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Abstract

Background: Thermal pasteurization and sterilization are predominantly used in the dairy industry due to their efficacy in improving the product safety and shelf life. However, heat treatment can cause undesirable protein denaturation, non-enzymatic browning, loss of vitamins and volatile flavor compounds, freezing point depression, and flavour changes. Cold plasma is a non-thermal technology that has gained attention in recent years as a potential alternative method for chemical and thermal disinfection in foods using ambient or moderate temperatures and short treatment times.

Scope and approach: This review aims to describe the fundamentals, parameters, and technology on cold plasma, discussing the critical processing factors involved in this technology. Also, it describes the mechanisms of microbial inactivation and provides an overview of the effects of non-thermal plasma on the quality of dairy products, considering a physicochemical, sensory and microbiology perspective.

Key findings and conclusions: Cold plasma uses less aggressive mechanisms of action to the milk matrix when compared to the techniques currently used, and has shown an excellent performance on the elimination of pathogenic and spoilage microorganisms besides maintaining, in many cases, the nutritional, functional, and sensory characteristics of the product.

Keywords: emerging technologies; cold plasma; dairy foods; processing; microbial; food safety.

Cold Plasma processing of milk and dairy products

Nathalia M. Coutinho[1], Marcelo R. Silveira[1], Ramon S. Rocha[2], Jeremias Moraes[2], Marcus Vinicius S. Ferreira[3], Tatiana C. Pimentel[4], Monica Q. Freitas[1], Marcia C. Silva[2], Renata S.L. Raices[2], C. Senaka Ranadheera[5], Fábio O. Borges[6], Simone P. Mathias[3], Fabiano A.N. Fernandes[7], Sueli Rodrigues[8], A.G. Cruz[2]*

[1] Universidade Federal Fluminense (UFF), Faculdade de Medicina Veterinária, 24230-340, Niterói, Brazil

[2] Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Departamento de Alimentos, 20270-021, Rio de Janeiro, Brazil

[3] Universidade Federal Rural do Rio de Janeiro (UFRRJ), Instituto de Tecnologia (IT), Seropédica, 23890-000, Rio de Janeiro, Brazil

[4] Instituto Federal do Paraná (IFPR), Paranavaí, 87703-536, Paraná, Brazil

[5] The University of Melbourne, Faculty of Veterinary & Agricultural Sciences, School of Agriculture & Food, Melbourne, VIC 3010, Australia

[6] Universidade Federal Fluminense (UFF), Instituto de Física. Laboratório de Plasma e Espectroscopia Atômica, 24210-340, Niteroi, Brazil

[7] Universidade Federal do Ceará (UFC), Departamento de Engenharia Química, 60440-900 Fortaleza, Ceará, Brazil

[8] Universidade Federal do Ceará (UFC), Departamento de Engenharia de Alimentos 60440-900 Fortaleza, Ceará, Brazil

* Email:food@globo.com (A.G.Cruz)

1. Introduction

Milk contains carbohydrates (lactose), fatty acids, high-quality protein, and various micronutrients, such as vitamins, minerals, and trace elements (European Milk Forum, 2017). According to the Food and Agriculture Organization (FAO), global per capita dairy consumption will increase 12.5% by 2025 (IDFA, 2016). In addition to the nutritional value, dairy products are considered health-promoting foods for the prevention or amelioration of osteoporosis, sarcopenia, metabolic syndrome, cardiovascular disease, cognitive decline, and digestive disorders (Hess, Jonnalagadda & Slavin, 2016).

To prevent pathogenic bacteria, toxic substances, and off flavours in dairy products, fresh whole milk should be submitted to thermal processing before commercialization (Amaral et al., 2017). This step eliminates pathogenic microorganisms, and provides low counts of spoilage microorganisms, acceptable aroma and flavour and adequate physicochemical characteristics (Barba et al., 2017).

Consumers have searched for dairy products that are safe, nutritious, practical, minimally processed, environmentally friendly, healthy, appetizing, economical, and personalized but that also have a long shelf life (Mir, Shah & Mir, 2016). Thermal processing improves the microbiological safety (Misra et al., 2017a), but they extensively damage sensory, nutritional, and physicochemical properties (Mosqueda-Melgar, Elez-Martínez, Raybaudi-Massilia & Martín-Belloso, 2008, Barba et al., 2012), resulting in non-enzymatic browning, loss of vitamins and volatile flavour compounds, freezing point depression, and flavour changes in dairy products (Gurol, Ekinci, Aslan &

Korachi, 2012). In addition, they require high-energy consumption, which compromises with the final product value to guarantee the profitability of the industry (Barba et al., 2017).

Non-thermal processes can meet microbial food safety standards and improve the physical, nutritional and sensory characteristics of the products, preserving unstable bioactive compounds and modulating enzyme activity (Amaral et al., 2017). These methods include ohmic heating (Cappato et al., 2017), high hydrostatic pressure (Barba, Esteve & Frígola, 2012), pulsed electric field (Cruz et al., 2010), pulsed-light technology (Abida, Rayees & Massodi, 2014), ultrasound (Ashokkumar et al., 2010), supercritical carbon dioxide technology (Amaral et al., 2017), and irradiation (Odueke et al., 2015). However, scientific knowledge on the utilization of cold plasma as an emerging non-thermal technology for dairy products is scattered and none of the previous studies reviewed these aspects.

This review aims to describe the fundamentals, process parameters, and technology involved in cold plasma. It also describes the mechanisms of microbial inactivation and provides an overview of the effects of non-thermal plasma on the quality of dairy products, considering the physicochemical, sensory and microbial characteristics.

2. Cold Plasma

2.1 Fundamentals

Plasma is known as the fourth state of matter, and is an electrically energized matter in a gaseous state composed of charged particles, free radicals, and some radiation. It is obtained by an electrical discharge (Fernández & Thompson, 2012), in which a partially or completely ionized gas is formed that is composed of photons (essentially), ions, free electrons, and atoms in their fundamental or excited states. These species are designated light species (photons and electrons) or "heavy" species (remaining constituents) (Misra, Tiwari, Raghavarao, & Cullen, 2011, Misra et al., 2017a).

The plasma is roughly electrically neutral in a global sense, i.e., the number of positive charges and negative charges are equal (Ekezie et al., 2017, Pankaj & Keener, 2017). Matter can reach this plasma state through the application of magnetic, thermal or electric energy at radio or microwave frequencies that increase the kinetic energy of the electrons. The process parameters and gas used will determine the nature of the plasma (Phan et al., 2017).

There are two plasma classes—denominated non-thermal plasma (NTP) or cold plasma and thermal plasma—which are distinguished by the thermodynamic equilibrium between electrons and ions. Cold plasma is generated at 30-60 °C under atmospheric or reduced pressure (vacuum), requires less power, exhibits electron temperatures much higher than the corresponding gas (macroscopic temperature), and does not present a local thermodynamic equilibrium (Thirumdas, Sarangapani, & Annapure, 2015). It is suitable for treatment of heat-sensitive food products because the ions and uncharged molecules gain only a little energy and remain at a low temperature (Phan et al., 2017, Pankaj, Shi & Keener, 2018). Thermodynamics, transport phenomena, and electrochemistry of external field assisted nonthermal food technologies (Misra et al.,2017b). In contrast, thermal plasma is generated at higher pressures (>10^5 Pa) and requires high power (up to 50 MW); there is almost a local thermodynamic equilibrium between electrons and heavy species. The gas temperature is nearly the same for all plasma components and is very high (4 to 20 x 10^3 °C) (Liao et al., 2017).

Plasma jets, dielectric barrier discharges (DBD), corona discharges, and microwave discharges (Figure 1) are common sources for the generation of cold plasma at atmospheric pressure (Surowsky, Schlüter, & Knorr, 2015).

A dielectric barrier discharge (DBD) (Figure 1a) is generated between two electrodes covered with dielectric layers, which stops electric currents and prevents the formation of sparks (Moreau, Orange & Feuilloley, 2008). The name "DBD" defines its configuration, in which the discharge is blocked by a dielectric barrier layer. It is a non-equilibrium alternating or direct current discharge and usually operates at frequencies between 0.05 and 500 kHz over a wide range of gas pressures (normally 10^4–10^6 Pa) (Zhang et al., 2017). The typical gap distance in DBDs varies from 0.1 mm to several centimetres. DBD operates under reasonably high-power levels and its efficiency depends on several factors, including the gas used, the operating voltage, and the distance between the electrodes (Ehlbeck et al., 2011). The major advantages of DBDs include the wide variety of gases that can be used for plasma generation (noble gases, air or water vapour, special mixtures of precursors and almost all combinations of gases), the low (< 100 sccm) or no gas flow needed, the homogeneous discharge over a large area, and the different possible geometries of electrodes, all of which lead to good adaptability (Phan et al., 2017). A disadvantage is the high ignition voltage of at least 10 kV, depending on the restricted electrode gap, which makes precautions or isolations essential. DBD is an ideal plasma source for large surfaces (Ehlbeck et al., 2011, Phan et al., 2017).

