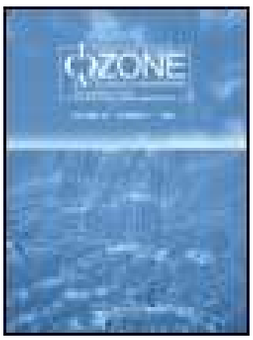

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Ozone Applications in Milk and Meat Industry

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ABSTRACT

Ozone is a GRAS certified cost-effective and eco-friendly technology whose application in food industry is gaining momentum in recent years. It is a powerful oxidizing agent and has a broad spectrum of anti-microbial property. The free radical generation of ozone treatment destroys bacterial cells and is responsible for its antimicrobial activity. This review provides an understanding of the underlying mechanism of microbial inactivation by ozone and its application in meat and dairy products. The impact of ozone on the physicochemical properties such as color, texture, lipid oxidation, protein functionality, and sensory attributes are reviewed along with its inactivation potential on microbes such as *Staphylococcus* sp., *Listeria monocytogenes, Enterobacteriaceae, Salmonella* sp. and yeasts and molds in food matrix. It is evident that ozone can improve the functionalities of food products while ensuring food safety. There are several researchers that have focused on the application of ozone technology in meat and dairy products. This review is a compilation of those works and can be used as a tool to select appropriate processing conditions for milk and dairy products for its improved safety and quality.

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KEYWORDS
Ozone; food safety; food quality; oxidation; meat and dairy products

Introduction

The recent years exhibit a change in matrix of consumer food preferences, which assisted in the progress of starter ideas and technologies. There is a swipe away from traditional pattern of food preferences to newer pattern, which includes larger proportion of animal products. According to studies, a similar pattern was also observed in Indian households, declining the importance of cereals in diets (Law, Fraser, and Piracha 2020). This change in demand elastics has contributed to the growth of both milk and meat industries. Milk, meat, meat products, and dairy foods belong to matrix of nutrient dense food products with high energy value profile. Milk and milk products are the sources of high-quality protein mainly casein, which exhibits excellent functional properties in food formulations. They are also the good source of calcium, zinc, phosphorous, magnesium, and vitamins such as B_{12}, B_2 etc. Meat is also considered to be significant source of a number of B vitamins, proteins etc. with high energy and biological value (Tomé, Dubarry, and Fromentin 2004). High lysine content of meat and meat products is important while considering the diet design to have a balanced intake. The nutrient content of meat varies accordingly with their type, cut of meat, food pattern, and the content of other ingredients. The presence of bioavailable minerals especially zinc and iron, makes meat an important dietary source. Prejudicious to this nutrient profile is the issues regarding the quality and level of fat in meat and milk products. Apprehensions were regarding the level of saturated fatty acids which in high intake may cause some serious health hazards in human populations. Even in midst of all these growing apprehensions, the consumption pattern of both the products is hastening making them a vital group among different food and food clusters.

Since the introduction of scope of food matrix on our diet, preservation of food is an obligatory arm for our survival. Any change associated with food product that makes it unacceptable to the consumer from a sensory point of view is characterized as spoilage. Microbial spoilage is one of the most common causes of spoilage in meat and milk products that leaves back detrimental effects on the quality. Meat is one of most fragile and favorable environments for the duplication of

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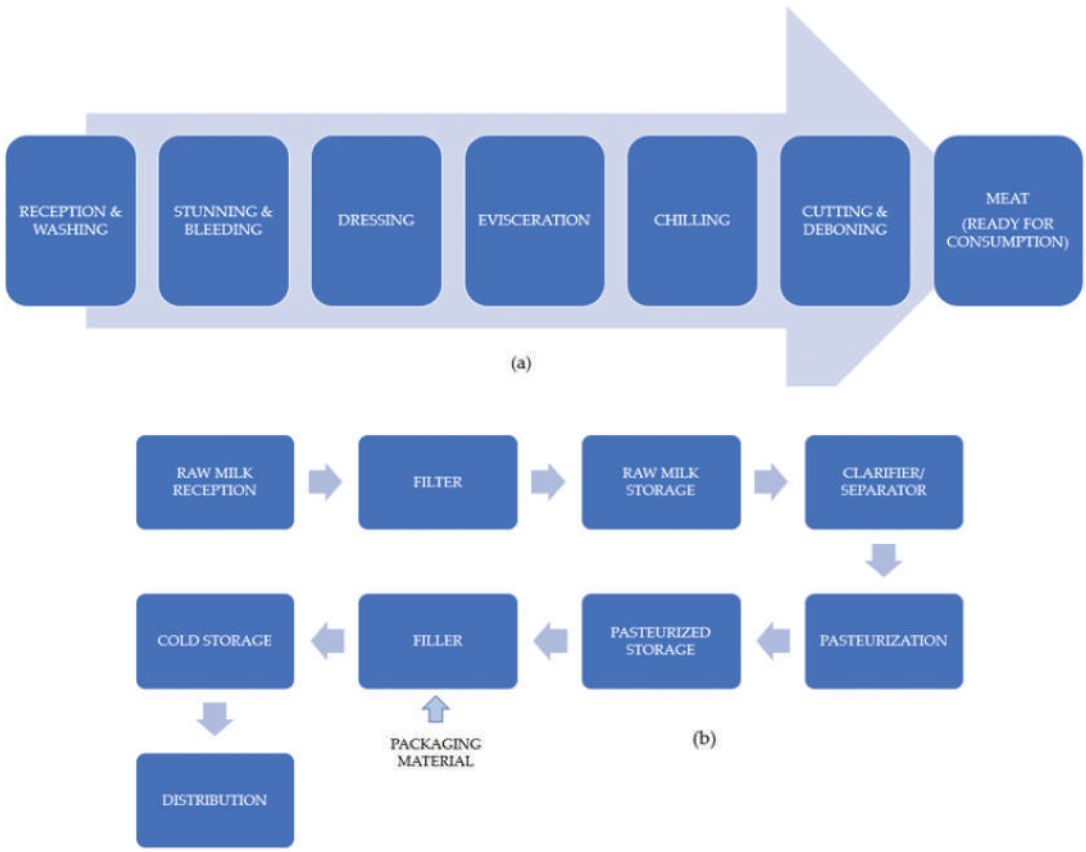

Figure 1. Flowchart depicting the contamination possibility in (a) meat and (b) milk.

microorganisms. Even though there are variations between each type, the most common spoilage organisms associated includes *Pseudomonas spp, lactic acid bacteria, Clostridium spp, Aspergillus*, and *Penicillium* etc (Odeyemi et al. 2020). Coming to milk and dairy products, the microbial populations of utmost importance with respect to spoilage includes *Clostridium spp, Total coliforms, Bacillus spp, Pseudomonas spp*, different types of yeasts and molds (Lu and Wang 2017). Both milk and meat acts as a favorable in-built environment for the growth of microbes due to their high nutritional content and other properties (Figure 1). The presence of these microbial populations appears as one of the important grounds for the spoilage associated with milk, meat, and their products. Control of these populations is therefore something that is of supreme importance. In addition to microbial spoilage, autolytic enzymatic spoilage and lipid oxidation also cause detrimental effects on the quality of meat and meat products (Dave and Ghaly 2011). The common methods employed in controlling the spoilage in meat and meat products are low temperature storage and chemical techniques. While in the case of milk and dairy products the common method of preservation includes high temperatures treatments such as Ultra High Temperature (UHT), High Temperature Short Time (HTST), Low Temperature Long Time (LTLT) etc, nisin addition and microfiltration (Lu and Wang 2017). Even though methods are effective to an extent, certain limitations such as high cost of operation, reliability, average shelf life, and less durability is associated with them. These limitations recommend the application of novel and emerging technologies in endorsing the quality and safety of meat and milk without entailing any safety concerns. This prerequisite undoes the application possibility of ozone on extending the shelf life of meat, milk and their products.

The application of ozone in the food industry has gained interest because of its high oxidizing power and superior antimicrobial activity. The extended consumption pattern of consumer ranging from fresh foods to processed food stuffs with minimum shelf-life guarantee increases the potential of ozone usage. Ozone was registered as Generally Recognized as Safe (GRAS) chemical in 1997 and thereby subsequently classified into a food additive by US FDA in 2001. It is a powerful disinfectant and a strong sanitizer, which leaves no toxic residues on food or processing equipment such as other chlorine-

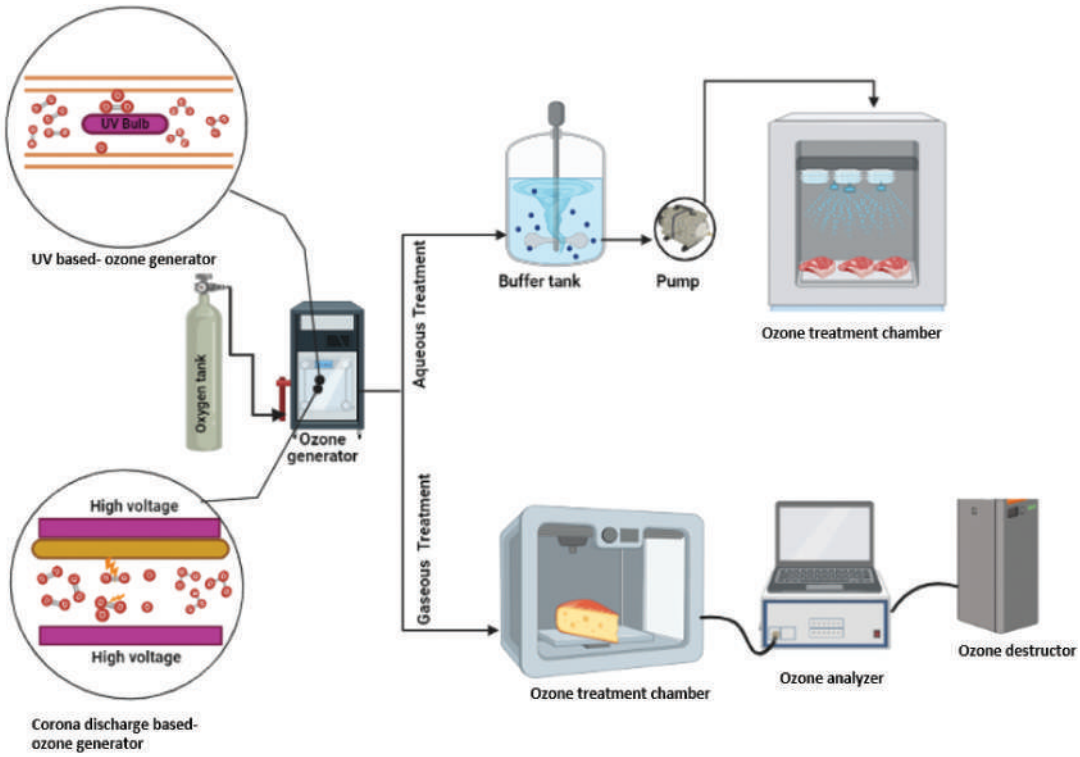

Figure 2. Schematic diagram for ozone treatment setup for meat and dairy products.

