International Journal of Innovation and Scientific Research
ISSN 2351-8014 Vol. 5 No. 2 Jul. 2014, pp. 194-203

http://www.ijisr.issr-journals.org/

Modeling of Total Psychrophilic Bacterial Population on the Sliced Chicken as a Function of Period of Storage and Time of Cold Atmospheric Plasma Given

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ABSTRACT: This paper deals with the developing the most suggested model with R^2 = 0.8734 for Total Psychrophilic bacterial count as a function of the cold plasma treatment time and the storage period for a sliced chicken meat.

KEYWORDS: Cold Atmospheric Plasma, Molecular oxygen, Modeling, Ionized gas, Design Expert Software 8.0.

1 INTRODUCTION

Poultry carcasses are commonly contaminated with en-teric pathogens such as Salmonella, Campylobacter and *Listeria monocytogenes* (Jacobsreitsma et al., 1994; Murphy et al., 2004); the possibility of cross contamination of poultry carcasses post slaughter is high. Decontamination of poultry carcasses is therefore desirable. Various decontamination technologies have been proposed including the use of various chemical agents such as alkali (Rodriguez et al., 1996), physical methods such as steam treatment (James et al., 2007) and biological control with bacteriophages (Carvalho et al., 2010), but only treat-ment with water supplemented with chlorine or a chlorine-ting agent is used commercially. The effects of such decontaminating treatments are limited (Oyarzabal, 2005; Russell and Axtell, 2005).

1.1 PLASMA SCIENCE

1.1.1 PLASMA- DEFINITION, PHYSICS AND CHEMISTRY

In 1922, the American scientist Irving Langmuir proposed that the electrons, ions and neutrals in an ionized gas could be considered as corpuscular material entrained in some kind of fluid medium and termed this entraining medium *"plasma",* similar to the plasma, introduced by the Czech physiologist Jan Evangelista Purkinje to denote the clear fluid which remains after removal of all the corpuscular material in blood. However, it emerged that there was no "fluid medium" entraining the electrons, ions, and neutrals in an ionized gas (Bellan 2006), nevertheless the name prevailed. The term "plasma" refers to a partially or wholly ionized gas composed essentially of photons, ions and free electrons as well as atoms in their fundamental or excited states possessing a net neutral charge. The plasma possesses a net neutral charge because the number of positive charge carriers is equal to the number of negative ones (Kudra and Mujumdar 2009). Electrons and photons are usually designated as "light" species in contrast to the rest of the constituents designated as "heavy" species. Due to its unique properties plasma is often referred to as the fourth state of matter according to a scheme expressing an increase in the energy level from solid to liquid to gas and ultimately to plasma.

1.1.2 TYPES OF PLASMA

Two classes of plasma, namely thermal and NTP can be distinguished on the basis of conditions in which they are generated. This classification of plasma is based on the relative energetic levels of electrons and heavy species of the plasma. NTP (near ambient temperatures of 30-60oC) is obtained at atmospheric or reduced pressures (vacuum) and requires less

power. NTPs are characterised by an electron temperature much above that of the gas (macroscopic temperature) and consequently do not present a local thermodynamic equilibrium. NTP can be generated by an electric discharge in a gas at lower pressure or using microwaves. Typical illustrations for plasma generation at atmospheric pressure include the corona discharge, Dielectric barrier discharges (DBD), Radio-frequency plasmas (RFP) and the gliding arc discharge. In contrast, thermal plasmas are generated at higher pressures, require high power, and an almost thermal equilibrium exists between the electrons and the heavy species. Plasma generation at atmospheric pressure is of interest, both technically and industrially for the food industries because this does not require extreme conditions.

