Optimization of high voltage sterilization machine based on dielectric barrier discharge plasma against *Salmonella sp.* with response surface methodology on (*Gallus gallus domesticus*) chicken egg

E Widyastuti, D Mashitoh and Yunianta

Department of Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia

E-mail: endrika_w@ub.ac.id

**Abstract.** Egg consumption experiences a substantial growth year by year. But on the other, eggs are susceptible to physical, chemical, or biological damage due to their high nutrients. Biological damage occurs when *Salmonella* infected eggs while still in the parent body or contamination from the outside. The technology used today to reduce *Salmonella* is ineffective and takes a long time in the process. Therefore, many new technologies were developed to eliminate *Salmonella sp.* like plasma technology. Dielectric Barrier Discharge Plasma is an electrical discharge between two separate electrodes with a dielectric insulator. Plasma reactivity can cause oxidative effect on the outer surface of microbial cells. Aim of this research is to determine the optimal condition of sterilization process in egg using sterilization machine based on Dielectric Barrier Discharge Plasma to reduce *Salmonella sp.* in egg. Central Composite Design in Responses Surface Methodology with two independent variables are Input Voltage and time was used in this research. The results show variable have a significant effect on the response with optimum point of each variable is 26.97 volt for input voltage and 377.64 for time. The optimum condition for reduce *Salmonella sp.* is 4.98 Log CFU mL⁻¹. Higher voltages and longer processing times result in much reactive oxygen produced by Dielectric Barrier Discharge Plasma so that microbial reduction is more effective. This research demonstrates the feasibility of Dielectric Barrier Discharge plasma as an intervention to further reduce foodborne pathogens on chicken egg.

1. **Introduction**
World egg production and consumption experience massive improvement during recent decades. Egg production in the world rose on average by 2.4% per year during 2001-2011. According to FAO [1], the egg consumption level in the world was 8.9 kg per person in 2009, which is to note about 15 eggs. Eggs have registered the most important growth in the coverage of protein needs for the world population in the past 20 years. High protein in eggs unfortunately causes eggs to easily be contaminated by pathogenic microorganisms such as *Salmonella typhimurium* [2], *Alcaligenes, Bacillus, Pseudomonas, Proteus, Listeria monocytogenes* [3]. According to Kouam et al. [4], the prevalence of *Salmonella* in the sampled eggs in commercial layer farms in West region of Cameroon was 88.6% (124 out of 140).
High prevalence of *Salmonella* in eggs can cause various cases of poisoning in the world. Manjowicz et al. [5] using Monte Carlo simulation estimated that 93.8 million cases of gastroenteritis were due to *Salmonella* species occurring globally each year, amounted to 155,000 deaths. In solving that problem many researchers concern about reducing *Salmonella* in food use various ways.

The technology used today to reduce *Salmonella sp.* on eggs is heat pasteurization resulting in a total *E. coli* reduction of 4-5 log [3]. Non-thermal technology is needed because of the high consumption rate of raw eggs. Moreover, thermal treatment can reduce functional properties such as emulsifying activity and stability of egg yolk. The other technology is UV technology which can reduce *Salmonella sp.* effectively. The efficacy of UV-C light in inactivating *Salmonella* on green tomato can decreased by 3.22 logs after treatment for 30 seconds [6]. Other non-thermal energy is Thyme Oil by plasma-assisted process that can reduced *Salmonella sp.* until 10 CFU/egg. However, thyme oil is accelerated the loss of the intracellular protein [7].

Technology that have inactivation effect at atmospheric pressure without increasing temperature is dielectric barrier discharge (DBD) Plasma [2]. The atmospheric pressure of non-thermal DBD plasma is a type of plasma which has been studied for biological and medical applications to select inactivate unhealthy cells. DBD Plasma was previously shown effective in killing microorganisms without destruction of living tissue or damage to heat-sensitive materials. The bactericidal effects of plasma are assumed to be caused by several factors as active radicals (mainly those involving oxygen). DBD Plasma can reduce *Salmonella* number until 6 log CFU/egg for humid (RH=80%) gas [2]. This research is part of on-going research aimed at eggs decontamination by using dielectric barrier discharge plasma. The previous published experiment for DBD Plasma machine showed the optimum process parameters are voltage, frequency, and treatment time [2] and optimization model for inactivation *Salmonella* used central composite design of the responses surface methodology [8]. The goal of this research is to find out the optimal condition of input voltage and treatment time for egg decontamination compared with control (egg without DBD Plasma sterilization). The eggs were artificially contaminated with *Salmonella enterica*.

2. Materials and Methods
2.1. Microorganism and media
This experiment were used *Salmonella enterica* obtained from the Medical Faculty of Universitas Brawijaya. Culture of *Salmonella enterica* were prepared by incubating the cultures in Lactose Broth (LB) at 37°C for 17 minutes [9].

