Effect of treatment by non-thermal plasma jet on the growth of various food spoilage bacteria in superfluous

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Summary

The efficiency of gas phase plasma at atmospheric pressure by using an electrical discharge in gas argon on the inactivation of microorganisms was examined. The gas phase plasma was applied to suspensions of pure cultures *Escherichia coli* 3014, *Staphylococcus aureus* 3048, *Salmonella* sp. 3064, *Listeria monocytogenes* ATCC 23074 and *Bacillus cereus* 30. The experiments were planned and performed according to a statistical experimental design, specifically central composite design, which considered three independent variables: volume (2, 3 and 4 mL), gas flow (0.75, 1 and 1.25 L/min) and treatment time (3, 4 and 5 min). Two studied parameters, volume and treatment time, substantially affected the inactivation. For plasma treatment, the inactivation can be attributed to UV radiation and plasma reactive oxygen species (ROS). It was found that Gram-negative bacteria were more susceptible to the plasma treatment than Gram-positive bacteria, and that the susceptibility of Gram-positive bacteria was remarkably species-dependent. Complete inactivation of *Escherichia coli*, *Salmonella* sp., and *Listeria monocytogenes* was achieved when optimal combination of parameters was applied.

Keywords: gas-phase plasma; atmospheric pressure; food spoilage bacteria; response surface methodology

Introduction

Technology and innovation have always played a key role in improving food industry through their focus on final product quality, as well as on safety and environmental issues.

Thermal processing is still the most common method for ensuring the microbiological safety of processed food industry products. Thermal damages caused by overheating and considerable losses of heat on the equipment surfaces represent serious limits of thermal processing methods. Another important aspect to be considered is the quality attributes (flavor and odor, visual appearance, color and texture, nutrition value, absence of additives). Many food ingredients and products are well known to be thermally sensitive and therefore undesirable chemical and physical changes can occur. These limitations have generated the continuous interest in developing industrial alternative, innovative techniques in food processing and preservation, which could be used to replace the severe heat-based methods that are commonly used. Recent advances in search for such non-thermal processing methods have led

researchers to investigate the application of non-thermal gas phase plasma at atmospheric pressure (NTP). The advantages of atmospheric pressure plasma over classical heat treatment for sterilizing are numerous and they offer a promising new way to fight opportunistic and pathogenic microorganisms. Plasma, defined as an ionized quasi-neutral gas, is the fourth state of matter that can be generated by applying an electrical field to an initially electrically neutral gas (Gaunt et al., 2006; Wan et al., 2009).

Plasma is a source of different antimicrobial species including UV photons, charged particles, and reactive species such as superoxide, hydroxyl radicals, nitric oxide and ozone (Daeschlein et al., 2010; Deng et al., 2006; Laroussi and Leipold, 2004; Priya Arjunan and Morss Clyne, 2011; Wan et al., 2009). Several studies indicate that reactive oxygen species are the most important agents in the microbial inactivation by non-thermal plasmas (Hähnel et al., 2010; Lee et al., 2006). NTPs are capable of inactivating a range of microorganisms including gram-positive and gram-negative bacteria, bacterial endospores, fungi, and viruses on different surfaces (Fridman et al., 2007; Herceg et al., 2015; Kelly-Wintenberg et al.,

1999, 1998; Korachi et al., 2009; Rød et al., 2012; Yasuda et al., 2010).

Mathematical modeling is a tool for the analysis of interaction between the investigated parameters that cannot be considered using simple statistical analysis. Response surface methodology (RSM) may be employed to optimize critical processing parameters by estimating interactive and quadratic effects. A further benefit of using RSM is a reduction in the number of experiments needed as compared to a full experimental design (Myers et al., 2009). RSM has been successfully employed to optimize food processing operations (Herceg and Jambrak, 2012; Herceg et al., 2012). In order to optimize processes by combination of technologies more research has to be done, particularly for optimizing practical applications.

The purpose of this study was to evaluate the effect of gas-phase plasmas treatment on two species of Gram-negative (*Escherichia coli*, *Salmonella* sp.) and three Gram-positive bacteria (*Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus*). The effect of various parameters (volume of aqueous suspensions of pure culture microorganisms, gas flow and treatment time) on the inactivation of the bacteria was studied and according to the statistical design.