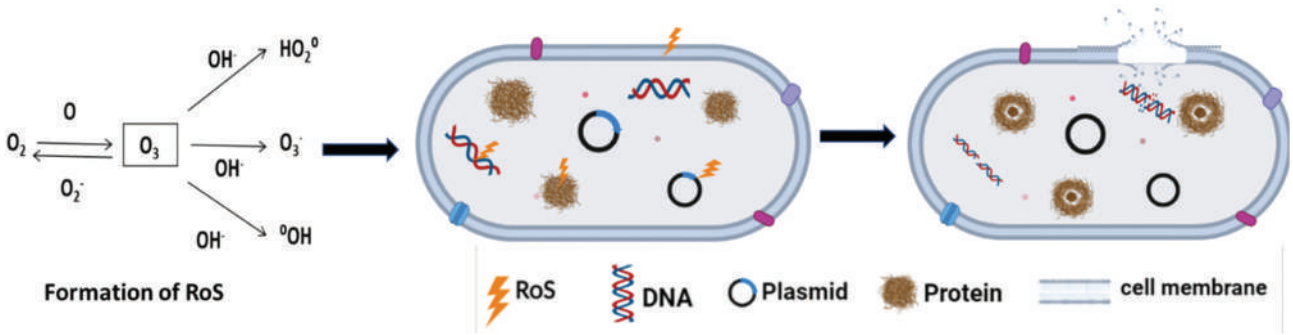

Figure 3. Bacterial inactivation by ozone.

based chemicals making it a greener environment friendly technology. Currently, ozone has found its relevance in the food industry as a disinfectant and an antimicrobial agent with proven efficacy against microbial populations. The ease of usage, higher efficacy, and on-site generation with a comparatively lower cost make the technology an easy alternative to many conventional as well as novel applied technologies. The on-site production of ozone also eliminates the need for transportation, storage and other inventory issues. The application of ozone can follow direct or indirect method in gaseous or aqueous forms (Figure 2). As ozone solubility increases with decrease in temperature and pH, it also allows us to use ozone in meat and poultry processing facilities where cool, damp, and refrigerated conditions are to be maintained. In this context, the current study covers the possibility of ozone application in meat and milk industry, effectiveness of the technology on controlling spoilage as well as maintaining the final quality of food matrix.

Methodology

The articles used to prepare the present paper were collected from internet sources. More than 80 research articles were collected out of which recent articles that were published after the year 2008 were shortlisted.

However, the articles published before 2008 were also considered to explain the mode of action of ozone on microorganism. The collection of articles was done through google scholar, PubMed and science direct by using key words such as ozone application, dairy products, ozone in meat, ozone and food safety etc. The collected articles were studied, and the results were systematically arranged and critically analyzed in the review format.

Mechanism behind the microbial inactivation by ozone

Ozone is formed when an oxygen-free radical combines with an oxygen molecule to form triatomic oxygen. The ozone molecule thus formed is highly unstable in both gaseous and aqueous phase. This structural instability causes the ozone to later decompose into various reactive oxygen specious (RoS) such as oxygen free radical (O^-), O_3^-, HO_2^-, O_2^- etc. (Figure 3). This decomposition occurs in three stages; the first stage or the initiation stage is where the free radicals are generated. The second stage is the promotion stage where ozone reacts with various promoters such as primary alcohols, aryl groups etc. to regenerate superoxide and hydroperoxide radicals. The final stage is the inhibition step, which occurs when the free radicals react with carbonates, bicarbonate etc. where no regeneration of RoS occurs. Ozone is a broad spectrum antimicrobial agent with proven efficacy against bacteria, virus, protozoa, fungi, and endospores (Jin-Gab Kim, Dave, and DAVE 1999).This broad spectral antimicrobial effect of ozone is mainly due to the formation of this RoS, as they are capable of undergoing a verity of complex reactions with organic molecules. Ozone can react with S-H and C-H bonds in alkanes, alkenes, sulfhydryls (SH), amines etc. either directly or, indirectly by free radical chain reaction (Adachi 2001). When ozone comes in contact with the bacterial cell, the primary target of ozone will be the cell wall and cell membrane of the bacteria (Zhang et al. 2011). As these structures are made of phospholipids, ozone will react with the polyunsaturated fatty acids present in the phospholipids and undergoes peroxidation process resulting in the formation of ozonide, which later decomposes to lipid peroxides. This will alter the cell wall permeability and leads to the leakage of intercellular components. The progressive degradation will then lead to loss of cellular integrity followed by lysis, which corresponds to cell death. Even though ozone shows no difference on cell viability or deformation between gram positive and gram negative bacteria, ozone does demonstrate a higher damage and severity to gram positive species (Thanomsub et al. 2002). This difference is mainly due to the difference in the cell wall structure of gram positive and gram-negative bacteria. As the gram-positive bacteria have a thick layer of peptidoglycan, forming a rigid structure around the cell, the outer membrane of gram-negative bacteria is mainly lipoproteins and lipopolysaccharides with a thin layer of peptidoglycan. Another theory that is used to explain the bactericidal activity of ozone is that, ozone inactivate the microbes by attacking their genetic material(Hunt and Mariñas 1999). Even though the exact mechanism of DNA damage by ozone is not well known, it is suggested that the reaction of ozone with lipids present in the cell membrane leads to the production of secondary reactive species which later travel to the nuclei of the host cell, damaging its genetic material. The reaction of ozone with purenes and pyrimidines - the building blocks of DNA can result in the release of carbohydrates and

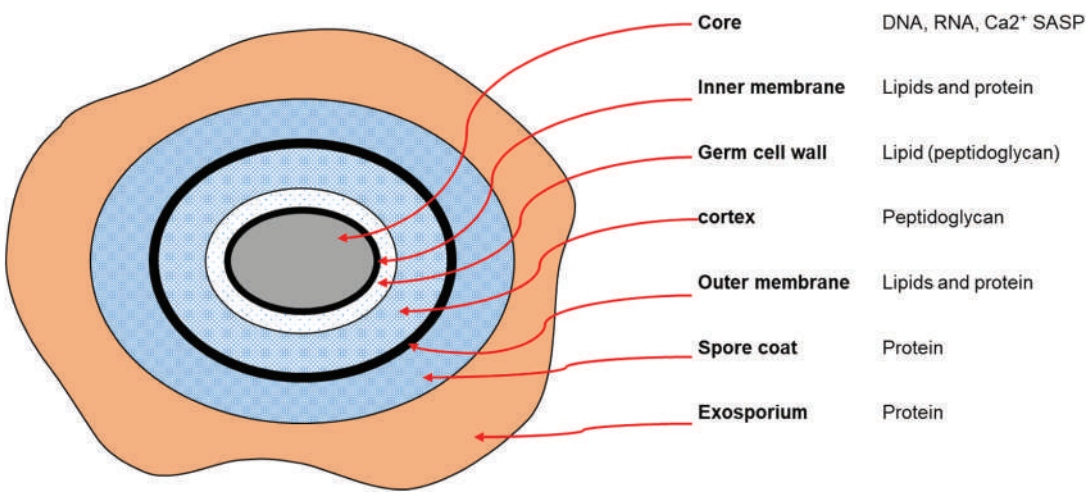

Figure 4. Structure of bacterial endospore.

phosphate ions. The studies have shown that ozone modifies pyrimidine bases with higher sensitivity to thymine than cytosine or uracil (Ito et al. 2005). Besides the chromosomal DNA, ozone can also react with cytoplasmic substances including the plasmid DNA of prokaryotes and can alter their structure (Asfahl and Savin 2012). In addition, ozone can also react with the R- group or the primary amine group of amino acids and alter the primary and secondary structures of protein and enzymes which are associated with cell metabolism (Cataldo 2003; Takamoto, Maeba, and Kamimura 1992). Ozone is found to inactivate a verity of cytosolic enzymes such as glyceraldehydes3-phosphate dehydrogenase and NAD-alcohol dehydrogenase (Dizengremel et al. 2009; Iriti and Faoro 2008), alkaline phosphatase and β–galactosidase (Takamoto, Maeba, and Kamimura 1992), etc, leading to a fatal changes in cell cytology. Even though Catalase and Superoxide Dismutase had a protective role against oxidative chemicals,1 ppm ozone as was powerful enough to inactivate *Listeria monocytogenes* cells within 5 min of exposure (Fisher et al. 2000).

