Ozone Processing in the Dairy Sector: A review of applications, quality impact and implementation challenges.

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Abstract

The impact on the natural characteristics of dairy products during thermal processing warrants the investigation of non-thermal techniques. Ozone has not only arisen as an inactivation treatment for milk and its products with minimal effects on the quality parameters. But has also been proven efficient for reducing biofilms, antibiotics, and aflatoxins, and used for equipment sterilization, air disinfection, sanitization, and de-sludge treatments. This review discusses the updates on the effect of ozone processing on the microbiological, physiochemical, nutri-functional, and sensory quality of milk and milk products. Other applications of ozone in the dairy processing sector (storage rooms disinfection, wastewater treatment, benefits in CIP, toxin reduction), current industrial scenario, and regulatory and safety requirements in the facility dealing with ozone have also been discussed alongside the research gaps and challenges.

Keywords: non-thermal processing, ozonation, milk, dairy processing, milk safety

1. Introduction

Dairy products are a potential source of nutrients and have numerous benefits to human health (Vashisht, 2021). Abundance of nutrients makes them susceptible to microbial growth, thus categorizing them as a perishable commodity. The microbes in dairy products can range from pathogenic vegetative bacteria to spoilage-causing bacterial spores (Bandla et al., 2012). Approximately 12% of animal outbreaks and 4% of worldwide foodborne outbreaks are because of the consumption of milk and milk products (Grace et al., 2020). This necessitates stringent processing requirements to ensure their safety (Delorme et al., 2020). Thermal processing techniques, including pasteurization, sterilization, and Ultra High Treatment (UHT), are majorly used for these products (Munir et al., 2019; Vashisht et al., 2021). Literature has reported their effectiveness against foodborne bacteria, bacterial spores, yeast, mold, and viruses. However, exposure to high temperatures leads to the loss of heat-labile components and alters the natural characteristics of dairy products (Cappozzo et al., 2015). Denaturation of proteins, loss of ascorbic acid content, undesirable changes in fat globules, and deprivation of typical sensorial characteristics are some of the major alterations due to the thermal processing of milk reported in the literature (Braun-Fahrländer and Von Mutius, 2011; Vashisht et al., 2022). These disadvantages have pushed the researchers to explore non-thermal technologies (Vashisht, 2021; Mohammadi et al., 2017; del Carmen Razola-Díaz et al., 2023). Among them, there has been considerable interest in ozone treatment, and it is seen as an affordable and sustainable alternative (Khanashyam et al., 2022; Varga and Szigeti, 2016).

Being a powerful oxidizing agent, ozone permeates the cell walls of the bacteria and inactivates them by damaging cell constituents (Afonso et al., 2022; Islam et al., 2022). Previous literature has proved the ozone's potential to increase the shelf life of dairy commodities as an independent treatment and with other processing techniques (hurdle technology) (Brodowska et al., 2018; Genecya et al., 2020; Sheelamary and Muthukumar, 2011). In addition, some studies have reported minimal effect of this processing technique on the natural parameters of these products (Khanashyam et al., 2022; Sert and Mercan, 2021a). However, optimizing factors, such as ozone concentration and treatment time, is necessary for each product. Other than the inactivation treatment, additional applications of ozone treatment include de-sludge treatment, wastewater processing, food contact surface and air disinfection, biofilm elimination, and reduction of toxins and antibiotics in milk (Khoori et al., 2020; Masotti et al., 2019; Sert and Mercan, 2021b; Suresh et al., 2021).

Many regulatory bodies in different regions of the world have shown a tremendous interest in the ozone processing of food products (O'Donnell et al., 2012). Regulations demonstrates that milk processing sector can potentially use ozonation in sanitation, cold storage, and direct applications on products (Alexopoulos et al., 2017; Sert and Mercan, 2021a). Despite that, significant industrialization efforts have yet to be made for its usage in dairy manufacturing plants and farms. More information regarding the ozone processing of milk and milk products is crucial for industrial expansion.

This review provides a comprehensive examination of the latest developments in the influence of ozone processing on the microbiological, physicochemical, nutri-functional, and sensory characteristics of milk and its derivatives. Additionally, the review delves into various ozone applications within the dairy processing sector, such as disinfection of storage rooms, wastewater treatment, advantages in Clean-in-Place (CIP) systems, and toxin reduction. The current industrial landscape, regulatory and safety requirements for facilities utilizing ozone, as well as existing research gaps and challenges, are thoroughly explored.

For the compilation of this study, a comprehensive review of 70 articles was undertaken, drawing from diverse internet sources such as Google Scholar, Science Direct, and PubMed using the following keywords (ozone processing, milk products, milk, ozone treatment). Each article was rigorously analyzed regarding the reported findings and critical insights. Subsequently, these studies were systematically organized, leading to a judicious conclusion.

2. Fundamentals of ozone processing

Ozone production is a simple process that originates due to a reaction between free oxygen radicals and oxygen molecules (Varga and Szigeti, 2016). The low stability of ozone molecules leads to their dissociation into reactive oxygen species (ROS), including O_3^-, O^-, O_2^- and HO_2^- (Kim et al., 1999). The generated ROS species efficiently undergo free radical chain reactions with numerous organic molecules, including alkanes, alkenes, amines, carbon-hydrogen bonds, and sulfhydryl groups (Khanashyam et al., 2022). As per Zhang et al. (2011), ROS species initially alter the permeability of cell membranes and walls of bacteria, followed by the leaching of nutrients and components of bacteria, which ultimately leads to its inactivation (**Fig. 1)**.

Corona discharge and photochemical methodology (UV irradiation) are the two common techniques that are commercially used for ozone production (Alexopoulos et al., 2017; Prabha et al., 2015). The schematic diagram of the lab scale set up for the ozone processing is depicted in **Fig. 2**. The corona processor consists of two electrodes (ceramic material, 6-7 eV) known as high tension and low tension parted by dielectric medium (**Fig. 3a**). The air is passed through the gap between these electrodes where oxygen molecules get segregated and form ozone molecules due to collision. In the food industries, high-frequency corona discharge generators (>1000 Hz) are considered suitable for high-capacity operation.

