Atmospheric Cold Plasma Nano Titanium in Cathode and Microbial Load of *Escherichia Coli* (*E. Coli*) in Ground Meat: Examinaation and Analysis

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Abstract
Application of cold plasma as a non-thermal treatment in food processing industry to reduce microbial load and cold sterilization has been extensively studied by researchers in the last decade. Accordingly, the purpose of thisstudy was to investigate effect of atmospheric cold plasma flow generated by two electrodes with respective diameters of 8 cm (A) and 15 cm (B) equipped with nano titanium in cathode at three varying intervals on the reduction of the exponential phase of **E. coli** ***in ground meat. After the preparation of young culture and inoculation on ground meat,*** **E. coli** ***bacteria were counted, before and after treatment. The reduction of*** **E. coli** ***bacteria in the electrode with large and small diameters were 80% and 50% respectively, and with increasing time of 4, 6, and 10 minutes, the exponential phase was reduced by 0.8, 1, and 1.2, respectively. As such, the results implied that the reduction of the exponential phase of*** **E. Coli** ***was greater for the electrode with a larger diameter compared to the smaller one, while increasing the duration of treatment reduced the exponential phase to greater effect.***
Keywords: Atmospheric cold plasma, Escherichia coli, Disinfection

1. Introduction

Both increase in global meat consumption and on the other hand, presences of microbial hazards in meat products have caused more importance of production chains in meat industry. Therefore, meat spoilage taken place because of microbial growth, fat oxidation, and enzymatic autolysis should be under controlled (8). Decontamination of bacteria can be performed using various methods, including the use of furnaces, autoclaves, dry heat, ethylene oxide, and gamma radiation, among others.Each of the methods mentioned abovehas certain limitations owing to some disadvantages such as temperature, high humidity and degradation, and even leaving toxic waste (8, 9).

In recent years, plasma has been employed to disinfect bacteria and other microorganisms, the results of which have been highly significant, to the extent of garnering the attention of a wide range of researchers (10, 11).Cold plasma or non-thermal plasma is a type of plasma which has been recently employed for bacterial disinfection (12,13).

Non-thermal plasma suitable for disinfection can be produced at low and atmospheric pressure. However, because a large volume of plasma at low fluxes is highly costly, more attention should be devoted to the production of plasmas at atmospheric pressure (14, 16). In this context, purpose of this study was to investigate the germicidal effect of atmospheric plasma flux produced by nano titanium (tio2) with two electrodes of 8 and 15 cm in 3 different durations of 4, 6, and 10 minutes on reducing exponential phase of *E. Coli*.

2. Material and Methods

2.1. Preparation of *E. coli* for Inoculation

Lyophilized *E. coli* (ATCC® 25922™) was supplied and preparation of bacteria for inoculation was performed by transferring bacteria from sterile microtubules to Brain-Head infusion (BHI) broth (Merck, Germany), which was then stored for 24 hours at 35 °C and then the second culture was prepared from the former, from which different amounts were added to the cuvette tubes containing 5 ml of sterile BHI broth and using a spectrophotometer, their light absorption was determined at a wavelength of 600 nm. The bacterial count was performed simultaneously with the aforementioned procedure (7).

2.2. Preparing meat samples

the meat was transferred from the meat distribution and supply center to the laboratory under hygienic conditions and accompanied with ice. The outer surface of the meat was sterilized by immersing it in 95% ethanol and then the remaining ethanol was ignited on the surface of the meat. After separating the outer surface of the meat under aseptic conditions, the inner part of the meat was grounded by a sterile meat grinder. (5). After inoculating the ground meat with 25 µl bacteria from 0.5 McFarland suspension, 108 bacterial cells were produced per gram of meat. Samples were stored at 4 °C until the day of cold plasma application (5).

2.3. Enumeration of the *E. coli*

Counting of*E. coli* in ground meat samples in VRB culture medium at 35±1 ° C (1).

Statistical analysis method

The results were obtained using SPSS-18 software and for statistical analysis, an average of three replications was performed. the significance level for all tests was considered as ($p < 0.05$).

