DECONTAMINATION OF *ESCHERICHIA COLI* O157:H7 AND

SALMONELLA IN LETTUCE, CHICKEN, AND APPLES

BY CHLORINE DIOXIDE AND ULTRASOUND

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THESIS ABSTRACT

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BY CHLORINE DIOXIDE AND ULTRASOUND

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Chlorine dioxide (ClO_2) has been used for many years by the food industry and public water suppliers as a sanitizing and disinfecting agents. The mechanisms of microbial inactivation by ultrasonication are mainly due to thinning of the cell membranes, localized heating, production of free radicals and formation of hydrogen peroxide. Non-thermal disinfectants combined with ultrasonication treatments may be relatively more effective for pathogen removal and inactivation.

In this study, decontamination efficiencies of ClO_2 alone and ClO_2 combined with ultrasonication on foodborne pathogens inoculated foods at different times were performed. Lettuce, chicken breasts, and apples were inoculated with *E. coli* O157:H7 and *Salmonella*, and treated with the combination of ClO_2 at 5, 10, 20,

and 40 ppm, and with ultrasonication at 120 and 170 kHz for 1, 3, 6, and 10 min. The efficacies of removing and inactivating *E. coli* or *Salmonella* on lettuce were mainly dependant on ClO_2 concentration but not on the treatment time and ultrasonic frequency. Various ClO_2 concentrations had significantly different log reductions in this study. The efficacies of removing and inactivating *E. coli* or *Salmonella* on chicken breasts were mainly based on treatment time and not on ClO_2 concentration and ultrasonication. Longer treatment times had significantly higher log reductions for both high and low inoculation levels of the pathogens, especially for *Salmonella*. The efficacies of removing and inactivating *Salmonella* on apples were mainly dependant on the ClO_2 concentration and ultrasonication but not on treatment times; for *E. coli* O157:H7, it mainly depended on the ClO_2 concentration, not treatment time and ultrasound.

The ClO_2 residual and temperature change after treatment were also investigated. For chicken breasts, the ClO_2 residuals dropped dramatically with longer treatment times, while, ClO_2 residuals only dropped a little for lettuce and apples. ClO_2 residual dropped more dramatically in combination treatments, than in ClO_2 treatments alone. However, no significant differences were found between these two treatments on lettuce and apples. These results indicated that ultrasonication accelerated the reaction of ClO_2 with chicken breast tissue but not with lettuce and apples. In different foods, temperature changes were similar for the same treatments. Temperature increased to near 60°C after the application of ultrasonication, and it elevated more dramatically with ClO_2 combined with ultrasonication than with ClO_2 treatment alone.

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INTRODUCTION

Fresh produce contaminated with pathogens is one of the major avenues contributing to foodborne diseases. There have been numerous reports regarding the occurrence of foodborne diseases caused by pathogenic microorganisms such as *Escherichia coli* O157:H7 (Ackers and others 1996; Mermin and Griffin 1999) and *Salmonella* (D'Aoust 1997; Ries and others 1990; Mahon and others 1997) in different foods.

Bacteria have been shown to enter fruits and vegetables through various pathways such as through the stomata, stem, stem scar, or calyx (Samish and Etinger-Tulczynska, 1963; Samish and others 1963; Zhuang and others 1995; Seo and Frank 1999). Microorganisms can enter physically damaged fruits and vegetables through punctures, wounds, cuts, and splits during maturation, harvesting, or processing. Bacterial soft rot of fruits and vegetables can also increase the likelihood of contamination with pathogens. It has been shown that *Salmonella* spp. were present in 18 - 20% of soft-rotted samples of vegetables. This nearly doubles the rate (9 - 10%), which was found on intact, healthy samples of the same vegetables (Wells and Butterfield 1997). *E. coli* is a common microflora in the intestinal tracts of humans and other warm-blooded animals. Fruits and vegetables can be contaminated with *E. coli* in the field or during

post-harvest handling. Traveler's diarrhea has been associated with consumption of salads (Merson and others 1976; Mintz 1994) and carrots (CDC 1994). *E. coli* O157:H7 is a strain of enterohemorrhagic *E. coli*. The growth of this strain in the human intestine produces a large quantity of toxins that can cause severe damage to the lining of the intestine and other organs of the body. Cattle are one of the major reservoirs for the pathogen; therefore, most outbreaks of illness have been associated with the consumption of contaminated, undercooked beef and dairy products. However, outbreaks have also been linked to lettuce (Ackers and others 1996; Mermin and Griffin 1999), apple cider (Besser and others 1993; CDC 1996a; Steele and others 1982;), radish sprouts, (Nathan 1997) and alfalfa sprouts (CDC 1997). Enterohemorrhagic *E. coli* can grow on cantaloupe and watermelon cubes (del Rosario and Beuchat 1995), shredded lettuce (Diaz and Hotchkiss 1996), sliced cucumbers (Abdul-Raouf and others 1993), and apple cider (Zhao and others 1993; Hilborn and others 2000) causing health problems.

Salmonella is widely distributed in nature, and humans and animals are their primary reservoirs. *Salmonella* has been isolated from many types of raw fruits and vegetables (Beuchat 1996; Wells and Butterfield 1997). *Salmonella* food poisoning is caused by consumption of foods that contain appropriate strains of this genus in significant numbers (Jay 2000). *Salmonella* contamination also has been reported on raw pork meat (Buchholz and others 2005), fish (Guerin and others 2004), horse meat (Espie and others 2005), and on chicken and eggs (Davies and Breslin 2003). Outbreaks of salmonellosis have been linked to a diversity of fruits, vegetables and raw meat including tomatoes (CDC 1993; Hedberg and others 1994; Wood and others 1991), bean sprouts (Mahon and others 1997; O' Mahony and others 1990; Van Beneden and others 1999),

melons (Blostein 1991; CDC, 1979; 1991; Gayler and others 1955; Ries and others 1990), unpasteurized orange juice (Krause and others 2001) and apple juice (CDC 1975).

There are many methods available to disinfect pathogenic microorganisms, which include non-thermal disinfection methods such as application of ClO_2, ozone, hydrogen peroxide, iodine, acidic compounds, alkaline compounds, and quaternary ammonium compounds. In this study, the combined effectiveness of ClO_2 and ultrasound in decontaminating *E. coli* O157:H7 and *Salmonella* on lettuce, chicken breasts and apples was evaluated. In order to explain the different effectiveness of these treatments on different foods, we also monitored the temperature change and ClO_2 residual after application of ultrasonication and ClO_2.

LITERATURE REVIEW

Foodborne Pathogens

Escherichia coli

Escherichia coli (E. coli) is a common microorganism in the normal microflora of humans and other warm-blooded animals. Strains of *E. coli* that cause diarrhea are categorized into groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes and antigenic characteristics. The major groups are designated as enterotoxigenic, enterohaemorrhagic, enteropathogenic, enteroinvasive, diffuse-adhering and enteroaggregative (Doyle and others 1997). These strains of *E. coli* that cause diarrhea include traveler's diarrhea (enterotoxigenic *E. coli*), persistent diarrhea (enteroaggregative *E. coli*), watery diarrhea of infants (enteropathogenic *E. coli*), hemorrhagic colitis (bloody diarrhea), and hemolytic uremic syndrome (enterohemorrhagic *E. coli*). Some *E. coli* strains even have the ability to cause diseases of the gastrointestinal, urinary, or central nervous system in even healthy people (Doyle and others 1989; FDA. 1993. HACCP; Nataro and Kaper 1998; Paton JC and Paton, AW 1998).

Fruits and vegetables can become contaminated with *E. coli* in the field or during post-harvest handling. Traveler’s diarrhea has been associated with the consumption

of salads (Merson and others 1976; Mintz 1994) and carrots (CDC 1994). *E. coli* O157:H7 is a strain of enterohemorrhagic *E. coli* [EHEC] group. The growth of this strain in the human intestine produces a large quantity of toxin(s) that causes severe damage to the lining of the intestine and other organs of the body. These toxins are very similar to the toxins produced by *Shigella dysenteriae* (FDA. 1993). Cattle appear to be a natural reservoir for the pathogen; therefore most outbreaks of illness have been associated with the consumption of contaminated, undercooked beef and dairy products (Renter and others 2004). However, outbreaks have also been linked to lettuce (Ackers and others 1996), apple cider (Besser and others 1993; CDC 1996a; Steele and others 1982), radish sprouts (Nathan 1997) and alfalfa sprouts (CDC, 1997). Enterohemorrhagic *E. coli* can also survive and grow on cantaloupe and watermelon cubes (del Rosario and others 1995), shredded lettuce (Diaz and others 1996), sliced cucumbers (Abdul-Raouf and others 1993), and apple cider (Zhao and others 1993; Hilborn and others 2000).

Contamination of raw fruits and vegetables with enterohaemorrhagic *E. coli* O157:H7 can occur when ruminant animals enter fields, or when improperly composted cow manure has been applied as fertilizer in the fields. The contamination can occur or may be enhanced when fallen fruits or vegetables are used during handling and processing. The contaminated manure forming dust particles may also become the contamination source of fruits and vegetables. Workers on farms or in packinghouses can also be a source of *E. coli* O157:H7. These mechanisms of contamination are somewhat speculative at present and must be thoroughly investigated before appropriate interventions can be introduced to reduce the risk (Beuchat 1999).

Salmonella

Salmonellae are widely distributed in nature, and humans and animals are their primary reservoirs. *Salmonella* food poisoning results from the ingestion of foods containing appropriate strains of this genus in significant numbers (Jay 2000). The general symptoms associated with most types of *Salmonella* poisoning include diarrhea and intestinal symptoms. In some cases, these *Salmonella* symptoms are accompanied by a mild fever. The symptoms of a diarrhea *Salmonella* poisoning usually appear 6 to 72 hours after initial contact with the bacteria. Most people with diarrhea recover completely without treatment (CDC 1996b).

Salmonella. *typhimurium*. can cause an illness called typhoid fever in human. Typhoid is life-threatening, and the infection is persistent with high fevers. Other common symptoms include headache, malaise, anorexia, splenomegaly, and relative bradycardia. Many mild and atypical infections occur. Although typhoid fever is common in the developing world, there are only about 400 cases reported in the U.S. each year. The vast majority of those cases are acquired while traveling in foreign countries. Vaccine for typhoid fever is available and is recommended for people traveling to developing countries. People who are diagnosed with typhoid are given a treatment of antibiotics (CDC 1996b).

The antigenic scheme for classifying salmonellae recognizes more than 2,300 serovars and, only about 200 of which are associated with human illness. Animal husbandry practices in the poultry, meat and fish industries, and the recycling of offal and inedible raw materials into animal feeds, have helped the continued prominence of

Salmonella in the global food chain (D'Aoust 1997). There are reports of human salmonellosis linked to cantaloupe (Ries and others 1990) and sprouts produced from alfalfa seeds (Mahon and others 1997) imported to the United States. Hygienic conditions during the production, harvesting, transport and distribution of raw fruits and vegetables from some countries may not always meet minimum hygienic requirements, thus facilitating contamination on arrival. Application of night soil, untreated sewage sludge or effluents, or irrigation water containing untreated sewage to fields and gardens can result in contamination of fruits and vegetables with *Salmonella* and other pathogens. Washing fruits and vegetables with contaminated water and handling of produce by infected workers, vendors and consumers in the marketplace helps the spread of pathogenic microorganisms, including *Salmonella*.

Salmonellae have been isolated from many types of raw fruits and vegetables (Beuchat 1996; Wells and Butterfield 1997). Outbreaks of salmonellosis have been linked to a diversity of fruits, vegetables and raw meat including tomatoes (CDC 1993; Hedberg and others 1994; Wood *and others* 1991), bean sprouts (Mahon *and others* 1997; O' Mahony and others 1990; Van Beneden and others 1999), melons (Blostein 1991; CDC 1979 and 1991; Gayler and others 1955; Ries and others 1990), unpasteurized orange juice (Krause and others 2001) and apple juice (CDC 1975). This pathogen can grow on the surface of alfalfa sprouts (Jaquette and others 1996), and on tomatoes (Zhuang and others 1995). *Salmonella* contamination also has been reported on raw pork meat (Buchholz and others 2005), fish (Guerin and others 2004), horse meat (Espie and others 2005), chicken and eggs (Davies and Breslin 2003) and perhaps on

other mature raw fruits, vegetables and meat, making it imperative to use hygienic practices during handling.

Pathogens in Food

Bacteria have been shown to enter produce through various pathways. Bacteria can enter leaves of plants through the stomata and enter fruit through the stem, stem scar, or calyx (Samish and Etinger-Tulczynska 1963; Samish and others 1963; Zhuang and others 1995). Seo and Frank (1999) used confocal scanning laser microscopy to show that lettuce leaves dipped in a suspension of *E. coli* O157:H7 absorbed the pathogen through the stomata and cut surfaces on the leaves.

Microorganisms can also enter fruits and vegetables through tissue damage such as punctures, wounds, cuts, and splits. These injuries can occur during maturation, harvesting, or processing. Ballinger and Nesbitt (1982) found that grapes with torn stem scars had 6-10 times more internal decay than grapes with dry stem scars. Bartz and Showalter (1981) reported that tomatoes with fresh stem scars are more vulnerable to infiltration than tomatoes with old stem scars and also showed that green and pink tomatoes are more susceptible to water infiltration and became diseased earlier than did similarly treated red fruits.

Bacterial soft rot in fruits and vegetables may increase the likelihood of contamination of the fruit or vegetable with pathogens of concern. Wells and Butterfield (1997) demonstrated that *Salmonella* spp. were present in 18-20 % of soft-rotted samples of vegetables. This is nearly double the rate (9-10 %) found on intact, healthy samples of the same vegetables.

Non-thermal Disinfection Methods

Chlorine and Chlorine Dioxide

Chlorine as sodium, potassium, or calcium hypochlorite has been used for many years by the food industry and public water suppliers as their principal sanitizing and disinfecting agent (Kirk and Mitchell 1980; Reina and others 1995). Hypochlorites are powerful disinfectants, which are active against a wide spectrum of organisms, and they are nontoxic to humans at low concentrations (Dychdala 1991). Many organic compounds present in foods and water treated with chlorine are subjected to chlorination reactions. When chlorine is applied onto organic molecules, their hydrophobicity or lipophilic nature increases. This will in turn increase the toxicity and bioaccumulation of these compounds. There are potential health hazards connected with the use of chlorine because some reaction products have toxic activity such as mutagenicity, tetratogenicity, or carcinogenicity (Croue and Reckhow 1989).

Chlorine dioxide (ClO_2) is an effective disinfectant and an oxidant that is widely used in water treatment. At ambient temperatures, chlorine dioxide is a reactive gas that is potentially explosive and unstable at concentrations above 10% by volume in air. It is normally generated on site and used as a dilute aqueous solution (von Heijne and Teder 1973).

Chlorine dioxide is soluble in water. This offers many advantages over chlorine as a biocide in water systems. The major advantages of ClO_2 over HOCl include reduced reactivity with organic matter and greater activity at neutral pH; however, stability of chlorine dioxide may be a problem (Pei and others 2003). ClO_2 forms fewer

organohalogens than HOCl, although its oxidizing power is reported as 2.5 times that of chlorine (Bernarde and others 1967). A maximum of 200 ppm ClO_2 is allowed for sanitizing of processing equipment and 3 ppm maximum is allowable for contact with whole produce. Only 1 ppm maximum is permitted for peeled potatoes (FDA 1995).

Chlorine dioxide, like chlorine, is an oxidant, but its redox potential in aqueous solution, 1.15V ($ClO_2 + e^- = ClO^-$), is less than that of hypochlorous acid, 1.49V ($HClO + H^+ + 2e^- = Cl^- + H_2O$) (White, 1986). Chlorine dioxide is, therefore, likely to be less reactive and produce fewer byproducts. For example, the chlorine atom in chlorine dioxide incorporates much more slowly with unsaturated lipids than chlorine itself in aqueous solution (Ghanbari and others 1982). Chlorine dioxide is also relatively inert compared to chlorine in reacting with individual amino acids (Tan and others 1987a; 1987b). Chlorine reacts with organic materials to form chloroform and trihalomethane. In contrast, chlorine dioxide is not a chlorinating reagent so no chloroform or other trihalomethanes are formed (Suh and others 1984).

Chlorine dioxide used as a disinfectant has drawn much attention by the food industry in recent years. The bactericidal efficacy of ClO_2 is seven times higher than that of aqueous chlorine in poultry processing chiller water (Lillard 1979). Chlorine dioxide can be decomposed to chlorite, chlorate and chloride in water, but both chlorite and chlorate have negative effects on human health (Johanna et al., 1993). That is why standards for concentration of chlorine dioxide and its by-products are established in many countries (Aieta and Berg, 1986). In March 1995, the FDA approved chlorine dioxide to be used as an antimicrobial agent in poultry processing at a residual level not

to exceed 3 mg/l (FDA 1995). Further studies have shown that chlorine dioxide is able to serve as a bactericidal, viricidal, and fungicidal agent for seafood, poultry, red meat, and peeled vegetables and for fruits to enhance freshness, reduce organic pesticides residual and extend shelf life (Kim and others 1999). Chlorine dioxide has proved to be useful for washing intact fruits and vegetables at a concentration not to exceed 5 ppm (FDA, 1998). The latest regulation amended has stipulated that chlorine dioxide (21 CFR 173.300) can be used as an antimicrobial agent in water used to wash fruits and vegetables that are not raw agricultural commodities in an amount not to exceed 3 ppm residual chlorine dioxide. Treatment of the fruits and vegetables with chlorine dioxide shall be followed by a potable water rinse or by blanching, cooking, or canning (FDA, 2005).

Gross physical damage to bacterial cells or viral capsids has not been observed at the low concentrations of chlorine dioxide typically used to disinfect drinking water. Therefore, studies have focused primarily on two more subtle mechanisms that lead to the inactivation of microorganisms: determining specific chemical reactions between chlorine dioxide and biomolecules and observing the effect of chlorine dioxide on physiological functions.

With the first disinfection mechanism, chlorine dioxide reacts readily with amino acids cysteine, tryptophan, and tyrosine, but not with viral ribonucleic acid (RNA) (Noss and others 1983; Olivieri and others 1985). From this research, it was concluded that chlorine dioxide inactivated viruses by altering the viral capsid proteins (Alvarez and others 1982; Li 2004). However, chlorine dioxide reacts with poliovirus RNA and impairs RNA synthesis (Alvarez and others 1982; Li 2004). It has also been shown that chlorine dioxide reacts with free fatty acids (Ghandbari and others 1983). At this time, it

is unclear whether the primary mode of inactivation for chlorine dioxide lies in the peripheral structures or nucleic acids. Perhaps reactions in both regions contribute to pathogen inactivation.

The second type of disinfection mechanism focuses on the effect of chlorine dioxide on physiological functions. It has been suggested that the primary mechanism for inactivation was the disruption of protein synthesis (Bernarde and others 1967). However, later studies reported the inhibition of protein synthesis might not be the primary inactivation mechanism (Roller and others 1980). A more recent study reported that chlorine dioxide disrupted the permeability of the outer membrane (Aieta and others 1986). The action of chlorine dioxide against bacteria involves the loss of permeability control with nonspecific oxidative damage to the outer membrane and subsequent destruction of the transmembrane ionic gradient (Berg and others 1986). The results of this study were supported by the findings of Olivieri and others (1985) and Ghandbari and others (1983), who found that the outer membrane proteins and lipids were sufficiently altered by chlorine dioxide to increase permeability.

Ozone

The utilization of ozone as an antimicrobial agent in food processing was reviewed in detail (Kim and others 1999b; Xu 1999). Ozone is effective in inactivating bacteria, fungi, viruses, and protozoa (Peeters and others 1989; Korich and others 1990; Finch and Fairbairn 1991; Restaino and others 1995), and bacterial pathogens such as *S. typhimurium*, *S. aureus*. and *L. monocytogenes* which are sensitive to 20 ppm ozone in water (Finch and Fairbairn 1991; Restaino and others 1995). It was reported that

Salmonella and *E. coli* populations were reduced 3 to 4 log/g in ground black pepper after 60 min treatments with ozonated air (Zhao and Cranston 1995).

Beuchat (1998) reported that treatment with ozonated water could extend the shelf life of apples, grapes, oranges, pears, raspberries, and strawberries by reducing microbial populations and by oxidation of the ethylene to retard ripening. Microbial populations on berries and oranges were reduced by treating with 2-3 ppm and 40 ppm, respectively. A 2 log/g reduction in total counts was seen for shredded lettuce suspended in water ozonated with 1.3 mM ozone at a flow rate of 0.5 L/min (Kim and others 1999a). Ozone gas has also been investigated to prolong the shelf-life of various foods, such as fish (Haraguchi and others 1969), poultry (Sheldon and Brown 1995), peanuts and cottonseed meal (Dwankanath 1968), pork, beef, dairy products, eggs, mushrooms, potatoes, and fruits (Kaess and Weidemann 1968; Gammon and Kerelak 1973).

Apples, lettuce, strawberries, and cantaloupe submerged in sanitizer solutions containing 3 ppm ozone were reported to reduce spoilage (Rodgers and others 2004). Fungal growth during storage of blackberries was inhibited by 0.1 to 0.3 ppm ozone (Barth and others 1995). Ozone on grapes increased shelf life and reduced fungal growth (Sarig and others 1996). Ozone has an excellent ability to penetrate foods and does not leave a residue; it may have usefulness for treatment of processed water, food contact surfaces, or whole produce. However, ozone has disadvantages due to its strong oxidizing activity, such as causing physiological injury to bananas and carrots (Horvath and others 1985; Liew and Prange 1994), inducing corrosion of metals and other materials in processing equipment, or having safety concerns for employees.

Hydrogen peroxide

The bactericidal and inhibitory activity of hydrogen peroxide (H_2O_2) result from its properties as an oxidant and its ability to generate other cytotoxic oxidizing species such as hydroxyl radicals. The sporicidal activity of H_2O_2 and its property of rapid breakdown make it as a desirable sterilant for use on both food contact surfaces and packaging materials in aseptic filling operations.

H_2O_2 has been recently applied on whole and fresh-cut produce. *Salmonella* populations on alfalfa sprouts were reduced approximately 2 log CFU/g after treated with 2% H_2O_2 for 2 min or 200 ppm chlorine, and less than 1 log CFU/g reduction was observed on cantaloupe cubes under similar conditions (Beuchat and Ryu 1997). Treatment with 1% H_2O_2 to inoculated salmonellae and *E. coli* O157:H7 on whole cantaloupes, honeydew melons, and asparagus spears was less effective at reducing levels than hypochlorite, acidified sodium chlorite or a peracetic acid-containing sanitizer (Park and Beuchat 1999). Treatment with H_2O_2 vapor significantly reduced microbial populations on whole cantaloupes, grapes, prunes, raisins, walnuts, and pistachios (Sapers and Simmons 1998). By dipping in H_2O_2 solutions, it reduced microbial populations on fresh-cut bell peppers, cucumber, zucchini, cantaloupe, and honeydew melon, without altering sensory characteristics. Treatment on other produce was not as successful as above, further research is necessary to determine the usefulness of H_2O_2 treatment on other fruits and vegetables (Sapers and others 1999).

Iodine

Iodophors, combinations of elemental iodine and nonionic surfactants or carriers, have the advantages of broad spectrum of antimicrobial activity, being less corrosive than

chlorine at low temperatures and less volatile and irritating to skin than other types of iodine solutions (Lawrence and others 1957). However, iodine-containing sanitizer solutions also have limitations, such as being corrosive (upon vaporization above 50 °C), reduced efficacy at low temperature, and staining equipment, clothes, and skin. Iodine-containing solutions may react with starch that results in a blue purple color. Nevertheless, iodine solutions such as iodophors have been widely used as sanitizers for food contact surfaces and equipment in the food processing industry (Bartlett and Schmidt, 1957; Hays and others 1967; Mosley and others 1976; Lacey 1979; Jilbert 1988; Bianchi and others 1994)

Iodophors show more activity against bacterial vegetative cells than spores, and decimal reduction values for vegetative bacterial cells between 3 and 15 seconds at 6 to 13 ppm available iodine at neutral pH (Hays and others 1967; Mosley and others 1976; Gray and Hsu 1979). D values for spores of *Bacillus cereus*, *Bacillus subtilis*, and *Clostridium botulinum* Type A treated with 10 to 100 ppm of iodophor are 10- to 1000-fold greater than for vegetative cells (Odlaug 1981).

Acidic compounds

Since most pathogens generally do not grow at a pH lower than 4.5, acidification may act to prevent microbial proliferation. Therefore, organic acids are commonly used as antimicrobial acidulants to preserve foods either by direct addition or through microbiological fermentation (Foegeding and Busta 1991). Organic acids could reduce pH of the environment, disrupt membrane transport and/or permeability, induce anion accumulation, or even reduce the internal cellular pH by the dissociation of hydrogen ions from the acid. Many types of produce, including melons and papayas, naturally

possess significant concentrations of organic acids such as acetic, benzoic, citric, malic, sorbic, succinic, and tartaric acids, which negatively affect the viability of contaminating bacteria (Alakomi and others 2000).

Lactic acid, a type of organic acid, is successfully used as a sanitizer on food animal carcasses and may have the potential to reduce microorganisms on produce surfaces. Treatment with citric acid has been shown to reduce *S. typhi*murium on inoculated cubes of papaya and jicama (Fernandez Escartin and others 1989). Castillo and Escartin (1994) investigated survival of *Campylobacter jejuni* on cubes of watermelon and papaya treated at room temperature with lemon juice. Concentration of *Campylobacter jejuni* was reduced by 86.7% to 100% of the original inoculum on cubes treated with lemon juice, and from 38.2 to 92.3% on cubes not treated with lemon juice six hours after treatment.

The use of acetic acid to inactivate pathogenic bacteria on fresh parsley was studied (Karapinar and Gonul 1992). Populations of *Yersinia enterocolitica* inoculated onto parsley leaves were reduced more than 7 logs after washing for 15 min in solutions of 2% acetic acid or 40% vinegar. Treatment in 5% acetic acid for 30 min did not result in any recovery of aerobic bacteria, while treatment with vinegar gave a 3 to 6 log decrease in aerobic counts, depending upon vinegar concentration and exposure time. Treatment of whole parsley leaves for 5 min at 21°C with vinegar (7.6% acetic acid) reduced populations of *Shigella sonnei* more than 7 log per gram (Wu and others 2000). Vinegar and lemon juice have potential as inexpensive, simple household sanitizers; however, possible negative sensory effects when used on produce would be a disadvantage.

