

# Cold Plasma Technology and Its Effects on Some Properties of Milk and Dairy Products

## Soğuk Plazma Teknolojisinin Süt ve Süt Ürünlerinin Bazı Özellikleri Üzerine Etkileri

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### ABSTRACT

This review covers cold plasma techniques as a non-thermal processing technique and their effects on the microbiological and chemical properties of some dairy products, as well as sensory properties. Beforehand, the techniques used to generate cold plasma and its types and mode of action were also mentioned to make the reader become familiar with the subject. So far, limited results have shown that cold plasma techniques are able to reduce the number of some pathogens important to dairy technology such as *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, etc., depending on the type of technique and application time. However, the effect of cold plasma application on physical, chemical, and sensory properties is still controversial. More research needs to be conducted to reveal the extent of the effectiveness of cold plasma techniques on the quality of dairy products.

**Keywords:** Cold plasma, corona discharge, dielectric barrier discharge, microwave discharge, plasma jet

### ÖZ

Bu derleme, termal olmayan bir işleme tekniği olan soğuk plazma tekniklerini ve bunun bazı süt ürünlerinin mikrobiyolojik ve kimyasal özellikleri ile duyu özellikleri üzerindeki etkilerini kapsamaktadır. Çalışmada öncelikle soğuk plazma üretmek için kullanılan tekniklerden ve soğuk plazma türlerinden ve etki biçimlerinden bahsedilmiştir. Şimdiye kadar gerçekleştirilen çalışmalardan elde edilen sınırlı sonuçlar, soğuk plazma tekniklerinin, tekniğin türüne ve uygulama süresine bağlı olarak *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* gibi süt teknolojisi için oldukça önemli olan bazı patojenlerin sayısını azaltabildiğini göstermiştir. Ancak soğuk plazma uygulamasının fiziksel, kimyasal ve duyu özellikleri üzerindeki etkisi halen tartışmalıdır. Soğuk plazma tekniklerinin süt ürünlerinin kalitesi üzerindeki etkinliğinin kapsamını ortaya koyabilmek için daha fazla araştırma yapılması gerekmektedir.

**Anahtar Kelimeler:** Soğuk plazma, korona deşarji, dielektrik bariyer deşarji, mikrodalga deşarji, plazma jeti

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## Introduction

Milk and dairy products are foods with high nutritional value, containing carbohydrates (lactose), fatty acids, and high-quality protein, as well as important micronutrients such as vitamins, minerals, and trace elements. The initial microbial load of the milk and the environmental factors such as the equipment used during milking and the environment in which the milking takes place can cause the raw milk to contain microorganisms at a level that may pose a health risk during the period until it is processed into the product. This microorganism load may also cause some sensory defects in milk and dairy products (Rathod et al., 2021). In addition, some enzymes such as lipase and alkaline protease in raw milk can cause structural and sensorial defects in milk and dairy products, leading to significant quality losses during processing, ripening, and storage of the product (Thirumdas & Annappure, 2020). Therefore, microorganisms and enzymes in raw milk must be inactivated at a certain level in order to ensure food safety, minimize sensory defects, and extend the shelf life of the product (Coutinho et al., 2018).

In the dairy industry, heat treatment techniques such as pasteurization, HTST (High temperature short time), LTLT (Low temperature long time), and UHT (Ultra high temperature) are generally applied for microorganism and enzyme inactivation, depending on the dairy product to be processed (Rathod et al., 2021). Such heat treatment applications are known as the most energy-consuming technologies in the food industry (Picart-Palmade et al., 2019) and may cause not only some nutritional losses in milk but the formation of undesirable sensory qualities such as bitterness and gelling in the final product (Misra et al., 2016; Rathod et al., 2021). In recent years, some novel techniques such as ohmic heating, high hydrostatic pressure, pulse electric field, and ultrasound have been developed in order to reduce the abovementioned effects (Coutinho et al., 2018). These technologies, which are generally called non-thermal techniques, aim at minimizing the negative effects on the nutritional value and quality characteristics of the products while meeting the necessary food safety and shelf life demands (Misra et al., 2016b). Cold plasma technology, on the other hand, is one of the newest techniques among these techniques. Unlike other non-thermal processes, it has some important advantages such as the need for shorter times in treatment, the need for no chemicals and water (Lee et al., 2021), and being applied at ambient temperature (Misra et al., 2016b).

