



## The Effect of Dielectric Barrier Discharge Plasma Treatment on the Microorganisms Found in Raw Cow's Milk

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**Abstract:** Milk is an essential source of nutrition especially for the breastfed infants. Sterilization of milk is necessary because it can be contaminated by microorganisms due to unhygienic collection and storage conditions. In this study, the sterilization of raw cow milk was performed by using dielectric barrier discharge (DBD) plasma method. Raw milk was transferred to the plasma reactor and dielectric barrier discharge cold plasma was performed by changing various parameters including voltage, exposure time and frequency. It was found that dielectric barrier discharge cold plasma is very effective at sterilization of raw cow milk particularly at room temperature. The optimum parameters that were demonstrated to completely kill the bacteria in raw milk were experimentally determined to be a 3 kV application voltage, 3 min exposure time and 500 Hz frequency. Additionally, there was almost no important change in pH value of cow milk after DBD plasma treatment (The average pH was 6.2). Pathogen microorganisms found in milk produces metabolites (during storage and transport) that have adverse effects on health. The method developed by us in this study will be used in a future study to develop a prototype of a sterilization device that can be integrated into the current milking system and can be continuously applied. Thus, the sterilization of milk during milking process could potentially be an extremely effective method for maintaining its quality and nutritional value. Furthermore, since the DBD plasma method is an ultra fast process that operates under ambient temperatures (ideal for thermolabile products) at a low running cost and is environment-friendly, it can be used for the sterilization of a wide range of liquid food products.

**Keywords:** Cold plasma, dielectric barrier discharge plasma, sterilization, milk

### 1. Introduction

Milk is essential source of nutrition especially for the breast-fed infants. However, raw milk may contain hazardous microorganisms, such as *E.coli*, *Streptococcus*, *Staphylococcus* and *Micrococcus sp.* under unsuitable conditions (Srujana et al., 2011). Furthermore, it can be contaminated due to unhygienic collection and storage conditions (Bali et al., 2013). The microorganisms contaminating raw milk adversely affect the quality of milk and dairy products as well as the health of consumers. Tuberculosis, brucellosis, typhoid are some of the serious milk-borne diseases (Azevêdo et al., 2014).

Several applications to perform sterilization have been employed, including thermal applications (e.g. low-temperature pasteurization and ultra-high temperature pasteurization) and non-thermal applications (e.g. UV and ozone

treatments, power ultrasound, beta and gamma irradiations, pulsed electric field and high hydrostatic pressure). However, unfortunately the present thermal methods have some disadvantages, such as loss of nutritional value and an adverse effect on organoleptic quality. On the other hand, non-thermal applications are limited in practice and require an expensive initial investment.

Pasteurization of milk is most common method among these. However, while pasteurization temperature (65 °C to 70 °C) is sufficient to kill all pathogen bacteria, there are a number of germs which not affected by this temperature, and some which even multiply readily at 70 °C (De Schweinitz, 1895). On the other hand while the sterilization of milk all pathogen bacteria and their spores are completely killed. Thus, they can't multiply at suitable conditions. Therefore the

sterilization of raw milk will be more effective method to prevent these diseases.

Plasma is the fourth state of matter and consists of electrically charged or ionized atoms and molecules. It does not have a regular shape or volume and can be formed filaments and beams under magnetic fields. It is found in natural phenomena, such as stars and lightning as well as man-made productions of fluorescent and neon lightings, plasma televisions and, many other products. Rapidly growing plasma technology presents a wide range of research areas and is widely studied for use in biomedical materials and devices (Laroussi, 2005; Rossi et al., 2006; Kogelschatz, 2007), modification of textiles surfaces (Morent et al., 2008), decontamination of heat sensitive materials (Montie et al., 2000), water sterilization (Korachi et al., 2009), wound healing, and food decontamination (Barbosa-Canovas et al., 1997; Nastuta et al., 2011).

There are two types of plasma depending on the method of generation; cold or non-thermal plasma (NTP), and thermal plasma (TP). Cold plasma consists of gas molecules with moderate temperatures and electrons with higher temperatures. In contrast, the TP consists of electrons and gas molecules with several thousands of degrees Kelvin temperature, and these species are found in equilibrium. Cold plasma techniques have attracted food scientists and researchers (Misra et al., 2011) and recently been studied for potential inactivation of microorganisms (Akishev et al., 2008). Cold plasma is an ultra fast method of sterilization that operates under ambient temperatures (ideal for thermolabile products) at a low running cost and is environment-friendly. There are various plasma techniques related to the method of generation (Banu et al., 2012). Dielectric barrier discharge (DBD) plasma is one of these techniques. It has been used to sterilize raw milk; however, anaerobic bacteria have been surviving after DBD plasma treatment (Kim et al., 2015).