1.2 ACTION OF PLASMA ON MICROORGANISMS

1.2.1 ACTION ON CELL COMPONENTS AND FUNCTIONS

The use of sterilizing properties of plasma was first introduced towards the end of 60s, patented in 1968 (Menashi 1968) and first works with plasma made from oxygen were proposed in 1989. Thereafter, considerable research has been performed on the mechanism of microbial inactivation by plasma agents. The plasma agents contribute to the lethal action by interacting with the biological material. Nelson and Berger (1989) have shown that O2 plasma could be a very efficient biocidal against bacteria. Plasma treatment can effectively inactivate a wide range of micro-organisms including spores (Kelly-Wintenberg et al. 1999; Feichtinger et al. 2003; Lee et al. 2006) and viruses (Terrier et al. 2009). Effect of plasma can be quite selective, meaning tuneable between damage to pathogenic organisms without damage to the host, or activation of different pathways in different organisms (Dobrynin et al. 2009). Low-pressure oxygen plasma has been shown to degrade lipids, proteins and DNA of cells (Mogul et al. 2003). The reactive species in plasma have been widely associated to the direct oxidative effects on the outer surface of microbial cells. As an example, commonly used oxygen and nitrogen gas plasma are excellent sources of reactive oxygen-based and nitrogen-based species, such as O•, O^2, O^3, OH•, NO•, NO_2 etc.

Atomic oxygen is potentially a very effective sterilizing agent, with a chemical rate constant for oxidation at room temperature of about 106 times that of molecular oxygen (Critzer et al. 2007). These act on the unsaturated fatty acids of the lipid bilayer of the cell membrane, thereby impeding the transport of bio-molecules across it. The double bonds of unsaturated lipids are particularly vulnerable to ozone attack (Guzel-Seydim et al. 2004). Membrane lipids are assumed to be more significantly affected by the reactive oxygen species (ROS) due to their location along the surface of bacterial cell, which allows them to be bombarded by these strong oxidizing agents (Montie et al. 2002). The proteins of the cells and the spores are equally vulnerable to the action of these species, causing denaturation and cell leakage. Oxidation of amino acids and nucleic acids may also cause changes that result in microbial death or injury (Critzer et al. 2007). Micro-organisms in plasma are exposed to an intense bombardment by the radicals most likely provoking surface lesions that the living cell cannot repair sufficiently faster. This may partially explain the observations wherein cells are in many cases destroyed very quickly. This process is termed “etching” (Pelletier 1992). The cell wall rupture has been additionally attributed by Laroussi et al., (2003) and Mendis et al., (2002) to electrostatic forces due to accumulation of charges at the outer surface of cell membranes. The morphological changes in *E. coli* cells treated with atmospheric plasma at 75W for 2 min as observed under an electron microscope by (Hong et al. 2009), clearly revealed that the treated cells had severe cytoplasmic deformations and leakage of bacterial chromosome. These observations demonstrate the loss of viability of bacterial cells after plasma treatment. An analogy between plasma and pulsed electric field has also been drawn to explain the action of plasma on the membranes (Pothakamury et al. 1995; Spilimbergo et al. 2003). It is well established that electroporation of membranes is induced by pulsed electric fields and it appears that plasma acts on similar lines inducing perforations in the membranes of micro-organisms (Sale and Hamilton 1967; Pothakamury et al. 1995; Wouters and Smelt 1997). In addition to generating pores, humid air plasma additionally provokes a marked acidification of the medium (Moreau et al. 2005; Moreau et al. 2007).

1.2.2 ROLE OF UV PHOTONS AND CHARGED PARTICLES

The production of UV photons of different wavelengths has been proposed to be involved in dimerizing the thymine bases of DNA including that of spores (Munakata et al. 1991). The role of UV photons in bacterial death when they are submitted to a plasma treatment was reviewed in detail by (Boudam et al. 2006). Recently, by exclusion of reactive particles and spectral fractions of UV radiation from access to the spores Roth et al., (2010) revealed that UV-C radiation is the most effective inactivation agent in the plasma. Ultraviolet (UV) photons play a less important role in atmospheric pressure glow discharge (APGD) because they are easily absorbed by gas atoms and molecules at atmospheric pressure (Vleugels et al. 2005). The role of the charged particles in the bacterial inactivation process was recently investigated by Lu *et al.* (2009). Their work revealed that the charged particles play a minor role in the inactivation process when He/N2 (3%) is used as working gas than when He/O2 (3%) is used. Also, they concluded that heat and UV play no or minor roles in the inactivation

process. Similar results were earlier obtained by (Perni et al. 2007) who interplayed bacterial inactivation kinetics with optical emission spectroscopy, and identified oxygen atoms as major contributor in plasma inactivation with minor contributions from UV photons, OH radicals, singlet oxygen metastables and nitric oxide. Thus, a contradiction over the role of UV photons in plasma exists and future studies must be directed to get a clear picture.

