ANTIBACTERIAL PROPERTIES OF A NON-THERMAL, ATMOSPHERIC, OPENAIR®, PLASMA JET IN SURFACE DECONTAMINATION OF EGGS IN SHELL

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Summary: In the European Union, eggs may not be washed or cleaned. So, in order to reduce food safety risks, several techniques for eggs in shell decontamination have been developed. The current study was undertaken to determine the potential of the Non-thermal, Atmospheric, Openair® Plasma Jet for surface decontamination of eggs in shell. In the experiment Polyethylene Theraphtalate plates and the eggshells of table eggs were exposed to single or multiple treatments with a Non-thermal, Atmospheric, Openair® Plasma Jet. This resulted in up to > 3 log reduction of *Staphylococcus aureus* (*S. aureus*) (NCTC 8325) on Polyethylene Theraphtalate and 1.8–2.5 log reduction of a common number of native aerobic mesophylic bacteria and S. aureus (NCTC 8325) on eggshells in a treatment time of 10–60 seconds. Ionising gas of the plasma jet was obviously not harmful to eggshell cuticle, since no significant alterations to plasma-treated eggs were found, or to physico-chemical properties of the contents of plasma-treated eggs, such as: air cell height, pH, the whole weight of eggs, or height of the thickness of egg white, which did not significantly differ from the untreated eggs during 54 days of aging. The results of the experiment indicate that the treatment of eggshells with the plasma jet has the potential for egg in shell decontamination with no side effects on egg quality, which is important as far as food safety and quality characteristics that are acceptable to consumers.

Key words: egg in shell; non-thermal; openair; atmospheric plasma jet; aerobic mesophylic bacteria; *S. aureus*; decontamination

Introduction

Salmonella, Listeria, Escherichia coli and *Campylobacter* are the most often found food contaminants (1, 2, 3, 4, 5, 6). Contaminated food can pose a considerable risk. For this reason, besides obligatory bio-security measures in the food industry, new approaches, additional, and alternative methods for food decontamination are still developing (7, 8). Among numerous methods of decontamination, plasma is one of the most important innovations for preventing food contamination (9, 10, 11). The main function of the plasma decontamination activity is in its diffusion of highly energetic reactive species (OH radicals and NO), oxygen atoms, and UV photons in a bacterial cell, resulting in irreversible damages to DNA and vital cell macromolecules (1, 12, 13, 14). Therefore, novel methods for plasma generation, and above all the development of technology by which the plasma is generated at atmospheric pressure in ambiental temperatures (15, 16, 17), are contributing to new approaches in safe

and environmental-friendly food processing. Due to the demands for absolute cleanliness also in food processing plants, different types of plasma were examined as alternatives to chemical and certain biological decontamination procedures and decontaminants. For these purposes plasma, as a non-chemical agent, used without water or other solvents, leaving no residue, has an important potential in terms of food safety and environmental protection (15, 18, 19). Whereas, the use of plasma was primarily directed on the surface decontamination of food premises and packing material, some recent investigations have been directed to using plasma directly on foods. Some studies of the non-thermal plasma surface treatment of chicken meat and chicken skin, in order to prevent *Campylobacter* contamination, have been already conducted (20, 21, 22, 17). Similar investigations have been made for inactivation of *Listeria innocua* in ready-to-eat meat products (22), meanwhile investigations, concerning the advantages and weaknesses of plasma used directly on food matrices, especially for decontamination of *Salmonella* spp., *Campylobacter, Streptococci, E.coli*, and *Lactobacillus*, were conducted as well (6, 21, 22, 10, 1). Nevertheless, investigations of plasma used for food decontamination, and above the all, for decontamination of commercial table egg in shell (14), are not numerous. This is a lack of important information, because until the beginning of the last decade, table eggs in shell were among the most important *Salmonella*-contaminated foods in the EU and USA. Therefore, in order to protect consumers from *Salmonella*, an integrated approach to food safety from the farm to the fork has been adopted in last five years in EU. However, the risk of contamination still exists, which is why some recent investigations focused on searching for innovative methods of table egg decontamination (23). For example given the ban of hen battery cages rearing in the EU (European Union Council Directive 1999/74/EC), several studies (RESCAPE project) have been conducted, searching how to diminish risk factors for potential egg contamination (24) by introducing the alternative egg-laying hen rearing systems. In recent years some innovative methods for eggshell decontamination, which do not include washing or chemical sterilization (25, 26), were investigated. Among the different methods, also non-thermal plasma for eggshell decontamination was explored,

to ensure safer egg handling, and to prevent the penetration of (not necessary pathogenic) micro flora from the eggshell to its contents (27). However, only a few studies in this field have achieved this goal. Amongst them, only Ragni *et al.* (2010) (28), and Vaninni *et al.* (29) reported about eggshell decontamination (*Salmonella enteritidis*) with the Resistive Barrier Discharge (RBD) gas plasma under atmospheric conditions. So, with respect to the lack of information in this field, the aim of our study was to establish possible implications of the Non-thermal, Atmospheric, Openair® Plasma Jet (Plasmatreat) for egg in shell decontamination. The most important goal of this research was to determine whether it is possible to decontaminate eggshells in ambiental conditions without damaging of the eggshell cuticle, and contemporary retaining all the characteristics of high-quality table eggs in shell.

