Application of Non-Thermal Plasma for Milk Sterilization: A Review

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Abstract. Milk is an optimal liquid medium for the growth of microbial contaminants due to the high nutritional content. Therefore, the sterilization process is needed to make the milk save to be consumed as well as to extend the milk’s shelf life by inactivating microorganisms. A non-thermal plasma system has been utilized as a new alternative sterilization method for food products. Non-thermal plasma generates free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) that contribute to bacterial cells inactivation. This review focused on the factors that affect the non-thermal plasma efficacy to microbial inactivation during the development of milk cold sterilization. The plasma effectiveness in inactivating bacteria could be affected by the attributes of plasma treatment time, the applied voltage, working gas and the type of plasma system. The non-thermal plasma system successfully applied to reduce the number of total bacteria in milk and prolong the shelf-life.

1. Introduction

Raw milk is milk that does not undergo any processing processes such as pasteurization and sterilization. Milk consists of water, non-fat solids, lipids, lactose, protein, vitamins, and minerals. Due to its composition, milk is considered as a functional food that provides health benefits [1,2]. In contrast, raw milk can be an optimal medium for the contaminant bacteria to grow. Therefore, it causes a reduction in milk quality and harmful for human consumption [3].

Milk-contaminant bacteria consist of spoilage and pathogenic bacteria. Poor-handling processes and improper storage conditions may change the chemical conditions of milk to be ideal as a growth medium for contaminating bacteria [3]. Some bacteria that generally have been isolated from milk include Escherichia coli, Staphylococcus aureus, Salmonella, Bacillus sp, Lactobacillus, Listeria, Enterobacter, Brucella, Streptococcus, Mycobacterium, and Pseudomonas [3,4].

Sterilization is a process that can reduce the number of bacterial contaminations by inactivating and killing the bacteria [5]. The common sterilization process of milk is carried out by heating methods, namely pasteurization, usually carried out by using thermal heating and Ultra High Temperature (UHT)
methods. The thermal heating pasteurization process uses a temperature of 72°C for 15 seconds to kill the pathogenic bacteria and inactive the microbial enzymes that could damage milk quality. The UHT process uses a higher temperature of 120–130°C for 2 seconds to kill all bacteria cells and spores present in milk. However, both of those sterilization processes may remove the nutritional content of the milk such as vitamins and cause milk coagulation because of protein denaturation [5,6].

Non-thermal plasma occurs in a state of thermal imbalance (non-thermal equilibrium) between the temperature of electrons and gases. Non-thermal plasma has been used as a novel sterilization technology for a wide range of fields, such as in biomedical, food, and water treatment applications [7-9]. Non-thermal plasma uses physical processes, including high hydrostatic pressure, and pulsed electric fields aiming to inactivate microorganisms instantly at low temperatures. The plasma generates UV photons, electric fields, electrons, physical forces, charged particles, free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS). [7,8] The formation of ionization and chemical reactions in non-thermal plasma is dependent on the temperature of the electrons compared to the temperature of the gas particles [7,9,10]. Those radical components can inactivate bacteria and other microorganisms such as yeast, mold, and airborne viruses by inducing the disruption on the cell membrane or outer layer through lipid peroxidation and protein denaturation [10].

Non-thermal plasma has been applied either to solid and liquid food because the non-thermal plasma sterilization process can maintain food nutritional and functional characteristics [8,11,12]. The application of non-thermal plasma in fresh vegetables and fruits successfully inactive the targeted microorganisms (i.e., E. coli, L. monocytogenes, Salmonella enterica) showed by log-reduction [13,14]. Similar results were also observed in poultry products such as chicken meat and eggshells [13-15]. Non-thermal plasma has been applied to various liquid foods, including apple juice, orange juice, white grape juice, and milk [13, 16,17]. The reaction between reactive species, generated by the plasma system, and water will produce more oxidizing agents such as hydroxyl radicals, hydrogen peroxide, ozone, and more oxygen base radicals. Those radical components that make the water become activated are potent in inactivating bacteria and other microorganisms such as yeast, mold, and viruses [10,18,19]. The non-thermal plasma application in fruit juices resulted in the log-reduction of the targeted bacteria such as E. coli and Salmonella enterica [13].