1.2.3 EFFECT OF PROCESS PARAMETERS

The concentrations in which the plasma agents occur in plasma depend greatly on the device set-up (reactor geometry), operating conditions (gas pressure, type, flow, frequency and power of plasma excitation) and gas composition which affect their efficacy in a process when employed. To cite an example, the destructive efficiency of various gas plasma sources and temperatures on *Bacillus spp.* spores were compared by (Hury et al. 1998). This group demonstrated that oxygen-based plasma is more efficient than pure argon plasma. Another deciding criterion is whether the substrate to be sterilized is in direct contact with the plasma (*Direct Exposure*) or located remote from it (*Remote Exposure*) (Moisan et al. 2001; Laroussi 2005; Boudam et al. 2006). If exposed remotely, the quantum of heat transmitted to a sample is reduced, the charged particles do not play a role since they recombine before reaching the sample, and many of the short-lived neutral reactive species also do not reach the sample. Since, the components of the plasma are reactive and self-quenching, with a relatively short half-life, decreased time of flight would be expected to be one of the major factors in antimicrobial efficacy in this case (Niemira and Sites 2008). By varying the process parameters involved in plasma generation, a multitude of mechanisms can be actuated which may act individually or synergistically.

Nevertheless, the details of interaction of the different plasma agents with the different components of bacterial cells or spores are currently very limited. The interactions which occur between plasma agents and biological materials, ultimately leading to sterilization are still under investigation.

Mathematical modeling is an effective way of representing a particular process. It can help us to understand and explore the relationship between the process parameters. Mathematical modeling can help to understand and quantitative behavior of a system. Mathematical models are useful representation of the complete system which is based on visualizations. Mathematical modeling is an important method of translating problems from real life systems to conformable and manageable mathematical expressions whose analytical consideration determines an insight and orientation for solving a problem and provides us with a technique for better development of the system.

The objective of the study is modeling of the total Psychrophilic bacterial count as a function of the cold plasma treatment time and the storage period of treated sliced chicken meat.

2 MATERIALS AND METHODS

[26]Sliced chicken were purchased from local market (Benha, Qaliobia governorate, Egypt). All samples were transported to our laboratory food irradiation unit, Nuclear Research Center in ice-box (0°C) and surveyed for microbiological counts for counts of total bacteria, psychrophilic bacteria, spore forming bacteria, total molds and yeasts. Then, sliced chicken samples were packed in tightly sealed polyethylene pouches and divided into seven groups and stored in freezing till irradiation treatments.

2.1 PLASMA TREATMENTS CHARACTER OF EXPOSURE MACHINE

The plasma generator consisted of a negative dc source, a Blumlein-type pulse-forming network (E-PFN), and a dynamic spark gap switch. A triggered spark gap switch was used as a closing switch of E-PFN. E-PFN had four stages of LC ladder, which were composed of 5 nF of capacitor and 3 μH of inductor. The characteristic impedance (2√L/C) and the pulse width (2N√LC) of E-PFN, calculated from capacitance (C) and inductance (L) of the LC ladder, and number (N) of LC ladder stages were approximately 49 Ω and 1.0 μs, respectively.