Bacterial endospore is one of the most resistant life forms known to man. The high resistance property of the spores is mainly due to its well-structured multilayer morphology. The spore layer includes an outer most layer called the exosporium, followed by spore coat, outer membrane, cortex, germ cell wall, inner membrane, and the spore core (Figure 4). Spore coat comprises about 80% of spore protein and helps the cell from the action of lytic enzymes, predative protozoan's, UV radiation and detoxifying oxidative chemicals(Driks 1999; Riesenman and Nicholson 2000; Setlow 2006). Spores with damaged spore coat were found to be more highly susceptible to ozone, than spores with intact spore coat (Young and Setlow 2004). When ozone comes in contact with the spore, it reacts with the lipoproteins and polysaccharides present in the spore coat causing heavy disruption and shrinkage of spores (Khadre and E. 2001; Wanqing Ding et al. 2019). In addition, the ozone can later penetrate in to the spore core, and affect the α/β – type small, acid soluble spore protein (α/β – SASP), which protect spore from damage by many genotoxic chemicals by binding to spore DNA. Though ozone does not kill spores by DNA damage, mutated spores which lack SASP were found significantly sensitive to ozone than wild types (Young and Setlow 2004). Recombinational repair is the process by which spores repair their damaged DNA. RecA is an important protein in this type of repair. Chemicals which inactivate spores by DNA damage is found to be more efficient on killing spores which lack RecA- dependent DNA repair. Studies have shown no significant difference in ozone sensitivity for spores that lack RecA- dependent DNA repair and wild types. The study also did not identify any mutations to the treated and untreated spore DNA (Young and Setlow 2004). Studies using real-time qPCR have shown that nearly one fourth of spore DNA remained unaltered even after exposure to high ozone concentration (Wanqing Ding et al. 2019). All of these lead to a conclusion that ozone doesn't kill spores by DNA damage. Even though antioxidant enzymes such as superoxide dismutase, catalase and reductase are found in bacterial spores no effect of these enzymes were reported on spore resistance against oxidizing agents as they are inactive within dormant spores (Casillas-Martinez and Setlow 1997).

Application of ozone in milk industry

Dairy industry is one of the most rapidly developing industries in both large and small scale. Ozone is used in the dairy industry for a verity of purposes such as surface decontamination, remove soil from processing surfaces, to ensure microbial safety etc. Heacox (2014) recently filed a patent for the method in which ozone at low concentrations (0.04–1.2 ppm) was used to disinfect dairy equipment and other infrastructure. As the dairy industry usually employs a hot water and chemical wash for this purpose, the above method decreased the chemical usage and almost completely eliminated the hot water cost on cleaning in dairy industry. Ozone was also used in dairy farms to treat bovine mastitis, with 60% recovery on infected cows without antibiotic administration (Ogata and Nagahata 2000). Ozone can also be used as a pre-treatment of fluid milk before pasteurization to maintain its shelf life (Varga; and Szigeti 2016). In this section, we focus on the application of ozone on ensuring microbial safety in the dairy industry (Table 1).

A present study has used ozone in inactivating *Listeria monocytogenes* in fluid milk (Sheelamary; and Muthukumar 2011). In the study, fluid milk was treated with 0.2 g/L of ozone for 5, 10, and 15 min, with a complete inactivation of *L. monocytogenes* achieved at the end of 15 min. A similar study was conducted by Munhõs et al. (2019) where ozone was used to inactivate *Pseudomonas aeruginosa* on skimmed and whole milk. As *Pseudomonas* is a psychotropic organism, it can survive under refrigerated conditions in milk and compromise the food safety. The study showed that ozonation of fluid milk at 28 mg/L for 5 min could reduce the *P. aeruginosa* count by 1 log. The microbial inactivation was found to be time dependent, and an increase in treatment time to 10 and 15 min significantly enhanced the microbial inactivation by ozone. Interestingly, the inactivation of *Pseudomonas* was more pronounced in

Table 1. Effect of ozone on microbial populations of different dairy products.

Targeted samples	Targeted organism	Ozone parameters	Observations	References
Fluid milk	*Listeria monocytogenes*	Concentration: 2 g/L Time: 0,5,10 and 15 min	A complete in activation of *Listeria* was observed with a slight change in nutritional composition (carbohydrate and proteins) at the end of 15 min.	(Sheelamary; and Muthukumar 2011)
Whole and skim milk	*Pseudomonas aeruginosa*	Concentration: 28 mg/L Time: 5,10 and 15 min	The inactivation was found to be depended on time and composition milk dependent	(Munhõs et al. 2019)
Whole and skim milk	*Staphylococcus aureus*	Concentration: 34.7 mg/L and 44.8 mg/L Time: 5,10,15,20,and 25 min	At 5 min there was no reduction in microbial load, but for treatment time above 5 min there was a steady decrease in log count. The ozone efficacy was also found to be dependent on fat content	(Couto et al. 2016)
Raw milk	Total mesophilic aerobic bacteria (TMA), psychrotrophic bacteria, *Staphylococcus* sp., *Enterobacteriaceae, Salmonella* sp. and yeasts and molds.	Concentration: 1.5 mg/L Time: 5, 10 and 15 min Gaseous ozone were bubbled.	Even though there was no microbial reduction after first 5 min of treatment, a 0.4–1.0 log reduction of microbial count was observed after15 min of ozone bubbling	(M. Cavalcante et al., 2013b)
Raw milk	Coliform, and *Staphylococcusaureus*	Concentration:0.5 ppm Time: 5, 10 an d 15	The study found that ozone treatment at 0.5 ppm for 5, 10 an d 15 min is not sufficient to achieve roper shelf life and quality of raw milk	(Khudhir and Mahmood 2017)
Raw milk	Total bacterial count, yeast and molds, enterobacteriacae, psychrotrophes, *S. aureus, Bacillus cerues, E. coli, S. typhimurium* and *Sh. Flexneri*	Concentration: 400 mg/h Time: 0,5,10,15,20,25 and 30 min	The ozone treatment for 20 min at 400 mg/h was found to be significantly improve the microbial quality of milk.	(Younis et al. 2019)
Raw milk	Total bacterial count	Concentration: 0.5 g/L Time: 5, 15 and 30 min	Ozone treatment was found to be an alternative for conventional thermal processing	(Azhar and ALmosowy 2020)
Yogurt and cheese brine	Total mesophilic count, coliforms, molds and yeasts, staphylococci, enterococci and lactic acid bacteria	Concentration: 2.5–3 ppm Time: 0, 10, 20 and 30 seconds for yogurt 0, 10, 20 and 30 min for brine solution	Ozone flushing for 30s significantly increased the shelf-life of the yogurt. The protein content in cheese brine interfered with the ozone and decreased it antimicrobial efficiency.	(Alexopoulos et al. 2017)
Cheese brine	Total viable count, microstaphylococci, yeasts	Concentration: 0.20, 0.40 and 2.0 mg/L Time: 30 and 60 min	The microbial inactivation by ozone were time and concentration depended, the application of 0.40 mg/L of ozone for a prolonged period of 240 min were able to reduce the Total microbial count >2 log CFU/ml and yeast > 1 log CFU/ml.	`(Marilena Marino et al. 2015)
Cheese	Total bacterial count, coliform and yeast and mold	Concentration: 0.5 ppm Time: 10, 15 and 20 min	Ozone treatment for 20 min was able to reduce the microbial count in soft cheese by 6 log cycles	(Zinasaab Khudhir and Mahdi 2017)
Cheese	*Pseudomonas* spp., Lactic acid bacteria, *E. coli* and coliforms	Concentration: 2, 10, 20 an d30 mg/L Time: 30 and 60 min	The results showed that ozone treatment cannot be used to recover the product which is already contaminated with microbial load, but is effective in reducing the contamination in treatment water which intern could help in increasing the product shelf life	(Segat et al. 2014)
Italian Cheese	*L. monocytogenes*	Concentration: 4 ppm Time: 8 min	*L. monocytogenes* counts were brought down from 103 CFU/g to 10 CFU/g	(Morandi et al. 2009)
Brazilan Cheese (*Minas Frescal*)	Lactic acid bacteria, Yeast and mold, Total mesophilic count	Concentration: 3 mg/L Time: 1–2 min	Approximately 2 log reduction were observed	(D. Cavalcante et al. 2013a)
Butter	*Coliform, Salmonella, Staphylococci, Yeast* and*mould, Lactobacillus, Streptococcus.*	Concentration: 3.5 g/h Time: 5, 15, 30 and 60 min	Ozone was successful in inactivation all tested micro organism	(Durmuş Sert, Mercan, and Kara 2020)

(*Continued*)

Table 1. (Continued).

Targeted samples	Targeted organism	Ozone parameters	Observations	References
Butter	*Coliform, Salmonella, Staphylococci, Yeast* and*mould, Lactobacillus, Streptococcus.*	Concentration: 0.15, 0.20, 0.25, and 0.30 mg/L	Ozone was successful in inactivation all tested micro organism	(Durmus, Sert and Mercan 2020)
Milk powder	*Cronobacter*	Concentration: 2.8 and 5.3 mg/L Time: 120 min	Ozone were successful in inactivating the selected microorganism with causing significant lipid oxidation, though the fat content of the milk played a major role in determining ozone efficacy	(Emrah Torlak and Sert 2013)
Milk concentrate an whey protein cincentrates	*Coliforms, Enterobacteriacaea, Staphylococi, yeast* and *mold*	Concentration 3.5 g/L Time: 0, 5, 10, 15, 30, and 60 min	Inactivation of microbes ranged from 0.6–1 log CFU/ml	(Sert and Mercan 2021)