In this review, the principle, mode of action of cold plasma technology, and its effects on microbiological, physicochemical, biochemical, and sensory properties of milk and dairy products are discussed.

### Plasma Technology and Cold Plasma

Plasma application is based on exposing food or food surfaces to plasma, which is accepted as the fourth state of matter (Lee et al., 2021). Plasma is obtained by transforming a gas into an ionized

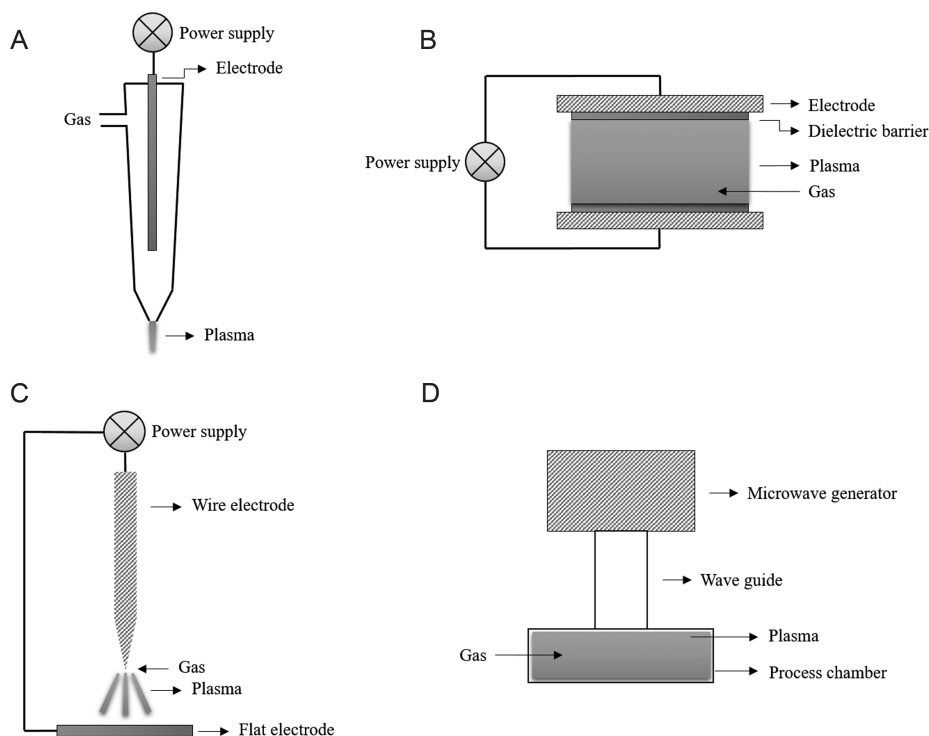
gas containing atoms, ions, and electrons by providing sufficient energy (Misra et al., 2016a). There are several plasma techniques, each of which has different advantages or disadvantages.

By application temperature, it can be classified as a thermal or cold (low temperature) plasma technique. In the former technique, highly ionized species are in thermodynamic equilibrium with each other (Pankaj et al., 2018). Latter, on the other hand, is defined as plasma at room temperature due to the non-equilibrium between ions and unionized species, although the temperature of the electrons is high (Sharma & Singh, 2020). High pressure ( $>10^5$  Pa) and very high energy (up to 50 MW) are needed to obtain thermal plasma. Besides, cold plasma is produced at 30–60°C and requires lower energy consumption compared to thermal plasma (Coutinho et al., 2018; Misra et al., 2016b). It is preferred especially in heat treatment-sensitive food products since ions and neutral molecules gain very low energy and remain stable at low temperatures in this application (Phan et al., 2017).

Considering the pressure conditions, this technique can be classified as high pressure, atmospheric pressure, and low-pressure plasma. Atmospheric plasma is generally preferred in practice because it eliminates the energy and cost required to create low or high pressure (Pankaj et al., 2018).