Various combinations of acetic acid, lactic acid and chlorine were observed to reduce populations of *L. monocytogenes* on shredded lettuce (Zhang and Farber 1996). Lactic or acetic acids in combination with 100 ppm chlorine were slightly more antagonistic toward *L. monocytogenes* than either acid or chlorine alone; however, the increased antagonism might be due to an additive effect of the combined compounds or due to an increase in hypochlorous acid at the reduced pH levels of the acid combinations. A 2 min dip in 5% acetic acid at room temperature was the most effective treatment of several treatments investigated for reducing populations of *E. coli* O157:H7 inoculated onto apples surfaces (Wright and others 2000). The 5% acetic acid treatment reduced the population more than 3 log CFU/cm2 as compared to less than a 3 log reduction for a commercial preparation with 80 ppm peroxyacetic acid. It was noteworthy that the 2 min dip treatment with a commercial 0.3% phosphoric acid-based fruit wash caused sublethal injury to *E. coli* O157:H7 as measured by a comparison of counts on selective and nonselective media.

Antimicrobial activity varies among the organic acids. Citric acid was much less effective than tartaric acid in preventing growth of microorganisms on salad vegetables (Shapiro and Holder 1960). A concentration of 1500 ppm citric acid did not affect bacterial growth, while treatment with 1500 ppm tartaric acid resulted in a 10-fold reduction in counts after 4 days at 10 °C (50 °F). Priepke and others (1976) reported that microbial populations of cut lettuce, endive, carrots, celery, radishes, and green onions treated with 2000 ppm sorbate and/or 10,000 ppm ascorbate, then stored 10 days at 4.4 °C (40 °F), were not effectively controlled. Coliforms and fecal coliforms were reduced about 2 and 1 log/g, respectively, on mixed salad vegetables treated with 1% lactic acid

(Torriani and others 1997). In the same study, treatment of the mixed vegetables with a 3% sterile permeate from a culture of *Lactobacillus casei* reduced the total mesophilic count about 5 log/g and prevented growth of coliforms, enterococci, and *Aeromonas hydrophila* after 6 days at 8 °C (46.4 °F).

Orthophosphoric acid with added surfactants is commonly used in the citrus processing industry for both cleaning and sanitizing purposes. Pao and Davis (1999) demonstrated that immersion of oranges in a 200 ppm phosphoric acid/surfactant solution decreased *E. coli* populations only slightly better than immersion in deionized water alone. Winniczuk (1994) determined that dipping oranges for 15 seconds in 500 ppm of a commercial phosphoric acid surfactant solution after brush-washing in water reduced surface populations approximately 85%, as compared to 60% for brush-washing alone.

Alkaline compounds

After a 30-second treatment with 1% trisodium phosphate (TSP) at 10 °C and room temperature, *E. coli* O157:H7 populations were reduced 5 and 6 logs respectively. *Campylobacter jejuni* was almost as sensitive as *E. coli* O157:H7 to TSP (Somers and others 1994). Resistance of *L. monocytogenes* to TSP was also reported, 8% TSP decreased populations of *L. monocytogenes* only 1 log cycle on fresh-cut vegetables (Zhang and Farber 1996). *Salmonella montevideo* populations on the surface of tomatoes were reduced from 5.2 log CFU/cm^2 to nondetectable levels after 15 seconds in 15% TSP. Populations of *S. montevideo* within the core tissue of tomatoes were less affected by TSP, although significant reductions were observed (Zhuang and Beuchat 1996). The numbers of *E. coli* O157:H7 on alfalfa seeds was reduced from 2.5 log CFU/g to nondetectable levels (<0.30 log CFU/g) under a 30-second treatment of 4% TSP

(Taormina and Beuchat 1999). Reductions of *E. coli* on inoculated orange surfaces were not significantly different after immersion in 2% TSP for 8 min as compared to immersion in deionized water (Pao and others 1999). Pao and others also found that various high pH cleaners containing sodium hydroxide, potassium hydroxide, sodium bicarbonate, and/or sodium orthophenylphenate (with or without surfactants) reduced populations of *E. coli* on orange surfaces (Pao and others 2000); reported that high pH waxes used on fresh market citrus provided substantial inactivation of *E. coli* on orange fruit surfaces (Pao and others 1999). Certain alkaline compounds were limited to use on produce because of high pH (11 to 12) of wash solutions and concerns about environmental discharge of phosphates.

Quaternary ammonium compounds

Quaternary ammonium compounds, commonly called "quats", are cationic surfactants which are odorless, colorless, stable at high temperatures, non-corrosive to equipment, nonirritating to skin, and able to penetrate food contact surfaces more readily than other sanitizers (Walker and LaGrange 1991). Due to their high surface-active capability, the mechanism of antimicrobial activity for quats possibly involves a breakdown of the cell membrane/wall complex (Marriott 1999). The antimicrobial activity of quats is greater against the fungi and Gram-positive bacteria such as *L. monocytogenes* than Gram-negative bacteria including coliforms, *Salmonella*, *E. coli*, or pseudomonads.

When quats are applied to most hard surfaces, they form a residual antimicrobial film, which is relatively stable to organic compounds. They are most effective when used at pH 6 to 10. As with iodine compounds, direct food contact would require regulatory

approval and a demonstration that produce treated with quats is safe for consumption (Duran and Marshall 2002). Quats may have some limitation applied to whole produce that must be peeled prior to consumption. Thus they are not approved for direct food contact.

A 500 ppm quat solution treatment for 30-second on oranges reduced *Xanthomonas campestris* pv. *vesicatoria* as effectively as 150 to 250 ppm chlorine for 2 min (Brown and Schubert 1987). The surface microflora of oranges which were brushwashed in water and dipped in 200 ppm quat for 15 seconds was reduced about 95% compared to 60% for washed oranges dipped in plain water (Winniczuk 1994).

Physical removal of microorganisms

Physical removal of soil and microorganisms is often started with a detergent treatment followed by a rinse of potable water. Brushing also removes a portion of the natural waxy cuticle on the surface that acts as a barrier to microorganisms. Commercial waxes are occasionally added to the produce surface after washing to replace the natural waxes that are removed. It is relevant to comment that microorganisms could become enmeshed within waxy materials on produce making their removal more difficult (Kenney and others 2001; Pao and others 1999). Washing efficiency varies with the different commodity, washing system, soil, contact time, detergent, and water temperature. It was reported that with chemical sanitizers, simple rinsing of produce in plain water reduces the surface populations although the reduction is usually well less than 1 log. Brush-washing of oranges in plain water reduced the surface microbial population approximately 60 to 70% compared to 90% reduction when a sanitizer was included (Winniczuk 1994).

Ultrasound

Effective washing and decontamination of fruits and vegetables is difficult because attached or entrapped bacteria are not readily accessible to disinfectant. Since the surfaces of raw produce may provide additional protection against contact with chemical antimicrobials (Simons and Sanguansri 1997), investigation of ultrasound has recently been proposed for preservation purposes in the food industry (Piyasena and others 2003).

Ultrasound, in its most basic definition, refers to pressure waves with a frequency of 20 kHz or more (Brondum and others 1998; Butz and Tauscher 2002). Generally, ultrasound equipment uses frequencies from 20 kHz to 10 MHz. The mechanism of microbial inactivation by ultrasonication is mainly due to thinning of cell membranes, localized heating, production of free radicals (e.g., ·OH, HOO·, and O·) (Butz and Tauscher 2002; Fellows 2000) and formation of hydrogen peroxide (Weissler 1959). During the sonication process, longitudinal waves are created when a sonic wave meets a liquid medium, thereby creating regions of alternating compression and expansion. These regions of pressure change cause cavitation to occur, and gas bubbles are formed in the medium (Sala and others 1995). The growth, collapse and oscillation of these bubbles generate the mechanical energy which has a 'cleaning' action on surfaces (Kinsloe and others 1954; Roberts 1991; Scherba and others 1991; Schett-Abraham and others 1992; Earnshaw and others 1995; Sala and others 1995; Raso and others 1998).

Using ultrasonication to separate *E. coli* from suspensions has been explored (Miles and others 1995). The mechanism of separation is not well established (Miles and others 1995). Coakley and others (1989) regarded the net force on a particle in a

stationary acoustic field to be composed of three components: a radiation force, a viscous force derived from acoustic streaming and a vertical gravitational force. The forces depend on some or all of the following properties (i.e. particle size and shape, sound frequency, the square of the sound pressure amplitude) and on the differences between the density and compressibility of the particles and those of the suspending phase. According to these authors, when particles are levitated into bands, these three forces must be balanced, and the net force is zero. By manipulating the frequency and intensity of applied ultrasound, these forces could be balanced so that particles or bacteria clump together could be separated by different densities and compressibilities in the sound field or to support cell away from solid surfaces and settle in a band near half wavelength of the ultrasound used.

The use of ultrasound to promote decontamination of raw vegetables has been described (Seymour and others 2002). The cleaning action of cavitation appeared to remove *Salmonella typhimurium* cells, rendering the pathogen more susceptible to chlorine. The combined effects of chemical, heat and ultrasound treatments in killing or removing *Salmonella* and *E. coli* O157:H7 on alfalfa seed has confirmed the hypothesis that combined stresses and enhanced exposure of cells to chemicals would result in higher lethality (Scouten and Beuchat 2002). A combination of sodium hypochlorite, copper ion, and sonication treatment to reduce populations of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on apples and in apple cider has been studied (Rodgers and Ryser 2004). However, sonication of copper ion with sodium hypochlorite solution at 22 to 44 kHz did not further improve pathogen reduction on apples.

Briefly, it is broadly accepted that ultrasound alone is not effective in inactivating bacteria on food (Piyasena and others 2003). Non-thermal decontamination methods combined with mechanical (i.e. ultrasonic) treatments may be relatively more effective for pathogen removal and inactivation. Also, these combination treatments help retain vitamins and other heat-sensitive ingredients.

MATERIALS AND METHODS

Bacterial strains

Three strains of *E. coli* O157:H7 (*204P, 301C, 505B*) with nalidixic acid and novobiocin resistance (obtained from Dr. Michael Doyle, Center for Food Safety, University of Georgia, Griffin, Georgia; adapted in Dr. D. E. Conner's laboratory, Poultry Science Department, Auburn University), and three strains of *Salmonella enterica*, serotypes Enteritidis, Typhimurim, and Mission with nalidixic acid resistant (obtained from N. A. Cox, Athens, GA) were used in this study. Bacteria were stored at –80°C in Trypticase® soy broth (TSB, Becton, Dickinson and Company Sparks, MD) containing 10% dimethyl sulfoxide (DMSO) until needed. Each strain of *E. coli* O157:H7 and *Salmonella* was subcultured at two successive 24-h intervals at 37°C in TSB (15 ml), and then was then streaked on master plates with Trypticase® soy agar (TSA, Becton, Dickinson and Company, Sparks, MD) plates. The plates were kept at 4°C for use.

Preparation of inocula

Each strain of *E. coli* O157:H7 (*204P, 301C, 505B*) or *Salmonella enterica*, serotypes Enteritidis, Typhimurim, and Mission was transferred to TSB and the cultures were incubated in a reciprocal shaking water bath (VWR International, Inc., West Chester, PA) at 37°C with 100 rpm overnight. Equal volumes of three cultures containing

10^8~10^9 CFU/ml, respectively, were combined to produce three-strain cocktails of *E. coli* O157:H7 or *Salmonella*. The cocktails were then washed with Butterfield's Phosphate Buffer (BPB) twice and centrifuged at 3,500g for 10 min, which was re-suspended in BPB to original volume. The optical density (OD) of the suspension was read with a spectrophotometer (DU7400 Beckman Coulter Inc. Fullerton, CA) at A_{640nm} and used to estimate the bacterial concentration compared to an established standard curve. Then the bacterial suspension was diluted to the designated working concentrations of ~ 10^7 CFU/ml (high level) or ~ 10^4 CFU/ml (low level).

Sample preparation

Chicken breasts were ordered from a local supermarket, and cut in 25 g cubes and kept at 4 °C before use. Unwaxed Gala apples (*Royal Gala*) were purchased from a local farm and stored in a cool room before use. Romaine lettuce (*Lactuca sativa*) was grown in Auburn University green house.

Sample inoculation and drying

Dip inoculation

Eight liters of 10^7 cfu/ml or 10^4 cfu/ml inoculum were prepared by washing the cell suspension as described above. The 8 L inoculum was transferred into a 20 L stainless steel container. Chicken breasts and lettuce were dipped into the bacteria solution with gentle agitation for 2 min, and then were drained to remove excess bacterial solution.

Spot inoculation

Apples were placed stem end up on aluminum foil inside a biosafety hood (Forma Scientific, Inc., San Diego, CA) and each apples was inoculated by spreading 25 µl of bacterial suspension at 10^8 CFU/ml or 10^5 CFU/ml on the surface of the stem end.

After inoculation, chicken breasts, lettuce, and apples were held in a biosafety hood to allow the inoculum to dry at static condition. Chicken breasts and lettuce were hooked by paper clips and hung on a metal bar for drying 10 min and 30 min, respectively. The apples were dried for 40 min.

Preparation of chlorine dioxide stock and working solutions

Deionized water, produced from a water purification units (US Filter D-56235 Ransbach-Baumbach, Germany), was used for the preparation of ClO_2 stock and working solutions. Two methods were used to generate chlorine dioxide stock solution.

In the first method, ClO_2 solutions were produced from Z-Series 200-S (ICA TriNova in Forest Park, Georgia). The Z-Series 200-S is designed for aqueous ClO_2 applications and ClO_2 was made on site. The chemical and activator were packed inside the same sachet and separated without contact. The ClO_2 stock solution was prepared by unrolling the Z-Series 200-S sachet and shaking thoroughly to mix the media for 5 sec, it was then submerged into 2 L of deionized water for 12 h of stored in the dark with aluminum foil wrapped. In the second method, 150 ml of Zep Dominion Sanitizer (Zep Manufacturing Company Inc™, Atlanta, GA) was mixed with 24 ml of Zep Dominion Activator (Zep Manufacturing Company Inc™, Atlanta, GA) for 5 min in an aluminum

foil covered flask after 5 min 1000 ml deionized water was added. The reaction solution contained around 2500 ppm total available ClO_2.

For the first method, ClO_2 concentrations were determined by iodometric and DPD methods (APHA 1989); for the second one, only the DPD method was to determine ClO_2 concentrations. Various working solutions (5, 10, 20, 40 ppm) were prepared from the stock solution. Both the ClO_2 stock and working solutions were freshly prepared on the day of the experiment.

Determination of ClO_2 concentrations in solutions

The iodometric method was used to determine the total available chlorine (TAC), which is the sum of free available chlorine (FAC) and combined available chlorine (CAC) (Jolley and Carpenter 1983). The CAC is the chlorine present as NH_2Cl, $NHCl_2$, and organic N-chloro compounds, and FAC is the chlorine present as hypochlorous acid and hypochlorite ion. The DPD titration method using ferrous ammonium sulfate was employed to differentiate individual chlorine species, including ClO_2, free chlorine, monochloramine and dichloramine, and chlorite (Jolley and Carpenter 1983; APHA, 1989). Since the optimal range of DPD titration was 0.5–5 ppm TAC, iodometric titration was conducted first to estimate TAC in test solutions. After the solutions were diluted to contain 5 ppm TAC, DPD titration was conducted.

Iodometric titration

The titration was performed away from direct sunlight. In a flask, an aliquot of chlorinating solution was mixed with excessive potassium iodide (KI, Fisher Scientific), which had been acidified by reacting with 5 ml of glacial acetic acid. The iodine liberated from KI, which was quantitatively proportional to the amount of chlorine present, was

then titrated with 0.01 N sodium thiosulfate ($Na_2S_2O_3$, Fisher Scientific) until the yellow color of the liberated iodine was almost discharged. Then 1 ml of 0.5% starch solution was added, and titration with $Na_2S_2O_3$ was continued until the blue color discharged. A blank titration was performed also. An aliquot of distilled water corresponding to the sample used for titration in a flask conducted same as above steps.

The TAC was calculated as following formula:

TAC (µg/ml , ppm) = (A±B) x N x 35450/ volume of sample (ml)

Where:

A = ml of titration sample

B = ml of titration blank (positive or negative), and

N = normality of $Na_2S_2O_3$

DPD method

The DPD indicator solution was prepared by dissolving 1.1 gram anhydrous DPD sulfate (Sigma Chemical Co.) in 1 L deionized water containing 8 ml 1 : 3 H_2SO_4 and 200 mg disodium ethylenediamine tetraacetate (EDTA, Fisher Scientific). The DPD indicator solution was stored in a brown glass-stoppered bottle at room temperature. Disodium EDTA enhances the stability of DPD solution by retarding deterioration due to oxidation. Furthermore, EDTA suppresses dissolved oxygen errors by preventing trace metal catalysis (APHA 1989).

The phosphate buffer solution was prepared by dissolving 24 g sodium phosphate dibasic anhydrous (Na_2HPO_4, Fisher Scientific) and 46 g potassium phosphate monobasic anhydrous (KH_2PO_4, Fisher Scientific) in deionized water, combining with 100 ml distilled water in which 800 mg disodium EDTA have been dissolved, and diluting to 1 L

with distilled water and adding 20 mg mercuric chloride ($HgCl_2$, Sigma Chemical Co.) to prevent mold growth and interference in the free chlorine test caused by any trace amounts of iodide in the reagents.

The standard ferrous ammonium sulfate (FAS) titrant was prepared by dissolving 1.106 g ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2.6H_2O$, Sigma Chemical Co.) in deionized water containing 1 ml 1 : 3 H_2SO_4 and made up to 1 L with freshly boiled and cooled distilled water. The FAS titrant is equivalent to 100 µg Cl as Cl_2/1.00 ml.

To quantify the chlorine dioxide content in the sample, 2 ml of 10% glycine (Acros Organics, New Jersey) solution was added to 100 ml of sample and mixed. Five ml each of a phosphate buffer reagent (pH 6.2 – 6.5) and DPD indicator solution as well as 200 mg disodium EDTA were mixed in a separate titration flask, and then the glycine-treated sample was added and mixed. The mixture was titrated rapidly with standard FAS titrant to a colorless endpoint (Reading G). The presence of glycine allowed instantaneous conversion of free chlorine into chloroaminoacetic acid. Therefore, only ClO_2 was left for reaction with DPD reagent. However, since ClO_2 was reduced to chlorite ion instead of chlorite at neutral pH, the titration represented only one-fifth of its potential oxidizing power. The concentration of ClO_2 (µg/ml as Cl_2) contained in this diluted sample was therefore 5G.

The ClO_2 concentration was calculated as the following formula:

ClO_2 (µg/ml or ppm) = 5G (or 1.9G when expressed as µg/ml ClO_2)

Chlorine dioxide treatment on chicken breasts, lettuce, and apples

Triplicate inoculated sample portions were each treated for 1, 3, 6 and 10 min with ClO_2 (0, 5, 10, 20 and 40 ppm) with gently stirring at 3L solution. Un-inoculated

and inoculated controls were used as non-treated sample portions. After draining excess liquid, all treated samples were placed in sterile Whirl-Pak® bags (Nasco, Fort Atkinson, WI) and placed on an automatic diluter (Dilumat 3, AES Laboratories, Combourg, France) for a 1:10 dilution using BPB buffer (pH = 7.0 ~ 7.2). The bag was then agitated for 2 min at high speed using a stomacher (Stomacher 400, Seward Inc., London, England). Twenty five grams cubes of chicken breast was used in this study, and the stem scar area of treated apples were cut and placed into a sterile stomacher bag for further processing. For treated lettuce, each leaf was folded twice and placed into a sterile stomacher bag.

Combination of ultrasound and ClO_2 treatment on chicken breasts, lettuce, and apples

The whole process of ultrasound and ClO_2 treatment on chicken breasts, lettuce, and apples are described in Fig. 1. In brief, the inoculated samples were loaded into the 8-liter rectangular stainless steel containers containing 3 liters of aqueous chlorine dioxide to cover the samples completely. The containers were mounted with six transducers on the long-sides of the container to generate the ultrasonic waves. Each concentration of ClO_2 (0, 5, 10, 20 and 40 ppm) was combined with different frequencies of ultrasound (120 kHz, 170 kHz) treated for 1, 3, 6 and 10 min. The following processing was the same as above described.

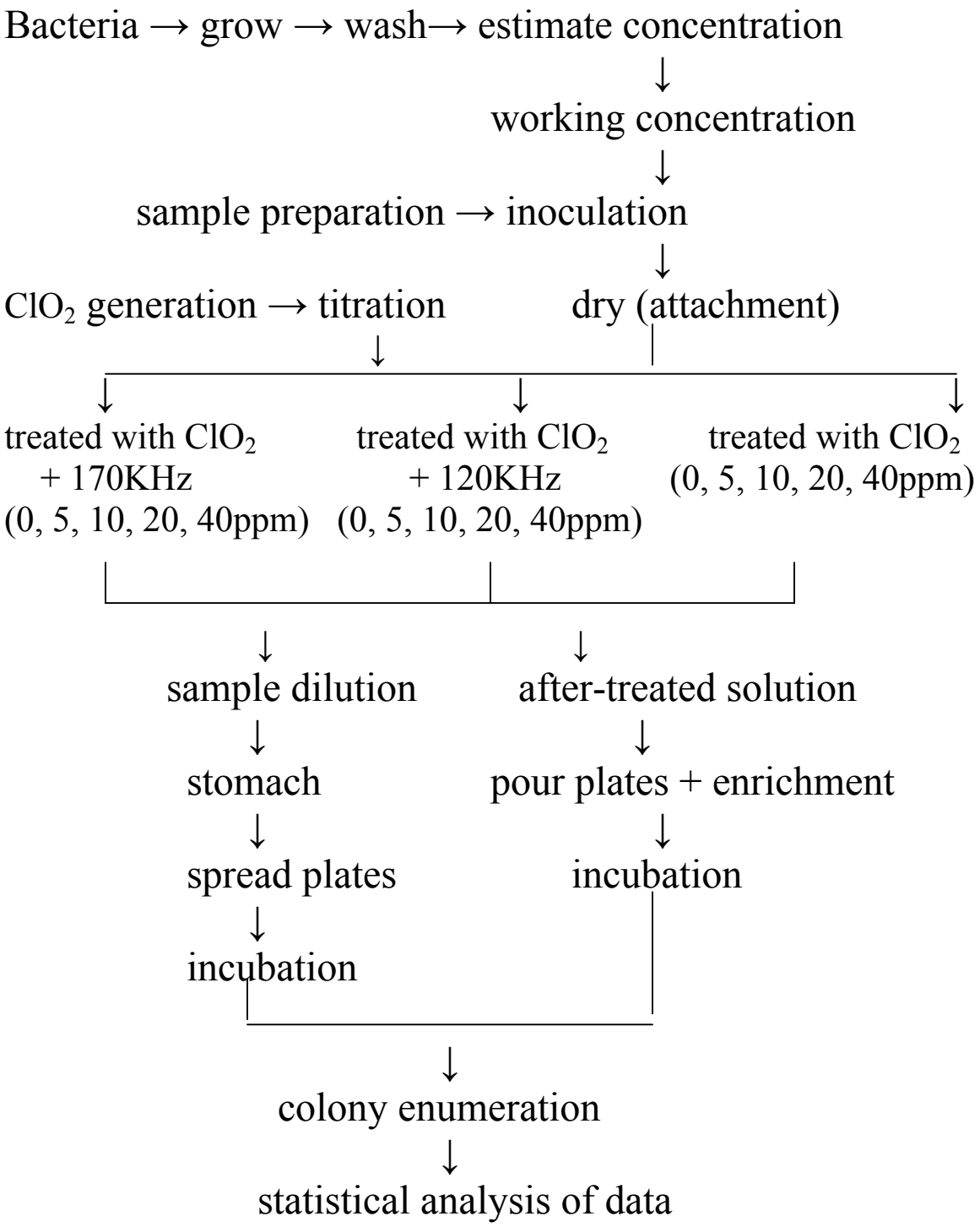

Figure 1-Flow chart for the study of decontamination of chicken breasts, lettuce and apples by ClO_2 and ultrasound.

Microbiological analyses

Populations (CFU/ml) of test pathogens in inocula with multi-strains were determined by colony count. After homogenizing in a stomacher for 2 min, triplicate samples, diluent was serially diluted in BPB buffer and 0.1 ml surface plated in duplicate on TSA with antibiotics. The medium for *E. coli* O157:H7 was TSA supplemented with 100 µg/ml nalidixic acid (Acros Organics, New Jersey) and 10 µg/ml novobiocin (Sigma-Aldrich Co.). The medium for *Salmonella* spp. was TSA supplemented with 100 µg/ml nalidixic acid. After the sample treatment, pathogens in the solution were detected by pour plates techniques or by enrichment streak plates. The pre-enriched broth was half strength TSB, and the selective enrichment broth for *E. coli* O157:H7 was EZ® coli enrichment broth, and the selective enrichment broth for *Salmonella* spp. was Salmosyst® broth base with Salmosyst® tablets (E M Science). Twenty-five ml of the treated solution was adding to 225 ml of half strength TSB, the pre-enrichment broth wax incubated at 37°C of 100 rpm for 6 h in a water-bath. Then a small aliquote was transferred to selective enrichment broth, and was incubated at 37°C at 100 rpm for 13 h (*E. coli* and *Salmonella*) in a water-bath. After incubation all samples were streaked onto antibiotic containing TSA agar plates for each bacterial recovery. Bacterial colonies on plates were counted after incubation at 37°C for 18-24 h, and the data were recorded.

Chlorine dioxide temperature record and residual detection

The temperature was monitored in various combination treatments (Table 1). Each of the treatments for chicken breasts, lettuce and apples was applied three times respectively with duplicate detection.