skimmed milk than whole milk. This was due to the difference in milk composition, as they can directly react with ozone, affecting its efficiency. A similar study was also conducted by Couto et al. (2016) on *Staphylococcus aureus*. The study was done to evaluate the efficacy of ozone inactivation of *S. aureus* in whole and skimmed milk (fat content >3.0% and <0.6%, respectively). When an ozone concentration of 34.7 mg/L and 44.8 mg/L was used in the time interval of 5 to 25 min, a log reduction of 0.42 was observed for skimmed milk at the end of 25 min. Whereas, a lower inactivation efficacy of 0.19 and 0.21 log reductions was observed for concentrations of 34.7 mg/L and 44.8 mg/L, respectively, in whole milk. This observation was due to the interference of milk fat with ozone. Cavalcante et al. (2013b) evaluated the effect of gaseous ozone bubbling on raw milk with a slightly modified method, where tween was initially added to the raw milk to improve the contact with ozone. Ozone was later bubbled to raw milk for 5, 10, and 15 min at a concentration of 0.5 mg/L. This was followed by microbial analysis for total mesophilic aerobic bacteria (TMA), psychrotrophic bacteria, *Staphylococcus* sp., *Enterobacteriaceae, Salmonella* sp. and yeasts and molds. Even though there was no significant reduction for the first 5 min of ozone treatment, there were a 0.60, 0.13, 1.02, 0.96, and 0.48 log of bacterial reduction for TMA, psychrotrophic bacteria, *Staphylococcus* sp., *Enterobacteriaceae*, and yeast and molds respectively after 15 min of ozone exposure. Another study by Khudhir and Mahmood (2017) evaluated the effect of ozone treatment on the microbial quality of milk collected from various markets in Baghdad. The study found the prevalence of coliforms in 100% of the collected milk and almost 60% of the milk samples were contaminated by *Staphylococusaereus*. The samples were then treated with ozone at 0.5 ppm for 10, 15 and 20 min followed by evaluation of ozone efficacy after storing in refrigeration and ambient temperature for 1 day. The study showed that storage temperature plays a significant role in the keeping quality of milk, as a reduction from 8.8 CFU/ml to 3.8 CFU/ml and 5.2 log CFU/ml to 2.1 CFU/ml were observed for Total aerobic bacteria and *S. aureus*, respectively, after 24 h of storage at 30 °C. Whereas for the milk samples stored at 4 °C, a final log CFU/ml of 2.9 and 1.8 were respectively recorded. A recent study have compared the effect of ozone treatment with pasteurization (72 °C for 15 s) and concluded ozonation treatment to be a possible substitute to conventional thermal treatments (Younis, Fayed, Elbatawy, & Elsisi, 2019). In the study, ozone was bubbled at a concentration of 400 mg/h, and the microbial qualities were evaluated for *S. aureus, Bacillus cerues, E. coli, S. typhimurium* and *Sh. Flexneri*.For pathogens such as *E. coli* and *S. typhimurium*, a microbial reduction below 1 log CFU was observed within 20 min of ozone treatment. *S. flexneri* was the most resistant pathogen to ozone treatment, as a reduction below 1 log was observed only at 30 min of exposure. A significant reduction in Total bacterial count, yeast, and molds, *Enterobacteriacae* and psychrotrophes count as compared to heat treatment was also reported by the study. Ozone treatment at 0.5 g/h for 30 min was also found to be a suitable alternative for pasteurization at 63 °C for 30 min (Azhar and ALmosowy 2020). The substantial reduction in psychrotrophic and mesopilic bacterial count was also reported by Mohammadi et al. (2017), where ~1 log reduction in microbial counts were obtained within 10 min of ozonation (80 mg/min). The study also showed that ozone treatment for 5 min can significantly reduce the aflatoxin M_1 (AFM_1) content in milk by 50%.

Researchers have also attempted to apply ozone to other processed dairy products such as yogurt, cheese, butter, and milk powders. Alexopoulos et al. (2017) used a filtered air stream of ozone at 2.5–3 ppm concentration to inactivate surface molds on packed yogurt. The cups

were analyzed for visual spoilage up to 90 days. Visual spoilage of the control samples was observed from day 30, whereas for samples ozonated for 60 s, the spoilage was first observed at day 45. All samples treated with ozone lasted longer than the control samples with a delay in spoilage of 5 days as compared to the control. This study also analyzed the effect of ozonation of brine solution used in the ripening of feta cheese, a special type of cheese produced by fermenting goat and sheep milk. But due to the high organic content in the cheese brine, the ozone was inactivated in the initial stages of application and has not shown any significant difference from control. The feasibility of using ozone for microbial inactivation of used brine was also evaluate by Marilena Marino et al. (2015). The brine samples were treated with 0.20, 0.40, and 2.0 mg/L of ozone for 30 and 60 min. Though the lower concentrations treatments of 0.20 and 0.40 for 30 min were not sufficient to give a significant decrease in the microbial load and prolonging the exposure time give a significantly higher inactivation for all microbial groups. Increasing the ozone treatment and time to 2.0 mg/L for 60 min gave a significant reduction in microbial count, as there were a 4.61, 3.37 and 2.70 log CFU/mL reduction, respectively, for Total bacterial counts, *microstaphylococci*, and yeast and mold counts. Ozone was also used for the inactivation of coliforms, yeast and molds in soft cheese (Zinasaab Khudhir and Mahdi 2017). Bubbling of 0.5 ppm ozone for 20 min gave a 6 log reduction in the total bacterial count. There was a reduction in coliform counts from 7.8 log CFU/g to 2.6 CFU/g for cheese stored at 4 °C, and the yeast and mold counts were reduced to a non-detectable level. Segat et al. (2014) used ozone as an anti-microbial agent at various production stage of mozzarella cheese. As the major source of microbial contamination in cheese processing occurs from the cooling water and the preservation liquid, experiments were conducted to evaluate the ozone efficacy in these processing steps. In the initial experiment, cheese was packed in ozonated water (2 mg/L) and stored for 21 days. The microbial analysis showed no significant difference for both treated and untreated samples due to the presence of high organic content. Similar data were also obtained when aqueous ozone (2, 5 and 10 mg/L) was used to cool the curd during cheese processing. Furthermore, the treatment of processed cheese with gaseous ozone (10, 20, and 30 mg/m^3) was also not suitable to inactivate the microorganism. However, ozone treatment reduced the cross contamination of product from treatment water which intern could help in increasing the product shelf life. Ozone treatment of raw cream at 3.5 g/h of ozone in decreasing the microbial load in butter was studied by Sert, Mercan, and Kara (2020). Ozonation of cream for 15 min decreased the *Salmonella* and yeast and mold counts in butter to an undetectable level. A complete inactivation of coliforms was observed at 30 min of ozonation. At prolonged exposure above 60 min, ozone decreased the staphylococci count from 5.01 log to 3.0 log, and log reduction of 5.3 to 1.4 and 5.6 to 2.1 log units, respectively, for *lactobacillus* and *streptococcus* were also observed. The effects of churning butter at different ozone concentrations were analyzed for its microbial quality (Durmus¸ Sert and Mercan 2020). The study showed that the butter produced by churning in ozonated water decreased the microbial count ranging from 0.07 to 1.56 log depending on the concentration used. Ozone was also successful in inactivating *Cronobacter* in milk powder (Emrah Torlak and Sert 2013). Whole milk and skimmed milk powders were individually exposed to ozone concentrations at 2.8 and 5.3 mg/L for 120 min. Even though ozone inactivated the *Cronobacter* in milk powder, there was a significant difference by 1 log between skimmed milk powder and whole milk powder. The *Cronobacter* count were reduced from 6 log to 2.71 and 3.28 log respectively for skim milk and whole milk powder respectively after 120 min of ozonation at 5.3 mg/L. The results suggested gaseous ozone to be an effective microbial reduction technique for milk powders. Exposure of milk and whey concentrates contaminated by aflatoxin (AFM1) to ozone concentrations of 3.5 g/L for 60 min decreased the aflatoxin content by 18.9% and 9.9%, respectively (Sert and Mercan 2021). A complete inactivation of *staphylococci* and yeast and mold were also observed for whey protein isolates at the end of 60 min ozone treatment.

Effect of ozone treatment on milk products quality

Ozone is a powerful oxidizing agent and, when comes in contact with protein, it can affect the peptide back bone, cause bond cleavage and modification of amino acid side chain. This reaction can later alter the functional properties of proteins such as foaming and emulsifying ability. Uzun et al. (2012) studied the effect of ozone treatment on the functional properties such as emulsifying properties, solubility, and foaming properties of whey protein isolates (WPI). When WPI was treated with 4.5 ppm ozone in aqueous medium, there was an oxidation in the amino acid side chain, which altered the protein structure. This leads to an increase in local flexibility or rigidity in protein chain causing a significant change in foaming properties of WPI. An increase in foam volume by 2.25 times and foam stability by 15 times as compared to the control samples were reported after the ozone

treatment. This study also analyzed the effects of ozone treatments on protein solubility, as solubility can be a direct measurement of the protein denaturation. The result revealed that the method used for ozonation can significantly affect the protein solubility, as the decrease in solubility by gaseous (60 g/h) and aqueous ozone was 18.85% and 16.50%, respectively. The study also measured the emulsifying properties of treated and untreated samples in terms of emulsion stability index (ESI) and emulsifying activity index (EAI). Even though there was no significant difference between treated and untreated samples in terms of EAI, there was a significant decrease in ESI of ozone-treated samples. Changes in the functional properties of WPI after exposure to high concentration of ozone (20000 mg/m^3) was studied by Annalisa Annalisa Segat et al. (2014). The study analyzed the functional properties such as hydrophobicity, free sulfhydryl (SH) content, solubility, and foaming properties of WPI after 30–480 min of ozone treatment. The FTIR analysis from the study showed a significant increase in the α-helix structure of proteins. Moreover, there was also decreased in the free SH groups during the ozone exposure, both of which resulted in an increased surface hydrophobicity. This increased hydrophobicity directly affected the solubility value of the WPI and decreased the solubility value as a function of ozone treatment. As ozone treatment lead to more flexible protein structures, the foaming properties of WPI were increased after ozone treatment. Both the foaming capacity and foam stability were significantly increased after ozonation.