Cold plasma is produced by multiple techniques such as plasma jets, dielectric barrier discharge, corona discharge and microwave discharge (Corradini, 2020). All these discharges are initiated and sustained by electron collision processes under the influence of certain electric or electromagnetic fields (Misra et al., 2016a).

Although plasma jets (Figure 1A) may consist of a single electrode, they usually contain two electrodes and produce small “plasma flames” in the radio frequency range. The electrode gap



**Figure 1.** Different Cold Plasma Systems: (A) Plasma Jet, (B) Dielectric Barrier Discharge, (C) Corona Discharge, and (D) Microwave Discharge (Modified from Coutinho et al., 2018; Misra et al., 2016a; Surowsky et al., 2015).

is usually a few millimeters, and the process gas (usually noble gases) is ignited at voltages of ~100 V. The biggest advantage of plasma jets is that they are small in size and can penetrate into narrow spaces (Surowsky et al., 2015).

In the dielectric barrier discharge (Figure 1B), plasma is produced between two electrodes separated by a dielectric. In this system, the process gas used, the distance between the electrodes and the electrical operation of the discharge are important process parameters. The biggest advantages of dielectric barrier discharges are that many different gases can be used to obtain this sort of plasma, relatively low gas flow is required, homogeneous discharges can be ignited for several meters, and can be adapted to different electrode geometries. However, depending on the distance between the electrodes, relatively high ignition voltages (10 kV) may be required in some cases. In such cases, it is necessary to take important measures by providing isolation (Surowsky et al., 2015). The dielectric barrier discharge is especially ideal for large surfaces (Coutinho et al., 2018).

Corona discharges (Figure 1C) are seen near large sharp electrode geometries under atmospheric pressure. There is an electric field large enough to accelerate the ionization level of the atoms or molecules of the gas surrounding the electrons. Cylindrical geometries or sharp, curved electrodes and flat electrodes are generally used in corona discharges. Being produced with simple devices with very low initial investment and operating costs appears to be an important advantage of this technique. However, it can be applied in small areas and non-uniformly is considered as a significant disadvantage (Coutinho et al., 2018; Surowsky et al., 2015).

Microwave discharges (Figure 1D) are produced without an electrode, unlike plasma jets, dielectric barrier discharges, and corona discharges. The microwaves produced by a magnetron are directed into the process chamber via a waveguide or a coaxial cable. Electrons in the process gas absorb these microwaves, leading to an increase in kinetic energy and, therefore, to the formation of ionization reactions with the collisions that occur. Depending on the microwave energy consumed here, neutral gas temperatures ranging from room temperature to about 1000 K can be reached. The biggest advantages of microwave discharges are that they can be installed without electrodes and can be ignited in the air or even with water vapor. In addition, their gas consumption is moderate and they can produce a high amount of reactive species depending on the discharge gas used. However, the necessity of using a series of discharges to ensure its applicability in large areas is a disadvantage of microwave discharges (Surowsky et al., 2015).

The properties of the produced plasma vary depending on factors such as the power source used, the parameters applied, and the composition of the gases (Pedrow et al., 2020). However, since dielectric barrier discharge and plasma jets are easier to construct, can operate continuously, and some configurations are commercially available, the use of cold plasma obtained from them in food products is more emphasized (Corradini, 2020; Misra et al., 2016).

### Mode of Action of Cold Plasma

The effectiveness of cold plasma is basically based on the production of ultraviolet radiation, reactive oxygen species (ozone, hydrogen peroxide, singlet oxygen, peroxy and hydroxyl radicals, etc.), and reactive nitrogen species (nitric oxide, peroxy nitrite, peroxy nitrous acid, etc.) (Misra & Jo, 2017; Misra et al., 2019). These reactive species formed cause some important physical, chemical, and microbiological changes in milk and dairy products.

One of the most important changes is the deformation of the microbial cell surface, damage to the intracellular genetic material, and ultimately the death of the cell by lysis (Coutinho et al., 2018; Timmons et al., 2018). In addition, many different atoms, metastable, radical, electronically, and vibrationally excited molecules, including short- and long-lived neutral reactive species, can also contribute to the antimicrobial effect (Misra & Jo, 2017).