The aim of this study was to show the effect of DBD plasma treatment on the all microorganisms found in raw milk.

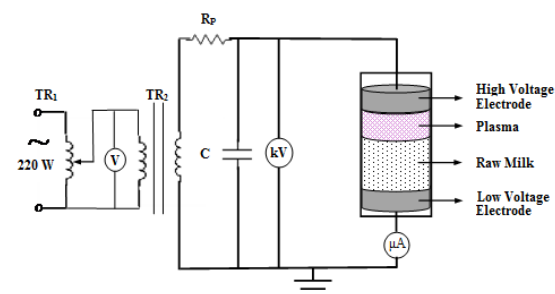
## 2. Materials and Methods

Raw milk was purchased from a local supermarket. The petri dishes (90 mm × 15 mm) was purchased from Inlab, São Paulo, Brazil. Standard TTC I mode growth medium (is rich in nutrients and vitamins and promotes the growth of lactic acid bacteria, yeasts and molds) was purchased from Sartorius acquires U.S. Digital power supply (GWINSTEK APS-9501 type, 0-500 Hz variable

frequency and voltage capable and can be adjusted from 0-400 V) was purchased from Good Will Instrument Co., Ltd, New Taipei, Taiwan. Transformer in 220/33000 V 800 W power (Plasma Prep 5, Typ 4656 N Model) was purchased from Gala Instrumente, Hayward, CA US.

The experimental studies were performed using raw cow milk at 30 °C. The raw and diluted milk samples were filtered in order to extract coarse particles with a cheesecloth and then poured into a clean can. Experimental studies were conducted by two different methods: (1) the use of raw milk without dilution and (2) the second way was the use of 10 % diluted (using distilled water) milk. For each method using a syringe, 5 mL raw or diluted milk was placed into the cold plasma reactor and exposed to DBD cold plasma under various conditions.

Figure 1 is a schematic diagram of the experimental setup used for raw and diluted milk sterilization. There were two transformers in the experimental setup. The first (TR<sub>1</sub>) was used for the arrangement of input line voltage from 0 to 220 VAC. The second (TR<sub>2</sub>) was used to improve output voltage from 0 to 33,000 VAC, and it was controlled by TR<sub>1</sub>. The resistance (R<sub>p</sub>) and capacitance (C) constitute an RC noise filter that eliminates noise signals from the line.



**Figure 1.** The schematic diagram of the experimental setup

Plasma was generated by high-voltage alternating current transformer (TR<sub>2</sub>) that could apply various voltages (0 kV-33 kV) and frequencies (50 Hz-500 Hz). The plasma reactor was composed of a smooth plastic pipe with a 25 mm inner diameter. Two disc-shaped electrodes (25 mm diameter and 5 mm thickness approx.) made from stainless bronze were mounted on both ends of the cylindrical reactor to transfer the plasma current the surface of these electrodes was covered by a thin polyethylene terephthalate film layer acting as a barrier. Moreover, these electrodes were sealed against to prevent leakage

of milk. While mounting the electrodes to the reactor, a 5 mm air gap was left between the upper electrode and the milk level to avoid a short circuit. Subsequently, various voltages with different frequencies were separately applied to each electrode to create different cold plasmas.

The plasmas occurred between the upper and lower electrodes by applying varied voltages, such as 1.5 kV, 3 kV, and 5 kV while DBD plasma treatment time was 3 min and frequency was 500 Hz in the presence of an air medium.

The plasma application time were chosen as 1 min, 3 min, and 5 min intervals while applied voltage 3 kV and frequency was 500 Hz.

The frequency rates of the application voltages were chosen as 50 Hz, 200 Hz, 350 Hz, and 500 Hz while DBD plasma treatment time was 3 min and applied voltage 3 kV. The medium temperature was approximately 30 °C.