In UV generators, ozone is generated through the photodissociation of oxygen molecules (Marino et al., 2018) (**Fig. 3b**). A low-pressure mercury lamp with peak emission at 185 nm is used in these generators [34]. In these systems, the air is allowed to go through a radiation field generated by the lamp in its ambiance. Photo-dissociation of oxygen (O_2) leads to the generation of free oxygen (O) radicals. This unstable radical promptly forms a bond with O_2 and generates ozone (O_3) molecules.

Both aqueous and gaseous forms of ozone have been widely explored for different applications in the dairy industry and farms (Islam et al., 2022; Marino et al., 2018). Careful choice according to the needs and processing plant structure is crucial. Foodborne pathogens are more sensitive toward the aqueous form rather than gaseous. Therefore, obtaining significant inactivation through the gaseous phase requires prolonged treatment time and higher ozone concentration (Marino et al., 2018; Pascual et al., 2007). However, gaseous phase molecules are known for extended half-life and more diffusivity than aqueous phase ones (Giménez et al., 2021). This makes them suitable for removing the biofilms from the dead ends in the processing facilities (Baggio et al., 2020).

3. Impact of ozone treatment on the microbiological quality of dairy products

Milk is a primary base of all dairy products, and its abundant nutrients and high water activity make it susceptible to microbial growth (Vashisht, 2021). Hence, it is crucial to subject milk to a safety treatment before manufacturing its products. As per Brodowska et al. (2018), ozone can be mixed directly in raw milk to prevent and cease microbial growth and subsequently improve its shelf life. Literature studies have demonstrated the potential of ozone processing against numerous dairy-borne pathogens and spoilage microorganisms inoculated in milk and milk products (Cavalcante et al., 2013b; Genecya et al., 2020; Wang et al., 2021). However, only a few studies have demonstrated a 5-log reduction of targeted microorganisms (Sheelamary and Muthukumar, 2011). Recent works that delve into the microbial inactivation potential of ozone in milk and milk products are summarized in **Table 1**.

In one of the large-scale industrial applications, ozone was used in combination with bubbling technology at a concentration of 1.5 mg/L for 15 min. The processing led to a 1 log reduction of bacterial as well as fungi count in raw milk. Also, in a study conducted by Sheelamary and Muthukumar (2011), ozone treatment (0.2 g/L concentration for 15 min) of raw milk accomplished an absolute inactivation of *Listeria monocytogenes*. Genecya et al. (2020) carried out ozone processing of skim and whole milk for 10.8 min at concentrations of 0.36 L/min and 5.01 L/min, respectively. It resulted in 1.70 and 2.16 log-reduction, respectively. Cavalcante et al. (2013b) used ozone gas at 1.5 mg/L concentration in raw milk for 5, 10 and 15 min. The effect of ozone on different microbes, including *Enterobacteriaceae*, total mesophilic aerobic (TMA), psychotropics, yeast and molds, and *Staphylococcus* in the milk samples before and immediately after treatment were evaluated. Ten min of processing led to the 0.58, 0.52, and 0.25 log inactivation of *Enterobacteriaceae*, *Staphylococcus*, and yeast and mold, respectively; however, in case of 15 min treatment, the *Enterobacteriaceae*, psychotropics, *Staphylococcus*, and yeast and molds reduction were 0.60, 0.96, 0.13, 1.02, and 0.48, respectively. Sert and Mercan (2021b) investigated the ozones' potential to inactivate coliforms inoculated in milk and whey concentrates. The treatment was given for 0, 5, 15, 30, and 60 min. It was observed that 60 min of treatment could reduce the coliforms by 0.87 log and 0.75 log for the milk and whey concentrates, respectively. Similarly, treatment of raw milk with 28 mg/L for 5 min reduced 1 log of *Pseudomonas aeruginosa* prior to powder (skim and whole) production (Munhõs et al., 2019). Torlak and Sert (2013) discovered that the application of gaseous ozone at a concentration of 2.8 mg/L for durations ranging from 0.5 to 2 h successfully deactivated 1.4 and 3 log *Cronobacter sakazakii* from whole and skim milk powder, respectively. Overall, ozone has proved to be an efficient processing technique for reducing microbial load in milk and milk products. However, additional studies are needed in this domain to demonstrate the 5-log inactivation of targeted microbes, thereby establishing its potential equivalence to thermal pasteurization.

4. Effect of ozone on the quality parameters of dairy products

While ozone treatment is a proven method of reducing a dairy product's microbial load, it may negatively affect its chemical composition if given in an unquantifiable manner. Hence, it is crucial to consider the effect on the product's chemical and microbiological quality before determining the exposure time or ozone concentration for the treatment of any dairy product (Akbas and Ozdemir, 2008; Perna et al., 2022). The impact of ozonation on the natural characteristics of numerous milk and milk products has been summarized in **Table 2**.

Any effects on the properties of fluid milk will affect the properties of dairy products produced from it. As per the literature, ozone treatment significantly affects the fat, protein, and color content of milk (Uzun et al., 2012; Mahanta et al., 2022). The strong oxidation potential of ozone is known to affect milk protein and fat. According to Uzun et al. (2012), it impact the peptide backbone of amino acids, causing breakage of bonds and alteration of the side chain structure causing alterations in functional properties like foaming and emulsifying ability (Uzun et al., 2012). Direct contact of ROS species generated during the process initiates milk protein oxidation, increasing surface hydrophobicity and decreasing free sulfhydryl (-SH) groups. The loss of -SH groups generates disulfide cross-links that can result in protein aggregation, forming larger protein particles (Habib et al., 2022; Nikmaram and Keener, 2022). In addition, an increase in aldehydes may be attributed to the auto-oxidation or spontaneous breakdown of some unsaturated fatty acids in milk, such as oleic and linoleic acids. These two are unsaturated fatty acids with double bonds found in milk (Nikmaram and Keener, 2022).

Additionally, treatment of 10 min at a concentration of 80 mg/min has been shown to influence the breakdown of β-carotene in milk. The carotenoid component declined by almost 50%, resulting in elevated lightness and a decline in yellowness (Mohammadi et al., 2017). Carotenoids are associated with the fat component of the product, and ozone oxidation is thought to occur in the double-bond structure of these molecules, thereby impacting the product's color (Perna et al., 2022). The currently available studies suggest that optimizing ozone concentrations and treatment time are essential in its application on fluid milk.