Milk and whey protein concentrates were analyzed for its textural properties after a 60 min exposure to 3.5 g/L ozone by Sert and Mercan (2021). The study found that ozone treatment significantly decreases the firmness and consistency values of the product. The particle size analysis showed an increase in particle diameter, which was suggested due to the aggregation of milk proteins. The aggregation occurs as a result of destabilization of protein structure by ozone. This destabilization causes unfolding or polymerization leading to protein–protein interaction. The viscosity character of the milk and whey isolates were significantly affected by ozone, as in the case of milk concentrate there was an increase in viscosity index whereas for whey isolates the viscosity index decreased. This increase in milk concentrate viscosity by ozone can be put to use in the production of dairy powders as viscosity because the viscosity of the concentrates affects the powder particle size by spray drying. Additionally, due to the carotenoid degradation after ozone treatment, an increase in lightness (L*) and a decrease in the yellowness of the product was also observed. Similarly, a decreased firmness and increased lightness values were also observed in butter when ozonated water were used to churn cream (Durmus¸ Sert and Mercan 2020). There was also a significant reduction in particle size, which resulted in decreased spreadability. Due to the high oxidation potential of ozone on milk fat, the oxidative stability of the butter churned with the ozonated water was also analyzed in the study. The study revealed that the oxidative stability of butter decreased with increasing ozone concentration and about 38% decrease in oxidative stability were observed when the butter was churned with 0.30 mg/L ozone. Durmuş Sert, Mercan, and Kara (2020) studied the effect of ozonation of cream before churning on their color, texture, and particle size. The results showed an increase in fat particle size in cream after ozonation, which was suggested to be due to partial coalescence or aggregation of milk fat. This in turn increased the firmness and consistency values of ozone-treated cream. Ozone treatment also decreased churning time of cream resulting in larger particle size in butter. A decrease in the oxidative stability of butter was also reported after ozone treatment. The lipid oxidation of whole and skimmed milk powder were analyzed by thiobarbituric acid reactive substances (TBARS) assay by Emrah Torlak and Sert (2013). The milk powders were treated with ozone for 2.8 mg/L and 5.3 mg/L for 30, 60, 90 and 120 min. In the case of skim milk powder, the TBARS values remained relatively constant, and even though non-significant, there was a slight increase in the TBARS values for whole milk powder, indicating a lower oxidative stability. In contrast, ozone treatment did not have any significant effect on the oxidative stability of cheese (Segat et al. 2014). There was no significant difference between control and treated samples in terms of peroxide value or TBARS values even after treating the cheese with 30 mg/m^3 of ozone for 2, 5 or 10 min. Application of gaseous ozone (2.5–3 ppm) has found to have no significant effect on sensory characters such as flavor, texture, color, and overall acceptability of yogurt. Whereas, exposure of ozone for 60 min significantly rescued the overall acceptability of feta cheese (Alexopoulos et al. 2017).

Ozone treatment has found to significantly reduce the β-carotene content of the milk (Hesam Mohammadi et al. 2017). The carotenoid content decreased from 5.11 ppm to 2.58 ppm within 10 min of ozone exposure at 80 mg/min. This resulted in a significant increase in the lightness value and decreased the yellowness of milk.

Table 2. Effect of ozone on microbial populations of different meat products.

Food matrix	Targeted organism	Treatment conditions	Observations	References
Raw chicken	*L. monocytogenes*	Ozone level- 33 mg/min Exposure time – 1 to 9 min	Specific dose of 33 mg/min for 9 min in gaseous phase could was effective in incapacitating 2×10^6 CFU/g of *L. monocytogenes* on chicken samples	(Muthukumar and Muthuchamy 2013)
Beef	*E. coli* and total aerobic mesophilic heterotrophic microorganism	Ozone level- 154×10^{-6} kg m^3 Exposure time – 3 h & 24 h Temperature – 0°C & 4°C	Specific dose at 0°C for 24-h, abridged a decrease of 0.7 and 2.0 $\log_{10}$ cycles in E. coli and total aerobic mesophilic heterotrophic microorganism counts. Shorter exposure times (3 h) at both temperatures reduced 0.6–1.0 $\log_{10}$ cycles and 0.5 $\log_{10}$ cycles the counts of E.coli and latter microbial populations.	(Cárdenas et al. 2011)
Chicken fillets	Aerobic mesophilic bacteria	Ozone levels – 0.21 and 0.38 mg/l Exposure time – 40, 80, and 120 min Temperature – 3, 26, and 37 °C	0.38 mg/l ozone concentration for 120 minutes at 3°C reduced the aerobic mesophilic bacteria by 1 unit log CFU/g. Ozone concentrations of 0.21 and 0.38 were able to disinfect total aerobic mesophilic bacteria of 0.42 and 0.89 log CFU/g respectively	(Karamah and Wajdi 2018)
Chilled chicken breasts	Total aerobic bacterial count, coliforms, and total mold	Ozone levels – 40, 60 & 70ppm Exposure time – 20 min	Reduction of total aerobic bacterial count, coliforms, and total mold counts with a prolonged the shelf life of more than 9 days.	(EL-Dahshan, Hafez, T. A., and Ghayaty, H. A 2013)
Chicken and duck breast meat	Coliform, aerobic and anaerobic bacteria	Ozone level- 10×10^{-6} kg O_3/m^3/h (4 ± 1 °C) Exposure time – 4 days	Reduction in growth of coliform, aerobic and anaerobic bacteria in both chicken and duck breast	(Muhlisin et al. 2016)
Pork	*L. monocytogenes*	Pre-treated with volume ratios of potassium lactate and ozone and stored at 8 °C for 15 days Ozone levels: 200, 500 and 1,000 mg/h	*L. monocytogenes* were sensitive to high concentrations of KL and ozone.	(Piachin and Trachoo 2011)
Chicken legs	*Pseudomonas spp.*,LAB, Yeasts and molds	Combined effect of ozonation and vacuum packaging on shelf-life extension Ozone levels: 2, 5, & 10 mg/L	Higher ozone dosages were more effective in controlling the microbial populations.	(Gertzou et al. 2017)
Chicken meat fillets	Total aerobic mesophilic bacteria and LAB	Ozone levels – 0.4, 0.6 & 0.72 ppm Exposure time –10, 30, 60 & 120 min Followed by freeze drying of samples.	Treatment blends promoted the shelf life up to 8 months, while lyophilization exhibited only 4-month shelf life. Ozone treatment of 0.6 ppm for 10 min showed better results in combination treatments	(Cantalejo, Zouaghi, and Pérez-Arnedo 2016)
Beef samples	*E. coli* and aerobic bacteria	Ozone levels – 12ppm Exposure time – 90 s of spray every 30 min for 12 h	Aqueous ozone spray chill reduction of *E. coli* and aerobic bacteria was 1.46 log and 0.99 log on surfaces of fresh beef compared to water spray chill which was not effective in case of aerobic bacteria.	(Kalchayanand, Worlie, and Wheeler 2019)
Chicken breasts	*Salmonellae*	Ozone level – 2000 ppm (Storage under 70% CO_2:30% N_2 at 7 °C) Exposure time – 30 min	Gaseous ozone reduced the counts of *salmonellae* by 97% and pseudomonads by 95%, but indigenous coliforms were unaffected.	(Al-Haddad, Al-Qassemi, and Robinson 2005)
Turkey breast meat	Total aerobic mesophilic bacteria	Ozone level: 1×10^{-2} kg m^{-3} Exposure time: 2 h, 4 h, 6 h and 8 h	1.5–3.0 log reductions in TAMB counts were obtained whereas reductions of 0.9–1.9 log were determined for yeast-mold. Prolonging ozone treatment increased the microbial inactivation up to 3 log reductions.	(Ayranci et al. 2020)
Beef	*Escherichia coli, Salmonella Typhimurium*, coliforms and total aerobic plate counts	Pre-treated with 1% ozonated water for 7 min and 15 min	15 min treatment reduced the populations of *Escherichia coli, Salmonella Typhimurium*, coliforms and total aerobic plate counts whereas 7 min treatment was effective only in case of certain populations	(Stivarius et al. 2002)

There was also an increase in peroxide value and TOTOX value with respect to the exposure time, indicating an increase in oxidation products in milk.

Application of ozone in the meat industry

The spoilage of meat and meat products is associated majorly with the activity of microorganisms and its own enzymes to a certain extent. Ozone is found to have a positive hand on controlling the microbial populations that were present internally or added to the system viva poor handling and processing steps. The effect of ozone is not only limited to fresh carcass but also to the processed set of meat and meat products. Among the different meat products involved in market, chicken meat is having utmost importance with regard to their consumption rate, utilization matrix and spoilage degree. The effectiveness of ozone in the preservation of chilled chicken breasts studied by Dahshan,

Hafez, T. A., and Ghayaty, H. A (2013) promoted the use of ozone in controlling microbial populations in chicken. The study states that the treatment of chicken breasts with different degree of ozone exposures was successful in controlling the aerobic plate count, coliforms, and total mold count for a period of more than 9 days. Raw poultry is considered to be a common source of *Listeria monocytogenes* in chicken plants due to poor handling protocols. Controlling this potential threat found in ready to eat processed meat and poultry products as well as cooked meats using ozone technology was premeditated by Muthukumar and Muthuchamy (2013). The study set forth the use of gaseous phase application of ozone in raw chicken samples before they reach the consumer chain. Ozonation exposure time was found to be specific in controlling the microbial populations in these raw chicken samples. Similar observations were obtained in analyzing the effectiveness of ozone in controlling the total aerobic and anaerobic bacterial counts in chicken and duck meat (Muhlisin et al. 2016). The study divulges the fact that the ozone treatment was effective in diminishing the population of coliforms in chicken and duck meat and also exposing ozone at certain concentrations has effect on harmful pathogens such as *E. coli*. The possibility of using ozone as a replacement of chlorine in sanitizing the chicken carcass before processing techniques was studied by Trindade. Ozone can be used for disinfecting chicken carcass in immersion chilling, which was effective in controlling the populations of *salmonella, staphylococci, E. coli* and total coliform counts. This indicates the use of ozone as an alternative or substitute for chlorine in poultry slaughter houses. Effectiveness of ozone treatment can be correlated with the exposure time and temperature of system. Increase in the number of disinfected bacteria was observed with longer contact duration and lower temperatures (Karamah and Wajdi 2018). But increase in contact duration may have a detrimental effect on the final quality of the product which needs to be closely examined. The selection of proper and suitable time and temperature combinations is something that should be given utmost importance. Microbial efficacy is positively influenced by low temperatures and high period of exposure (Cárdenas et al. 2011). The microbial disinfection of chicken and beef was reported to be effective at this particular condition of temperature and time (Cárdenas et al. 2011; Muhlisin et al. 2016). The optimization of the treatment should be done to obtain proper degree of treatment for particular item which maintains overall quality and safety of the product. The effectiveness of this treatment shows a similar trend in the case of products derived from meat. The microbial populations isolated from spoiled chicken rolls was found to be reduced during an exposure levels of 1 to 4 hin total (Naito and Takahara 2006).