Also, plasma-reactive species (free radicals) have the potential to inactivate enzymes (Thirumdas et al., 2015). These reactive species cause modifications in amino acids through chemical reactions such as oxidation, sulfonation, and hydroxylation. It is stated that cold plasma specifically targets the secondary structure of enzymes ( $\alpha$ -helix and  $\beta$ -sheet) (Thirumdas & Annapure, 2020). Binding and catalysis are inhibited due to the structural change seen in enzyme active sites with the exposure of proteins to radicals (Bubler et al., 2017; Khani et al., 2017). Rodacka et al. (2016) reported that the greatest effect on enzyme inactivation was seen in the presence of reactive oxygen species.

Plasma-produced reactive oxygen species, such as hydroxyl radicals, hydrogen peroxide, and superoxide anions (Attri et al., 2015), can also interact with lipids in foods and cause lipid oxidation (Gavahian et al., 2018). This situation has the potential to cause some undesirable changes such as deterioration of sensory properties, especially in dairy products with high fat content such as cream and butter. The primary target of reactive oxygen species is the methyl groups of fatty acids. Especially fatty acids with double bonds are more sensitive to reactive oxygen species. Linoleic acid (18:2) containing two double bonds and  $\alpha$ -linolenic acid (18:3) containing three double bonds are the most sensitive fatty acids (Gavahian et al., 2018).

Some researchers expect an increase in the acidity of the product due to the chemical interactions between reactive species such as hydrogen peroxide and nitric acid formed during plasma production (Thirumdas & Annapure, 2020). However, no acidity change was observed in other studies (Gurol et al., 2012; Segat et al., 2016). This is thought to be due to the difference in the plasma source used and the applied process parameters.

### The Effects of Cold Plasma on Milk and Dairy Products

There are a limited number of studies examining the effects of cold plasma application on the physical, chemical, and microbiological properties of milk and dairy products (Table 1).

The majority of these studies are on the inhibition of the most common pathogens in milk and dairy products. Cold plasma significantly reduces the number of pathogens such as *E. coli* (Gurol et al., 2012; Kim et al., 2015; Lee et al., 2012), *E. coli* O157:H7 (Yong et al., 2015), *Staphylococcus aureus* (Lee et al., 2012), *Listeria monocytogenes* (Kim et al., 2015; Yong et al., 2015), *Salmonella* Typhimurium (Kim et al., 2015; Yong et al., 2015), *Listeria innocua* (Wan et al., 2019), and *Cronobacter sakazakii* (Chen et al., 2019) in products such as milk, cheese, milk powder, and whey beverages, depending on the technique applied, the type of gas used, and the application time. In products with high surface roughness, such as cheese, it is thought that the microstructure provides a suitable environment for bacterial cells to adhere to the surface and reduces the effectiveness of the process by protecting the bacteria from the effects of cold plasma (Wan et al., 2019).