4.1. Fat rich dairy products (cream milk, cream, and butter)

Fat-rich dairy products are more prone to lipid peroxidation when subjected to ozone processing. Cream milk is a fat-rich dairy product known for its creaminess and mouth feel. Perna et al. (2022) observed the increased lipid peroxidation and deterioration of color attributes of cream milk by increasing the ozone exposure time from 0 to 60 min (300 mg/h concentration). The main reason for color degradation in this product was the degradation of carotenoids from ozone treatment. The impact of ozonation on the cream was observed by Sert et al. (2020), where the increased size of fat particles was observed, where partial coalescence and cluster formation of milk fat could be the primary reason for it.

Butter is another popular dairy product which has approximately 80% milk fat and its spreadability plays a crucial role in deciding the consumer acceptability (Mahanta et al., 2022). Sert and Mercan (2020) conducted an interesting study where butter was churned using ozonated water (ozone concentrations: 0.15, 0.20, 0.25, 0.30, and 0.35 mg/L). This treatment resulted in elevated lightness and redness and the declined yellowness of the product. The results were more pronounced at the higher concentrations. Because ozone significantly impacted the fat's oxidative potential, that declined the oxidative stability of butter. The impact was worse when churning was conducted in the presence of higher concentrated ozonated water, which led to fat agglomeration and decreased spreadability. Therefore, selecting optimal ozone concentration and treatment time is crucial to reducing the detrimental effects of ozone on fat-rich products.

4.2. Cheese and yogurt

Cheese and yogurt are popular dairy products known for their typical sensory characteristics and health benefits (A EL DAHSHAN et al., 2013; Azhar and ALmosowy, 2020; Chandan et al., 2017). Earlier studies have reported no significant impact of ozone processing on the natural attributes of these products. Alexopoulos et al. (2017) investigated the effect of ozonation (2.5-3 ppm) on feta cheese and yogurt quality. No significant impact on the overall sensory quality was observed for the yogurt after 60 sec of exposure. The brine solution (ozonated for 60 min) applied to the feta cheese also had no significant impact on the overall acceptability. Segat et al. (2014) analyzed the impact of ozonation on the peroxidation of mozzarella cheese. Processing for 2 h at a concentration of 30 mg/min had no significant impact on the peroxide value. Botondi et al. (2023) also reported no significant effects on the chemical and sensory characteristics of Russian and Swiss-type cheese stored in the refrigeration ambiance disinfected by ozone. The above studies indicate ozone as an efficient treatment that does not affect the native functional and physicochemical properties of cheese and yogurt.

4.3. Milk powders and concentrates

The current global milk powder market has reached a market value of 20 billion, mainly attributed to its health benefits (Steinitz, 2023). The ozone process has been reported to have a negative effect on the sensorial attributes of the dried milk powders; however, amelioration in the physical properties like wettability, flowability, and cohesiveness was observed. Spray-dried skim milk powders given an ozone pretreatment at higher levels showed considerably poorer sensory scores than those produced at lower ozone levels. Furthermore, it was determined that whole milk powders were more susceptible to ozone damage than skim milk powders (Khanashyam et al., 2022). This suggests that milk fat and ozone interaction may be responsible for forming undesirable flavor components.

Another study suggested that the ozone treatment could improve the wettability of milk powder (Sert and Mercan, 2021a). The experiment results were based on gaseous ozone treatment at 3.5 g/h for 60-120 min. Similar trends were observed for dispersibility when treated with ozone at similar concentrations for 30-60 min. Additionally, the foaming capability of the ozone-treated skim milk powder was in the range of 41% to 48.5%, which was also significantly higher than the control samples. This might be due to the modifications of protein structure and oxidative radicals that enhanced foaming ability due to increased surface-active properties. Powder flow speed dependency and compaction coefficients of ozonated samples were analyzed after treatment with gaseous ozone at a 3.5 g/h rate for 60-120 min. The compaction coefficient decreased as flow speed increased, i.e., the free-flowing ability of the ozonated samples increased at a higher flow rate. Milk powders with free-flowing properties have several advantages in the dairy sector, aiding in easy transport and less energy use. When the ozone treatment time was extended relative to untreated samples, the cohesiveness coefficients increased.

Whey protein isolates exposed to ozone in an aqueous medium at a concentration of 4.5 ppm had alterations in the amino acid side chains and protein structure, where enhanced flexibility or rigidity of the protein chain was observed. This considerably improved whey protein isolate's foaming properties. Changes in protein structure affect other functional qualities such as hydrophobicity, -SH concentration, and solubility, which occurred after ozone treatment for 30-480 min. The α-helix structure of the protein molecules enlarged, whereas free-SH content declined, resulting in increased surface hydrophobicity of the proteins (Uzun et al., 2012). Textural properties of whey protein and milk protein isolates were further studied, where ozone treatment of 3.5 g/L reduced the firmness and consistency of the products made from them. This was most likely due to protein molecule aggregation caused by the destabilization of amino acid side chains. However, ozone treatment at 3.5 g/h elevated the volume mean diameter of milk concentrate while it decreased in case of whey concentrate. Sert and Mercan (2021b) also obtained similar results with 3.5 g/h of gaseous ozone treatment for 30 min, possibly due to the formation of whey-casein clusters with ozone treatment. Overall, ozone processing had a negative impact on the sensory attributes while the physical attributes of the milk powders enhanced.

5. Additional applications of ozone in the dairy sector

In addition to the microbial inactivation treatment, ozone has proved its effectiveness against the aflatoxins (AF) and antibiotics content in milk and its products (Sert and Mercan, 2021b). Studies have also observed its applications (as individual and hurdle techniques) for air purification or disinfection, biofilm inactivation, sterilization of dairy equipment, and sanitation of dairy farms and processing areas (Masotti et al., 2019; Suresh et al., 2021; Heacox, 2014).