Table 1-Various combination treatments for temperature detection.

Treatment	Ultrasound (kHz)	Time (min)
1	0	1
2		3
3		6
4		10
5	120	1
6		3
7		6
8		10
9	170	1
10		3
11		6
12		10

Full combinations of different ClO_2 concentrations and ultrasound frequencies were performed for residual detection in different time points as seen in Table 2. Each of the treatments for chicken breasts, lettuce and apples was applied three times respectively with duplicate titration. The DPD method was used for ClO_2 residual detection.

Table 2-Various combination treatments for ClO_2 residual detection.

Treatment	ClO2 (ppm)	Ultrasound (kHz)	Time (min)
1	5	0	1
2			3
3			6
4			10
5		120	1
6			3
7			6
8			10
9		170	1
10			3
11			6
12			10
13	10	0	1
14			3
15			6
16			10
17		120	1
18			3
19			6
20			10
21		170	1
22			3
23			6
24			10
25	20	0	1
26			3
27			6
28			10
29		120	1
30			3
31			6
32			10
33		170	1
34			3
35			6
36			10
37	40	0	1
38			3
39			6
40			10
41		120	1
42			3
43			6
44			10
45		170	1
46			3
47			6
48			10

Statistical analysis

Two trials were performed for each experiment, and triplicate experiments for each set of treatments were conducted. All statistical analyses were performed with the Statistica 7.0 software package (StatSoft, In., Tulsa, OK). Data of bacterial colony forming units (CFU) /gram were Log_{10} transformed. All the mean data were evaluated by mean ± standard deviation (SD) or mean ± 95% confidence interval (CI), and comparisons of means between different experimental treatments were performed by the Tukey honest significant difference (HSD) test. The plots for individual analysis were done by SigmaPlot 8.0 software package (SPSS Inc. Chicago, Illinois).

The general strategy for the multivariate analysis of several factors on the Log reduction of bacteria is: the analysis with possibly two-way, three-way, four-way and five-way analysis relating the factors of time, initial ClO_2 concentration, ultrasound frequency, high or low inoculum, and species of bacteria first, followed by main factors analysis were performed.

RESULTS AND DISCUSSIONS

In this study, four series of experiments were conducted. In the first three series of experiments, the efficacies of ClO_2 treatments combined with ultrasound to decontaminate bacteria on lettuce, chicken breasts, and apples were measured, respectively. The last study was to evaluate the effect of different ClO_2 concentrations, treatment times, and ultrasonication on ClO_2 residuals and the temperature change.

Lettuce treated with ClO_2 and ultrasonication

In the first series of studies, the efficacies of ClO_2 treatments at different concentrations alone or combined with ultrasound at 120 kHz or 170 kHz in the decontamination of *E. coli* O157:H7 or *Salmonella* inoculated lettuce were investigated. The results of the decontamination for *E. coli* O157:H7 on lettuce are shown in Figures 2-7. The log reductions of *E. coli* O157:H7 increased from 1 to 10 min of treatment and ClO_2 concentrations from 0 to 40 ppm. The log reductions between high and low inoculums of *E. coli* O157:H7 on lettuce showed significant differences. The lethality of *E. coli* O157:H7 on low inoculum treatments was significantly higher than those in high inoculum treatments, and it was about 0.6 log difference. When comparing the ClO_2 treatments alone to ClO_2 combined with ultrasound treatments, the results showed that ultrasonication did not have a synergistic effect on *E. coli* O157:H7 reduction in either

high or low inoculum treatments. The most effective treatment was 40 ppm ClO_2 combined with 120 kHz ultrasonication for 6 min with low inoculum, which resulted in a 2.4 log reduction (Figure 5).

In the decontamination of *Salmonella* on inoculated lettuce, log reductions were similar to those for the decontamination of *E. coli* O157:H7 in different treatments (Figures 8-13). With the high inoculum of *Salmonella* inoculated lettuce, the log reduction for ClO_2 combined with ultrasonication was significantly higher than that of the ClO_2 treatment alone (Figures 8, 10, 12). However, similar results of the same treatments were not shown on the low inoculum of *Salmonella* (Figures 9, 11, 13) and high or low inoculum of *E. coli* O157:H7 inoculated lettuce. The most effective treatment was at 40 ppm ClO_2 combined with 120 kHz ultrasonication for 6 min on high inoculum, which was 2.7 log reductions (Figure 10).

The decontamination effectiveness of *E. coli* O157:H7 and *Salmonella* on lettuce and chicken breasts by chlorine dioxide concentrations, treatment time, and ultrasonication are summarized in Figures 14-16. The bacterial removal and inactivation of *E. coli* or *Salmonella* on lettuce mainly depended on ClO_2 concentration rather than on treatment time and ultrasonic frequency. Various ClO_2 concentrations caused significant difference in log reductions on both the high and low inocula. The water treatments resulted in a 1.1 log reduction in both *E. coli* O157:H7 or *Salmonella* inoculated lettuce of high or low inocula (Figures 2-13).

Chicken breasts treated with ClO_2 and ultrasonication

The second series of studies were done to understand the efficacies of ClO_2 treatments alone or ClO_2 combined with ultrasound treatment on *E. coli* O157:H7 or *Salmonella* inoculated chicken breasts. The results of decontaminating *E. coli* O157:H7 on inoculated chicken breasts are shown in Figures 17-22. The increased log reductions with higher ClO_2 concentrations and longer times were shown only for ClO_2 treatments alone (Figures 17-18). However, the log reductions in the groups of ClO_2 combined with ultrasonication treatments were lower than those in ClO_2 treatments alone (Figures 19-22). The chlorine dioxide treatments combined with 170 kHz ultrasonication resulted in lower log reductions than when combined with 120 kHz ultrasonication.

Similar results were attained when chicken breasts were inoculated with *Salmonella* (Figures 23-28). For the chlorine dioxide treatments alone, the highest log reduction of approximately 0.9 was noted for 40 ppm of ClO_2 for the 6 min treatment with the high inoculum of *Salmonella* inoculated chicken breasts. While, the highest log reduction was about 0.6 in the low inoculum samples at 40 ppm ClO_2 for the 10 min treatment. The ClO_2 treatments combined with ultrasonication did not cause a significant increase in log reductions for *Salmonella* on the high inoculum samples (Figures 25 & 27). However, a significant decrease in log reductions was observed on the low inoculum of *Salmonella* inoculated chicken breasts (Figures 26 & 28).

Based on main factor analysis (Figures 14-16), the efficacies of removing and inactivating *E. coli* or *Salmonella* on chicken breasts mainly depended on the treatment time, not on the ClO_2 concentration and ultrasonication. A longer treatment time resulted in significantly higher log reductions on both high and low inocula samples, especially

for *Salmonella*. Treatments of various ClO_2 concentrations did not result in significant log reductions in either high or low inocula chicken breasts. The log reductions of water treatments were around 0.3 on both *E. coli* O157:H7 or *Salmonella* inoculated chicken breasts.

Salmonella and *E. coli* O157:H7 are two important foodborne pathogens in poultry. Lillard (1988a) found that bacteria are firmly attached to or entrapped in chicken skin or muscle, even when broilers first arrive at the processing plant (Lillard 1989a; 1990). Attached or entrapped bacteria are difficult to remove during processing and do not seem easily accessible to bactericides (Lillard 1989b). After 40 consecutive whole carcass rinses, levels of bacteria recovered were still within 1 log of those recovered by the first rinse, indicating that a gradual shedding of bacteria from the surface does occur during rinsing (Lillard 1988b; 1989b). Lillard (1989b) and James and others (1992) confirmed that bactericides are lethal to *Salmonella* in processing water but do not access the bacteria which are firmly attached or entrapped on the surface of meat. Similar results were found in this study with limited log reductions of *E. coli* O157:H7 and *Salmonella* on chicken breasts

Sams and Feria (1991) reported that no decrease in total aerobic bacterial counts was found for drumsticks which were sonicated with and without heat. It was explained that the irregular or rough surface might provide higher levels of physical protection for bacteria against cavitation. Stumpf and others (1946) and Miller (1982) observed that ultrasonic waves are transmitted most efficiently over flat surfaces; irregular surfaces reflect or refract the waves, creating stationary waves, which greatly reduced cavitation.

Results from our experiments suggested that this may be the reason why ultrasonication had no synergistic effect with ClO_2 on bacterial decontamination.

Comparison of chicken breasts and lettuce treated with ClO_2 and ultrasonication

When comparing the log reductions of the water control groups on chicken breasts and lettuce, the decontamination by the water treatments on lettuce was significantly more effective than on the chicken breasts. This indicates that the surface of lettuce is different from the surface of chicken breasts. Bacteria attached or entrapped on the surface of chicken breasts were not easily removed or inactivated by disinfectant liquids; while, the bacteria on lettuce were easily removed and inactivated by disinfectant liquids.

No significant differences were found between *E. coli* O157:H7 and *Salmonella* on chicken breasts. However, on the lettuce surface, *Salmonella* was more sensitive to chlorine dioxide alone or when combined with ultrasonic treatments when compared to *E. coli* O157:H7. Therefore, it was concluded that the sensitivity of the *E. coli* O157:H7 and *Salmonella* was different to the treatments on the surfaces of the lettuce, but no differences were observed on the surface of the chicken breasts.

The decontamination effectiveness was different using the same treatments with high and low *E. coli* O157:H7 inoculated chicken breasts or lettuce. There is a significantly higher log reduction in the high inoculum of *E. coli* O157:H7 on chicken breasts when compared to the low inoculum with the same treatment; However, the opposite was found with lettuce. The log reduction for the *E. coli* O157:H7 low inoculum sample was significantly higher than for the high inoculum on the lettuce. The

decontamination effectiveness of *Salmonella* on high and low inoculum chicken breasts or lettuce was similar to each other under the same treatment conditions.

Investigating the role of ultrasonication combined with chlorine dioxide treatments in the decontamination of *E. coli* O157:H7 and *Salmonella* on chicken breasts and lettuce, showed opposite results. Ultrasonication has an adverse effect on the decontamination of chicken breasts by ClO_2 treatments, but has a synergistic effect on lettuce especially in removing *Salmonella* on lettuce.

Bacterial attachment is affected by many factors, such as the chemical and physical properties of the microorganism, substratum surfaces, and the composition of the surrounding medium (Dalton and March 1998, Gilbert et al. 1991). Population density, stress or nutrient limitations as external stimuli are responsible for bacterial surface protein expression and extracellular polymeric substance production (Dalton and March 1998, Gilbert et al. 1991, Pratt and Kolter 1998). A critical surface tension value promotes bacterial adhesion. Maximal attachment of bacterial cells depends upon the high free surface energy or the wettability of a surface. Surface roughness and defects are also associated with increase in bacterial attachment (Bos et al., 2000; Bower et al., 1996; Butler et al., 1979).

Apples treated with ClO_2 and ultrasonication

The third series of studies was undertaken to compare the efficacies of ClO_2 treatment and ClO_2 combined with 170 kHz ultrasonic treatment on *E. coli* O157:H7 or *Salmonella* inoculated apples. The results of decontamination are shown in Figures 29 and 30 for *E. coli* O157:H7 and in Figures 31 and 32 for *Salmonella*.

The treatment results for apples were different from those seen on chicken breasts or lettuce. For apples, a low concentration of chlorine dioxide (5 ppm) was enough to kill 99 % of *E. coli* O157:H7 and *Salmonella* attached to apples (Figures 29-33). A significant difference was observed between ClO_2 treatments combined with ultrasound and ClO_2 treatments alone for *Salmonella* attached on apples, but this result wasn't seen for *E. coli* O157:H7 inoculated apples (Figure 34). Log reductions increased with longer treatment times for both bacteria on apples, but no significant differences were observed (Figure 35). The 10 min, 5 ppm ClO_2 treatment combined with 170 kHz ultrasonication on apples inoculated with *E. coli* O157:H7, was the most effective with a 2.4 log reduction (Figure 30); for *Salmonella*, the most effective treatment was observed at 10 min, 40 ppm ClO_2 treatment combined with 170 kHz ultrasonication, which resulted in a 3.3 log reduction (Figure 32). For the water treatment, around 0.5 log reduction was observed for both *E. coli* O157:H7 and *Salmonella* inoculated on apples (Figures 29-33).

Based on the main factor analysis (Figures 33-35), *Salmonella* was more sensitive to ClO_2 alone or combined with ultrasonic treatments than *E. coli* O157:H7 on apples. In comparing the log reductions of water control groups on chicken breasts, lettuce and apples, the bacteria adhesion appeared to be stronger on apples than lettuce, but less than chicken breasts. In this study, the inoculated area on the apple was stem scar, which is difficult to expose to washing water or disinfectant. Combination treatments with ultrasound had the synergistic effect, which helped ClO_2 reach the inoculated area, detach the bacteria into liquid, and inactivate the bacteria. In apples, the efficacies of bacterial removal and inactivating *Salmonella* on apples mostly depended on the ClO_2

concentration and ultrasound, not treatment time. *E. coli* O157:H7 mainly depended on the ClO_2 concentration, not treatment time and ultrasound treatments.

Chlrine dioxide residual after ultrasound treatment

The fourth series of studies was conducted to monitor the change of ClO_2 concentration and temperature in the treatments of ClO_2 only or ClO_2 combined with ultrasonication on chicken breasts, lettuce and apples. The results of ClO_2 residual and temperature change after treatments are shown in Figures 36-44 and Figures 48-50, respectively.

The ClO_2 residuals dropped dramatically with the increase of treatment time on chicken breasts, but it only dropped slightly on the lettuce and apples (Figures 36, 39, 42). The trends of chlorine dioxide residual declines were similar between lettuce and apples under the same treatments (Figures. 39 & 42). This suggests that ClO_2 probably reacts more with the chicken components than with the vegetable samples of lettuce and apples.

When comparing the ClO_2 residuals under treatment of ClO_2 only or ClO_2 combined with 120 kHz or 170 kHz ultrasonication on chicken breasts, the ClO_2 residual decreased more dramatically in the combination treatment than in the ClO_2 treatment alone (Figures 36-38). However, there was no significant difference between these two treatments on lettuce (Figures 39-41) or apples (Figures 42-44).

The results for the main factor analysis are seen in Figures 45-47. With the increasing ClO_2 concentrations, the ClO_2 residuals increased in all three samples (Figure 45). The application of ultrasonication resulted in significantly lower ClO_2 residuals only in chicken, but not in lettuce and apples (Figure 46). Three samples had similar declining

trends of ClO_2 residual when treatment times were increased; however, the ClO_2 residual decreased significantly on chicken compared to lettuce and apples (Figure 47). Therefore, ultrasonication appeared to accelerate the reaction of chlorine dioxide with chicken components but not with lettuce and apples, or the decomposition of chlorine dioxide.

Temperature change after ultrasound treatment

Temperature changes were monitored under different treatments on various samples of chicken breasts, lettuce, and apples. The temperature increased to almost 60°C after the application of ultrasound, and the temperature changes were similar for different samples with the same treatments. Temperature increased faster when the ClO_2 treatment was combined with ultrasound when compared to the ClO_2 treatment alone (Figures48-50).

As in the Arrhenius equation, the rate of a chemical reaction is seriously affected by reaction temperature. The rate of reaction at a 10°C higher temperature can be expected to be twice as fast as that at a primary temperature. The ultrasonic treatment induced significantly higher temperatures, thus the reaction rate of chlorine dioxide with organic matter of chicken breasts may increase several times faster than that at a primary temperature. So the ClO_2 in chicken breasts treated by combination treatments quickly disappeared by reacting with organic matter or being decomposed under high temperatures.

Chlorine dioxide is soluble in water but remains as a dissolved gas, which can be removed from dilute aqueous solutions by aeration or by heat. Chlorine dioxide can be decomposed to chlorite, chlorate and chloride in water, and it is decomposed faster in the

presence of light, or at temperatures greater than 50°C; this is the reason that on-site ClO_2 generation is recommended (Gates and Harrington, 1995).

By manipulating the frequency and intensity of applied ultrasound, bacterial clumps could be separated by different densities and compressibilities in the sound field, or put cells away from solid surfaces and settle in a band near half the wavelength of the ultrasound used (Miles et al., 1995; Coakley et al. 1989). Bacteria detached from the surfaces of meat, vegetables and fruits by cavitations will be released into the wash water or the bacterial clumps will be separated and result in higher population than those without ultrasonication. In the treatment with chlorine dioxide combined with ultrasonication, cavitations helped to detach cells from surfaces and helped to separate bacterial clumps or particles from surface, which are more susceptible to the sanitizers resulting in high lethality of bacteria.

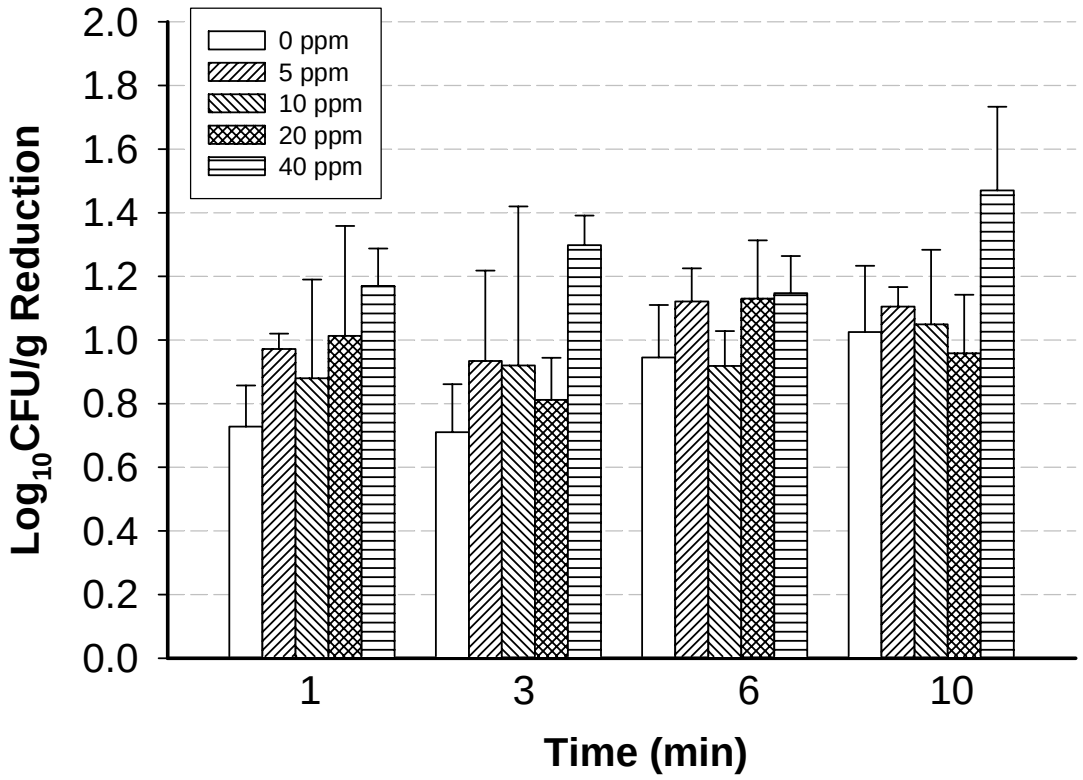

Figure 2-Log reduction of *E. coli* O157:H7 on high inoculum (10^7 CFU/ml) lettuce treated by various concentrations and times of ClO_2. Data are shown as means of log reduction ± SD.

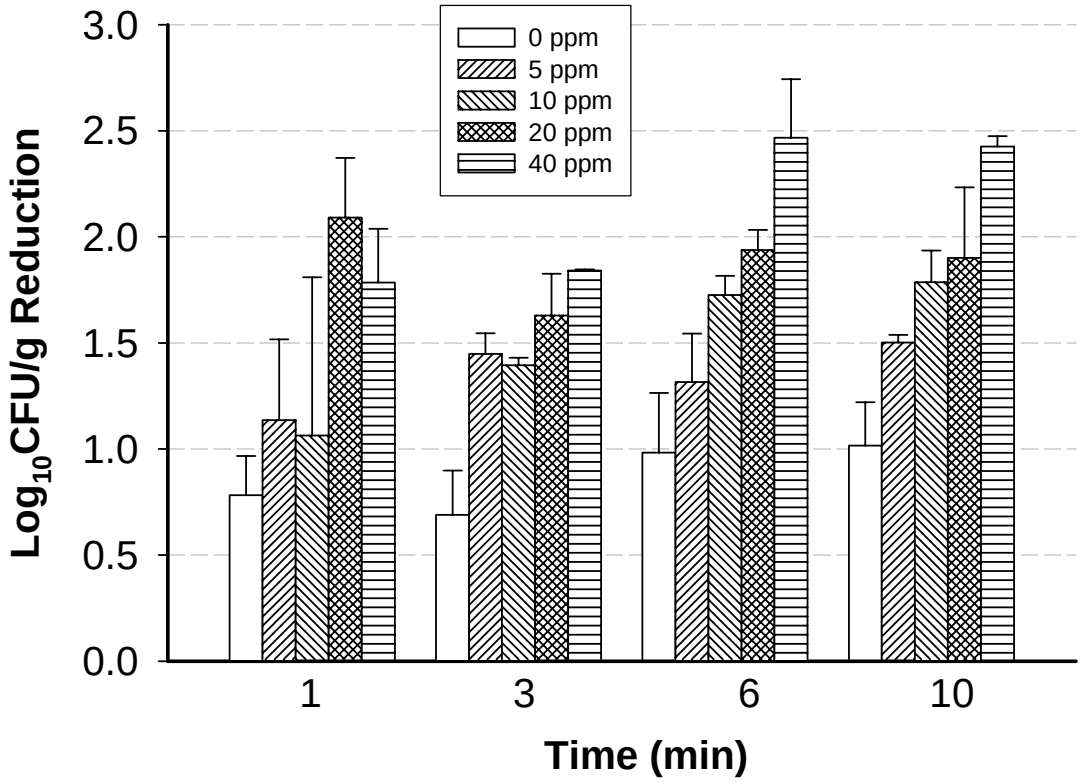

Figure 3-Log reduction of *E. coli* O157:H7 on low inoculum (10^4 CFU/ml) lettuce treated by various concentrations and times of ClO_2. Data are shown as means of log reduction ± SD.

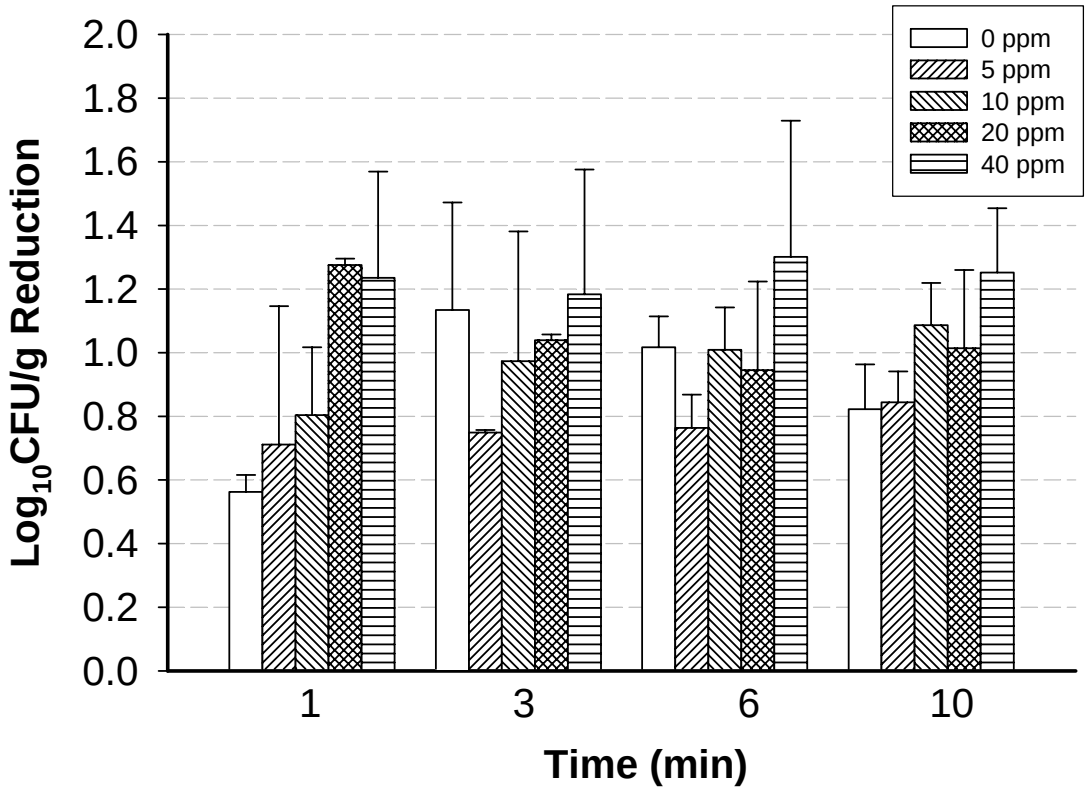

Figure 4-Log reduction of *E. coli* O157:H7 on high inoculum (10^7 CFU/ml) lettuce treated by various concentrations and times of ClO_2 combined with ultrasound 120 kHz. Data are shown as means of log reduction ± SD.