Plasma jets (Fig. 1b) cover various configurations that enable the operation of gas discharge in a non-sealed ("open") electrode arrangement and the projection of the discharge plasma species in an open environment (Nishime et al., 2017). Small "plasma flames" that are typically generated in the radio frequency range are produced by non-thermal plasma jets at atmospheric pressure, which are capacitively coupled devices consisting of two coaxial electrodes, between which gas flows at high rates. The outer electrode is grounded, while the central electrode is excited by radio frequency (RF) power at 13.56 MHz. The free electrons are accelerated by the RF field and collide with molecules of background gas. These inelastic collisions can produce various reactive species (excited atoms and molecules, free radicals) that exit the nozzle at high velocity (Misra et al., 2016). Common gases used in the process include noble gases such as helium or argon at a high flow rate (> 10 slm). This is an expensive approach, because of the high cost requirement due to the gas flow, is acceptable for some biomedical applications, rather than food processing, in which cost is an important consideration. Furthermore, the treatment rate is overstepped by plasma jets, and the small dimension is also a disadvantage. The advantages of this type of plasma include its direct applicability and ability to penetrate into narrow gaps (Weltmann et al., 2008).

Corona discharge (Fig. 1c) is a weakly luminous discharge that usually appears at atmospheric pressure near sharp electrode geometries (points, edges or thin wires).

This is where electric field is large enough to accelerate electrons produced randomly to the level of ionization energy of the atoms or molecules of the surrounding gas (Phan et al., 2017). Typical geometries are point-to-plate geometries (sharply curved electrode arranged counterpart to a flat one) and cylindrical configurations (Elbeck et al., 2011). Ionization and luminosity are mainly located in the sharp electrode. These arrangements can work in direct current or pulsed voltage mode, and the sharp electrode can have a positive or negative potential (Pekarek, 2010). The corona system does not require a complex apparatus and can be generated in a simple device that does not require large implementation and operating expenses. Its main limitation is that it acts only on a small area, with non-uniform treatment (Scholtz, Julak & Kriha, 2010).

Microwave-driven (MW-driven) discharges (Fig. 1d) are generated without electrodes; emanated by a magnetron; typically occur at 2.45 GHz; and guided to the process chamber by a waveguide or a coaxial cable, where they reach the electrons present in the process gas (Tolouie et al., 2017). The electrons absorb the microwaves, leading to an increase in kinetic energy and thus in ionization reactions due to inelastic collisions (Schlüter & Fröhling, 2014). The major advantage of MW-driven discharges is the electrode-less setup, which is easy to handle. They can be ignited in an air environment, even with special admixtures, precursors or water vapour. However, the spatial limitation is the disadvantage. An array of discharges must be used for direct decontamination of large areas (Ehlbeck et al., 2011).

The ability of plasma to work at moderate operating temperatures has opened up the possibility of using cold plasma for disinfection and sterilization of heat sensitive materials for which thermal plasma cannot be used (Misra et al., 2016). Plasma processing modifies the food material to the desired characteristics, maintains texture and nutritional properties, and promotes microbial decontamination. In addition, a wide range of microorganisms, including spores and viruses (Misra & Jo, 2017, Barba et al., 2017) can be inactivated by plasma treatment.

2.2 Effectiveness of cold plasma in microbial inactivation

Cold plasma is a relatively new non-thermal technology that efficiently inactivates microorganisms, including bacteria, bacterial spores, fungi, and biofilms (Segat et al., 2016). Figure 2 presents an overview of cold plasma microbial inactivation. Three basic mechanisms are triggered by the plasma and contribute to cell death, including etching of cell surfaces induced by reactive species formed during plasma generation, volatilization of compounds and intrinsic photodesorption of ultraviolet (UV) photons, and destruction of genetic material (Moisan et al., 2002; Laroussi, 2005).

Many agents can act during plasma application, such as radicals and chemical products, e.g., NxOy, atomic oxygen (O), ozone (O_3), hydroxyl (OH), reactive oxygen (ROS) and nitrogen species (RNS), high-energy UV radiation, radiation in the visible and infrared spectral range, charged particles, alternating electric fields, and physical and chemical etch processes. The combination of these agents makes cold plasma attractive because it is almost impossible for pathogens to develop resistance against these plasma stress factors (Ehlbeck et al., 2011).

Intrinsic photodesorption can be induced by UV irradiation and leads to the breakage of chemical bonds in microorganisms, followed by the formation of volatile by-products such as CO and CHx from the intrinsic atoms of the microorganisms (Schlüter & Fröhling, 2014). The apparent contribution of UV from plasma sources is dependent on the configuration of the plasma source, the operating pressure, and the scale of the plasma discharge, among others (Misra & Jo, 2017).

Microorganisms are exposed to intense bombardment by plasma radicals (OH and NO), which are absorbed onto the bacterial surface and form volatile compounds (CO_2 and H_2O). These compounds provoke lesions on the surface that the cell cannot repair, resulting in cell death. Furthermore, the cell membrane is exposed to intense electric fields that can provoke rupture due to the electrostatic tension experienced from the high electrical charge developed within (Misra & Jo, 2017).

The formation of pores results in higher membrane permeability, which directly affects the cell transmembrane potential and regulation of intracellular pH, with acidification provoked by the humid air plasma. However, many microorganisms have very dense cytoplasm and cytoplasmic proteins with buffer capacity, resulting in maintenance of the pH. Thus, this parameter is a non-essential factor for bacterial inactivation (Moreau, Orange & Feuilloley, 2008). Furthermore, the formation of pores contributes to release of the inner fluid, precluding stimulation of anti-stress action, and repairing microorganismal processes. Subsequently, the active species are transported into the cell, where internal cell damage can occur through destruction of DNA, proteins, and other internal cell components (Phan et al., 2017).

Changes in membrane integrity can directly affect DNA mainly through the breakdown of interactions between membrane proteins and DNA, as well as pore formation that results in the release of DNA from the cell. Furthermore, partial fragmentation of the DNA due to exposure to electrical discharges can be observed. The effect of plasma on DNA is related to a combination of activities of free radicals (NO and OH) and UV, which can form thymine dimers and strand breaks, thus inhibiting the ability of bacteria to replicate (Moreau, Orange & Feuilloley, 2008).

A reaction between reactive oxygen species (ROS) and the cellular macromolecules is observed after bacterial exposure to plasma. ROS cause protein denaturation and cell leakage at equal amounts in cells and spores (Critzer, Kelly-Winterberg, South, & Golden, 2007). The ROS reacts with membrane lipids, resulting in the formation of unsaturated fatty acid peroxides, while the amino acids and nucleic acids are oxidized to 2-oxo-histidine and 8-hydroxy-2 deoxyguanosine, respectively. It is believed that the lipids are the most vulnerable macromolecules in cell membranes, likely due to their location near the surface (Liao et al., 2017). Alteration of membrane lipids results in leakage of macromolecules (Schlüter & Fröhling, 2014). The inactivation mechanisms cause several reactions in bacterial cells, including lipid peroxidation of polyunsaturated fatty acids and oxidation of amino acids and DNA (Misra & Jo, 2017).

Table 1 presents the important parameters for the efficiency of cold plasma treatment in microbial inactivation, considering the processing conditions, product, and equipment parameters. The gas or gas mixture used for plasma generation is important for considering their effects on antimicrobial efficacy and process cost (Misra & Jo, 2017). Plasma discharges with a higher oxygen concentration are associated with increased levels of microbial inhibition, due to higher levels of oxygen-based active species, including atomic oxygen and ozone (Misra & Jo, 2017).

In the context of gas-liquid environments, the most important reducing agents are hydrogen radicals (H) and superoxide radicals (O_2^-) due to their degradation of compounds containing nitro groups and compounds with high electron affinity (Misra et al., 2016). Noble gases are traditionally used in cold plasma due to several reasons, including high thermal conductivity (for heat removal), rich ultraviolet emission spectra, and lower operating discharge voltage at atmospheric pressure. However, they are more expensive than air, making air a better option (Misra & Jo, 2017).

Gas flow is an important parameter that determines the velocity with which active species are driven to the target location. Depending on the flow rate, some short-lived species may not reach the sample (Nishime et al., 2017). Gas flow rate can influence the operation of discharge, the retention time and the mass transfer process of reactive species. Increasing the flow rate can enhance the collision and reaction possibilities of the reactive species (Zhang et al., 2017).

The reactive species generated in the discharge also depend on the electrical input (voltage, frequency, and power) used in the process. Greater electrical input and longer treatment lead to higher process efficiency (Liao et al., 2017). However, food quality parameters must be evaluated in the selection of electrical input parameters.

Furthermore, the improvement of efficacy with increasing time increases until saturation because the limited lifetime of the active species does not allow coverage of larger sample areas, even with increased treatment time (Nishime et al., 2017).

Plasma sources can be operated in either direct or indirect mode (also known as a remote or afterglow). Direct plasma application is characterized by the inclusion of a greater variety of reactive species, most with very short lifetimes (milliseconds). There may also be surface plasma reactions, such as etching and deposition (Misra & Jo, 2017). In indirect or remote plasmas, only "plasma exhaust" containing longer-living reactive species such as nitric oxide or ozone contact the food, and plasma is generated in a separate chamber (Surowsky, Schlüter, & Knorr, 2015). In indirect operation mode, the quantum of heat transmitted to a sample is reduced, and the charged particles do not play a role since they recombine before reaching the sample. In addition, many short-lived neutral reactive species do not reach the sample (Misra et al., 2011). In general, direct exposure is more efficient than indirect exposure. However, it can be somewhat more challenging to build and operate compared to indirect treatment systems (Niemira, 2012).

