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**The effects of atmospheric pressure cold plasma treatment on microbiological, physical-chemical and sensory characteristics of vacuum packaged beef loin**

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**Abstract**

Effects on vacuum packaged and non-packaged beef longissimus samples exposed to atmospheric cold plasma (ACP) generated at different powers over a 10 day period of vacuum- , and a subsequent 3 day period of aerobic storage were studied. Exposure of non-covered beef samples under high power ACP conditions resulted in increased a\*, b\*, Chroma and Hue values, but ACP treatment of packaged loins did not impact colour (L\*, a\*, b\*, Chroma, Hue), lipid peroxidation, sarcoplasmic protein denaturation, nitrate/nitrite uptake, or myoglobin isoform distribution. Colour values measured after 3 days of aerobic storage following unpackaging (i.e. 20 days *post mortem*) were similar and all compliant with consumer acceptability standards. Exposure to ACP of the polyamide-polyethylene packaging film inoculated with *Staphylococcus aureus*, *Listeria monocytogenes* and two *Escherichia coli* strains resulted in >2 log reduction without affecting the integrity of the packaging matrix. Results indicate that ACP can reduce microbial numbers on surfaces of beef packages without affecting characteristics of the packaged beef.

**Keywords:** Cold plasma; package decontamination; colour stability; beef quality

## 1. Introduction

In recent years it has been repeatedly suggested that the application of 'Atmospheric Cold Plasma' (ACP) in the food industry should be considered as an effective means to decontaminate and pasteurise food matrices for the production of minimally processed, high quality and microbiologically safe foods (e.g. Vijaya Nirmala, Lavanya, & Darsana, 2015). Antimicrobial (including antiviral) effects of ACP have in past years been reported for various food items (particularly carbohydrate-based foods such as soft fruits and salads). Yet, data on its application for muscle foods is relatively scarce (see Misra, Schlüter, & Cullen, 2016).

ACP is a technology that effectively achieves food preservation at ambient or sub-lethal temperatures, thus minimizing negative thermal effects on nutritional and quality parameters. ACP generation yields a rich mixture of reactive neutral species, energetic charged particles, UV photons and intense transient electric fields, which can interact simultaneously and synergistically at the food surface (see Guo, Huang, & Wang, 2015). Its antimicrobial action has been attributed to multiple pathways including ACP-induced DNA damage, photodesorption, lipid peroxidation leading to bacterial membrane disintegration, and through etching by radicals (Deng, Shi, & Kong, 2006; Korachi & Aslan, 2011; Mogul et al., 2003; O'Connell et al., 2011). Depending on the operating pressure, working gas and how the electrical energy is applied, the properties of ACP can vary enormously in terms of temperature and composition. The use of noble gas and low pressure plasma is widespread; however, in the area of plasma-food treatment, atmospheric pressure conditions using ambient air can almost be considered prerequisites given the financial constraints imposed. When generated in ambient air, ACP produces a wealth of antimicrobial Reactive Oxygen and Nitrogen Species (RONS) that are free to interact with the food surface. The readership is referred to Misra et al. (2016) for more details on the process.

Although applying ACP on an unpackaged food matrix would seem to effectuate the most potent antimicrobial action, such 'direct' treatment currently meets with difficulties as - to date - the technology is not certified for use in industrial practice. For instance, the fact that it remains unclear which particular foods are suitable for ACP treatment in the first place, and the lack of sufficient microbiological,

physical-chemical, toxicological and allergological data on foods subjected to particular and clearly defined ACP scenarios have been listed as major arguments against certification in the immediate future (German Research Community, DFG, 2012).

However, in the food sector ACP can also be applied for disinfecting surfaces and equipment. Given that ACP only requires air and electricity to operate and can be generated directly at the point of need, it can offer several advantages over existing disinfection methods. It has been widely reported that ACP can achieve a significant bacterial reduction (up to 6 log units in some reports) on a wide range of biotic and abiotic surfaces (e.g. Gadri et al., 2000; Muranyi, Wunderlich, & Heise, 2007; Yun et al., 2010). Given that British studies have indicated that the external surfaces of meat packages can act as a potential vehicle for the transfer of pathogens such as *Salmonella* and *Campylobacter* (e.g. Harrison, Griffith, Tennant, & Peters, 2001), it would seem entirely sensible to contemplate using ACP treatment for the purpose of decontamination on the outside of packed food items - at least until the accumulation of convincing data that would justify certification of 'direct' ACP treatment of the food matrix.

In this study, we first consider ACP decontamination of vacuum packaged beef, to confirm (1) a significant reduction of the microbial load prevalent on packaging film, (2) the integrity of the packaging matrix is maintained, and (3) that ACP does not breach the packaging barrier (possibly rendering the packaged food item to be classified as a 'novel food' and consequently to be subjected to the associated legislation). To add further evidence to the growing body of knowledge on the use of direct ACP exposure on the food matrix the major physical-chemical and sensory food quality parameters under differing ACP scenarios were considered. Certainly, several of the RONS produced in ACP, particularly  $N_yO_x$ , Hydrogen Peroxide and Ozone are known to affect the physical-chemical and sensory traits of muscle foods (e.g. Kanner, German, Kinsella, & Hultin, 1987; Min & Ahn, 2005) and their effects need to be further investigated.

## 2. Materials and Methods

### 2.1 Animals and sampling plan

Beef loins (left and right thoracic and lumbar longissimus muscle) with a normal ultimate muscle core pH were excised from the carcass of three 17-18 months old Fleckvieh bulls, which had been slaughtered, subsequently refrigerated at  $2\pm2$  °C, and sectioned to primal cuts, which were vacuum packed and further refrigerated. At 3 days *post mortem*, loin sub-primals were transported to the laboratory in refrigerated containers, and upon arrival portioned in approximately 2 x 5 x 5 cm cross sections to be assigned to ACP or control treatment.

The experimental setup aimed at - whenever feasible - comparing the effects of various treatments on adjacent areas within a muscle of a single animal, for which we followed a strict portioning plan. Per type of ACP treatment ('high power'- 'medium power' – 'low power'), including controls, one loin was used. Each loin was divided into 18 slices of 15 mm thickness. The first (i.e. most cranial) three slices per loin were cut in a medial and lateral half of comparable area. Assignment of these meat cuts to treatments was as follows: slice 1, lateral half: ACP-treatment, then vacuum-packaging; medial half: vacuum-packaging, then ACP ('treated control'); slice 2: lateral half, vacuum-packaging without ACP ('untreated control'). These three meat cuts constituted replicate 1. Replicate 2 was formed by the medial half of slice 2 ('untreated control'), the lateral half of slice 3 (ACP-treatment, then vacuum-packaging) and the medial half of slice 3 (vacuum-packaging, then ACP; 'treated control'). Since 6 replicates were tested, 3 sets of three slices each were needed for colour measurement and storage trials. Likewise, 3 x 3 slices were needed for chemical examination. Per loin, the first three slices formed replicates 1&2 for colour measurement and storage trials, followed by three slices for replicates 1&2 for chemical tests, and this sequence was repeated for replicates 3&4 and 5&6, respectively.

The rationale for this scheme was, that fibre type distribution is not homogeneous throughout skeletal muscle and this may lead, for instance, to different myoglobin concentrations (thus affecting light absorption), and different rates of glycolysis and hence protein denaturation (thus affecting light reflection) [e.g. Klont, Brocks, & Eikelenboom, 1998; Lundström & Malmfors, 1985].

## 2.2 ACP system

The non-thermal atmospheric pressure plasma system used in this study was developed at the University of Liverpool and is similar in operation to those reported by Olszewski, Li, Liu, & Walsh (2014) and Ni, Lynch, Modic, Whalley, & Walsh (2016). The system comprised of a surface DBD electrode unit, fabricated using a grounded metallic mesh electrode adherent to a quartz dielectric surface; on the opposing side of the quartz dielectric a metallic sheet was adhered to form a counter electrode, shown schematically in Figure 1(a). On application of a high voltage sinusoidal signal at an operating frequency of 9 kHz, a plasma was formed on the dielectric surface as shown in Figure 1(b). The surface DBD electrode unit was capable of generating air plasma over an 8 x 8 cm area of the hexagonal mesh electrode. Photographs of the system under differing operating conditions were used to calculate the surface coverage of the plasma to obtain power densities (W/cm<sup>2</sup>). The surface DBD electrode unit was stationed 2 cm from the sample under test. For all tests, the electrode enclosure loosely covered the sample, enabling some diffusion of ACP generated species away from the treatment area.

