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Effect of cold atmospheric pressure plasma treatment on inactivation of *Campylobacter jejuni* on chicken skin and breast fillet

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ABSTRACT

The applicability of an atmospheric pressure plasma jet to reduce *Campylobacter jejuni* on chicken skin and breast fillet was evaluated. The efficacy of plasma treatment against two *C. jejuni* strains was examined using different feed gases (argon, air), exposure times (30–180 s) and distances from plasma source to sample (5, 8 or 12 mm). Furthermore, changes in color and temperature of treated samples were measured. Maximum mean *C. jejuni* reductions using argon or air as feed gases ranged from 0.78 to 2.55 and 0.65 to 1.42 log CFU/cm^2, respectively. Highest *C. jejuni* reductions were observed when using argon as feed gas, especially at longer treatment times. Most experimental settings had low and insignificant impact on color values. However, when using argon combined with a shorter distance of 8 mm, the lightness (L^*) of chicken breast samples increased significantly after 120 s treatment time. Temperature measurement revealed a maximum surface temperature of 61 °C, suggesting that denaturation could have affected inactivation efficacy and L^* values. This study shows that *C. jejuni* can effectively be reduced on chicken skin and breast fillet using an atmospheric pressure plasma jet.

1. Introduction

Campylobacteriosis is a common foodborne disease with over 200,000 reported cases in year 2015 in Europe (EFSA, 2016). Poultry meat, especially broiler chicken meat is considered the most important source of human *Campylobacter* infection, contributing to an estimated 20–30% of illnesses (EFSA, 2011). As measures to control the occurrence of *Campylobacter* in the primary production or during slaughter have not been sufficient, decontamination treatments are increasingly considered as another option for reduction.

Cold atmospheric plasma (CAP), which is a partly ionised gas consisting of electrons, ions, free radicals, excited species and UV-light has the advantage of reducing pathogen or spoilage organisms on food while having little impact on product quality (Scholtz, Pazlarova, Souskova, Khun, & Julak, 2015). In recent years a lot of research has been done investigating the potential of CAP to inactivate a variety of microorganisms on foods. For example, *Listeria innocua* was reduced by > 3 log CFU on chicken muscle after 4 min treatment using a CAP-pen (Noriega, Shama, Laca, Díaz, & Kong, 2011). Dirks et al. (2012) showed that *C. jejuni* can be reduced on raw poultry by up to 3 log CFU after 3 min treatment time using nonthermal dielectric barrier discharge (DBD) plasma. Radio-frequency (RF) plasma reduced *C. jejuni* on chicken hams by 0.5 or 1.5 log CFU after 3 min, depending on the strain tested (Kim, Lee, Cho, & Kim, 2013). These previous studies suggest that the method of plasma generation and application have an influence on the efficacy of CAP against pathogenic bacteria on chicken meat and -products. However, more information is needed on which plasma processing parameters are best for inactivation of *Campylobacter* on raw chicken meat while maintaining good product quality.

In this study, an atmospheric pressure plasma jet was used to inactivate *C. jejuni* on chicken skin and breast meat and to assess the impact of plasma treatment on product color quality. The aim was to evaluate the impact of different feed gases (argon, air), treatment distances and exposure times in regard to applicability for decontamination of chicken skin and meat.

2. Material and methods

2.1. Bacterial strains

Two different *C. jejuni* strains were used in this study. The strains were previously isolated from chicken (strain F2372, Hamedy, 2012) or chicken carcasses at slaughter (strain Ca144/*flaA* 49, Rossow, 2015). Frozen stocks (−80 °C) of *C. jejuni* were retrieved and grown on Modified Cefoperazone-Charcoal-Deoxycholate Agar (mCCDA, Oxoid Ltd.) for 24 h at 42 °C under microaerobic conditions (Anaerocult® C, Merck KGaA). For preparation of the inocula one colony was transferred from the agar plates to 10 ml Brain Heart Infusion Broth (BHI, Oxoid Ltd.)

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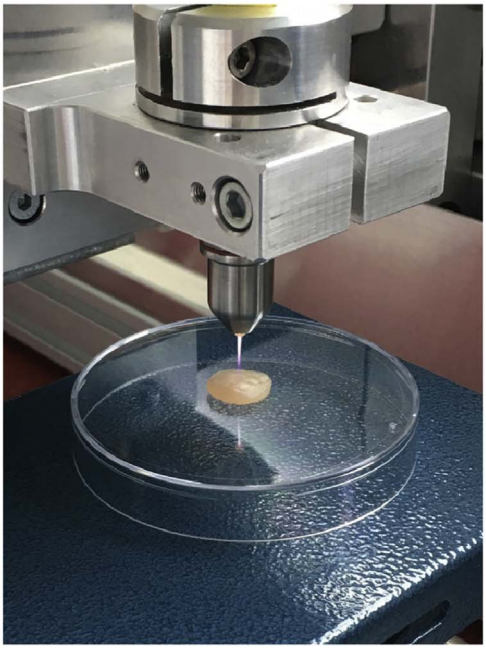