2.2. Sample preparation
Chicken eggs (*Gallus gallus domesticus*) were collected from Traditional Farm at Malang which aged 0 days old and the weight of eggs were 51 gr. Eggs were carefully cracked on tube vials which were sterile and homogenized using vortex in laminar air flow (LAF). The samples were added with 1 ml *Salmonella enterica* 5 log CFU mL⁻¹ on Lactose Broth (LB) and moved to 13 vial tubes, each tube has 2 ml of sample [10].

2.3. DBD plasma
Previous DBD Plasma process which placed sample (egg) close to plasma discharge and adjusted voltage and treatment time based on what was suggested by Design Expert 11 [7]. Microorganism such as *Salmonella sp.* has damaged during process. The charged particles, mainly ions from the plasma can act as bond by breaking agents in the cell proteins and nucleic acids while oxygen atoms could interact with bacteria by oxidation reactions, damaging the integrity of DNA molecules, which further results in bacteria destruction [11].

2.4. Experimental
This research was conducted to determine the number of residual *Salmonella sp.* About 45 ml eggs were aseptically transferred to sterile tube vial and was added with 1 ml of *Salmonella sp* in Lactose
Broth (LB). Sample was diluted in sterile of 0.1% peptone water and 1 ml of each was diluted with suspensions to pour on petri dish added with Salmonella Shigella Agar (SSA) and incubated at 37°C for 48 hour [9].

2.5. Design of experiment
The data obtained from this research were processed using Central Composite Design (CCD) in Response Surface Methodology (RSM) then Design Expert 11 program to obtain the optimal value of the input voltage and treatment time from the DBD Plasma usage. Central Composite Design (CCD) consists of embedded factorial design, star point to estimate the curvature, and center points to evaluate the experimental reproducibility [12].

3. Result and Discussion
3.1. Salmonella sp. concentration
Reduction of Salmonella sp. numbers on egg under various conditions converted through RSM central composite design. This research was used two independent variable consist of input voltage (X1) and treatment time (X2). DBD Plasma at input voltage 28 volt for 120 seconds resulted in the maximum reduction in concentration Salmonella sp. numbers (Table 1). The reduction of concentration Salmonella sp. number under this condition is 4.98 log CFU mL\(^{-1}\). The minimum reduction in Salmonella sp. at input voltage 18.34 volt for 300 seconds resulted in reduction 0.30 log CFU mL\(^{-1}\) (Table 1).

Table 1. Variable response of (X1=input voltage, X2=treatment time) Salmonella analysis in egg added with Salmonella sp.

| Std | Run | Code Variable X1 | Actual Variable X1 | Code Variable X2 | Actual Variable X2 | Salmonella Reduction (Log CFU mL\(^{-1}\)) |
|-----|-----|------------------|-------------------|------------------|-------------------|------------------------------------------|
| 9   | 1   | 0.000            | 0.000             | 24.00            | 300.00            | 4.02                                     |
| 10  | 2   | 0.000            | 0.000             | 24.00            | 300.00            | 3.98                                     |
| 8   | 3   | 0.000            | 1.414             | 24.00            | 554.56            | 4.51                                     |
| 1   | 4   | -1.000           | -1.000            | 20.00            | 120.00            | 0.70                                     |
| 12  | 5   | 0.000            | 0.000             | 24.00            | 300.00            | 3.68                                     |
| 13  | 6   | 0.000            | 0.000             | 24.00            | 300.00            | 3.51                                     |
| 3   | 7   | -1.000           | 1.000             | 20.00            | 480.00            | 1.08                                     |
| 5   | 8   | -1.414           | 0.000             | 18.34            | 300.00            | 0.30                                     |
| 2   | 9   | 1.000            | -1.000            | 28.00            | 120.00            | 2.99                                     |
| 4   | 10  | 1.000            | 1.000             | 28.00            | 480.00            | 4.98                                     |
| 6   | 11  | 1.414            | 0.000             | 29.66            | 300.00            | 4.68                                     |
| 11  | 12  | 0.000            | 0.000             | 24.00            | 300.00            | 3.81                                     |
| 7   | 13  | 0.000            | -1.414            | 24               | 45.442            | 1.00                                     |

3.2. Analysis response of Salmonella sp.
The data of Salmonella sp. reduction, input voltage, and treatment time are fit to the polynomial model used in the RSM. The values for the P-value is 0.0024 and coefficient of determination (R\(^2\)) is 0.97. This result shows that the response is close to the ideal value due to getting closer to 1 which is supported by Raissi and Farsani [13] statement that if the response falls within the ideal intervals or the response reaches its ideal value, the desirability is 1. The desirability lies between 0 and 1. The polynomial model obtained from the results of Salmonella sp. number is in the equation of \(Y= -29.79710 + 2.32173X_1 + 0.002190X_2 + 0.000561X_1X_2 - 0.043812X_1^2 - 0.000018X_2^2.\)
Table 2. Analysis of variance response of *Salmonella* sp. variable input voltage and treatment time.