Here Figure 1

In the surface DBD ACP configuration the power dissipated within the plasma has a major impact on the nature of the RONS generated. Three different operating conditions were considered to provide contrasting gas phase chemistries, highlighted in Table 1.

Here Table 1

## 2.3 Gas phase species analysis

Key gas phase species generated by the plasma were analysed using a Fourier Transform InfraRed (FTIR) spectrometer (Jasco Analytical Instruments FT/IR-4000 series; JASCO Europe s.r.l., Cremella, Italy). Using a mid-IR optical bench from 7800 cm<sup>-1</sup> to 500 cm<sup>-1</sup> and resolution of 1 cm<sup>-1</sup> the absorption spectrum from the gas phase, under the operating conditions highlighted in Table 1, was obtained. For

analysis purposes, the effluent generated by the plasma system was drawn in to an FTIR gas cell (4 m path length) using an air pump at a rate <1 l/m, each measurement was repeated 5 times to obtain a mean.

In order to quantify species, densities from the FTIR absorption spectra, standard reference spectrum profiles obtained from the Pacific Northwest National Laboratory were used for spectra fitting (Ni et al., 2016). The HITRAN database was used as a reference for gaseous chemical identification and quantification. To complement the FTIR analysis, a commercial ozone monitor (106-M; 2B Technologies, Boulder, USA) was employed, the device had a measurement range from 0.1 to 1000 ppm and a resolution of 0.01 ppm.

#### *2.4 Loin sample treatments*

Three trials were conducted. In a Pre-Trial we compared untreated with ACP treated samples before these were packaged, to examine ‘immediate’ effects of plasma on muscle colour. In Trial 1 the effects of treating vacuum packaged loins with high and low power ACP scenarios were studied over a vacuum storage period of 10 days. Trial 2 also included treatment with medium power ACP and aimed at studying ‘delayed’ effects possibly observed after a subsequent final 3 day period of aerobic storage in a display refrigerator (fitted with a glass door, i.e. samples were exposed to day light) at 3±2 °C.

Animal is the experimental unit, i.e. to each of the treatment groups to be compared 6 cross section samples (taken from adjacent areas in the loins of a single animal; see above) were assigned. Table 2 includes a diagrammatic presentation to clarify the sequence of events, and time points of physical-chemical analysis during the various trials.

Here Table 2

Note that the experimental design included two control groups. The ‘untreated control’ reflects the industrial standard currently achieved in commercial practice. The ‘treated control’ samples were subjected to ‘direct’ plasma treatment before packaging, allowing the generation of reference values for any physical-

chemical/sensory change that may result from exposure of lean muscle to the ACP scenarios under study. These values should serve to establish whether or not plasma breaches the packaging matrix and consequently may produce similar effects in ACP-exposed packaged items.

## 2.5 Microbiological analysis

Antibacterial efficacy of the ACP device was assessed for four bacterial strains, i.e. *Staphylococcus aureus* DSM 1104, *Listeria monocytogenes* DSM 19094, *Escherichia coli* DSM 1103 and an isogenic mutant of *E. coli* O157:H7 EHEC strain EDL 933 (constructed by Gobert et al., 2007), provided by C. Martin, Unité Microbiologie, INRA Clermont-Ferrand-Theix; France.

Bacteria were kept in 20% glycerol at -80 °C. Before use, strains were separately cultivated in LB broth acc. to Miller (Merck, Darmstadt, Germany) and incubated overnight at 30 °C to reach the stationary phase. The overnight cultures were diluted in 0.85% saline to ca. 5 log cfu/ml using McFarland standards (BioMerieux, Marcy l'Etoile, France). A volume of 0.1 ml of this suspension was spread on Plate-Count-Agar (PCA, Merck, Germany; supplemented with 0.5% Lab Lemco Powder, and 0.3% yeast extract; Oxoid, Basingstoke, UK). After 5-7 minutes, plates were subjected to atmospheric plasma treatment (2 cm distance from the mesh electrode to agar surface, 1 minute duration); with high and low power settings (Trial 1). Plates were exposed to the atmospheric plasma source either directly or after having been covered with the packaging film (with ca. 7 mm distance from the agar surface). Controls consisted of PCA plates inoculated with the original suspensions and decimal dilutions thereof. Plates were incubated for 48 h at 30 °C and colonies per plate then recorded. In order to exclude growth of contaminant bacteria, colonies were examined for morphology and per plate, identity of colonies was confirmed by testing three colonies with Gram stain and biochemical testing (API 20E, Staph, Listeria schemes; BioMerieux). To plates with no colony growth, a value of 1 cfu/plate was assigned. For each strain and device setting, six replicates were processed.

In order to study the efficacy of the treatment on bacteria contaminating a packaging film surface, suspensions (10 µl droplets) of the aforementioned bacteria were placed on a polyamide-polyethylene film and treatment with atmospheric

plasma followed immediately. For determination of microbial numbers, the inoculated film was excised under sterile conditions and then vortexed with sterile glass beads in 10 ml of 0.85 % saline, and the suspension processed as indicated above.

## 2.6 Analysis of packaging matrix

The packaging film used was a 90 µm thick polyamide-polyethylene (PAPE) food grade packaging film [Combivac, foil type 20/70, O<sub>2</sub> permeability of 50 m<sup>3</sup>/(m<sup>2</sup> 24 h bar), CO<sub>2</sub> permeability of 150 cm<sup>3</sup>/(m<sup>2</sup> 24 h bar), N<sub>2</sub> permeability of 10 cm<sup>3</sup>/(m<sup>2</sup> 24 h bar), and steam permeability of 2.6 g/(m<sup>2</sup> 24 h bar)]. Its surface roughness and morphology was assessed with atomic force microscopy (Solver PRO, NT-MDT, Spectrum Instruments, Moscow, Russia). Silicon cantilevers with a typical resonant frequency of 240 kHz and a spring constant of 11.8 N/m were used to acquire images in semi-contact mode at room temperature under ambient conditions. The scanning rate was 1.5 Hz. Flattening of the raw images was performed before surface roughness analysis. The average surface roughness was determined from images with an area of 3 x 3 µm.

The chemical composition of the packaging matrix was determined by XPS analysis. A TFA XPS spectrometer (Physical Electronics Inc., Chanhassen, USA) operating under ultra-high vacuum (10<sup>-7</sup> Pa) and equipped with a monochromated Al K $\alpha$  X-ray source (1486.6 eV) was used. On each sample, an area of 0.4 mm in diameter was analysed to a depth of 3-5 nm. Two different locations were analysed on each sample and the data averaged. Quantification of the surface composition was performed from XPS peak intensities taking into account the relative sensitivity factors provided by the instrument manufacturer (Moulder, Stickle, Sobol, & Bomben, 1995).

## 2.7 Physical-chemical analysis of muscle samples

### 2.7.1 Effects of ACP on surface-temperature and -pH

In Trial 2, surface-pH and -temperature immediately before and after samples were subjected to a particular ACP scenario (high, medium, low power) were measured using a pH meter (Testo 230, Testo AG, Lenzkirch, Germany) combined with a

surface pH electrode calibrated at pH 4 and pH 7 (Blue Line SI Analytics, Schott, Mainz, Germany), and an infrared thermometer (Testo 831, Germany).

### 2.7.2 Colourimetry

To monitor changes in meat surface colour (as reflected by L\*, a\*, b\*, Chroma and Hue values), samples were measured (averaging the results of 2 scans) with a double beam spectrophotometer with an aperture radius of 8 mm (Phyma Codec 400, Phyma, Gießhübl, Austria) on day 0 (before and immediately after ACP treatment) as well as after a further 3 and 10 day vacuum storage period at 2±2 °C according to the protocols of Faustman & Phillips (2001). For the latter two measurements samples were unpacked, re-measured and subsequently repacked. Time interval between unpacking and repacking was approximately 1 minute. It should be noted that, consequently, samples were not allowed a ‘blooming’ period and thus these values are relevant for the packaged product only. Colour values after further storage under atmospheric conditions were only measured during Trial 2 (see below). During all trials hygienic sample handling was assured by wearing surgical gloves.

It would seem relevant to establish whether or not colour parameters of vacuum packaged ACP-treated items change after unpacking and subsequent exposure to atmospheric conditions, as practised in retail display or in the domestic kitchen. These data would provide information whether the final fresh product would be sufficiently appealing for the consumer (Bekhit & Faustman, 2005). To this end in Trial 2, loins were treated as described above, except that samples were unpackaged after 10 days of vacuum storage, wrapped in O<sub>2</sub> permeable foil and further stored in the display refrigerator, whereafter colour was measured 13 days after ACP treatment.

