

Assessment of the efficacy of a low-temperature gas plasma prototype for superficial decontamination of table eggs

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Abbreviated title: Gas plasma treatment of table eggs

Summary

A resistive barrier discharge (RBD) prototype was set-up in order to decontaminate table eggs surface from *Salmonella* Enteritidis and *Listeria monocytogenes*. The eggs were treated in the discharge afterglow where the gas temperature was similar to room temperature, thus minimizing thus the risk of thermal alterations of the samples.

The examined prototype appeared to be very effective for the inactivation of both the pathogens deliberately inoculated onto the surface of egg shells. Its efficacy was significantly increased when treatments were performed in the presence of humid air: in particular, reductions ranging between 4 and 5 Log CFU/egg shell in relation to the time of treatment were achieved for both the pathogens with the highest relative humidity in the treatment chamber.

Moreover, a gas plasma treatment of 90 minutes only slightly affected the egg cuticle without other modifications of the egg

Keywords: Superficial decontamination, low-temperature gas plasma, *Salmonella* Enteritidis, *Listeria monocytogenes*, safety, quality, table eggs.

Introduction

The consumption of eggs has increased during years due to their low cost and high nutritional value. Also the World Health Organisation has designated eggs as the reference protein source for children because of their ideal amino acid balance. However, *Salmonella enterica* serovars Enteritidis and Typhimurium are considered as the primary zoonotic agents for the egg sector as it is recognized that eggs are one of the main sources of food-borne outbreaks in Europe (Regulation EC No 2160/2003). Big efforts have been made both at national and European level to identify efficient means to reduce any potential or additional risks for the consumers due to eggs consumption. However, egg shells cleaning and washing, which are practised in some countries (e. g. USA and Japan) for reducing bacterial contamination in table eggs, are not allowed in Europe except Sweden and the Netherlands.

Cold gas plasmas are partial ionized gases consisting of ions and electrons, uncharged particles such as atoms, molecules and radicals collectively called neutrals. Man-made plasmas are usually produced by subjecting gases to an electric field either of constant or alternating amplitude resulting in the formation of numerous reactive species that are in excited states and can lose their internal energy by emitting a photon or through collisions with other particles or a surface. Such reactive species and the UV radiation can cause damages to microbial DNA and membranes (Moreau *et al.*, 2008). Due to its intrinsic characteristics gas plasma offers an original alternative to conventional decontamination techniques of food mainly due to the atmospheric operating temperature, which results in minimal degradation of nutrients, changes in organoleptic properties as well as formation of new potentially dangerous molecules (Moisan *et al.*, 2002; Laroussi and Leipold, 2004). However, most of the applications of this innovative technique are limited to medical devices (Machala *et al.*, 2007).

The principal aims of this work were: i) to explore the effectiveness of a resistive barrier discharge prototype (RBD), able to generate gas plasma at atmospheric pressure, for the surface decontamination of table eggs deliberately contaminated with pathogens; ii) to assess the effects of gas plasma treatments on eggs quality.

Materials and methods

RBD prototype In the selected prototype (70 dm³ of maximum volume of the treatment chamber) the discharge is generated between three parallel plate reactors made of brass and the glass (5 mm thick) is used as high resistive materials to prevent arcs (Figure 1). The voltage at the electrodes (about 15 kV, with an input voltage of 19 V) is produced by high voltage transformers and power transistors. The decontamination treatments were achieved in the plasma afterglow where the temperature was about 21-23°C.

Figure 1. The RBD gas plasma prototype.



Inoculum of shell eggs and gas plasma treatments Shell eggs have been deliberately inoculated with *Salmonella* Enteritidis (strain MB2509) or *Listeria monocytogenes* (strain ATCC 13932) and then treated with the RBD prototype for 0, 10, 20, 30, 45, 60 and 90 minutes. Each treatment, characterized by 5 repetitions, was conducted by considering two different levels of relative humidity (%) of the device chamber: 21% and 66-70%.