Khoori et al. (2020) investigated the combined impact of ozonation, ultraviolet (UV) treatment, and pulsed electric field (PEF) against the total AFs and AF M_1 levels in the acidophilus milk. The maximum reduction of AF M_1 (96.1%) and total AF content (98.81%) was observed at the optimized conditions: pulse duration – 13.15 µsec, ozone concentration – 9.99 mg/min, and UV intensity – 4990 mJ/cm^2. In another study by Mohammadi et al. (2017), milk samples were exposed to gaseous ozone at an 80 mg/min rate within containers for varying durations: 0, 0.5, 1-, 2-, 5-, and 10-min. Results revealed that 5 min of treatment could reduce AF M_1 by 50%.

Suresh et al. (2021) investigated the combined application of ozone, UV irradiation, and H_2O_2 for treating wastewater in the dairy industry. Significant reductions were observed at the combination of Ozone/H_2O_2, Ozone/UV, UV/H_2O_2, and Ozone/UV/H_2O_2 conducted at pH 7 and 50 mL/h of flow rate. Processes achieved COD reductions of 32.5%, 35.2%, 25%, and 83%, as well as lactose reductions of 40.6%, 43.6%, 38.2%, and 80%, respectively., Maximum reduction of COD (88%) and lactose (93.4%) was observed with the UV/H_2O_2/Ozone process under the conditions of pH 5, 50 mL/h circulation, and 180 min of treatment time. Packyam et al. (2015) used a combination of ultrasonication and ozonation to ameliorate the sludge breakdown in dairy effluent. The combined process with a specific energy of 76.4 kJ/kg total solids and ozone dosage of 0.0011 mg/mg of soluble solids resulted in improvements in COD solubilization and soluble solids reduction compared to pre-ozonation alone. Furthermore, it enhanced the potential for anaerobic biodegradation of the dairy waste-activated sludge.

Masotti et al. (2019) applied ozone treatment (1.5 g/h, 40 L/min, 3 h) for air disinfection in the cheese packaging facility, where no bacterial isolates were observed on plates throughout the five weeks of the experiments. The periodic ozonation of processing areas in dairy factories (with similar or lower concentration), effectively prevented the mold growth with no impact on the quality parameters of cheese. Botondi et al. (2023) also stated the high effectiveness of ozone in deactivating mold in the ambiance of cheese aging chamber.

A patented method by Heacox (2014) used ozone at low concentrations (ranging from 0.04 to 1.2 ppm) to decontaminate dairy instruments and facilities. This innovative approach deviates from the traditional practice in the dairy industry, which typically involves hot water and chemicals usage for cleaning. It reduces the need for chemicals and almost eliminates the hot water costs associated with cleaning in the dairy industry. Ozone can also be mixed directly in water to be used for cleaning. It reduces the CIP cost, along with improved cleaning and removal of milk residues. The same water may be used for more repetitive cleaning cycles, reducing the water requirements, and improving cleaning results. Additionally, no chemical residues are left as in conventional methods. Chlorine residues can potentially stay in the pipes, which later mix into the products and generate hurdles in product manufacturing, affecting sensory acceptability and being hazardous to consumers' health (Varga and Szigeti, 2016).

Botondi et al. (2021) and Wang et al. (2021) reported the effectiveness of ozone as a sanitizer in dairy farms as well as plants attributed to its oxidation-reduction potential and ecofriendly and cost-effective nature. This has a potential application in dairy farms for cleaning pipelines and food contact surfaces. Biofilms or bacterial fouling has always been a major concern for the dairy industry (Panebianco et al., 2022). It can occur at any point, from raw milk collection to wastewater treatment in the plant. However, Marino et al. (2018) proved that the usage of aqueous and gaseous ozone can be effective in getting rid of biofilms. This study suggested that ozonation can be used as a sanitizer for daily cleaning practices. However, gaseous ozone was more suitable for usage in confined spaces as it is more reactive and can harm the operators if used in open spaces. Similarly, Panebianco et al. (2022) studied ozonation (50 ppm concentration) for 6 h on biofilms formed by twenty-one strains of *Pseudomonas* spp and isolated from dairy. Based on the impact of ozone on eradication and inhibition treatment, it was suggested that ozone treatment could be used as an effective method to increase the existing sanitation procedures. However, its use was inadequate to contrast established biofilms of *Pseudomonas* spp. Overall, ozone is an effective processing technique for the treatment of antibiotics, toxins, disinfection of storage rooms, equipment, and processing lines. However, for dairy waste it is technically effective but economically it is eighteen times more expensive than coagulation processing conducted using $FeSO_4$ (excluding sludge disposal and labor costs (Varga and Szigeti, 2016).

6. Regulations, industrial status, and safety concerns for ozone processing

Electric Power Research Institute's specialists' approval during the 1997 meeting about the Generally Regarded as Safe (GRAS) classification of ozone as a potential disinfectant for foods under the conditions of appropriate amount of usage and compliance with good manufacturing practices leads to the significant interest of food manufacturers in this technology (EPRI, 1997). At present, ozone usage in food facilities has received legal approval in regions including North America, Australia, New Zealand, Japan, and multiple European nations (O'Donnell et al., 2012; Varga and Szigeti, 2016). In June 2001, the FDA classified it as a food additive, issued a ruling, and approved the ozonation application, in both gaseous and aqueous phases, against the foodborne microbes within the processing facility (Food and Drug Administration, 2001; Khanashyam et al., 2022). Ozone was first used at a Swedish milk plant as a pretreatment of fluid milk prior to thermal pasteurization. The product preserved lipid and protein contents for a longer period, resulting in an elongated shelf-life of milk and no sign of oxidation occurring during storage (Varga and Szigeti, 2016). However, after that, no significant industrial efforts have been made to apply ozone as a safety treatment for milk or milk products. Under standard working conditions, ozone exposure should not be more than 0.1 ppm by volume (0.2 mg/m^3 NTP) for 8 h every day, or 40 h per week. Another limitation includes 0.3 ppm by volume (0.6 mg/m^3 NTP) for 15 min, and not more than 4 times every day with at least 1 h intervals between small time exposure (Brodowska et al., 2018). The specific residual and recommended limits for ozone can vary depending on the country, product, and regulatory authority. Generally, ozone residual limits are set to ensure that ozone-treated food products do not contain harmful or excessive levels of residual ozone that could impact the quality and safety of the product. It has a lot of potential for its application in the food industry as a powerful sanitizer. There are no safety issues with its residual limits in this application as it breaks down into simple oxygen (Kim et al., 1999).