### 2.7.3 Sarcoplasmic protein solubility

At 20 days *post mortem* (i.e. 13 days after ACP treatment) 5 g samples were taken from the treated surface of all vacuum packaged cross sections to establish sarcoplasmic protein solubility using the method of Hart, described by Swatland (1995). Samples that contain a higher amount of denatured protein, will produce

filtrates containing less soluble proteins (less precipitation) than samples with a lower amount of denatured proteins and consequently will render higher transmission values. Although Hart's 1962 test was primarily developed for studying intrinsic denaturation in PSE pork, the method has been proven equally useful as an indicator for increased sarcoplasmic protein denaturation in bovine muscle, e.g. as resulting from electrical stimulation (Eikelenboom & Smulders, 1986).

#### 2.7.4 Nitrate/nitrite analysis

Ca. 10 g of the homogenized meat samples were mixed with 40 ml distilled water, heated for 15 minutes and filtrated through a folded filter paper (MN 625 1/4; Macherey Nagel, Düren, Germany) as well as a membrane filter (0,2 µm cellulose acetate) according to Schmidt & Schwedt (1984). For analysis aliquot parts of the filtrate were used. Nitrate levels were determined by HPLC (Waters 600s Controller, 626 Pump Bio-Inert, PDA 996; Waters, Milford, USA). Nitrate was separated on a Spherisorb-NH<sub>2</sub>, 5 µm, 250 x 4.6 mm anion-exchange column, equilibrated at 20 °C. The eluent was composed of 95% K<sub>2</sub>HPO<sub>4</sub> solution (10 g/1000 ml, adjusted to pH = 3 with orthophosphoric acid) and 5% acetonitrile, with a flow rate of 1ml/min. Total run time was 5 min; detection was at 205 nm.

#### 2.7.5 TBARS analysis

To assess the extent of lipid oxidation and its consequent possible effects on colour (Faustman, Sun, Mancini, & Suman, 2010), the content of Thiobarbituric Acid Reactive Substances (TBARS) was determined according to Witte, Krause, & Bailey (1970).

#### 2.7.6 Mb isoform analysis

Spectral analysis was conducted on meat surface shavings, so as to assess any changes in the distribution of myoglobin isoforms following ACP treatment. To this end the procedure described by Hamm (1975) was followed. In brief, in Trial 2, 10 g homogenized sample were mixed with either 50 ml distilled water, or (for nitrosomyoglobin analysis; Trial 1) with 50 ml acetone (Grau, 1969) and then cooled

for 1 h. After filtration through a folded filter paper (MN 625 1/4; Macherey Nagel), light absorption was recorded at 700-450 nm (Hitachi U3000; Hitachi, Tokyo, Japan).

## 2.8 Statistical analysis

Changes in bacterial numbers were evaluated by one-way ANOVA (Statgraphics 3.0; Statistical Graphics Corp., Warrenton, USA), with Scheffé's *post hoc* test to discriminate among means. Levels of TBARS, sarcoplasmic protein denaturation, and those of  $\text{NO}_3^-$  (before and immediately after atmospheric plasma treatment) were examined with paired t-test.

Imbalances in the experimental design of Trials 1 and 2 necessitated a slightly different mathematical approach for evaluating colour parameters. Before each trial, the colour values of the sample groups were analysed with one-way ANOVA to assess whether or not significant differences existed in colour before starting of the treatment. In Trial 1, the analyses for high and low power settings were done separately. Per setting, the colour values immediately after cold plasma treatment were compared using an unpaired t-test. Per storage day, i) untreated vacuum packaged controls, ii) ACP-exposed and then vacuum packaged samples and iii) vacuum packaged and then ACP-exposed samples were compared with one-way ANOVA and Scheffé's *post hoc* test to discriminate among means. This allowed ascertaining if during storage, colour properties of ACP treated samples would differ from the control. Trial 2 was designed to confirm the major finding of Trial 1, i.e. that ACP treatment of vacuum-packed beef would not induce colour changes during extended storage. For this purpose vacuum-packed untreated controls and samples vacuum packaged and then exposed to ACP were compared. Each device setting (high, medium and low power) was compared separately to untreated controls at days 3, 10 (assessed immediately after removing vacuum) and 13 days (assessed after 3 days of further aerobic storage), by means of a non-paired t-test. Level of significance was at  $P \leq 0.05$ .

## 3. Results

### 3.1 ACP gas phase species

Figure 2 shows the steady-state FTIR absorption spectra of the discharge effluent under the low, medium and high power operating conditions highlighted in Table 1. Under low power conditions (Figure 2a), Ozone is the predominate species produced. As the discharge power was increased Ozone production is inhibited due to accelerated thermal degradation and quenching reactions with NO (Figure 2b & 2c). Under the highest power level considered (Figure 2c),  $\text{NO}_2$  is the dominant species, resulting from the oxidation of NO in the plasma region. The low power operating point was determined as the minimum dissipated power capable of sustaining plasma over the entire electrode area. The high power operating condition was determined from the FTIR data as the point at which Ozone production is completely inhibited. The medium power operating point was chosen as the mid-point between the high and low power conditions.

Here Figure 2

It is worth noting that FTIR analysis of the gas phase species generated by the plasma cannot be considered a comprehensive characterisation. Several species that are known to be generated by ACP either do not actively absorb in the IR region or have a short lifetime, preventing them from being transported in to the FTIR gas cell for measurement. For a comprehensive discussion on the production and transport of plasma species from an air DBD the reader is referred to the computational study of Hasan & Walsh (2016). In addition, when the RONS produced by ACP are free to interact with the sample surface a wide variety of chemical reactions can occur. In the context of this investigation, the potential for moisture to be present on the surface adds to the complexity of the situation. ACP interaction with liquid has been the subject of intense investigation recently and it is well known that such interactions can produce a wide variety of RONS directly in the bulk liquid, this includes the production of high levels of nitrate and nitrite (for a detailed discussion on reaction pathways, see Liu et al., 2016).

### 3.2 Microbiological effects

Exposure of inoculated PCA plates to ACP effectuated a significant reduction of the four test strains, with low power yielding higher reduction than high power. However, when inoculated PCA plates had been covered with a 90 µm polyamide-polyethylene film before treatment, no significant reduction in microbial numbers was observed (Table 3), indicating ACP is incapable of penetrating the packing film. A significant reduction of bacteria was also observed when bacteria suspended in 0.85% saline were placed on a packaging film and – before drying of the liquid – exposed to atmospheric plasma. This scenario, mimicking a ‘fresh’ contamination as could occur during packaging, resulted in a >1.5 log reduction at ‘high’ and a >2 log reduction at low power (Table 4). Again, low power demonstrated better bacterial reduction than high power.

Here Table 3

Here Table 4

### 3.3 Package integrity

The effect of ACP exposure on PAPE packaging film was considered in terms of surface morphology and composition. AFM analysis was conducted on PAPE films exposed to 8 minutes of LP ACP and 2 minutes of HP ACP treatment, respectively. It should be noted that the choice of 2 and 8 minute exposure times was motivated by a need to consider the ‘worst case scenario’ and are not indicative of the treatment times used to achieve a significant level of bacterial inactivation on the surface.

Figure 3 shows AFM images of the treated and untreated PAPE films and reveals little change to the morphology of the exposed PA surface. The mean surface roughness of the untreated and treated films was observed to vary by less than 1 nm and no obvious changes of the surface on the nanoscale were observed.

Here Figure 3

To probe changes in the surface composition of the PAPE film following ACP exposure, X-Ray Photoelectron Spectroscopy (XPS) was performed on the PA surface of both treated and untreated films. Figure 4 indicates that ACP exposure led to minor changes in surface composition of the packaging matrix. Silicon was found to be present on the untreated film and is a likely result of contamination remaining from the manufacturing process. Following plasma exposure, both silicon and nitrogen concentrations were seen to reduce whilst oxygen content was seen to increase (<2 %).

Here Figure 4

The use of plasma for the cleaning of materials is widespread (Goossens, Dekempeneer, Vangeneugden, Van de Leest, & Leys, 2001), and it is not surprising that ACP treatment resulted in the removal of surface contaminants from the film. Additionally, surface oxidation as a result of ACP exposure has been widely observed (Shenton & Stevens, 2001). The addition of oxygen containing functional groups to the surface has the potential to impact key properties of the film such as contact angle and should be investigated further. However, it is worth noting that the results were obtained under worst case scenario conditions. Despite this, only minor changes to the surface composition of the film were observed.

