Turkish Journal of Food and Agriculture Sciences

TURJFAS

7(2): 138-160 (2025)

Review

DOI: https://doi.org/10.53663/turjfas.1735346

Advanced physical techniques to prevent microorganisms in food

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ARTICLE INFO

HISTORY

Received: 5 July 2025
Revised: 29 July 2025
Accepted: 6 September 2025
Online Published: 30 December 2025

KEYWORDS

Advanced techniques
Cold plasma
Decontamination
High pressure processing
Microorganisms

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ABSTRACT

In the food industry the quality and safety of products are vital concerns, necessitating the development and implementation of effective microbial mitigation strategies. Traditional methods, such as thermal processing, are effective but, often compromise the nutritional value and sensory attributes of food. This review focuses on advanced physical techniques that offer alternative or complementary approaches to conventional methods. Non-thermal technologies, including high-pressure processing (HPP), pulsed electric fields (PEF), cold plasma, and ultraviolet (UV) light, have emerged as promising tools in enhancing food safety without significantly altering food quality. These methods are explored in terms of their mode of action and efficacy against various pathogens. The review also addresses the challenges and limitations related with the industrial adoption of these technologies, alongside future perspectives for their optimization and integration into food processing chains. By advancing the understanding of these innovative techniques, the review aims to support the production of safer and higher-quality food products.

Citation: Ahmad, A.M. (2025). Advanced physical techniques to prevent microorganisms in food. *Turkish Journal of Food and Agriculture Sciences*, 7(2), 138-160.

ORCID> 0009-0009-1800-8354 (AMA)

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Introduction

Microbial contamination in food is a significant concern globally, impacting public health, food security, and economic stability. Microorganisms, including bacteria, viruses, fungi, and parasites, can infiltrate the food supply chain at various points from the production of food and its processing to its storage and distribution (Alexa et al., 2024; Anupma and Sumanshu, 2024). These contaminants pose risks such as foodborne illnesses, spoilage, and reduced shelf life, leading to substantial losses in the food industry and posing serious health risks to consumers (Karanth et al., 2023).

Effective microbial mitigation is crucial in assuring the safety, quality, and longevity of food items. Microbial contamination not only threatens public health but also undermines consumer confidence, leading to significant economic losses through product recalls, waste, and legal liabilities. Foodborne infections, brought about by pathogens like *Salmonella*, *E. coli*, and *Listeria*, are a serious public health concern, resulting in millions of cases of illness and thousands of deaths annually worldwide (Oladunjoye and Awani-Aguma, 2024). Beyond health risks, microbial spoilage contributes to the decline of food quality, affecting texture, flavor, and appearance, which are critical factors in consumer acceptance. As global food supply chains become more complex, the risk of contamination at various stages increases, necessitating robust microbial control measures.

Conventional techniques of microbial control, such as thermal treatment, chemical preservatives, and refrigeration, have been widely used to combat these threats. However, these methods often come with limitations, including potential loss of nutritional and sensory attributes, the emergence of resistant microbial strains, and demand by consumers for lightly processed and free from additive foods. Thermal treatment can lead to nutrient loss and undesirable alterations in sensory attributes. Meanwhile, the use of chemical preservatives is increasingly scrutinized by consumers who demand clean-label products with fewer additives (Thomas-Popo, 2021; Zuo et al., 2024). These challenges have driven the need for advanced physical techniques that can effectively reduce or eliminate microbial load without compromising food quality.

Since the last decade, innovative physical techniques have been introduced to address these challenges, offering promising alternatives to traditional techniques. These advanced methods leverage physical principles such as high pressure, electric fields, and non-thermal plasma to inactivate or destroy microorganisms in food products while preserving their quality (Thomas-Popo, 2021; Dangal et al., 2024; Sridipta Paul, 2024). These methods aim to mitigate microbial contamination while maintaining the nutritional and sensory characters of food, aligning with consumer preferences for lightly processed, safe, and high-quality food products. The adoption of these advanced techniques not only enhances food safety but also supports the food industry's efforts to meet regulatory standards, extend product shelf life, and reduce food waste. As such, understanding and implementing these technologies are of paramount importance in modern food processing and preservation. This review provides a comprehensive examination of advanced physical techniques used to mitigate microbial contamination in food.

Advanced Physical Techniques

Various advanced physical techniques like High pressure processing, UV irradiation, pulsed- electric field, ozone and cold plasma are discussed below. Figure 1 gives an overview of advanced physical techniques for microbial mitigation along with their advantages and disadvantages.

1. High-Pressure Processing (HPP)

High-pressure processing (HPP) is a technique that applies high hydrostatic pressure to sustain food quality, freshness, and safety, meeting consumer demands for additive-free and healthy products (Woldemariam and Emire, 2019). It retains the nutritional character as well as sensory appeal of food while additionally prolonging shelf life and enhancing food safety

1.1. Mechanism of microbial inactivation

HPP retains food quality and prolongs shelf life by disrupting microbial cell structures without affecting covalent bond (Linton et al., 2000). The efficacy of HPP is dependent upon factors like pressure level, duration

