ANTIMICROBIAL EFFECT OF NON-THERMAL PLASMA ACTIVATED WATER AGAINST FOOD-BORNE PATHOGENS

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Abstract: Pathogenic microorganisms are a serious threat to global health, that involves high costs of the treatment and their fight. We proposed to evaluate PAW antimicrobial activity against the microorganisms food-borne pathogens. PAW It was observed that due to the reactive species in water shows a post-discharge an antimicrobial effect on food-borne pathogens showed until a few days after discontinuation of plasma discharge in water. The logarithmic reduction was higher than 5log10 after an exposure time of 5-min, exhibiting a powerful bactericidal effect. Significant reduction in bacterial population were achieved in all type strains, demonstrating the effectiveness of this new approach to treat the food borne pathogens. Thus, non-thermal plasma activated water can be a promising alternative to traditional disinfection applied in food industry.

Keywords: decontamination, food-borne pathogens, plasma activated-water

Introduction

Broiler chicken meat is incriminated to be an important vector for human infections, including the most common causes of foodborne diseases (*Campylobacter* spp. and *Salmonella* spp.). During the last years, it was noticed a high incidence for the extended spectrum beta-lactamases (ESBL) producing *E. coli* strains both in humans and in broiler (WHO, 2012). Moreover, chicken meat contamination on technologic food line with enteric bacteria may lead to economic losses due to short availability and induction of unwanted organoleptic changes in meat or it may have health repercussions because of food-borne pathogens.

Non-thermal plasma is the fourth state of matter existence and it consists of: positive and negative ions, electrons, neutral particles (atoms and molecules in the ground or excited state), photons, and free radicals. Recently, the non-thermal plasma is more often used in various biomedical domains such as: decontamination of the medical equipment, blood coagulation, devitalized tissue healing, air and surfaces decontamination, implant treatment, etc. (Deng *et al*., 2007; Deilmann *et al*., 2008; Farin *et al*., 1994; Fridman *et al*., 2008; Grundmann *et al*., 2006; Moreau *et al*., 2008; Morrison *et al*., 1977; Selcuk *et al*., 2008). Non-thermal plasma discharge has both direct antimicrobial efficacy (by plasma flow treatment of the microorganisms from various surfaces and culture media) and indirect antimicrobial efficacy by transfer of the oxygen and nitrogen reactive species in water that become activated (PAW). The activated water means a "post-discharge" antimicrobial effect present up to few days after interrupting the plasma discharge in water and determined by both reactive species from water and its intrinsic changes (the water structure changes, the cluster size is decreased or water becomes monomolecular; modified energy state of water characterized by considerably changed absorption spectrums; decrease of pH, modified

redox potential). It is generally accepted that reactive oxygen species such atomic oxygen or hydroxyl radicals are key factors in the direct inactivation and oxidative stress, which result in impairment and destruction of the microbial cells (Larouss and Leipold, 2004; Nagatsu *et al*., 2005; Perni *et al*., 2007; Kim *et al*., 2009; Helmke *et al*., 2011; Zhang *et al*., 2012; Zhang *et al*., 2013).

Non-thermal plasma-activated water (PAW) has been widely considered to be an effective method for decontamination of foods. (Ma *et al*., 2015).

The aim of this paper was to systematically investigate the antimicrobial potential of PAW against most encountered food-borne pathogens, which would substantially improve scientific knowledge in the field.

Materials and methods

In order to produce PAW, a GlidArc plasma reactor was used. The working parameters were as follow: room temperature and atmospheric pressure, air as gas carrier (40 liters per minute). In this experiment, distilled water was treated 20 minutes in order to obtain PAW (pH 2.7-2.9, conductivity 465±20 μS/cm).

As testing microorganisms, we used 9 type strains: *Salmonella typhimurium* ATCC 1866, *Salmonella anatum* RTCC 1415, *Salmonella derby* RTCC 1868, *Salmonella enteritidis* ATCC 13076, *Listeria monocytogenes* ATCC 19114, *Listeria welshimeri* RTCC 1265, *Staphylococcus aureus* ATCC 6583, *Escherichia coli* ATCC 25922 and *Campylobacter coli* ATCC 33559.