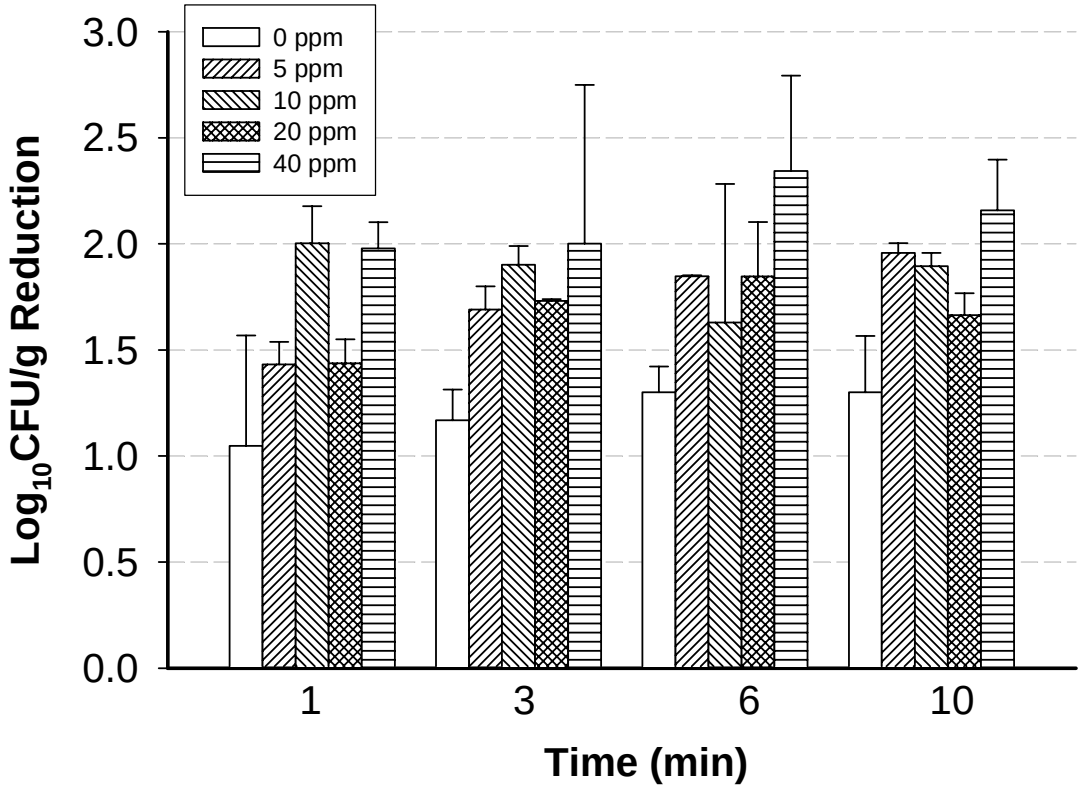

Figure 5-Log reduction of *E. coli* O157:H7 on low inoculum (10^4 CFU/ml) lettuce treated by various concentrations and times of ClO_2 combined with ultrasound 120 kHz. Data are shown as means of log reduction ± SD.

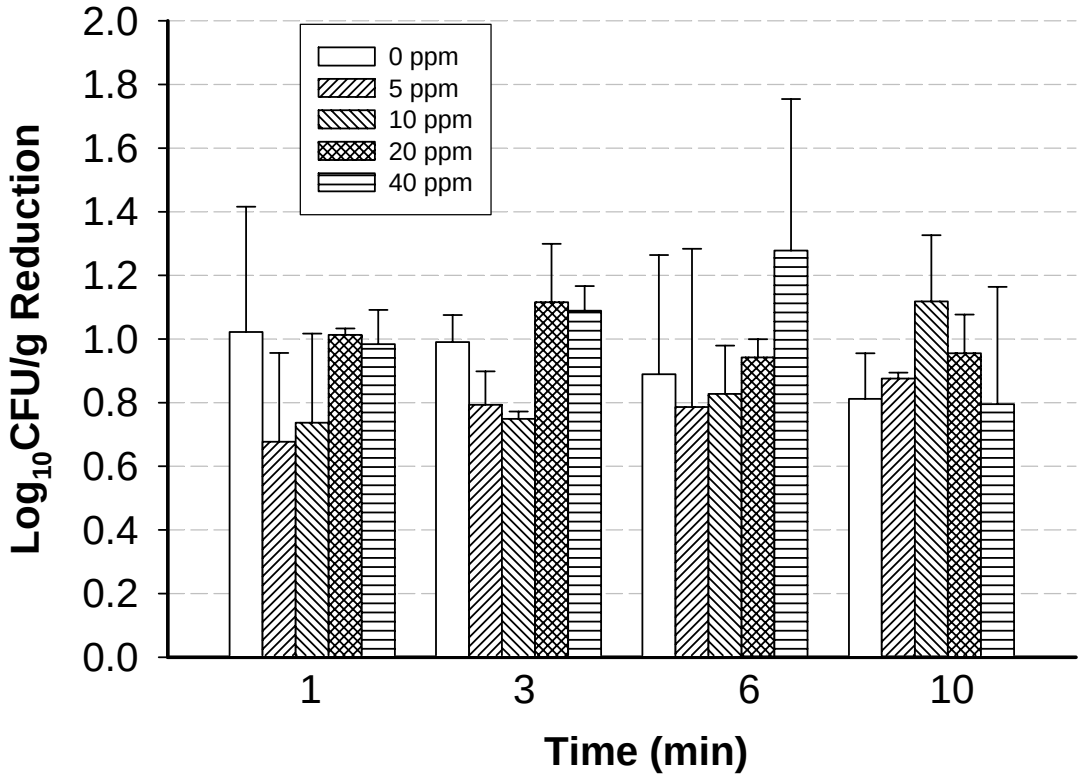

Figure 6-Log reduction of *E. coli* O157:H7 on high inoculum (10^7 CFU/ml) lettuce treated by various concentrations and times of ClO_2 combined with ultrasound 170 kHz. Data are shown as means of log reduction ± SD.

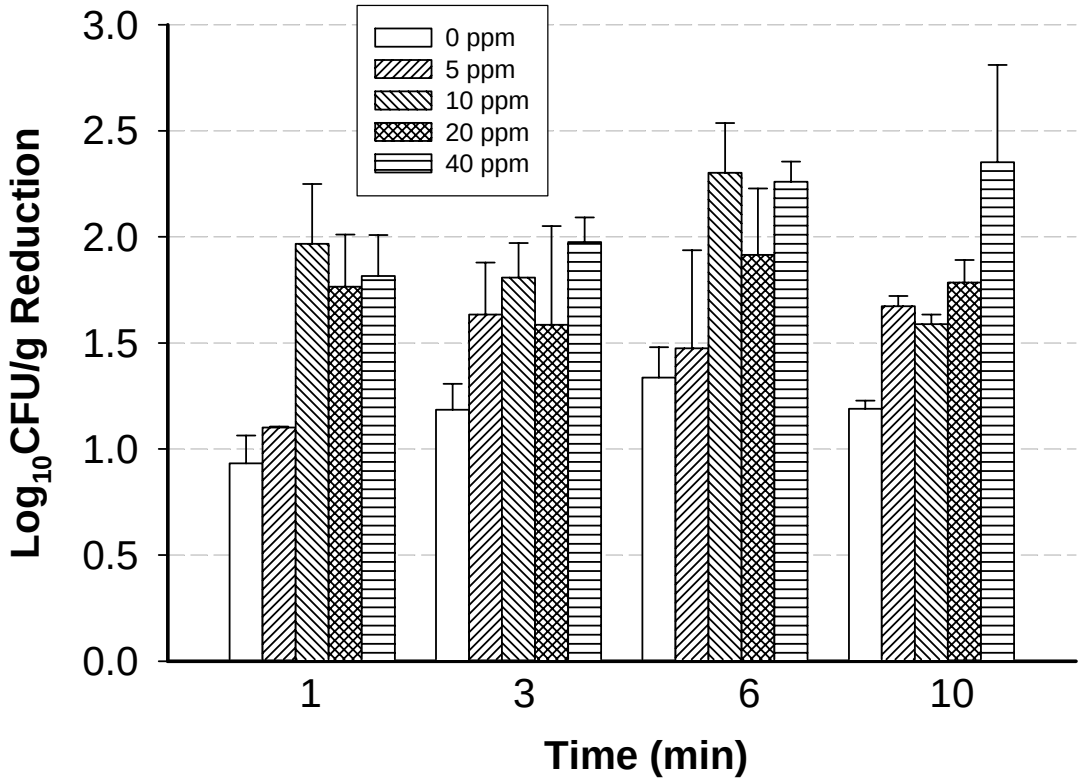

Figure 7-Log reduction of *E. coli* O157:H7 on low inoculum (10^4 CFU/ml) lettuce treated by various concentrations and times of ClO_2 combined with ultrasound 170 kHz. Data are shown as means of log reduction ± SD.

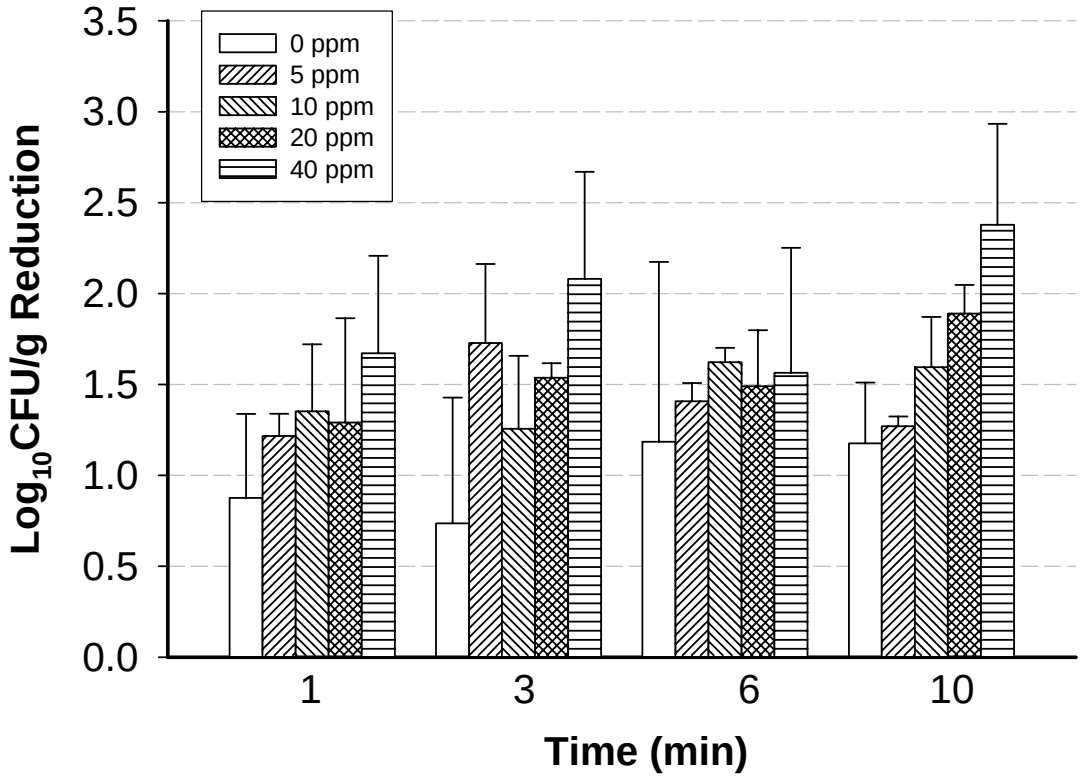

Figure 8-Log reduction of *Salmonella* on high inoculum (10^7 CFU/ml) lettuce treated by various concentrations and times of ClO_2. Data are shown as means of log reduction ± SD.

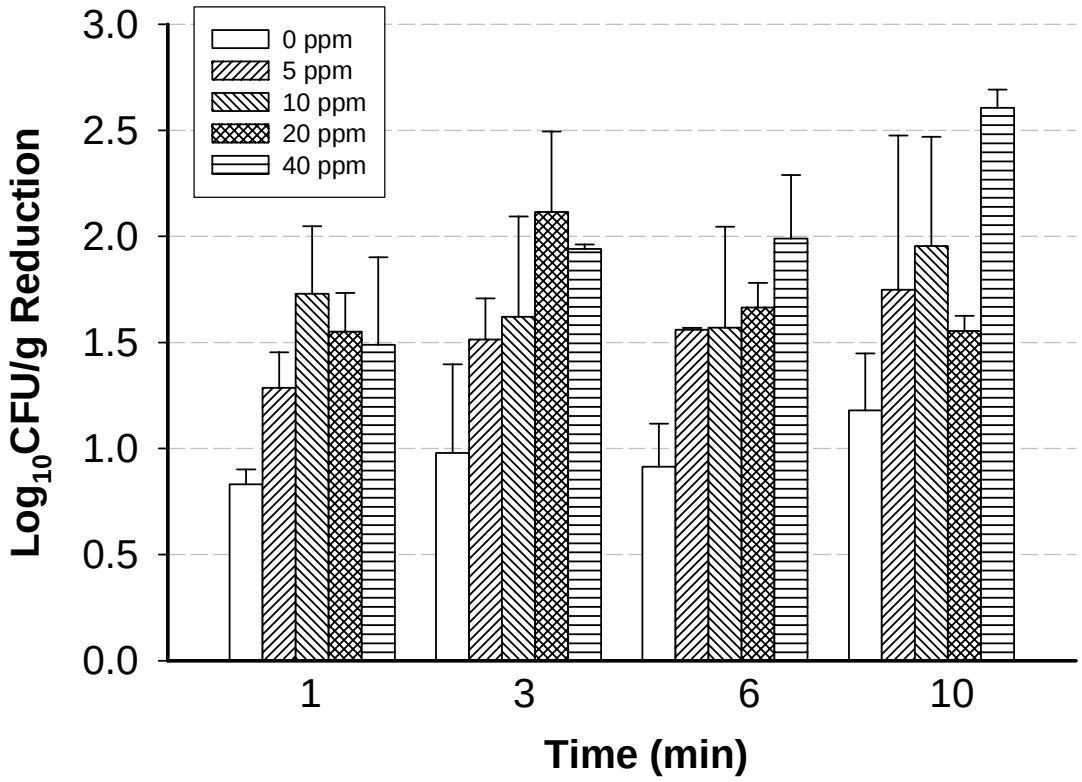

Figure 9-Log reduction of *Salmonella* on low inoculum (10^4 CFU/ml) lettuce treated by various concentrations and times of ClO_2. Data are shown as means of log reduction ± SD.

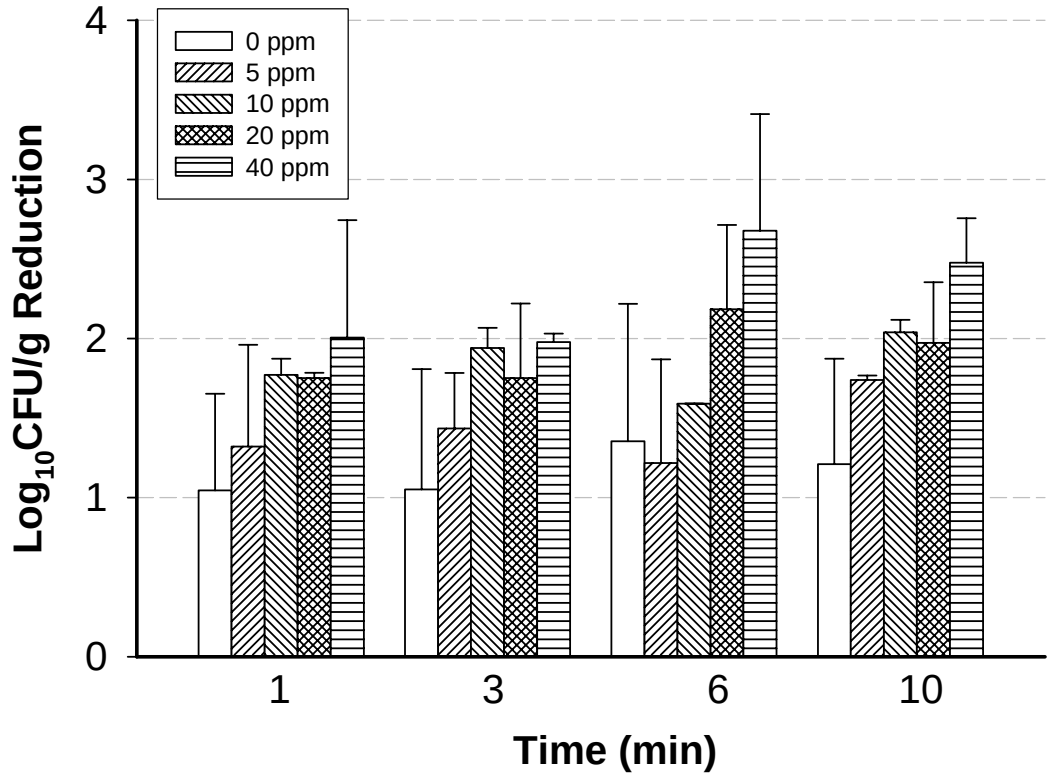

Figure 10-Log reduction of *Salmonella* on high inoculum (10^7 CFU/ml) lettuce treated by various concentrations and times of ClO_2 combined with ultrasound 120 kHz. Data are shown as means of log reduction ± SD.

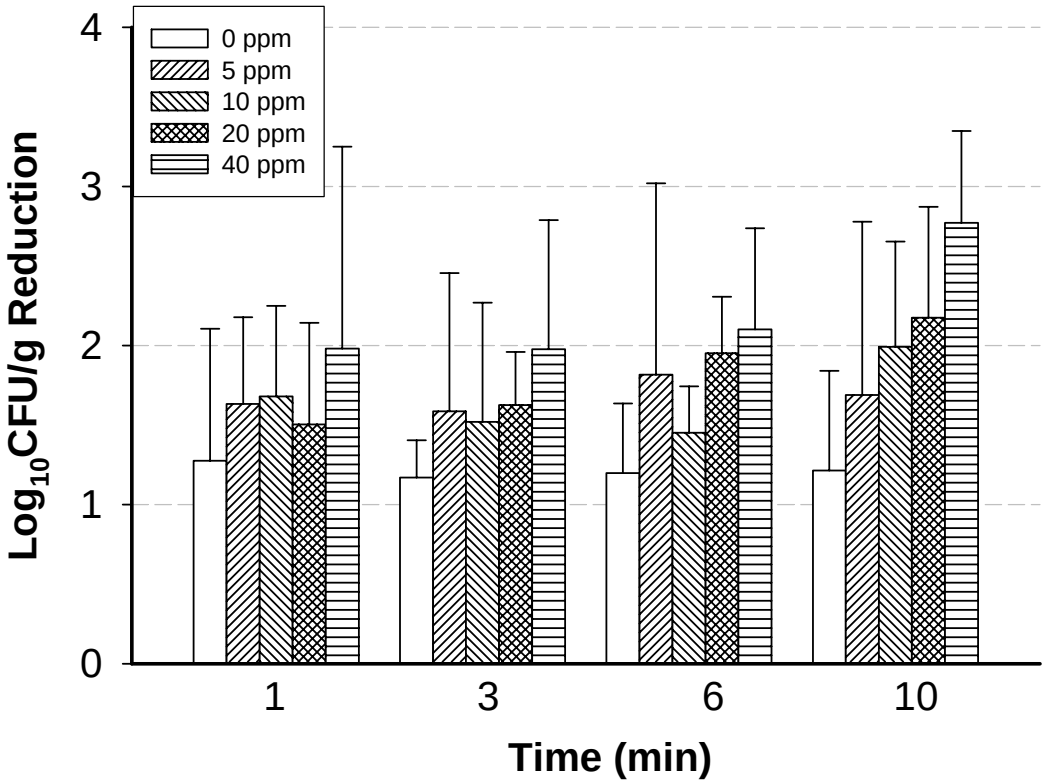

Figure 11-Log reduction of *Salmonella* on low inoculum (10^4 CFU/ml) lettuce treated by various concentrations and times of ClO_2 combined with ultrasound 120 kHz. Data are shown as means of log reduction ± SD.

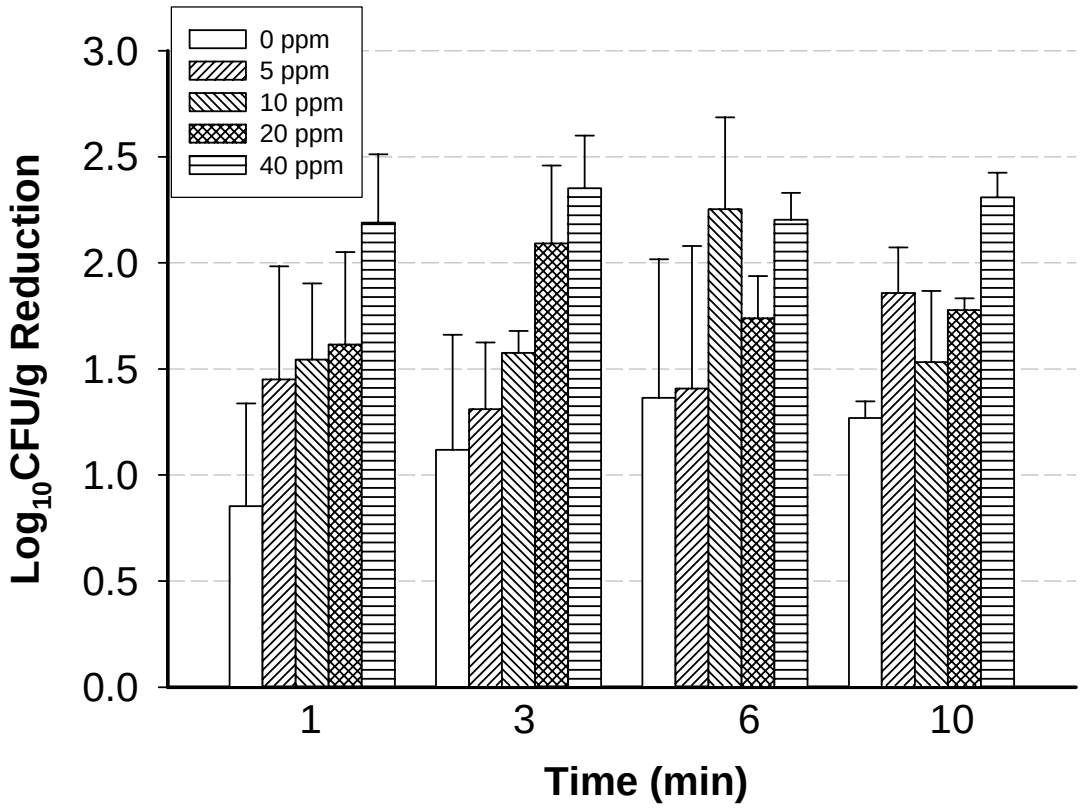

Figure 12-Log reduction of *Salmonella* on high inoculum (10^7 CFU/ml) lettuce treated by various concentrations and times of ClO_2 combined with ultrasound 170 kHz. Data are shown as means of log reduction ± SD.

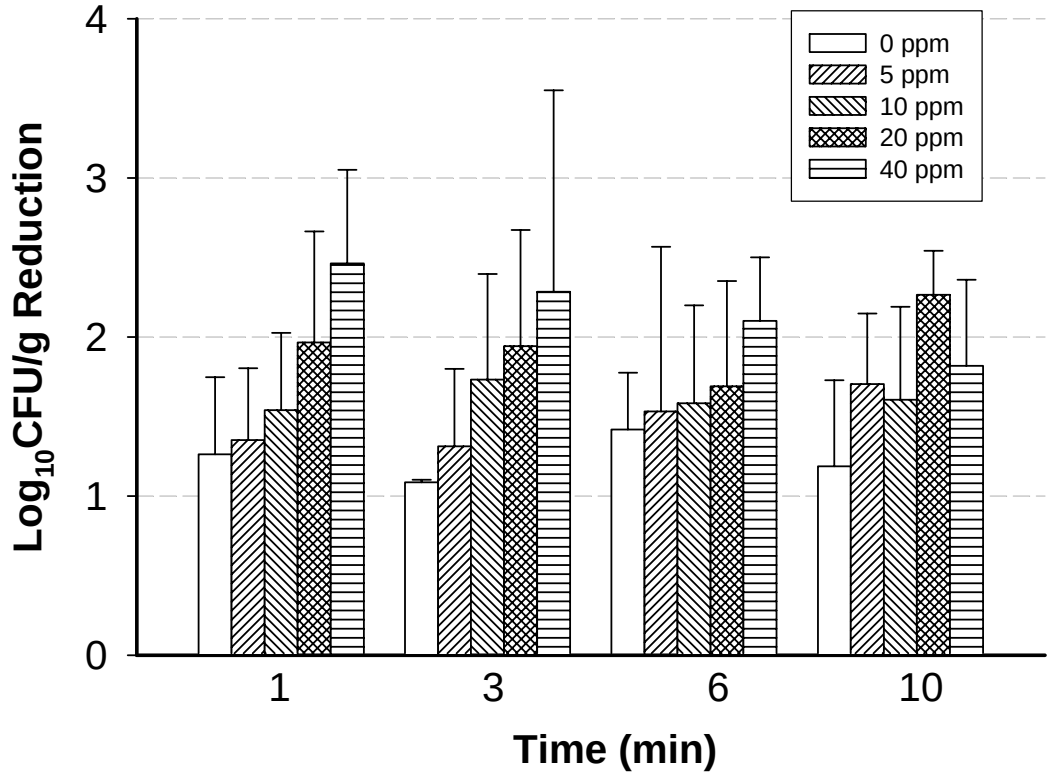

Figure 13-Log reduction of *Salmonella* on low inoculum (10^4 CFU/ml) lettuce treated by various concentrations and times of ClO_2 combined with ultrasound 170 kHz. Data are shown as means of log reduction ± SD.