Materials and methods

Non-thermal, Atmospheric, Openair® Plasma Jet and surface treatments

A Non-thermal, Atmospheric, Openair® Plasma Jet (Plasmatreat) (AOPJ) is a special type of highly energized plasma (30, 16, 17, 31, 27) generated in the process of compressed gas discharging in high electricity voltage and in a pulsed electric arc. This type of plasma is especially useful in industrial processes where absolute surface purity is needed, such as micro-cleaning of plastic, metal, glass, ceramic, and other materials (30). An AOPJ is generated in the neutral atmospheric air pressure at room temperature inside the reaction chamber between the electrode and dielectric barriers, producing a non-equilibrium discharge (normally 5–10 kV; in our experiment 1 kV) at a working frequency of 21 kHz, and at a power range from 500 to 1000 W. The plasma jet carrier is compressed (6 bar), oil free (max. concentration of oil in air: 0.1 mg/m^3 at 20°C), and filtered air (99.9% particles reduction in diameter up to <0.03 µm), which streams on the surface as a jet through the reaction chamber of the jet head (Figure 1). Consequently, the plasma jet treatment results in strong activation of material surface (30), so, in the present experiment an AOPJ was used as a source of plasma jet energy for surface eggshell decontamination.
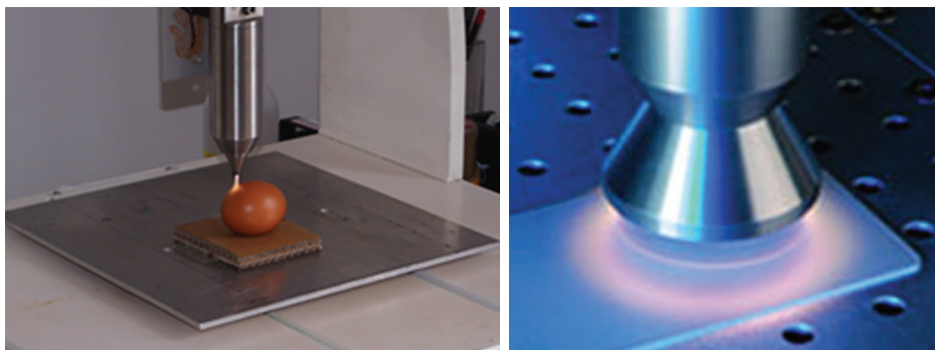
Figure 1: AOPJ head A (Left); OPJ head B (Right) (Plasmatreat, 2014)

Two types of AOPJ head nozzles were used in experiment. AOPJ head A is a static (firm) nozzle intended for the linear surface treatment in the width of 8–16 mm, which depends on the distance between the nozzle and treated surface (from 4 to 20 mm) (Figure 1, left). AOPJ head B is a rotary nozzle which rotates at 2000 RPM, forming a ring of spinning plasma jet in an outlet angle of 25°, enabling a circular surface treatment in the width of 40 mm, regarding the distance between the nozzle and treated surface (from 5 up to 20 mm) (Figure 1, right).

AOPJ heads A and B were constructed to move above the treated surfaces, following the component geometry with exact precision, driven by the software programmed robot. In the experiment they were regulated to follow the curve line of experimental eggs in shell surface (Figure 1, left) and/or the flat shape (Figure 1, right) of experimental Polyethylene Theraphtalate (PET).

In order to find the optimal decontamination efficiency, an AOPJ was tested in different experimental conditions, e.g. with regard to: distances of the heads A and B to the treated surface (10 mm, 15 mm, 20 mm), head speeds (5 cm/s and 10 cm/s, which is equivalent to 1/50 s/mm and 1/100 s/mm), and treatments recurrences (single or triple). Treatment times were in range from 10 – 60 s depending on the width of the AOPJ stream and area of tested surfaces. Therefore, with head A, PET and eggshells need to be treated in 5 parallel lines, or in 3 parallel lines when head B was employed.

An antimicrobial test of an AOPJ was performed in a preliminary test on PET, and in an experiment on eggs in shell. The antimicrobial efficiency of an AOPJ was experimentally tested on native aerobic mesophylic microorganisms on eggshells, and on a test microorganism *S. aureus* (NCTC 8325). The reasoning behind this is in farm egg production *S. aureus* is among the most common eggs contaminants (32). Thus *S. aureus* was used as the test microorganism on PET, wherein irrespective to the variability in quantity of native aerobic mesophylic microorganisms on eggshells from egg to egg, part of the eggs were additionally contaminated with the suspension of *S. aureus* of a known concentration (10^6 CFU/ml) in order to get a more accurate AOPJ antibacterial efficiency evaluation. So, the common number of bacteria on eggshells consists of common counts of native aerobic mesophylic microorganisms and *S. aureus*.

Preliminary test of AOPJ treatment of Polyethylene Theraphtalate (PET)

In order to investigate the potential antimicrobial activity of an AOPJ on PET, flat surfaces of PET plates (6 cm x 3 cm) were covered with suspension of *S. aureus* of known concentration (10^6 CFU/ml) and treated with head A and head B. *S. aureus* was chosen as a reference material for aerobic mesophylic bacteria. For this purpose, 65 PET plates were artificially coated with *S. aureus* on the surface of 18 cm^2. Thirty PET plates were treated with head A, and 30 with head B, while 5 PET plates were used as a control (Table 1). The results were presented as a total count of *S. aureus* (TC *S.a.*).

