HIGH VOLTAGE ATMOSPHERIC COLD PLASMA TECHNOLOGY AS A FOOD SAFETY INTERVENTION FOR DECONTAMINATION OF CUTTING TOOLS DURING RTE POULTRY MEAT SLICING

Vanessa R Souza[a]*, Alba E Illera[a], Kevin M Keener[a]*

[a] School of Engineering, University of Guelph, 50 Stone Road East, NIG 2W1, Guelph, ON, Canada.

* Corresponding author: vanessardsouza@gmail.com

E-mail addresses: vanessardsouza@gmail.com (V. R. Souza), aeillera@ubu.es (Alba. E. Illera), kkeener@uoguelph.ca (K. Keener)

Declarations of interest: none

Abstract

This study aimed to build a prototype that integrates a cold plasma capability into a manual meat slicer while it is in operation to minimize cross-contamination from the circular cutting blade. A high voltage atmospheric cold plasma (HVACP) generator was attached to a meat slicer with a rotating 22.5 cm diameter stainless steel slicing blade. The meat slicer with the installed cold plasma generator was contained within a sealed, clear acrylic box. The HVACP generator consisted of a dielectric barrier discharge (DBD) configuration with 15 cm diameter aluminum electrodes and was operated at 50 kV with an average current of 4 mA (200 W). *Listeria innocua* (LI) was selected as the test organism, and initial testing was performed on stainless-steel coupons in room air at a relative humidity ranging from 30 to 90% for treatment times up to 240 s. Results found that 240 s and 90% RH showed a 3.0 log reduction of LI. These conditions were then replicated using "dirty" stainless-steel knife while rotating. The results showed only a 0.5 log LI reduction. The gas was changed from air to MA65 gas blend and then a 1.91 log LI reduction was achieved on the "dirty" knife.

Industrial Relevance

Due to the rapid processing speeds required in the food industry, it is difficult to assure the complete cleanliness of the tools and surfaces used at all times, and then, operations like the cutting /slicing step are still one of the critical points for cross-contamination during meat processing. High voltage atmospheric cold plasma (HVACP) is a practical intervention that has the potential to deliver a continuous, in-situ decontamination of the knife during operation to mitigate the risk of cross-contamination. The developed prototype, as shown in this study, can

deliver beneficial results in short treatment time and with minimal inputs of air and electricity, without heating effects, minimizing the use of water, or the use of sanitizers.

Keywords: cutting tool, knife, cross-contamination, meat industry, ready-to-eat poultry products, *Listeria innocua*, high voltage atmospheric cold plasma (HVACP).

1. Introduction

Despite the current sanitary regimes in food production, contamination and outbreaks are still a real and recurrent problem. In the case of the meat industry, a major concern is related to the re- or cross-contamination directly resulted from product-to-product contact, or indirectly following repeated contact with common surfaces (Bacon, 2005). The cutting step, by using the same knife for several batches, is usually one of the critical points for cross-contamination by pathogenic bacteria during meat processing (Leipold, Kusano, Hansen, & Jacobsen, 2010). If one piece of meat exhibits microbial contamination, it is likely that the cutting tool used for the slicing will disperse that microorganism onto other portions of the food. Due to the rapid processing speeds required in the food industry, it is difficult to assure the complete cleanliness of the tools and surfaces used at all times. It is impractical to decontaminate cutting tools between each meat "log" or batch due to its continuous operation. So, in the case of a contaminated cutting tool, the contamination would be carried into subsequent meat batches.

Poultry carcasses are commonly contaminated with enteric pathogens such as *Salmonella* spp., *Campylobacter* spp. and *Listeria monocytogenes*, and hence there is high risk of those pathogens to re-contaminate or cross-contaminate the meat throughout processing (Rouger, Tresse, & Zagorec, 2017). *Listeria monocytogenes* is a human pathogen of concern that has been isolated and linked to foodborne outbreak of ready-to-eat (RTE) foods, including processed meats as cooked chicken (Kurpas, Wieczorek, & Osek, 2018; WHO, 2018). This bacteria is relatively heat sensitive and contamination, in the case of cooked poultry, normally occurs after cooking, during any subsequent slicing and packaging operations (Patterson, Mackle, & Linton, 2011). Outbreaks

of listeriosis linked to cooked poultry products are still very common (USDC, 2021; Centers for Disease Control and Prevention, 2021; Health Canada, 2019), and according to Murphy, Duncan, Beard, & Driscoll (2003), outbreaks caused by pathogens associated with RTE poultry products are presumably caused by post-cook cross-contamination prior to packaging.