This study described the literature review in the scope of non-thermal plasma application for milk sterilization. We reviewed the effect of non-thermal plasma to the microbiology quality such as total bacterial count and the presence of pathogenic bacteria such as L. monocytogenes, S. aureus, E. coli, and Salmonella sp. The study aimed to review the factors that affect non-thermal plasma effectiveness for bacterial inactivation and milk sterilization.

2. Bacterial inactivation through non-thermal plasma
Microbiological contamination of milk varies due to milk handling practices ranging from the milking process to storage. Microbial contamination through the environment comes from milk direct contact with soil or air contamination due to dirty bedding. Microbial contamination through milking equipment occurs when microorganisms stick on the surface of milking equipment. Besides, the contamination can be through milk residue that remains in the appliance after the cleaning cycle. The cow health condition also affects the diversity of microbial contaminant in milk. Mastitis, causing by infection of S. aureus, can alsocontaminate the milk products [20,21].

There are two groups of bacterial contaminants in milk, the pathogenic and spoilage bacteria. Pathogenic bacteria produce toxins in raw milk, causing diseases, in which the severity depends on the number of bacteria, the infectious dose, and consumer health. Examples of pathogenic bacteria in raw milk are Campylobacter spp., S. aureus, Salmonella sp., L. monocytogenes, and E. coli. Milk spoilage occurs when the milk components are degraded due to microbial activity due to the condition is optimal
for microbial growth besides it may produce the unwanted by-products. The rapid breakdown of milk composition as a result from exponential microbial growth will affect the rheology, aroma, taste and chemical characteristic. The degree of milk degradation is affected by the cell number, the bacteria species, handling processes, and storage conditions. Examples of spoilage bacteria are *Enterococcus faecalis*, *Bacillus subtilis*, and *Bacillus cereus* [22].

The plasma glow is generated when a gas, such as air or working gas mixture, is partly or fully ionized due to the interaction with an electrical discharge. Therefore, the plasma glow contains free electrons, ions, protons, and active free radicals. Helium (He) and Argon (Ar) are the noble gas used as the carrier gas in plasma technology due to their excellent heat transfer properties and the sensitivity to control the plasma energy. Air as working gas and the addition of oxygen can produce more free radicals such as ROS, atomic oxygen, superoxide anion, singlet oxygen, hydroxyl radical, ozone, and RNS that lead to intense antimicrobial activity [8,13].

Non-thermal plasma can inactivate bacterial cells through three-postulated mechanism. First, UV radiation induces DNA fragmentation that leads to replicate inhibition and cell death. Second, charged particles disturb the electrostatic condition on the cell membrane. The potential difference in cell membranes results in pore formation that induces the releasing of ions. Moreover, cell inactivation is caused by reactive species through lipid oxidation, DNA damage, protein oxidation, and cell perforation. Lipid peroxidation disturbs the integrity of cell membrane composition that leads to cell burst. Cell perforation allows the reactive species to pass the membrane and induce intracellular damage [10,23].

A literature study found some previous reports of non-thermal plasma inactivation to *E. coli*, *S. aureus*, *S. enterica* Serovar Typhimurium, *Pseudomonas aeruginosa*, *L. monocytogenes* and *B. subtilis* (Table 1). Those studies were conducted at atmospheric pressure, in which non-thermal plasma was generated in a variety of plasma treatment types such as indirect corona discharge, dielectric barrier discharge (DBD), and surface micro discharges. The variables of non-thermal plasma processing treatment were exposure time and voltage [17-24].

| Treatment Type               | Targeted Bacteria       | Working Gas       | Processing Condition | Result                              | References |
|-----------------------------|-------------------------|-------------------|----------------------|-------------------------------------|------------|
| Indirect Corona Discharge   | *Salmonella* sp.        | Atmospheric air    | Plasma exposure at interval of 30, 60, 120, 180, and 240 s | Reduction of 0.5-5.5 log<sub>10</sub> CFU/cm<sup>2</sup> | [24]       |
|                             | *E. coli*               |                   |                      | Reduction of 1.3-55.3 log<sub>10</sub> CFU/cm<sup>2</sup> |            |
| Dielectric Barrier Discharge (DBD) | *P. aeruginosa*        | Atmospheric air    | 120 kV at 50 Hz; plasma treatment time at interval of 0, 60, 120, and 300 s | Reduction of 5.4 log<sub>10</sub> CFU/ml after 60 s of treatment | [25]       |
| Dielectric Barrier Discharge Plasma Jet | *S. aureus*           | Helium            | 7.5 kV at 12 kHz; plasma treatment at interval of 1, 3, 5, 10, and | Increment of inhibition zone; reduction of 5-6 log<sub>10</sub> CFU/ml | [26]       |
The effectiveness of non-thermal plasma for bacterial inactivation is influenced by the attributes of reactor types, treatment types, working gas, and the processing parameters such as exposure duration and voltage [24-27]. The dielectric barrier discharge (DBD) non-thermal plasma is the most common treatment type that has been reported in several scientific papers. The system consists of two metal electrodes with at least one electrode covers with a dielectric barrier. The equipment produces microdischarges plasma that providing stable and homogenous treatment besides preventing the plasma arcs. The DBD type can work with several working gas under atmospheric conditions [11,13].