A defined volume of bacterial suspension (10 mL) with 3 McFarland density (approximately 10^9 CFU/mL) was mixed with 90 mL PAW and after various contact times (3, 5, 7 and 10 minutes) known volumes (0.1 and 1.0 mL, respectively) were transferred to Plate Count Agar plates in order to determine the number of CFU/mL. The plates were incubacted at 37ºC in aerobic conditions for 24h. The initial concentration of the bacterial suspension determined on Plate Count agar was used as control.

The reduction of bacterial burden was evaluated using colony-forming unit (CFU) count and the formula: Log Reduction = $\log_{10}$ (CFU $_{\text{before PAW treatment}}$ / CFU $_{\text{after PAW treatment}}$).

Also, the experiment was performed on *liquid* culture media to demonstrated the effect sterilizing of PAW by incubating the type strains treated with PAW on BacT/ALERT bottles.

In order to assess the PAW interactions with bacterial cell wall Dynamic Light Scattering (DLS) was used on *S. aures* ATCC 6583.

Results and discussion

The present study emphasizes the bactericidal effect of plasma-activated water against food-borne pathogens. The summative results concerning bacterial load reduction for 9 type strains belonging to food-borne pathogens after contact with PAW are presented in Table 1.

A powerful antimicrobial effect after the PAW contact was documented for each strain and each time of contact (Figure 1).

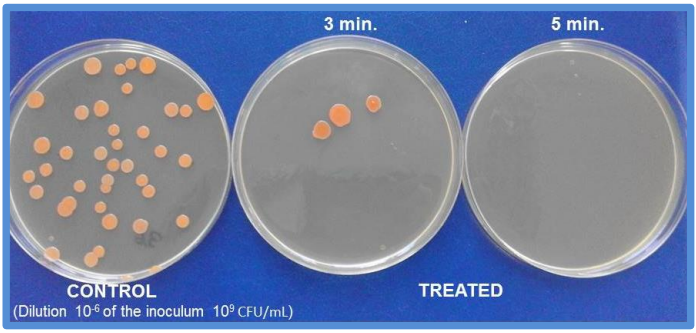

Fig. 1. S. aureus ATCC 13076 before and after treatment with PAW on Plate Count Agar

Fig. 2. S. enteritidis before and after treatment with PAW on BacT/ALERT bottles

After cultivation the strains of food-borne pathogens on BacT/ALERT bottles and incubated in automated BacT/ALERT 3D Microbial Identification System, it was confirmed the sterilization effect of treatment with PAW (Fig. 2). After an exposure time of 3-min (Fig.2), the complete inactivation of bacteria was achieved (sterilization).

Table 1 Bacterial load reduction after PAW treatment

Strain	**CFU T0/ inoculum**	PCA			BacT/ALERT
		Log_{10} T1	**$Log1_0$ T3**	**Log_{10} T5**	**Sterilizing activity**
S. typhimurium	5.2 (10^8)	5,716	5,716	5,716	3 min.
S. anatum	7.7 (10^8)	4,509	5,889	5,889	1 min.
S. derby	7.5 (10^8)	4,036	5,875	5,875	3 min.
S. enteritidis	5.5 (10^8)	5,74	5,740	5,740	1 min.
L. monocytogenes	1.2 (10^8)	4,778	4,778	5,079	10 min.
L. welshimeri	3.4 (10^8)	3.033	3.033	5,230	7 min.
S. aureus	3.2 (10^8)	4,397	5,511	5,511	5 min.
E. coli	2.4 (10^8)	5,088	5,088	5,389	3 min.
C. coli	3.7 (10^8)	5,748	5,748	5,748	3 min.

Significant reductions in bacterial populations were achieved in all strains of tested bacteria, demonstrating the effectiveness of this new approach to treat the bacteria.