High voltage atmospheric cold plasma (HVACP) is a practical intervention that has the potential to deliver a continuous, in-situ decontamination of the knife during operation to mitigate the risk of cross-contamination. HVACP consists of a partially ionized gas, primarily composed of electrons, ions and elements in their fundamental or excited states generated after the application of a high voltage electric field into any gas such as air. For discharge to open-air atmospheres, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the most important active plasma agents formed and extensively evaluated for their anti-microbial effects, playing a crucial role on the inactivation over a broad range of sporulating and spoilage/pathogenic microorganisms (Sarangapani, Patange, Bourke, Keener, & Cullen, 2018), including *E. coli*, *Salmonella* spp., *Listeria* spp., among others on a wide variety of food products and food contact surfaces (Leipold et al., 2010; Noriega, Shama, Laca, Díaz, & Kong, 2011; Wan, Chen, Pankaj, & Keener, 2017; Wan, Pankaj, Mosher, & Keener, 2019a; Ziuzina, Patil, Cullen, Keener, & Bourke, 2014,). HVACP can deliver beneficial results in short treatment time and with minimal inputs of air and electricity, without heating effects, minimizing the use of water, or the use of sanitizers. The plasma generation conditions (voltage, humidity, dielectric material, electrode geometry, etc.), which influence the reactive gas species generated and hence the mechanisms of sterilization, can be easily tailored to achieve selected organism destruction without damaging the quality or performance of the food product and/or food surface.

The main goal of this study is to build a prototype that integrates a cold plasma generator into a meat slicer that can continuously generate cold plasma while it is in operation to minimize cross-contamination during meat slicing, particularly cooked poultry products. This study aims to demonstrate the effectiveness of HVACP coupled with the rotating tool for inactivating *Listeria innocua*, a non-pathogenic surrogate for *L. monocytogenes* (Vaz-Velho, Fonseca, Silva, & Gibbs, 2001) – a pathogen of concern in ready-to-eat (RTE) chicken products, on the stainless-steel surface. Treatment time, relative humidity and gas composition were the variable conditions investigated in this study.

2. Materials and Methods

2.1 HVACP prototype set-up

The high voltage atmospheric cold plasma (HVACP) was generated using a dielectric barrier discharge (DBD) integrated into the meat slicer. The system comprised a high voltage transformer (Phoenix Technologies, Inc., USA) with a voltage output of 30-130 kV at a frequency of 60 Hz, a voltage regulator (0-100%) and two circular aluminum electrodes of 15 cm diameter between which the plasma was generated.

An electrical meat slicer (Weston, Hubert Company, USA), with a manual feed and belt driven with a 9" stainless steel blade with dimensions of 29.21 cm (L) x 49.53 cm (W) 35.56 cm (H) (150 W) was used for this prototype. The HVACP DBD was installed in the top of an acrylic box of 42.50 cm (L) x 61.50 cm (W) x 65.00 cm (H) with the meat slicer located directly below. The center of the knife was positioned approximately 20 cm below the DBD. Each of the two

electrodes were attached to a polypropylene dielectric layer of 1 mm thickness with a gap of 2 cm where plasma was generated. The acrylic box has a front door to allow the access to the system and it has a few sealed holes for the meat slicer and electrodes' cables. Figure 1 shows the prototype unit.

2.2 Characterization of reactive gaseous species (RGS)

Optical Emission Spectroscopy (OES)**,** performed to identify the electronic transitions in the gaseous species during plasma treatment, was carried out using an optical fibre (Ocean Optics, Inc., USA) with a core diameter of 400 μm that was fixed 10 cm away from the middle of the electrode (plasma field). The characteristic light emission was transferred by the fibre, that would come out of the system through a sealed hole, to a custom-built high-resolution (0.3459 ± 0.0265 nm) spectrometer HR 2000+, controlled through the OceanView software (Ocean Optics, Inc., USA). The integration time for spectral recording was set to 5 s and an average of 10 scans was recorded. The light emission was measured during the 240 s plasma treatment (every 60 s), at low (30%) and high (90%) relative humidity conditions. Each spectrum was automatically corrected by the software for dark current and background noise via subtraction. The electronic transitions of the gaseous reactive species were identified based on literature (Mahnot, Mahanta, Keener, & Misra, 2019; Misra et al., 2014; Moutiq, Misra, Mendonça, & Keener, 2020).

The ozone concentration was measured immediately after each treatment using an ozone monitor (2B Technologies, 106-MH Model, USA). The peroxide species measurements were carried out using a hydrogen peroxide detection tube obtained from pre-calibrated Dräger® gas detection tubes. Briefly, immediately after the HVACP treatments, 20 to 100 mL of gas samples were

accurately pulled out of the box using the Gastec gas sampling pump. The concentration was calculated based on the color change (white to brown) approximately 3 minutes after sampling.