| Source       | Sum of Squares | Df | Mean Square | F-value | p-value    | Statement  |
|--------------|----------------|----|-------------|---------|------------|------------|
| Model        | 31.59          | 5  | 6.32        | 40.66   | <0.0001    | Significant|
| A-Voltage    | 19.17          | 1  | 19.17       | 123.41  | <0.0001    | Significant|
| B-Time       | 6.72           | 1  | 6.72        | 43.26   | 0.0003     | Significant|
| AB           | 0.65           | 1  | 0.65        | 4.20    | 0.0796     | not significant|
| A²           | 3.42           | 1  | 3.42        | 22.00   | 0.0022     | Significant|
| B²           | 2.26           | 1  | 2.26        | 14.56   | 0.0066     | Significant|
| Residual     | 1.09           | 7  | 0.16        |         |            |            |
| Lack of Fit  | 0.90           | 3  | 0.30        | 6.45    | 0.0518     | not significant|
| Pure Error   | 0.19           | 4  | 0.05        |         |            |            |
| Cor Total    | 32.68          | 12 |             |         |            |            |

3.3. Normal plot of residuals

Figure 1 shows that all dropping points of *Salmonella* sp. are along the midline chart. This result indicates that the decrease in *Salmonella* sp. has a good normal mode. Data points which are increasingly close to the normal line indicate that the data spreading is normal. There is one data that is far spread over other responses but remains still in normal. This result is in accordance with Zangeneh [14] pointing out that the response has significant interaction with other responses. This is in accordance also with the Normal Plot of Residuals curve criteria; therefore, the normality of the model is met.

![Normal plot of residuals](image)

**Figure 1.** Normal plot of residuals curve *Salmonella* sp. reduction responses of variable input voltage (X1) and treatment (X2).

3.4. Response surface *Salmonella* sp.

Input Voltage and treatment time are factors that affect the *Salmonella* sp. reduction. The number of reduced *Salmonella* sp. is related to the characteristics of each type of microorganism depend on the strength of each cell wall [11]. Input voltage and treatment time response to the *Salmonella* sp. reduction in shown in Figure 2. Figure 2a shows that the voltage variable is the axis (X) and the time
variable of sterilization is the axis (Y). The curved lines indicate the response. The outer line in the figure shows a decrease in the smallest value response of Salmonella sp., while the deeper line shows the largest response value. Thus, it is apparent that the optimum response based on the image above is located on the top right corner of the contour plot as marked by the red part. Figure 2b present the response surface curve of the variables (voltage and treatment time) to the response of Salmonella sp. which produces a convex cultivar model. This result indicates the maximum decreased response of Salmonella sp. higher voltage of the sterilization process with a certain time of sterilization will damage the cell membrane of Salmonella sp. Therefore, the decrease rate of Salmonella sp. also increases to the optimum point. This is caused by a DBD Plasma which produces reactive oxygen and destroys the chemical bonds of the Salmonella sp cell wall. The diffusion of reactive substances released by the plasma causes lesions and perforations of the cell membrane and results in local damage. The damage will cause the cell to become lysis [15].

![Figure 2](image)

**Figure 2.** Response surface of Salmonella sp. Reduction of variable input voltage (X1) and treatment time (X2) (a) contour Plot (b) curve.

### 3.5. Comparison of Egg Control and DBD Plasma Treatment
In this analysis, the comparison is conducted for eggs contain Salmonella enterica before treated with sterilized DBD Plasma and after treated with sterilized DBD Plasma at SSA Media. The colour of SSA Media changes from red to yellow, due to the ability of Salmonella sp. in fermenting lactose. The result present that control egg has Salmonella sp. counted to 9.57 log CFU mL⁻¹ log that shown in Figure 3a, the yellow colour illustrates that the lactose in the media are absorbed by microorganism. After using DBD Plasma sterilization, the Salmonella sp. activity decreased to 5.00 log CFU mL⁻¹ which can be seen in Figure 3b.
Figure 3. Number of *Salmonella sp.* in egg added with *Salmonella enterica* at concentration $10^{-5}$ CFU mL$^{-1}$ (a) control (b) treatment with DBD plasma at SSA media.

4. Conclusion
The optimum condition of sterilization process of *Salmonella sp.* is achieved by using Dielectric Barrier Discharge Plasma with input voltage 26.97 volt for 377.64 s. From the optimum point, the optimum condition for the *salmonella sp.* is 4.98 log CFU mL$^{-1}$. Differences in response values decrease the amount of *Salmonella sp.* The result of verification with Design Expert calculation is 0.42 log CFU mL$^{-1}$. Differences in *Salmonella sp.* is not more than 5% indicating the appropriate model. This research demonstrates the feasibility of DBD plasma as an intervention to further reduce foodborne pathogens on chicken egg.

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