AOPJ treatment of eggs in shell

The antibacterial efficiency of AOPJ on the medium-sized table eggs (54-62 g) in shell from the same age stable and rearing technology was analysed. So, eggshell surfaces of naturally contaminated eggs with native aerobic mesophylic

bacteria and eggs additionally coated with *S. aureus* were treated with head A and head B (Table 1). In order to perform tests on 120 eggs, two test surfaces (each 4 cm^2) were marked on each egg on the opposite sides of the eggshell (direction east-west) (Figure 2). One side of the egg was AOPJ-treated, while the opposite side was not, and served as a control. Immediately after an AOPJ treatment, swabs were taken (test surfaces 4 cm^2) from both sides of egg for microbiological analysis. The results of the total number of native aerobic mesophylic microorganisms were presented as a total viable count (TVC). Meanwhile, when *S. aureus* was additionally coated, the common counts of bacteria on eggshells were presented as the sum of native aerobic mesophylic microorganisms and *S. aureus* (TVC + TC *S.a.*). The experiment was performed in conditions of the air temperature 22°C and 55% relative humidity, which were monitored using Testo 350 M/XL

454. Temperatures of the treated surfaces were monitored using an infra-red camera (Testo 881).

Microbiological and physico-chemical properties of eggs during the period of 54 days (in 7 days intervals starting from day the 4th) after AOPJ treatment

For determining the differences in microbial and physico-chemical properties of AOPJ-treated and untreated eggs, eggshell surfaces of naturally contaminated, medium-sized table eggs (n=189) with native aerobic mesophylic bacteria, and eggs (n=19) additionally coated with *S. aureus* (were treated with head A (Table 2). Sampling was performed in 7 days intervals, starting from 4th day thus after the treatment, the TVC on eggshells was analysed on the 4th, 11th, 18th, 25th, 32nd, and 54th day, meanwhile the TVC + TC *S.a.* in egg contents was determined on the 18th and 32nd day.

Table 1: AOPJ treatment of PET and eggshell and microbiological analysis

| AOPJ surface treatment	No. of samples (PET/egg)	Tested surface	Head A	Head B	Aerobic mesophylic bacteria analysis	Additional coating and *S. aureus* analysis
PET test surface	30	18 cm^2	yes			yes
	30	18 cm^2		yes		yes
PET control surface	5	18 cm^2	no	no		yes
Eggshell test surface	50	4 cm^2	yes	no	yes	no
control surface	50	4 cm^2	no		yes	no
Eggshell test surface	30	4 cm^2	yes	no	no	yes
control surface	30	4 cm^2	no			yes
Eggshell test surface	40	4 cm^2	no	yes	no	yes
control surface	40	4 cm^2		no		yes
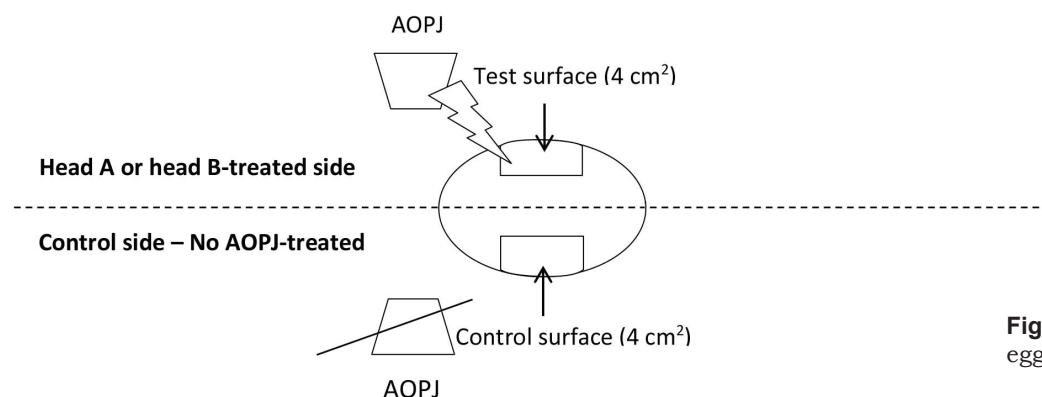
Figure 2: AOPJ treatment of eggshell

Table 2: AOPJ treatments (head A) of eggshell and tests for 54 days after treatment

Head A eggshell surface treatment	No. of eggs for TVC on eggshell	No. of eggs with additional coating *S. aureus*	No. of eggs for TVC+ TC *S.a.* in egg content	No. of eggs for egg weight	No. of eggs for egg pH values	No. of eggs for air cell height	No. of eggs for thick egg white height
test eggs	80	19	33	10	6	30	30
control eggs	80	19	33	10	6	30	30

Eggs were weighed on an analytical balance (XP 205, Mettler Toledo, accuracy of 0.1 mg weekly one the 4th, 11th, 18th, 25th, 32nd, and 54th day after treatment. The air cell height of eggs (mm) were determined weekly at 4th, 11th, 18th, 25th, and 32nd day after treatment, using the air cell measurer and bright light to 'candle' the egg. For the analysis of the egg contents we cracked the eggs under sterile conditions. The pH values of albumen were measured using a pH meter (PHM210 MeterLab, Radiometer analytical, and accuracy of 0.01) on the 32nd day of experiment. The height (mm) of a thick egg white was measured using a micrometer at 4th, 11th, 18th, 25th, and 32nd day after treatment. During the experiment eggs were kept in conditions with a constant temperature of 22°C and a relative humidity of 60%.