Microbiological investigations Shell eggs were diluted 1:10 in NaCl (0.9%, w/v) solutions and the surviving cells of the target microorganisms were enumerated by plate countings onto selective media, i.e. Brilliant Green Agar for *Salmonella* Enteritidis and Listeria Selective Agar for *L. monocytogenes*.

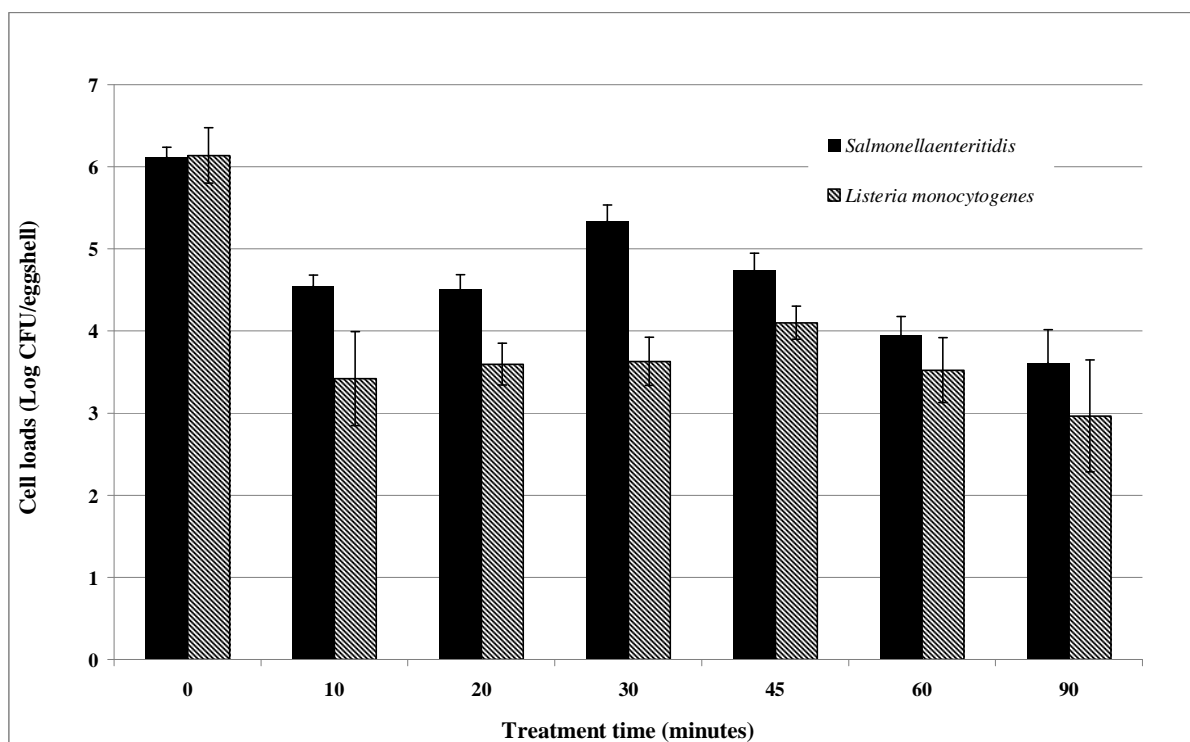
Egg quality assessment The possible negative effects of 90 minutes treatments on the eggs quality were investigated on samples of 50 eggs each. The following parameters were measured: the cuticle presence, the shell colour and the albumen pH (immediately after the treatment), the shell membranes, the shell dynamic stiffness and breaking strength and the yolk index (during a storage of 28 days at 25°C).

Results and discussion

Influence of gas plasma treatments on cell viability

In figures 2 and 3 the effects of different gas plasma treatment times and relative humidity on the viability of *Salmonella* Enteritidis and *Listeria monocytogenes* are reported.