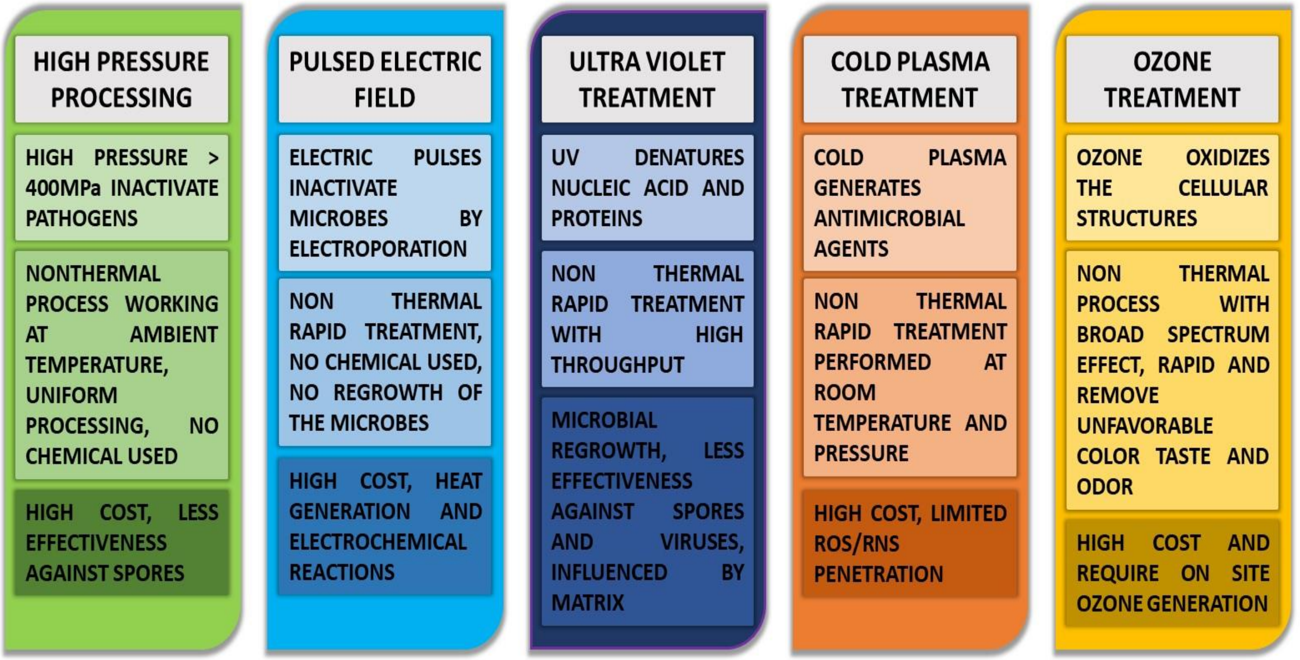

Figure 1. An overview of advanced physical techniques for microbial mitigation along with their advantages and disadvantages

temperature and food properties (Bilbao-Sáinz et al., 2009; Perera et al., 2010; Ps et al., 2011). HPP primarily damages cell membranes, altering permeability and disrupting cellular functions (Kato et al., 2002). It inactivates vegetative bacteria, yeast and molds, but bacterial spores are highly resistant requiring extreme conditions for inactivation (Wilson et al., 2008). Table 1 shows microorganisms mitigated by advanced physical techniques. Combining HPP with heat treatment enhances microbial inactivation, especially for spores (Paidhungat et al., 2002). For pasteurization purposes, treatment typically takes place at 300-600 MPa for a short duration, effectively inactivating spoilage and vegetative pathogenic microorganisms by approximately 4-log units. On the other hand, harmful bacteria react differently to high-pressure (HP) treatments depending on the temperature at which they are applied (Woldemariam and Emire, 2019).

1.2. Pressure–temperature interactions and pathogen response

Studies on *E. coli* O157 in chicken meat demonstrated a 1-log decline after 15 min of treatment at 20°C and 400 MPa, similar to the effect of only a 50°C heat treatment. However, combining the 400 MPa treatment with a temperature of 50°C led to a decline of 6-log (Patterson and Kilpatrick, 1998). The inactivation of bacterial spores is the main obstacle in high-pressure processing, and reaction varies significantly between species and even strains of the same species (Heinz and Knorr, 2001). In order to substantially (5-log) diminish Clostridium sporogeneses spores in fresh chicken breast, 680 MPa of pressure was applied for one hour (Crawford et al., 1996), Nevertheless, further investigation exhibited that treating *C. sporogenes* in liquid media at 1500 MPa only resulted in a 1.5-log decline (Maggi et al., 1996).

Inactivation of yeast and molds through pressure treatment has been observed in citrus juices. For example, juices treated with high-pressure processing at 400 MPa for 10 min at 40 °C remained microbiologically stable during storage for 2–3 months (Ogawa et al., 1990). These results demonstrate how HPP can limit microbial development and prolong food product shelf life. The results reported for *Penicillium roqueforti* in cheese slurry at 20 and 30°C are in line with these observations. Generally, in contrast to prokaryotic bacteria, eukaryotic microorganisms are more susceptible to pressure (Hoover et al., 1989). Studies show that pressures above 200 MPa typically inactivate vegetative bacteria, yeast, and molds, with treatments up to 700 MPa and several minutes' duration. However, spores require additional strategies, such as higher pressures or moderate temperatures.

Table 1. Microbes mitigated by advanced physical techniques; High Pressure Processing