### *3.4 Physical-chemical effects on muscle tissue*

#### *3.4.1 Effects of ACP on surface-pH and -temperature*

The pH and temperature measurements revealed that ACP treatment had no effects on surface pH, which were 5.8 in all treatment groups, whilst surface temperature increase was on average 1.5 to 2 °C under all ACP scenarios.

#### *3.4.2 Colour and colour stability*

In the Pre-Trial exposure of vacuum-packed beef to ACP at high or low power had no significant effect on colour parameters. When fresh beef cut surfaces were directly exposed to ACP at high power, statistically significant increases of  $2.7 \pm 0.9$  for  $a^*$ , of  $5.3 \pm 1.3$  for Hue angle and a decrease of  $2.2 \pm 1.1$  for Chroma were observed at day 0; conversely, no significant changes were seen for direct exposure to low power ACP. This observation motivated us to study if such differences would persist when the meat was stored in vacuum-package for up to 10 days (Trials 1 and 2; Tables 5 and 6).

Here Table 5

Before treatment with high power ACP, no significant differences were found for the colour parameters of the three sample groups. After 3 and 10 days storage, only the HP ACP-exposed and then vacuum packaged group had significantly higher  $a^*$ ,  $b^*$ , Chroma and Hue angle values than those of the untreated control, or those of the first packaged and then exposed group (Table 5), whereas for low power treatment in Trial 1, no significant differences were found.

Results for Trial 2 are shown in Table 6. Comparisons of vacuum-packed beef with ACP treated vacuum-packed beef, at three power levels, showed few significant differences ( $L^*$  at day 3 for high power ACP;  $a^*$  and Chroma pre-treatment at low power ACP).

Here Table 6

### *3.4.3 Analysis of chemical parameters*

#### *3.4.3.1 Nitrate/nitrite uptake, lipid peroxidation (TBARS)*

Content of nitrite (Trial 1) was consistently below the limit of quantification (1.4 mg/kg NaNO<sub>2</sub>), whereas nitrate contents, expressed as KNO<sub>3</sub>, were in the range of 0.1 to

0.23 mg/kg. Also, in Trial 1, contents of TBARS were – with the exception of a single sample (0.11 mg/kg) below 0.1 mg malondialdehyde/kg. Therefore, the above data are not further considered. TBARS values observed after 3 days aerobic storage (Trial 2) are included in Table 7.

Here Table 7

#### *3.4.3.2 Sarcoplasmic protein solubility*

No significant difference in transmission values existed between treatment groups, indicating a similar degree of sarcoplasmic protein denaturation (see Table 7). The variation in values is similar to that recorded by Den Hertog-Meischke, Klont, Smulders, & van Logtestijn (1997), who sampled refrigerated veal loin samples at two days *post mortem* and (relying on the same method used in this study) also observed mean transmission values to range from roughly 54 to 59% (with considerable standard deviations).

#### *3.4.4 Myoglobin isoforms*

In Figure 5 the results of spectrometric analysis of myoglobin isoforms are presented graphically.

Here Figure 5

The spectrometric data of treated and untreated samples were very similar. To illustrate this, the spectrographs of one individual sample per treatment (analysed in an aquadest mixture) and each representative for the four treatment groups have been superposed in Figure 5B. The spectrum of acetone-mixed homogenates is shown in Figure 5A.

Characteristic extinction peaks (AMSA, 2001) for MbO (typically at 544 and 582 nm) can be clearly distinguished while the characteristic spectral profiles of deoxymyoglobin (DeMb; typically peaking at 525 nm) and metmyoglobin [MMb: typically peaking at 503 (and at 650 nm according to Hamm (1975)] cannot. Figure 5A provides evidence that nitrosomyoglobin (in cured items characteristically

distinguishable between 500 and 600 nm; see the reference graph insert) is not prevalent in ACP treated samples.

#### 4. Discussion

##### 4.1 Microbiology

Our study indicates that direct exposure to the DBD device effectuates significant reductions of bacteria suspended on PCA, or inoculated as droplets onto a food packaging film, whereas no reduction is observed when a PAPE film is placed between the plasma source and the bacterial suspensions. Our observation that low power plasma treatment reduced bacterial numbers better than did high power treatment is explained by the relative abundance of reactive oxygen species, i.e. Ozone, in the plasma (Pavlovich et al., 2013). The antibacterial effect of Ozone is well-known and - for short-time treatment - is largely explained by cell membrane destruction and extrusion of cytoplasm (see e.g. Thanomsu et al., 2002). Its efficacy is not only dependent on the amount of Ozone produced, but also on its diffusion in the liquid phase, the composition of the latter, and on the concentration and physiological condition of the bacterial cells. At least for one particular *E. coli* strain it has been proven that the antibacterial action is largely due to the interaction with the reactive species generated in liquids (Hänsch, Mann, Weltmann, & von Woedtke, 2015). Also, it would appear that the capacity of the liquid phase to buffer reactive Oxygen or  $N_yO_x$  species (Traylor et al., 2011) or secondary reactive compounds (Lukes, Dolezalova, Sisrova, & Clupek, 2014) affects antibacterial efficacy. Thus, whilst our results are a proof-of-concept, further studies are required to explore the full potential of the DBD device in meat packaging environments.

Most studies reported in literature concentrate on the (antimicrobial) effects on foods directly treated with ACP, few studies, however, regard meat and fewer indeed fresh beef. Meat is a rather complex buffer system compared to saline, thus, effects could be less pronounced than in saline or liquids with low protein content. The antimicrobial potential of 'direct' ACP treatment of beef has recently been addressed in a study by Jayasena et al. (2015), who inoculated beef loin with *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium and

reported reductions of 1.90, 2.57 and 2.58 log units, respectively, after a 10 min treatment with a thin-layer DBD plasma system, relying on using ambient air at atmospheric pressure. Recent studies report similar antibacterial effects for raw pork surfaces, although these effects were generated by different ACP systems [corona discharge jet stream (Choi, Puligundla, & Mok, 2016); low pressure plasma system using Helium as the working gas (Ulbin-Figlewicz, Jarmoluk, & Marycz, 2015)].

Our study did not include microbiological analysis of ACP treated beef surfaces, but its results show a marked antimicrobial potential of ACP for purposes of decontaminating packaging film surfaces. Reference values from comparable studies were not found in literature; however, the application of alternative ACP configurations for packaging decontamination has been considered previously. Yun et al. (2010) demonstrated the utility of a plasma jet-like device for effectively decontaminating a range of packing materials; however, it should be noted that the device used by these authors relied on the use of a noble gas and operated at power levels almost an order of magnitude higher than those considered in our study.

#### *4.2 Suitability of the ACP set-up*

The ACP surface DBD system employed in this investigation is known to be capable of producing a large density of RONS that are free to interact with the sample surface. The transport of species from the plasma region to the sample is primarily driven by diffusion and as a result, many of the shorter-lived radical species that are known to be produced in ACP (e.g. OH, O and O<sub>2</sub><sup>-</sup>) are not able to reach the surface. Given that short-lived RONS have the most pronounced antimicrobial effect, it is reasonable to assume that the surface DBD ACP system used may not be as efficient as localised treatments provided by alternative ACP systems where the plasma is able to directly impinge on the sample. Examples of localised ACP systems include plasma jet systems where a stream of plasma flows directly on to the sample and large area Dielectric Barrier Discharges (DBDs) where the sample is sandwiched between the plasma generating electrodes.

Whilst the antimicrobial efficiency of remote ACP systems is likely to be lower than that of their localised counterparts, remote systems do offer several other key advantages. Critically, direct exposure of the sample (either the food or packaging matrix) has the potential to cause significant physical and chemical changes.

Localised heating can occur in filamentary plasmas and direct contact with polymeric materials can result in a significant change to both the surface morphology and composition (see Walsh & Kong, 2007). In this investigation, the results from both XPS and AFM analysis confirm that remote plasma treatment causes little change to the composition or morphology of the PAPE film, even under worst case scenario conditions. In addition, the generation of plasma directly on or in packaged foods could potentially lead to the undesirable transport of polymeric material from the packaging film to the food surface. Finally, the variety of reactive species reaching the surface in a remote ACP system is considerably less complex than what would be expected in a localised treatment; a factor that could ease and accelerate the certification of ACP technology.