Results and Discussions

The count of *E. Coli* before and after treatment revealed that *E. coli* was respectively reduced by 80% and 50% in the electrode with the larger diameter and the one with the smaller diameter, while with the treatment durations 4, 6, and 10 minutes, the exponential phase was reduced respectively by 0.7, 1 and 2.1. Because of its higher cost-effectivity, production of less pollution and environmental friendly, atmospheric plasma has been extensively employed in sterilization and germicidal applications as well as sterilizing living tissues on-site to remove contamination and tissue infections. Atmospheric plasma is one of the techniques that been growing rapidly in the world in the last decade, garnering the attention of the researchers from a plethora of disciplines (18, 19, 20). Gaseous plasma can be used to eliminate vegetative forms such as bacteria, fungi, and even viruses (21, 22). Ruth et al. reported that using a 25-minute atmospheric plasma source at room temperature can reduce the population of *Staphylococcus aureus* and *Escherichia coli* (21). Zingmin et al. examined the effect of non-thermal plasma flux on *Escherichia coli*, *Staphylococcus aureus,* and *Bacillus subtilis* at different intervals of 30, 60, 90, and 120 minutes. They then grew the damaged bacteria on a solid culture medium and counted the colonies, concluding that at the time of the study, the number of *Escherichia coli* colonies grown was less than those of *Staphylococcus aureus*, itself being less than those of *Bacillus subtilis* (19). Wilger et al. studied the effect of inactivating the flow of non-thermal plasma produced using O_2 gas mixture on *Escherichia coli,* the results of which revealed that, after a treatment of 25 minutes, almost 100% of the bacteria were killed, confirming it by colony counting in solid culture medium (23). Saba et al., examined the application of cold plasma for the disinfection of *E. coli* bacteria with a DBD device at a voltage of 10 kV and a frequency of 20 kHz. The results obtaained by the researchers indicated that this device completely sterilized all samples in 2 seconds without causing heat effects (3). Increasing the irradiation duration improved the germicidal properties of cold plasma. Increasing the capacitance improved the germicidal properties of DBD (3). Deilami, examined the application of cold plasma as a non-thermal treatment in the food industry, in which the effects of plasma on the growth of gram-negative *E. coli* bacteria in solid culture medium were investigated and then the effects of plasma flow on the removal of *E. coli* bacteria inoculated into milk were measured (2).Mazloum (2013) investigated the effect of atmospheric cold plasma on the inactivation of microorganisms in the liquid-sensitive liquid medium of an egg (6). Sohbatzadeh et al. (2013) designed and fabricated a triple atmospheric cold plasma jet compromised of argon, air, oxygen, and nitrogen gases, for which the bactericidal effect on gram-negative and positive bacteria was investigated in sterilizing solid and liquid surfaces to remove gram-negative and gram-positive *Escherichia coli* and *Streptococcus pyogenes*(4).The results showed that the triple jet device is more effective in the disinfection of the bacteria. Comparing the results of this experiment with the other researches (18, 21, 24, and 23) indicates that the volume of plasma produced with larger diameter electrodes might be owing to electric field distribution used to break the air on the surface of the ground meat. Therefore, the volume of plasma and possibly the active radicals produced by the larger diameter electrode is increased in the plasma compared to the smaller diameter electrode. However, the destructive effect of UV on DNA is considered one of the main factors influencing the germicidal properties of the flux. These produced radicals cause DNA damage and severe disruptions in the systems of replication and transcription of bacterial proteins, resulting in their annihilation.

Conclusion

The results of this study revealed that the geometric structure of the electrodes and the flow duration of the produced plasma gas are of the utmost value for bacterial disinfection. Accordingly, it is widely assumed that atmospheric plasma is less expensive, and results in less pollution and environmental damage. Hence, it is recommended that the germicidal properties of atmospheric plasma might be used as an effective method in the management of contamination prevention and disinfection of food surfaces. Consequently, the effective parameters in the production of atmospheric cold plasma such as power supply, geometric structure of electrodes, and the effect of reducing their microbial load on other bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* are highly significant.

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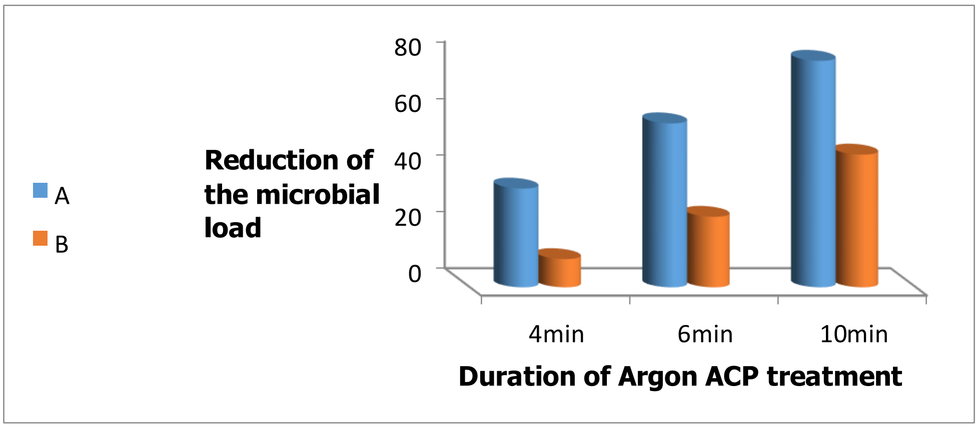

****Figure had better become 2 dimensions with black-white. Also please mention figure in text.**