Figure 2. Effects of treatment time on cell viability of *Salmonella* Enteritidis and *Listeria monocytogenes*. Relative humidity: 21%



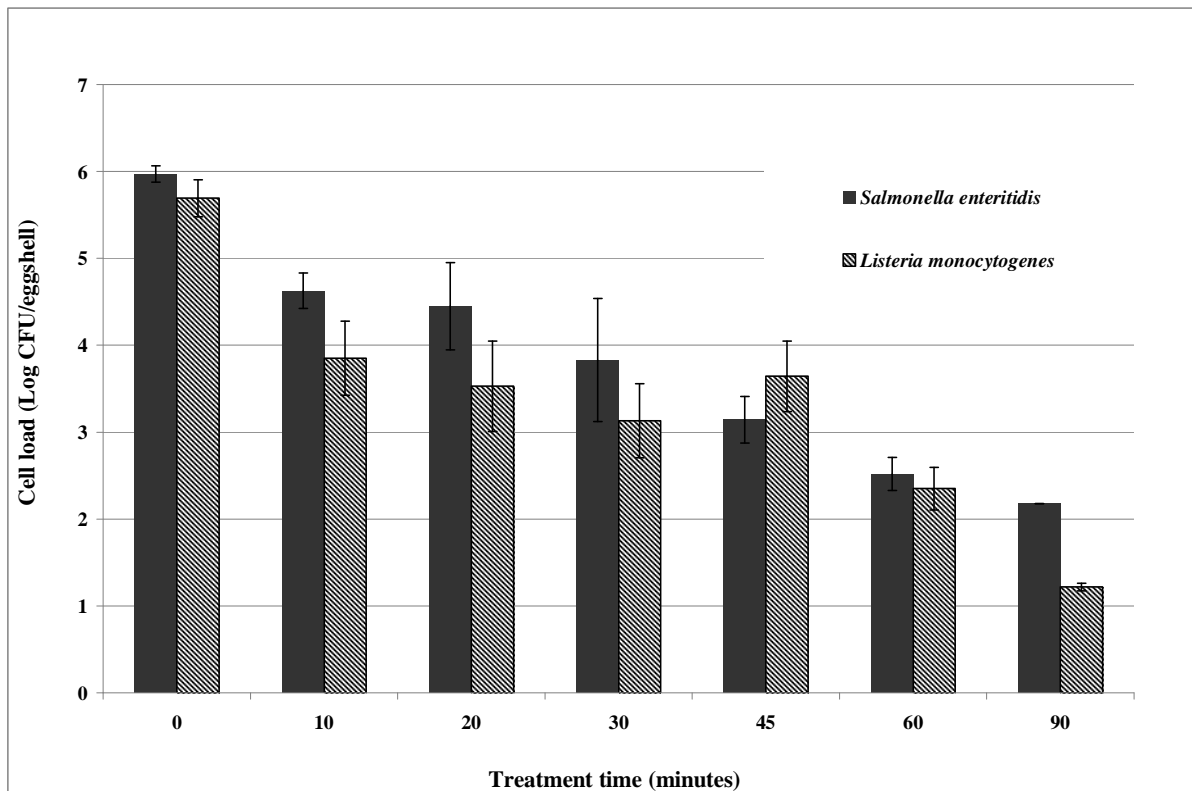
All the survival curves obtained were non-linear and presented a biphasic behaviour with a tail due to the presence of surviving cells, regardless the initial contamination level and the microbial species taken into consideration.

The resistive barrier discharge prototype used to generate cold plasma proved to have a good decontamination power towards both *Salmonella* Enteritidis and *Listeria monocytogenes*: in fact also after the shortest treatment times, a 1-1.5 Log CFU/egg reduction was obtained for *Salmonella* Enteritidis, while a 2-2.5 Log CFU/egg viability loss was observed for *L. monocytogenes* when treatments were performed with the lowest relative humidity value (Figure 2).

The maximum deactivation levels observed did not exceed 2.5 and 3 Log CFU/egg for *Salmonella* and *Listeria monocytogenes*, respectively. Only a 1.5 Log units reduction was observed for total mesophilic bacteria following 45 minutes of exposure to the gas plasma (data not shown).

The increase of the relative humidity in the device chamber from 21% up to 66-70% significantly affected the treatment efficacy: in fact the maximum reductions achieved after the longest treatment corresponded to 4 and 5 Log CFU/eggs for *Salmonella* Enteritidis and *Listeria monocytogenes*, respectively (Figure 3).