Materials and methods

Bacterial strains

Bacterial strains used in this study: *Escherichia coli* 3014, *Salmonella* sp. 3064, *Staphylococcus aureus* 3048, *Listeria monocytogenes* ATCC 23074 and *Bacillus cereus* 30 were obtained from the Collection of Microorganisms of the Laboratory of General Microbiology and Food Microbiology, Faculty of Food Technology and Biotechnology, University of Zagreb (Zagreb, Croatia). The cultures were stored on nutrient agar (Biolife, Milan, Italy) at 4 °C.

Preparation of inoculum

To prepare the inoculum, the investigated bacteria were incubated on nutrient agar (Biolife) for 24/48 hours at 37 °C, and then a loopful of young cells were suspended in sterile water solution. The total viable cell count (TVC) was performed by standard dilution method on nutrient agar after incubation at 37 °C for 48 h. The bacterial colonies were counted and reported as log colony forming units per mL (log CFU/mL). The initial cell concentrations were obtained: 8.19 log CFU/mL for *E. coli* 3014, 8.49 log CFU/mL for *Salmonella* sp., 8.37 log CFU/mL for *S. aureus*, 8.06 log CFU/mL for *L. monocytogenes* and 7.47 log CFU/mL for *B. cereus* (Table 2 - Sample A0). All samples were analyzed in triplicate and given score is the mean value of three determinations.

The plasma source

In its most basic form, the non-thermal plasma jet is produced using a gas-fed dielectric tube which houses an internally powered electrode. The application of a high-voltage gives rise to the formation of an ionization front which emanates from the electrode and travels along the jet of existing gas, ionizing and exciting the working gas along the way. The plasma source used was a single-electrode atmospheric jet (End-field Jet type), designed at the Institute of Physics (Zagreb, Croatia) (Fig. 1). It consists of Teflon body to which a glass capillary tube of 7.5 cm length and 0.15/0.1 cm outer/inner diameter is attached. Cu wire of 1×10^{-4} m in diameter is placed inside the capillary tube. Tube is connected to the High Voltage source through the vacuum tight connector. High voltage source of nominal 6 W provides 2.5 kV at 25 kHz. The actual current through the electrode was measured to be typically 3 mA. The actual power of plasma was about 4 W as determined from the voltage and current waveforms (Kregar et al., 2011). The capillary tube is also connected to the argon gas input, the flow of which was regulated by means of a flow meter (rotameter). Argon was used as operating gas for the generation of atmospheric-pressure non-thermal plasmas. Plasma source was mounted on the z-axis translator to maintain a fixed distance of 1.5 cm between the plasma electrode and the surface of the liquid (Fig. 1).

Optical emission spectroscopy (OES) of Ar plasma jet in the region from 200-1000 nm was performed by means of a miniature fiber spectrometer (Avantes 3600, Leatherhead, Surrey, UK) of 0.8 nm spectral resolution. The light was collected from the region near the capillary tube exit by means of a quartz lens and a solar resistant optical fiber. Spectrometer was calibrated for the spectral response by means of a deuterium-halogen calibration lamp.
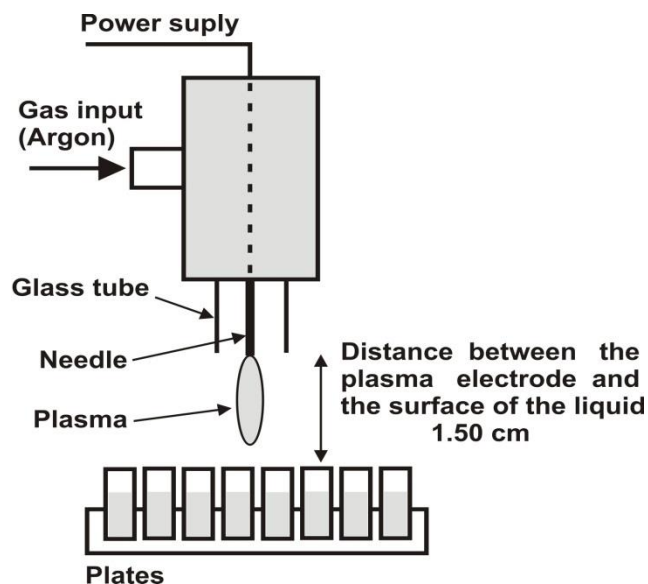
Fig. 1. Schematic representation of the plasma source

Gas-phase plasma treatments

The non-thermal plasma jet was generated in argon (purity 99.99%; Messer, Sulzbach, Germany) by applying a strong electric field through the electrode. For the sample treatments, plasma was running at a constant power of 4 W, varying the gas flow, treatment time and volume of treated sample according the experimental design (Table 1).