A charging resistance value of 50 kΩ was chosen in the present case which corresponds to a charging RC time constant of 1 ms, which is 40 times faster compared to the repetition rate of the pulse. A schematic of the pulsed atmospheric-pressure plasma jet (PAPPJ) device for generating high voltage pulsed, cold atmospheric plasma jets is shown in Figure 1. The high voltage (HV) wire electrode, which is made of a copper wire, is inserted into a hollow barrel of a syringe. The distance between the tip of the HV electrode and the nozzle is 0.5 cm. When HV pulsed, DC voltage (amplitudes up to 25 kV, repetition rate up to 25 Hz), was applied to the HV electrode and helium gas was injected into the hollow barrel. This device was made using medical syringe (made out of an insulating material cylinder). The gas was fed into the system via flow

meter. The applied voltage to and the discharge current through the discharge chamber were measured using a voltage divider (Homemade), which was connected between the two electrodes, and a current monitor, which can be located upon returning to the ground. The signals from the voltage divider and the current monitor were recorded in a digitizing oscilloscope (Lecroy, USA) with a 200-MHz bandwidth. The high voltage pulses are applied between the needle electrode positioned inside a dielectric cylinder (a simple medical syringe) and a metal ring placed on the exterior of this cylinder. In order to obtain electric discharges at atmospheric pressure, a high voltage pulses (tens of kV) which have limited duration (hundreds of nanoseconds) and are repeated (tens of pulses per second), in addition to an inert gas (argon) is introduced in the cylinder.

The gas flows were in the range 0.5-10 l/min. The discharge takes place between the metallic needle top and a metallic ring fit on the outer surface of the syringe. Under optimal conditions, plasma is emitted as centimeter-long jets, just millimeters in diameter or even smaller. The working gases are supplied by high-pressure cylinders. Gas pressure regulators are used to reduce the pressure of gases to a workable level. Then, gas flow controllers deliver the gases with the desired flow. For voltage amplitudes of 15-18 kV, the plasma jet is very weak. The plasma jet disappears for voltage amplitudes lower than 15 kV. When argon is injected from the gas inlet and high voltage pulses, 26 kV voltages is applied to the electrode, the plasma jet is generated and a plasma plume reaching length of 21 mm is launched through the end of the tube and in the surrounding air. The length of the plasma plume can be adjusted by the gas flow rate and the applied voltage. Three bags from each of sliced chicken were exposured to plasma at 0.5, 1.0 and 1.5 min in Plasma Physics and Nuclear Fusion Department, Nuclear Research Center, Atomic Energy Authority, Inshas, Cairo, Egypt. After the exposure time of plasma, all samples were stored at 4±1°C.

2.2 MICROBIAL ANALYSIS[26]

psychrophilic bacterial count according to (FDA, 2002).

2.3 STATISTICAL ANALYSIS[26]

The statistical evaluation of the mean data was compared using one-way analysis of variance (ANOVA) according to Zar (1984). The chosen level of significance was P≤ 0.05.

The experimental data[26] obtain using the previous procedures were analyzed by the response surface regression procedure using the following higher-order polynomial equations: like, $y = \beta 0 + \sum \beta_i x_i + \sum \beta_{ii} x^2_i + \sum \beta_j x_j + \sum \beta_{jj} x^2_j + \sum \beta_{ij} x_i x_j$, where y is the response, xi and xj are the uncoded independent variables (factors), and $\beta 0$, β_i & β_j, β_{ii} & β_{jj} and β_{ij} are intercept, linear, quadratic, and interaction constant coefficients, respectively. Design Expert software package 8.0 was used for regression analysis, analysis of variance (ANOVA) and developing of models of different forms by transformation (linear and of higher order) based on above mentioned principles of forming a functions. Confirmatory experiments were carried out to validate the equations using the combinations of independent variables which were not part of the original experimental design but were within the experimental region. Various models were compared for the best fit summary and there R^2 values were compared to choose the best appropriated model for particular data design and selected runs. In this the Total Psychrophilic bacterial count was the response and the dependent two factors were the Storage time and the cold atmospheric plasma treatment time given to the sliced chicken.