The contribution of ozone in long-term stability of meat and meat products is an area that needs equal importance as fresh and processed products (Table 2). The combination of ozone with several technologies was found to be effective in controlling the microbial populations and thereby increasing the shelf life of the produce. The effectiveness of employing freeze drying and ozone treatment as hurdles in developing a chicken product from broiler chicken breasts was evaluated by Cantalejo, Zouaghi, and Pérez-Arnedo (2016). The combination of ozone and lyophilization suggestively reduced the total aerobic mesophilic bacteria compared with those treated only with lyophilization. This fact may be attributed to the antimicrobial effects of ozone to destroy wide bacterial populations in food. The combination of ozone treatments with other possible options was also found to be effective in increasing the shelf life of meat and meat products. The study also states the correlation between time and microbial count as there was a decreasing trend in mesophilic counts with the increase in ozonation time. Analogous to the effectiveness of combination treatments, Lyu et al. (2016) studied the effectiveness of color stabilizing effect of CO and sterilization effect of ozone in the storage of beef. Alike to the reported results, the combination treatments showed better control on microbial populations. This set forth a new dimension of application in the case of meat and meat products eliminating the negatives of individual treatments in particular food product.

Effect of ozone treatment on meat products quality

The reported issues with ozone treatment are the effect of the treatment on the final product quality. EL-Dahshan, Hafez, T. A., and Ghayaty, H. A (2013), states that the product quality of chicken breasts was comparable with that of control samples and with extension of storage period the ozonated samples exhibited better quality parameters. Contrary to these results, the color of chicken and duck meat was found to be affected by the exposure of particular degree of ozone to fresh meat surfaces (Muhlisin et al. 2016). The redness of the samples reduced significantly with the storage time and decline rate was higher in the case

of duck meat compared to chicken. Degradation of color parameter is allied with the oxidation of myoglobin and oxymyoglobin with respect to the exposures. The presence of these highly reactive oxygen species leads to the development of meta myoglobin which is depicted by lower redness. This emphasizes the importance of degree of ozonation on the quality of the products. Importance should be given on various factors like exposure degrees, period etc. in regulating the effectiveness of ozone application on meat and meat products. Lipid oxidation is one of the primary factors that reduces the quality of meat and meat products leading to discolorations which is highly problematic in customer point of view. Ozone is reported to have an effect on these lipid oxidations in certain cases leading to unfavorable conditions affecting the product quality. Muhlisin et al. (2016) studies the possibility of the influence of lipid oxidation in chicken and duck meat by exposing the meat to a particular flux of ozone concentration (10×10^{-6} kg $O_3/m^3/h$). The lipid oxidation was found to be affected by the presence of ozone treatment. The change in the parameter may possibly be correlated between high oxidizing power of ozone and characteristic lipid profile of chicken which is exalted in unsaturated fatty acids. The reduction activity of antioxidants such as catalase and glutathione peroxidase present in meat was found to be a counting factor to high lipid oxidation rates in treated samples. Trindade also upholds the chance of problems related to high levels of liquid oxidation rates during the storage period of ozonated chicken. But while comparing with chlorine which is widely used as a sanitizing agent in the

Table 3. Effect of ozone on different quality parameters of meat and meat products.

Food matrix	Treatment conditions	Parameters	Observations	References
Beef	Ozone level- 154×10^{-6} kg m^3 Exposure time – 3 h & 24 h Temperature – 0 °C & 4 °C	• Surface color • Oxidative rancidity	Short exposure timing maintained the color and rancidity characteristics whereas 24 h exposures exhibited detrimental effect.	(Cárdenas et al. 2011)
Chicken and duck breast meat	Ozone level- 10×10^{-6} kg O_3/m^3 /h (4 ± 1 °C) Exposure time – 4 days	• Color • Lipid oxidation	Undesirable brown color in case of duck meat while chicken meat remaining unaffected. Influence on lipid oxidation rate was observed and antioxidant enzyme activity decline rates were observed.	(Muhlisin et al. 2016)
Chilled chicken breasts	Ozone levels – 40, 60 & 70ppm Exposure time – 20 min	• Texture • Color • Odor	No negative effects on the quality parameters, but also prolonged retainment of attributes by more than 9 days	(EL-Dahshan, Hafez, T. A., and Ghayaty, H. A 2013)
Chicken fillets	Ozone levels – 0.21 and 0.38 mg/l Exposure time – 40, 80, and 120 min Temperature – 3, 26, and 37 °C	• Protein • Water content	Exhibited little effect on the protein and water content of samples.	(Karamah and Wajdi 2018)
Beef	Pre-treated with 1% ozonated water for 7 min and 15 min	• Color • Odor	Lightness in color was observed in treated samples but was not to unacceptable range. Lightness was more observed in case of 15 min exposure.	(Stivarius et al. 2002)
Chicken meat fillets	Ozone levels – 0.4, 0.6 & 0.72 ppm Exposure time –10, 30, 60 & 120 min Followed by freeze drying of samples.	• Texture • Color • Rehydration ratio	Ozone treatment at 0.6 ppm for 10 min followed by lyophilization promoted the enhancement of quality parameters. 0.4 ppm ozone concentration had negative effect on increasing both the hardness and chewiness of chicken meat.	(Cantalejo, Zouaghi, and Pérez-Arnedo 2016)
Chicken legs	Combined effect of ozonation and vacuum packaging on shelf-life extension Ozone levels: 2, 5, & 10 mg/L	• Color • Sensory parameters	With increase in storage time, decline of sensory scores was observed but within the acceptable limits. Ozonation dosage also has a hand on the parameters.	(Gertzou et al. 2017)
Turkey breast meat	Ozone level: 1×10^{-2} kg m^{-3} Exposure time: 2 h, 4 h, 6 h and 8 h	• Color • TBARS analysis • Water holding capacity • Cooking yield	Significant changes in color TBARS values was observed in treated samples and were higher than those of untreated. But the values of none of treated samples exceed the acceptable sensory threshold limit for exhibiting rancid flavor. Increased water holding capacity and cooking yield was observed.	(Ayranci et al. 2020)
Beef	Pre-treated with volume ratios of carbon monoxide and ozone for 1.5 hours, vacuum-packaged and stored in 0 °C for 46 days (1) 100% CO (T1), (2) 2%O_3 + 98%CO (T2), (3) 5%O_3 + 95%CO (T3) (4) 10%O_3 + 90%CO (T4)	• Surface color • Sensory parameters	Evaluated parameters maintained better values than control during storage period. In later storage period, T3 and T4 showed a higher a* value than T1 which signifies the role in color stability of beef meat	(Lyu et al. 2016)
Pork	Pre-treated with volume ratios of potassium lactate and ozone and stored at 8 °C for 15 days Ozone levels: 200, 500 and 1,000 mg/h	• Color	Color stability was maintained due to the combined action of both the treatments. Exhibited a light change in a* values during days 10–15.	(Piachin and Trachoo 2011)

meat industry, the sensory characteristics of ozonated chicken did not show any significant difference which promotes the chance of ozone usage in the meat industry. It is found that both the treatments did not differ much in acceptance score maintaining an average score of 6 (liked slightly) throughout the storage period. Similarly, while considering the possibility of longer storage period of meat and meat products, the quality parameters of treated samples show higher acceptance. Greater acceptability of ozone-treated freeze-dried samples for a period of 8 months upsurges the possibility of usage of ozone in the industry (Table 3). This also shows the effectiveness of the treatment with respect to product quality by diversifying the application matrix. While evaluating the effectiveness of treatment on meat and meat products, it is important to have a close look on the factors affecting the efficacy and their relation with the product quality. Observations regarding the effect of factors like contact time, ozone concentration, temperature etc. on quality of different types of meat and meat products exists (Karamah and Wajdi 2018; Muhlisin et al. 2016). The period of exposure or contact time is one of the important factors of that sort, which has great influence on product quality. While comparing the effect of ozonated water treatment in chicken with that of water, it was found that the decrease in protein content was less than 1% which is considered to be less (Karamah and Wajdi 2018). But it is important that according to the change in the type of meat handled there will be change in suitable exposure timings. There was a similar observation in the case of ozone concentration treated on chicken and duck meat. The discoloration in case of duck meat was high while comparing that with chicken which was treated at the same concentration (Muhlisin et al. 2016). Beef samples also showed similar results where shorter exposure time was effective in controlling the discoloration and lipid oxidation (Cárdenas et al. 2011). Maintaining lower temperatures during the exposure period was not found to be effective in avoiding the discoloration and oxidation reactions. The so-called discussed issue of discoloration is somewhat controllable in the case of combination treatments of ozone with other existing methods or technologies. Contrary to reported results about the effect of ozone on color, the combination showed a better retention of color during the storage period compared to untreated samples. Improved color stabilization was observable in case of pork (Piachin and Trachoo 2011) and beef portions treated with ozone in combination with potassium lactate in former and carbon monoxide in latter. The lactate contributes to color value of the meat sample via augmented lactic hydrogenase activity which in turn promotes the reducing activity of meta myoglobin producing either oxy or deoxy myoglobin. Effective and intelligent use of ozone treatment in combinations can be used to eliminate and, in some cases, enhance the negative effects of the treatment.