High Pressure Processing				
Mitigated Microbe	**Pressure**	**Temperature**	**Reduction**	**Reference**
Fusarium graminearum	380 MPa	60 °C	100%	(Kalagatur et al., 2018)
C. perfringens	600 MPa	75 °C	2.2-log	(Evelyn et al., 2017)
Neosartorya fischeri	600 MPa	75 °C	4.3-log	
B. Nivea	600 MPa	75 °C	2-log	
B. cereus spores	600 MPa	70 °C	4-log	
Yeast/Molds Acid tolerant microorganism	600 MPa	15 °C	3 log	(Moussa-Ayoub et al., 2017)
Yeasts/Molds	550 MPa	Room temperature	4.8 log	(Chai et al., 2014)
B. stearothermophilus	600 MPa	105 °C	3-log	(Devatkal et al., 2015)
C. perfringens	600 MPa	65 °C	2.54-log	(Gao et al., 2011)
Total aerobic bacteria	600 MPa	20 °C	5.2 log	(Liu et al., 2014)
Psychrotrophic bacteria	400 MPa	20 °C	3.31 log	(Landl et al., 2010)
A. acidoterrestris	600 MPa	60 °C	3.5 log	(Vercammen et al., 2012)
B. coagulans	600 MPa	60 °C	2.0-log	
Total mold	250 MPa	25 °C	90%	(Tokuşoğlu et al., 2010)
	250 MPa	4 °C	100%	
C. perfringens	900 MPa	100 °C	4-log	(Shao et al., 2010)
Aerobic mesophilic bacteria	450–650 MPa	20 °C	3 log	(Andrés et al., 2016)
Yeasts/Molds	550 MPa	20 °C	2.5 log	(Li et al., 2015)
L. monocytogenes	500 MPa	25 °C	4.8	(Hafiz Muhammad Shahbaz et al., 2016)
S. aureus	500 MPa	25 °C	2.4	
E. coli	500 MPa	25 °C	5.0	
S. tiphimurium	500 MPa	25 °C	7.0	
S. cerevisiae	500 MPa	25 °C	5.8	
Total aerobic bacteria	300–500 MPa	8-15 °C	1–3.3 log	(Mukhopadhyay et al., 2017)
Salmonella enterica serovar Anatum, serovar Typhimurium, serovar Enteritidis, serovar Newport, and serovar Montevideo	35 MPa 241 MPa 380 MPa	25 °C	0.75 log 0.53-1.88 log 2.76-7.0 log	(Allison et al., 2018)
Total aerobic bacteria	400-500 MPa		1.64-3.28 log	(Liu et al.
Yeast and molds	400 MPa		1.96 log	
Salmonella enterica serovar Stanley H0558, serovar Newport H1275, and *serovar Poona RM 2350*	300-500 MPa	8-15 °C	2.7-7.0 log	(Mukhopadhyay et al., 2016)

1.3. Combinations with other technologies

Nonthermal technologies, like HPP, aim to sustain the quality of the food while eradicating microorganism. Combining HPP with moderate temperatures increases microbial inactivation, although challenges exist in scaling up industrial processes (Raso and Barbosa-Cánovas, 2003). For example, a combination of 690 MPa pressure and 80°C temperature for 20 min effectively reduced the spore count of *Clostridium sporogenes* (Crawford et al., 1996). The combination of moderate temperatures (about 70°C) and pressure has been noted to be efficient at treating Bacillus stearothermophilus spores (Hayakawa et al., 1994).

The combination of pulsed electric fields (PEF) and high-pressure processing (HPP) demonstrates potential for spore inactivation, with the sequence of application influencing efficacy due to their distinct mechanisms of action. In a study on *Bacillus subtilis* spores, HPP and PEF were tested alone and combined to assess spore inactivation. HPP at 700 MPa and 55°C reduced spore counts to some extent, while PEF alone was ineffective. When combined, applying PEF before HPP resulted in up to a 7-log decline in spore counts, particularly effective in buffer solutions. This effectiveness was attributed to PEF generating cracks on the spore surface, followed by cell rupture due to subsequent HPP. Conversely, applying HPP before PEF did not reduce spore counts and led to spore reactivation. H-spores, observed during this treatment, were likely formed due to ion replacement with hydrogen under low pH conditions during HPP (Sasagawa et al., 2006).

2. Ultraviolet (UV) Irradiation

Ultraviolet (UV) irradiation, especially UV-C light, is emerging as a highly effective and non-toxic food preservation techniques that deactivates pathogenic microorganisms by damaging their DNA, reducing the need for chemical disinfectants (Yin et al., 2013). Traditionally utilized for sanitizing surfaces, air and water (Ledy et al., 2020), UV-C light is now applied in the food sector to treat liquids like juices and milk, process ready-to-eat meats and prolong the lifespan of fresh commodities (Keklik et al., 2012). Engineering innovations have improved the efficacy and penetration of UV-C systems (Singh et al., 2021). The food industry values UV irradiation for its broad-spectrum microbial inactivation, conservation of sensory and nutritive qualities, lack of toxic residues and low energy consumption (Gayan et al., 2014). The germicidal impact of UV-C light, peaking at wavelengths of 260-265 nm, relies on forming DNA photoproducts that prevent transcription and replication (Kowalski, 2009). Effectiveness depends on the microorganisms DNA repair mechanisms (López-Malo and Palou, 2004) and various factors like microbial physiology, growth conditions, and recovery environments (Sommer et al., 2000). Consequently, UV light technology is becoming an increasingly attractive and balanced option for food decontamination.

2.1. Applications of UV-C irradiation across food categories

UV-C irradiation-based preservation techniques are increasingly utilized for an array of foods including vegetable and fruit juices, meat products, dairy and milk items and fresh produce. UV-C light is favored for its capacity to inactivate microorganism without the drawbacks of heat processing, such as loss of nutrient and heat damage. The FDA recognizes UV-C light as a safe technique for pasteurizing fruit juices, requiring a significant reduction in pathogens to ensure safety. Studies have shown that UV-C treatment effectively prolongs the shelf life of liquid foods while retaining safety standards (Caminiti et al., 2012; Zhu et al., 2014). Microbial inactivation parameters vary widely, highlighting the need for tailored applications to different food types. In the dairy industry, UV-C light offers a substitute to thermal pasteurization and chemical preservatives, preserving the quality of milk and cheese while ensuring safety through rigorous control protocols (Jose Miguel Pestana, 2015). Additionally, UV-C light is utilized to sanitize packaging materials and the air in production areas in dairy plants, and its efficacy in various applications is documented in multiple studies (Christen et al., 2013).