A lower ozone concentration is also safe for personnel, food products, and machinery. However, a higher concentration is linked to adverse effects on the health of personnel, the acceptability and quality of dairy and food products, and the lifespan and performance of the processing and machinery. Ozone poses health risks to humans when they encounter it in higher concentrations and for a longer duration, where 0.1 mg/L exposure can cause headaches, mild irritations in the eyes, throat, and nose, and severe illness. However, when concentration reaches up to 95 mg/L, the consequences become irreversibly hazardous, with the worst-case scenario being fatal (Brodowska et al., 2018; Cullen and Norton, 2012; Pirani, 2010). Effective systems for the detection and subsequent catalytic or thermal degradation of ozone are needed to ensure the safety of workers in the facility. This is of particular concern when gaseous ozone is employed. In such scenarios, the installation of a continuous ozone analyzer is essential. This analyzer should be designed to activate a comprehensive alarm system, comprising both auditory and visual warning signals, as soon as the ozone concentration surpasses 0.1 ppm (equivalent to 0.2 mg/m^3) in the room's atmosphere (Cullen and Norton, 2012; Langlais et al., 2019). While using oxygen as a feed gas to produce ozone, workers need to be aware that many organic compounds might become highly flammable in the presence of oxygen. Furthermore, certain organic materials decompose when exposed to oxygen. Therefore, some pertinent safety measures should be considered if oxygen is used to produce ozone, typically used in most food processing industries, to prevent accidental fires produced by stray sparks or flames caused by oxygen leaks (Rice, 2012).

Apart from humans, ozone may also interact with surfaces of equipment and processing machinery. Therefore, it is of utmost importance to use only materials compatible with ozone for the fabrication. Most materials are resistant to ozone at concentrations of 1-3 ppm, but this can cause a corrosive effect on the equipment at high concentrations. Food-grade plastics like Teflon, Kynay, PVC, and Halar exhibit acceptable behavior and corrosion resistance during exposure (Brodowska et al., 2018). However, ozone pulsed into the water at the concentration of 0.4-0.5 ppm, at 21-23°C for 20 min regularly for over seven days caused noticeable weight reduction in aluminum, copper, stainless steel, and carbon steel material (Varga and Szigeti, 2016).

7. Current challenges and gaps

Despite regulatory approvals and numerous potential applications of ozone processing in the dairy sector, product manufacturers have explored it at a limited level. Most of the literature on microbial inactivation has failed to demonstrate a significant (5-log) reduction of targeted microorganisms in milk and milk products. Bacterial spores and unwanted enzymes are another significant problem of the dairy sector. If they remain active, they can quickly spoil dairy products (Charles, 2021, 2022; Julakanti et al., 2023; Pendyala et al., 2022; Rathnakumar et al., 2023). The potential of ozone in the inactivation of dairy spores or enzymes has yet to be explored.

ROS species generated during the processing have significant effects on the oxidative stability of milk fat. Also, the variation in the fat content can have a detrimental effect on the efficacy of ozone treatment. Investigating how the ozone treatment inactivation efficiency varies with the amount of fat can be another potential area of research. Prolonged exposure to ozone or ozone-treated water has been reported to negatively impact dairy products' nutritional, functional, and sensory parameters. This is attributed to its strong oxidation potential. There is a need for a better understanding of ozone effects on these parameters, and processing optimization is needed to obtain significant microbial inactivation with minimal effect on the quality parameters.

Though ozone is a potential processing alternative for dairy processors, there is hesitation to use this process in the facilities. This is attributed to the complexity of the present equipment and the need for high funds for their setup. Limited capacity makes it unfeasible for larger capacity dairies. Additionally, there is a need for extra safety precautions for the employees dealing with ozone. Therefore, more focus is needed to improve the current processing equipment and its safety.

8. Conclusion

Ozone processing emerged as a potential alternative to thermal treatment to ensure the safety of dairy products. Recent literature demonstrated the bacterial inactivation potential in milk and its products through ozone processing. However, a limited number of authors have shown the five-log reduction. Further, process optimization is needed to avoid negative impacts on the quality parameters of treated dairy products; common ones include lipid and protein oxidation and color alternations. Ozone also has applications in aflatoxin and antibiotic reduction, biofilm prevention, disinfection of equipment, sludge treatment, and air disinfection in dairy farms and industry. Despite regulatory approvals in different countries and various ozone applications in the dairy sector, its usage in the industry is insignificant. The major reasons are higher setup costs, the sophistication of equipment, and the need for continuous treatment with commercially relevant flow rates. Further, higher ozone concentrations can be a hazard to workers; hence, facilities need extra caution. Future studies need to test ozone's inactivation potential against indicator microorganisms of pasteurization, spores, and enzymes. Evaluation of environmental impact of ozone processing as compared to thermal processing, development of continuous and affordable reactors and strict safety plans for ozone working environments are other potential areas to focus.

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Table 1: Ozone-based microbial inactivation on microbial strains present in dairy products.

Milk and milk product	Treatment conditions	Targeted microbe	Results	Reference
Pecorino cheese	C: 200 ppb; T: 8 h, for 150 days	Mites and molds	• Inhibitory effect on mites up to 25^{th} days of storage. • Mold inhibition up to 75^{th} day of storage.	(Grasso et al., 2023)
Soft Cheese	C: 2 and 6 mg/m^3 (gaseous ozone), during ripening.	*M. plumbeus*	• 2-log reduction	(Tabla and Roa, 2022)
Cream (Used for butter making)	C: 300 mg/h; T: 5, 15, 30 and 60 min	*Staphylococci*, yeast and mold and *Salmonella*	• 2-log reduction of *Staphylococci* after 60 min. • Yeast, mold, and *Salmonella* were eliminated after 15 min.	(Sert et al., 2020)
Whey Concentrate (used for whey powder manufacturing)	C: NA T: 30, 60, 90 and 120 min	*Staphylococci*	• *Staphylococci* population reached to non-detectible level after 30 min of treatments.	(Sert and Mercan, 2022)
Minas Frescal Cheese	Ozonated water, C: 2 mg/L, T:1-2 min	*Enterobacteriaceae, Staphylococcus sp.* and total thermotolerant, total	• *Enterobacteriaceae, Staphylococcus sp.* and total thermotolerant were eliminated.	(Cavalcante et al., 2013a)