Moreover, the inactivation with direct treatment gives a remarkable result than samples with the indirect treatment, in which this correlates to the charged particles-inactivation mechanism. The charged particles induce cell membrane disruption through direct contact [28]. The use of working gas also affects the bacterial inactivation process, in which the result of non-thermal plasma inactivation with oxygen was greater than air or nitrogen. It produces higher oxygen-based reactive species and generates ozone. The prolonged plasma exposure and higher voltage also may produce higher reactive species. Therefore, the bacterial inactivation mechanism is due to the strong oxidative effect in the cell membrane and cellular respiration inhibition [23,29].

### 3. Non-thermal plasma as milk sterilization method

Non-thermal plasma has been applied to milk to investigate its potential as an alternative method to the milk sterilization process in non-thermal condition. The plasma processing condition was at atmospheric pressure using various treatment types, working gas, exposure duration, and voltage. Most of the previous reports were focused on the effect of non-thermal plasma to the milk microbiology quality. Some of the previous reports concluded that non-thermal plasma treatment has been reduced the microbial cell numbers in the milk samples as shown at Table 2.

| Treatment Types | Working gas | Processing condition | Result | Reference |
|-----------------|-------------|----------------------|--------|-----------|
| Gas Bubble Discharge | Argon | Plasma frequency of 2, 3, and 4 kHz; Treatment time varied from 30-120 s | Reduction of 4.6 log<sub>10</sub> CFU/mL; Prolonged shelf life up to 6 weeks. | [30] |
| Dielectric Barrier Discharge | Air | Plasma frequency of 2, 3, and 4 kHz; Treatment time at interval of 0, 15, 30, 60, 90, and 120 s, with the voltage of 70 V; A variation of voltage between 0-80 V at 120s. | The bacterial cell number decreased alongside the higher voltage and longer treatment time | [29] |
Previous studies reported that variation of treatment time and voltage in the processing condition affects non-thermal plasma pasteurization efficacy. A significant bacterial cell reduction occurred in a longer duration of plasma exposure, high voltage, and high frequency [29-32]. The bacterial inactivation resulted from cell membrane disruption, cellular metabolism reduction, and DNA fragmentation [10,23,29]. It is worth to notice that non-thermal plasma could prolong milk shelf-life, which was demonstrated by no bacterial cell number increment until a six-week storage period. The studies suggested that non-thermal plasma either work to inactive or kill the viable cells and inactive bacterial spores [30,31].

A limited study reported the effect of non-thermal plasma to milk physicochemical characteristics and nutritional values. Wu et al. [29] and Gurol et al. [31] reported no significant effects of non-thermal plasma treatment to milk color as well as its pH. Moreover, the voltage of the plasma condition affects milk viscosity. Low-voltage (<60 V) treated milk has lower viscosity compared to high-voltage treated milk because low-intensity plasma induces the shear thinning of fluid [29]. Other studies concluded that non-thermal plasma treatment did not affect fatty acid composition in milk samples [32,33].

4. Conclusion
Non-thermal plasma treatment for bacterial inactivation and milk sterilization is influenced by various factors such as reactor types, treatment types of non-thermal plasma, working gas, treatment time, and applied voltage. Higher bacterial cell number reduction occurred in a high-voltage plasma system with longer treatment time. Non-thermal plasma produces charged particles and reactive species that cause bacterial cell death due to membrane rupture, DNA damage, and cellular enzyme inactivation. Furthermore, a further study about the non-thermal effect of milk nutrition composition and physicochemical characteristics is needed to determine the optimal plasma processing parameter for future application as milk sterilization.

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