UV-C light is employed in the meat industry to prevent microbial contamination on meat surfaces, notably in ready-to-eat products, due to strict food safety standards (Chen et al., 2012). UV-C light has been employed to attain substantial reductions in pathogens such as *Listeria monocytogenes* and *Salmonella spp.* (Singh et al., 2021). Despite its limited penetration, UV-C is effective as a surface treatment and can be used synergistically with other technologies to enhance antimicrobial action (Ha et al., 2015). Fresh produce which is prone to microbial contamination from field to table benefits from UV-C treatment to reduce surface

Table 2. Microbes mitigated by advanced physical techniques; Ultraviolet (UV) Irradiation

Ultraviolet (UV) Irradiation			
Mitigated Microbe	**Wavelength**	**Dose**	**References**
S. bayanus, S. cerevisiae D. anomala, D. bruxellensis, Z. bailii	254 nm	9.8 – 22.8 J ml^{-1}	(Gouma et al., 2015)
Bacillus subtilis spores	254 nm	2.37 J/mL	(Ansari et al., 2019)
S. typhimurium TISTR 292	254 nm	13.75 mJ cm^{-2}	(Mansor et al., 2014)
Staphylococcus aureus, Listeria monocytogenes, Salmonella	254 nm	5 – 40 kJ m^{-2}	(Sommers et al., 2010)
Aerobic mesophyll, bacteria and molds	254 nm	2.16 J m^{-2}	(Türkmen and Takci, 2018)
Aspergillus flavus, Aspergillus niger	254 nm	203 kJ m^{-2}	(Flores-Cervantes et al., 2013)
E. coli O157:H7	254 nm	152.17 mJ cm^{-2}	(Gabriel and Musni, 2019)
Yeast	254nm	0.2, 0.4, 0.6 and 4.8 kJ m^{-2}	(Manzocco et al., 2016)
S. Enteritidis	254 nm	0 -10 J cm^{-2}	(Possas et al., 2018)
E. coli DH5α, S. *subterranea, L. innocua* WS 2258	254 nm	243 mJ cm^{-2}	(de Souza et al., 2014)
Psychrotrophic bacteria	254 nm	880 J l^{-1}	(Rossitto et al., 2012)
Mesophilic bacteria	254 nm	1,760 J l^{-1}	
Listeria innocua, Escherichia coli, Staphylococcus aureus, Salmonella Enteritidis	-	12 – 72 kJ m^{-2}	(Birmpa et al., 2013)
E. coli ATCC 25922, *Pseudomonas fluorescens, Listeria innocua*	254 nm	1.02 to 12.29 J/cm^2	(Proulx et al., 2015)
L. Monocytogenes	254 nm	195 mJ cm^{-2}	(Hamidi-Oskouei et al., 2015)
Salmonella, E. Coli Campylobacter	200–1,100 Nm	1.27 J cm^{-2}	(Cassar et al., 2018)
B. thermosphacta, C. divergens, S. aureus, S. enteritidis, L. monocytogenes, Pseudomonas spp., enterohemorrhagic E. Coli	253.7 nm	0.05 – 3 J cm^{-2}	(McLeod et al., 2018)
Salmonella Typhimurium ATCC 14028	254nm	25 mW/cm^2	(Kim et al., 2009)
Mesophilic bacteria, coliforms, yeasts, molds	254 nm	6.22 kJ m^{-2}	(Jemni et al., 2014)
Murine norovirus	254 nm	12,000 J m^{-2}	(Liu et al., 2015)
Saccharomyces cerevisiae	-	8.45 J/cm^2	(Hafiz M Shahbaz et al., 2016)

microbial loads. Studies have demonstrated the commercial potential of UV-C for treating freshly cut fruits and enhancing the antimicrobial properties of natural components (Pinela et al., 2017). This technology is increasingly recognized for its role in improving the safety of food, extending shelf life and maintaining the quality of food items without giving in their nutritional and sensory attributes.

2.2. Limitations and future directions for UV-C technology

While UV treatment presents a viable substitute for heat pasteurization, it is not without its drawbacks, including inconsistent dose administration, extended treatment durations and optical attenuation. It is also less effective against viruses and spores (Koutchma, 2009; Falguera et al., 2011). Research is ongoing to understand the interaction of UV light with complex food matrices. It is necessary to critically assess challenges pertaining to technology, safety of food, and quality in order to efficiently develop and optimize UV treatment parameters for a variety of microorganisms in a range of food matrices (Ramesh et al., 2016).

3. Pulsed Electric Fields (PEF)

Pulsed Electric Field (PEF) technology is a promising non-thermal food preservation method that uses short, high-voltage electric pulses to eliminate microorganisms while maintaining food quality (Syed et al., 2017). Unlike thermal processing, PEF minimizes detrimental alterations in food's physical, sensory and nutritional attributes, making it an advantageous alternative for delivering high-quality products to consumers. PEF has been widely applied to various foods, particularly liquids and semi-solids, for microbial control however, recent studies have also looked into how it might improve the effectiveness of food processing steps like dehydration and juice extraction. (Qin et al., 1995; Vorobiev et al., 2004). Although pulsed electric fields (PEF) are effective in inactivating vegetative bacterial and yeast cells, their limited efficacy against bacterial spores restricts their application primarily to the control of pathogenic and spoilage microorganisms. PEF technology presents a competitive substitute to traditional thermal techniques, offering energy-efficient, economical food preservation while preserving sensory attributes and nutritional value (Hodgins et al., 2002).

3.1. Mechanism of action

The mode of inactivation of microorganism by PEF technology involves the destabilization of microbial membranes through the generation of an electric field, leading to electromechanical compression and pore formation (Barbosa-Canovas et al., 1999). According to the degree of membrane breakage, this process, called electro-permeabilization, can be either reversible or irreversible and eventually lead to cell death (Rowan et al., 2000). While the exact mode remains unclear, increased electric field strength enhances membrane permeability, thereby improving microbial inactivation. The efficiency of PEF is influenced by the nature of the substance, the characteristics of microbial cells, and various process parameters such as electric field intensity, pulse duration, and temperature (Raso et al., 2000; Wouters et al., 2001). The efficacy of microbial mitigation is also considerably impacted by the food's chemical and physical traits like pH, water activity, and electrical conductivity (Alvarez et al., 2000). Higher pH and water activity generally improve microbial reduction, while high electrical conductivity tends to reduce the efficacy of PEF (Wouters et al., 1999).