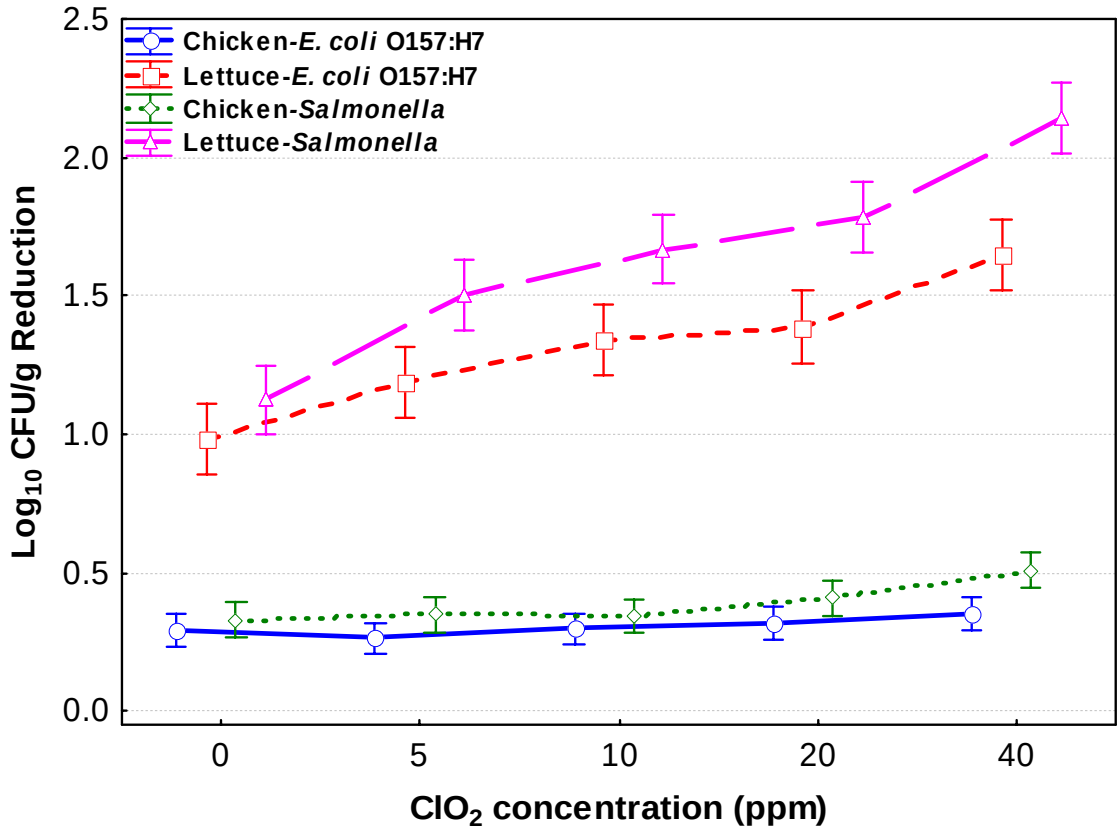

Figure 14-Summary of the effects of ClO_2 concentrations to *E. coli* O157:H7 or *Salmonella* log reduction on chicken and lettuce. Data are shown as means of log reduction ± 95% CI.

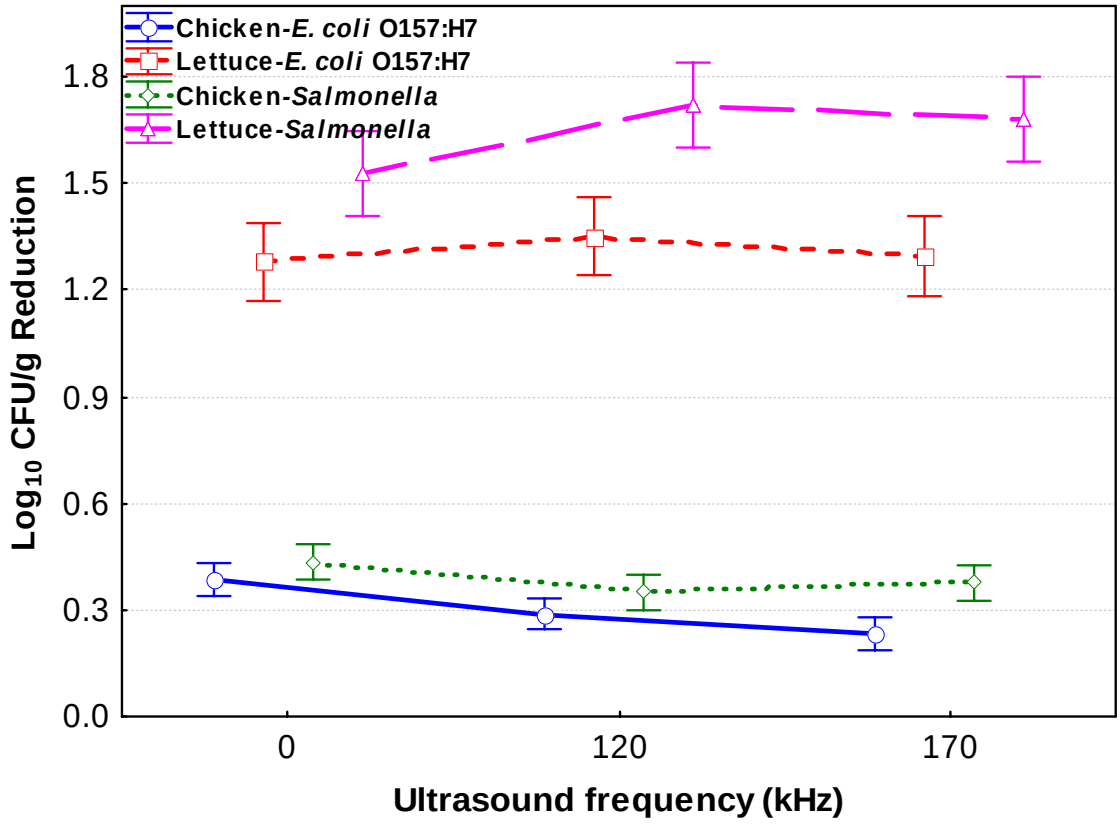

Figure 15-Summary of the effects of ultrasound frequencies to *E. coli* O157:H7 or *Salmonella* log reduction on chicken and lettuce. Data are shown as means of log reduction ± 95% CI.

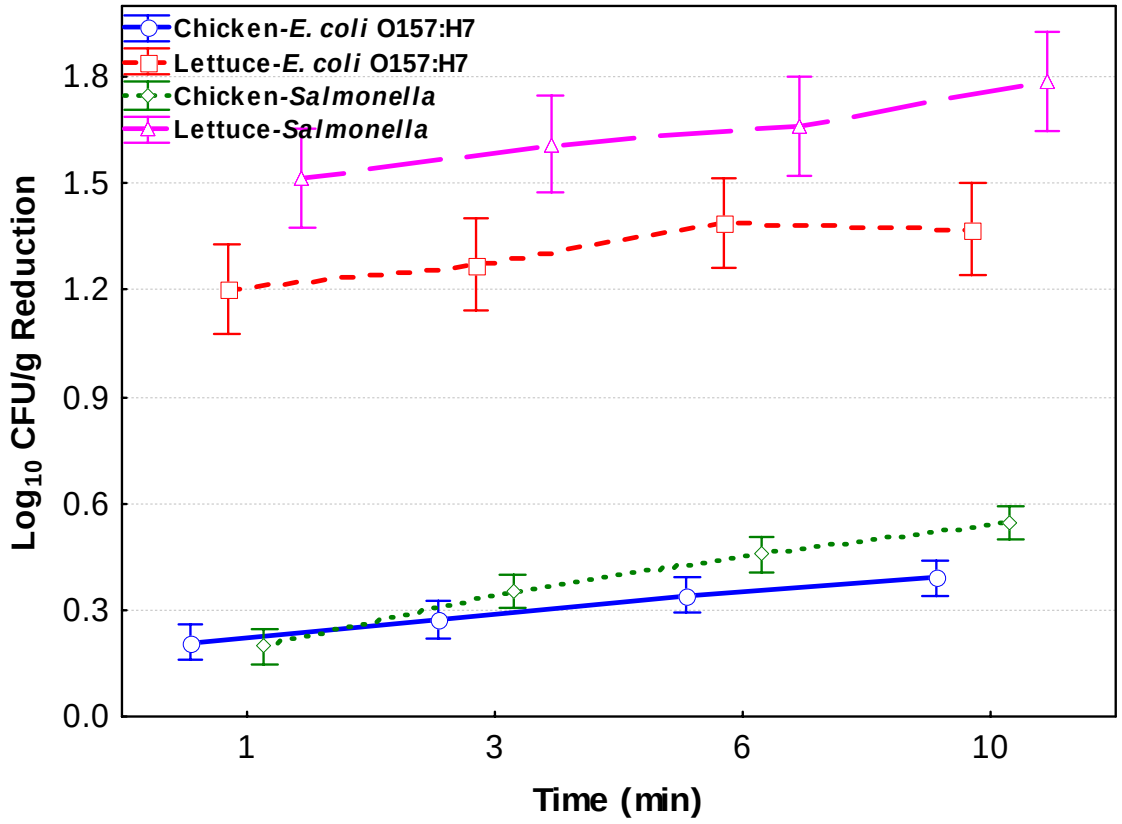

Figure 16-Summary of the effects of treatment times to *E. coli* O157:H7 or *Salmonella* on log reduction chicken and lettuce. Data are shown as means of log reduction ± 95% CI.

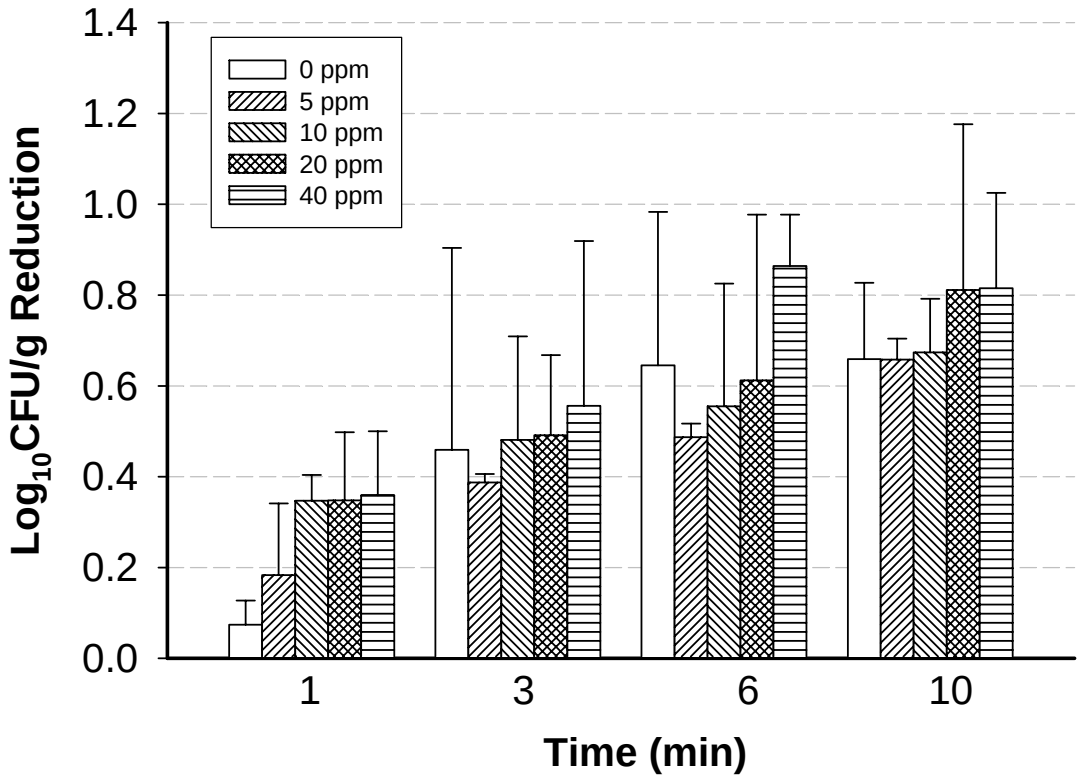

Figure 17-Log reduction of *E. coli* O157:H7 on high inoculum (10^7 CFU/ml) chicken breasts treated with various concentrations and times of ClO_2. Data are shown as means of log reduction ± SD.

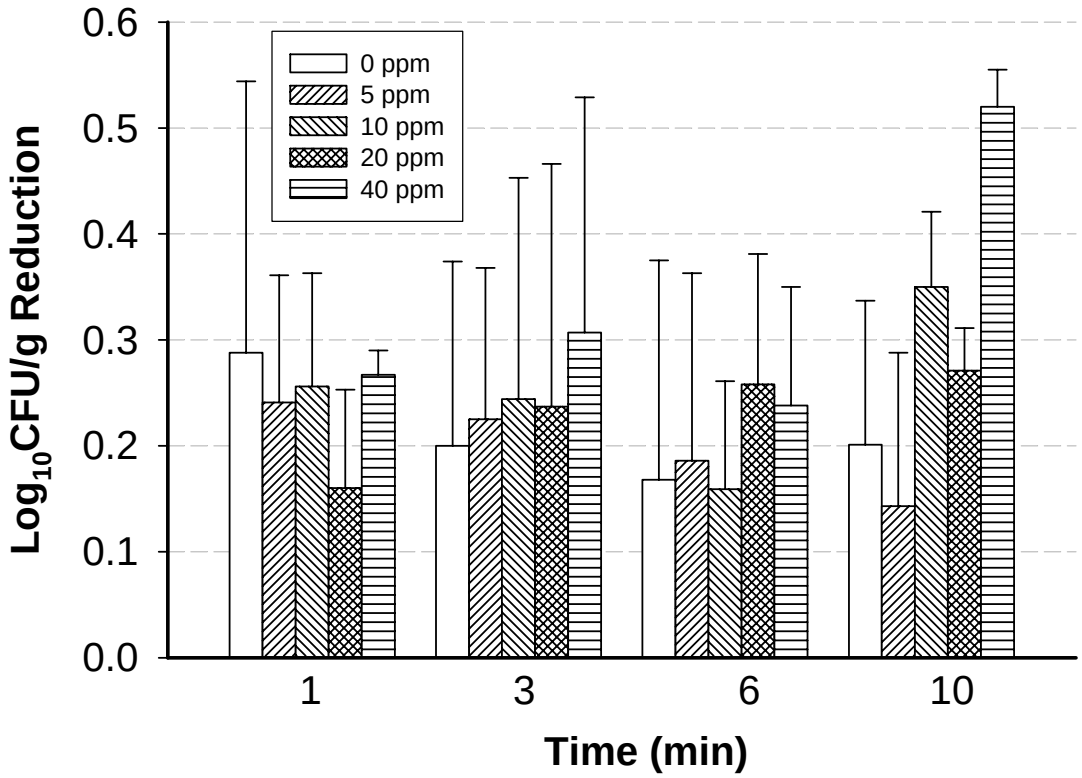

Figure 18-Log reduction of *E. coli* O157:H7 on low inoculum (10^4 CFU/ml) chicken breasts treated with various concentrations and times of ClO_2. Data are shown as means of log reduction ± SD.

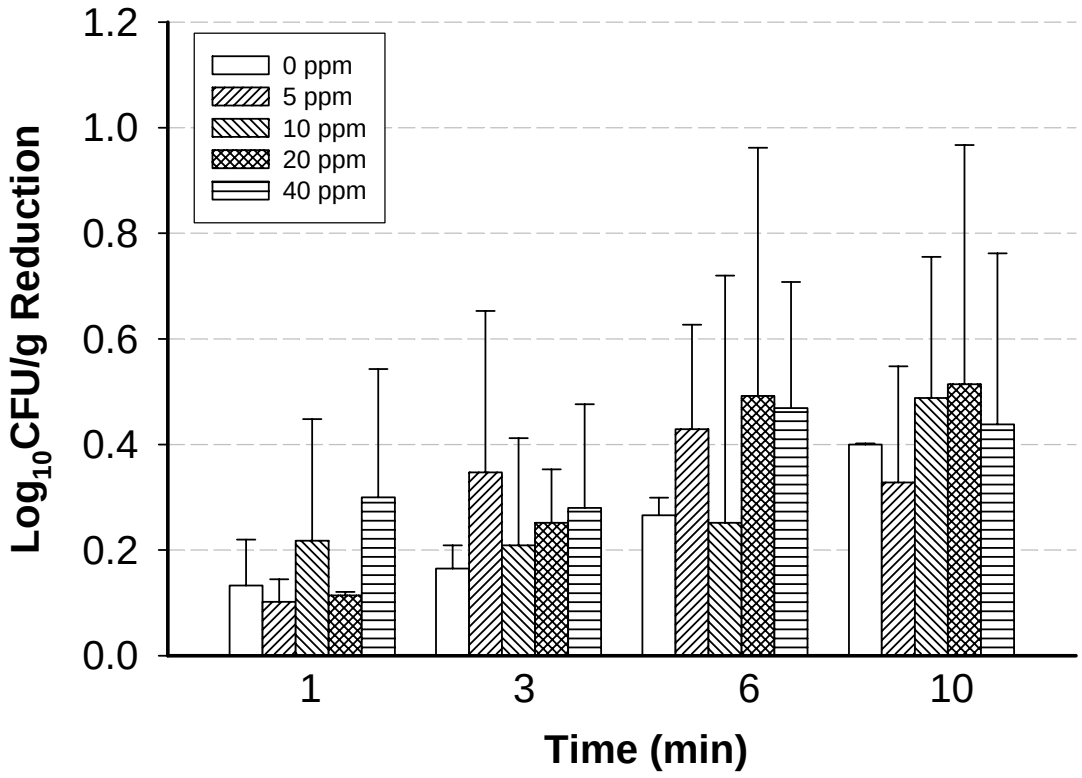

Figure 19-Log reduction of *E. coli* O157:H7 on high inoculum (10^7 CFU/ml) chicken breasts treated by various concentrations and times of ClO_2 combined with ultrasound 120 kHz. Data are shown as means of log reduction ± SD.

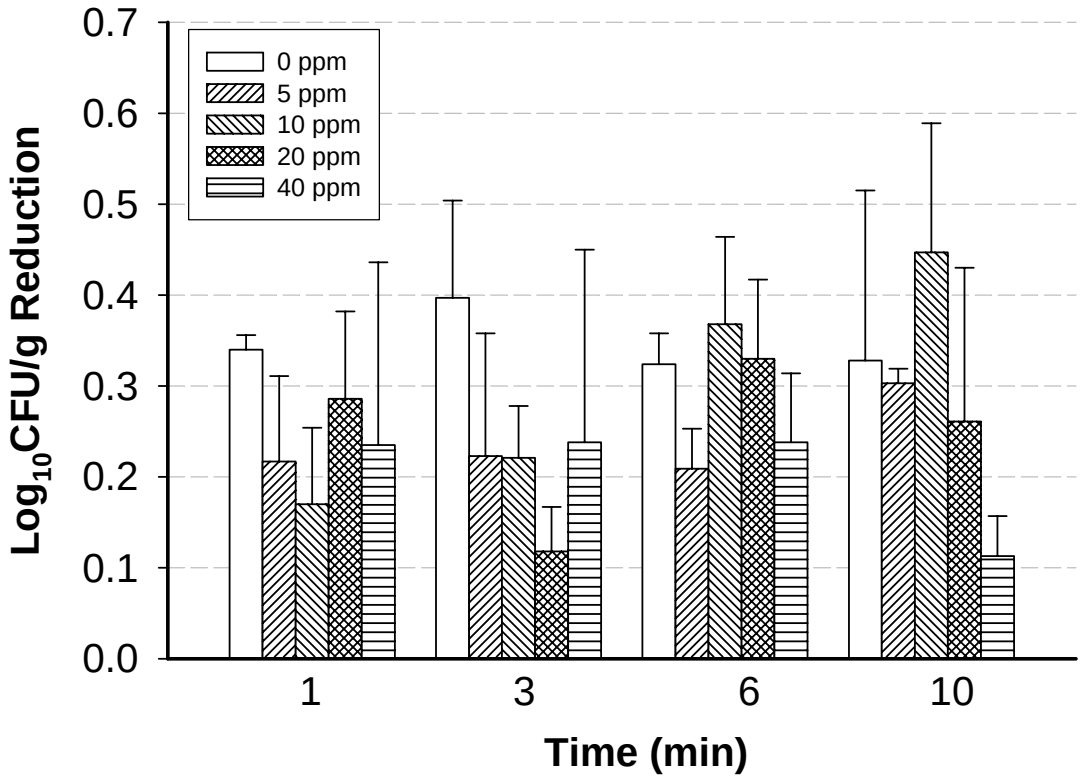

Figure 20-Log reduction of *E. coli* O157:H7 on low inoculum (10^4 CFU/ml) chicken breasts treated by various concentrations and times of ClO_2 combined with ultrasound 120 kHz. Data are shown as means of log reduction ± SD.

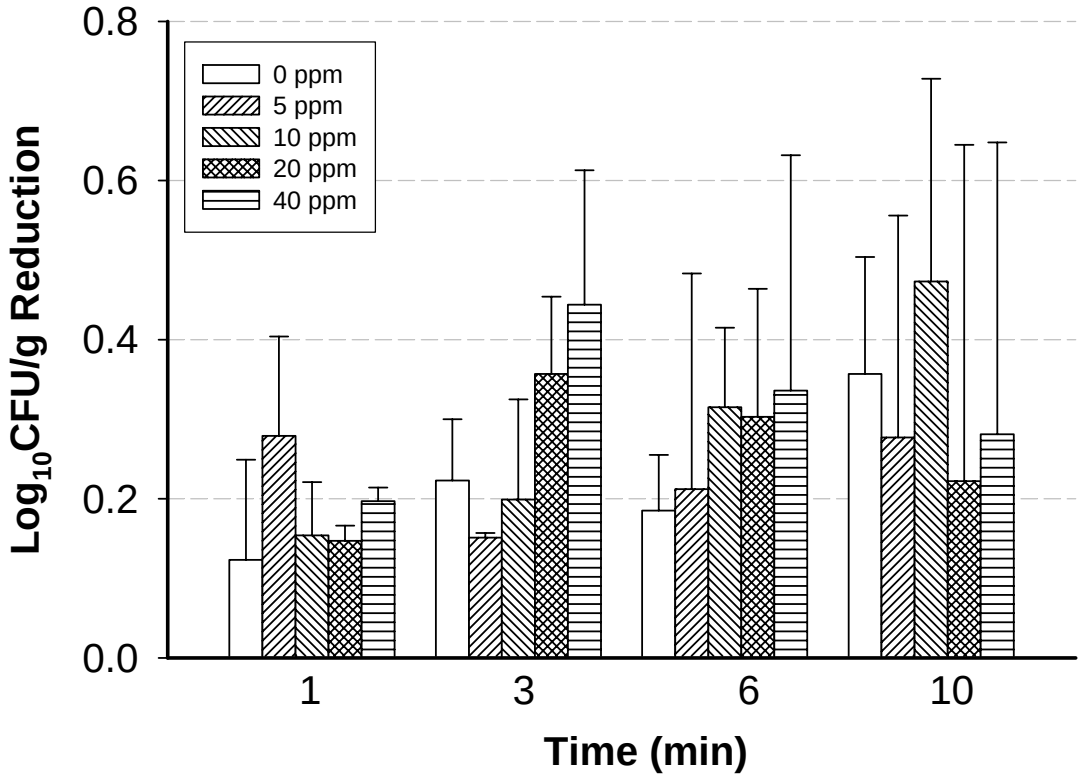

Figure 21-Log reduction of *E. coli* O157:H7 on high inoculum (10^7 CFU/ml) chicken breasts treated by various concentrations and times of ClO_2 combined with ultrasound 170 kHz. Data are shown as means of log reduction ± SD.

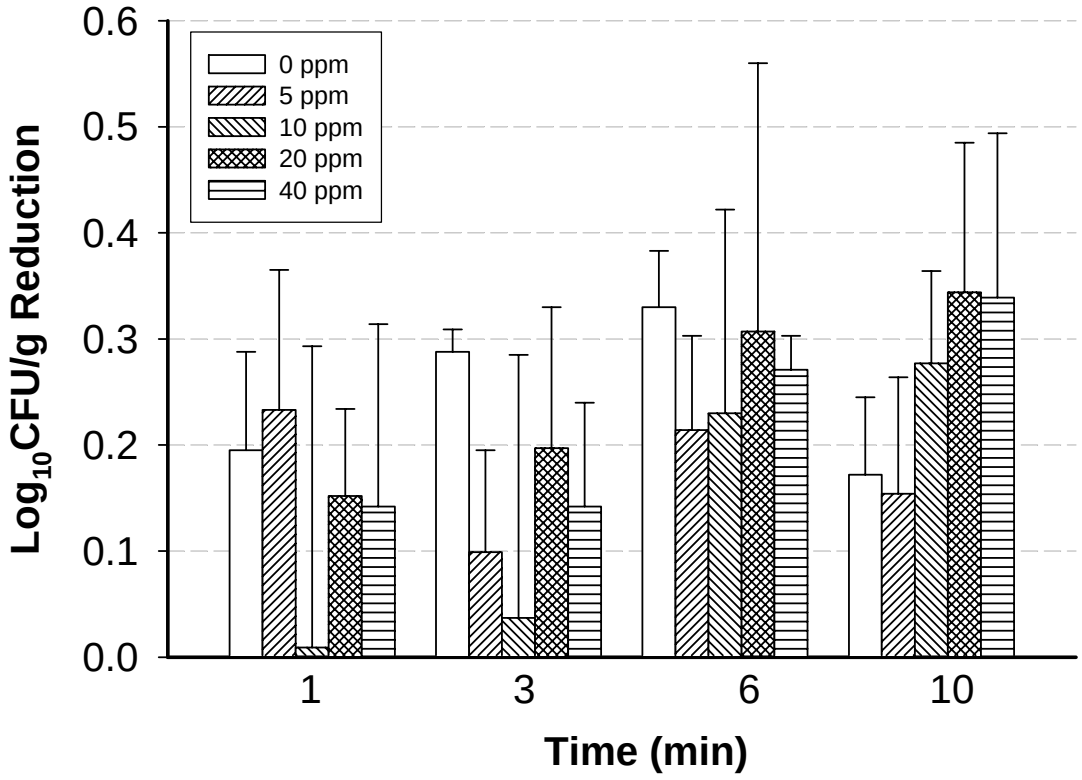

Figure 22-Log reduction of *E. coli* O157:H7 on low inoculum (10^4 CFU/ml) chicken breasts treated by various concentrations and times of ClO_2 combined with ultrasound 170 kHz. Data are shown as means of log reduction ± SD.