Relative humidity (RH) plays an important role in the microbial effect of the cold plasma because it influences the generation of reactive species, thus affecting the overall process. Studies have shown that an optimal amount of water can lead to higher inactivation efficiency, and vice versa; excess water leads to dilution of the effects. With increased RH, there is an increase in peroxyl acid groups and OH production due to the decomposition of additional water molecules (Guo, Huang & Wang, 2015, Liao et al., 2017). Therefore, the addition of water vapour to the system can originate atomic species that enhance the antimicrobial effect.

The characteristics of the microorganism are important for the process effectiveness because sensitivity to treatment can differ among microorganisms, even for similar species or strains. Microorganisms in the stationary phase or sporulated form are more resistant than those in the exponential phase or vegetative form. In addition, fungal resistance against cold plasma is commonly higher than in bacteria because the cell wall of fungi consists of chitin, which is more rigid than the peptidoglycan of bacterial cell walls (Liao et al., 2017). Studies have shown greater efficiency of cold plasma in the inactivation of gram-negative bacteria compared to gram-positive bacteria (Schlüter & Fröhling, 2014; Liao et al., 2017). This is due to the presence of a thick peptidoglycan structure on the outside of gram-positive cells that is resistant to chemical changes; in contrast, gram-negative bacteria are more susceptible to membrane rupture since they have a roughened, thin membrane and electrostatic force can overcome the tensile strength of this outer membrane (Nishime et al., 2017).

The initial microorganism concentration in food is an important parameter in determining the efficiency of cold plasma processing. A higher initial concentration decreases the inactivation effect of the cold plasma likely because higher microorganism counts cluster more cells together, decreasing the ability of plasma active ingredients to reach cell. This parameter should be considered when selecting the process parameters of the cold plasma apparatus, so that the microbial load can decrease to suitable values (Liao et al., 2017).

The performance of cold plasma also depends on the raw materials used in processing. For solid foods, such as cheeses, the treatment is usually limited to the product surface. The ability of plasma and reactive species to penetrate solid foods is dependent on several factors, including physicochemical composition, water content, and porosity. However, in general, cold plasma has limited penetration depth (Surowsky, Bußler & Schlüter, 2016). Short-lived reactive plasma species can directly react with the external cell membrane and be transported into the cell, where internal cell damage can occur. In liquid foods, such as milk, yoghurt, dairy drinks, etc., each volume element comes into contact with the applied plasma (or at least subsequent reaction products), so that the penetration depth is of minor importance. In this case, microorganisms and all other surrounding components are affected. Therefore, optimization is required for good antimicrobial efficacy and retention of other food constituents (Surowsky, Bußler & Schlüter, 2016).

The physicochemical parameters of the dairy product are also important for microbial inactivation by cold plasma. The acidity of the food matrix has been found to affect bacterial resistance towards some stress, with lower microbial tolerance in more acidic products. In addition, higher moisture content of the product is related to an increased effect of cold plasma on microbial inactivation, likely due to increased decomposition of additional water molecules into hydroxyl radicals (Liao et al., 2017).

Food shape and porosity can influence antimicrobial treatment with cold plasma. It is difficult to treat bulky, irregularly shaped food, once the rough surface provides numerous sites for microorganisms to attach and potentially escape antimicrobial treatment (Misra & Jo, 2017). For packaged food, in-package plasma treatments, through-package, or open treatments can also be applied. In in-package cold plasma treatment, bactericidal molecules are generated and contained in the package, allowing higher exposure to pathogenic microbes while reverting to the original gas within a few hours of storage. Antimicrobial treatment inside a sealed package can ensure the prevention of post-processing contamination (Misra et al., 2013). In addition, the composition and surface characteristics of electrodes are important because they guide discharge and can evolve during the process. The distance between the electrodes where the plasma is generated, and the target must be evaluated, as greater distance leads to the less effective treatment (Moreau, Orange & Feuilloley, 2008). Studies on the efficacy of cold plasma on microbial inactivation in dairy foods have mainly focused on DBD or corona discharge plasma sources. The first is preferred because it is easy to design, generates more reactive compounds, and works well in air or oxygen/nitrogen mixtures (Misra & Jo, 2017).

3. Impact of Cold plasma on the quality of dairy foods

3.1 Microbiological aspects

Infectious diseases caused by the ingestion of pathogenic bacteria in contaminated milk are still a major health concern, especially for children. The most predominant infectious diseases caused by contaminated milk include campylobacteriosis, salmonellosis, yersiniosis, listeriosis, tuberculosis, brucellosis, staphylococcal enterotoxin poisoning, streptococcal infections and *Escherichia coli* 0157:

H7 (Gurol, Ekinci, Aslan & Korachi, 2012; Ranadheera, Prasanna, Vidanarachchi, McConchie, Naumovski, & Mellor, 2017).

Although many studies have focused on the decontaminating ability of plasma technology, there are limited investigations into the effect of cold plasma on food products themselves, especially dairy foods (Gurol et al., 2012, Lee et al., 2012, Song et al., 2009, Yong et al., 2015a,b, Kim et al., 2015). The application of cold plasma to microorganism control has not been fully studied, as this high-complexity technology with a diversity of devices was not developed for biological applications (Moreau, Orange & Feuilloley, 2008). Table 2 shows the published studies using cold plasma in milk and dairy products, including experimental conditions for the microbial inactivation of cold plasma in several dairy foods, such as sliced cheese (Song et al., 2009; Lee et al., 2012, Yong et al., 2015a,b); whole, semi-skimmed, and skimmed UHT milk (Gurol et al., 2012); and milk samples (Kim et al., 2015). All studies confirm the potential of cold plasma to inactivate harmful microorganisms in milk and dairy products. The antimicrobial effects of plasma are mainly due to interactions between the reactive oxygen species (ROS) and reactive nitrogen species, leading to strong oxidative effects on double bonds in the lipid bi-layer of the microbial cell, and damaging the transport of macromolecules inside and outside the cell. These reactive species can be considered the most important agents that participate in pathogen inactivation (Phan et al., 2017). In dairy products, the antimicrobial efficiency of cold plasma technology depends on several factors, including the species of the target microorganism, input power, treatment time, gas composition, and food composition.

3.2 Physicochemical and sensory aspects

Physicochemical and sensory characteristics are important parameters for the quality and preservation of dairy products. Although the ions present in cold plasma give rise to an antimicrobial effect, studies of the possible negative effects on the physicochemical and sensory characteristics of the treated product, especially those with high nutritious value, are required (Korachi et al., 2015). There are few studies in the literature that evaluate the effect of cold plasma technology on the physicochemical and/or sensory characteristics of dairy products (Gurol et al., 2012, Lee et al., 2012, Korachi et al., 2015, Segat et al., 2015, 2016, Yong et al., 2015b, Kim et al., 2015).

Table 3 shows published studies using cold plasma technology in milk and dairy products, including experimental conditions for the quality standards of dairy foods. Inconclusive findings were reported for milk pH (Gurol et al., 2012), while interesting findings were observed for the instrumental colour parameters of sliced cheese (Lee et al., 2012; Yong et al., 2015b); for the fatty acid profile, volatile compounds and protein content of milk (Kim et al., 2015); for milk fat (Sarangapani et al., 2017); and for whey protein isolate (Segat, Misra, Cullen & Innocent, 2015), although these differences were not observed by trained panellists. The results indicate that cold plasma could be a prospective alternative to traditional thermal food pasteurization methods, because of the minimized colour changes (Maillard browning) and formation of off-flavours and losses of nutritional value. However, the disparate findings suggest the need for optimization of cold plasma parameters for industrial applications.

The action of plasma on endogenous milk enzymes is similar to that occurring in microorganisms. Enzymes are inactivated by oxidation reactions of peptides that change the conformation of proteins, thus decreasing their enzymatic activity. This technology is currently used in milk and dairy products. Recent studies by Segat, Misra, Cullen & Innocent (2016) evaluated the effect of ACP on the activity and structure of alkaline phosphatase (ALP), an indigenous milk enzyme. ALP in solution was subjected to ACP at three discrete high voltages (40, 50, and 60 kV) for durations of 15 s to 5 min. The results demonstrated that dielectric barrier discharge-based plasma technology was able to inactivate the enzyme within a few seconds. The dichroic spectra suggested that the enzyme was characterized by a predominant α-helix structure, and the helical content showed a decreasing tendency with increasing treatment time and voltage. The maximum temperature recorded for the most intense treatments was on the order of only 30°C, with no changes in pH.

Studies in the literature have shown that the physicochemical characteristics of milk and dairy products are not extensively affected by cold plasma treatment. Despite some observed instrumental colour differences, many studies have reported no differences that are detectable by the human eye. Cold plasma-treated milk can have higher acidity, which may be due to the multistep reactions of plasma-generated reactive species—including NOx, O, and O_3—with water at the gas-water interface. Differences in the acidity of plasma-treated liquids may arise from several factors, including the volume treated, the buffering capacity, and the plasma source and inducer gas used. For cheese, an increase in lipid oxidation can occur, which may induce off flavours and affect consumer acceptance. Thus, the application of this technology to high-fat products should be carefully evaluated. Cold plasma can be successfully used to selectively modify protein structure and improve WPI functionality. It was also able to inactivate alkaline phosphatase (ALP) within a few seconds.