2.3 Inoculum Preparation and Inoculation

Listeria innocua ATCC 33090, obtained from the American Type Culture Collection (Manassas, VA, USA), was activated by transferring a loopful of the stock culture, stored with 50% glycerol at −80 °C, to 40 mL of Tryptic Soy plus Broth Yeast Extract (TSBYE) (Oxoid, Thermo Fisher Scientific, Canada) and incubating overnight at 37ºC. The working culture was then spread plated on Tryptic Soy plus Agar Yeast Extract (TSAYE) and after incubation at 37°C for 48 h, one isolated colony was transferred into 40 mL of TSBYE. After incubation, the bacteria cells were harvested by centrifuging at 3,500 x g for 20 min, at 4ºC (Thermo Scientific, Model Sorvall ST 40R, EUA). The obtained pellet was washed in 0.9% saline and then re-suspended in this solution to a final concentration of 10^8 cells.ml^{-1}. The stock suspension was kept refrigerated at 4ºC for up to one week. The bacteria concentration was determined by measurement of its optical density at 600 nm using a UV-VIS spectrophotometer (Thermo Scientific, Model Genesys 10S UV-Vis, EUA).

For a better control and convenience, stainless-steel coupons with the dimension of 1 cm x 5 cm and with a thickness of 2 mm were initially used for optimization of the HVACP conditions over *L. innocua* inactivation. Afterwards, a polished stainless steel round knife with a diameter of 22 cm was utilized. For this purpose, two different scenarios were investigated: *1)* Sterilized "Clean" Knife and *2)* Sterilized "Dirty" Knife. In the first scenario, the knife was cleaned and disinfected with ethanol 70% before inoculation, but for the second scenario, after cleaning and

disinfection, cooked chicken breast was rubbed around the knife's edge to mimic a knife in use within the poultry industry before the inoculation.

L. innocua suspension (100 µL) was spot inoculated onto the coupons or knife surface (1 x 10^7 cells), taking care not to allow the suspension to overflow the edges. The coupons and knife were kept at room temperature inside the Biosafety Cabin (BSC) and air-dried for around 45 min.

2.5 HVACP Treatment

The plasma treatment voltage was fixed at 50 kV and all experiments were carried out under room temperature conditions. The working gas used was atmospheric air (22% O_2 and 78% N_2), and in the case of the "dirty" knife, enriched oxygen modified air (MA65-65% O_2, 30% CO_2 and 5% N_2).

In summary, the coupons, in triplicate, were attached to the knife (in the center, in the middle and close to the border of the knife) using a double side tape and they were initially enclosed with a plastic container and the system was warmed up for 5 minutes to increase the reactive gas species, simulating a more real industry situation. After this warming up time, the cover of the coupons was carefully and quickly removed to expose them to the antimicrobial species generated during the plasma treatment, the knife was switched on to be constantly rotating and the plasma treatment started to be counted. In the case of the knife, after the warming-up, the door was quickly opened, and the inoculated knife attached to the meat slicer. The treatment time was considered as the time after the 5 minutes warm up.

189 The variables investigated in this study were treatment time (plasma treatment time after the 5
190 minutes warming up time) – 30, 60, 120 and 240 s and the relative humidity of the air – low
191 (30%) and high (90%) humidity. The experiment consisted in a complete factorial design
192 (Treatment Time *X* Relative Humidity), with 8 treatments that were performed in 3 repetitions.
193 The positive (inoculated coupons) and negative (non-inoculated coupons) controls have been
194 performed using the same procedure without switching on the plasma. The best condition (90%
195 RH and 240 s) was replicated in the actual knife ("clean" and "dirty" scenario). As in the
196 coupons, a positive (inoculated knife) and negative (non-inoculated knife) controls have been
197 performed using the same procedure without switching on the plasma.
198
199 The power drawn by the system during the operation was monitored using a wall power meter.
200 The temperature of the knife was also recorded after each treatment.
201
202 **2.6 Microbial Recovery and Enumeration**
203 Immediately after the treatment time, the enumeration of the surviving *L. innocua* was
204 performed. Briefly, the coupons were carefully removed from the knife and placed inside a
205 centrifuge tube containing 10 mL of Maximum Recovery Diluent (MRD) and vortexed for 2
206 min. Surviving cells on the surface of the stainless-steel knife were swabbed using a round cotton
207 previously hydrated with 2 mL of MRD + 0.01% Tween 20. After swabbing, the cotton was
208 transferred to a sterile stomacher bag, and after adding 18 mL of MRD (final volume of 20 mL),
209 the samples were pummeled at high speed for 120s in stomacher Seward Laboratory blender 400
210 (UAC House, London, England). The diluent obtained after the coupons or knife recovery was
211 serially diluted in 0.9% saline and spread plated on Tryptic Soy plus Yeast Extract Agar