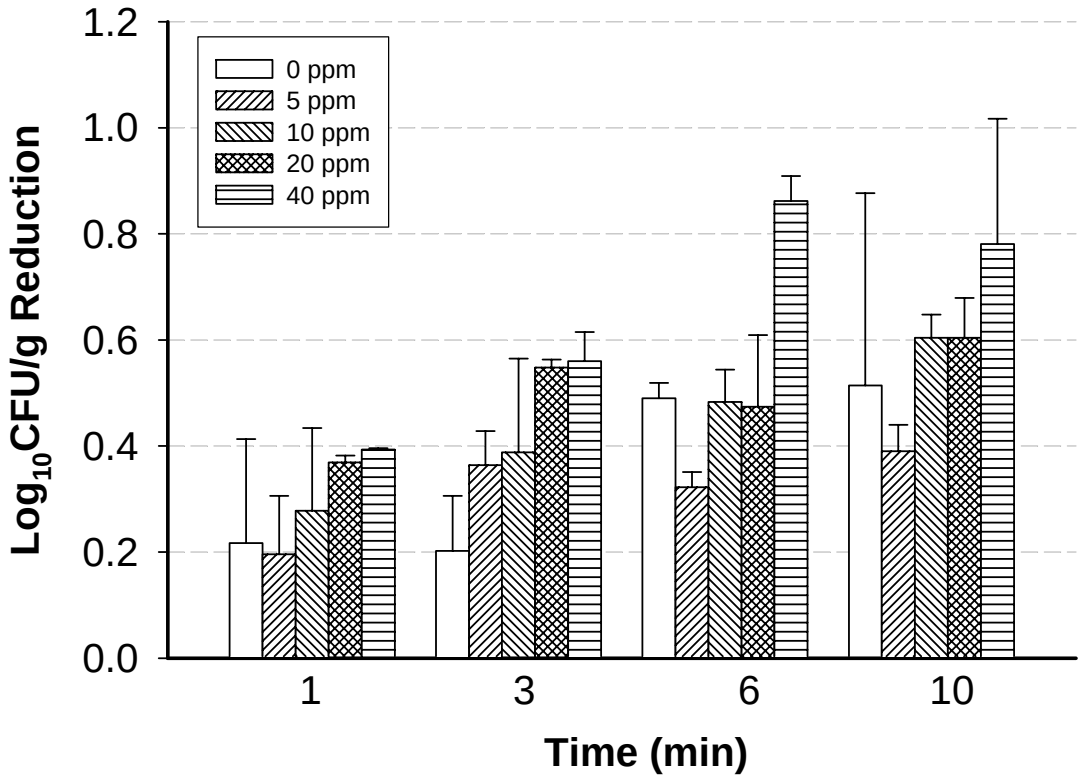

Figure 23-Log reduction of *Salmonella* on high inoculum (10^7 CFU/ml) chicken breasts treated by various concentrations and times of ClO_2. Data are shown as means of log reduction ± SD.

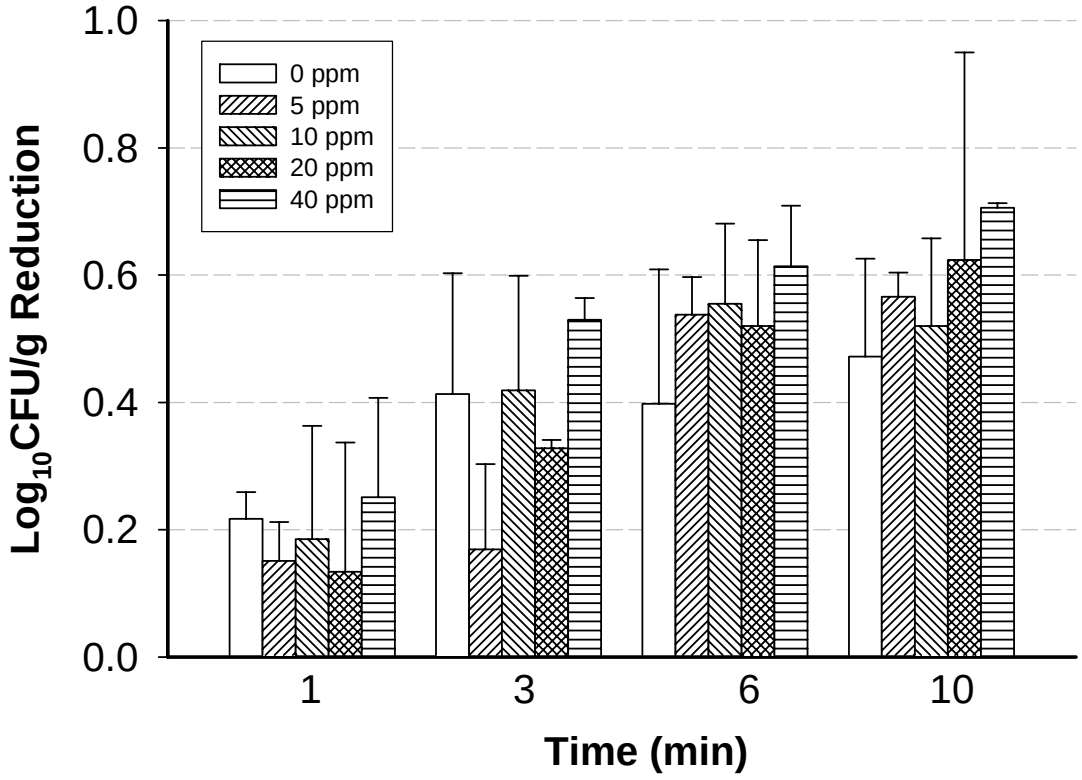

Figure 24-Log reduction of *Salmonella* on low inoculum (10^4 CFU/ml) chicken breasts treated by various concentrations and times of ClO_2. Data are shown as means of log reduction ± SD.

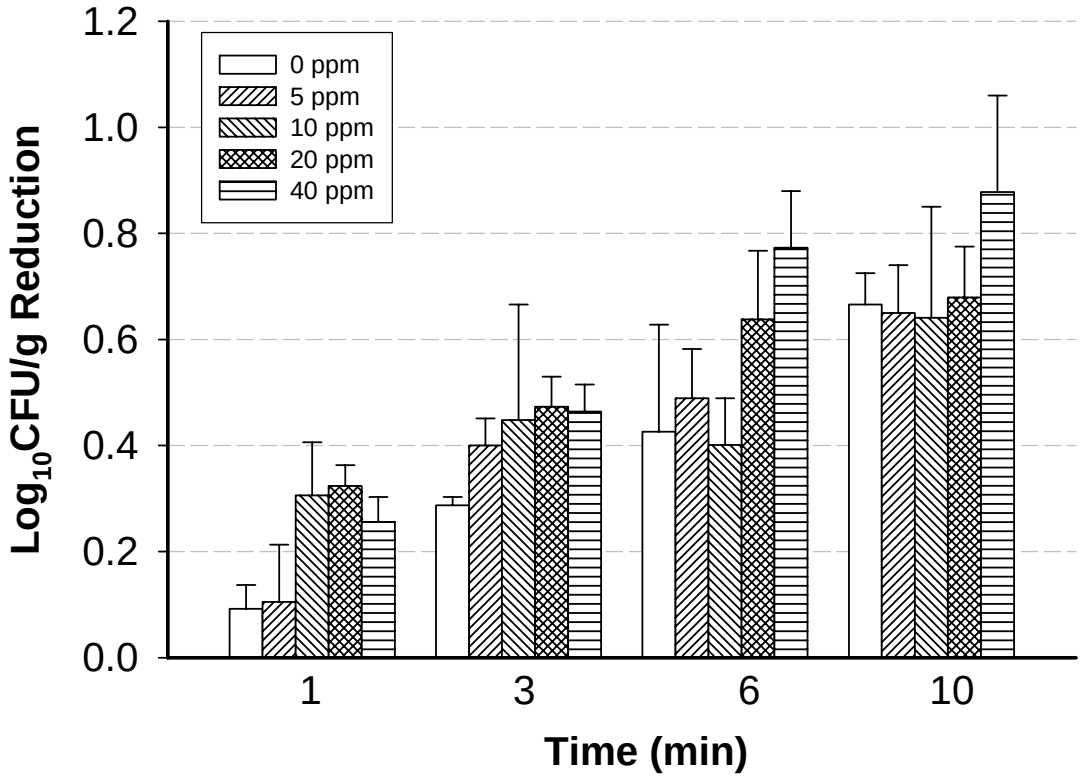

Figure 25-Log reduction of *Salmonella* on high inoculum (10^7 CFU/ml) chicken breasts treated by various concentrations and times of ClO_2 combined with ultrasound 120 kHz. Data are shown as means of log reduction ± SD.

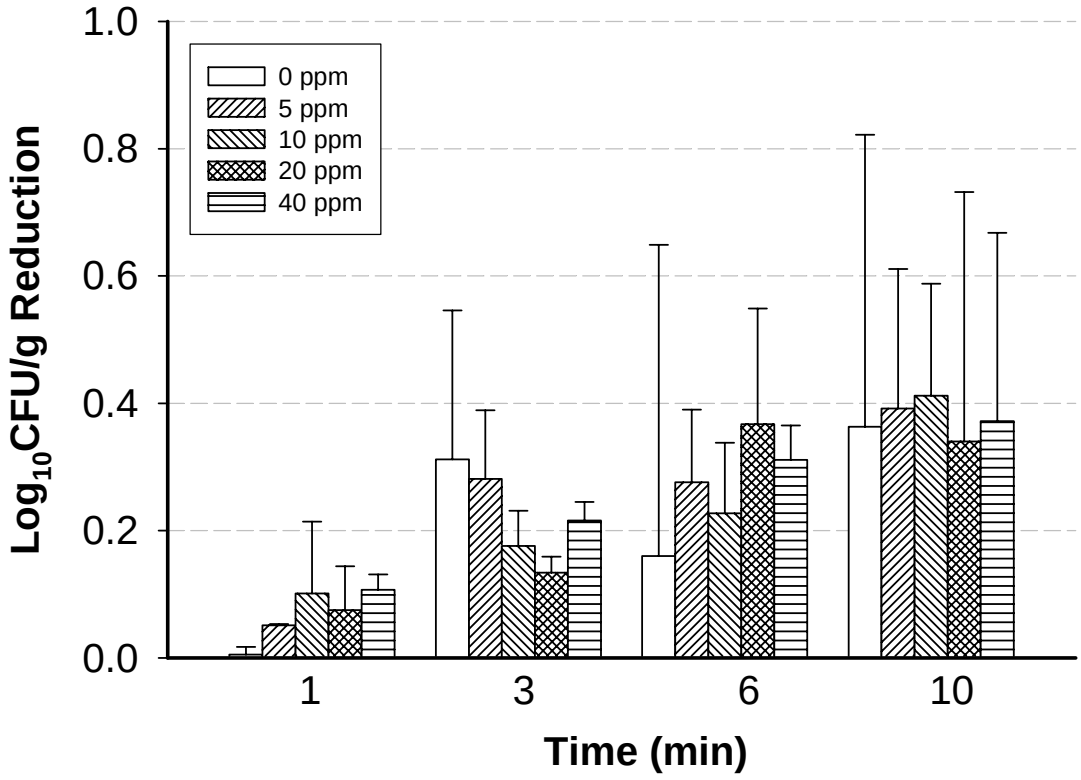

Figure 26-Log reduction of *Salmonella* on low inoculum (10^4 CFU/ml) chicken breasts treated by various concentrations and times of ClO_2 combined with ultrasound 120 kHz. Data are shown as means of log reduction ± SD.

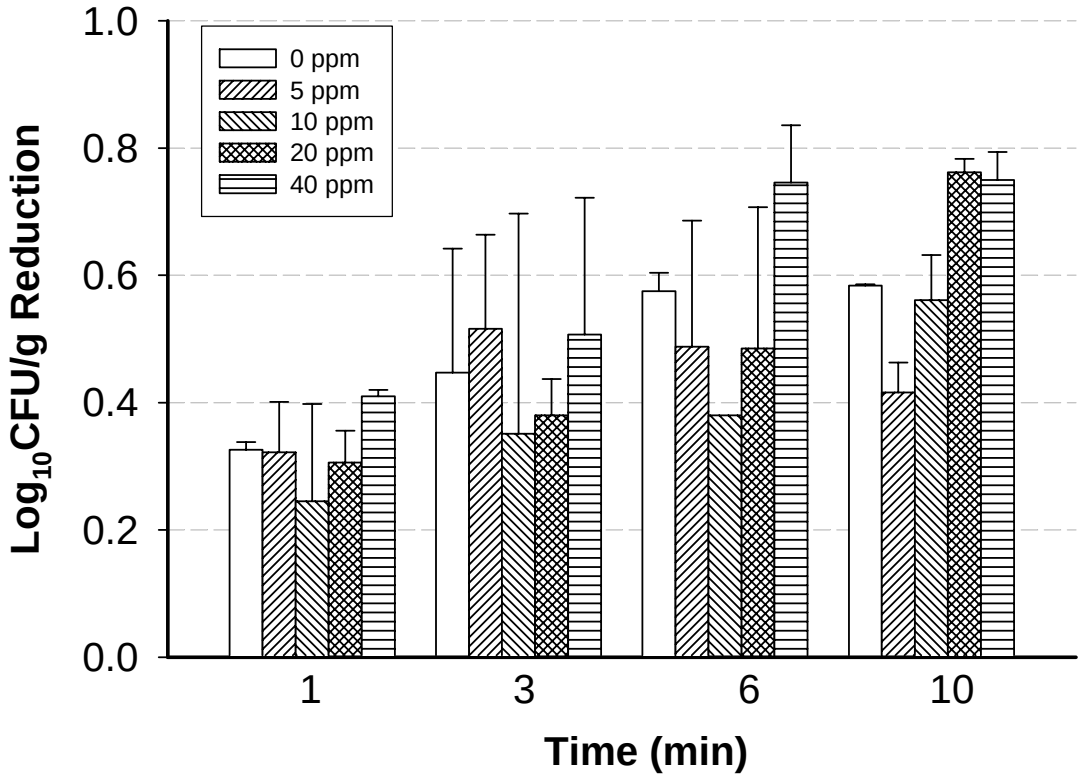

Figure 27-Log reduction of *Salmonella* on high inoculum (10^7 CFU/ml) chicken breasts treated by various concentrations and times of ClO_2 combined with ultrasound 170 kHz. Data are shown as means of log reduction ± SD.

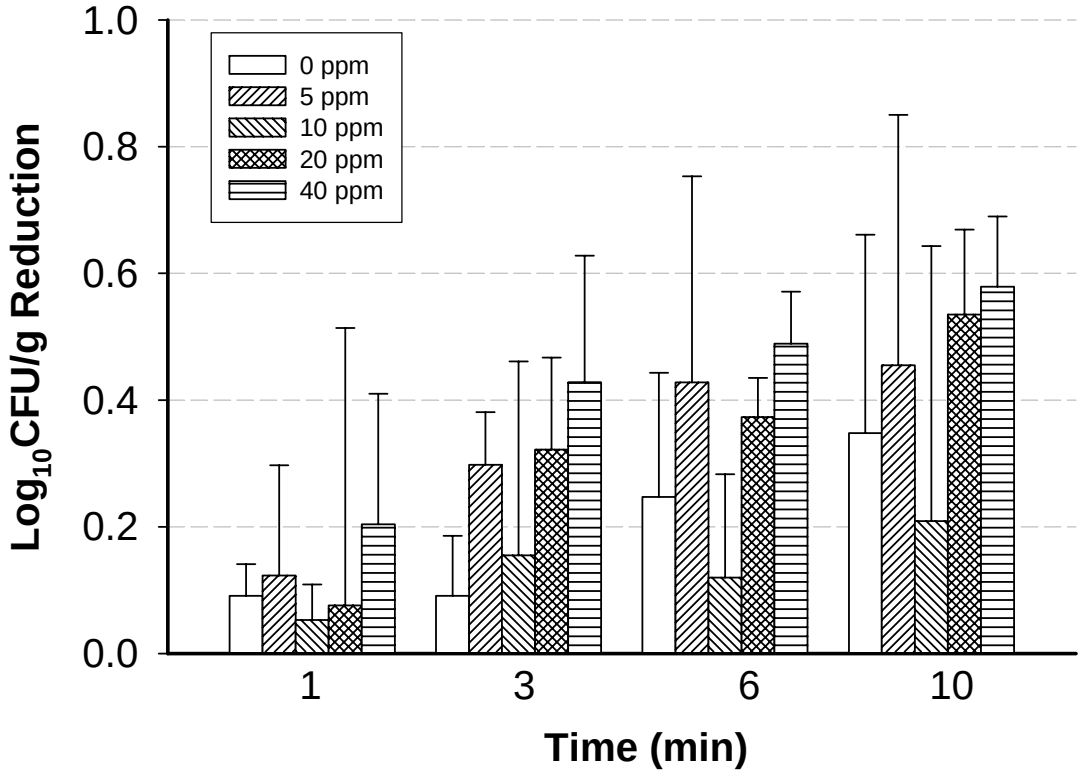

Figure 28-Log reduction of *Salmonella* on low inoculum (10^4 CFU/ml) chicken breasts treated by various concentrations and times of ClO_2 combined with ultrasound 170 kHz. Data are shown as means of log reduction ± SD.

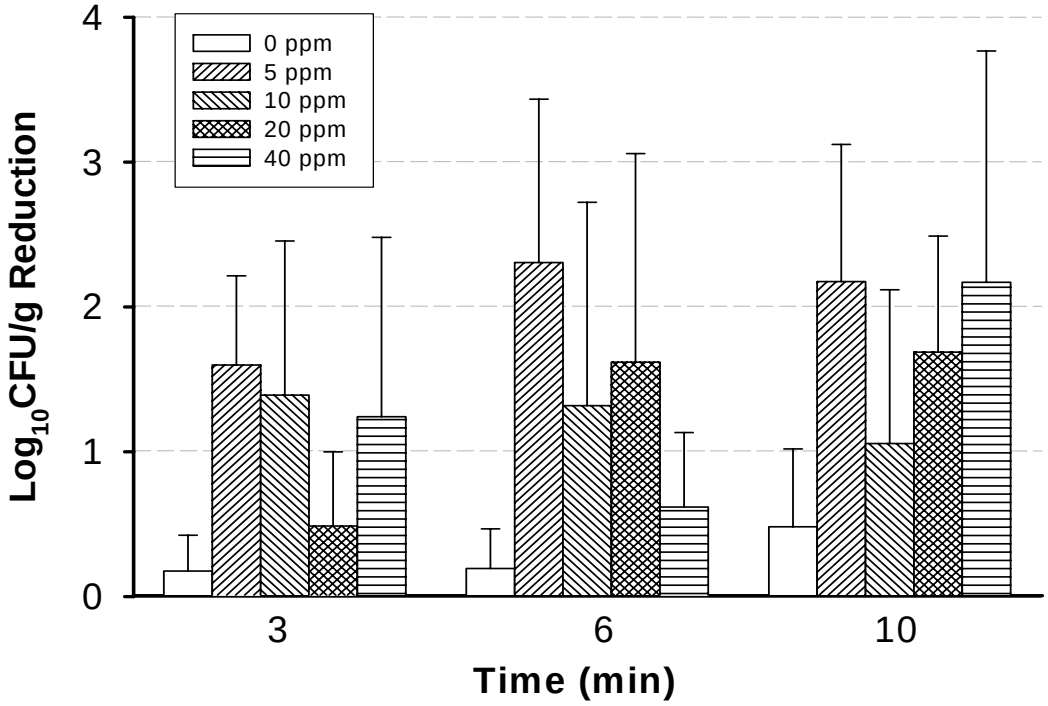

Figure 29-Log reduction of *E. coli* O157:H7 on high inoculum (10^8 CFU/ml) apples treated by various concentrations and times of ClO_2. Data are shown as means of log reduction ± SD.

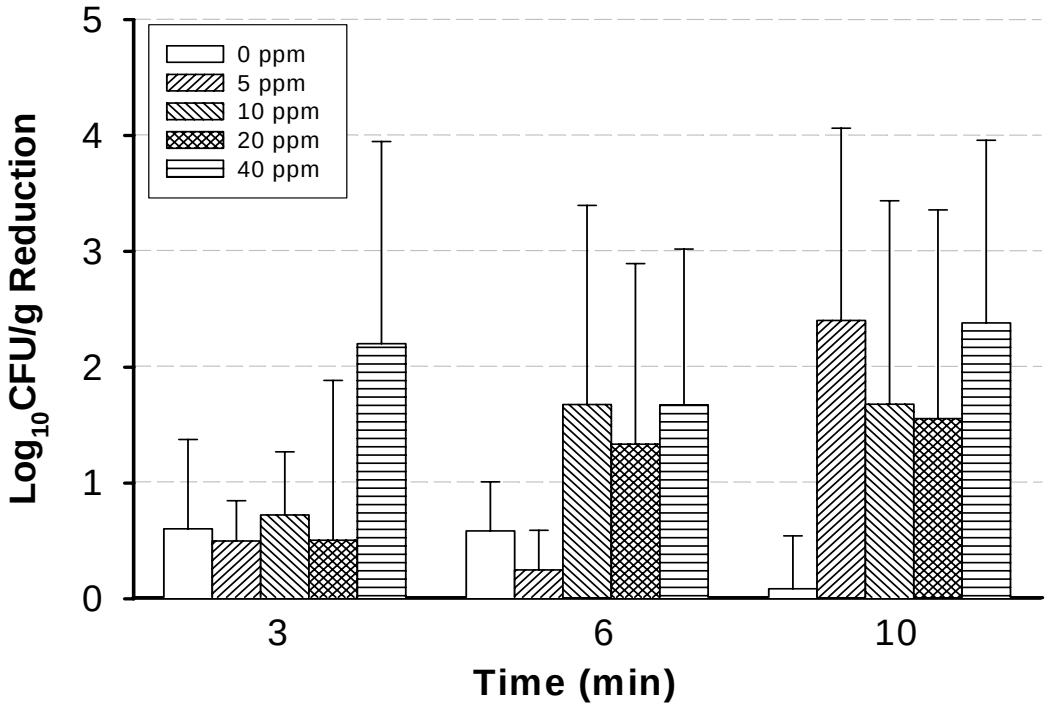

Figure 30-Log reduction of *E. coli* O157:H7 on high inoculum (10^8 CFU/ml) apples treated by various concentrations and times of ClO_2 combined with ultrasound 170 kHz. Data are shown as means of log reduction ± SD.

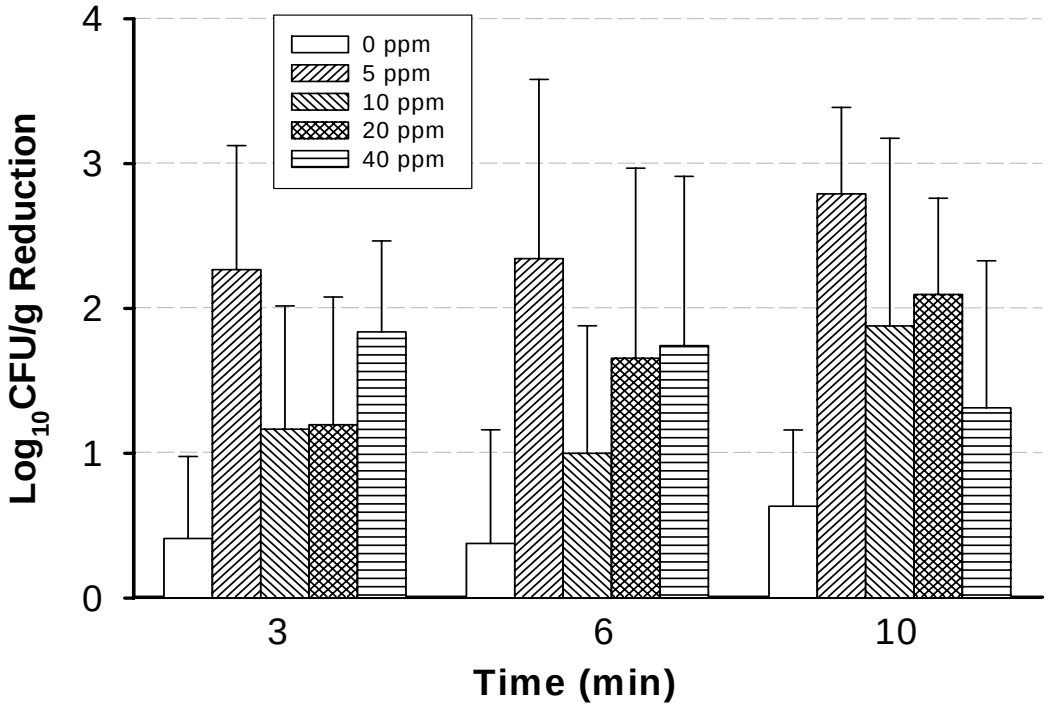

Figure 31-Log reduction of *Salmonella* on high inoculum (10^8 CFU/ml) apples treated by various concentrations and times of ClO_2. Data are shown as means of log reduction ± SD.

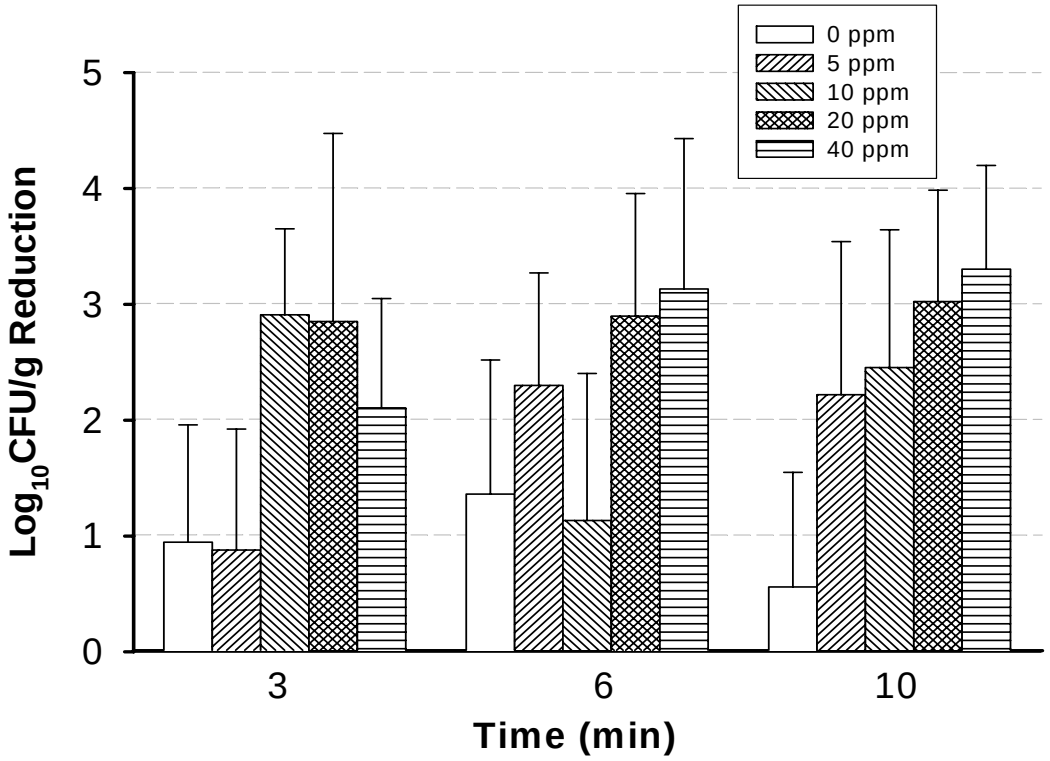

Figure 32-Log reduction of *Salmonella* on high inoculum (10^8 CFU/ml) apples treated by various concentrations and times of ClO_2 combined with ultrasound 170 kHz. Data are shown as means of log reduction ± SD.