4. Advantages, Disadvantages, and Limitations of cold plasma

Cold plasma is a newcomer to the food technology field. Like all techniques, it presents advantages and disadvantages. Table 4 shows the main advantages, disadvantages, and limitations of cold plasma treatment for food preservation.

The advantages of plasma processes include high microbial inactivation efficiency at low temperatures; low impact on the internal product matrix; on-demand production of the acting agent; precise generation of plasmas suitable for the intended use; and the absence of water, solvents or residues; it is also a resource-efficient technology. Plasma processing is environmentally safe and can fulfil all ecological standards, once the active species disappear after the plasma power is turned off (Misra, Tiwari, & Cullen, 2011). However, the disadvantages of cold plasma process for food sterilization are evidenced in the treatment of bulky and irregularly shaped food. The volume and size of food should be considered because microbial inactivation occurs on the food surface and thus reactive plasma species can only penetrate foods to a limited extent (Song et al., 2009). The rough surface of some products provides numerous sites for microorganisms to attach and potentially escape antimicrobial treatment (Yong et al., 2015b).

In addition, there are other limitations for the widespread adoption of cold plasma by the dairy industry (Deeth & Datta, 2011). First, thermal processing is well established and has served the industry very well. Any new technology to replace a thermal process must offer clear advantages in terms of costs and product quality, or it must be able to perform additional functions that cannot be performed by heat alone. Furthermore, suitable tests of efficacy equivalent to the alkaline phosphatase test for thermal processing of milk must be conducted, and based on the results, regulatory authorities can approve the technology for a "Pasteurization" purpose. In addition, some potential pathogenic organisms have been shown to be resistant to certain technologies, but there are studies investigating these organisms and cold plasma technology. One of the main difficulties is precisely defining the operating conditions, which precludes comparison across studies and the scale-up from laboratory to industry. Finally, the sensory acceptance of treated products and the production of undesirable flavours have not been extensively studied.

Considering the applicability of cold plasma in food industry, the economic cost associated with the use of the new technology in comparison with the heat treatment should be assessed. The cost mainly depends on the investment of the equipment, energy cost of the treatment and general production costs (Barba et al., 2017). The machine should be inexpensive, process continuously at high speed with least maintenance and operates with a variety of gases. Therefore, it is of primordial importance to avoid using costly noble gases due to the low operating margins. Ideally, plasma sources capable of ionizing air at large gaps will be suitable. Furthermore, plasma sources operating at line frequency rather than radio-frequency power sources could result in cost management (Keener & Misra, 2016). The non-thermal methodologies usually have higher costs than the thermal processes, but these costs are expected to decrease as more efforts are made to commercializing them. Moreover, the advantages on the sensory and quality properties of the products could outweigh the relatively higher cost (Li & Farid, 2016).

5. Perspectives

Cold plasma technology can be utilized as a novel antimicrobial intervention for the inactivation of pathogens and improvement of dairy product safety. The technique is classified as environmentally safe, fits all ecological standards, has high microbial inactivation efficiency at low temperatures, and a low impact on the product matrix. It creates on-demand production of the acting agent and precise generation of plasma suitable for the intended use, and importantly, requires no water or solvents, generates no residue, and is resource-efficient. However, studies performed to date have also demonstrated certain limitations of cold plasma treatment in dairy products, such as the acceleration of lipid oxidation and a negative impact on the sensory characteristics of processed products. Further investigations are required to elucidate the effects of cold plasma on the quality parameters of dairy products, including sensory characteristics, the retention of nutritional value, and the storage stability of the products. In addition, the effect of non-thermal plasma technology compared to conventional heat treatments should be studied.

Currently, plasma systems are not commercially available as a sterilizing tool in the food industry, mainly because they come in many size, shape, and state, and the area has not attracted the interest of physicists and engineers to a sufficient degree. Medical scientists and physicists have already established a good collaboration, and now some commercial scale results can be observed. Therefore, important aspects of this technology are still immature, particularly concerning its use in food. The application of NTP to food products must be studied in depth to supply a basis for the feasibility of plasma for large-scale commercial production. Once food security concerns are clarified, plasma processing must be scaled up for industrial applications.

References

Abida, J., Rayees, B.& Masoodi, F. A. (2014). Pulsed light technology: a novel method for food preservation. *International Food Research Journal*, 21, 839-848.

Amaral, G.V., Silva, E.K, Cavalcanti, R.N., Cappato, L.P., Guimaraes, J.T., Alvarenga, V.O., Esmerino, E.A., Portela, J.B., Sant' Ana, A.S., Freitas, M.Q., Silva, M.C., Raices, R.S.L., Meireles, M.A.A., Cruz, A.G. (2017). Dairy processing using supercritical carbon dioxide technology: Theoretical fundamentals, quality, and safety aspects. *Trends in Food Science & Technology*, 64, 94-101.

Ashokkumar, M., Bhaskaracharya, R., Kentish, S., Lee, J., Palmer, M., & Zisu, B. (2010). The ultrasonic processing of dairy products– An overview. *Dairy Science Technology*, 90, 147–168.

Barba, F. J., Esteve, M. J., & Frígola, A. (2012). High-pressure treatment effect on physicochemical and nutritional properties of fluid foods during storage: a review. *Comprehensive Reviews in Food Science and Food Safety*, 11, 307-322.

Barba, F. J., Koubaa, M., Prado-Silva, L., Orlien, V. & Sant'Ana, A. (2017). Mild processing applied to the inactivation of the main foodborne bacterial pathogens: A review. *Trends in Food Science & Technology*, 66, 20-35.

Calvo, T., Alvarez-Ordóñez, A., Prieto, M., Bernardo, A. & López, M. (2017). Stress adaptation has a minor impact on the effectivity of Non-Thermal Atmospheric Plasma (NTAP) against *Salmonella* spp. *Food Research International,* 102, 519-525.

Cappato, L. P., Ferreira, M. V. S., Guimarães, J. T., Portela, J. B., Costa, A. L. R., Freitas, M. Q., Cunha, R. L., Oliveira, C. A. F., Mercali, G. D., Marzack, L. D. F., & Cruz, A. G. (2017). Ohmic heating in dairy processing: Relevant aspects for safety and quality. *Trends in Food Science & Technology*, 62, 104-112.

Critzer, F., Kelly-Wintenberg, K., South, S., & Golden, D. (2007). Atmospheric plasma inactivation of foodborne pathogens on fresh produce surfaces. *Journal of Food Protection*, 70, 2290-2296.

Cruz, A. G., Faria, J. A. F., Saad, M. I. S., Bolini, H. M. A., Sant'Ana, A. S., & Cristianini, M. (2010). High-pressure processing and pulsed electric fields: potential use in probiotic dairy foods processing. *Trends in Food Science & Technology*, 21, 483-493.

Deeth, H. C. & Datta, N. (2011). Heat Treatment of Milk | Non-Thermal Technologies: Introduction. In: Fuquay, J. W, Encyclopedia of Dairy Sciences. 2nd Ed. Academic Press, p.725-731.

Ehlbeck, J., Schnabel, U., Polak, M., Winter, J., Von Woedtke, T., Brandenburg, R., Weltmann, KD. (2011). Low-temperature atmospheric pressure plasma sources for microbial decontamination. *Journal of Physics D: Applied Physics*, 44, 013002.

Ekezie, F-G. C., Sun, D-W., Cheng, J-H. (2017). A review on recent advances in cold plasma technology for the food industry: Current applications and future trends. *Trends in Food Science and Technology*, 69, Part A, 46-58.

European Milk Forum. (2017). Milk facts nutritional info. Nutrient richness. Available in: http://www.milknutritiousbynature.eu/milk-facts/nutritional-info/. Accessed in: 19/07/2017.

Fernández, A., & Thompson, A. (2012). The inactivation of Salmonella by cold atmospheric plasma treatment. *Food Research International*, 45, 678-684.

Guo J., Huang K., Wang J. (2015). Bactericidal effect of various non-thermal plasma agents and the influence of experimental conditions in microbial inactivation: A review, *Food Control*, 50, 482-490.

Gurol, C., Ekinci, F. Y., Aslan, N., Korachi, M. (2012). Low-Temperature Plasma for decontamination of *E. coli* in milk. *International Journal of Food Microbiology*, 157, 1–5.

Hess, J. M., Jonnalagadda, S. S., & Slavin, J. L. (2016). Dairy foods: current evidence of their effects on bone, cardiometabolic, cognitive, and digestive health. *Comprehensive Reviews in Food Science and Food Safety*, 15, 251-268.

IDFA. (2016). Bulletin of the International Dairy Federation 485/2016. 1-6.

Keener, K. M. & Misra, N. N. (2016). Future of cold plasma in food processing. In: Misra, N. N., Schlüter, O. K, Cullen, P. J. Cold plasma in food and agriculture. Fundamentals and application. London: Elsevier, Chapter 14, p. 343-360.

Kim, H-J., Yong, H. I., Park, S., Kim, K., Choe, W., & Jo, C. (2015). Microbial safety and quality attributes of milk following treatment with atmospheric pressure encapsulated dielectric barrier discharge plasma. *Food Control*, 47, 451-456.

Korachi, M., Ozen, F., Aslan, N., Vannini, L., Guerzonic, M. E., Gottardi, D., & Ekinci, F. Y. (2015). Biochemical changes to milk following treatment by a novel, cold atmospheric plasma system. *International Dairy Journal*, 42, 64-69.