3 RESULT AND DISCUSSION

The result of statistical Analysis are shown below:

Table 1. shows the fit summary the models

Response 1 Psychrophili Transform: None

*** WARNING: The Cubic Model and higher are Aliased! ***

Summary (detailed tables shown below)

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Squared	Predicted R-Squared	
Linear	0.0087		0.4435	0.1295	
2FI	< 0.0001		0.8418	0.6749	Suggested
Quadratic	0.5490		0.8316	0.5806	
Cubic	0.1802		0.8752	0.1948	Aliased

Table 2. Shows the model sum of square

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	3.714E+008	1	3.714E+008			
Linear vs Mear	4.697E+008	2	2.348E+008	6.98	0.0087	
2FI vs Linear	3.227E+008	1	3.227E+008	33.72	< 0.0001	Suggested
Quadratic vs 2	1.298E+007	2	6.490E+006	0.64	0.5490	
Cubic vs Quad	4.903E+007	3	1.634E+007	2.17	0.1802	Aliased
Residual	5.283E+007	7	7.548E+006			
Total	1.279E+009	16	7.991E+007			

Table 3. Model summary Statistics

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	5801.32	0.5177	0.4435	0.1295	7.897E+008	
2FI	3093.55	0.8734	0.8418	0.6749	2.949E+008	Suggested
Quadratic	3191.55	0.8877	0.8316	0.5806	3.805E+008	
Cubic	2747.28	0.9418	0.8752	0.1948	7.305E+008	Aliased

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

Table 4. Shows the ANNOVA tables

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	7.924E+008	3	2.641E+008	27.60	< 0.0001	significant
A-storage pe	*3.727E+008*	*1*	*3.727E+008*	*38.95*	*< 0.0001*	
B-TCAP	*7.819E+008*	*1*	*7.819E+008*	*81.70*	*< 0.0001*	
AB	*3.227E+008*	*1*	*3.227E+008*	*33.72*	*< 0.0001*	
Residual	1.148E+008	12	9.570E+006			
Cor Total	9.072E+008	15				

The Model F-value of 27.60 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

Table 5. ANNOVA Summary

Std. Dev.	3093.55	R-Squared	0.8734
Mean	4818.13	Adj R-Squared	0.8418
C.V. %	64.21	Pred R-Square	0.6749
PRESS	2.949E+008	Adeq Precisior	14.911

The "Pred R-Squared" of 0.6749 is in reasonable agreement with the "Adj R-Squared" of 0.8418; i.e. the difference is less than 0.2.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 14.911 indicates an adequate signal. This model can be used to navigate the design space.

The developed Fit Suggested required Model in term of Coded Factors:

Final Equation in Terms of Coded Factors:

Psychrophilic bacterial count =

+11388.01

+11566.68 * A

-11813.74 * B

-11977.58 * AB

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

The developed Fit Suggested required Model in term of Actual Factors:

Final Equation in Terms of Actual Factors:

