 ± 0.03 ^d _C	ND	24.2 ± 0.8 ^{c,e} *108.5 ± 10.2 ^f
V _(b)	1.0	8.0	38.5	5.0	0.0	2.23 ± 0.23 _B	ND	ND	ND	ND	4.5 ± 0.5 ^e
VI _(b, c)	1.0	9.0	23.0	5.0	25.0	3.02 ± 0.51 _C	ND	ND	ND	ND	3.4 ± 0.6 ^e
VII _(c)	1.0	11.0	23.0	5.0	25.0	3.26 ± 0.37 _C	ND	ND	ND	ND	3.1 ± 0.3 ^e

ND: not detectable (detection limit, 1 x 10³ CFU/cm²)

¹ Initial viable counts of experiments lacking common letters are significantly different (P ≤ 0.05).

² Within the same row (same experiment), log-reductions bearing different superscripts are significantly different (P ≤ 0.05).

³ Within the same column (same exposure time), log-reductions bearing different subscripts are significantly different (P ≤ 0.05).

⁴ D-values bearing different superscripts are significantly different (P ≤ 0.05).

*D-values of the second phase (biphasic survival curves)

TABLE 2. Inactivation of *Listeria innocua* and total microflora on the skin of chicken breasts

Exp.	CAP-Pen Treatment Conditions ⁺					Log-reductions (log CFU/cm ²) ^{1,2,3}				D-values ^{4,5} (min.) ($\frac{\text{Listeria}}{\text{Total}}$) ^{a,d}
	Gap (cm)	Voltage (kV)	Frequency (kHz)	O ₂ (mL/min)	Skin type	4 min exposure		8 min exposure		
						Total	<i>Listeria</i>	Total	<i>Listeria</i>	
I	1.0	8.0	38.5	0.0	Rough	(a)0.16 ± 0.05 ^a _A	(a)0.14 ± 0.14 ^a _A	(b)0.49 ± 0.09 ^a _A	(b)0.56 ± 0.12 ^a _{A,D}	16.9 ± 4.3 ^{a,d} _A 18.2 ± 3.5 ^a _A
II	1.0	11.0	23.0	25.0	Rough	(a)0.02 ± 0.04 ^a _B	(a)0.00 ± 0.11 ^a _A	(a)0.01 ± 0.21 ^a _B	(a)0.22 ± 0.18 ^A _B	28.6 ± 1.9 ^b _B 52.8 ± 15.3 ^b _A
III	1.0	11.0	23.0	25.0	Smooth	(a)0.05 ± 0.01 ^a _B	(a)0.05 ± 0.04 ^a _A	(a)0.01 ± 0.02 ^a _B	(b)0.18 ± 0.01 ^b _B	47.7 ± 2.7 ^c _B 89.2 ± 18.9 ^c _A
IV	1.0	11.0	30.0	25.0	Rough			0.48 ± 0.10 ^a _A	0.50 ± 0.06 ^a _A	16.3 ± 2.1 ^{a,d} _A 17.2 ± 4.0 ^a _A
V	1.0	16.0	30.0	25.0	Rough			0.69 ± 0.03 ^a _{A,C}	0.40 ± 0.17 ^b _{A,B}	21.8 ± 7.3 ^d _A 11.7 ± 0.6 ^a _A
VI	1.0	16.0	30.0	50.0	Rough			0.50 ± 0.04 ^a _A	0.55 ± 0.00 ^a _{A,D}	14.6 ± 0.1 ^{a,e} _A 16.1 ± 1.2 ^a _A
VII	1.0	16.0	30.0	100.0	Rough			0.91 ± 0.23 ^a _C	0.94 ± 0.20 ^A _C	8.8 ± 1.7 ^e _A 9.1 ± 2.0 ^a _A
VIII	0.5	16.0	30.0	100.0	Rough			0.57 ± 0.20 ^a _A	0.60 ± 0.13 ^a _{A,D}	13.9 ± 3.3 ^{a,e} _A 15.1 ± 4.7 ^a _A
IX*	1.0	16.0	30.0	100.0	Rough			0.77 ± 0.25 ^a _{C,D}	0.77 ± 0.25 ^A _{C,D}	11.1 ± 3.9 ^{a,e} _A 15.7 ± 1.5 ^a _A

⁺ Helium flow (5 L/min)

* Samples were not incubated to allow cell attachment, but treated immediately after cell deposition

¹ Within the same column (4 or 8 min exposure) and the same type of count (Total or *Listeria*), log-reductions lacking common letters (subscripts) are significantly different ($P \leq 0.05$).

² Within the same row (same experiment) and same treatment (4 or 8 min), log-reductions bearing different superscripts (uppercase letters on the right) are significantly different ($P \leq 0.05$).

³ Exp. I to III: within the same row (same experiment) and same type of count (Total or *Listeria*), log-reductions bearing different subscripts (brackets on the left) are significantly different ($P \leq 0.05$).

⁴ For like comparisons (either *Listeria* or Total), D-values lacking common superscripts are significantly different ($P \leq 0.05$).

⁵ For comparisons between *Listeria* and total counts for the same experiment, D-values lacking common subscripts (capital letters) are significantly different ($P \leq 0.05$).

TABLE 3. Inactivation of *Listeria innocua* on chicken breast muscle

Exp. ¹	CAP-Pen Treatment Conditions						Log-reductions ^{2,3} (log CFU/cm ²)						D-values ⁴ (min)
	Gap (cm)	Vol. (kV)	Freq. (kHz)	He (L min ⁻¹)	O ₂ (mL min ⁻¹)	Inoculum	10 s	30 s	1 min	2 min	4 min	8 min	
I_(a)	1.0	8.0	38.5	5.0	0.0	Aliquot	--	--	--	--	--	0.06±0.04 _A	162.2 ± 95.8 ^a
II_(b)	1.0	11.0	30.0	5.0	25.0	Aliquot	--	--	--	--	--	0.41±0.02 _B	19.5 ± 0.8 ^b
III_(a)	1.0	16.0	30.0	5.0	100.0	Filter	1.25±0.26 ^a	1.58±0.34 ^a	1.61±0.03 ^{a,b}	2.17±0.56 ^{b,c}	2.71±0.54 ^c	3.30±0.14 ^d _C	0.1 ± 0.0 ^b *3.4 ± 0.3 ^b

¹ Initial viable counts of experiments lacking common letters are significantly different ($P \leq 0.05$).

² Within the same row, log-reductions bearing different superscripts are significantly different ($P \leq 0.05$).

³ Within the same column, log-reductions bearing different subscripts are significantly different ($P \leq 0.05$).

⁴ D-values bearing different superscripts are significantly different ($P \leq 0.05$)

*D-values of the second phase (dual-slope survival curves)

A)

B)

FIGURE 4

A)

20 μm

B)

20 μm

C)

D)