212 (TSAYE) (Oxoid, Thermo Fisher Scientific, Canada). The plates were incubated at 37ºC for 48 h
213 and the resulting colony forming units (CFU) were counted.
214
215 **2.7 Statistical Analysis**
216 The data were represented as mean ± standard deviation. The results were evaluated by analysis
217 of variance (ANOVA) and Tukey's post-hoc test with a 95.0% significance level using SPSS
218 software Statistics Premium 27 ver. (IBM® SPSS® Statistics 20).
219
220 **3. Results and Discussion**
221 **3.1 Characterization of the reactive gaseous species**
222 Emission spectroscopy is a technique that is used to identify the electronic transitions in the
223 gaseous species during the time of high voltage plasma generation (Moutiq et al., 2020). The
224 emission spectrums of the plasma discharge (in absorbance) for the tested conditions (low and
225 high relative humidity) are shown in Figure 2. This spectrum refers to the emission spectrums
226 collected immediately after the 240 seconds treatment at 50 kV, at low (30%) and high (90%)
227 relative humidity.
228
229 Regardless of the relative humidity (RH), the strongest emission was observed on the wavelength
230 range from 315 to 405 which represent mostly excited nitrogen species including nitrogen
231 second positive system N_2(C-B) and first negative system N_2^+ (B-X) (Figure 2). This intense
232 spectral signature indicates the occurrence of energetic collisions of electrons with molecular
233 nitrogen in air. According to Moutiq et al. (2020), during the electrical discharge process,
234 electrons acquire enough energy to ionize dominant air molecules such as N_2 and O_2 and form

the excited N_2^* molecules. These air plasma emission spectrum obtained in this study is similar to the spectrum presented by different authors (Mahnot, Mahanta, Keener, et al., 2019; Moutiq et al., 2020; Wan et al., 2019a)

The hydroxyl molecular band (OH) was recorded at a very low intensity with a band-head around 300 nm, corroborating with previous papers (Mahnot, Mahanta, Keener, et al., 2019; Misra et al., 2014). There was not a clear difference in the intensity of this peak at 30 or 90% humidity. The optical transition of the O atom was captured also at a low intensity and only at the wavelength around 777 nm ($2s^2p^3 3p5P \rightarrow 2s^2 2p^3 3s5S$). The relatively lower intensity or even indetectable peaks associated with oxygen comparing to the intensity of reactive nitrogen species is likely due to self-quenching of O (^3p) or O(^5p) in the air plasma (Walsh, Liu, Iza, Rong, & Kong, 2010). Moreover, the singlet oxygen formed in plasma also undergoes self-recombination or react with ozone (O_3) to produce molecular oxygen (Moutiq et al., 2020).

Ozone and hydrogen peroxide concentration after 30-, 60-, 120- and 240-seconds plasma treatment at 50 kV, for the tested conditions (Air 30% humidity and Air 90% humidity) are shown in Table 1.

Table 1. Ozone and hydrogen peroxide (mean ± standard deviation) for HVACP treatment at 50 kV immediately after 30, 60, 120 and 240 s minutes plasma treatment with air 30% humidity and 90% humidity.

Treatments	Dry Conditions (30% RH)		Wet Conditions (90% RH)	
	Ozone (ppm)	H_2O_2 (ppm)	Ozone (ppm)	H_2O_2 (ppm)

30s	235.5 ± 4.5	21.0 ± 1.0	78.5 ± 0.5	7.5 ± 0.5
60s	239.5 ± 13.5	31.0 ± 1.0	97.0 ± 7.0	8.5 ± 0.5
120s	267.0 ± 3.0	41.0 ± 1.0	111.0 ± 3.0	9.5 ± 1.0
240s	329.5 ± 14.5	52.0 ± 6.0	136.5 ± 5.5	10.5 ± 0.5

The ozone concentrations increased significantly with the plasma exposure time (30–240s), regardless of the relative humidity (Table 1). It can also be noticed that the increase in humidity promoted a decrease in the ozone levels generated. The low humidity conditions (30% RH) generated ozone levels ranging from 225.5 to 329.5 ppm, while high humidity conditions (90% RH) produced levels from 78.5 to 136.5 ppm. As discussed by Patil et al. (2014), this trend can be attributed both to quenching by increased levels of water dissociation, and to O_3 direct reaction to water molecules, leading to an increase in OH, peroxide, and nitrogen oxide levels.