Samples of bacterial suspension were placed in a sterile glass positions (TPP Techno Plastic Products AG, Trasadingen, Switzerland), which was used as the treatment vessel (Fig. 1). The plasma source was fixed at distance of 1.5 cm between the plasma electrode and the surface of the liquid and placed in the 'center' of the sample. Plasma treatment was carried out at gas flow (0.75, 1 and 1.25 L/min). Samples were treated with plasma for 3, 4 and 5 min at 20 ºC. For this study, 16 samples of basal medium with a determined initial number of bacterial cells were gas plasma treated.

Table 1. Treatment time (X_1), volume of microbial suspensions (X_2), gas flow (X_3) during plasma treatments

Sample	X_1 (min)	X_2 (mL)	X_3 (L /min)
A0	-	-	-
A1	3	2	0.75
A2	3	2	1.25
A3	3	3	1.00
A4	3	4	0.75
A5	3	4	1.25
A6	4	2	1.00
A7	4	3	0.75
A8	4	3	1.00
A9	4	3	1.00
A10	4	3	1.25
A11	4	4	1.00
A12	5	2	0.75
A13	5	2	1.25
A14	5	3	1.00
A15	5	4	0.75
A16	5	4	1.25

Experimental methodology

In order to determine the influence of the operational parameters on the count of food spoilage bacteria, central composite design (CCD; STATGRAPHICS Centurion, Stat Point Technologies, Inc., Richmond, VA, USA) and face-centered model were used (Jones, 2002). Because CCD requires the choice of operational parameters, the authors have chosen to study the effects of treatment time, volume of aqueous suspensions of pure culture microorganisms and gas flow. Analysis of variance (ANOVA) was carried out to determine any significant differences ($p<0.05$) among the applied treatments. The operating variables were considered at three levels, namely low (-1), central (0) and high (1). Accordingly, 16 experiments were conducted organized in a factorial design (including factorial points, axial points and center point), and the remaining experiment involving the replication of the central point to get a good estimate of experimental error. Response (output) values were total count of food spoilage bacteria expressed as log CFU/mL.

The designs were based on a two-level full factorial design and augmented with center and star points (Kuehl, 2000). The total number of experiments of the designs (N) was calculated as follows:

$$N = N_i + N_o + N_j \qquad (1)$$

where $N_i = 2^n$ is the number of experiments of the two-level full factorial design, N_o is the number of center points, and $N_j = 2n$ is the number of star points.

Response surface methodology

The experimental results were analyzed by response surface methodology (RSM) using the STATGRAPHICS Centurion software. Specifically, the RSM was used to study the effect of three different parameters; X_1 - treatment time (min), X_2 - volume of aqueous suspensions of pure culture microorganisms (mL) and X_3 - gas (Ar) flow (L/min). In order to optimize the NTPs treatment and to investigate the effects of the three independent variables on the count of food spoilage bacteria, a central composite rotatable design with the variables at three levels was used in the experiments (Table 1). Design matrix for the experiment as well as the regression model proposed for the response is given by the following *Eq.* (2) (Kuehl, 2000):

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i\langle j}^{4} \beta_{ij} X_i X_j \qquad (2)$$

where β_0 is the value of the fixed response at the central point of the experiment (point 0, 0, 0), β_i, β_{ii}

and β_{ij} are the linear, quadratic and cross-product coefficients, respectively (Myers et al., 2009). The model was fitted by multiple linear regressions (MLR). The validity of the quadratic empirical model was tested with the analysis of variance (ANOVA) with the confidence level of 95%. Durbin-Watson statistical analysis was also conducted, which enabled autocorrelation and prediction errors after the statistical regression analysis was completed.

Results and discussion

The count of *Salmonella* sp., *E. coli*, *S. aureus, L. monocytogenes* and *B. cereus* after gas plasma treatments was analyzed by RSM using the Statgraphics Centurion software. In these various treatments, the combination effect of treatment time (X_1), volume (X_2) and gas flow (X_3) was studied.