Laroussi, M. (2005). Low-temperature plasma-based sterilization: Overview and state-of-the-art. *Plasma Processes and Polymers*, 2, 391–400.

Lee, H. J., Jung, S., Jung, H., Park, S., Choe, W., Ham, J. S., & Jo, C. (2012). Evaluation of a dielectric barrier discharge plasma system for inactivating pathogens on cheese slices. *Journal of Animal Science and Technology*, 54, 191-198.

Li, X. & Farid, M. (2016). A review on recent development in non-conventional food sterilization technologies. *Journal of Food Engineering,* 182, 33-45.

Liao, X., Liu, D., Xiang, Q., Ahn, J., Chen, S., Ye, X. & Ding, T. (2017). Inactivation mechanisms of non-thermal plasma on microbes: A review. *Food Control*, 75, 83-91.

Miao, H., &Yun, G. (2011). The sterilization of Escherichia coli by dielectric-barrier discharge plasma at atmospheric pressure. *Applied Surface Science*, 257, 7065–7070.

Mir, S. A., Shah, M. A., & Mir, M. M. (2016). Understanding the role of plasma technology in food industry. *Food Bioprocess Technology*, 9, 734-750.

Misra, N. N., Tiwari, B. K., Raghavarao, K. S. M. S., & Cullen, P. J. (2011). Nonthermal Plasma Inactivation of Food-Borne Pathogens. *Food Engineering Reviews*, 3, 159-170.

Misra, N.N., Ziuzina, D., Cullen, P.J., & Keener, K.M. (2013). Characterization of a novel atmospheric air cold plasma system for treatment of packaged biomaterials. *Trans. ASABE*, 56, 1011–1016.

Misra, N.N., Schlüter, O. & Culler, P. J. (2016). Plasma in food and agriculture. In: Misra, N. N., Schlüter, O. K, Cullen, P. J. Cold plasma in food and agriculture. Fundamentals and application. London: Elsevier, Chapter 7, p.1-16.

Misra, N. N., Pankaj, S. K., Segat, A., Ishikawa, K. (2016). Cold plasma interactions with enzymes in foods and model systems. *Trends in Food Science & Technology*, 55, 39-47.

Misra, N. N. & Jo, C. (2017). Applications of cold plasma technology for microbiological safety in meat industry. *Trends in Food Science & Technology*, 64, 74-86.

Misra, N. N., Koubaa, M., Roohinejad, S., Juliano, P., Alpas, H., Inácio, R. S., Saraiva, J. A., & Barba, F. J. (2017a). Landmarks in the historical development of twenty-first century food processing technologies. *Food Research International*, 97, 318-339.

Misra, N. N., Martynenko, A., Chemat, F., Paniwnyk, L., Barba, F. J., & Jambrak, A. R. (2017b). Thermodynamics, transport phenomena, and electrochemistry of external field-assisted nonthermal food technologies. *Critical Reviews in Food Science and Nutrition,* 31, 1-32

Moisan, M., Barbeau, J., Crevier, M.C., Pelletier, J., Philip, N., &Saoudi, B. (2002). Plasma sterilization. Methods and mechanisms. *Pure and Applied Chemistry*, 74,349–358.

Moreau, M., Orange, N., & Feuilloley, M. G. J. (2008). Non-thermal plasma technologies: New tools for bio-decontamination. *Biotechnology Advances*, 26, 610-617.

Mosqueda-Melgar, J., Elez-Martínez, P., Raybaudi-Massilia, R.M., & Martín-Belloso, O. (2008). Effects of Pulsed Electric Fields on Pathogenic Microorganisms of Major Concern in Fluid Foods: A Review. *Critical Reviews in Food Science and Nutrition*, 48, 747–759.

Niemira, B.A. (2012). Cold plasma reduction of *Salmonella* and *Escherichia coli* O157: H7 on almonds using ambient pressure gases. *Journal of Food Science*, 77, M171–M175.

Nishime, T. M. C., Borges, A. C., Koga-Ito, C. Y., Machida, M., Hein, L. R. O. & Kostov, K. G. (2017). Non-thermal atmospheric pressure plasma jet applied to inactivation of different microorganisms. *Surface and Coatings Technology*, 312, 19-24.

Odueke, O. B., Farag, K. W., Baines, R. N. & Chadd, S. A. (2016). Irradiation Applications in Dairy Products: a Review. *Food and Bioprocess Technology*, 9, 751-767.

Pankaj, S. K. & Kenner, K. M. (2017). Cold plasma: background, applications and current trends. *Current Opinion in Food Science.* 16, 49-52.

Pankaj, S. K., Shi, H. & Kenner, K. M. (2018). A review of novel physical and chemical decontamination technologies for aflatoxin in food. *Trends in Food Science & Technology.* 71, 73-83.

Phan, K. T. K., Phan, H. T., Brennan, C. S. & Phimolsiripol, Y. (2017). Nonthermal plasma for pesticide and microbial elimination on fruits and vegetables: an overview. *International Journal of Food Science and Technology*. 52, 2127–2137.

Pekarek, S. (2010). DC corona discharge ozone production enhanced by a magnetic field. *The European Physical Journal D*, 56, 91–98.

Ranadheera, C. S., Prasanna, P. H. P., Vidanarachchi, J. K., McConchie, R., Naumovski, N., & Mellor, D. (2017). Nanotechnology in microbial food safety. In A. E. Opera & A. M. Grumezescu (Eds.), *Nanotechnology applications in food* (pp. 245–265). London: Elsevier.

Saragapani, C., Keogh, D. R., Dunne, J., Bourke, P., & Cullen, P. J. (2017). Characterisation of cold plasma treated beef and dairy lipids using spectroscopic and chromatographic methods. *Food Chemistry*, 235, 324-333.

Scholtz, V., Julak, J., & Krıha, V. (2010). The microbicidal effect of low-temperature plasma generated by corona discharge: comparison of various microorganisms on an agar surface or in aqueous suspension. *Plasma Processes Polymers*, 7, 237–243.

Schlüter, O. & Fröhling, A. (2014). Cold Plasma for Bioefficient Food Processing. In: Batt, C. A., Tortorello, M-L. Encyclopedia of Food Microbiology, v. 2, London: Academic Press, p.948-953.

Segat, A., Misra, N. N., Cullen, P. J., & Innocente, N. (2015). Atmospheric pressure cold plasma (ACP) treatment of whey protein isolate model solution. *Innovative Food Science and Emerging Technologies*, 29, 247–254.

Segat, A., Misra, N. N., Cullen, P. J., & Innocente, N. (2016). Effect of atmospheric pressure cold plasma (ACP) on activity and structure of alkaline phosphatase. *Food and Bioproducts Processing*, 98, 181-188.

Song, H. P., Kim, B., Choe, J. H., Jung, S., Moon, S. Y., Choe, W., et al. (2009). Evaluation of atmospheric pressure plasma to improve the safety of sliced cheese and ham inoculated by 3-strain cocktail *Listeria monocytogenes*. *Food Microbiology*, 26, 432-436.

Surowsky, B., Schlüter, O., Knorr, D. (2015). Interactions of Non-Thermal Atmospheric Pressure Plasma with Solid and Liquid Food Systems: A Review. *Food Engineering Reviews*, 7, 82–108.

Surowsky, B., Bußler, S., & Schlüter, O. K. (2016). Cold plasma interactions with food constituents in liquid and solid food matrices. In: Misra, N. N., Schlüter, O. K, Cullen, P. J. Cold plasma in food and agriculture. Fundamentals and application. London: Elsevier, Chapter 7, p.179-204.

Thirumdas, R., Sarangapani, C., & Annapure, U. S. (2015). Cold Plasma: A novel Non-Thermal Technology for Food Processing. *Food Biophysics*, 10,1–11.

Tolouie, H., Hashemi, M., Mohammadifar, A. M. &Ghomi, H. (2017). Cold atmospheric plasma manipulation of proteins in food systems. Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2017.1335689.

Weltmann K. D., Brandenburg, R., Von Woedtke, T., Ehlbeck, J., Foest, R., Stieber, M., & Kindel, E. (2008). Antimicrobial treatment of heat sensitive products by miniaturized atmospheric pressure plasma jets (APPJs). *Journal of Physics D: Applied Physics*, 41, 194008, 1-6.

Yong, H. I., Kim, H-J., Park, S., Alahakoon, A. U., Kim, K., Choeb, W., & Jo, C. (2015a). Evaluation of pathogen inactivation on sliced cheese induced by encapsulated atmospheric pressure dielectric barrier discharge plasma. *Food Microbiology*, 46, 46-50.

Yong, H. I., Kim, H-J., Park, S., Kim, K., Choeb, W., Yu, S. J. & Jo, C. (2015b). Pathogen inactivation and quality changes in sliced cheddar cheese treated using flexible thin-layer dielectric barrier discharge plasma. *Food Research International*, 69, 57-63.

Zhang, H., Ma, D., Qiu, R., Tang, Y. & Du, C. (2017). Non-thermal plasma technology for organically contaminated soil remediation: A review. *Chemical Engineering Journal*, 313, 157-170.