Commercial meat processing and packaging lines operate at rather high speeds. Consequently, there are limitations as to treatment duration. In our study treatment time was limited to 60 s which approaches the maximum time a packaged product may remain on a conveyor belt. Obviously at longer durations antimicrobial and physical-chemical effects may be intensified, as may the physical effects on the packaging matrix. However, as in our study the latter were tested up to 8 times higher duration without significant adverse effects being noticed, our approach would appear to be feasible in practice.

#### *4.3 Physical chemical effects*

Our study design allowed to determine if ACP breaches the packaging barrier as well as to generate important information on physical-chemical/sensory effects of 'direct' ACP exposure on muscle quality characteristics.

##### *4.3.1 Surface colour changes*

Our results show that meat surfaces covered by the packaging film before being exposed to ACP did not differ from those not ACP treated ('untreated controls'). Major physical-chemical indications for sensory changes might have been signs of (sarcoplasmic) protein denaturation [NOT observed in our study but possibly leading to higher L\* values as reported by Eikelenboom & Smulders (1986) and by Joo, Kauffman, Kim, & Park (1999)] or oxidation, the latter leading to shifts in the

myoglobin isoform distribution pattern, and both would affect meat colour (Faustman & Cassens, 1990). The Hue angles in Trial 1, observed for HP 'direct' treatment to be consistently higher over time (Table 5), would appear to suggest that ACP may have induced some oxidation. Yet, the results of both TBARS analysis (indicative for lipid oxidation) and Mb isoform analysis (indicative for oxidation of Mb to MMb) failed to substantiate such.

Surprisingly, low power ACP [which is primarily composed of Ozone known to initiate oxidation reactions (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013)] did not result in colour changes in any of the trials. Considering that (ground) beef - over a 3 day refrigerated storage period intermittently exposed to Ozone for as long as 18 hrs - only exhibited minor discolouration (reduced  $a^*$  values; Cho, Choi, Hahn, & Lee, 2014), it is unlikely that a mere one minute low power ACP treatment of (non-commminuted) fresh beef has sufficient oxidative capacity to produce similar results.

As, under high power operating conditions, ACP treatment can generate nitrites (Olszewski, Li, Liu, & Walsh, 2014), we wanted to establish if treated samples would take up this substance in high enough quantities to produce even a minor superficial curing effect. Should this have been the case the product would, according to international standards (e.g. European Union, 2004), no longer classify as 'fresh' meat. As nitrite-uptake of treated muscle surface tissue was shown not to occur and thus nitrosomyoglobin formation indeed not observed, such concerns are unjustifiable. However, in plasma-treated water, nitrite formation will occur. Thus, when a meat matrix is mixed with such water, curing is observed indeed, as demonstrated for pasteurized comminuted sausage by Jung et al. (2015).

#### *4.3.2 Oxidative changes in lipids*

ACP generated RONS are free to interact with the surface tissue containing intramuscular fat. Reactive oxygen species particularly interact with the unsaturated fatty acids fraction and may thus cause lipid peroxidation (Min & Ahn, 2005), whilst secondary oxidation products (e.g. aldehydes) may i) initiate conformational changes in myoglobin causing increased heme oxidation and brown off-colours (Alderton, Faustman, Liebler, & Hill, 2003) and/or ii) result in off-odours and iii) rancid off-flavours (Shahidi & Wanasundara, 2008). The latter occurs at TBARS threshold

values of 0.5-2.0 mg MDA/kg (Chang, Younathan, & Watts, 1961; Gray, Gomaa, & Buckley, 1996).

In our study the TBARS values, measured after 3 and 10 days of vacuum storage (data not shown), were with one exception below the limit of detection of 0.10 mg MDA/kg. This was not an unexpected finding as i) the amount of intramuscular fat in beef is low [around 2% according to Souci, Fachmann, & Kraut, 2016], which ii) predominantly contains saturated and mono unsaturated fatty acids, but relatively little polyunsaturated fatty acids (PUFA) which are known to be particularly vulnerable to oxidative changes (Souci, Fachmann, & Kraut, 2016) and iii) TBARS values are primarily expected to increase in a packaging environment with a high oxygen content and at extended storage times as discussed by Ulbin-Figlewicz & Jarmoluk (2016).

In our Trial 2, samples had been continually stored in vacuum until exposed to atmospheric oxygen and daylight at day 10 for a final 3 days. We observed that TBARS values increased to around  $0.15 \pm 0.03$  mg MDA/kg, i.e. below the threshold values for off-odours and off-flavours, and values were not different for ACP-treated and untreated samples.

#### *4.3.3 Mb Isoform distribution*

The approximately 70 min sample preparation time (homogenisation and filtration) preceding myoglobin analysis, allows for ample exposure to oxygen of the substrate in the final solution. At such high pO<sub>2</sub> values (and assuming intact reductase activity) deoxymyoglobin (DeMb) will have been oxygenated to oxymyoglobin (MbO), and - if at all - there should only be traces of metmyoglobin (MMb) and deoxymyoglobin (DeMb) (Skibstedt, 1996). This explains the findings in Figure 5. Hence, our data suggest - although we did not specifically quantify such - that in all (treated or untreated) samples, Metmyoglobin Reducing Activity (MRA) (Bekhit & Faustman, 2005) is still largely intact. Consequently, in this study 'display life' does not seem to be adversely affected by the ACP treatment applied.

The latter supposition is also corroborated by comparing our colourimetric data with 'colour acceptability standards' suggested by Farouk, Bekhit, Dobbie, & Waller (2007), who conducted consumer panel studies involving over 500 panelists evaluating the appearance of beef loin steaks displayed under atmospheric

conditions. These authors determined the 'cut off' colour values above or below which a consumer is no longer willing to buy the product and consequently 'display life' has ended. Considering the colour measurement equipment we used, desirable values would be:  $a^* \geq 14$  and  $33 \leq \text{Hue} \leq 41$  (Farouk, pers.comm.). Table 6 indeed shows that - at 20 days *post mortem* - samples (aerobically stored for the final 3 days) still qualify for being displayed, regardless of the ACP scenario applied.

#### *4.3.4 Sensory ramifications of 'direct' ACP treatment*

Physical-chemical analysis in this study primarily concentrated on ACP effects on colour. It could be argued that other major sensory attributes may also be subject to changes resulting from such treatment. For instance a) ACP-induced (sarcoplasmic) protein denaturation [as reported e.g. for LDH by Zhang et al., 2015] would (marginally) affect water-holding (Smulders, Hofbauer, & Geesink, 2014), b) oxidative environments resulting from ACP treatment could conceivably decrease tenderization through inactivation of  $\mu$ -calpain (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004), or c) ACP could affect the degree of oxidation [e.g. an increase in fat oxidation as reported for sliced cheese by Yong et al. (2015), or a suppression of oxidation following inactivation of peroxidases as found by Surowsky, Fisher, Schlueter, & Knorr (2013) and might thus affect flavour (see above). Although the action of ACP on enzymes has been studied well, there are limited data on meat matrices, since most studies on enzyme inactivation by ACP deal with model systems or non-meat samples (reviewed by Misra, Pankaj, Segat, & Ishikawa, 2016).

However, one should confront the fact that - should such indeed be observed in meat directly subjected to ACP - these biochemical effects would be restricted to a very thin surface layer. Although the latter needs to be confirmed in further studies, it is questionable if such strictly localised effects would afford significant changes in waterholding/tenderness/flavour traits of the overall product. In contrast to treatment of meat with irradiation [depending on the wavelengths achieving a penetration depth of 3-6 cm (Beta-rays) up to 1-2 m (Gamma-rays) (CSIRO, 2003)], ACP is a technology that restricts its action to the immediate surface only. Even in set-ups that achieve the highest penetration (e.g. jet stream devices) ACP penetrates the tissue in the micrometer range. For instance, an effective tissue penetration depth of maximally 60  $\mu\text{m}$  has been recorded for high rate flow ACP treatment (Hoffmann,

Berganza, & Zhang, 2013). Consequently, it is likely that the direct treatment of meat with plasmas potentially affects only those quality traits that are principally related to surface-associated phenomena.

## Conclusions

Our observations show that a standard food grade packaging film functions as a barrier that is not breached by RONS generated under the ACP scenarios studied, and hence, that such treatment does not adversely affect major physical-chemical beef quality characteristics, whilst ACP has marked potential for eliminating or significantly reducing the microbial load of package surfaces. The potential to sanitize food packaging materials or other food contact surfaces warrants further research.