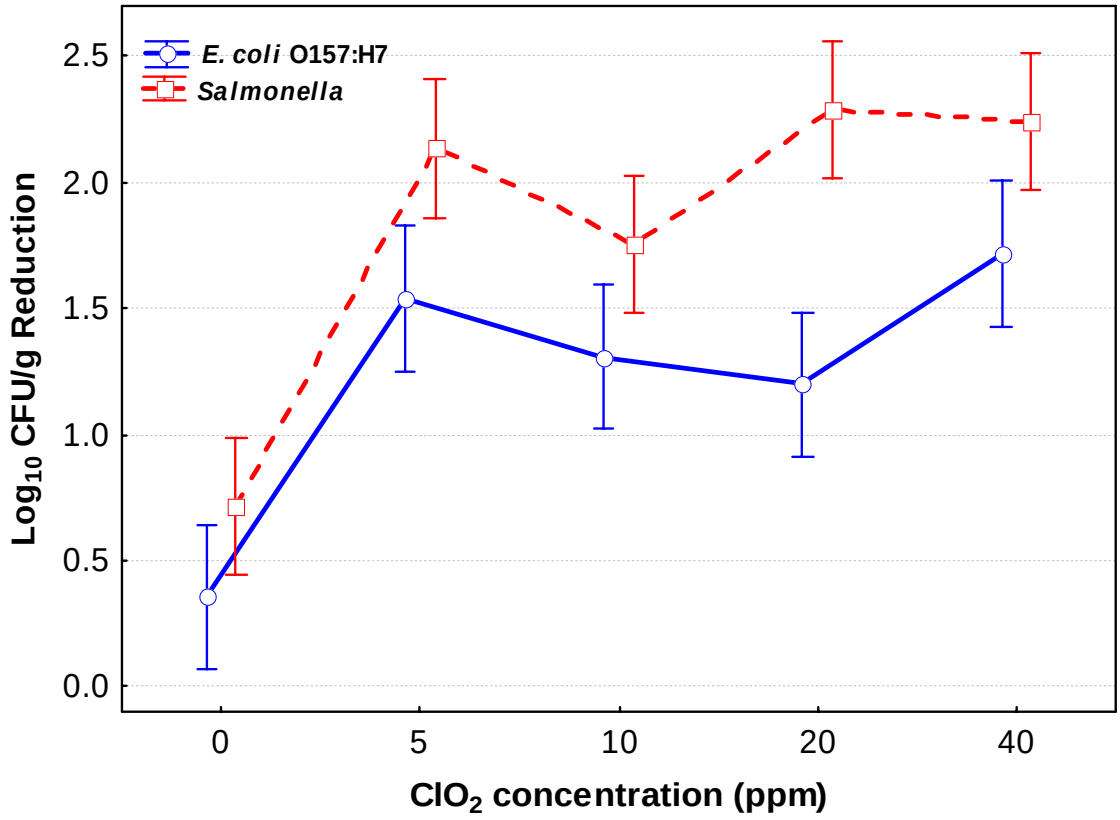

Figure 33-Summary of the effects of ClO_2 concentrations to *E. coli* O157:H7 or *Salmonella* log reduction on apples. Data are shown as means of log reduction ± 95% CI.

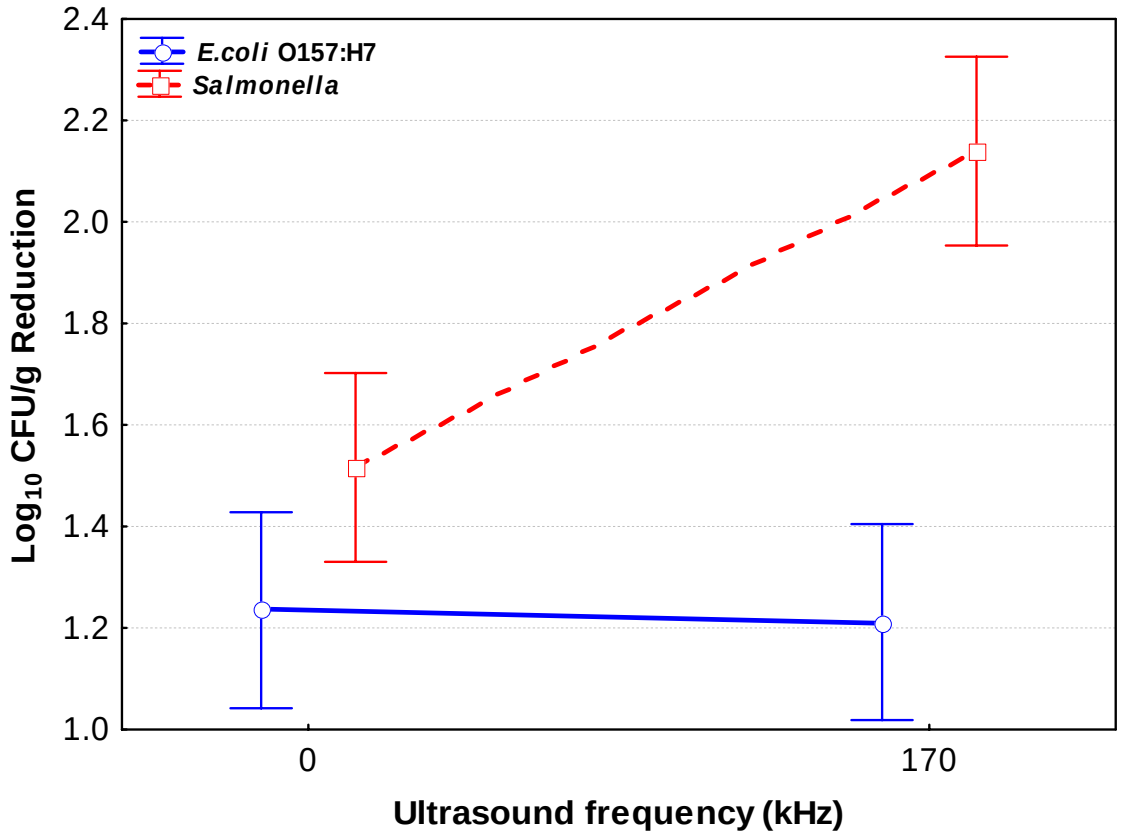

Figure 34-Summary of the effects of ultrasound frequencies to *E. coli* O157:H7 or *Salmonella* log reduction on apples. Data are shown as means of log reduction ± 95% CI.

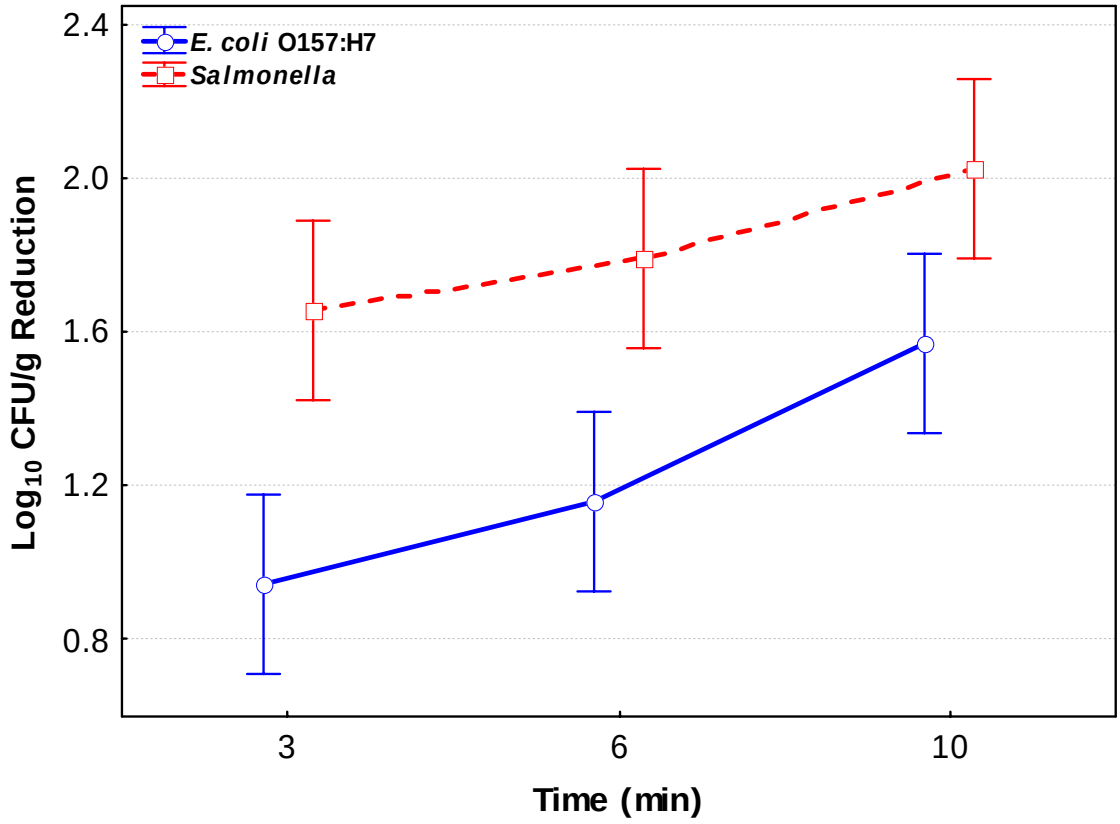

Figure 35-Summary of the effects of treatment times to *E. coli* O157:H7 or *Salmonella* log reduction on apples. Data are shown as means of log reduction ± 95% CI.

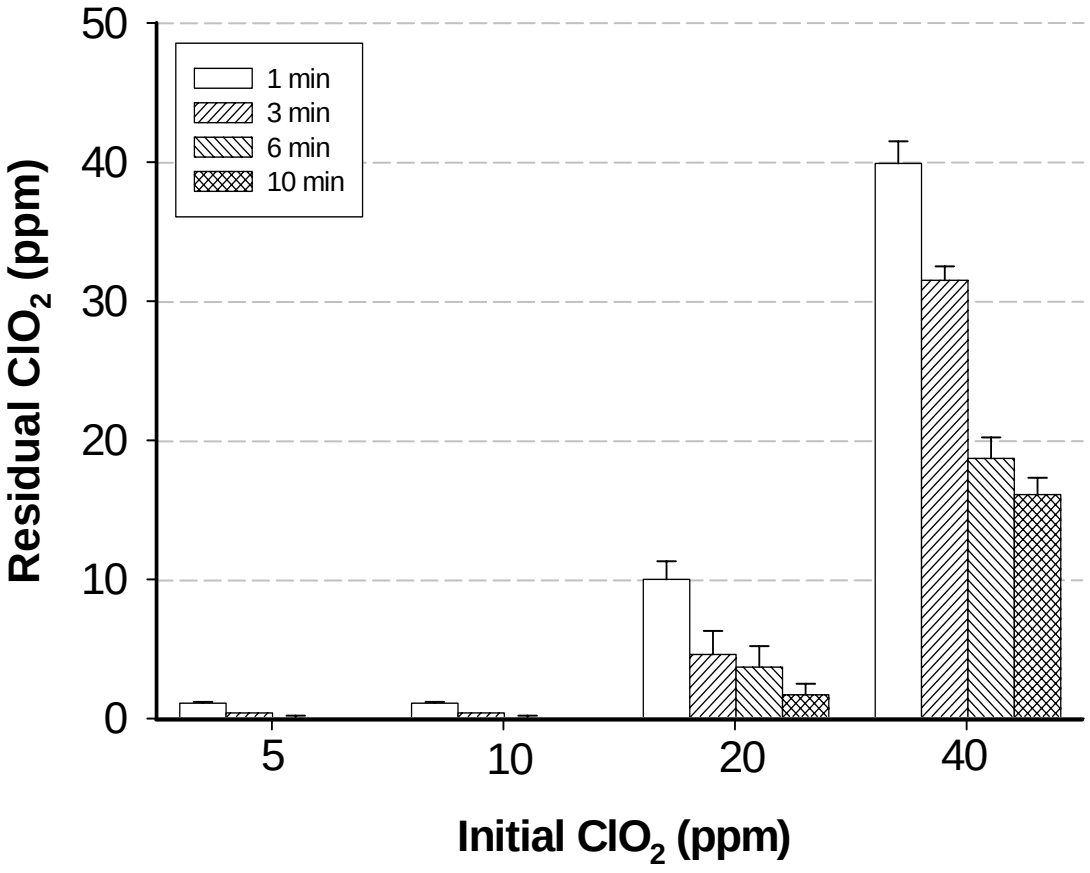

Figure 36-Chlorine dioxide residual change with various initial concentrations (5, 10, 20 & 40 ppm) and different times (1, 3, 6 & 10 min) in the treating solution of chicken breasts. Data are shown as means of the concentration of residual ClO_2 ± SD.

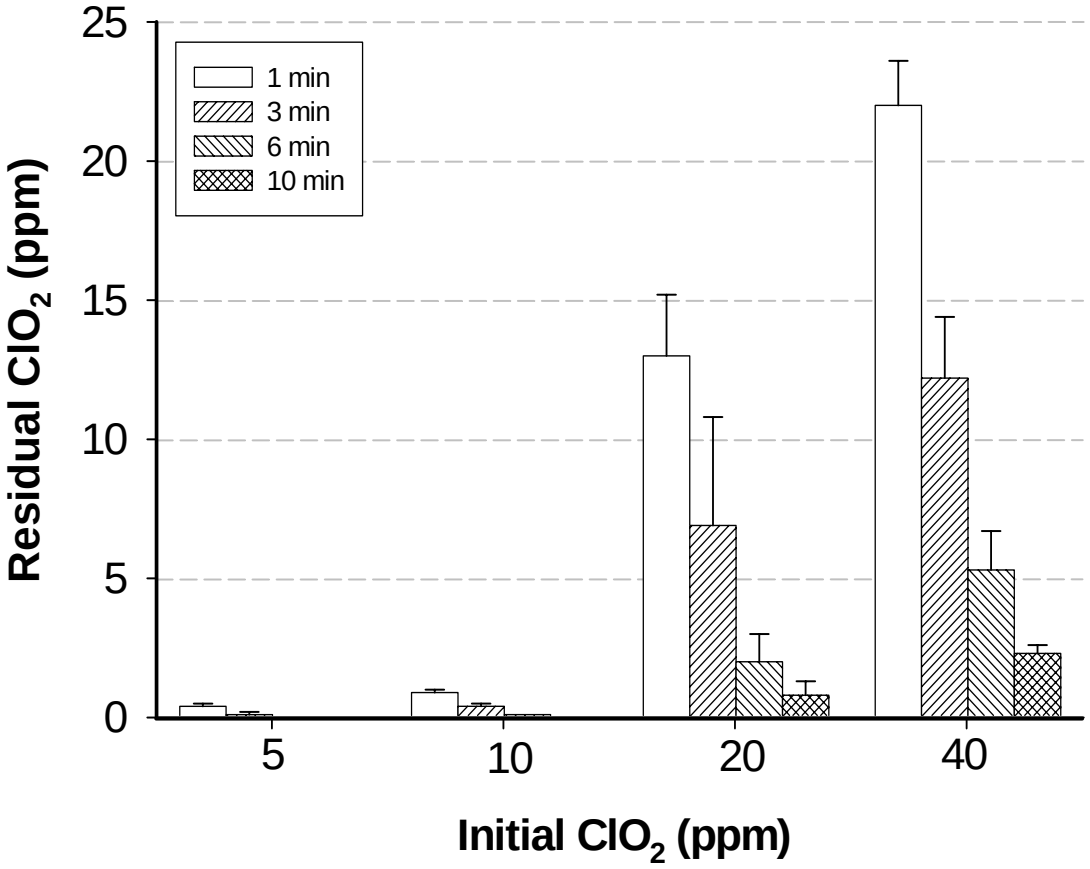

Figure 37-Chlorine dioxide residual change in the treating solution of chicken breasts at various times (1, 3, 6 & 10 min) and initial concentrations (5, 10, 20 & 40 ppm) combined with ultrasound 120 kHz. Data are shown as means of the concentration of residual ClO_2 ± SD.

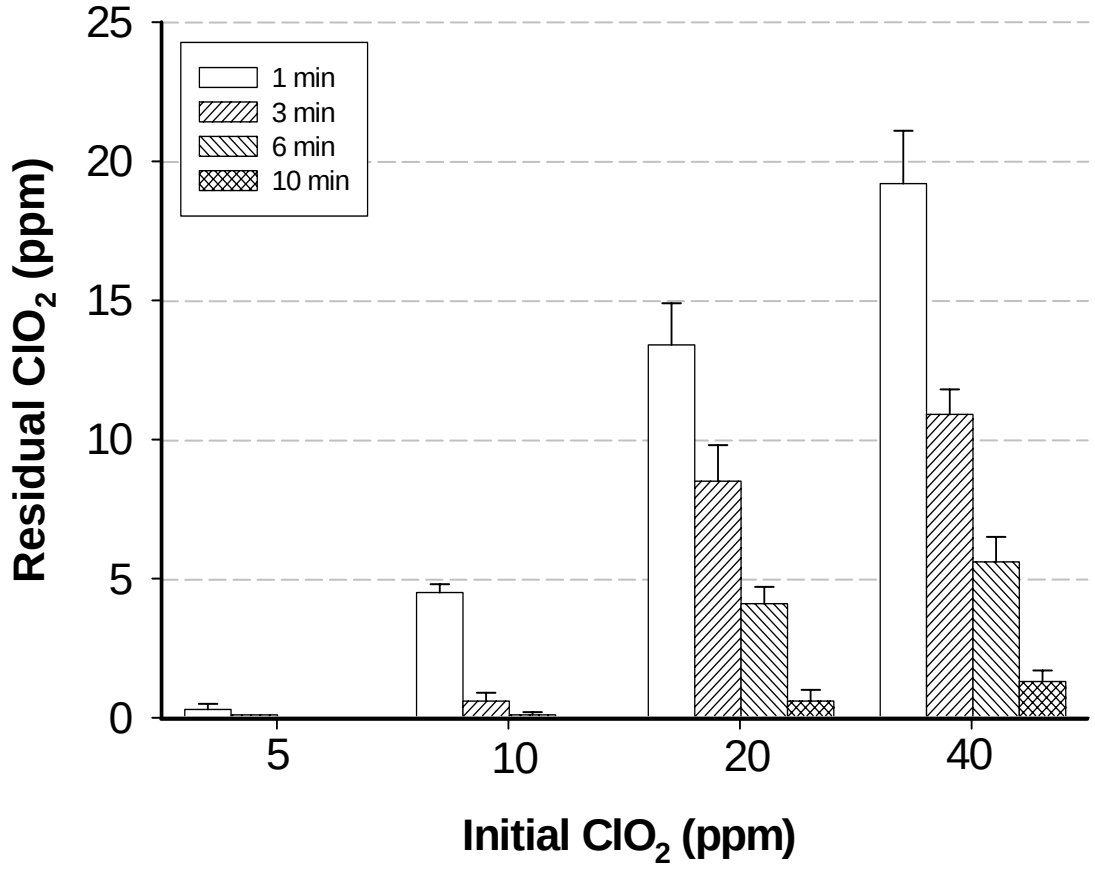

Figure 38-Chlorine dioxide residual change in the treating solution of chicken breasts at various times (1, 3, 6 & 10 min) and initial concentrations (5, 10, 20 & 40 ppm) combined with ultrasound 170 kHz. Data are shown as means of the concentration of residual ClO_2 ± SD.

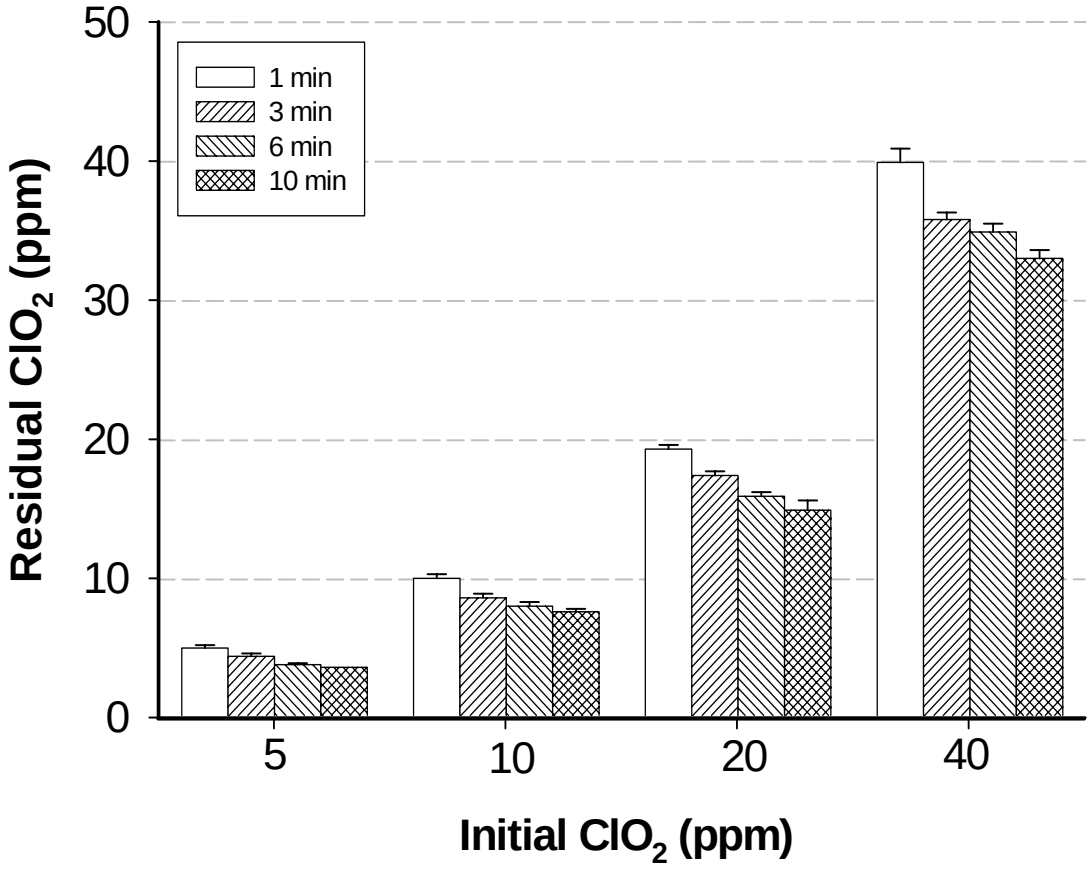

Figure 39-Chlorine dioxide residual change with various initial concentrations (5, 10, 20 & 40 ppm) and different times (1, 3, 6 & 10 min) in the treating solution of lettuce. Data are shown as means of the concentration of residual ClO_2 ± SD.

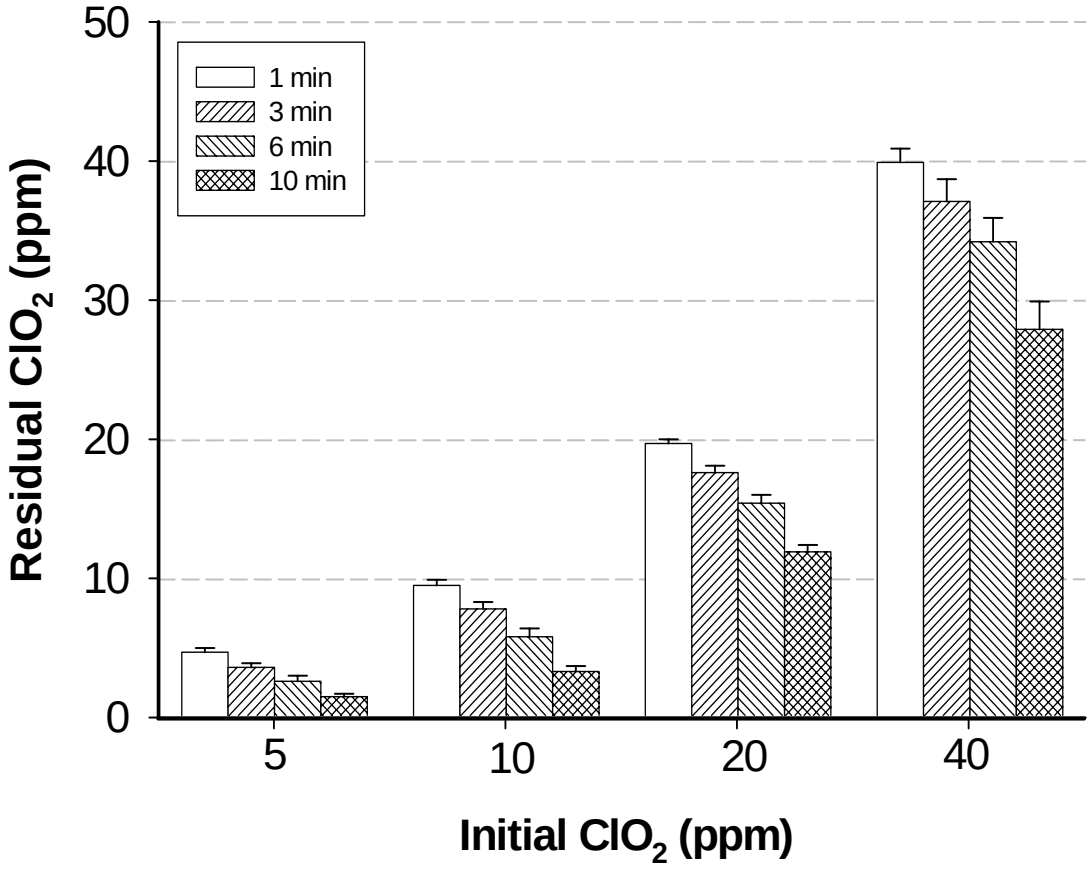

Figure 40-Chlorine dioxide residual change in the treating solution of lettuce at various times (1, 3, 6 & 10 min) and initial concentrations (5, 10, 20 & 40 ppm) combined with ultrasound 120 kHz. Data are shown as means of the concentration of residual ClO_2 ± SD.