		aerobic mesophiles, lactic acid bacteria, and yeast and molds	• Aerobic mesophiles, lactic acid bacteria, yeast, and molds were reduced by 1.64, 1.91, and 2.14 log, respectively.	
Milk (used for yogurt manufacturing)	C:0, 40, 60, and 70 ppm, T: 10 min.	*Staphylococcus aureus,* total coliforms, and mold counts	• All the microbes were significantly reduced at a ozone concentration of 70 ppm.	(El-Dahshan et al.)
Milk	C: 0.5 g/L T:5, 10 and 15 min.	Total plate count	• Equivalent microbial reduction to LTLT pasteurization treatment (63℃ for 30 min)	(Azhar and ALmosowy, 2020)
Whole milk and skim milk	C: 0.36 L/min (whole) and 5.01 L/ min (skim)	Total plate count	• 1.7-log (whole milk) and 2.16-log (skim milk) reduction was obtained.	(Genecya et al., 2020)
Milk and whey concentrate	C: NA T: 60 min	Coliform count	• 0.87-log (milk concentrate) and 0.75-log reduction (whey concentrate) was obtained.	(Sert and Mercan, 2021b)

Note: C refers to the ozone concentration and T refers to the treatment time.

Table 2. Change in quality parameters observed due to ozone processing of dairy products.

Product	Treatment conditions	Results	Reference
Fluid Milk	C: 80 mg/min; T: 10 min	• Enhanced shelf life with intact nutritional attributes. • Increased brightness. • Reduced yellowness.	(Mohammadi et al., 2017)
Pecorino cheese	C: 200 ppb; T: NA	• No effect on the physicochemical attributes.	(Grasso et al., 2023)
High-moisture Mozzarella cheese	C: 30 mg/min; T: 2 h	• No elevation in the production of primary and secondary lipid oxidation products.	(Segat et al., 2014)
Butter churned from ozonated cream	C: 300 mg/h; T: 5-60 min	• Elevated firmness, consistency, and fat particle size of the cream. • Declined average fat particle size of butter. • Decreased yellowness of butter with increased exposure time. • Increased spreadability of butter with exposure time. • Declined oxidative stability of butter with treatment time.	(Sert et al., 2020)
Soft Cheese (Torta del Casar PDO)	C: 2 and 6 mg/m^3 (ozonated air)	• Improved rind's look and color. • No impact on other quality attributes.	(Tabla and Roa, 2022)
Butter	C: 0.15-0.35 mg/L (water used for churning)	• Increased lightness and redness. • Decreased yellowness. • Increased firmness with ozone concentration. • Decreased mean fat particle size and oxidative stability with increased ozone concentration.	(Sert and Mercan, 2020)

Whey powder	C: NA T: 30, 60, 90 and 120 min (whey concentrate treatment)	• Declined in the cohesiveness of concentrated whey. • Improved the foaming capacity. • Decreased foaming stability and bulk density. • Increased mean particle size. • Decreased caking ability and improved flow stability of whey powder.	(Sert and Mercan, 2022)
Whey Protein Isolate		• Increased surface hydrophobicity. • Improved foaming capacity and foam stability.	(Segat et al., 2014)
Cream Milk	C: 300 mg/h; T: 0-60 min.	• Increased lipid oxidation with the increase in exposure time.	(Perna et al., 2022)
Skim Milk Powder	C: 3.5 g/h	• No alternation in the particle size of the powder. • Enhanced dispersibility and foaming capacity. • Decreased wettability time. • Decreased Cohesion index with increased exposure. • Decreased caking, cohesion and compaction. • Improved flow stability.	(Sert and Mercan, 2021a)
Milk concentrate	C: 3.5 g/h	• Increased mean diameter. • Declined consistency and firmness. • Declined cohesivity. • Increased lightness and decreased yellowness.	(Sert and Mercan, 2021a)
Whey concentrate	C: 3.5 g/h	• Decreased mean diameter. • Declined consistency and firmness. • Increased lightness and decreased yellowness.	(Sert and Mercan, 2021a)

Note: C refers to the ozone concentration and T refers to the treatment time.