The logarithmic reduction was 5.6 $\log_{10}$ after an exposure time of 5-min, exhibiting a powerful biocide effect for all strains of foodborne pathogens.

DLS analysis exhibits the degree of peripheral electronegativity influences overall cell surface polarity (zeta potential) which is most often determined by estimating the electrophoretic mobility of cells in an electric field. The growth phase of bacteria only affects the zeta potential giving a more negative potential when bacteria are in stationary phase (Ursache *et al*., 2014).

The Zeta potential of the staphylococcus ranged from -25.02 mV for the untreated sample to +1.25 mV for the sample treated with PAW. This change can be attributed to the death of bacteria (Fig. 3).

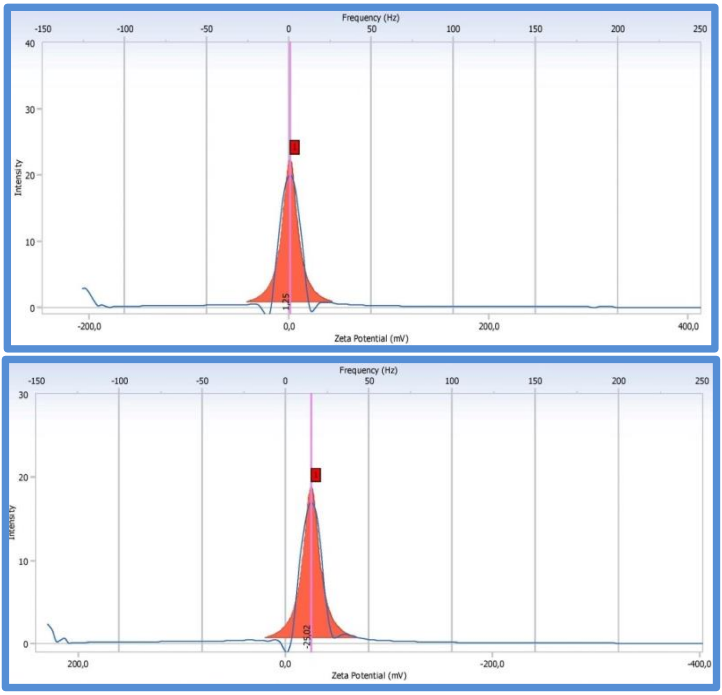

Fig. 3. DLS of *S. aureus* ATCC 6538 before (a) and after treatment with PAW (b)

Recent research has reported that PAW can also efficiently inactivate a wide diversity of microorganisms. Non-thermal plasma activated water is a new technique to improving microbiological safety also maintaining the sensory attributes of the treated foods with PAW.

One recently published paper presents the successful decontamination of fresh fruits (strawberries) using PAW (Zhang *et al*. 2015), giving us a supplementary argument for the extension of studies focused on PAW antimicrobial effect against food-borne pathogens.

At the same time, no significant change was observed in the color, pH, or antioxidant properties of foods fresh treated with plasma activated water (Xu *et al*., 2016).

Conclusions

This study demonstrates PAW has the potential as disinfectant in a large-scale industrial application.

Effect non-discriminatory against tested strains with plasma activated water, both Gram positive (*Listeria monocytogenes* ATCC 19114, *Listeria welshimeri* RTCC 1265, *Staphylococcus aureus* ATCC 6583,) and Gram negative (*almonella typhimurium* ATCC 1866, *Salmonella anatum* RTCC 1415, *Salmonella derby* RTCC 1868, *Salmonella enteritidis* ATCC 13076, *Escherichia coli* ATCC 25922, *Campylobacter coli* ATCC 33559) is a promising agent disinfectant for applications in food industry.

A significant logarithmic reduction (more than 8 log reduction) compared to the initial suspension concentration was observed after treatment time of 5 minutes exhibiting a powerful biocide effect for Gram positive and Gram negative bacteria.

Thus, PAW as cost-effective disinfectant useful to applied in slaughterhouses and food processing plants.

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