Diagnostics of aerobic mesophylic bacteria (TVC)

Samples for aerobic mesophylic bacteria enumeration were taken as swabs from the PET plates (18 cm^2) and the eggshell test surfaces (4 cm^2), and in egg contents (20 ml) by egg cracking in sterile conditions. Laboratory analysis of mesophylic microorganisms and *S. aureus* (TVC + TC *S.a.*) were performed with the same procedures in accordance with the ISO standard 4833-1:2013, while a results interpretation was performed in accordance with the ISO standard 7218:2007/ A1:2013. The TVC was calculated as CFU/cm^2 or CFU/g, depending on the sampling matrix.

Scanning electron microscopy (SEM) of eggshell

Since a plasma jet contains highly energetic species which can be potentially harmful for the eggshell cuticle, the scanning electron microscopy (Field emission SEM microscope JEOL 7600F) was used to screen the surface of the eggshell. Prior to the SEM investigation the surface of the eggs was coated with 3nm thick layer of carbon using a Precision Etching Coating System (Gatan, model 682). The microscope was operating at 10 kV and working distances were from 8.0 to 4.5 mm. For images taken at low magnification (LM-from 25 to 80000 times), a lower secondary detector for lower resolved secondary electrons (LEI) was used, while an upper secondary detector (SEI) was used for images taken at higher magnifications. Cuticle surface damage was estimated by the appearance of differentiation among eggshell images of the AOPJ-treated and untreated (control) eggs at x400, x1000 and x20,000 magnifications. The evaluation was based on the density of visible cuticle cracks, their width, and the edges sharpness, while at high magnifications the density and distribution of visible Ca, Mg, P spherules (80–300 nm in diameter) were evaluated.

Results evaluation

Statistical evaluation of results was carried out by ANOVA, t-test, and correlation analyses using the GraphPad Prism 6 computer programme (GraphPad Software, Inc., USA, 2014). The Pearson product-moment correlation and linear regressions ABS versus time were accepted for r >0.95, and values of the slopes less than $P<0.05$ were considered statistically significant. Counts of the mean TVC were calculated in common logarithms ($\log_{10}$) ± SD, while the percentage (%) of TVC reduction was calculated in the absolute numbers of TVC. The term 'log reduction' is used as the total reduction of microorganisms determined by the following formula: log reduction = $\log_{10}$ initial population – $\log_{10}$ final population (e.g. 3 log reductions = 99.9% kill rate).

Results

The antibacterial effects of the AOPJ treatments on Polyethylene Theraphtalate (PET)

The results depicted in Figure 3 and Table 3 show the bactericidal activity of the AOPJ head A and B treatments on the PET. In a mutual comparison head A showed a 24% higher mean bactericidal efficiency than head B. The median values of the total count ($\log_{10}$) of *S. aureus* (TC *S.a.*) showed significant (P<0.0001) difference (1.73±0.89, r=0.15) in bactericidal activity between treatments with head A and head B (Figure 3). The highest bacterial efficiency of 3.15 log reductions was achieved using head A at a distance of 20 mm from the surface, with a speed of 5 cm/s and triple successive treatments (Table 3).
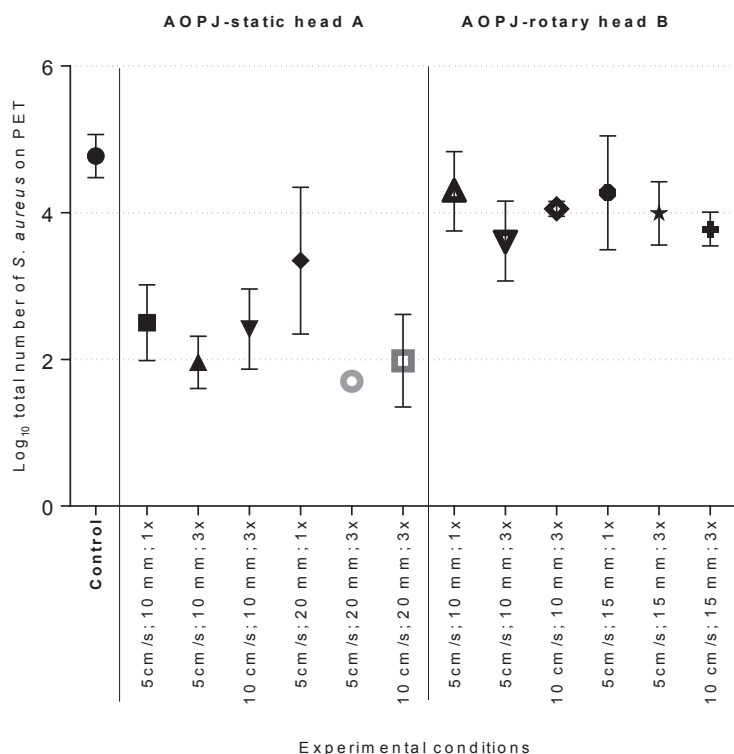

Figure 3: Mean of total number (with SD in error bars) of the TC *S.a.* ($\log_{10}$ CFU/cm^2) after head A and B treatments of PET plates coated with *S. aureus* with regard to the untreated PET plates (control) in different experimental conditions

Table 3: Mean differences in total number of the TC *S.a.* ($\log_{10}$ CFU/cm^2) after jet head A and B treatments of PET plates with regard to untreated PET plates (control), considering the mean of experimental conditions and in experimental conditions in which the highest differences of *S. aureus* were recorded and the log reduction of the TC *S.a*