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**Table 1:** Plasma operating conditions

Power	Power input ( $P_{in}$ ) [W]	Output Voltage ( $V_{pp}$ ) [kV]	Dissipated	Power Density
			Power ( $P_{out}$ ) [W]	[W/cm <sup>2</sup> ]
Low	20.7	8.16	17.87	0.48
Medium	25.4	8.88	21.73	0.56
High	29.9	9.44	25.38	0.67

**Table 2:** Sample treatments at day 0 (top) and time points of physical-chemical analyses (bottom) of beef loin cross sections, either 'directly' exposed to cold plasma [ACP, at different power settings: ● = High Power (HP), ● = Medium Power (MP), ● = Low Power (LP)] or after these had been vacuum packaged (■ = HP, □ = MP, ○ = LP). Note that days (Day<sub>n</sub>) are expressed as days after treatment.

Sequence of events at day 0	Control*	Treated control: ACP, then pack (ACP-Vac)			Pack, then ACP (Vac-ACP)		
		HP	MP	LP	HP	MP	LP
Portion to samples; measure 'initial' colour	○	○	○	○	○	○	○
Power setting	-	HP	MP	LP	-	-	-
ACP; measure colour after treatment	-	●	●	●	-	-	-
Vacuum pack (Vac)	○	■	■	■	○	○	○
Power setting	-	-	-	-	HP	MP	LP
ACP	-	-	-	-	■	■	■
Unpack, measure colour after treatment, repack and reseal the (original) package	-	-	-	-	+	+	+
Store at 2±2 °C	+	+	+	+	+	+	+

pH/T	Colour	Sarcoplasmic		NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup>	Lipid	
		Protein	Denaturation		oxidation	Mb isoforms
Day 0	* <sub>2</sub>	* <sub>PT,1,2</sub>		* <sub>1</sub>	* <sub>1,2</sub>	
Day 3		* <sub>1,2</sub>			* <sub>1,2</sub>	* <sub>1</sub>
Day 10		* <sub>1,2</sub>			* <sub>1,2</sub>	* <sub>2#</sub>
Day 13	* <sub>2</sub>		* <sub>2</sub>		* <sub>2</sub>	

\*n subscripts indicate if applicable to PreTrial and/or Trials 1 and 2 (1,2)

# i.e. samples were unpacked at Day 9, wrapped in O<sub>2</sub> permeable foil, aerobically stored overnight at 2±2 °C in a dark chill room and analysed at Day 10

**Table 3:** Effect of treatment with atmospheric cold plasma (ACP 1 min, 2 cm distance) on bacterial test strains spread onto plate count agar (n = 6). Numbers are expressed as log cfu per plate (Trial 1).

	Untreated control	Treated with low power ACP		Treated with high power ACP	
		Direct	Covered*	Direct	Covered
<i>Staph. aureus</i> DSM 1104	3.8±0.1 <sup>a</sup>	0.4±0.4 <sup>b</sup>	3.6±0.1 <sup>a</sup>	0.8±0.4 <sup>c</sup>	3.6±0.0 <sup>a</sup>
<i>Listeria monocytogenes</i>					
DSM 19094	3.4±0.1 <sup>a</sup>	0.2±0.3 <sup>b</sup>	3.5±0.2 <sup>a</sup>	0.3±0.3 <sup>c</sup>	3.5±0.3 <sup>a</sup>
<i>E. coli</i> DSM 1103	3.6±0.1 <sup>a</sup>	0.1±0.2 <sup>b</sup>	3.6±0.0 <sup>a</sup>	0.8±0.2 <sup>c</sup>	3.7±0.1 <sup>a</sup>
<i>E. coli</i> O157	3.4±0.0 <sup>a</sup>	0.1±0.2 <sup>b</sup>	3.5±0.2 <sup>a</sup>	0.8±0.2 <sup>c</sup>	3.4±0.3 <sup>a</sup>

\*= plate covered with a 90 µm polyamide-polyethylene film. Within rows, Figures with different superscript differ significantly (P<0.05).

**Table 4:** Effect of treatment with atmospheric cold plasma (ACP 1 min, 2 cm distance) on bacterial test strains (suspended in 0.85% saline) inoculated onto a polyamide-polyethylene film ( $n = 6$ ). Unless indicated otherwise, figures are expressed as log cfu/10  $\mu$ l.

	Native (not inoculated) film (log cfu/cm <sup>2</sup> )	Untreated Control*	Treated with low power ACP	Treated with high power ACP
<i>Staph.aureus</i> DSM 1104	<2 <sup>a**</sup>	7.2±0.1 <sup>b</sup>	4.2±0.2 <sup>c</sup>	5.5±0.2 <sup>d</sup>
<i>Listeria monocytogenes</i>				
DSM 19094	<2 <sup>a</sup>	8.1±0.1 <sup>b</sup>	6.2±0.2 <sup>c</sup>	5.9±0.3 <sup>d</sup>
<i>E. coli</i> DSM 1103	<2 <sup>a</sup>	7.6±0.2 <sup>b</sup>	4.9±0.3 <sup>c</sup>	5.9±0.3 <sup>d</sup>
<i>E. coli</i> O157	<2 <sup>a</sup>	7.1±0.9 <sup>b</sup>	4.7±0.2 <sup>c</sup>	5.7±0.2 <sup>d</sup>

\* inoculated, but not treated with ACP; \*\* i.e. below limit of detection; within rows, figures with different superscripts differ significantly ( $P<0.05$ )

**Table 5:** TRIAL 1: The effects of subjecting (non-packaged/vacuum packaged) beef loin cross sections to ACP on surface colour parameters, as measured after 3 and 10 days storage in vacuum at  $2\pm2$  °C; means and standard deviations of 6 replicate measurements.

Initial (pre-treatment) values at day 0 (i.e. measured at 3 days *post mortem*).

	High power ACP			Low power ACP		
	Untreated control	ACP-Vac	Vac-ACP	Untreated control	ACP-Vac	Vac-ACP
L*	37.1±2.8	36.1±2.6	37.5±2.3	39.3±2.2	36.9±2.5	38.2±3.3
a*	14.5±1.7	14.0±1.0	14.3±2.3	11.2±1.1	11.3±1.2	10.7±1.0
b*	8.9±1.1	8.6±1.1	9.0±2.2	7.9±1.3	7.4±0.9	7.6±0.8
Chroma	17.0±1.9	16.4±1.6	17.0±3.1	13.7±1.6	13.3±1.6	12.9±1.8
Hue <sup>Δ</sup>	31.5±1.7	31.4±1.9	32.0±2.1	35.1±2.0	33.1±2.0	33.4±2.6

After 3 days of vacuum storage (i.e. measured at 6 days *post mortem*)

	High power ACP			Low power ACP		
	Untreated control	ACP-Vac	Vac-ACP	Untreated control	ACP-Vac	Vac-ACP
L*	34.7±2.4	38.6±2.4	38.6±3.0	36.8±2.6	36.3±2.7	35.6±3.7
a*	13.0±0.4 <sup>aΔΔ</sup>	15.6±0.8 <sup>b</sup>	13.5±1.5 <sup>a</sup>	9.6±1.1	10.5±1.0	10.2±1.6
b*	8.0±0.6 <sup>a</sup>	11.1±1.0 <sup>b</sup>	8.0±1.0 <sup>a</sup>	5.9±0.9	6.7±1.0	6.2±1.3
Chroma	15.3±2.2 <sup>a</sup>	19.1±1.0 <sup>b</sup>	15.7±1.7 <sup>a</sup>	11.3±2.1	12.4±1.3	11.9±2.0
Hue	31.3±2.0 <sup>a</sup>	35.6±1.8 <sup>b</sup>	30.6±0.6 <sup>a</sup>	31.4±2.1	31.8±2.2	30.6±1.4

After 10 days of vacuum storage (i.e. measured at 13 days *post mortem*)

	High power ACP			Low power ACP		
	Untreated control	ACP-Vac	Vac-ACP	Untreated control	ACP-Vac	Vac-ACP
L*	40.0±2.2	41.2±3.8	39.3±2.7	39.5±3.7	39.8±2.1	38.9±3.3
a*	12.2±0.9 <sup>a</sup>	14.0±1.3 <sup>b</sup>	13.0±0.7 <sup>a</sup>	10.8±0.9	10.9±1.1	10.5±1.4
b*	8.1±1.1 <sup>a</sup>	11.0±1.4 <sup>b</sup>	8.0±0.8 <sup>a</sup>	7.6±0.8	7.9±1.0	7.8±1.1
Chroma	14.7±1.2 <sup>a</sup>	17.6±1.4 <sup>b</sup>	15.6±0.9 <sup>a</sup>	13.2±1.1	13.5±1.4	13.1±1.7
Hue	33.4±2.5 <sup>a</sup>	37.0±2.9 <sup>b</sup>	33.4±1.7 <sup>a</sup>	34.9±1.7	35.5±1.3	35.7±0.8

Δ expressed as degrees; calculated as  $[ARCTAN(b^*/a^*) \times 180/\pi]$

ΔΔ In rows, figures with different superscripts differ significantly ( $P \leq 0.05$ ).