Figure 3. Effects of treatment time on cell viability of *Salmonella* Enteritidis and *Listeria monocytogenes*. Relative humidity: 66-70%



Influence of a 90 min gas plasma treatment on shell egg quality

After the cuticle assessment by a dipping method with the dye MST Cuticle Blue, significant differences emerged between the shell colour of the control and the treated eggs samples indicating a possible partial damage of the cuticle (Figure 4). Significant differences emerged also between the shell colour before and after the treatment (Table 1). On the contrary, no appreciable consequences for the quality of the albumen (evaluated in terms of pH), the shell membranes, the shell dynamic stiffness and breaking strength, and the yolk were observed.

Figure 4. The eggshell colour after the cuticle assessment: A) control eggs; B) treated eggs.

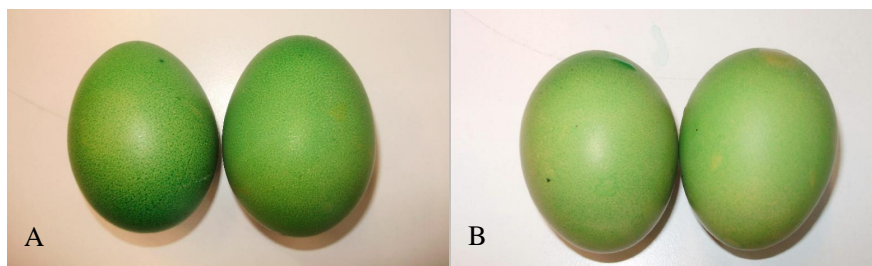


Table 1. Influence of the gas plasma treatment (90 minutes) on the cuticle and the shell colour.

	Shell colour after the cuticle assessment			Shell colour ^{§§}		
	L*	a*	b*	L*	a*	b*
Control	51.8 ^a (4.0) [§]	-10.0 ^a (10.2)	29.2 ^a (1.8)			
Before treatment				63.1 ^a (3.3)	17.2 ^a (2.1)	30.3 ^a (1.4)
After 90 min	52.4 ^a (4.3)	-14.4 ^b (6.4)	28.5 ^a (2.3)	63.7 ^a (3.1)	16.0 ^b (1.7)	29.4 ^b (1.1)

Differences between means with the same exponent letter within a column are not significant at p-level < 0.05 (ANOVA).

[§]Values into brackets are the standard deviations.

^{§§}The measurements of the shell colour were carried out on the same sample of 50 eggs before and after the gas plasma treatment.

Conclusions

The results obtained in this work evidenced that cold gas plasma treatment is a promising technique for superficial decontamination of table eggs being able to induce cell reductions up to 4-5 Log CFU/egg for both the target pathogens. On the other hand, a gas plasma treatment of 90 minutes only slightly affected the egg cuticle without inducing any other modifications of the eggs.

The efficacy of resistive barrier discharge prototype used resulted to be dependent on both the time of exposure to gas plasma and on the relative humidity in the device chamber. According to Benstaali *et al.* (2002) spectroscopic investigations of the gas plasma revealed that the main species formed in the non-thermal plasma are the radicals OH and NO: it is therefore presumable that the formation of gas plasma in humid air results in higher concentrations of such radicals that are highly oxidative and hence responsible for the bactericidal activity of the plasma. It is reported that the action of gas plasma seems to involve an etching mechanism, mainly affecting the outer bacterial membrane causing marked morphological changes, the release of constitutive proteins and the dissociation of membrane-linked DNA (Moreau *et al.*, 2007). However, all the biological and chemical effects of plasma need to be deeply investigated and characterised also in relation to the process parameters before proposing this technology as a valuable tool for superficial decontamination of different foods or packaging materials.

Acknowledgements

This work was funded by the European Community within the FP6 European project RESCAPE "Reducing Egg Susceptibility to Contaminations in Avian Production in Europe" (Food-CT-2006-036018).

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