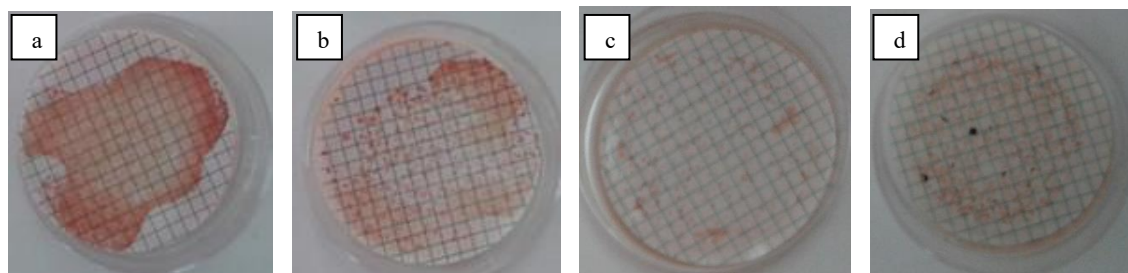
Finally, the plasma-treated raw and diluted milk obtained by methods mentioned above was separately aliquoted into the petri dishes that were partially filled with approximately 5 mL of DBD plasma-treated milk, leaving an air gap of about 5 mm. After preventing microbial contamination of the samples, the petri dishes were incubated at 37 °C for 24 h and to observe the degree of bacterial growth. Microbial densities of samples were evaluated in form of available-not available after incubation.

### 3. Results and Discussion

The effect of DBD on the sterilization of raw milk was investigated under different cold plasma conditions. By changing the voltage, frequency, and time parameters, the optimal conditions under which DBD cold plasma could completely kills the bacteria living in raw milk, were determined. All experiments were performed at room temperature, and thus, the plasma-treated milk could not spoil. The experimental studies were performed for both non-diluted and diluted raw milk. However, more

effective results were obtained using 10 % diluted raw milk. Using these conditions, average power consumption of the system was calculated to be 0.3 W.ml<sup>-1</sup>. No substantial change was observed in the pH value of raw milk after DBD the plasma treatment (average pH was 6.2) as shown in the study of Kim et al. (2015).

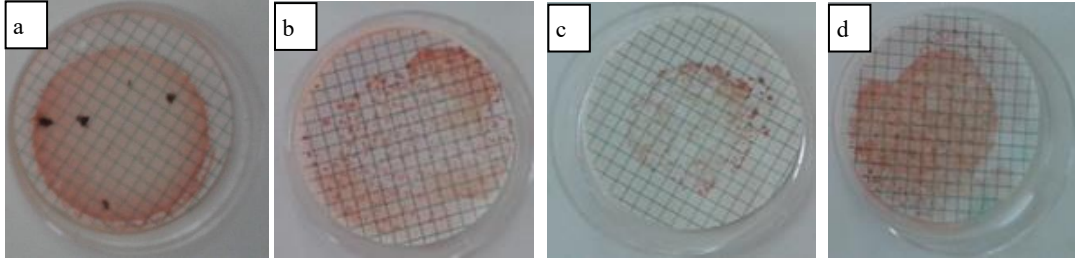
The effect of voltage on sterilization was investigated by changing the application voltages to 1.5 kV, 3 kV, and 5 kV separately with fixed frequency of 500 Hz for 3 min. As seen in the Figure 2, the optimal results were achieved when applied voltage of 3 kV. In Figures 2, our findings show that under the same application conditions the level of bacterial growth obtained at 3 kV voltages was significantly lower than that obtained at 1.5 kV and 5 kV. Thus, It can be inferred that the application of a low voltage to plasma seems to be too weak to kill all the bacteria present in the milk. Interestingly, Figure 2 also shows that applying a voltage can actually increase of the level of bacterial growth in plasma-treated and non-plasma treated raw milk under various conditions. In Figure 2c and d, it can be seen that when a high voltage (5 kV) was applied, plasma increased the number of bacteria in the raw milk, while a lower voltage (3 kV) decreased their number. Increasing the applied voltage resulted in a higher degree of ionization of the gas and thus increased the density of various reaction species which were the reactive agents in the inactivation of the bacterial cells (Duan et al., 2005; Yu et al., 2007). Hence, the plasma germicidal efficiency was improved and enhanced with a higher applied voltage. But on the other hand, when the applied voltage was too high, the glow discharge would turn into an arc discharge. And then the plasma shrank in the discharge channel and the cross-sectional area of the plasma was substantially reduced, which weakened the plasma germicidal efficiency (Miau and Yun, 2012). Therefore, it can be concluded that there is peak value of applied voltage of DBD plasma to achieve the highest level of bacterial clearance from the milk.