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Authors:

N.M. Coutinho, M.R. Silveira, R.S. Rocha, J. Moraes, L.C. Pimentel, M.Q. Freitas, M.C. Silva, , Renata S.L. Raices, Fabiano A.N. Fernandes, S. Rodrigues, A.G. Cruz

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Table 1. Important parameters of the cold plasma

Parameter	Factor	General characteristic	Cold plasma application	References
Processing parameters	Gas composition	The nature and the proportions of the active species in the discharge and the treatment efficiency depends on the type of gas used in the process.	Plasma discharges with a higher oxygen concentration, due to the higher levels of oxygen-based active species, such as atomic oxygen and ozone, have been associated with higher microbial inhibition.	Misra & Jo (2017)
	Gas flow	The gas flow determines the velocity with which the active species are driven to the target location.	The carrier gas flow influences the operation of discharge, the mass transfer, and the retention time of the reactive species. Increasing the flow rate can enhance the collision and reaction possibilities of the reactive species	Zhang et al. (2017), Calvo et al. (2017)
	Electrical input (voltage, frequency, power)	Electrical input has influence on the microbial inactivation by cold plasma	The greater the electrical input the higher the efficiency of the treatment. Food quality parameters must be evaluated in the selection of electrical input parameters values.	Liao et al. (2017), Nishime et al. (2017)
	Mode of plasma exposure	Exposure can be direct or indirect/remote	If exposed remotely, the quantum of heat transmitted to a sample is reduced, and the charged particles do not play a role since they recombine before reaching the sample; in addition, many of the short-lived neutral reactive species also do not reach the sample. Direct exposure is more efficient than indirect exposure.	Misra et al. (2011), Niemira, (2012)
	Treatment time	Time of treatment has an influence on the microbial inactivation.	The greater the treatment time the higher the efficiency of the treatment, until saturation.	Nishime et al. (2017)
	Relative humidity	Relative humidity (RH) influences the generation of reactive species, thus affecting the overall process.	RH accelerated the bacterial spore inactivation rate.	Guo, Huang & Wang (2015), Liao et al. (2017)
Product parameters	Type and initial concentration of microorganism	The sensitivity to the treatment can differ among microorganisms, even for similar species or strains. The initial load is important.	Microorganisms in both stationary phase and sporulated form are more resistant to destruction than those in exponential phase and vegetative form. Greater inactivation efficiency of gram-negative bacteria in comparison to the gram-positive bacteria. Higher initial concentration decreases the cold plasma inactivation effect.	Liao et al. (2017), Ekezie et al. (2017)
	Food raw material	There are two major differences between the application of plasma on solid/dry media and liquid media: the penetration depths or contact surface between plasma and food, and the chemistry/physics initiated by ROS.	The application on solid foods is usually limited to a treatment on their surface. In liquid foods, every volume element comes into contact with the plasma (or at least with subsequent reaction products). In this case, both the microorganisms and all other surrounding components are affected. Therefore, an optimization regarding a good antimicrobial efficacy and retention of other food constituents is required.	Surowsky, Bußler & Schlüter (2016)

	Food composition	Moisture, protein, fat and other components.	In high-fat dairy foods the reactive oxygen species formed can lead to oxidation. Cold plasma can result in protein and carbohydrate oxidation, amino acid oxidation, hydrogen bonding disruption and prosthetic group modification.	Sarangapani et al. (2017)
	Food shape and porosity	The foods shape and porosity can influence the antimicrobial treatment by cold plasma	Difficult with bulky and irregularly shaped food. The rough surface of some products provides numerous sites for the microorganisms to attach and potentially escape from the antimicrobial treatment.	Misra & Jo (2017)
	Package	In-package, through-package or open treatment	In-package cold plasma treatment, the bactericidal molecules are generated and contained in the package, allowing higher exposure to pathogenic microbes, while reverting back to the original gas within a few hours of storage. The antimicrobial treatment inside a sealed package ensures the prevention of post-processing contamination.	Misra et al. (2013)
Equipment parameters	Composition and surface characteristics of the electrodes	The electrodes guide the discharge and can evolve during the process. The selection of the electrode material played a role in terms of decontamination efficacy.	A layer of oxide can rapidly cover the electrode surface, leading to the formation of a dielectric barrier that can modify the electrode potential and consequently the properties of the discharge. Silver and brass electrodes can be more effective than stainless steel or glass/brass.	Moreau, Orange & Feuilloley (2008)
	Distance between the electrodes and the target location	Distance between the electrodes and the target influences the cold plasma antimicrobial efficiency	The greater the distance the less efficient the treatment	Moreau, Orange & Feuilloley (2008)
	Plasma source	There are many plasma sources (DBD, plasma jet, microwave plasma, corona discharge, etc). The type can influence the efficiency and the characteristics of the plasma produced	DBD and Corona discharge system are the main plasma sources applied in dairy products.	Misra & Jo, 2017

Table 2. Effect of cold plasma technology on the microbial inactivation in dairy products.

Strain	Dairy	Cold plasma treatment	Assay	Results and Conclusion	Reference
Escherichia coli ATCC 25922	UHT and raw milk	Corona discharge system	The corona discharge system consisted of a 9 kV AC power supply, two tungsten electrodes (0.8 mm radius) and a simple ballast circuit. A high voltage was applied between the upper electrode tip and the liquid surface. The tip of the electrode was kept at a distance of 8 mm from the milk surface. A current of 90 mA was measured to flow into in the corona system and the temperature was kept below 35^{o}C. The time dependent effect of atmospheric corona discharge on *E. coli* ATCC 25922 dispersed in whole, semi skimmed and skimmed milk was examined. Plasma was applied at time intervals of 0, 3, 6, 9,12, 15 and 20 min.	A significant 54% reduction in the population of *E. coli* cells after only 3 min was observed regardless of the milk fat content. The initial pre-plasma bacterial count of 7.78 Log CFU/mL in whole milk decreased to 3.63 Log CFU/mL after 20 min of plasma application. Low temperature plasma (LTP) did not cause any significant change in pH and color of raw milk samples. No viable cells were detected after one-week storage in whole milk samples and remained so over the 6-week storage period. The LTP was able to significantly reduce *E. coli* in milk by more than a 3-fold log reduction without significantly affect pH or color properties.	Gurol, Ekinci, Aslan & Koraci (2012)
Escherichia coli KCTC 1682 and Staphylococcus aureusKCTC 11764	Cheese	Dielectric barrier discharge (DBD)	DBD plasma at 3.5 kV$_{pp}$and a bipolar 50 kHz (low frequency range) square wave with a 50% duty cycle. Evaluation of the potential of a dielectric barrier discharge (DBD) plasma system, using helium and He/O_2 mixture gas to improve the inactivation of *Escherichia coli* and *Staphylococcus aureus.* The effect of DBD on color parameters (L*, a* e b*) and sensory characteristics of sliced cheese during 1, 5, 10, and 15 min was also evaluated.	Significant reductions were observed in *E. coli* ranging from 0.09 to 1.47 log and 0.05 to 1.98 log with helium and with He/O_2 mixture, respectively. The number of *S. aureus* also decreased ranging from 0.05 to 0.45 log and 0.08 to 0.91 log with helium and with He/O_2 mixture, respectively. Significant decrease in the L* value and an increase in the b* value. Cheese slices were damaged after 10 and 15 min of plasma treatment. Significant reductions in the sensory quality including flavor, odor, and acceptability. The results indicate that the addition of oxygen resulted in a significant increase in inactivation of both pathogens and has potential for use in sanitizing food products, although the effect was limited.	Lee et al. (2012)
Escherichia coli (KCTC 1682), *Salmonella Typhimurium (KCTC 1925)*, and *Listeria monocytogenes (KCTC 3569)*	Cheese	Dielectric barrier discharge (DBD)	Inactivation of *Escherichia coli*, *Salmonella Typhimurium*, and *Listeria monocytogenes* on sliced cheese by plasma DBD (250 W, 15 kHz) treatment. The effect of post-treatment storage time on the inactivation was also assessed.	When agar plates were subjected to plasma treatment, populations of Escherichia coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes showed 3.57, 6.69, and 6.53 decimal reductions at 60 s, 45 s, and 7 min, respectively. No viable cells of these pathogens were detected after treatments for 90 s, 60 s, and 10 min, respectively. When the pathogens were inoculated on cheese slices, 2.88, 3.11, and 2.26 decimal reductions were achieved after 15 min of treatment. No damage of cheese slices was detectable to the naked eye after 10 min of treatment.	Yong et al. (2015a)

				The post-treatment storage duration following plasma treatment potently affected further reduction of pathogen populations. After the plasma treatment, the populations of E. coli, S. typhimurium, and L. monocytogenes on cheese slice (approximately 5 Log CFU/g) decreased by 1.75,1.97, and 1.65 Log CFU/g, respectively, after 5 min of treatment. The results indicated that the DBD system decreased and inactivated successfully pathogens in cheese slices. Furthermore, increasing post-treatment duration can improve the applicability of this system.	
Escherichia coli O157:H7 (ATCC 43894), *Salmonella Typhimurium (KCTC 1925)*, and *Listeria monocytogenes (KCTC 3569)*	Cheddar	Dielectric barrier discharge (DBD)	Inactivation of *Escherichia coli*, *Salmonella Typhimurium*, and *Listeria monocytogenes* on sliced cheddar cheese by flexible thin-layer DBD plasma (100 W, 15 kHz) treatment during 0, 2.5, 5 and 10 min.	*Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella Typhimurium* populations on agar plates were significantly reduced by plasma treatment. The level of these microorganisms in sliced cheddar cheese in response to 10-min plasma treatment significantly decreased by 3.2, 2.1, and 5.8 Log CFU/g, respectively. These results indicate that cold plasma can be used to sanitize food products.	Yong et al. (2015b)
Escherichia coli (KCTC 1682), L. monocytogenes *(KCTC 3569), and* Salmonella *Typhimurium (KCTC 1925)*	Milk	Dielectric barrier discharge (DBD)	Encapsulated DBD plasma was generated in a plastic container (250 W, 15 kHz, ambient air) and DBD plasma treatment was applied to milk samples for periods of 5 and 10 min.	The total aerobic bacteria counts in the untreated control sample was 0.98 log CFU/mL. No viable cells were detected in the milk samples after plasma treatments. When milk samples were inoculated with *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* Typhimurium, plasma treatment for 10 min resulted in a reduction of bacterial counts by approximately 2.40 log CFU/mL. The results of this study indicate that encapsulated DBD plasma treatment for less than 10 min improved the microbial quality of milk.	Kim et al. (2015)