Vac-ACP ... vacuum packaged beef was subject to ACP treatment

ACP-Vac .... beef was exposed to ACP and then vacuum-packaged

**Table 6:** TRIAL 2: The effects of subjecting vacuum packaged cross sections from beef loins (that had been aged in vacuum for 7 days) to ACP on surface colour parameters, as measured after 3 and 10 days of further storage in vacuum at 2±2 °C and after a subsequent 3 day aerobic storage in a display refrigerator (3±2 °C); means and standard deviations of 6 replicate measurements.

Initial (pre-treatment) values at day 0 (i.e. measured at 7 days post mortem)						
	High power ACP		Medium power ACP		Low power ACP	
	Untreated control	Vac-ACP	Untreated control	Vac-ACP	Untreated control	Vac-ACP
L*	35.4±2.4	35.5±3.2	35.4±2.4	37.3±3.3	35.4±2.4	36.1±3.0
a*	13.5±1.3	14.6±1.9	13.5±1.3	13.6±1.6	13.5±1.3 <sup>aΔΔ</sup>	15.3±1.3 <sup>b</sup>
b*	8.4±1.5	8.9±1.4	8.4±1.5	7.8±1.5	8.4±1.5	9.8±1.1
Chroma	15.9±1.8	17.1±2.3	15.9±1.8	15.7±2.0	15.9±1.8 <sup>a</sup>	18.2±1.4 <sup>b</sup>
Hue <sup>Δ</sup>	31.7±2.4	31.5±0.5	31.7±2.4	29.5±2.6	31.7±2.4	32.7±2.5

After 3 days of further vacuum storage (i.e. measured at 10 days post mortem)						
	High power ACP		Medium power ACP		Low power ACP	
	Untreated control	Vac-ACP	Untreated control	Vac-ACP	Untreated control	Vac-ACP
L*	36.9±2.1 <sup>aΔΔ</sup>	39.0±2.4 <sup>b</sup>	36.9±2.1	37.5±2.2	36.9±2.1	37.2±2.9
a*	11.9±0.8	11.8±1.3	11.9±0.8	11.5±0.9	11.9±0.8	12.3±1.0
b*	7.6±0.8	7.4±1.1	7.6±0.8	6.9±0.7	7.6±0.8	7.4±0.6
Chroma	14.2±1.0	14.0±1.6	14.2±1.0	13.4±1.1	14.2±1.0	14.4±1.1
Hue	32.6±1.4	31.8±1.6	32.6±1.4	31.1±1.7	32.6±1.4	30.9±1.7

After 10 days of further vacuum storage (i.e. measured at 17 days post mortem)						
	High power ACP		Medium power ACP		Low power ACP	
	Untreated control	Vac-ACP	Untreated control	Vac-ACP	Untreated control	Vac-ACP
L*	38.7±2.3	40.7±1.4	38.7±2.3	40.7±2.5	38.7±2.3	41.4±3.3
a*	11.4±1.2	12.2±0.7	11.4±1.2	11.6±1.1	11.4±1.2	12.0±0.9
b*	7.4±1.1	8.2±0.7	7.4±1.1	7.2±0.9	7.4±1.1	7.4±0.9
Chroma	13.6±1.5	14.7±0.9	13.6±1.5	13.6±1.4	13.6±1.5	14.2±1.0
Hue	32.9±1.2	33.3±1.4	32.9±1.2	31.8±0.8	32.9±1.2	32.0±3.6

After a further 3 days of aerobic storage (i.e. measured at 20 days post mortem)						
	High power ACP		Medium power ACP		Low power ACP	
	Untreated control	Vac-ACP	Untreated control	Vac-ACP	Untreated control	Vac-ACP
L*	38.7±2.3	40.7±1.4	38.7±2.3	40.7±2.5	38.7±2.3	41.4±3.3
a*	11.4±1.2	12.2±0.7	11.4±1.2	11.6±1.1	11.4±1.2	12.0±0.9
b*	7.4±1.1	8.2±0.7	7.4±1.1	7.2±0.9	7.4±1.1	7.4±0.9
Chroma	13.6±1.5	14.7±0.9	13.6±1.5	13.6±1.4	13.6±1.5	14.2±1.0
Hue	32.9±1.2	33.3±1.4	32.9±1.2	31.8±0.8	32.9±1.2	32.0±3.6

	Untreated control	Vac-ACP	Untreated control	Vac-ACP	Untreated control	Vac-ACP
L*	40.6±2.9	42.8±2.5	40.6±2.9	41.9±3.0	40.6±2.9	42.5±2.2
a*	17.5±2.0	19.2±1.2	17.5±2.0	17.1±1.5	17.5±2.0	19.0±1.1
b*	13.9±1.8	15.1±1.6	13.9±1.8	13.7±1.4	13.9±1.8	15.3±1.1
Chroma	22.3±2.6	24.4±1.9	22.3±2.6	21.9±2.0	22.3±2.6	24.4±1.4
Hue	38.4±0.9	38.1±1.5	32.9±1.2	38.6±0.5	38.4±0.9	38.7±1.2

Δ expressed as degrees; calculated as [ARCTAN(b\*/a\*) x 180/π]

ΔΔ In rows and per ACP power setting, figures with different superscripts differ significantly ( $P<0.05$ ).

**Table 7:** Lipid peroxidation (TBARS) and sarcoplasmic protein solubility of beef loin samples measured 13 days after treatment with High (HP) , Medium (MP) and Low Power (LP) ACP (Trial 2; n=6 per treatment). Note that none of the figures differ significantly ( $P>0.05$ ).

Control	Exposed to ACP, then vacuum packaged			Vacuum packaged, then exposed to ACP		
	HP	MP	LP	HP	MP	LP
TBARS (mg/kg MDA)	0.17±0.03	0.15±0.03	0.14±0.02	0.15±0.02	0.14±0.02	0.15±0.03
% Transmission	52.6±7.9	56.9±15.0	-*	60.1±18.1	53.9±10.8	-
						58.2±12.5

\* not determined

## Figure captions

**Figure 1:** (a) Schematic showing surface DBD electrode and sample position, (b) Photograph showing plasma formed on hexagonal mesh electrode at a discharge power of  $0.67 \text{ W/cm}^2$ .

**Figure 2:** FTIR absorption spectra of the air plasma effluent under (a) Low power, (b) Medium power, and (c) High power operating conditions.

**Figure 3:** AFM images of the PA surface of PAPE packaging film: (a) untreated control, (b) 8 minute Low Power ACP exposure and (c) 2 minute High Power ACP exposure.

**Figure 4:** XPS analysis of ACP treated and untreated PA surface. Treated films subjected to 8 minute Low Power and 2 minute High Power ACP exposure, respectively.

**Figure 5:** Spectrographs of qualitative analyses for nitrosomyoglobin (in filtrates of acetone homogenates of ACP-treated and control beef loin samples in Trial 1; Figure A) and for myoglobin isoforms (in aquadest homogenates; Trial 2; Figure B).

1...vacuum packed, then low power ACP; 2...('direct') low power ACP, then vacuum packed; 3...vacuum packed, then high power ACP; 4...('direct') high power ACP, then vacuum packed; 5...untreated control. Note the insert graph in Figure A, showing a characteristic spectrograph for nitrosomyoglobin in a cured meat product.