Although it is expected that higher relative humidity will generate a higher amount of OH radicals and hence larger amounts of peroxides such as H_2O_2, this was not the trend observed in this study. Levels of hydrogen peroxide ranging from 21.0 to 52.0 ppm was generated at low humidity conditions (30% RH) whereas much lower levels, ranging from 7.5 to 10.5 ppm was observed at high humidity environment (90% RH). It is hypothesized that at high relative humidity, OH preferably generates other peroxides species – $OH^-(H_2O)_n$ and $H_3O^+(H_2O)_n$ or nitrogen species – HNO_x rather than H_2O_2.

3.2 *Listeria Innocua* Inactivation

Stainless-steel Coupons

The effect of HVACP treatment on *L. innocua* inactivation on stainless-steel coupons for the different treatment times and humidity levels is shown in Figure 3.

It can be noticed that regardless the treatment time, at low relative humidity conditions (30%), less than 1 log is inactivated (Figure 3). However, it is clear the critical role the humidity has on the *L. innocua* inactivation since reductions levels are 2-3 times greater at high humidity conditions (90% RH) than low humidity environment (30% RH), at the same treatment time. Also, at high humidity, the longest the treatment time, the greater the inactivation effect, being 3 logs inactivated after 240 s treatment.

Supporting our findings, previous studies have shown that the gas mixtures containing water results in an abundance of highly oxidizing species, such as $OH^{\cdot}$ radicals, H_2O_2 and hydronium ions H_3O^+, that have a strong bactericidal effect, yielding significantly the inactivation rate (Dobrynin, Friedman, Fridman, & Starikovskiy, 2011; X. Lu, 2014). Several studies have reported that the relative humidity plays a very important role on bacterial inactivation. In a study on the influence of high voltage atmospheric cold plasma process parameters on inactivation of *Bacillus strophaeus* spores, the authors concluded that increasing relative humidity had a significant benefit on spore inactivation (Patil et al., 2014).

Several studies have reported the effectiveness of cold plasma and the effect of the process conditions and matrix composition/surface on *L. innocua* / *L. monocytogenes* in food surface (Leipold et al., 2010) and a broad variety of food products such as chicken meat and chicken skin (Noriega et al., 2011), ready-to-eat meat (Rød, Hansen, Leipold, & Knøchel, 2012), cheese

298 (Wan, Pankaj, Mosher, & Keener, 2019b), fresh produce (Ziuzina et al., 2014), among many
299 others. The mechanism of inactivation of microorganisms by cold plasma is complex, but
300 previous studies have reported that the reactive oxygen species, such as O_3, O, $^1O_3$, H_2O_2 and
301 NO_x, attack both cell envelope, causing disruption of the cell and therefore resulting in leakage
302 and also damages to intracellular components (*e.g.*, DNA) (Han et al., 2016; H. Lu, Patil,
303 Keener, Cullen, & Bourke, 2014; Mahnot, Mahanta, Farkas, Keener, & Misra, 2019).
304
305 ***Stainless Steel Knife***
306 The effect of HVACP treatment on *L. innocua* inactivation on stainless steel knife (Clean and
307 Dirty) after 240s treatment at 90% RH is shown in Figure 4. The time of 240s was selected
308 because, although statistically similar to 120s, it was the treatment time that presented
309 statistically better reductions than 30 and 60s on stainless-steel coupons (Figure 3). Note that due
310 to the poor inactivation in the dirty knife using air, this scenario was also performed using
311 modified air (MA65). In those further experiments involving the modified air, the focus was
312 given only to "dirty" knife, as it represents a more realistic situation.
313
314 As expected, the *L. innocua* inactivation on the clean knife surface using air 90% RH after 240 s
315 treatment achieved 2.74 logs reduction, which is similar to the inactivation observed on the
316 stainless-steel coupons (3.00 logs reduction) at the same conditions. The inactivation on the
317 "dirty" knife surface however, was much lower at 0.5 log reduction. Studies evaluating the
318 effectiveness of HVACP on different surfaces/food matrix as cheese, cheese model and culture
319 medium and chicken muscle and skin, revealed that the surface features have a huge impact on