3.2. Applications and limitations in food processing

The food industry has substantially utilized PEF technology to pasteurize goods such liquid eggs, milk, juices, and soups. It is not without restrictions, though, as products must have particles smaller than the treatment gap, be low in electrical conductivity, and be free of air bubbles. Although generally unsuitable for solid foods, PEF has shown potential in enhancing bioactive component extraction and treating certain solid products (Siemer et al., 2014). In fruit processing, particularly with low-viscosity juices such as apple and citrus, PEF not only inactivates microorganisms but also preserves quality attributes like flavor and texture while extending shelf life (Dunn, 2019). Moreover, PEF has been used to improve drying efficiency, enzymatic activity, and even wastewater treatment (Ade-Omowaye et al., 2001).

In the dairy industry, PEF has demonstrated effectiveness in reducing microbial loads in milk, especially in products like skim milk and simulated milk ultrafiltrate (Sobrino-López and Martín-Belloso, 2010). However, its efficacy in whole milk is limited ascribed to the protective influence of protein and fat on bacterial cells

Table 3. Microbes mitigated by advanced physical techniques; Pulsed Electric Field

Pulsed Electric Field			
Mitigated Microbe	**Parameters**	**Results**	**Reference**
Brettanomyces	E = 31–50 kV/ cm, t = 21 or 51 µs	sterilization > 6 logs achieved	(Van Wyk et al., 2019)
Yeasts, fungi, and *Actinomycetes*	E = 30 kV/cm	No microbes detected after 400 pulses	(Dziadek et al., 2019)
E. coli	E = 4.5 kV/cm, t = 3 µs	Maximum bactericidal effect of 5.8 logs at 1500 pulses detected	(Arshad et al., 2022)
Coliforms, L. monocytogenes, and *the total number of bacteria*	E = 5–24 kV/ cm t = 25 µs	Sufficient reduction in the bacterial count in samples at 24 kV/cm, pulse duration 25 µs, 20 pulses.	(Šalaševičius et al., 2021)
Lactobacillus delbrueckii ssp. bulgaricus, Saccharomyces cerevisiae, Hansenula anomala, Candida lipolytica, and *E. coli* O157: H7	E = 17–31 kV/ cm t = 163–488 µs	Sufficient inactivation of all microbes	(Akdemir Evrendilek, 2022)
S. cerevisiae and *O. oen*	E = 15, 20, 25 kV/cm t = 10 µs	Eradication of *S. cerevisiae* and *O. oeni* up to 4 log achieved	(Delso et al., 2023)
Escherichia coli, Listeria innocua	E = 10–21 kV/cm f = 235–588 Hz t = 2 µs	*Escherichia coli* and *Listeria innocua* reduced by 5.0 and 3.9 log CFU/ mL, respectively.	(Yildiz et al., 2023)
Staphylococcus aureus, Escherichia coli	E = 20–40 kV/cm f = 1 Hz t = 10 µs Treatment time = 100–500 µs	Reduction of 5.891 to 5.924 log CFU/mL for *Staphylococcus aureus* and 5.876 to 5.949 for *Escherichia coli* detected.	(Kantala et al., 2022)
Escherichia coli, Salmonella Typhimurium.	E = 21–34 kV/cm f = 500–1500 Hz t = 2 µs Treatment time = 72–217 µs	More than 5.0 log CFU/mL of *Escherichia coli* and *Salmonella Typhimurium* reduced.	(Mendes-Oliveira et al., 2022)
Salmonella enteritidis	f = 193 Hz; Δt = 4 µs; E = 35 kV/cm Treatment time =1709 µs +2% citric acid/0.2% cinnamon bark oil	Greater than 5 log CFU/ml reduction	(Mosqueda-Melgar et al., 2012)
Salmonella senftenberg	f = 0.5 Hz Δt = 3 µs E = 25 kV/cm Temperature = 40 ◦C Treatment time = 3.5 min	About 3 log reduction detected	(Espina et al., 2014)
Saccharomyces, Lactobacillus plantarum, Salmonella Senftenberg, Listeria monocytogenes, and *E. coli*	E = 2.7 kV.cm-1, Δt = 15-1000 µs	Reduction of about 5-log noted	(Timmermans et al., 2019)
Enterobacter aerogenes	E = 50 kV cm-1, Δt = 590 ns, f = 1 Hz	2.4-log reduced	(Baba et al., 2018)
E. coli and *Listeria*	E = 2 kV cm-1, Δt = 1 µs, f = 100 Hz	About 3-log reduction	(Jin et al., 2017)

(Sharma et al., 2014). Research has indicated that the utilization of PEF in conjunction with modest heat treatments might augment microbial inactivation, leading to notable decline in bacteria such as Escherichia coli and Listeria spp. The implications of PEF on the functional attributes and quality of milk, however, is still being studied. In meat processing, PEF is utilized to enhance tenderness, reduce microbial load, and maintain meat quality during storage (Faridnia et al., 2015). While some studies report significant improvements in meat tenderness, the technology's overall effectiveness in muscle foods requires further exploration.