A general factorial design consisting of 16 experimental trials has been chosen to obtain overall observation of gas plasma treatment on the bacterial species (Table 1). Initial counts of treated bacterial strain (A0) and counts after the plasma treatments (A1-A16) are presented in Table 2.

The complete inactivation of both Gram-negative bacterial species was achieved with 4 various factor combinations: A2, A6, A12 and A13 for *E. coli*, and A6, A12, A13 and A14 for *Salmonella* sp. Among the Gram-positive species only *L. monocytogenes* turned out to be completely inactivated under some of the treatment condition combinations (A6, A12 and A13). The treatment combination A13, the combination of the maximal treatment time, minimal volume and maximal gas flow caused the best reduction effect with the other Gram-positive species too: the count of *S. aureus* was reduced from 8.37 to 1.60 log CFU/mL and *B. cereus* was reduced from 7.47 to 4.53 log CFU/mL. An overall insight into the Table 2 shows as expected that the level of inactivation was enhanced with the longer treatment, higher gas flow and with smaller volume.

Experimental work on the bacteria inactivation caused by non-thermal atmospheric plasma has shown that survivor curves (CFU versus treatment time) take different shapes from single to multi – slope survivor curves which indicates different physicochemical processes that occurred during inactivation (Critzer et al., 2007).

There are several mechanisms or a synergistic combination of these that cause microbe inactivation by non-thermal plasma. Mutation of DNA can be caused by UV irradiation, erosion of microorganism by photon-induced desorption, and chemical interaction of reactive species from plasma causes reaction with cell membranes and other cell components (Moisan et al.,

2002). The reactive species differ depending on the types of gas or gas-mixture used for plasma formation. If oxygen or air is used, the mechanism involves O, OH, HO_2 and other oxygen-based radicals, which may have good sterilization properties, but may have a negative impact on the material that should be sterilized. Use of inert gases such as argon offers better possibility in preserving functional requirements of material in the food industry. However, the mechanism of action different than those observed with oxygen is still not entirely clear. It seems that, due to the weak possibility of ionization of inert gasses, ions, electrons, radicals or even UV do not contribute significantly to the sterilization effect, and that the UV irradiation, below

200 nm should be considered as a possible factor (Deng et al., 2006; Lerouge et al., 2000). UV is known to inactivate cells only if its wavelength is within the range of 220 nm to 280 nm, because DNA has an absorption spectrum in this region, and the UV dose (expressed in watts s/cm) is high enough (Laroussi, 2002). However, if during plasma discharge occurs that generated UV light does not satisfy these conditions, it would be predictable to not have a noticeable effect. Also, it is already known several repair mechanisms in bacteria which are generally very efficient and rapid, meaning that cell death only follows after numerous UV photon hits, which crushes bacterial repair mechanisms (Gaunt et al., 2006).

Table 2. Influence of gas phase plasma treatment on the count of Gram-negative (*Salmonella* sp. and *E. coli*) and Gram-positive bacteria (*S. aureus, L. monocytogenes* and *B. cereus*)

Sample	*E.coli* log CFU/mL	*Salmonella* sp. log CFU/mL	*S. aureus* log CFU/mL	*L. monocytogenes* log CFU/mL	*B. cereus* log CFU/mL
A0	8.19±0.02	8.49±0.07	8.37±0.04	8.06±0.03	7.47±0.04
A1	4.01±0.05	3.12±0.03	4.53±0.03	3.47±0.02	5.11±0.02
A2	N/A*	3.03±0.05	4.59±0.06	2.98±0.04	5.05±0.03
A3	5.63±0.03	7.57±0.06	6.29±0.03	7.59±0.05	6.64±0.05
A4	7.63±0.05	7.80±0.02	6.92±0.06	7.65±0.06	6.87±0.04
A5	7.03±0.07	7.18±0.07	6.24±0.02	7.48±0.02	6.00±0.06
A6	N/A*	N/A*	3.92±0.06	N/A*	4.98±0.03
A7	5.80±0.04	6.84±0.05	6.21±0.02	7.05±0.04	5.72±0.05
A8	4.44±0.02	6.44±0.04	6.08±0.04	6.75±0.06	5.33±0.04
A9	4.46±0.02	6.31±0.02	5.99±0.05	6.87±0.06	5.37±0.06
A10	4.04±	5.49±0.03	4.26±0.07	5.36±0.06	5.20±0.02
A11	7.36±0.03	7.16±0.07	6.66±0.03	7.08±0.02	5.73±0.05
A12	N/A*	N/A*	2.10±0.05	N/A*	4.63±0.03
A13	N/A*	N/A*	1.60±0.06	N/A*	4.53±0.06
A14	3,04±0.02	N/A*	5.91±0.02	4.35±0.05	4.95±0.02
A15	5.12±0.03	4.38±0.04	6.09±0.03	5.34±0.06	5.22±0.06
A16	4.34±0.07	4.11±0.02	4.57±0.05	5.04±0.02	5.11±0.07