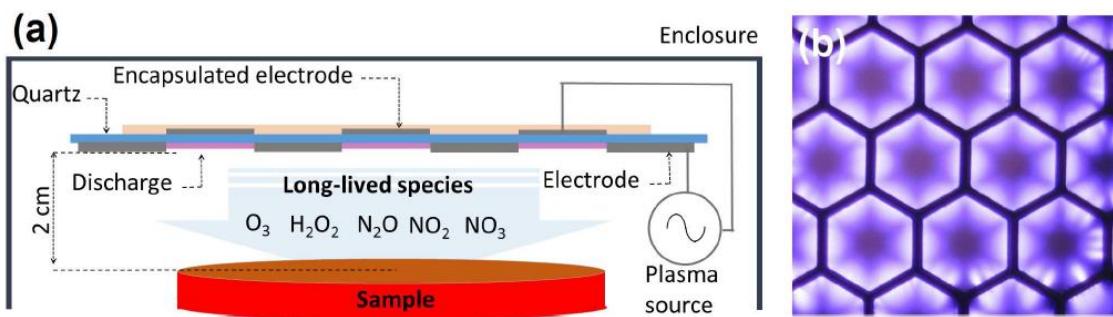


Figure 1

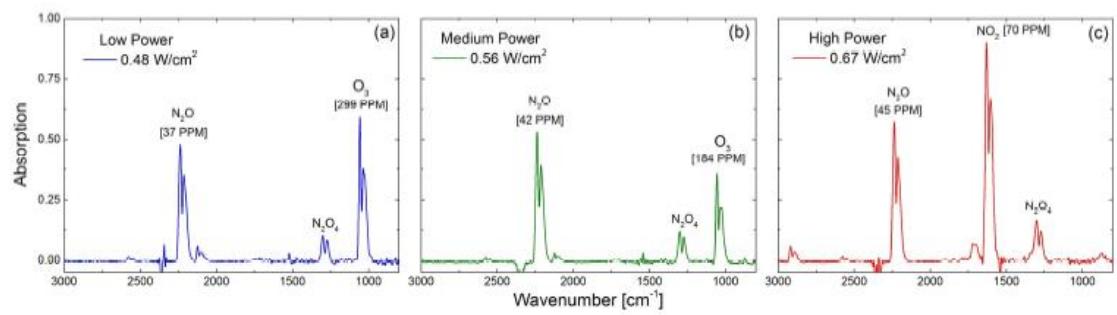


Figure 2

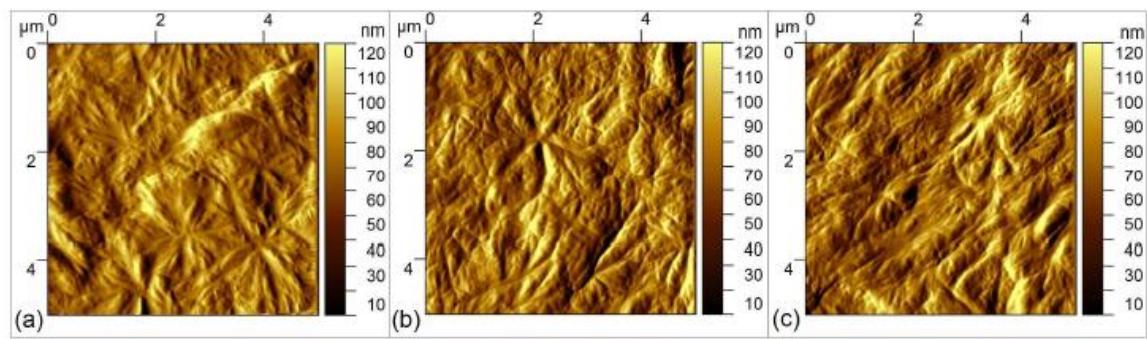


Figure 3

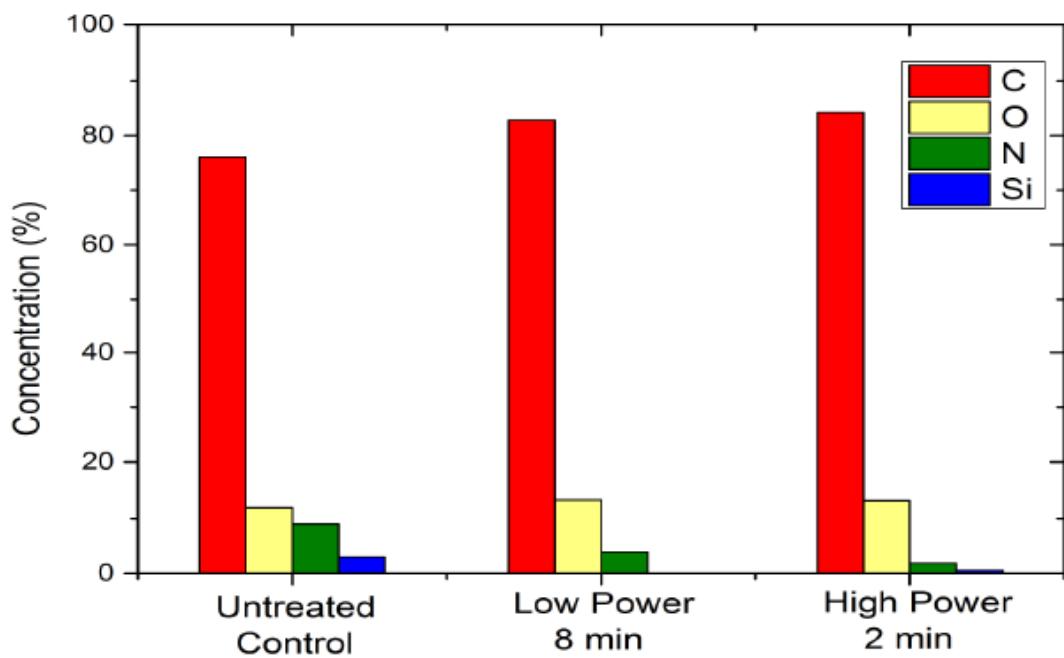


Figure 4

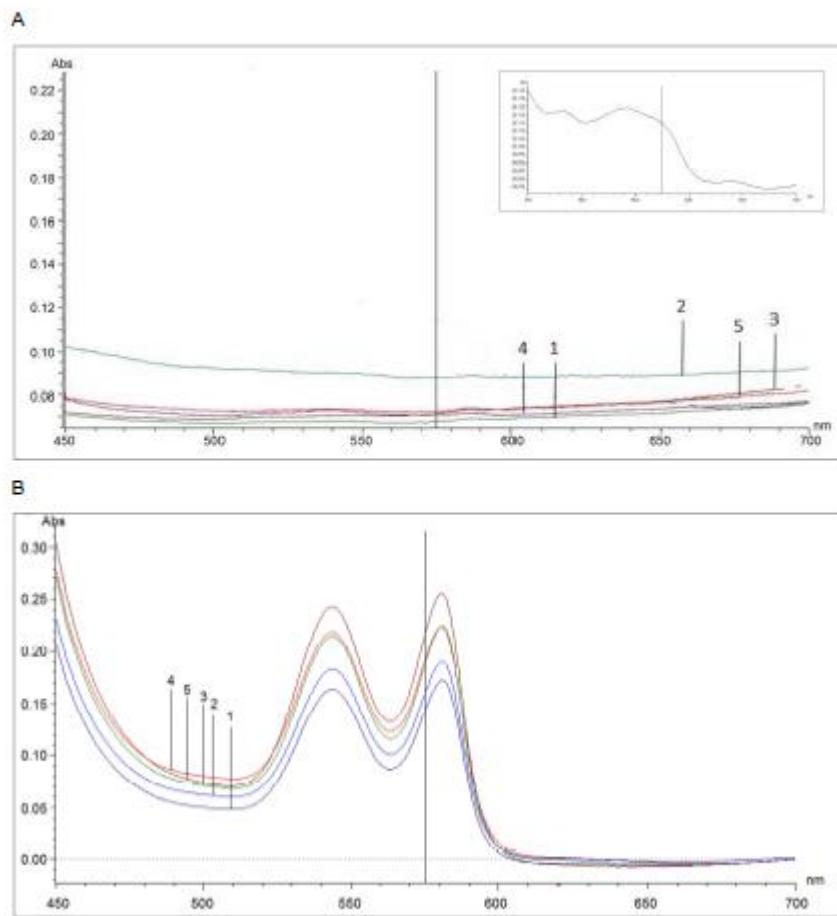


Figure 5

**Highlights:**

- Atmospheric pressure cold plasma treatment reduced numbers of contaminant bacteria on vacuum packaging film >2 log units.
- The integrity of the packaging matrix was not affected.
- No immediate effects on colour, Mb isoform distribution and degree of oxidation in vacuum packaged beef loins.
- No delayed effects were observed over 10 days vacuum- and 3 days aerobic storage.
- Cold plasma technology has the potential to sanitize surfaces in the meat packaging area.