4. Cold Plasma

The fourth state of matter, plasma is an ionized gas state with distinct features from solid, liquid, and gaseous phases. Cold plasma (CP) a kind of non-thermal plasma, is made up of an array of reactive species such electrons, ions, and electromagnetic radiation along with excited atomic, molecular, ionic, and radical species (Zhang et al., 2013). CP can be generated under atmospheric or low-pressure conditions, leading to similar microbial mitigation mechanisms ascribed to the production of comparable reactive species and electron densities (Niemira, 2012; Scholtz et al., 2015). The generation of thermal plasma involves heating gas to extremely high temperatures, resulting in thermodynamic equilibrium among the chemical species, while CP maintains a non-equilibrium state with cooler ions and uncharged molecules (Misra et al., 2011; Moreau et al., 2008; Wan et al., 2009). Commonly, cold plasma (CP) is generated by applying a strong electromagnetic field to a neutral gas through the utilization of various electrical discharges, such as plasma jet and dielectric barrier discharge (DBD). These methods are preferred for biological, environmental, and biomedical applications due to their adaptability and versatile design (Banu et al., 2012; Nehra et al., 2008).

4.1. Mechanism of action

CP's complicated chemical makeup includes a number of reactive substances that either work alone or in concert to inactivate microbial targets. A number of variables, including voltage, frequency, temperature, moisture level, flow rate, gas composition, and device design affect efficacy of CP (Dobrynin et al., 2009; Wan et al., 2009; Ehlbeck et al., 2010). Reactive species, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), are generated by atmospheric air CP and are essential for the inactivation of microorganisms (Stoffels et al., 2008). These species can diffuse through bacterial cell walls, causing damage by oxidizing cytoplasmic membranes, proteins, and DNA (Gallagher et al., 2007; Joshi et al., 2011). Additionally, charged particles in CP bombard cell walls, leading to the erosion of membranes and the formation of lesions that allow further penetration of toxic compounds (Moreau et al., 2008; Stoffels et al., 2008). The inactivation process is often more effective in Gram-negative bacteria ascribed to their thinner cell walls, although Gram-positive bacteria exhibit higher intracellular ROS levels, resulting in more pronounced damage (Han et al., 2016). The mechanical destruction of cell membranes by CP is categorized as direct or indirect, with direct contact leading to electrostatic stress and potential cell morphology changes (Mendis et al., 2000; Laroussi, 2009). This erosion impact, resulting from the breakage of chemical bonds, can also destroy microbial support structures like biofilms, with atomic oxygen and ozone accelerating the etching of molecules (Ermolaeva et al., 2011). Despite extensive research on CP's antimicrobial effects, the type of microbiological contamination and the particular situation must be taken into consideration while optimizing its application, such as food processing or clinical environments, to develop effective antimicrobial technologies (Graves, 2014).

4.2. Application parameters and factors influencing efficacy

The antimicrobial influence of CP treatments is sufficiently influenced by the mode of exposure and system configuration employed during application. For instance, Hertwig et al. (2015) indicated that remote air plasma treatments achieved higher bactericidal effects against Salmonella on whole black pepper compared to direct argon plasma jet treatments. Air plasmas generate both ROS and RNS, which can penetrate microbial surfaces and lower intracellular pH, adversely affecting essential cellular functions (Hertwig et al., 2015). The use of contained atmospheric cold plasma (ACP) systems has been shown to enhance antimicrobial efficacy by retaining reactive species post-treatment (Ziuzina et al., 2014). Factors such as the distance between the plasma emitter and the sample significantly influence inactivation efficiency, with closer proximity resulting in greater microbial reduction, as demonstrated by Kim HyunJoo et al. (2013) in

Table 4. Microbes mitigated by advanced physical techniques; Cold Plasma Technology

Cold Plasma Technology			
Mitigated Microbe	**Parameters**	**Results**	**Reference**
Psychrophiles	Dielectric discharge O_2 and CO_2 60, 70, or 80 kV for 60, 180, or 300 s	Reduction greater than 1.0 log achieved	(Zhuang et al., 2020)
Aspergillus flavus, Aspergillus parasiticus	Atmospheric pressure fluidized bed plasma, Air or nitrogen 18–25 kHz 5 min 5–10 kV	4 to 17 log Reduction of microbes	(Dasan et al., 2016)
Escherichia coli O157:H7 and *Listeria monocytogenes*	Corona discharge plasma jet, Air 20 kV, 58 kHz, for 0, 30, 60, 90, and 120 s	1.5 log reduction of *Escherichia coli* O157:H7 and > 1.0 log reduction of *Listeria monocytogenes*	(Choi et al., 2016)
Salmonella spp., Escherichia coli, Bacillus cereus	Corona discharge plasma jet, Air 20 kV, 1.5 A, and 58 kH	1.2–2.2 log reduction of microbes	(Puligundla et al., 2017)
S. aureus, E.coli, C. albicans	Dielectric barrier discharge Air 60 kHz 8, 12, 25s 30 kV	More than 5 log reduction achieved	(Shi et al., 2011)
Aerobic mesophilic total viable count, yeasts and molds	Diffuse coplanar surface barrier dis -charge, Nitrogen gas	Reduction of 0.68–1.25 log CFU/g	(Pathak et al., 2020)
Bacillus tequilensis	Dielectric barrier discharge Helium 10.3 kV and 22.1 min	3.4 log CFU/g 1.7 log spores/g Reduction	(Bang et al., 2020)
Pseudomonas spp., lactic acid bacteria (LAB), yeasts and molds	Dielectric barrier discharge Atmospheric air6 kV, 45 kHz	0.57 to 1.02 log CFU/g Reduction	(Giannoglou et al., 2020)
G. liquefaciens, P. agglomerans, S. cerevisiae	Atmospheric plasma jet Helium 30 kHz 2.5 - 30 s 12-16 kV	3 log reduction was noted	(Perni et al., 2008)
Staphylococcus spp., and *Salmonella sp.*	Dielectric barrier discharge, Atmospheric air 10 min, 500 Hz, 40 kV	1–2 log Reduction	(de Souza Silva, 2019)
Salmonella Typhimurium and *Listeria monocytogenes*	Dielectric barrier discharge, N_2, CO_2 10 kV, 2 kHz	1.14 log reduction of *Salmonella Typhimurium* And 1.02 log reduction of *Listeria monocytogenes*	(Lis et al., 2018)
Brochothrix thermosphacta	Dielectric barrier discharge, CO_2 + O_2 80 kV for 60, 120 or 300 s	2 Log Reduction noted	(Patange et al., 2017)
Penicillium italicum	Microwave plasma, N_2, He, N_2 + O_2 (4:1), 2.45 GHz, 900W, 1 L/min, 0.7 kPa, 10 min	84 % reduction detected	(Won et al., 2017)