PET surface AOPJ to control	head A	head B	head A	head B
Experimental conditions	mean	mean	5 cm/s; 20 mm; 3×	5 cm/s; 10 mm; 3×
Mean differences in TC *S.a.*	-2.27±0.49, r=0.35, P=0.0005	-0.48±0.58, r=0.10, P=0.14	-3.07±0.29, P<0.0001	-1.16±0.48, r=0.44, P=0.006
TC *S.a.* log reduction	1	<1	3.15	1
TC *S.a.* reduction (%)	98.2	74	99.93	89.4

Figure 4: Boxplot of the number of TVC + TC *S.a.* colonies ($\log_{10}$ CFU/cm^2) on the eggshells after the head A and B treatments considering the mean of experimental conditions, with regard to the untreated (control) eggshells

Comparisons are presented separately with respect to the treatment with head A or head B. The median value of each distribution is shown with a horizontal line within each box, while the + marks the mean. The boundary of the box closest to zero indicates the 25th percentile, and the boundary farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. The outliers are shown as dots.
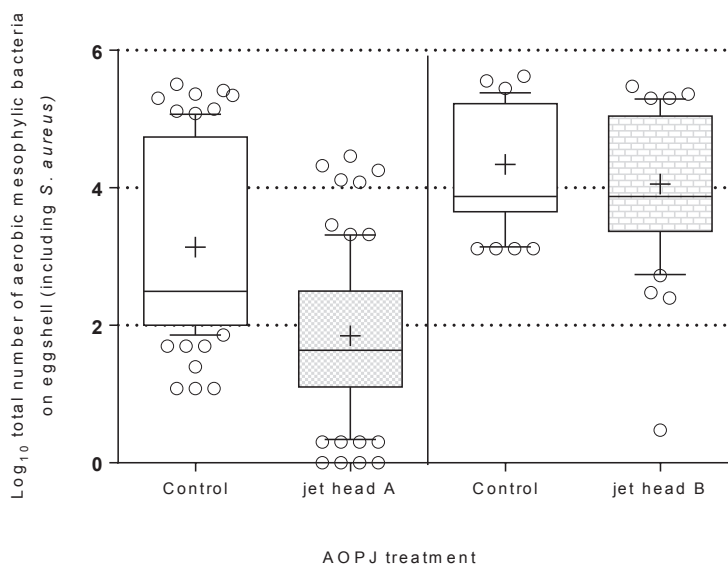

Table 4: Mean differences in the total number of the TVC + TC *S.a.* ($\log_{10}$ CFU/cm^2) after head A and B treatments of AOPJ-treated eggshells with regard to the untreated (control) eggshells considering the mean of experimental conditions and the log reduction of TVC and TC *S.a.* on the eggshells

Eggshell surface AOPJ to control	head A		head A	head B
Experimental conditions	mean		mean	mean
Mean differences in TVC + TC *S.a.*	-1.29±0.90, r=0.73, P<0001	mean differences in TC *S.a.*	-1.62±0.62, r=0.62, P<0001	-0.28±0.75, r=-0.72, P=0.02
TVC + TC *S.a.* log reduction	1.36	TC *S.a.* log reduction	1.33	< 1
TVC + TC *S.a.* reduction (%)	95.69	TC *S.a.* reduction (%)	95.35	29.1

The antibacterial effects of the AOPJ treatments (using head A and B) on the TVC on an eggshell surface

The results presented in Figure 4 show the bactericidal activity of the AOPJ treatments (head A, head B) on the eggshell surfaces. The median number ($\log_{10}$) of the TVC and TC *S.a.* colonies from eggs treated with head A and head B was significantly (P<0.0001) different (2.12±1.64, r=-0.22), wherein head A had a 66% higher antibacterial efficiency as was attained by the treatment with head B. Thus, in further assays only head A was still tested with regard to significant higher bactericidal efficiency, as was depicted by head B.

In Figure 5 and Table 5 the mean differences (log10 CFU/cm2) and the log reduction of TVC + TC S.a. after head A treatments in different experimental conditions were presented. Most significant 1.8 - 2.5 log reductions were depicted in experimental conditions when eggshells were head A-treated in triple successive treatments (Table 5).

Owing to head A treatments, the contact maximum temperatures (T_{max}) of the eggshells varied in the range 53-80°C (average 66°C) for not more than 1/50 or 1/100 sec, but never exceeded 80°C.

Microbiological and physico-chemical properties of the eggs during the 54 days after head A treatment

In the analysis of egg properties during the 54 days, the mean of TVC + TC *S.a.* on the eggshells after treatment (5 cm/s; 20 mm; 3x), was significantly (r=0.38, P<0.0001) 99.54% lower (0.72±0.64) than the number (1.85±1.05) on eggshells on plasma untreated (control) group of eggs. Furthermore, the mean number of TVC + TC *S.a.* in egg contents of plasma treated and untreated (control) group of eggs

was under the limit of confidentiality, meanwhile the results of TVC were negative or less than 40 CFU/ml, thus comparisons were not possible. The mean of differences (0.4 g) in whole weight between head A-treated eggs (58.66 g) and control eggs (59.08 g) was insignificant (-0.41±3.66, r=0.28, P=0.38). The mean of differences of air cell height values between the group of treated (4.27mm) and control group of eggs (4.35 mm) were insignificant as well (0.07±1.16, r=0.67, P=0.73), wherein almost no difference (-0.005±0.05, r=0.2, P=0.8) (mean = 9.31) was depicted in pH values between the egg contents of plasma-treated and untreated eggs. The height (mm) of a thick egg white was barely different (0.006±0.58, r=0.91, P=0.95) between treated (3.28 mm±0.26) and untreated eggs (3.27 mm±1.45).

