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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 were studied over a 10 day period of vacuum-, and a subsequent 3 day period of aerobic storage. 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.

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 Nirmaly, 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, Schlüter and Cullen (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

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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 ± 2 °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 × 5 × 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 × 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, and Walsh (2014) and Ni, Lynch, Modic, Whalley, and 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 Fig. 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 Fig. 1(b). The surface DBD electrode unit was capable of generating air plasma over an 8 × 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^2). 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.

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.

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^{-1} to 500 cm^{-1} and resolution of 1 cm^{-1} 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.

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