Table 3. Effect of cold plasma technology on quality parameters in dairy products

Parameter	Dairy	Cold plasma treatment	Assay	Results and Conclusion	Reference
pH and color parameters	UHT and raw milk	Corona discharge system	The corona discharge system consisted of a 9 kV AC power supply, two tungsten electrodes (0.8 mm radius) and a simple ballast circuit. A high voltage was applied between the upper electrode tip and the liquid surface. The tip of the electrode was kept at a distance of 8 mm from the milk surface. A current of 90 mA was measured to flow into in the corona system and the temperature was kept below 35^{o}C. The effect of atmospheric corona discharge on pH and color parameters (L*, a* and b*) of whole, semi skimmed and skimmed milk was examined. Plasma was applied at time intervals of 0, 3, 6, 9,12, 15 and 20 min.	Cold plasma did not cause any significant changes in pH and color of raw milk samples. Only a slight change in comparison with the untreated milk was observed after 20 min.	Gurol, Ekinci, Aslan &Koraci (2012)
Color (L*, a* and b*) and sensory acceptance	Cheese	Dielectric barrier discharge (DBD)	DBD plasma at 3.5 kV_{pp} and a bipolar 50 kHz (low frequency range) square wave with a 50% duty cycle. The effect of a dielectric barrier discharge (DBD) plasma system was evaluated, using helium and He/O_2 mixture gas on color parameters (L*, a* e b*) and sensory evaluation (overall acceptance, appearance, color, flavor, odor, and texture). in sliced cheese during 1, 5, 10, and 15 min.	Cold plasma technology decreased the L*-value and increased the b*-value. Cheese slices were damaged after 10 and 15 min of plasma treatment. Significant reductions in sensory quality including flavor, odor, and acceptability.	Lee et al. (2012)
Physicochemical and sensory evaluation	Cheddar	Dielectric barrier discharge (DBD)	Evaluation of DBD plasma (100 W, 15 kHz) during 0, 2.5, 5 and 10 min in sliced cheddar cheese by flexible thin-layer.	The pH and L* values decreased whereas thiobarbituric acid reactive substances and b* values increased significantly with a higher exposure of the sliced cheddar cheese to DBD plasma. No significant differences were observed for total color difference (ΔE), sensory appearance, and color scores of DBD plasma-treated and untreated sliced cheddar cheese. Significant reductions in flavor and overall acceptance as well as an increase in off-odor were observed. The results indicated that flexible thin-layer DBD plasma can be used, but conditions should be optimized for industrial applications.	Yong et al. (2015b)

pH, color, fatty acid composition and lipid peroxidation	Milk	Dielectric barrier discharge (DBD)	Encapsulated DBD plasma was generated in a plastic container (250 W, 15 kHz, ambient air) and DBD plasma treatment was applied to milk samples for periods of 5 and 10 min.	The pH of milk decreased after the 10-min plasma treatment. Hunter color *L** and *b** values of milk increased, and the *a** value decreased after the plasma treatment. The production of 2-thiobarbituric acid reactive substances increased slightly, but not significantly, after plasma treatment. The results indicated that the encapsulated DBD plasma treatment for less than 10 min resulted in slight changes in physicochemical quality of milk.	Kim et al. (2015)
Biochemical changes to the protein, free fatty acids and volatiles profiles	Milk	Corona discharge system	The corona discharge system consisted of a 9 kV AC power supply, two tungsten electrodes (0.8 mm radius) and a simple ballast circuit. A high voltage was applied between the upper electrode tip and the liquid surface. The tip of the electrode was kept at a distance of 8 mm from the milk surface. A current of 90 mA was measured to flow into in the corona system and the temperature was kept below 35 ^{o}C. Raw milk was treated with a cold plasma system at intervals of 0, 3, 6, 9, 12, 15 and 20 min.	Significant increase was observed for 1 octanol, 2 heptanone, 2 hexenal, 2 octenal, nonanal and benzaldehyde levels. Plasma treatment did not result in significant changes in the lipid composition, total ketone or alcohol levels. Exposure to cold plasma significantly increased the total aldehyde content after 20 min of treatment.	Korachi et al. (2015)
Physicochemical, protein oxidation and functional properties	Whey protein	Dielectric barrier discharge (DBD)	The interaction between atmospheric pressure cold plasma (70 kV, ambient air) applied from 1 to 60 min and whey protein isolate (WPI) was evaluated.	Increase in yellow color (b*) and decrease in pH value was observed. Protein oxidation occurred for 15 min. Protein structure modifications demonstrated a certain degree of unfolding, as confirmed by high performance liquid chromatography (HPLC) profiles and dynamic light scattering (DLS), which improved foaming and emulsifying capacity. The changes were quite pronounced for 30 and 60 min of treatment. Overall, the foaming and emulsifying capacities were affected by the process. However, the foam stability increased. This study demonstrated that plasma can be successfully applied to electively modify the protein structure and therefore, improve the WPI functionality.	Segat et al. (2015)
pH, temperature, CD spectroscopy, ALP inactivation kinetics.	Alkaline Phosphatase (ALP) (milk enzyme)	Dielectric barrier discharge (DBD)	The effect of DBD plasma, at 40, 50 and 60 kV, from 15 s and 5 min on the activity and structure of ALP was evaluated.	Enzyme inactivation within a few seconds. Enzyme was characterized by a predominance of α- helix structure. Helical content showed a tendency to decrease with an increase in treatment time and voltage. The maximum temperature for most intense treatments was in the order of only 30◦C and no change in pH was noticed. These results indicated that DBB plasma	Segat et al. (2016)

				treatments were significantly effective in inactivating the ALP enzyme.	
FTIR, [1] H NMR and chromatographic techniques	Dairy fat	Dielectric barrier discharge (DBD)	Plasma treatment was performed at variable voltage (60–80 kV) and treatment duration (3–30 min)	The formation of secondary oxidation products was only observed in extended plasma treatment times of 30 min. The increase in ozonide band at 1105 cm−1, 1195 cm−1, formation of aldehydes at 1725, 2950 cm−1 and 829, 969, 3470 cm−1 was due to the formation of hydroperoxides. These changes were dependent on treatment time and applied voltage. 1H NMR analysis identified the formation of several lipid oxidation products, including aldehydes, such as hexanal or pentanal, mixtures of trans-2-heptenal, -octenal or -nonenal and 4-hydroxy-trans-2-nonenal. For the first time the formation of hydroperoxides of oleic acid (9-hydroperoxy-trans-10-, 11-hydroperoxy-cis-9-, 10-hydroperoxy-trans-8-, 8-hydroperoxy-cis-9-octadecenoates) and linoleic acid (as 9- and 13-hydroperoxy-octadecadienoylglycerol species) was observed. Fatty acid composition analysis identified a reduction in oleic, palmitoleic and linoleic acids along with formation of the oxidation products 2-nonenal, azelaic acid, 9-oxononanoic acid, nonanoic acid and octanoic acid. Understanding cold plasma interactions with food lipids and the critical parameters governing lipid oxidation is required prior to the industrial adoption of this technology for high-fat food products.	Sarangapani et al. (2017)

Table 4. Advantages, disadvantages, and limitations of cold plasma treatment for dairy foods

Advantages	Disadvantages	Limitations
High microbial inactivation efficiency at low temperatures (generally<50°C), extending shelf life and improving the efficiency of the supply chain	Difficult to precisely control the chemistry of the gas plasma reactions, especially due to the different moisture levels of foods	Thermal processing is well established and has served the industry very well. There is a need for studies on the effective advantages of cold plasma in terms of costs and/or product quality over thermal processing. Alternatively, the performance of additional functions that cannot be performed by heat alone is of interest
Compatible with most existing packaging and modified atmospheres - Almost all plasma sources available allow in situ production of the acting agents, just on demand, and in a range of gases	High-fat dairy foods - the reactive oxygen species formed can lead to oxidation. Sensory characteristics must be evaluated.	There are few tests of efficacy equivalent to the alkaline phosphatase test for thermal processing of milk. This impairs the regulatory authorities to approve the technology for ‘pasteurization’ purpose
The active chemical species of plasma has high diffusivity and fast action and can access to the entire food surface (in most cases)	The overall process can turn out to be expensive if operated using noble gases, because the cost of the plasma processing is largely dictated by the cost of the gas or gas mixture	Absence of information about possible resistant microorganisms to cold plasma technology.
Generally, it has negligible impact on the product matrix and can reduce the use of preservatives	Requires additional safety measures when the plasma generation is carried out using very high voltages. Appropriate measures for destruction and exhaustion of gases are also required.	The difficulty in precisely defining the operation conditions and comparing the results from different studies. It also makes it difficult to utilize the results from different research laboratories for scale-up purposes.
Environmentally friendly - free of water or solvent	Difficult with bulky and irregularly shaped food - The rough surface of some products provides numerous sites for the microorganisms to attach and potentially escape antimicrobial treatment	Possible formation of undesirable flavor
No residues formation, given sufficient time is provided for the recombination reactions.	Possible alteration in the color characteristics of the products	
Energy efficient - require only a low energy input	No studies have been conducted on the formation of toxic compounds in plasma treated foods	---
Applied to solid or liquid foods	There are few studies concerning the projected cost of treatment for scaling up this technology in food industry, with no comparisons with the heat treatment process.	----

Source: Yong et al. (2015b), Misra, Schlüter& Cullen (2016), Deeth & Datta (2011)

Figure 1. Cold plasma systems. (a)Plasma jets, (b) dielectric barrier discharges (DBD) (c) corona discharges (d) and microwave discharges. Adapted from Surowsky, Schlüter & Knorr, 2015.