Scanning electron microscopy (SEM) of the plasma (AOPJ) treated eggshell cuticle

In general, after the AOPJ head A treatment (5 cm/s; 20 mm; 3x), the eggshell surfaces examined by Scanning Electron Microscopy (SEM) looked slightly cleaner and more polished (Figure 6). By SEM estimating at x400 magnification, 25% more cracks on the treated eggshell cuticle were noticed (image B) than on the untreated (control) eggs (image A). At x1000 magnification it was depicted that the cracks sharpness and width (0.25–0.3 µm) of edges did not differ between the plasma-treated (image D) and control eggs (image C). Individual grains of Ca, Mg, P spherules (80–300 nm) were observed at the highest magnification
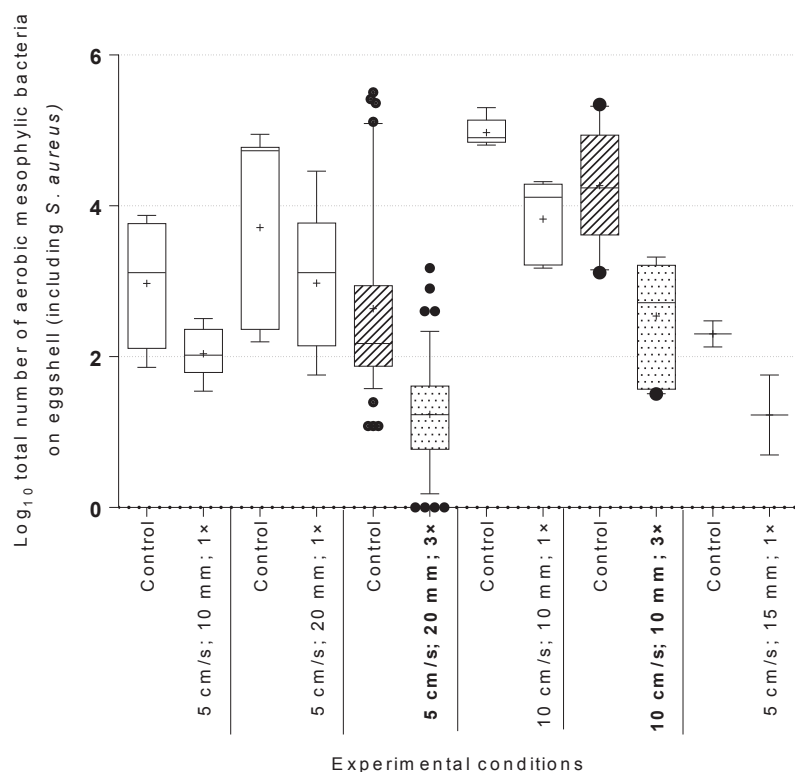

Figure 5: Boxplot of the number of TVC + TC *S.a.* colonies ($\log_{10}$ CFU/cm^2) on the eggshells after head A treatments in different experimental conditions with regard to the untreated (control) eggshells.

Comparisons are presented separately for the each experimental condition. Boxes with pattern present experimental conditions, where the most significant reductions of bacteria, were depicted. The median value of each distribution is shown with horizontal line within each box, while the + marks the mean. The boundary of the box closest to zero indicates the 25th percentile, and the boundary farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. The outliers are shown as dots.

Table 5: Mean differences in total number of the TVC + TC *S.a.* ($\log_{10}$ CFU/cm^2) after head A treatments of AOPJ-treated eggshells with regard to the untreated (control) eggshells in different experimental conditions and the log reduction of the TVC and TC *S.a.* on the eggshells

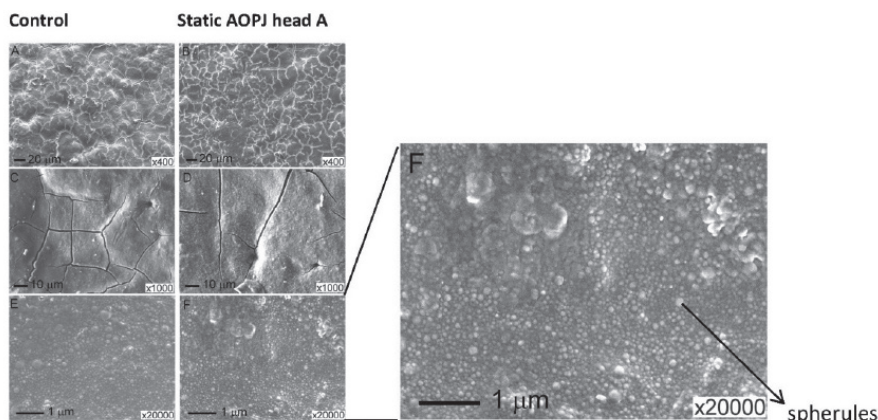| Eggshell surface jet head A to control	5 cm/s; 10 mm; 1×	5 cm/s; 20 mm; 1×	5 cm/s; 20 mm; 3×	10 cm/s; 10 mm; 1×	10 cm/s; 10 mm; 3×	5 cm/s; 15 mm; 1×
Exposition time (s)	20	20	60	10	30	20
Mean differences in TVC + TC *S.a.*	-0.93±0.63, r=0.68, P=0.002	-0.73±0.68, r=0.86, P=0.01	-1.40±1.05, r=0.56, P<0.0001	-1.14±0.50, r=0.43, P=0.00	-1.73±0.36, r=0.88, P<0.0001	-1.07±0.99, P=0.36
TVC + TC *S.a.* log reduction	1.3	1	2.5	1	1.8	1.5
TVC + TC *S.a.* reduction (%)	92.4	85.2	99.7	89.4	98.4	85.6