**Table 1.** The Studies on the Effects of Cold Plasma Application on Some Properties of Milk and Dairy Products

Sample	Gas	Cold Plasma	Treatment Time	Microbial Activity	Enzyme Inactivation	Physicochemical and Biochemical Properties	Color Values	Sensory Properties	Reference
Whole milk, semi-skimmed milk, skimmed milk	Air	Corona discharge	0, 3, 6, 9, 12, 15, and 20 minutes	<i>Escherichia coli</i> 3 minutes 54% ↓	n/a	pH ↔	L*, a*, b* ↔	n/a	Guroi et al. (2012)
Sliced cheese	He/He/O <sub>2</sub>	Dielectric barrier discharge	1–15 minutes	<i>E. coli</i> —0.05–1.98 log ↓ <i>S. aureus</i> —0.05–0.91 log ↓	n/a	n/a	L* ↓ b* ↑	Appearance, flavor, odor, total acceptability ↓	Lee et al. (2012)
Whole milk	Air	Dielectric barrier discharge	5 and 10 minutes	<i>E. coli</i> , <i>L. monocytogenes</i> , <i>Salmonella typhimurium</i> 10 minutes 2.40 log cfu/mL ↓	n/a	pH ↓ Lipid oxidation ↔	L*, b* ↑ a* ↓	n/a	Kim et al. (2015)
Raw milk	Air	Corona discharge	0, 3, 6, 9, 12, 15, and 20 minutes	n/a	n/a	FFA content ↔ Lipid composition ↔ Total aldehyde content—20 minutes ↑ Toplam ketone and alcohol content ↔	n/a	n/a	Korachi et al. (2015)
Sliced Cheddar cheese	Air	Dielectric barrier discharge	0, 2.5, 5, and 10 minutes	<i>E. coli</i> O157:H7, <i>L. monocytogenes</i> , <i>Salmonella typhimurium</i> 10 minutes 3.2, 2.1, and 5.8 log cfu/g ↓	n/a	pH ↓ Thiobarbituric acid-reactive species ↑	ΔE ↔ L* ↓ b* ↑	Appearance ↔ Odor-aroma and total acceptability ↓	Yong et al. (2015)
Alkaline phosphatase solution	Air	Dielectric barrier discharge	15 seconds–5 minutes	n/a	Alkaline phosphatase 180 seconds 90% ↓	pH ↔	n/a	n/a	Segat et al. (2016)
Süt yağı (tereyağı)	Air	Dielectric barrier discharge	3–30 minutes	n/a	n/a	Secondary oxidation products—30 minutes ↑ Oleic, palmitoleic, and linoleic acid—30 minutes ↓	n/a	n/a	Sarangapani et al. (2017)
Non-fat milk powder	N	Corona discharge	20–120 seconds	<i>C. sakazakii</i> 1:17–3:27 log ↓	n/a	Amino acid composition ↔ phenolic acid content ↔	L*, a*, b* ↔	n/a	Chen et al. (2019)
Whey beverage	N	Corona discharge	5, 10, and 15 minutes	n/a	n/a	Bioactive and volatile compound contents ↑ Short times: Vitamin C ↑ Antioxidant activity ↑ Carotenoid content ↓ Long times: Vitamin C and volatile compound ↓ Carotenoid content ↑ ACE inhibitor activity ↑	n/a	n/a	Silveira et al. (2019)
Tryptic soy agar (TSA), Queso Fresco cheese, cheese model	Air	Dielectric barrier discharge	5 minutes	<i>Listeria innocua</i> 1.6–5.0 log ↓	n/a	n/a	n/a	n/a	Wan et al. (2019)
Gel	Air	Plasma jet	0–120 seconds	<i>S. aureus</i> and <i>L. monocytogenes</i> 120 seconds 1–2 log ↓	n/a	n/a	n/a	n/a	Lee et al. (2021)
Whey beverage	Air	Corona discharge	0, 5, 10, and 15 minutes	n/a	n/a	HMF value ↓ Antioxidant activity ↑ ACE inhibitor activity ↑	L*, a*, b* ↔	Acceptability ↑	Ribeiro et al. (2021)

n/a: not applicable.



Cold plasma application is also effective on pH, minor components, enzyme, and volatile components of milk and dairy products mostly depending on the application time. The application of cold plasma (dielectric barrier discharge) for 5 and 10 minutes in whole milk decreased the pH values with no major changes in lipid oxidation (Kim et al., 2015). It was also observed that the application of cold plasma (dielectric barrier discharge) for different times (0, 3, 6, 9, 12, and 15 minutes) in raw milk increased the total aldehyde content after 20 minutes of application (Korachi et al., 2015). Similarly, the thiobarbituric acid reactive substance content, which is an indicator of lipid oxidation, increased in sliced Cheddar cheeses, by 30% after an additional 7.5 minutes of plasma application (Yong et al., 2015). In another study (Sarangapani et al., 2017), when milk fat was treated with atmospheric cold plasma (dielectric barrier discharge) for 3–30 minutes, it was observed that secondary oxidation products were released in the samples with only 30 minutes of application. With the release of oxidation products (2-nonenal, azelaic acid, 9-oxononanoic acid, nonanoic acid, and octanoic acid), a decrease in the amount of oleic, palmitoleic, and linoleic acids occurred. Cold plasma application increased the bioactive and volatile component content of guava-flavored whey samples (Silveira et al., 2019). Its application at low flow rates and for short periods increased the vitamin C content and antioxidant activity of the samples but decreased the carotenoid content, resulting in a less acceptable fatty acid profile. On the other hand, higher flow rate and application times decreased the vitamin C and volatile component content of the samples, while increasing the carotenoid content and ACE (angiotensin-I-converting enzyme)-inhibitory activity. Cold plasma application had a reducing effect on the HMF (hydroxymethylfurfural) value of the xylooligosaccharide-added whey beverage, but increased the antioxidant and ACE-inhibitory activities, compared to the pasteurization process, and these effects were enhanced with the increase of the application time (Ribeiro et al., 2021). In skimmed milk powder, no changes in the amino acid composition or phenolic acid content occurred with cold plasma application for 120 seconds (Chen et al., 2019).