studies on chicken and pork products. Low-pressure CP generation methods offer advantages like reduced risk of arcing and suitability for treating pre-packaged produce, leading to effective decontamination without compromising product integrity (Zhang et al., 2013). The effectiveness of CP is further impacted by operational specifications like humidity levels, where higher moisture content enhances the generation of ROS and improves microbial inactivation rates (Ragni et al., 2010). Research focusing on fresh produce safety has revealed that surface characteristics significantly impact CP effectiveness; smoother surfaces facilitate better inactivation outcomes compared to complex, textured surfaces that may shield microbes from reactive species (Fernández et al., 2013; Ziuzina et al., 2014). Additionally, treatment time and plasma energy density are vital parameters that determine the success of CP treatments across various food matrices (Zhang et al., 2013).

5. Ozone Treatment

Ozone has been extensively applied in various industries, particularly the food sector, due to its potent oxidative properties and ability to decompose into oxygen without leaving any toxic residues (Fundo et al., 2018; Holah et al., 2016). Authorized by the US FDA for utilization in food in 2001, ozone is a strong alternative to chlorine-based sanitizers, effectively eliminating an array of microbes such as Viruses, fungi, and bacteria making it suitable for use in food processing, sanitation, and water treatment (Guzel-Seydim et al., 2004; O'Donnell et al., 2012). The gaseous form of ozone is particularly effective ascribed to its greater solubility in water and stability, making it an ideal candidate for disinfecting fruits, vegetables, and other food products (Kim et al., 2003). Recent studies highlight ozone's capability to inactivate pathogens such as *E. coli*, contributing to its growing popularity as a non-thermal food preservation technique (Khadre et al., 2001; Pandiselvam et al., 2019). Additionally, the inactivation mechanisms of ozone, involving oxidative damage to cellular components, underscore its effectiveness as a disinfectant, particularly in comparison to other oxidizing agents (Khadre et al., 2001).

5.1. Antimicrobial efficacy and influencing factors

With efficacy against protozoa, bacteria, fungi, and viruses ozone is a powerful antibacterial agent that works by disrupting cell membranes and oxidizing biological components (Khadre et al., 2001). Studies have demonstrated ozone's effectiveness against both Gram-positive and Gram-negative bacteria, with *Escherichia coli* being particularly sensitive. The efficacy of ozone in inactivation of microbes varies with factors such as food type, microbial load, ozone concentration, and pH (Bialka and Demirci, 2008; Perry et al., 2011). For instance, ozone treatment has been noted to sufficiently diminish *E. coli* in apple juice and *Shigella sonnei* in water (Selma et al., 2007; Mukhopadhyay and Ramaswamy, 2012). Additionally, ozone has been effective against viruses and fungi, including the reduction of aflatoxin B1 in contaminated dried figs (Öztekin et al., 2006; Zorlugenç et al., 2008). However, viruses tend to be more resistant to ozone than bacteria (Rojas-Valencia, 2011).

5.2. Industrial applications and practical considerations

Ozone is widely utilized in the food industry for its powerful antimicrobial properties, making it efficient in preserving food, sanitizing surfaces, and treating processing environments. Its application ranges from sanitizing eggs, fruits, vegetables, poultry, and meats to treating water and storage atmospheres (Khadre et al., 2001; Kim et al., 2003). In the dairy industry, ozone has proven effective in removing biofilms from equipment, thereby reducing microbial contamination (O'Donnell et al., 2012). For instance, *Listeria monocytogenes* biofilms on stainless steel were significantly reduced with ozone treatment, especially when combined with sonication (Baumann et al., 2009). Additionally, ozone has been utilized to control mold in cheese ripening rooms, preventing contamination with aflatoxins (O'Donnell et al., 2012). In meat and poultry, ozone decontaminates surfaces and prevents bacterial growth, thereby extending shelf life (Kim et al., 2003; Ziyaina and Rasco, 2021). Furthermore, ozone is efficient in reducing *Salmonella* in eggs and *E. coli* in fresh produce, with studies showing significant microbial reductions under various treatment conditions (Perry et al., 2008; Chuajedton et al., 2017). In fruits and vegetables, ozone treatment has been noted to diminish microbial loads without compromising the quality of the produce (Alves et al., 2019; Loredo et al., 2015). In the meat industry, ozone application challenges include oxidative effects on meat quality, but it remains effective in microbial reduction (Cho et al., 2014; Degala et al., 2016). Ozone's efficacy extends to