Figure 6. SEM images of AOPJ head A treated (5 cm/s; 20 mm; 3x) (images B, D, F) and plasma untreated eggshells (control) (images A, C, E) taken at magnifications x400, x1000, and x20,000

(x20,000) which are of approximately same density on the treated (image F), as was on the untreated (control) eggs (image E) (Figure 6).

Discussion

Assuring the most effective conditions enabling the highest antimicrobial effectiveness of an AOPJ, and to prevent side effects on the eggshell cuticles, the antimicrobial activity of static (head A) and rotary (head B) heads in different experimental conditions was compared. Already from our experiments on PET it has been ascertained that head A had a significantly higher antibacterial efficiency than head B, whose antibacterial efficiency did not exceed 1 log reduction. Actually, the reduction of bacteria (*S. aureus*) on the PET treated with head A was in the range of 1 – 3.15 log reduction, and was achieved in 20–60 seconds. The main reason for such difference is presumably on the concentration of the plasma jet streaming out of the head A nozzle, directly targeted to the treated surface, forming an 90°angle, meanwhile head B forms a wider 25° angle, which presumably reduces the energy of the plasma jet on the treated surface. Similar experiments were made by Noeske *et al.* (15) and Lommatzsch *et al.* (31), who tested the physical functionality of a plasma jet on polymers and polyethylene surfaces, and by Noriega *et al.* (19) who investigated the antimicrobial properties of cold atmospheric gas plasma-pen in desinfection of membrane filters establishing more than 3 log reductions of *L. innocua* in 10 seconds. According to the results of the experiment on PET, it was shown that the bactericidal effectiveness of an AOPJ strongly depended on influential experimental conditions. Besides the type of jet heads, the

distance between the surface substrate and the exposition time are crucial for the optimal antimicrobial efficiency (16). We also showed that prolonging the exposition time and lowering the distance resulted in higher bactericidal activity, but it was also demonstrated higher risks for the eggshell cuticle alterations induced by the plasma's temperature, and the energy of ionised gas (reactive species OH radicals and NO). Therefore, in our experiment, the speed of the jet head was lowered presently with an increasing distance (10, 15, 20 mm) and *vice versa*, so the contact temperatures (T_{max}) of treated areas were in the average of 66°C. Baier *et al.* (33) reported about similar experiences in the experiment of decontamination efficiency of an atmospheric pressure plasma jet on fresh meats at distances of 13 and 18 mm, where the T_{max} never exceeded 25°C. Also, Liu *et al.* (34) studied an atmospheric plasma jet for the sterilisation of *S. aureus* on glass, where the surface temperature did not exceed 35°C. We showed in our experiment that neither surface temperatures nor the ionising gas of the plasma jet were obviously harmful to eggshell cuticle. Those findings were similar to experiments of Hyun *et al.* (3) who investigated a plasma jet for the inactivation of *Listeria monocytogenes* on agar and processed meat, at a distance of 40 mm, and have not reported about harmful effects on treated surfaces. The energy of plasma on surfaces can be moderated with shorter, but multiple plasma treatments, as was reported by Laroussi (35), who discussed the potential use of cold plasma on medical applications. Also, in our experiment it was found that for the successful reduction of bacteria with an AOPJ, multiple treatments were obligatory, since, in any experimental condition with a single application, the reduction

of bacteria was not greater than 1 log. This means that the sufficient treatment time for the AOPJ antimicrobial operation is needded, although the surface of eggs should not be continuously exposed to a plasma operation due the high intensity of plasma jet. Thus, we showed an intermediate time for cooling of the eggshell surface is needed. Owing to that, in our experiment, to improve antimicrobial efficiency, an AOPJ was applied in short, intermittent multiple treatments, which has been repeated in at least 20 second intervals, to avoid side effects on the treated surfaces. We also showed that the enlarging of the distances of the AOPJ head to the treated surfaces, in combination with the high AOPJ head speeds, can predict immoderate raisings of the surface temperatures. The same statements were also considered by Rod *et al.* (21), who tested the antimicrobial effects of cold atmospheric plasma on deli meat in multiple 10 minutes intervals. In addition, plasma's high excitation frequencies are responsible for the higher plasma energy and stability (36). Thus, in order to protect the eggshell cuticle against excessive energy of the plasma jet, a relatively low frequency (21 kHz) of AOPJ was used in our experiment. In other similar experiments, plasma was used in a higher frequency range of 30 – 38 kHz (22, 20).