320 HVACP efficacy by affecting the attachment of the bacteria cells and protecting the bacteria
321 from the chemical plasma species generated (Noriega et al., 2011; Wan et al., 2019a).
322
323 Residual food contamination on a food contact surface poses a greater difficulty for
324 decontamination than a surface without food contamination. We have not found any cold plasma
325 research on this topic, but other non-thermal technology studies on knife decontamination
326 including UV-C treatment and pulsed UV light found that the decontamination of knives is
327 affected by the presence of food residues like blood, protein and fat (Rajkovic et al., 2010;
328 Raschle, Zweifel, Zurfluh, & Stephan, 2019). According to Rajkovic et al. (2010), the
329 inactivation of pathogenic bacteria (*L. monocytogenes* and *Escherichia coli* O157:H7) depended
330 on the type of meat product that was in the contact with the treated knife and on the time
331 between the contamination and the treatment. Specifically, they found that the inactivation of *L.*
332 *monocytogenes* that was higher than 6 logs if treatment was performed after 1 min inoculation,
333 can be lower than 2 logs if treatment occurs 60 min after contamination.
334
335 To overcome this lower inactivation, the inoculated "dirty" knife was treated using a different
336 gas composition (MA65 - 65% O_2, 30% CO_2 and 5% N_2), at also 90% RH. After the same
337 treatment time (240 s), a much higher inactivation was observed (1.91 logs). At this condition, a
338 higher concentration of ozone is generated, around 198 ppm compared to approximately 137
339 ppm using atmospheric air. Besides the ozone, other reactive oxygen species, such as atomic
340 oxygen (O), excited oxygen (O_2), singlet oxygen ($^1O_3$), superoxide anion (O_3) and also the water
341 species as $OH^{\cdot}$ radicals, peroxides species and hydronium ions H_3O^+, will likely be generated at a
342 higher concentration.

In a similar study, where the authors also aimed to decontaminate a rotating cutting tool during operation by using cold plasma, a much higher reduction of *L. Innocua* in "clean" knife was observed in ambient air (unknown relative humidity) using a milder condition, but much simpler and in a much smaller scale than the one developed here (Leipold et al., 2010). The authors stated that after 340 s of treatment at 10.4 kV and 360 W, 5 logs were inactivated. It is hypothesized that the higher inactivation observed in this work, although using milder conditions, it due to the fact that the plasma was created on the knife surface, which means a direct type of exposure. In this way, the exposure of target to plasma occurs before active species recombine and are lost; therefore, this system maximizes the surface interaction with the short-lived reactive gas species, UV and electric fields (Niemira & Gutsol, 2011; Sarangapani et al., 2018). In this study, the air gap between the ceramic sheet, that holds the electrode, and the knife surface was very small, only 2 mm, which is impractical for industrial purposes. A study on the effect of HVACP on inactivation of *L. innocua* on queso fresco, is one of the several that have been reporting that direct treatment is in fact more efficient compared to indirect treatment, where only longer-lived reactive species would reach and interact with the microorganism. However, the direct mode of exposure system is more challenging to scale up where one would need to consider isolating any electrical charge accumulating on the knife from the other sensitive electronics on the machine and the operator.

The cold plasma generator-meat slicer prototype developed in this work shows promise as a sustainable, cost-effective, in-situ and continuous intervention to minimize cross-contamination in the meat slicing operation. Because it requires minimal power, it can be easily scaled up to

366 operate industrially Further testing is needed to evaluate the knife performance over a prolonged
367 time being exposed to the cold plasma species and also studies focusing on other pathogens of
368 concern (*E.g. Salmonella* and *Campylobacter*) and other types of food matrix (E.g. Beef and
369 Pork Meat).
370

371 **4. Conclusion**

372 This study shows that the developed HVACP DBD meat slicer prototype that integrates a cold
373 plasma capability into a meat slicer has great potential to reduce pathogen cross-contamination
374 during meat processing, particularly cooked poultry products. The results showed that the
375 relative humidity plays a crucial role in the *L. innocua* inactivation being that 90% relative
376 humidity and 340 s treatment, around 3 logs reduction was observed in the "clean knife". In the
377 case of the "dirty" knife, the chicken nutrients such as proteins and fat clearly posed a higher
378 level of complexity on the decontamination since at the same conditions, the *L. innocua*
379 inactivation was much lower, 0.5 logs. However, changing the gas composition to a gas richer in
380 oxygen (modified air – MA65 at 90% RH), a higher inactivation (1.91 logs) was observed after
381 the same treatment time (340 s).
382

References

(USDC), U. S. D. of A. (2021). *Tyson Foods Inc. Recalls Ready-To-Eat Chicken Products Due to Possible Listeria Contamination*. Retrieved from https://www.fsis.usda.gov/recalls-alerts/tyson-foods-inc.-recalls-ready-eat-chicken-products-due-possible-listeria

Bacon, R. T. (2005). Physical decontamination strategies for meat. In *Improving the Safety of Fresh Meat* (pp. 318–349). https://doi.org/10.1533/9781845691028.2.318