*N/A - viability of cells not confirmed after plasma treatment
All values are expressed as mean of three repetitions ± standard deviation

On the other hand interaction of argon with humid air in the vicinity of the plasma source, which is the case in our study, could provide a wider range of reactive species (Van Gaens and Bogaerts, 2013; Zhang et al., 2012) that include oxygen and nitrogen radicals. In this paper, optical emission spectroscopy (OES) measurements were also performed. The existence of excited NO, OH, O radicals within the plasma jet, as well as excited N_2 and Ar were observed as shown in Fig. 2. The most prominent spectral features are neutral Ar atomic lines in the region from 700-900 nm, OH molecular emission at 309 nm, molecular N_2 band from 315-400 nm, and atomic Oxygen lines at 777 nm and 844 nm. The signature of molecular NO bands

from 200-300 nm are present as well. When there are more electrons per time unit (current) flowing through the electrode and the plasma, there are more inelastic collisions of electrons with the plasma particles. In these collisions, electrons ionize, dissociate and excite molecules and atoms. Generally, we could say that more electrons per unit time lead to more excited particles and increase the intensity of the emission lines. Also the emission of molecular NO bands increases with increasing humidity around the plasma source (Zaplotnik et al., 2015, 2014).
Gweon et al. (2009) investigated the deactivation of *E. coli* by an atmospheric pressure radio frequency glow plasma with parameters varied in

order to understand the main contributors to deactivation. Contribution of the UV irradiation from the plasma was determined to be negligible. On the other hand, it was found that the sterilization was 40% more effective with only 0.15% oxygen added to the argon. It indicates that the inactivation process was dominantly controlled by oxygen radicals, rather than heat or UV photons.

The reduction in number of bacteria cells after NTP treatments are displayed by response surface methodology (RSM) using the STATGRAPHICS Centurion software. Statistical calculations were done at 95% confidence level using ANOVA. According to the RSM model, the inactivation of food spoilage bacteria can be described by predicted mathematical model for the count of *E. coli*, *S. aureus, Salmonella* sp., *L. monocytogenes* and *B. cereus* (Table 3).
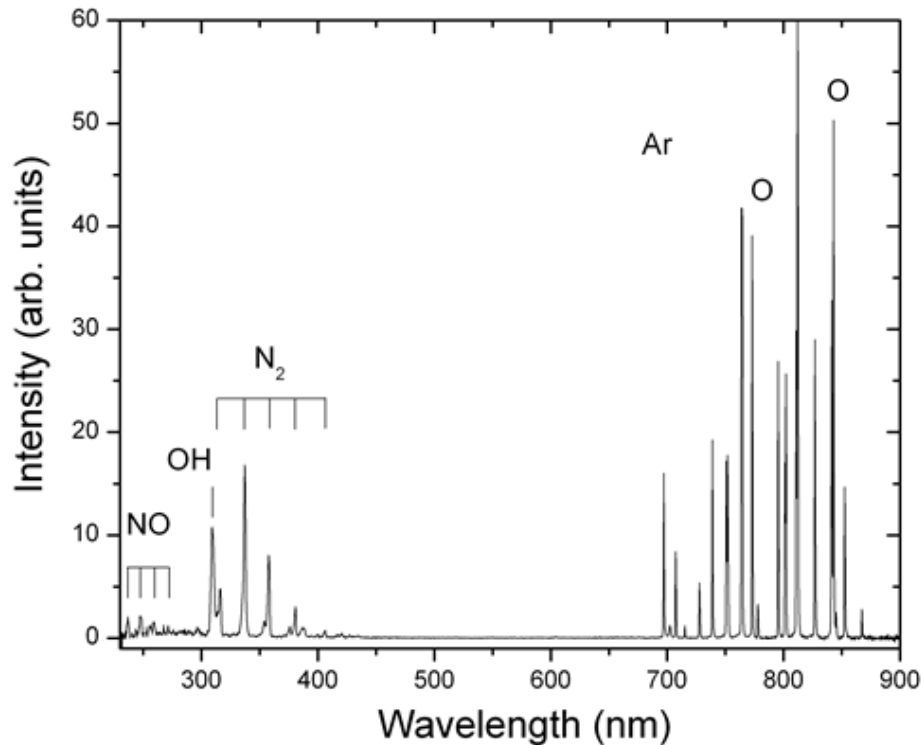
Fig. 2. Optical emission spectrum of atmospheric argon jet during interaction
with aqueous suspensions of pure culture of microorganisms