Therefore, the results of our experiment on the antibacterial properties of the AOPJ in surface decontamination of eggs in shell are represented in the <1−2.5 log reduction (29.1 – 99.7%) of TVC + TC *S.a.* with regard to untreated eggshells, and was achieved in 10−60 seconds, depending on the experimental conditions. Those results are similar to the studies of Ragni *et al.* (28), in which the eggshells were treated with non-thermal RBD (Resistive Barier Discharge) plasma (15 kV), and being found that the number of TVC was reduced in a range from 1 to 1.6 log reduction within an exposition time of 10−20 minutes, and even a 5.5 to 6.5 log reduction, although within 90 minutes of exposition. In addition, Liu *et al.* (34) reported about a 100% *S. aureus* reduction after an atmospheric non-thermal plasma jet treatment on a glass slide in 120 seconds, at the electro discharge of 18 kV. In both experiments a higher electric voltage and at least double exposition time for plasma jet treatments were used, as in our experiment. However, in our experiment the highest reduction of bacteria (up to 2.5 log) on eggshells was achieved with an AOPJ an electro discharge of 1 kV, within a treatment time of 60 seconds. Owing

to a SEM analyses of eggshell cuticles (37), we assumed that the plasma jet treatment did not leave significant changes on elemental composition of the eggshell (38). From SEM images it can be seen that the appearance of the surface of the AOPJ treated eggshells seemed more polished, which is logical considering the cleansing properties of an AOPJ. However, no significant microscopic damages to the eggshell cuticle were evidenced during the experiment, since no significant alterations were found, with the exception of a slightly higher number of cuticle cracks on plasma-treated *vs.* untreated eggshells, but this did not affect the aging or higher contamination of eggs contents after 54 days. Similar statements was also confirmed by Ragni *et al.* (28) and Vannini *et al.* (29), who did not find significant changes on cuticle after plasma treatment of eggshells. No significant side effects to chicken meat or skin exposed to cold atmospheric gas plasma was determined in the experiment of Noriega *et al.* as well (19). Supposing an AOPJ alters the functionality of the eggshell cuticle, as the first line of defence against soil and bacterial penetration, more microorganisms can penetrate the eggshell, and so could be found in egg contents (38, 39). However in the experiment, during 54 days, the total numbers of bacteria in the egg contents of both: AOPJ treated and untreated eggs, were negative or less than 40 CFU/ml, which is under the limit of confidentiality. This result is evidence of unchanged cuticles of AOPJ-treated eggs. Another indication that an AOPJ did not influence the cuticle egg protective properties was evident in the investigated physico-chemical properties of the AOPJ-treated eggs as air cell height, pH, whole weight of eggs, or height of the thick of egg white (40), which did not significantly differ from the untreated eggs during aging. Indeed, a slightly higher whole weight and lower air cell height of AOPJ treated eggs were obtained, meanwhile negligible differences among the other investigated physico-chemical egg properties were established. This is important due to the stability of the mechanical properties of the cuticle responsible for resisting water transmission, bacterial penetration, and CO_2 losses, which slow down the natural decline of egg internal quality, and indicates an unchanged functional operation of the cuticle after the AOPJ treatment (41). So, no important influence of the AOPJ on the functional operation of cuticle was established, considering the experimental conditions in which eggshells were treated.

Conclusion

The results of the experiment of antibacterial properties of AOPJ in surface decontamination of eggs in shell demonstrated anitimicrobial efficiency in short operating time with no significant side effects on eggs quality and that is the main advantages of an AOPJ in decontamination of table eggs in shell. The running system should be developed in further investigations; meanwhile the experiment contributes to the knowledge of new approaches on how to diminish contamination of table eggs, and thus on improving food safety.

Acknowledgements

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ANTIBAKTERIJSKE LASTNOSTI HLADNE ATMOSFERSKE PLAZME S CURKOM PRI POVRŠINSKI DEKONTAMINACIJI JAJC V LUPINI

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Povzetek: V državah Evropske unije konzumnih kokošjih jajc pred oddajo v prodajo ni dovoljeno prati ali jih mehanično čistiti, zato se za zmanjšanje tveganj glede varnosti živil razvija več tehnik za dekontaminacijo jajc v lupini. V poskusu smo testirali potencialno učinkovitost hladne atmosferske plazme za površinsko dekontaminacijo jajc v lupini. Jajčne lupine konzumnih jajc smo izpostavili enkratnemu ali večkratnemu vplivu atmosferskega plazemskega curka za 10 - 60 sekund. Zmanšanje prisotnosti *Staphylococcus aureus* (NCTC 8325) na ploščicah iz polietilen teraftalata je znašala > 3 log stopnje, medtem, ko je zmanjšanje prisotnosti števila aerobnih mezofilnih bakterij in *S. aureus* na površini s plazmo tretiranih jajčnih lupin znašala med 1,8 do 2,5 log stopnje. Povrhnjica jajčnih lupin s plazmo obdelanih jajc je ostala funkcionalno nepoškodovana, kljub fizikalnim in ionizirajočim lastnostim plina v plazmi. S plazmo obdelana jajca niso bila spremenjena glede senzoričnih in fizikalno-kemijskih lastnosti, tudi procesi staranja so bili enaki kot pri neobdelanih jajcih. Rezultati poskusa kažejo, da tretiranje jajčne lupine s curkom atmosferske plazme pozitivno vpliva na dekontaminacijo jajc v lupini in nima negativnih vplivov na kakovost in staranje jajc, kar je pomembno z vidika varnosti in kakovosti živil.

Ključne beside: jajca v lupini; atmosferska hladna plazma; aerobne mezofilne bakterije; *S. aureus*; dekontaminacija