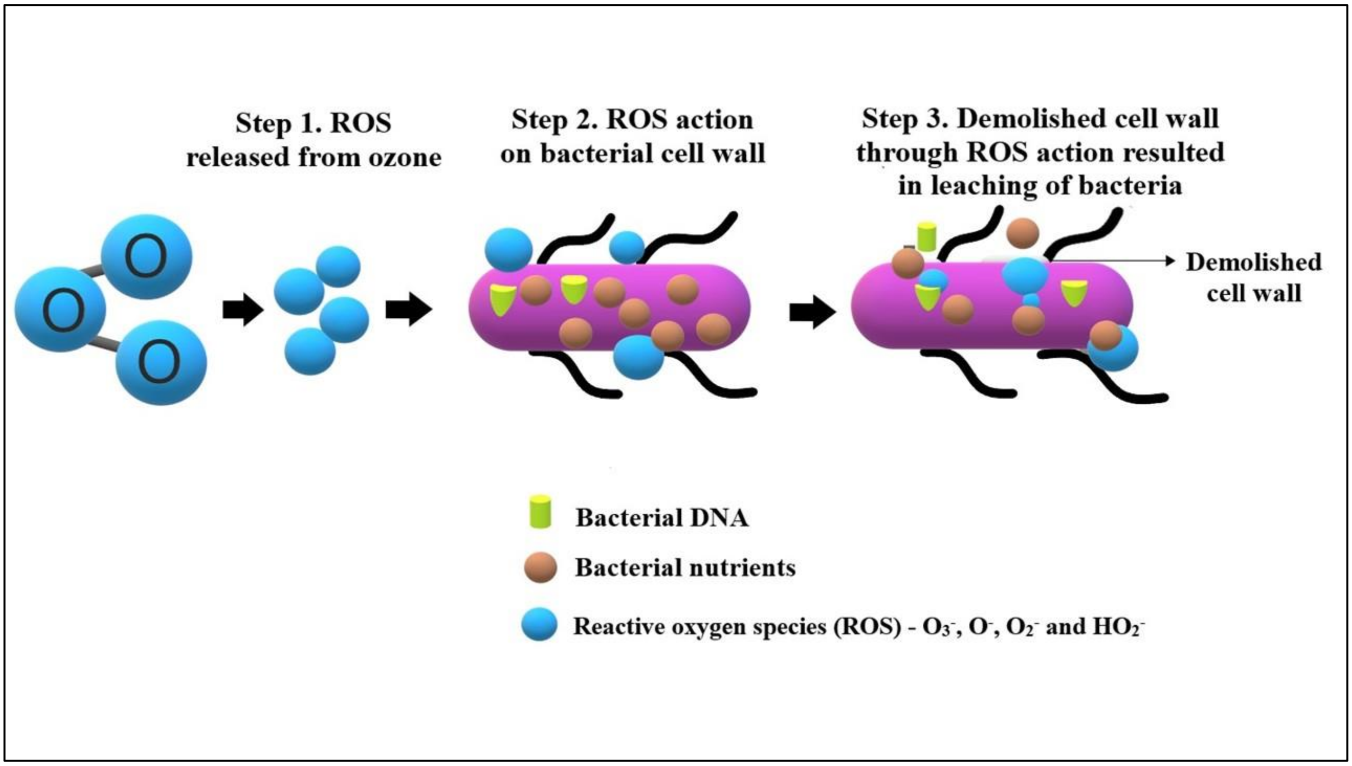


Fig. 1. Microbial inactivation through oxidative stress generated by the free radical of ozone.

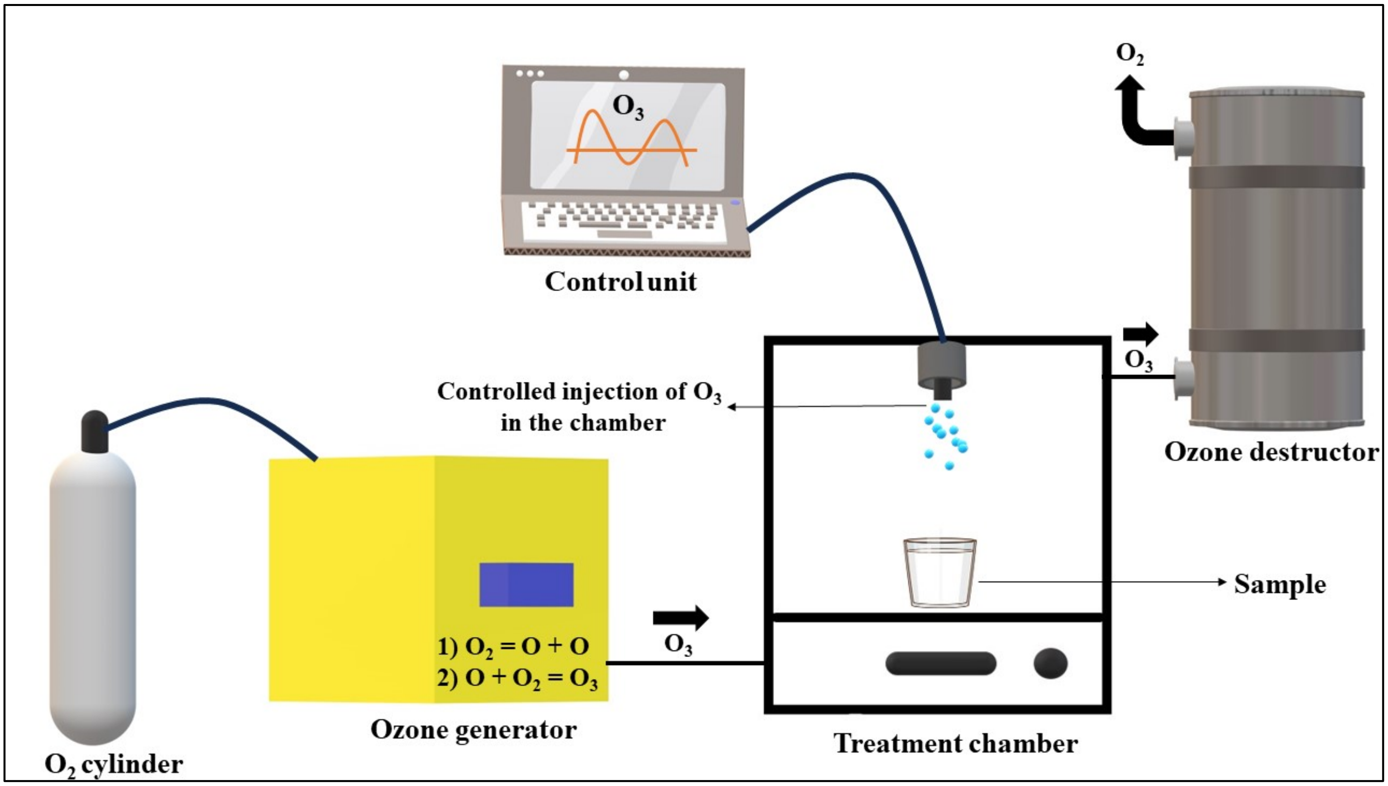


Fig. 2. Schematic diagram of lab scale set up for ozone processing of dairy products.