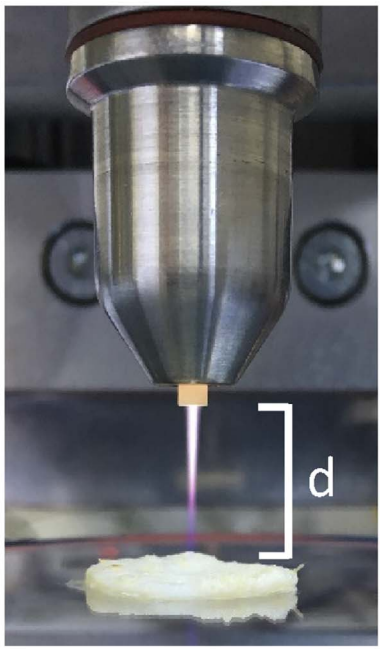

Fig. 1. Left, setup for plasma treatment of chicken skin and breast samples; right, adjustable distance (d) between plasma jet nozzle and sample surface.

and grown for an additional 24 h under the same conditions to obtain approximately 10^8 CFU/ml. The culture was then serially diluted in normal saline (0.85% w/v of NaCl, pH 7) solution and plated onto mCCDA to confirm counts.

2.2. Chicken skin and breast fillet

Chicken drumsticks with skin and breast fillets from conventional farming were purchased up to one week in advance of each experiment in a local supermarket and frozen at −20 °C. According to information obtained from the poultry processing company the products originated from strain Ross 308 broilers fed with complete feed pellets consisting mainly of wheat, maize and soya, and slaughtered at the age of 36 days. Before plasma treatment the products were thawed overnight at 4 °C and then punched into 2 cm^2 round pieces of skin or muscle. Care was taken in cutting equal pieces of approximately 1–2 mm thickness. The size of the samples was selected so that each piece at a time could be treated under the plasma jet under the same conditions (Fig. 1). For inactivation experiments the pieces were inoculated with 10 µl of inoculum (10^6–10^7 CFU/cm^2), which was uniformly spread over the surface of the samples with the pipette tip. The inoculated samples were allowed to sit at room temperature for approximately 20 min before plasma treatment.

2.3. Cold plasma treatment

For all experiments an atmospheric pressure plasma jet (kINPen 09®; neoplasm tools GmbH, Greifswald, Germany) was used. The device consists of a ceramic capillary tube with two electrodes: a stainless steel electrode, coupled with a high frequency voltage (1 MHz, 2–3 kV), in the centre and a grounded ring electrode at the distal end. The high frequency discharge between the electrodes results in plasma generation, when a feed gas flows through the capillary and expands to the surrounding air outside of the ceramic nozzle.

The efficacy of *Campylobacter* reduction was tested using different feed gases (argon or air), exposure times (30, 60, 120 or 180 s) and distances from plasma jet nozzle to sample surface (5, 8 or 12 mm). Pre-experiments showed that *C. jejuni* was inactivated on inoculated agar plates using the tested settings (data not shown). Argon was applied as a feed gas because of its well-known bacterial inactivation efficacy and low temperature when ionised in plasma jets using similar treatment times (Daeschlein et al., 2009; Lim, Uhm, & Li, 2007; Moritz, Wiacek, Köthe, & Braun, 2017). As an alternative feed gas air was chosen due to its low-cost and therefore high potential for industrial scale use. For a similar plasma jet as used in the present study Weltmann et al. (2009) recommends that the visible tip of the plasma plume touches the surface to be treated to achieve an effective antimicrobial effect. Therefore, the applied distances were adjusted in the same way depending on the length of the visible plasma plume (air: 5 mm, argon: 8 mm). In subsequent experiments the treatment distance for argon was extended to 12 mm to evaluate the impact on inactivation efficacy and product quality, when there was no visible contact between the sample and the tip of the plasma plume. The plasma jet was adjusted above the centre of the inoculated area (2 cm^2). The gas flow rate was kept constant at 5 standard liters per minute (slm).

Overall, 20 different treatment combinations (feed gas, exposure time, distance, *C. jejuni* strain) were tested for inactivation of *C. jejuni* on skin and breast. Each experiment was replicated three times resulting in a total of 60 independent experiments that were carried out at different days. Per experiment four skin and breast samples each were treated. For each experiment additionally two inoculated but untreated control skin and breast samples each were taken. The treatment distance 12 mm was tested only against strain F2372, since previous experiments showed no difference in susceptibility to argon plasma between the strains at a distance of 8 mm.

2.4. Bacterial recovery and enumeration

Immediately after the plasma treatment, each sample was vigorously vortexed in 10 ml of sterile 0.85% NaCl solution for 30 s. *Campylobacter* counts were determined by serial dilution (1:10) followed by plating onto mCCDA and microaerobic incubation for 24 h at 42 °C. For untreated inoculated controls the same procedure was applied. In each experiment negative control samples (skin, muscle) were tested equally by plating the lowest dilution on mCCDA to assure that no native *Campylobacter* contamination was present.

2.5. Surface color measurement

The color of uninoculated skin and breast samples was measured