Table 3. The predicted mathematical model for the count of *E. coli*, *S. aureus, Salmonella* sp., *L. monocytogenes* and *B. cereus*
after gas phase plasma treatments

Microorganism	Polynom*
E. coli	$3.70124 - 0.0115086 \cdot X_1 + 7.93493 \cdot X_2 - 22.636 \cdot X_3 - 0.329655 \cdot X_1^2 - 0.14875 \cdot X_1 \cdot X_2 + 1.915 \cdot X_1 \cdot X_3 - 0.984655 \cdot X_2^2 + 1.315 \cdot X_2 \cdot X_3 + 4.08552 \cdot X_3^2$
Salmonella sp.	$24.06 + 4.4575 \cdot X_1 + 12.8475 \cdot X_2 + 6.16 \cdot X3 - 1.0825 \cdot X_1^2 + 0.655 \cdot X_1 \cdot X_2 - 0.04 \cdot X_1 \cdot X_3 - 2.0775 \cdot X_2^2 - 0.14 \cdot X_2 \cdot X_3 - 3.36 \cdot X_3^2$
S. aureus	$12.0388 - 1.82776 \cdot X_1 + 5.20193 \cdot X_2 + 28.873 \cdot X_3 + 0.0753448 \cdot X_1^2 + 0.365 \cdot X_1 \cdot X_2 - 0.7 \cdot X_1 \cdot X_3 - 0.734655 \cdot X_2^2 - 0.88 \cdot X_2 \cdot X_3 - 12.6345 \cdot X_3^2$
L. monocytogenes	$20.1604 + 2.2677 \cdot X_1 + 11.8665 \cdot X_2 + 9.31245 \cdot X_3 - 0.577941 \cdot X_1^2 + 0.4325 \cdot X_1 \cdot X_2 - 0.561667 \cdot X_1 \cdot X_3 - 1.96125 \cdot X_2^2 + 0.751667 \cdot X_2 \cdot X_3 - 5.48706 \cdot X_3^2$
B. cereus	$3.61166 - 1.89584 \cdot X_1 + 3.09024 \cdot X_2 + 3.48462 \cdot X_3 + 0.198793 \cdot X_1^2 - 0.1925 \cdot X_1 \cdot X_2 + 0.36 \cdot X_1 \cdot X_3 - 0.241207 \cdot X_2^2 - 0.41 \cdot X_2 \cdot X_3 - 2.17931 \cdot X_3^2$

*X_1 is treatment time (min), X_2 is the volume of bacterial suspension of pure culture microorganisms (mL) and X_3 is gas flow (L/min)

The estimated effects of operating variables and an analysis of variance for the models are reported in Table 4. Investigated factors were combined in linear, cross-products and quadratic coefficients. According to the results, the fitted model was significant at the considered confidence level as the F-value was more than three times higher than that of the listed F-value. The p-values that are lower than 0.05 ($p< 0.05$) for X_1 (treatment time) and X_2 (volume) indicate that these factors are significant for the count of all tested food spoilage bacterial species. The finding that the gas-flow rate is not a significant factor is in agreement with findings in other researches (Ermolaeva et al., 2011; Feng et al., 2009).

The Durbin-Watson (DW) statistics is presented in Table 4. Generally, the values can range from 0 - 4. Positive serial correlation is associated with DW values below 2 and negative serial correlation with DW values above 2. Serial correlation, sometimes also called autocorrelation, defines how any value or variable relates to itself over a time interval. Durbin-Watson statistics (Table 4) showed that the Durbin-Watson number in all tested bacteria was between 1.56 and 1.77 which showed that there was a positive correlation, as well as high probability of the accuracy of mathematical models for the inactivation of food spoilage bacteria after NTP treatment (Table 3).