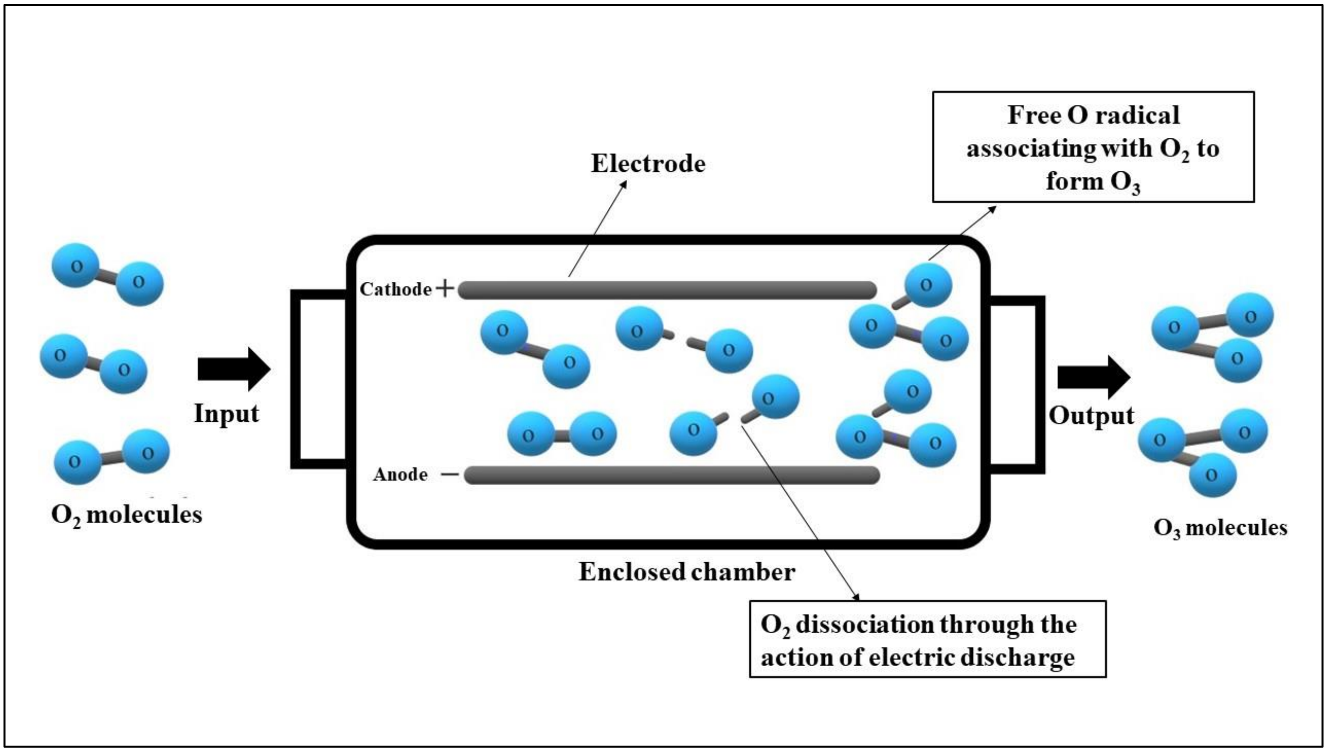
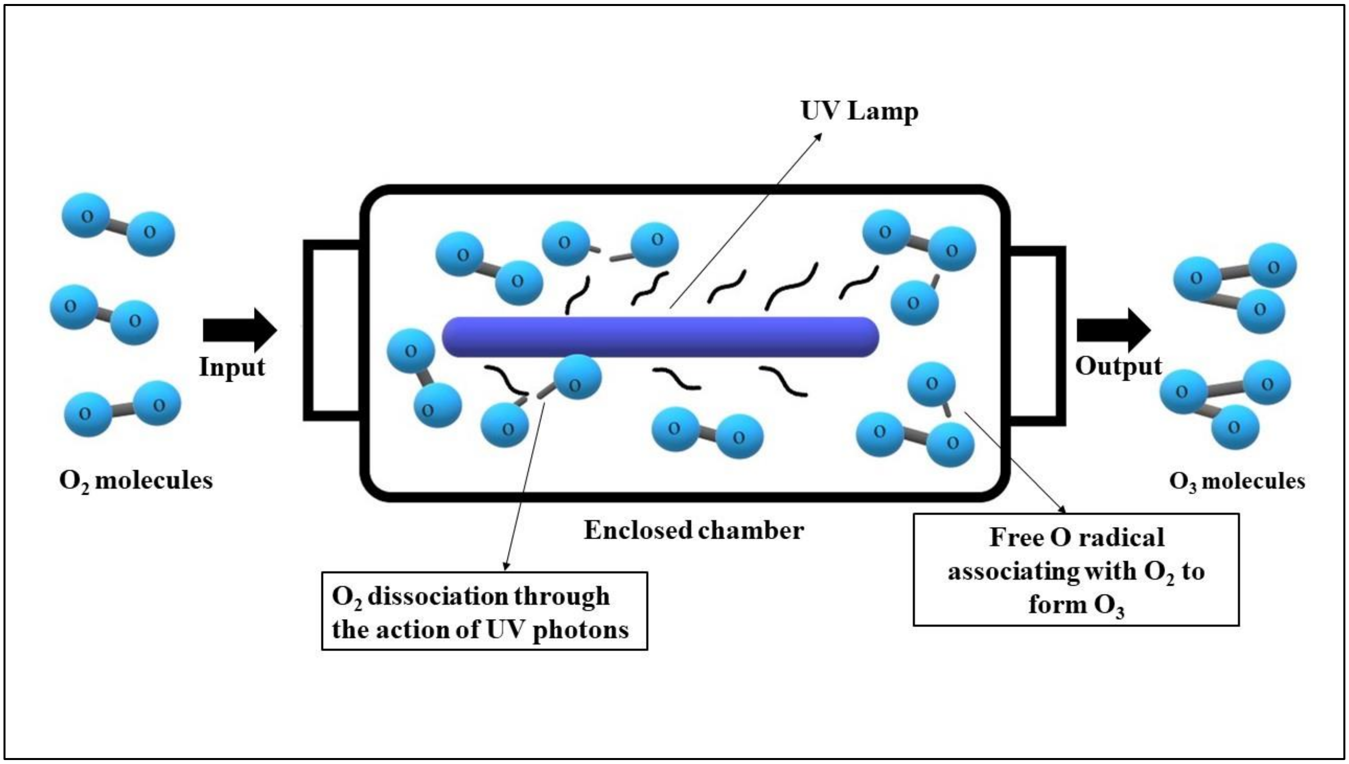


Fig. 3a. Ozone generation by passing oxygen through the corona discharge processor.



669
670 **Fig. 3b.** Ozone generation by passing oxygen through the UV generator.