Challenges associated with ozone technology

As a powerful oxidizing and an antimicrobial agent, ozone is widely used to extend the shelf life of different food groups (Pandiselvam et al. 2018). It does not leave any chemical residues and can be applied to food products in both aqueous and gaseous forms irrespective of the product state. Counter balancing this positive nature of ozone is the difference in sensitivity of various microorganism to ozone (Miller, Silva, and Brandão 2013). The sensitivity of ozone is also affected by other parameters such as organic content, temperature, physical state of ozone, initial microbial load etc. and the fact that there are so many external and internal factors that affect the efficiency of ozone remains as one of the major challenges in optimizing an effective ozone dosage. Adequate precautions should also be taken where high ozone doses are applied for obtaining optimal microbial inactivation as in some cases, prolonged contact periods as well as high dosages for attaining decline in microbial populations has affected the quality parameters of meat, milk, and their associated products. Discolorations, lipid oxidation and antioxidant enzyme activity were associated with prolonged exposures in the case of meat and meat products. Understanding the degree of ozonation that is capable of balancing both microbial decline and quality retention is very important. Studies with emphasis on how these ozonation dosages and contact time has effect on each type of food matrix is very limited. This limited knowledge on the reaction part of food groups acts as a hindrance in ensuring the final quality and safety of processed products. As ozone is highly unstable in nature, care should be taken to give adequate contact time for the removal of ozone-resistant compounds (pesticides) so that partial oxidation of the targeted compound may not take place. Economic viability of the ozonation system also acts as a limitation in their wide scale application. Although ozonation technology requires relatively less working capital, it fails to entice small-

scale entrepreneurs due exorbitant initial set up cost involved. Nevertheless, inadequate awareness among the consumers about ozone technology also plays a role in final consumer acceptance of the products (Brodowska, Nowak, and Śmigielski 2018)

Conclusion

Ozone being a strong antimicrobial agent is effective against controlling the microbial populations in milk, meat, and their products. But in certain cases, this efficacy was found to be affected by the decline in quality aspects of meat and milk products. With increasing sensory, health and safety concerns among the consumers it is very important to have a detailed study and knowledge on this aspect to promote the wide application of ozone technology.

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References

Adachi, D. 2001. "*Virus Inactivation by Ozone*". (Masters), University of Toronto,

Alexopoulos, A., S. Plessas, Y. Kourkoutas, C. Stefanis, S. Vavias, C. Voidarou, E. Bezirtzoglou, and E. Bezirtzoglou. 2017. "Experimental Effect of Ozone upon the Microbial Flora of Commercially Produced Dairy Fermented Products." *International Journal of Food Microbiology* 246:5–11. doi: 10.1016/j.ijfoodmicro.2017.01.018

Al-Haddad, K. S., R. A. Al-Qassemi, and R. Robinson. 2005. "The Use of Gaseous Ozone and Gas Packaging to Control Populations of *Salmonella Infantis* and *Pseudomonas Aeruginosa* on the Skin of Chicken Portions." *Food Control* 16 (5): 405–10. doi:10.1016/j.foodcont.2004.04.009

Annalisa Segat, N.N., Astrid Fabbro Misra, Federica Buchini, Patrick J. Cullen Giovanna Lippe, and N. Innocente. 2014. "Effects of Ozone Processing on Chemical, Structural and Functional Properties Ofwhey Protein Isolate." *Food Research International* doi: 10.1016/j.foodres.2014.10.002.

Asfahl, K. L., and M. C. Savin 2012. "Destruction of *Escherichia Coli* and Broad-host-range Plasmid DNA in Treated Wastewater by Dissolved Ozone Disinfection under Laboratory and Field Conditions".

Ayranci, U. G., O. Ozunlu, H. Ergezer, and H. Karaca. 2020. "Effects of Ozone Treatment on Microbiological Quality and Physicochemical Properties of Turkey Breast Meat." *Ozone: Science and Engineering* 42 (1): 95–103. doi:10.1080/01919512.2019.1653168

Azhar, J. ALmosowy, and Z. H. ALmosowy. 2020. "Study the Effect of Ozone Gas in Milk Treatment on Chemical and Microbial Properties of Soft Cheese." *Indian Journal of Ecology* 47 (7): 1–5.

Brodowska, A. J., A. Nowak, and K. Śmigielski. 2018. "Ozone in the Food Industry: Principles of Ozone Treatment, Mechanisms of Action, and Applications: An Overview." *Critical Reviews in Food Science and Nutrition* 58 (13): 2176–201. doi:10.1080/10408398.2017.1308313

Cantalejo, M. J., F. Zouaghi, and I. Pérez-Arnedo. 2016. "Combined Effects of Ozone and Freeze-drying on the Shelf-life of Broiler Chicken Meat." *LWT-Food Science and Technology* 68:400–07. doi: 10.1016/j.lwt.2015.12.058.

Cárdenas, F. C., S. Andres, L. Giannuzzi, and N. Zaritzky. 2011. "Antimicrobial Action and Effects on Beef Quality Attributes of a Gaseous Ozone Treatment at Refrigeration Temperatures." *Food Control* 22 (8): 1442–47. doi:10.1016/j.foodcont.2011.03.006

Casillas-Martinez, L., and P. Setlow. 1997. "Alkyl Hydroperoxide Reductase, Catalase, MrgA, and Superoxide Dismutase are Not Involved in Resistance of *Bacillus Subtilis* Spores to Heat or Oxidizing Agents." *Journal of Bacteriology* 179 (23): 7420–25. doi:10.1128/jb.179.23.7420-7425.1997

Cataldo, F. 2003. "On the Action of Ozone on Proteins." *Polymer Degradation and Stability* 82 (1): 105–14. doi:10.1016/S0141-3910(03)00170-8

Cavalcante, D., B. L. Júnior, A. Tribst, and M. Cristianini. 2013a. "Microbiological Quality of Minas Frescal Cheese Treated with Ozonated Water." *International Food Research Journal* 20 (5): 2911.

Cavalcante, M., B. Leite Júnior, A. Tribst, and M. Cristianini. 2013b. "Improvement of the Raw Milk Microbiological Quality by Ozone Treatment." *International Food Research Journal* 20 (4).

Couto, E. P., E. R. Alencar, V. S. Gonçalves, A. J. P. P., dos Santos, J. L. Ribeiro, and M. de Aguiar Ferreira. 2016. "Effect of Ozonation on the *Staphylococcus Aureus* Innoculated in Milk." *Semina: Ciências Agrárias* 37 (4): 1911–17.

Dahshan, EL-, H. A. Hafez, T. A., and EL- Ghayaty, H. A. 2013. "Effect of Ozone on Preservation of Chilled Chicken." *Assiut Vet. Med. J* 59 (136): 22–26.

Dave, D., and A. E. Ghaly. 2011. "Meat Spoilage Mechanisms and Preservation Techniques: A Critical Review." *American Journal of Agricultural and Biological Sciences* 6 (4): 486–510. doi:10.1128/MMBR.63.1.1-20.1999

Ding, Wanqing, Wenbiao Jin, Song Cao, Xu Zhou, Changping Wang, Qijun Jiang, Q. Wang, R. Tu, S-F. Han, and Q. Wang. 2019. "Ozone Disinfection of Chlorine-resistant Bacteria in Drinking Water." *Water Research* 160:339–49. doi: 10.1016/j.watres.2019.05.014

Dizengremel, P., D. Le Thiec, M. P. Hasenfratz-Sauder, M. N. Vaultier, M. Bagard, and Y. Jolivet. 2009. "Metabolic-dependent Changes in Plant Cell Redox Power after Ozone Exposure." *Plant Biology* 11:35–42. doi: 10.1111/j.1438-8677.2009.00261.x.

Driks, A. 1999. "*Bacillus Subtilis* Spore Coat." *Microbiology and Molecular Biology Reviews* 63 (1): 1–20.

Fisher, C. W., D. Lee, B.-A. Dodge, K. M. Hamman, J. B. Robbins, and S. E. Martin. 2000. "Influence of Catalase and Superoxide Dismutase on Ozone Inactivation of *Listeria Monocytogenes*." *Applied and Environmental Microbiology* 66 (4): 1405–09. doi:10.1128/AEM.66.4.1405-1409.2000

Gertzou, I. N., I. K. Karabagias, P. E. Drosos, and K. A. Riganakos. 2017. "Effect of Combination of Ozonation and Vacuum Packaging on Shelf Life Extension of Fresh Chicken Legs during Storage under Refrigeration." *Journal of Food Engineering* 213:18–26. doi: 10.1016/j.jfoodeng.2017.06.026.

Heacox, D. 2014. *Use and Generation of Ozone as a Disinfectant of Dairy Animal Tissues, Dairy Equipment, and Infrastructure*. U.S. Patent Application, 14/102,140.

Hunt, N. K., and B. J. Mariñas. 1999. "Inactivation of *Escherichia Coli* with Ozone: Chemical and Inactivation Kinetics." *Water Research* 33 (11): 2633–41. doi:10.1016/S0043-1354(99)00115-3

Iriti, M., and F. Faoro. 2008. "'Oxidative Stress, the Paradigm of Ozone Toxicity in Plants and Animals." *Water, Air, and Soil Pollution* 187 (1): 285–301. doi:10.1007/s11270-007-9517-7

Ito, K., S. Inoue, Y. Hiraku, and S. Kawanishi. 2005. "Mechanism of Site-specific DNA Damage Induced by Ozone." *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 585 (1–2): 60–70. doi:10.1016/j.mrgentox.2005.04.004

Jin-Gab Kim, Ahmed E. Yousef, S. Dave. 1999. "Application of Ozone for Enhancing the Microbiological Safety and Quality of Foods: A Review." *Journal of Food Protection* 62 (9): 1071–87. doi:10.4315/0362-028X-62.9.1071

Kalchayanand, N., D. Worlie, and T. Wheeler. 2019. "A Novel Aqueous Ozone Treatment as A Spray Chill Intervention against *Escherichia Coli* O157: H7 on Surfaces of Fresh Beef." *Journal of Food Protection* 82 (11): 1874–78. doi:10.4315/0362-028X.JFP-19-093

Karamah, E. F., and N. Wajdi 2018. "*Application of Ozonated Water to Maintain the Quality of Chicken Meat: Effect of Exposure Time, Temperature, and Ozone Concentration*". Paper presented at the E3S Web of Conferences, Indonesia, vol. 67, p. 04044.