Psychrophilic bacterial count =

-506.33363

+2841.74625 * storage period

+327.67142 * TCAP

-1916.41211 * storage period * TCAP

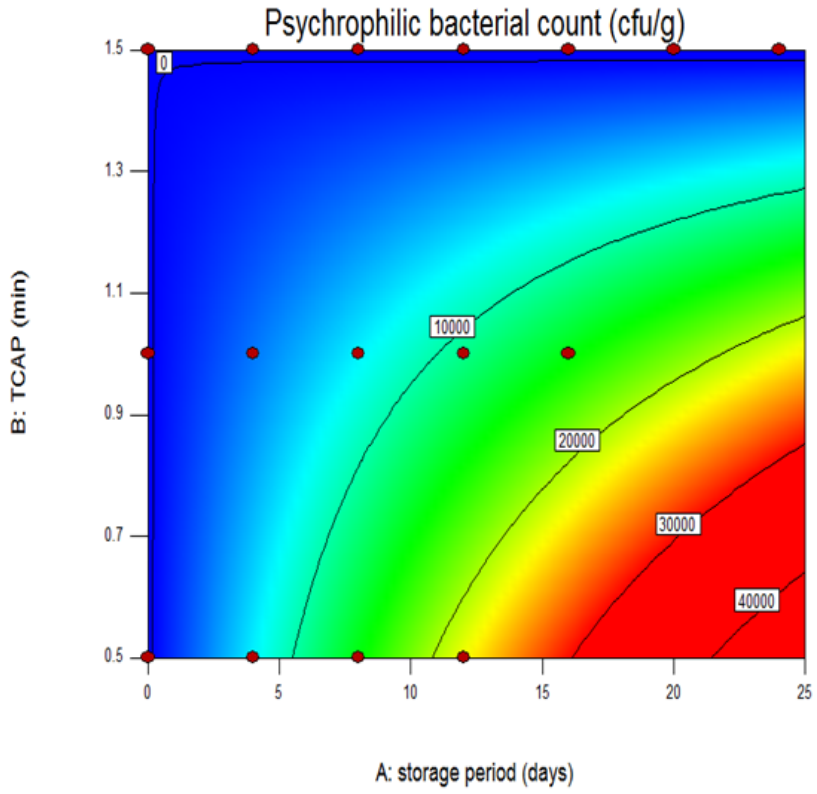

Fig 1. Shows the Contour Graph for the developed Model.

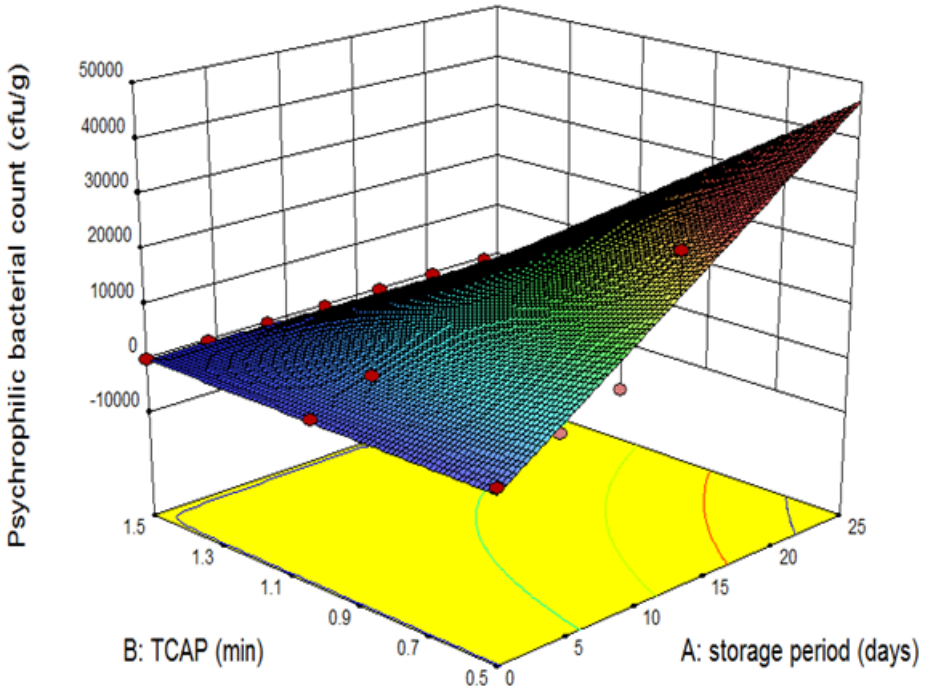

Fig 2. Shows the 3-D Graph Plotted between PBC, TCAP and SP. For the developed model.

4 CONCLUSION

Thus we get a most fitted model for the function of total bacterial count (PBC) with cold atmospheric plasma treatment time (TCAP) and storage period (SP) as the two variants, with R^2 = 0.8734, F value 27.60 and P value <0.0001, the suggested model is more significant for the given design data set.

ACKNOWLEDGEMENT

We are appreciative of the SHIATS University for its continuous support in the development of important technologies for the future use. The effort of higher authorities to promote the technologies has been very valuable in the promotion of new technologies. A special thanks goes to the dean and head of department for believing in our dream to develop new technologies. Many people have contributed either directly or in directly to make this work a reality.

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