Centers for Disease Control and Prevention, C. (2021). *Listeria Outbreak Linked to Fully Cooked Chicken*. Retrieved from https://www.cdc.gov/listeria/outbreaks/precooked-chicken-07-21/index.html

Dobrynin, D., Friedman, G., Fridman, A., & Starikovskiy, A. (2011). Inactivation of bacteria using dc corona discharge: Role of ions and humidity. *New Journal of Physics*, *13*. https://doi.org/10.1088/1367-2630/13/10/103033

Han, L., Patil, S., Boehm, D., Milosavljević, V., Cullen, P. J., & Bourke, P. (2016). Mechanisms of inactivation by high-voltage atmospheric cold plasma differ for Escherichia coli and Staphylococcus aureus. *Applied and Environmental Microbiology*, *82*(2), 450–458. https://doi.org/10.1128/AEM.02660-15

Health Canada. (2019). *Public Health Notice - Outbreak of Listeria infections linked to Rosemount brand cooked diced chicken*. Retrieved from https://www.canada.ca/en/public-health/services/public-health-notices/2019/outbreak-listeria-infections-cooked-diced-chicken.html

Kurpas, M., Wieczorek, K., & Osek, J. (2018, March 1). Ready-to-eat meat products as a source of Listeria monocytogenes. *Journal of Veterinary Research (Poland)*, Vol. 62, pp. 49–55.

https://doi.org/10.2478/jvetres-2018-0007

Leipold, F., Kusano, Y., Hansen, F., & Jacobsen, T. (2010). Decontamination of a rotating cutting tool during operation by means of atmospheric pressure plasmas. *Food Control*, *21*(8), 1194–1198. https://doi.org/10.1016/j.foodcont.2010.02.006

Lu, H., Patil, S., Keener, K. M., Cullen, P. J., & Bourke, P. (2014). Bacterial inactivation by high-voltage atmospheric cold plasma: Influence of process parameters and effects on cell leakage and DNA. *Journal of Applied Microbiology*, *116*(4), 784–794. https://doi.org/10.1111/jam.12426

Lu, X. (2014). On atmospheric pressure nonequilibrium plasma jets. In P. K. Chu & X. Lu (Eds.), *Low temperature plasma technology: Methods and applications* (pp. 173–206). CRC Press, Taylor and Francis Group.

Mahnot, N. K., Mahanta, C. L., Farkas, B. E., Keener, K. M., & Misra, N. N. (2019). Atmospheric cold plasma inactivation of Escherichia coli and Listeria monocytogenes in tender coconut water: Inoculation and accelerated shelf-life studies. *Food Control*, *106*, 106678. https://doi.org/10.1016/j.foodcont.2019.06.004

Mahnot, N. K., Mahanta, C. L., Keener, K. M., & Misra, N. N. (2019). Strategy to achieve a 5-log Salmonella inactivation in tender coconut water using high voltage atmospheric cold plasma (HVACP). *Food Chemistry*, *284*, 303–311. https://doi.org/10.1016/j.foodchem.2019.01.084

Misra, N. N., Sullivan, C., Pankaj, S. K., Alvarez-Jubete, L., Cama, R., Jacoby, F., & Cullen, P. J. (2014). Enhancement of oil spreadability of biscuit surface by nonthermal barrier discharge plasma. *Innovative Food Science and Emerging Technologies*, *26*, 456–461. https://doi.org/10.1016/j.ifset.2014.10.001

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

Murphy, R. Y., Duncan, L. K., Beard, B. L., & Driscoll, K. H. (2003). D and z values of Salmonella, Listeria innocua, and Listeria monocytogenes in fully cooked poultry products. *Journal of Food Science*, *68*(4), 1443–1447. https://doi.org/10.1111/j.1365-2621.2003.tb09664.x

Niemira, B. A., & Gutsol, A. (2011). Nonthermal plasma as a novel food processing technology. In *Nonthermal Processing Technologies for Food* (pp. 271–288). https://doi.org/10.1002/9780470958360.ch20

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

Patil, S., Moiseev, T., Misra, N. N., Cullen, P. J., Mosnier, J. P., Keener, K. M., & Bourke, P. (2014). Influence of high voltage atmospheric cold plasma process parameters and role of relative humidity on inactivation of Bacillus atrophaeus spores inside a sealed package. *Journal of Hospital Infection*, *88*(3), 162–169. https://doi.org/10.1016/j.jhin.2014.08.009

Patterson, M. F., Mackle, A., & Linton, M. (2011). Effect of high pressure, in combination with antilisterial agents, on the growth of Listeria monocytogenes during extended storage of cooked chicken. *Food Microbiology*, *28*(8), 1505–1508. https://doi.org/10.1016/j.fm.2011.08.006