Khadre, M. A., and Y. A E.. 2001. "Sporicidal Action of Ozone and Hydrogen Peroxide: A Comparative Study." *International Journal of Food Microbiology* 71 (2–3): 131–38. doi:10.1016/S0168-1605(01)00561-X

Khudhir, Zinasaab, and A. M. Mahdi. 2017. "The Efficacy of Ozonated Water on the Microbial Quality of Locally Produced Soft Cheese in Baghdad." *International Journal of Science and Research* 6 (10): 383–87. doi:10.21275/ART20176990.

Khudhir, Z. S., and M. R. Mahmood. 2017. "The Efficacy of Ozone Treatment on the Microbiological Quality of Raw Milk at Different Storage Temperatures." *Journal of Entomology and Zoology Studies* 5 (5): 930–34.

Law, C., I. Fraser, and M. Piracha. 2020. "Nutrition Transition and Changing Food Preferences in India." *Journal of Agricultural Economics* 71 (1): 118–43. doi:10.1111/1477-9552.12322

Lu, M., and N. S. Wang. 2017. "Spoilage of Milk and Dairy Products." In *The Microbiological Quality of Food (Eds: Antonio Bevilacqua, Maria Rosaria Corbo and Milena Sinigaglia),* 151–78. Woodhead Publishing.

Lyu, F., K. Shen, Y. Ding, and X. Ma. 2016. "Effect of Pretreatment with Carbon Monoxide and Ozone on the Quality of Vacuum Packaged Beef Meats." *Meat Science* 117: 137–46. doi: 10.1016/j.meatsci.2016.02.036.

Marino, Marilena, Annalisa Segat, Michela Maifreni, Francesca Frigo, Caterina Sepulcri, and N. Innocente. 2015. "Efficacy of Ozonation on Microbial Counts in Used Brines for Cheesemaking." *International Dairy Journal* 50: 9–14. doi: 10.1016/j.idairyj.2015.06.003

Miller, F. A., C. L. Silva, and T. R. Brandão. 2013. "A Review on Ozone-based Treatments for Fruit and Vegetables Preservation." *Food Engineering Reviews* 5 (2): 77–106. doi:10.1007/s12393-013-9064-5

Mohammadi, Hesam, Seyed Mohammad Mazloomi, Mohammad Hadi Eskandari, Mahmoud Aminlari, and M. Niakousar. 2017. "The Effect of Ozone on Aflatoxin M1, Oxidative Stability, Carotenoid Content and the Microbial Count of Milk." *Ozone: Science & Engineering* 39 (6): 447–53. doi:10.1080/01919512.2017.1329647.

Morandi, S., M. Brasca, R. Lodi, and G. Battelli. 2009. "Use of Ozone to Control *Listeria Monocytogenes* in Various Types of Cheese." *Scienza E Tecnica Lattiero-Casearia* 60:211–15.

Muhlisin, M., D. T. Utama, J. H. Lee, J. H. Choi, and S. K. Lee. 2016. "Effects of Gaseous Ozone Exposure on Bacterial Counts and Oxidative Properties in Chicken and Duck Breast Meat." *Korean Journal for Food Science of Animal Resources* 36 (3): 405. doi:10.5851/kosfa.2016.36.3.405

Munhõs, M., R. Navarro, S. Nunez, D. Kozusny-Andreani, and A. Baptista 2019. "*Reduction of Pseudomonas Inoculated into Whole Milk and Skin Milk by Ozonation.*" Paper presented at the XXVI Brazilian Congress on Biomedical Engineering, Armação de Buzios, RJ, Brazil, 21-25th October .

Muthukumar, A., and M. Muthuchamy. 2013. "Optimization of Ozone in Gaseous Phase to Inactivate *Listeria Monocytogenes* on Raw Chicken Samples." *Food Research International* 54 (1): 1128–30. doi:10.1016/j.foodres.2012.12.016

Naito, S., and H. Takahara. 2006. "Ozone Contribution in Food Industry in Japan." *Ozone: Science and Engineering* 28 (6): 425–29. doi:10.1080/01919510600987347

Odeyemi, O. A., O. O. Alegbeleye, M. Strateva, and D. Stratev. 2020. "Understanding Spoilage Microbial Community and Spoilage Mechanisms in Foods of Animal Origin." *Comprehensive Reviews in Food Science and Food Safety* 19 (2): 311–31. doi:10.1111/1541-4337.12526

Ogata, A., and H. Nagahata. 2000. "Intramammary Application of Ozone Therapy to Acute Clinical Mastitis in Dairy Cows." *Journal of Veterinary Medical Science* 62 (7): 681–86. doi:10.1292/jvms.62.681

Pandiselvam, R., S. Subhashini, E. P. Banuu Priya, A. Kothakota, S. V. Ramesh, and S. Shahir. 2018. "Ozone Based Food Preservation: A Promising Green Technology for Enhanced Food Safety." *Ozone: Science & Engineering* 41 (1): 17–34. doi:10.1080/01919512.2018.1490636.

Piachin, T., and N. Trachoo. 2011. "Effect of Ozone and Potassium Lactate on Lipid Oxidation and Survival of *Salmonella Typhimurium* on Fresh Pork." *Pakistan Journal of Biological Sciences: PJBS* 14 (3): 236–40. doi:10.3923/pjbs.2011.236.240

Riesenman, P. J., and W. L. Nicholson. 2000. "Role of the Spore Coat Layers in *Bacillus Subtilis* Spore Resistance to Hydrogen Peroxide, Artificial UV-C, UV-B, and Solar UV Radiation." *Applied and Environmental Microbiology* 66 (2): 620–26. doi:10.1128/AEM.66.2.620-626.2000

Segat, A., M. Biasutti, L. Iacumin, G. Comi, F. Baruzzi, C. Carboni, and N. Innocente. 2014. "Use of Ozone in Production Chain of High Moisture Mozzarella Cheese." *LWT' - Food Science and Technology* 55 (2): 513–20. doi:10.1016/j.lwt.2013.10.029.

Sert, D., and E. Mercan. 2021. "Effects of Ozone Treatment to Milk and Whey Concentrates on Degradation of Antibiotics and Aflatoxin and Physicochemical and Microbiological Characteristics." *LWT-Food Science and Technology* 144:111226. doi: 10.1016/j.lwt.2021.111226

Sert, Durmuş, and E. Mercan. 2020. "Effects of Churning with Different Concentrations of Ozonated Water on Particle Size, Texture, Oxidation, Melting and Microbiological Characteristics of Butter." *International Dairy Journal* 111:1–7. doi: 10.1016/j.idairyj.2020.104838

Sert, Durmuş, Emin Mercan, and Ü. Kara. 2020. "Butter Production from Ozone-treated Cream: Effects on Characteristics of Physicochemical, Microbiological, Thermal and Oxidative Stability." *LWT - Food Science and Technology* 131:1–8. doi: 10.1016/j.lwt.2020.109722

Setlow, P. 2006. "Spores of *Bacillus Subtilis*: Their Resistance to and Killing by Radiation, Heat and Chemicals." *Journal of Applied Microbiology* 101 (3): 514–25. doi:10.1111/j.1365-2672.2005.02736.x.

Sheelamary;, M., and M. Muthukumar. 2011. "Effectiveness of Ozone in Inactivating *Listeria Monocytogene*s from Milk Samples." *World Journal of Young Researchers* 1 (3): 40–44.

Stivarius, M., F. Pohlman, K. McElyea, and J. Apple. 2002. "Microbial, Instrumental Color and Sensory Color and Odor Characteristics of Ground Beef Produced from Beef Trimmings Treated with Ozone or Chlorine Dioxide." *Meat Science* 60 (3): 299–305. doi:10.1016/S0309-1740(01)00139-5

Takamoto, Y., H. Maeba, and M. Kamimura. 1992. "Changes in Survival Rate Enzyme Activities and in *Escherichia Coli* with Ozone." *Applied Microbiology and Biotechnology* 37 (3): 393–95. doi:10.1007/BF00210999

Thanomsub, B., V. Anupunpisit, S. Chanphetch, T. Watcharachaipong, R. Poonkhum, and C. Srisukonth. 2002. "Effects of Ozone Treatment on Cell Growth and Ultrastructural Changes in Bacteria." *The Journal of General Applied Microbiology and Biotechnology* 48 (4): 193–99. doi:10.2323/jgam.48.193

Tomé, D., M. Dubarry, and G. Fromentin. 2004. "Nutritional Value of Milk and Meat Products Derived from Cloning." *Cloning and Stem Cells* 6 (2): 172–77. doi:10.1089/1536230041372445

Torlak, Emrah, and D. Sert. 2013. "Inactivation of *Cronobacter* by Gaseous Ozone in Milk Powders with Different Fat Contents." *International Dairy Journal* 32 (2): 121–25. doi:10.1016/j.idairyj.2013.05.013.

Uzun, Hicran, Esra Ibanoglu, Hatice Catal, and S. Ibanoglu. 2012. "Effects of Ozone on Functional Properties of proteins." *Food Chemistry* 134 (2): 647–54. doi:10.1016/j.foodchem.2012.02.146.

Varga;, L., and J. Szigeti. 2016. "Use of Ozone in the Dairy Industry: A Review." *International Journal of Dairy Technology* 69 (2): 157–68. doi:10.1111/1471-0307.12302.

Young, S. B., and P. Setlow. 2004. "Mechanisms of *Bacillus Subtilis* Spore Resistance to and Killing by Aqueous Ozone." *Journal of Applied Microbiology* 96 (5): 1133–42. doi:10.1111/j.1365-2672.2004.02236.x.

Younis, F. I., A. E. Fayed, O. I. Elbatawy, and A. Elsisi. 2019. "A Comparison between Ozonation and Thermal Process in Relation to Cow's Milk Attributes with Emphasis on Pathogens." *Arab Universities Journal of Agricultural Sciences* 27 (5): 2593–600.

Zhang, Y., Q. Wu, J. Zhang, and X. Yang. 2011. "Effects of Ozone on Membrane Permeability and Ultrastructure in Pseudomonas Aeruginosa." *Journal of Applied Microbiology* 111 (4): 1006–15. doi:10.1111/j.1365-2672.2011.05113.x