**Figure 2.** The effect of applied voltages on sterilization of milk while frequency was 500 Hz and DBD plasma treatment time was 3 min. a) Non-plasma b) 1.5 kV c) 3 kV d) 5 kV

The effect of the DBD plasma application time was investigated by changing the time parameters of 1 min, 2 min, 3 min, and 5 min separately, while applying a fixed voltage of 3 kV and a fixed frequency of 500 Hz. Figure 3 shows the effect of the plasma application time on killing of bacteria in the raw milk. It is seen that the optimal DBD

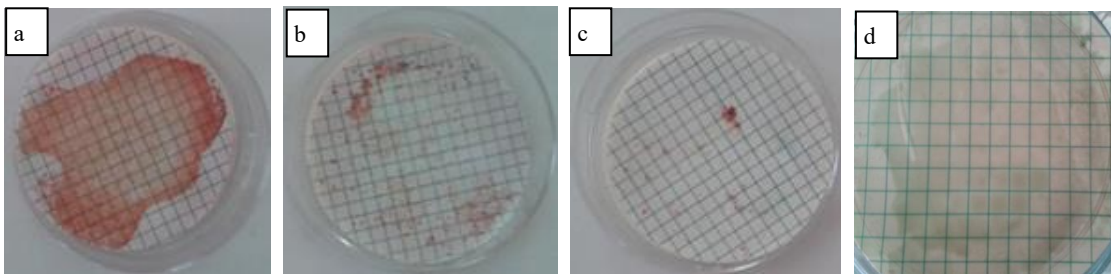
plasma application time was 3 min. In Figure 3, it can be seen that there is a peak value for plasma application time in killing bacteria present in raw milk. In this study, the optimal results were achieved with 3 min time interval. Therefore, the plasma applied time of 3 min was utilized for all subsequent experiments.



**Figure 3.** The effect of DBD plasma treatment time on sterilization of milk while applied voltage was 3 kV and applied frequency was 500 Hz. a) 1 min b) 2 min c) 3 min d) 5 min

The effect of frequency on the DBD application voltage was investigated by changing the frequency parameters to 200 Hz, 350 Hz, and 500 Hz separately, while remaining parameters were fixed to their optimal values. Figure 4 shows that the best frequency for DBD plasma was 500 Hz. Figure 4 shows the effects of various application frequencies on killing bacteria in DBD plasma-treated raw milk. It can be seen that the increase in frequency is significantly effective in killing bacteria. Furthermore, as seen in Figure 4d, by increasing the frequency up to 500 Hz, all the bacteria were completely killed by the DBD plasma method. These results are in agreement with the literature (Kim et al., 2015) that depending on the type of plasma, it is possible to inactivate a wide range of pathogens. However, the probiotic bacteria were also killed together with all

pathogens present in raw milk during DBD sterilization. It can be concluded that this result is a disadvantage of DBD plasma sterilization. Since raw milk contains only a few probiotic bacteria, it is already not one of major natural sources of probiotic bacteria. Therefore, killing of probiotic bacteria present in raw milk is not a disadvantage of DBD sterilization. Furthermore, since UHT milk also doesn't contain probiotic bacteria, dairy products such as probiotic milk and probiotic yogurt are commercially produced by fermenting UHT milk. Therefore, when probiotic bacteria added into UHT milk, they wouldn't compete against to pathogen bacteria for nutrition. Consequently, killing of probiotic bacteria during sterilization of raw milk is not a disadvantage, is an advantage contrary.



**Figure 4.** The effect of frequency on sterilization of milk while applied voltage was 3 kV and DBD plasma treatment time was 3 min. a) Non-plasma b) 200 Hz c) 350 Hz d) 500 Hz

#### 4. Conclusion

The efficacy of milk sterilization process was experimentally investigated using DBD plasma

method. Our results indicate that DBD cold plasma is quite effective in sterilizing milk process. In addition, compared to other parameters, the frequency parameter was found to be extremely

effective in killing the bacteria. Therefore, it was concluded that the parameters that could completely kill the bacteria living in raw milk were experimentally determined to be an application of a voltage of 3 kV for 3 min at a frequency of 500 Hz.

During storage and transport, pathogen microorganisms found in raw milk can produce metabolites that have adverse effects on human health. Therefore, the sterilization of milk during the milking process could potentially be more useful for its quality and nutritional value.

The method developed in the present study can be used in future studies to develop a sterilization device prototype that can be integrated into the current milking system based on a continuous process. Since average ten minutes DBD plasma treatment has not changed the nutritional quality and physicochemical properties of milk (Kim et al., 2015), DBD plasma sterilization of milk can be an extremely effective method for maintaining its quality and nutritional value.

Furthermore, cold plasma is an ultrafast sterilization process that operates under ambient temperatures (ideal for thermolabile products) at a low running cost, and is environment-friendly. Therefore, this technology can be used for the sterilization of wide range of liquid foods.

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