Surface plots showing the predicted counts of bacterial species as a function of factors that were confirmed as significant (treatment time and volume) under the gas flow of 1.0 L/min are given in Fig. 3 (Fig. 3a -3e).

All the plots showed that maximal treatment time and minimal volume led to the best reduction effect. The best effect is expected for *Salmonella* sp., where complete inactivation could be achieved in less than 5 min of treatment and/or in a volume higher than 2 mL. The complete inactivation is also expected for *E. coli* and *L. monocytogenes* which, altogether with the similar surface plots of these two bacteria species, indicates that gram-positive and gram-negative features do not determine the susceptibility to the treatment. On the other hand, it could be seen from the plots that gram-positive species *S. aureus* and *B. cereus* are much more susceptible to the treatment that the other ones. There are numerous researches on this topic, but because of lot of variation in plasma type, plasma devices and micro-environments in these researches, the results and conclusions are discrepant and incoherent. In addition, most of the researches are based on variation of conditions using single bacteria species, and not on comparison of behavior of various species under the same conditions.

Ermolaeva et al. (2011) have found that the sensitivity of *S. aureus* and *E. coli* to the treatment with non-thermal argon plasma was almost the same. Their conclusion that Gram-negative bacteria are generally more sensitive than the gram-positive ones, supported by findings of (Lee et al., 2006), is in line with our findings here, as well as with the indication that the susceptibility of the Gram-positive bacteria is species-dependent (Fig. 3). *L. monocytogenes* showed much lower ability than the other two Gram-positive species to prevail under the stressful conditions caused by the plasma treatment used in our study.

Table 4. Analysis of variance (ANOVA) for plasma treatments and viability of *E. coli; Salmonella* sp.; *S. aureus; B. cereus* and *L. monocytogenes*

Source	*E. coli*		*Salmonella* sp.		*S. aureus*		*B.* cereus		*L. monocytogenes*	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
X_1	20.26	0.0041	14.62	0.0450	14.79	0.0085	30.78	0.0014	18.58	0.0230
X_2	67.07	0.0002	27.42	0.0240	59.09	0.0003	25.23	0.0024	33.17	0.0104
X_3	2.59	0.1585	0.02	0.8987	0.80	0.4046	0.02	0.8840	0.03	0.8664
$X_1{:}X_1$	0.38	0.5612	2.24	0.2731	0.05	0.8260	1.30	0.2969	3.06	0.1786
$X_1{:}X_2$	0.23	0.6460	0.37	0.6052	3.75	0.1008	3.71	0.1023	1.08	0.3760
$X_1{:}X_3$	2.42	0.1708	0.00	0.9694	0.86	0.3888	0.81	0.4024	0.37	0.5855
$X_2{:}X_2$	3.37	0.1159	8.25	0.1028	5.01	0.0665	1.92	0.2151	13.96	0.0334
$X_2{:}X_3$	1.14	0.3265	0.02	0.8934	1.36	0.2872	1.05	0.3445	0.66	0.4747
$X_3{:}X_3$	0.23	0.6507	0.19	0.7056	5.79	0.0529	0.61	0.4636	1.08	0.3756