Figure 2. Overview of cold plasma mechanisms involved in microbial inactivation. Adapted from Schlüter & Fröhling, 2014.

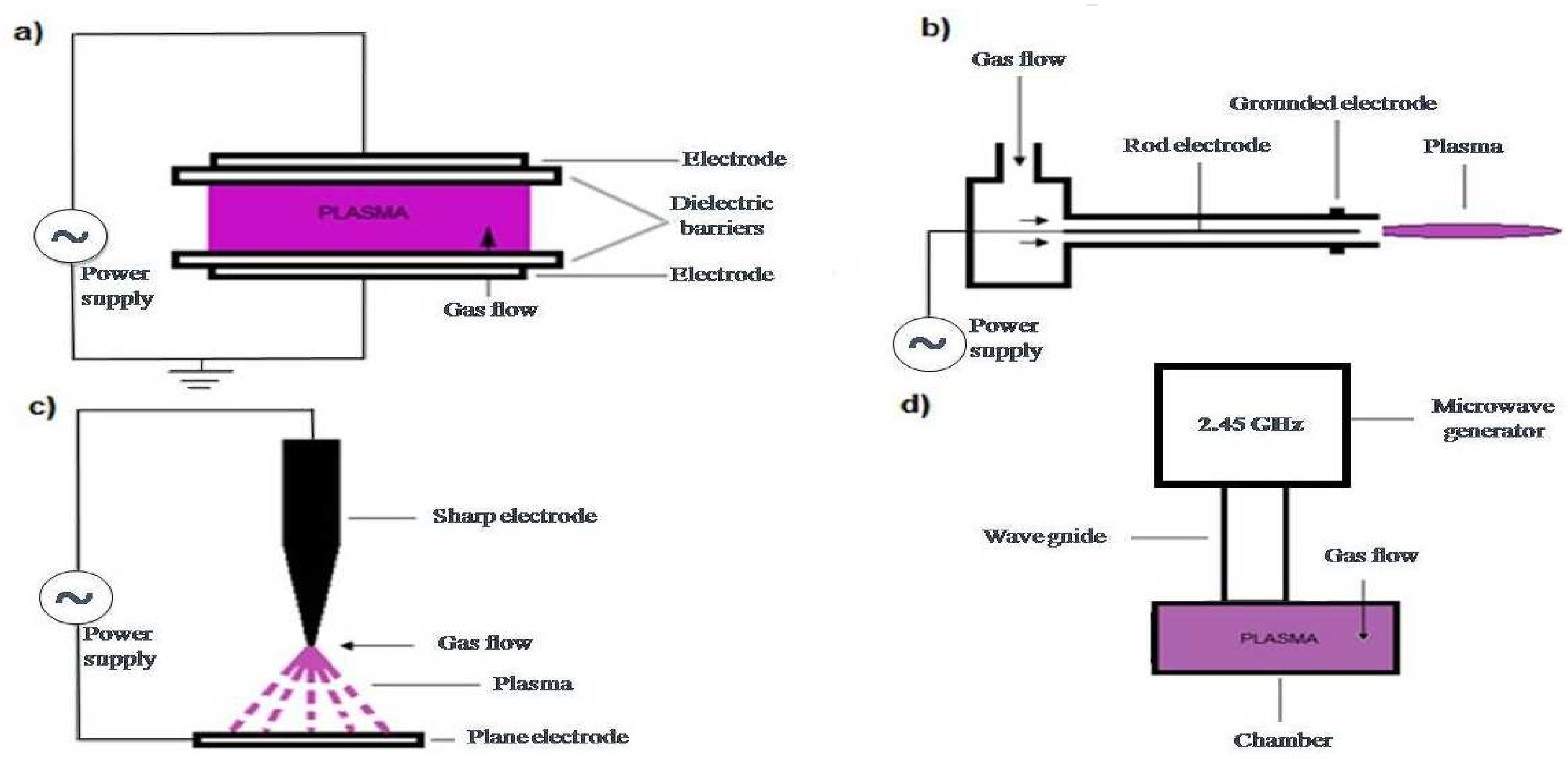

Figure 1. Plasma generation at atmospheric pressure. (a) Dielectric barrier discharges (DBD), (b) Plasma jets, (c) corona discharges (d) and microwave discharges. Adapted from Surowsky, Schlüter & Knorr, 2015.

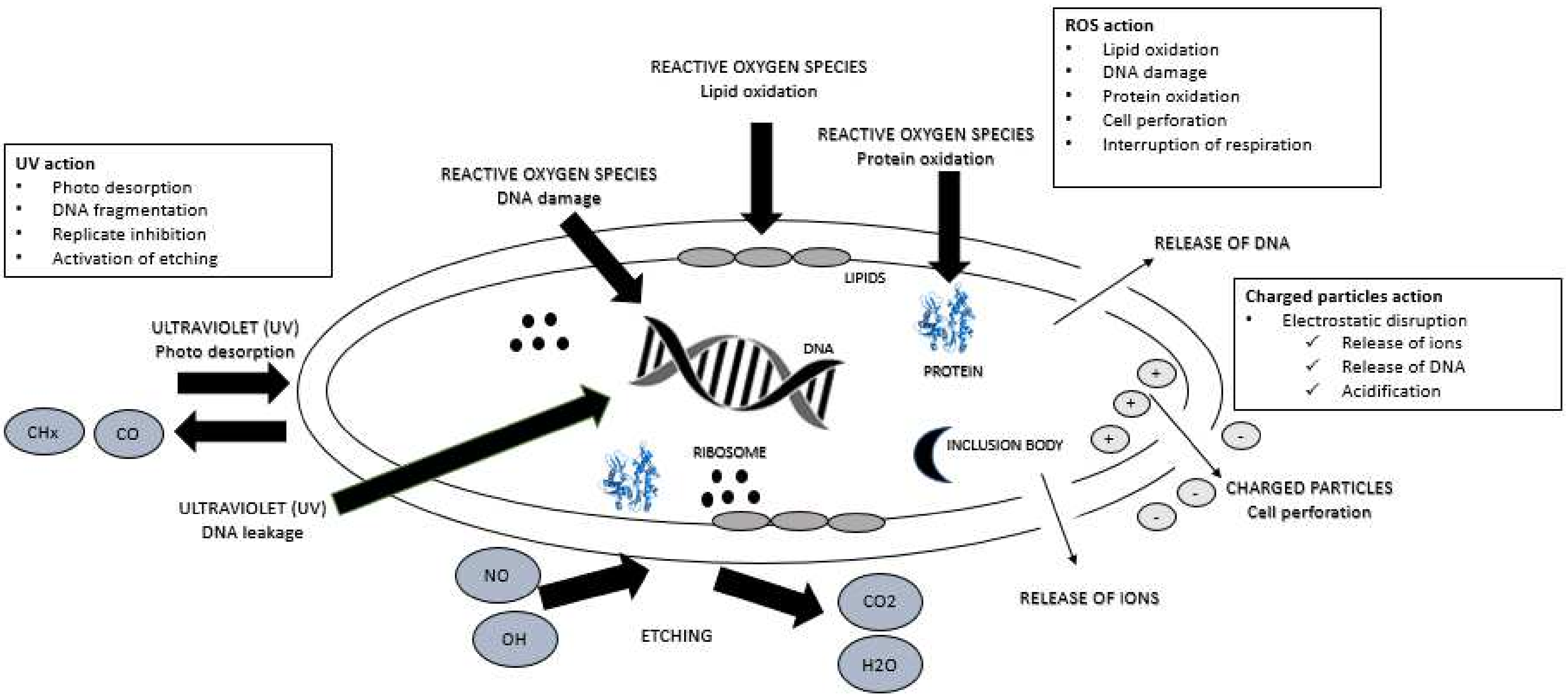

Figure 2. Overview of cold plasma mechanisms involved in microbial inactivation. Adapted from Schlüter & Fröhling, (2014).

- ✓ Cold plasma as non-thermal technology is reviewed;
- ✓ Diversity of systems, operational parameters and mechanisms are revised;
- ✓ Effects of cold plasma on the quality and safety of dairy foods are reported.