Rajkovic, A., Tomasevic, I., Smigic, N., Uyttendaele, M., Radovanovic, R., & Devlieghere, F. (2010). Pulsed UV light as an intervention strategy against Listeria monocytogenes and

Escherichia coli O157:H7 on the surface of a meat slicing knife. *Journal of Food Engineering*, *100*(3), 446–451. https://doi.org/10.1016/J.JFOODENG.2010.04.029

Raschle, S., Zweifel, C., Zurfluh, K., & Stephan, R. (2019). Decontamination of knives used in a slaughterhouse by a commercial non-thermal UV-C treatment. *Italian Journal of Food Safety*, *8*(2), 8107. https://doi.org/10.4081/IJFS.2019.8107

Rød, S. K., Hansen, F., Leipold, F., & Knøchel, S. (2012). Cold atmospheric pressure plasma treatment of ready-to-eat meat: Inactivation of Listeria innocua and changes in product quality. *Food Microbiology*, *30*(1), 233–238. https://doi.org/10.1016/j.fm.2011.12.018

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

Sarangapani, C., Patange, A., Bourke, P., Keener, K., & Cullen, P. J. (2018). Recent advances in the application of cold plasma technology in foods. *Annual Review of Food Science and Technology*, *9*(1), 609–629. https://doi.org/10.1146/annurev-food-030117-012517

Vaz-Velho, M., Fonseca, F., Silva, M., & Gibbs, P. (2001). Is Listeria innocua 2030c, a tetracycline-resistant strain, a suitable marker for replacing L. monocytogenes in challenge studies with cold-smoked fish? *Food Control*, *12*(6), 361–364. https://doi.org/10.1016/S0956-7135(01)00029-9

Walsh, J. L., Liu, D. X., Iza, F., Rong, M. Z., & Kong, M. G. (2010). Contrasting characteristics of sub-microsecond pulsed atmospheric air and atmospheric pressure helium-oxygen glow discharges. *Journal of Physics D: Applied Physics*, *43*(3). https://doi.org/10.1088/0022-3727/43/3/032001

Wan, Z., Chen, Y., Pankaj, S. K., & Keener, K. M. (2017). High voltage atmospheric cold

plasma treatment of refrigerated chicken eggs for control of Salmonella Enteritidis contamination on egg shell. *LWT - Food Science and Technology*, *76*, 124–130. https://doi.org/10.1016/j.lwt.2016.10.051

Wan, Z., Pankaj, S. K., Mosher, C., & Keener, K. M. (2019a). Effect of high voltage atmospheric cold plasma on inactivation of Listeria innocua on Queso Fresco cheese, cheese model and tryptic soy agar. *LWT*, *102*, 268–275. https://doi.org/10.1016/j.lwt.2018.11.096

Wan, Z., Pankaj, S. K., Mosher, C., & Keener, K. M. (2019b). Effect of high voltage atmospheric cold plasma on inactivation of Listeria innocua on Queso Fresco cheese, cheese model and tryptic soy agar. *LWT - Food Science and Technology*, *102*(July 2018), 268–275. https://doi.org/10.1016/j.lwt.2018.11.096

WHO. (2018). Listeriosis. Retrieved May 18, 2021, from https://www.who.int/news-room/fact-sheets/detail/listeriosis

Ziuzina, D., Patil, S., Cullen, P. J., Keener, K. M., & Bourke, P. (2014). Atmospheric cold plasma inactivation of Escherichia coli, Salmonella enterica serovar Typhimurium and Listeria monocytogenes inoculated on fresh produce. *Food Microbiology*, *42*, 109–116. https://doi.org/10.1016/j.fm.2014.02.007

Figure Captions

Figure 1. Meat Slicer coupled with high voltage cold plasma prototype.

Figure 2. Emission Absorption spectrum for HVACP treatment at 50 kV immediately after 240 s treatment using air 30% and 90% relative humidity.

Figure 3. *Listeria innocua* inactivation after exposed to HVACP treatments for 30, 60, 120 and 240 s using air at two different relative humidity levels (30 and 90%). The detection limit of the applied enumeration method was 1.0 $\log_{10}$ CFU/g.

Same capital letter on top of each bar indicated no significant difference among the same treatment time, at different humidity levels. Same small letter on top of each bar indicated no significant difference among the same humidity, at different treatment time ($p < 0.05$).

Figure 4. *Listeria innocua* inactivation after exposed to HVACP treatments for 240 s at 90%RH. The detection limit of the applied enumeration method was 1.0 $\log_{10}$ CFU/g.