Table 5. Microbes mitigated by advanced physical techniques; Ozone Treatment

Cold Plasma Technology			
Mitigated Microbe	**Parameters**	**Results**	**Reference**
E.coli	Ozone concentration <1 ppm, 15 min	Reduction of 4.2 log detected	(Gibson et al., 2019)
Escherichia coli O157:H7	Aqueous ozone 23–30 mg/L, 3 min	3.7 log reduction Observed	(Perry et al., 2008)
Yeasts and molds, *Staphylococcus sp.*, *Enterobacteriaceae*, *Salmonella sp.*, psychrotrophic bacteria, and Total mesophilic aerobic bacteria (TMA)	Concentration: 1.5 mg/L Time: 5, 10 and 15 min	0.4–1.0 log reduction, After 15 min	(Cavalcante et al., 2013)
Bacillus cereus spores	Gaseous ozone at 9 ppm, 360 min	1.5 log reduction observed	(Asill et al., 2013)
Clostridium perfringens spores	Aqueous ozone at 5 ppm followed by heating at 55°C	Spores reduced by 0.87 log	(Pohlman, 2012)
E.coli	Chilled water 4.6 ◦C, chilled aqueous ozone 5.6 ◦C, 90s, 12 ppm Oxidation reduction potential: 2.6 V	Aqueous ozone spray chill leading to 1.46 log reduction, water chilled causing 0.60 log reduction	(Kalchayanand et al., 2019)
Listeria monocytogenes	Aqueous ozone 21.8 ppm, 10–20 min followed by heating at 60°C for 3 h	1.48 log10 CFU/g reduction observed	(Wade et al., 2003)
Salmonellae	Ozone 2000 ppm, 30 min	*Salmonellae* reduced by 97% and pseudomonads by 95%, coliforms were not affected	(Al-Haddad et al., 2005)
Botrytis cinerea (gray mold)	Gaseous ozone 5000 µL/L, 60 min	50–60% reduction detected	(Ozkan et al., 2011)
Mesophilic Psychrotrophic Mold-yeast	Heating at 60°C for 10 min, 100°C for 5 min followed by gaseous ozone 5% wt/wt for 15–30 min	No growth occurred up to 4 weeks at 4°C	(Oner et al., 2011)
Pseudomonas aeruginosa	28 mg/L Ozone for 5,10 and 15 min	Microbe was inactivated	(Munhõs et al., 2019)
Coliform, aerobic and anaerobic bacteria	Ozone 10 × 10−6 kg O3/m3/h (4 ± 1 °C) for 4 days	Decline in growth of coliform, aerobic and anaerobic bacteria observed	(Muhlisin et al., 2016)

other food products like mushrooms and grains, demonstrating its versatility as a food safety tool (Akata et al., 2015; Mohammad et al., 2019).

Challenges and Future Prospects

The implementation of these advanced techniques is influenced by several factors. One of the primary technological challenges associated with the industrial adoption of advanced physical techniques is scalability and cost. Approaches such as High-Pressure Processing (HPP) and Pulsed Electric Fields (PEF) often require sophisticated and expensive equipment, which can be a significant barrier to implementation, particularly for smaller food producers (Juliano et al., 2023; Afraz et al., 2024). Scaling these technologies from a laboratory setting to full-scale industrial production involves substantial capital investment and operational costs, which may be prohibitive for some businesses. Process optimization also presents a challenge. Each advanced physical technique has specific operational parameters that must be carefully controlled to balance microbial inactivation with the preservation of food quality. For example, HPP requires precise pressure levels, while PEF needs accurate electric field strengths (Petrus et al., 2020; Ashrafudoulla et al., 2023). Achieving the optimal parameters for different types of food products can be complex and requires ongoing adjustment and fine-tuning to maintain efficacy without compromising sensory attributes or nutritional value. Uniform treatment of food products is another significant challenge. In methods like HPP, ensuring consistent pressure distribution across all food particles is crucial for effective microbial inactivation (Tsagkaropoulou et al., 2024). Inconsistent treatment can lead to areas of the product that are inadequately processed, reducing the overall effectiveness of the technology. Additionally, the complexity of advanced physical techniques necessitates skilled personnel for operation and maintenance. Regular calibration and upkeep are essential to ensure consistent performance, which can be resource-intensive and require specialized training.

Regulatory approval is a significant challenge for adopting advanced physical techniques, as these technologies often need extensive validation and certification, which can be both time-consuming and expensive. This process can delay adoption and increase financial risks for companies (Tallon and Kalman, 2024). Additionally, consumer perception is crucial; skepticism or resistance to new methods, especially if they seem to affect the naturalness of the food, can hinder acceptance. Effective communication and education about the safety and benefits of these technologies are essential to build consumer trust and facilitate wider adoption.

Looking ahead, integrating advanced physical techniques with other preservation methods, such as chemical or biological interventions, could enhance microbial control and food quality. Research is exploring new methods and improvements, like novel cold plasma and advanced ultrasound techniques, which may offer greater efficacy and cost benefits. Customizing these techniques for specific food products, aided by machine learning and data analytics, could optimize microbial control and processing efficiency. Sustainability will be a key focus, with future research aiming to make these technologies more energy-efficient and environmentally friendly. Harmonizing international regulations could also facilitate broader adoption. Addressing these challenges will enable the food industry to better use advanced techniques for improved safety and quality.

Conclusion

The advanced physical techniques in microbial mitigation have presented a significant leap forward in ensuring food safety without compromising quality. Non-thermal technologies such as high-pressure processing, pulsed electric fields, cold plasma, and ultraviolet light demonstrate considerable potential in inactivating a wide range of foodborne pathogens while preserving the nutritional value and sensory attributes of food products. Despite the promising outcomes, the full-scale industrial implementation of these methods faces limitations pertaining to cost, scalability and consumer acceptance. There is a need to optimize these technologies to overcome these barriers, integrating them into existing food processing systems, and ensuring regulatory frameworks that support their adoption. The continued advancement and refinement of these techniques are essential for meeting the growing demand for safer, higher-quality food products in a sustainable manner.

Compliance with Ethical Standards

Conflict of Interest

The authors confirm that there are no conflicts of interest.

Authors' Contributions

Abdul Mueez AHMAD: Conceptualization, Data Curation, Laboratory analysis, Writing-original Draft, Investigation, Methodology, Formal analysis, Visualization, Writing-review and Editing.

Ethical approval

Not applicable.

Funding

Not applicable.

Data availability

Not applicable.

Consent for publication

Not applicable.

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