Limited studies revealed that cold plasma applications have different effects on the sensory properties of milk and dairy products. On one hand, it negatively affects the taste, odor, and overall acceptability of cheese (Lee et al., 2012; Yong et al., 2015), on the other hand, there was a positive effect in whey beverages containing oligosaccharides (Ribeiro et al., 2021). Some researchers reported no noticeable changes in color parameters in milk samples containing different amounts of fat (Gurol et al., 2012), cheese (Lee et al., 2012; Yong et al., 2015), milk powder (Chen et al., 2019) and whey beverage (Ribeiro et al., 2021), the changes in, while others (Kim et al., 2015) reported an increase in the  $L^*$  and  $b^*$  values of the dairy products samples and a clear decrease in the  $a^*$  value in whole milk. In contrast, no noticeable effect of cold plasma was observed in color parameters in milk samples containing different amounts of fat (Gurol et al., 2012), cheese (Lee et al., 2012; Yong et al., 2015), milk powder (Chen et al., 2019) and whey beverage (Ribeiro et al., 2021).

When the cold plasma technique is considered as a sanitation application in the dairy industry, it has been shown to have a lower degree of effectiveness than peracetic acid, a widely used chemical in sanitation (Lee et al., 2021). With the application of peracetic acid, a 7-log reduction was recorded in the count of two microorganisms (three different strains of *S. aureus* and one strain

of *L. monocytogenes*) in a short time (10 seconds). Cold plasma application, on the other hand, yield a very low bactericidal effect, only a 1–2 log reduction was achieved after 120 seconds of application. It is pointed out that RNA and DNA damage occurred in cells and esterase activity decreased with the application of peracetic acid, while cold plasma application had no such effect on the mentioned parameters.

The enzyme inactivation efficiency of cold plasma was investigated in a solution prepared using a commercial alkaline phosphatase enzyme, which is specific to milk and obtained from bovine intestinal mucosa (Segat et al., 2016). The plasma obtained by dielectric barrier discharge was applied at three different voltages, 40, 50, and 60 kV, between 15 seconds and 5 minutes. The results indicated that enzyme inactivation was achieved by 45%–50% at the end of 120 seconds and by 90% at the end of 180 seconds at all applied voltages. In the meantime, no change in the pH of the solution was observed and the highest temperature recorded during the application was 30°C.

## Conclusion and Recommendations

Cold plasma technology, which is considered one of the newest among non-thermal (thermal) techniques, has special importance especially for milk and dairy products as it has significant advantages compared to thermal methods. However, studies on the subject have generally focused on the antimicrobial effect of cold plasma, and the changes in the physical, chemical, and sensory properties of the final product have not been adequately addressed until today. The results of the studies, some of which have been summarized in this review, showed that cold plasma has different effects on the microbiological, physicochemical, biochemical, and sensory properties of milk and dairy products. The type and concentration of reactive species that can be found in the plasma vary depending on many factors such as the gas or gas mixtures in which the plasma is induced, the configuration of the source used in the production, and the applied voltage and time. Therefore, obtaining different results in studies is closely related to the method of obtaining the applied plasma, the process parameters, and also the microorganism species examined.

As a result, more studies are still required to reveal the changes in milk and dairy products due to cold plasma application. In particular, the examination of fat oxidation and therefore the volatile compounds originating from fat emerges as a new research topic as a potential for acceleration of cheese ripening.

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