Durbin-Watson statistics (*E. coli*) = 1.87653
Durbin-Watson statistics (*Salmonella* sp.) = 1.66364
Durbin-Watson statistics (*S. aureus*) = 1.80432
Durbin-Watson statistics (*B. cereus*) = 1.5665
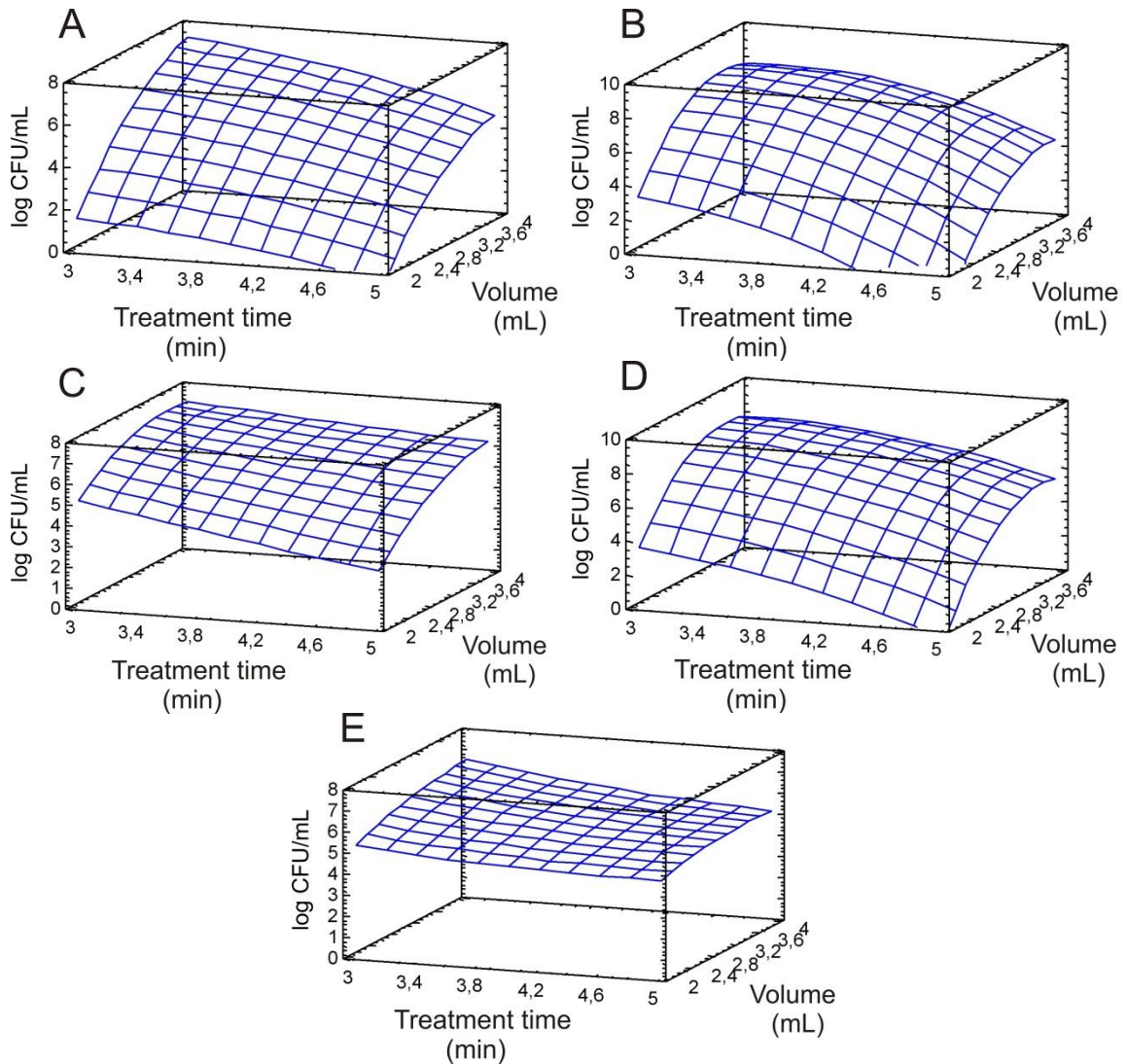Durbin-Watson statistics (*L. monocytogenes*) = 1.62655

Fig. 3. Surface plots for a) *E. coli,* b) *Salmonella* sp., c) *S. aureus*, d) *L. monocytogenes*, and e) *B. cereus* (log CFU/mL) count at the gas flow of 1.0 L/min

Conclusions

The results described in this work suggest that non-thermal plasma at atmospheric pressure, using an electrical discharge in a gas - argon, is a potent way of bacteria inactivation. It could provide a cheap, clean, safe and environmental friendly method for efficient removal of usual food contaminants. The parameters that seem to substantially affect the inactivation of food spoilage bacteria are the exposure/contact time with the microorganisms and the treated volume of aqueous suspension of microorganisms. It was found that Gram-negative bacteria (*E. coli* and *Salmonella* sp.) are more susceptible to the NTP treatment than the Gram-positive ones (*S. aureus*, *L. monocytogenes* and *B. cereus*).

Also, according to calculations of response surface methodology (95% of confidence level), the inactivation of food spoilage bacteria can be predicted by using a mathematical model for the bacterial count. Further study and close collaboration between food technologists and physicists is crucial to clarify antimicrobial mechanisms and to verify that no harmful by-products are generated by this future technology. A clear understanding of inactivation mechanisms, will increase application of plasma sterilization and thus accelerate the widespread adoption of this new method.

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