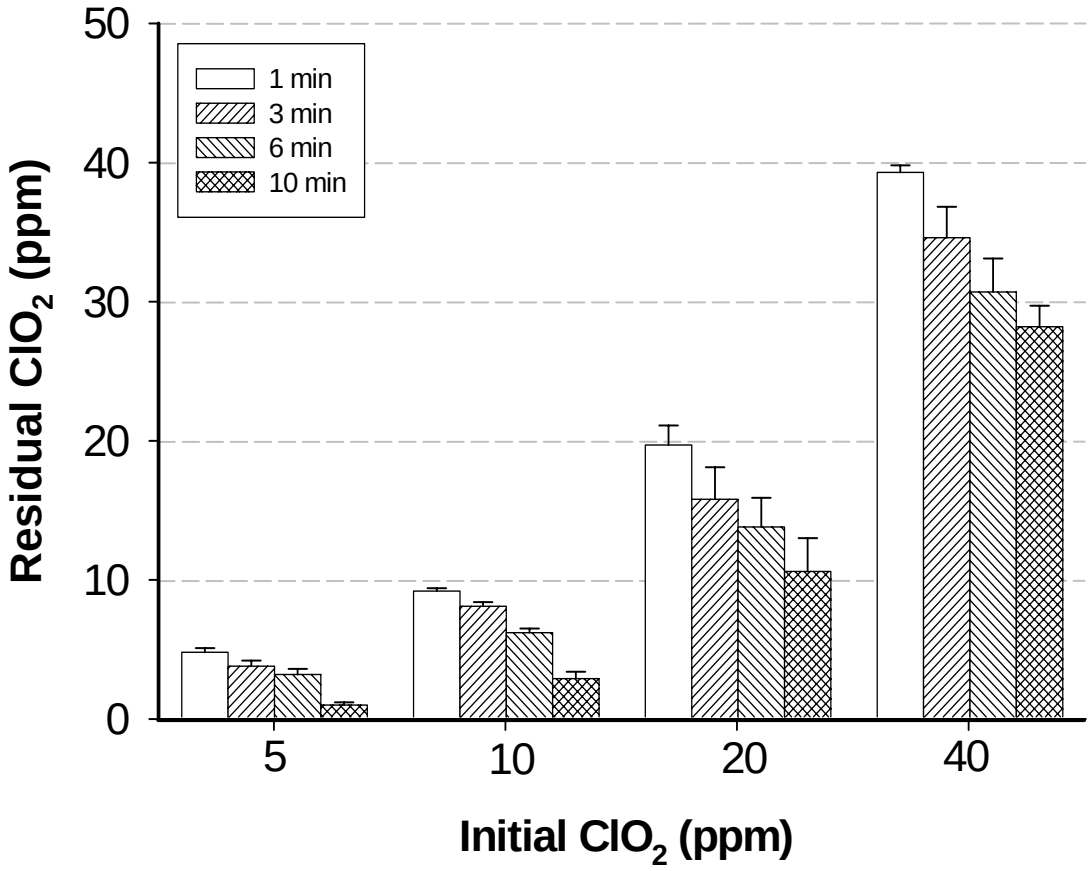

Figure 41-Chlorine dioxide residual change in the treating solution of lettuce at various times (1, 3, 6 & 10 min) and initial concentrations (5, 10, 20 & 40 ppm) combined with ultrasound 170 kHz. Data are shown as means of the concentration of residual ClO_2 ± SD.

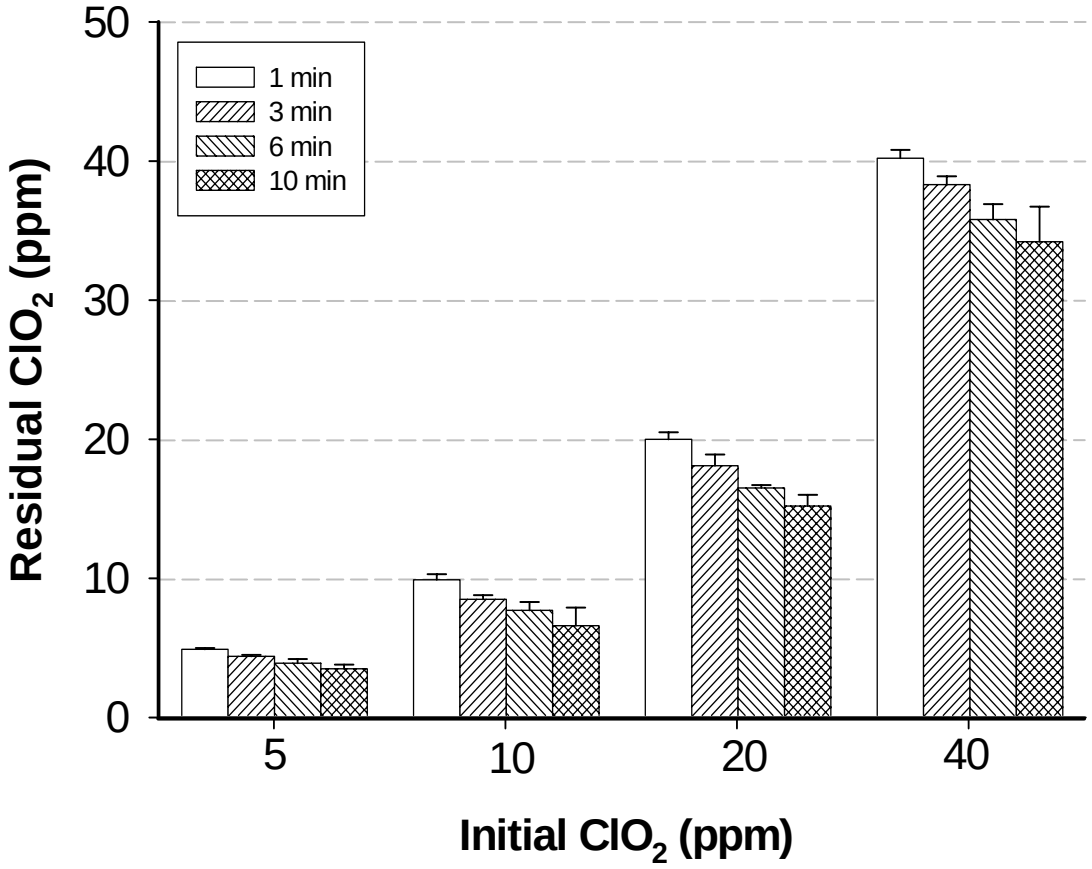

Figure 42-Chlorine dioxide residual change with various initial concentrations (5, 10, 20 & 40 ppm) and different times (1, 3, 6 & 10 min) in the treating solution of apples. Data are shown as means of the concentration of residual ClO_2 ± SD.

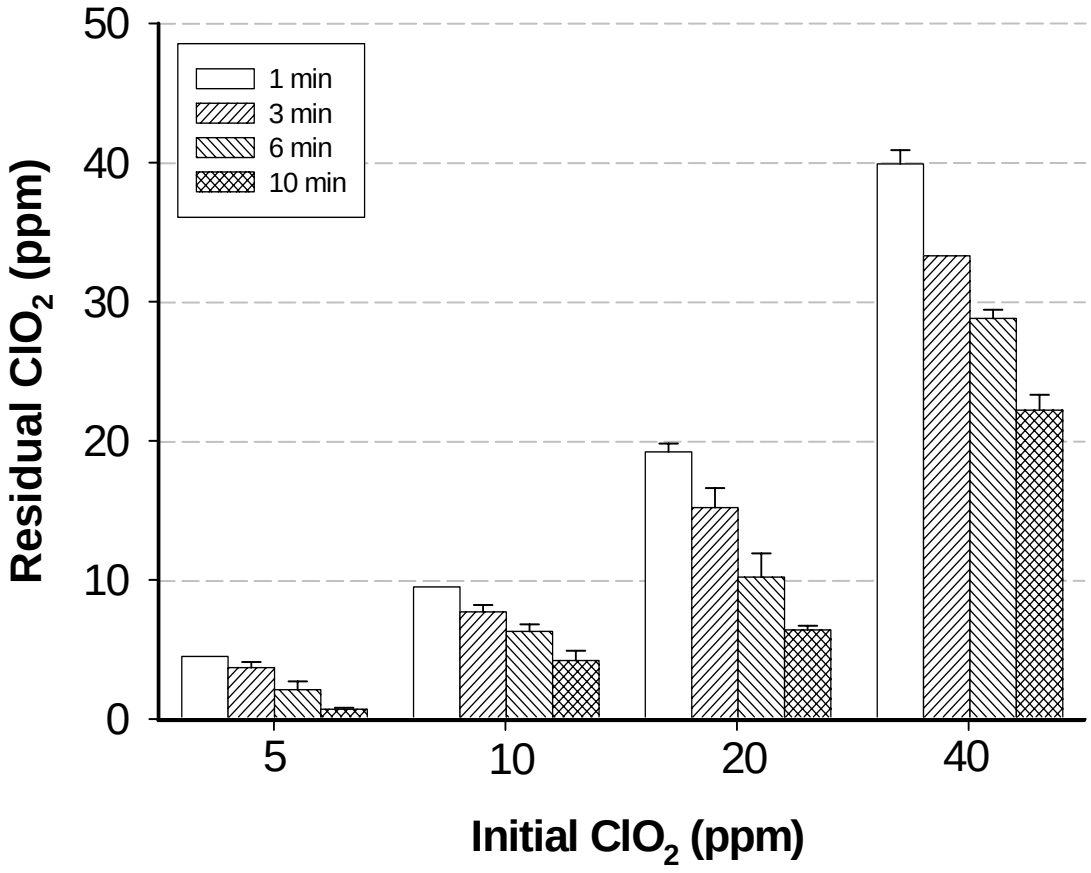

Figure 43-Chlorine dioxide residual change in the treating solution of apples at various times (1, 3, 6 & 10 min) and initial concentrations (5, 10, 20 & 40 ppm) combined with ultrasound 120 kHz. Data are shown as means of the concentration of residual ClO_2 ± SD.

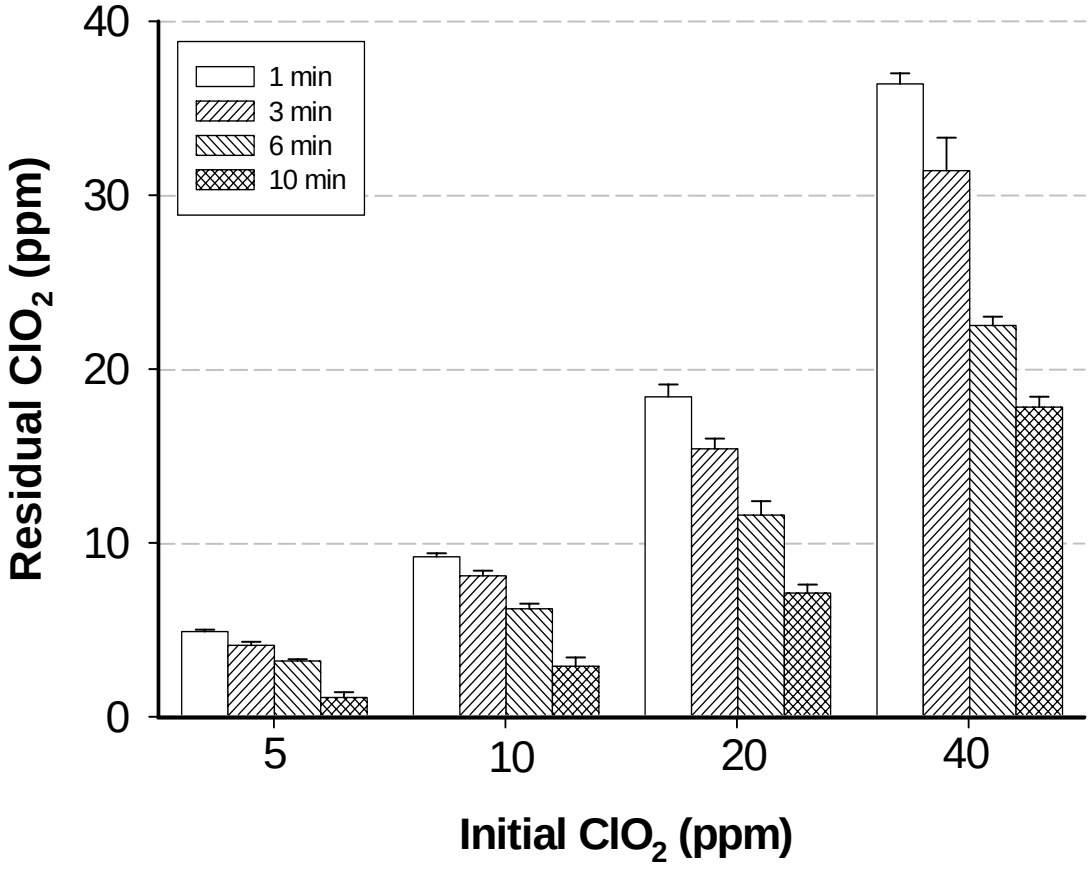

Figure 44-Chlorine dioxide residual change in the treating solution of apples at various times (1, 3, 6 & 10 min) and initial concentrations (5, 10, 20 & 40 ppm) combined with ultrasound 170 kHz. Data are shown as means of the concentration of residual ClO_2 ± SD.

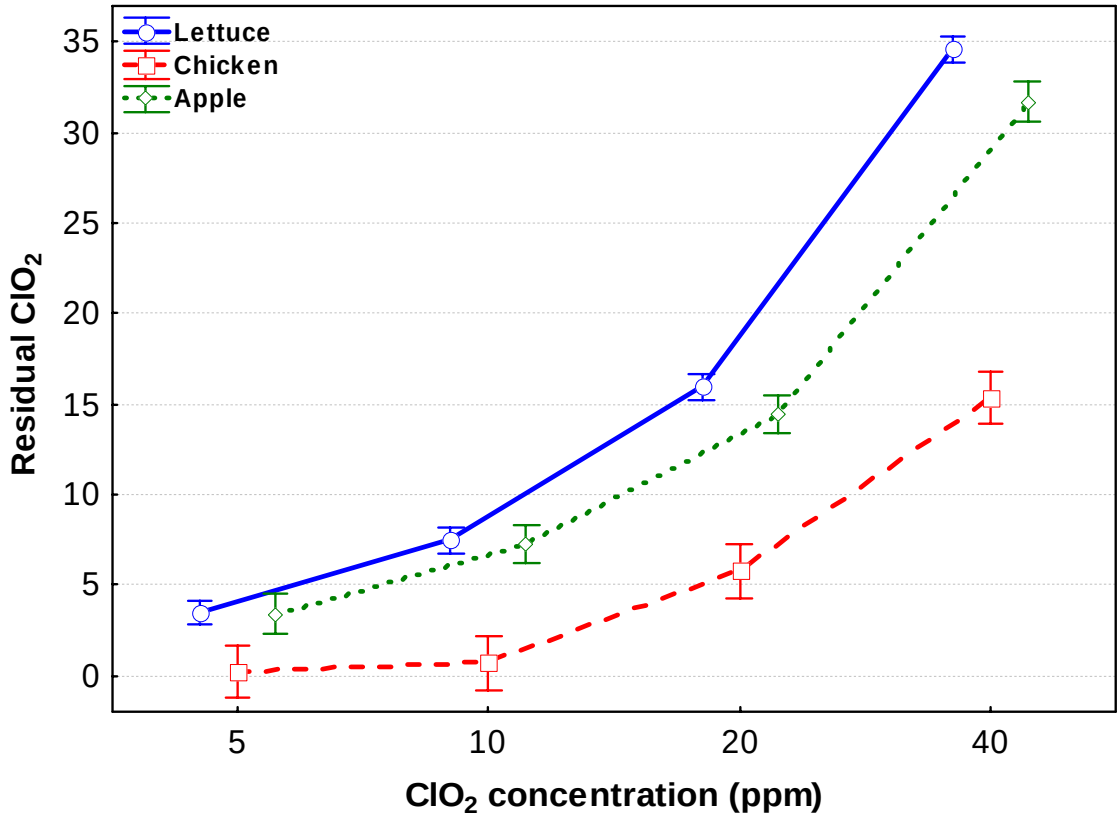

Figure 45-Summary of the effects of ClO_2 concentrations of the residual of ClO_2 on lettuce, chicken and apples. Data are shown as means of the concentration of residual ClO_2 ± 95% CI.

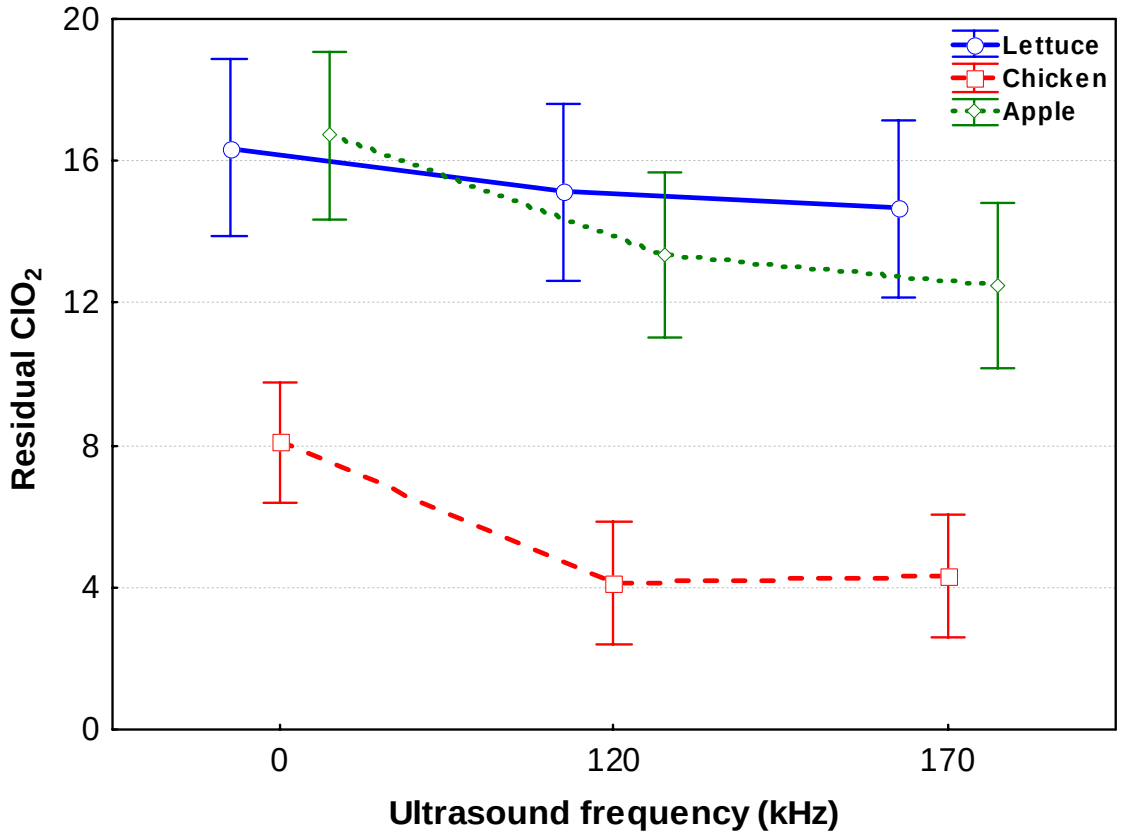

Figure 46-Summary of the effects of ultrasound frequencies of the residual of ClO_2 on lettuce, chicken and apples. Data are shown as means of the concentration of residual ClO_2 ± 95% CI.

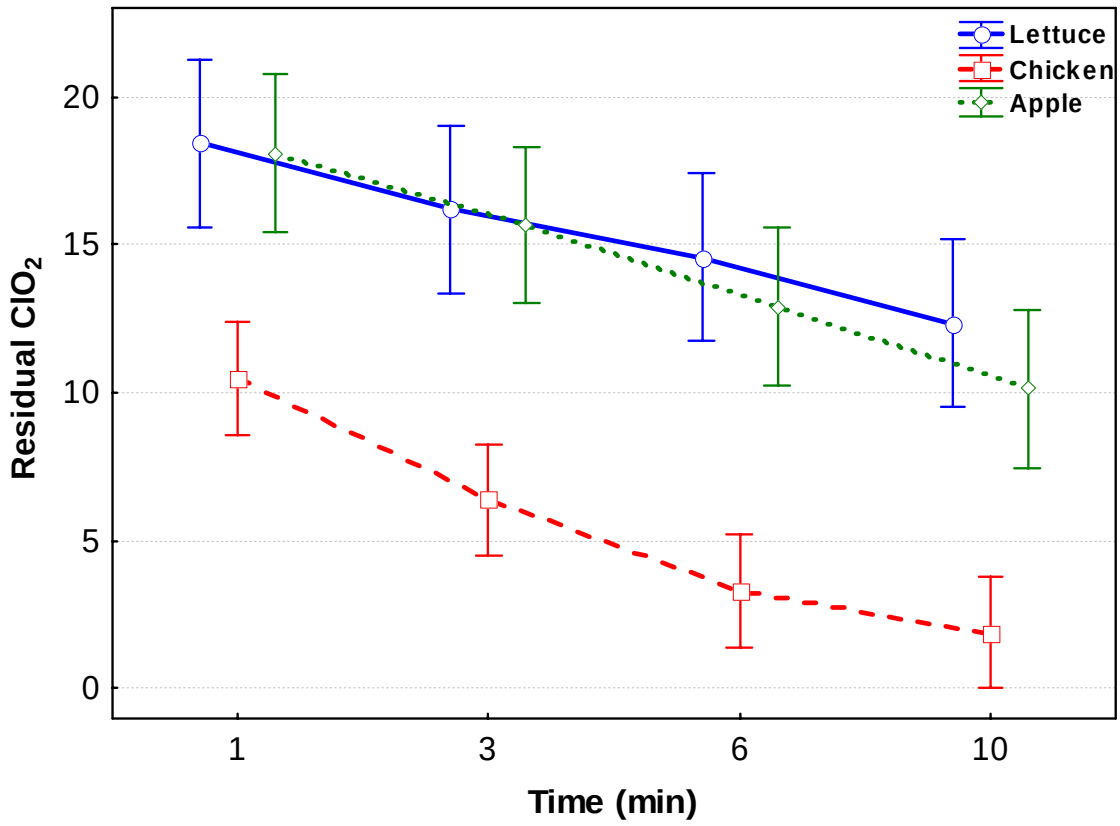

Figure 47-Summary of the effects of treatment times of the residual of ClO_2 on lettuce, chicken and apples. Data are shown as means of the concentration of residual ClO_2 ± 95% CI.

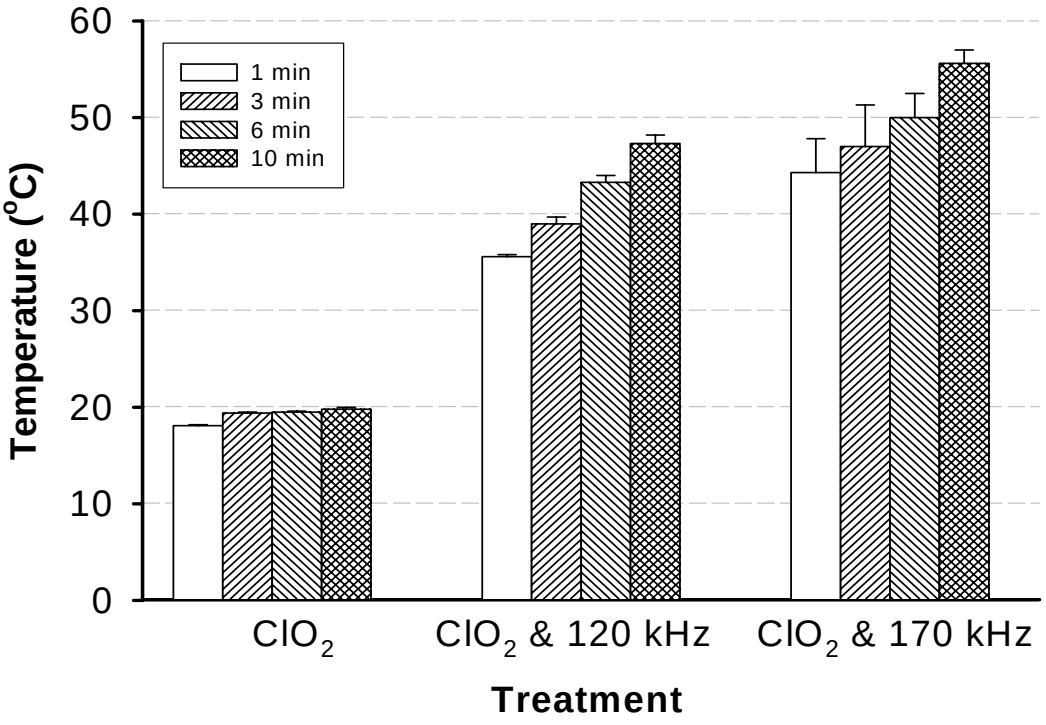

Figure 48-Temperature monitoring of ClO_2, ClO_2 and ultrasound 120 kHz, or ClO_2 and ultrasound 170 kHz at different times of 1, 3, 6, and 10 minutes in chicken breasts. Data are shown as means of temperature ± SD.

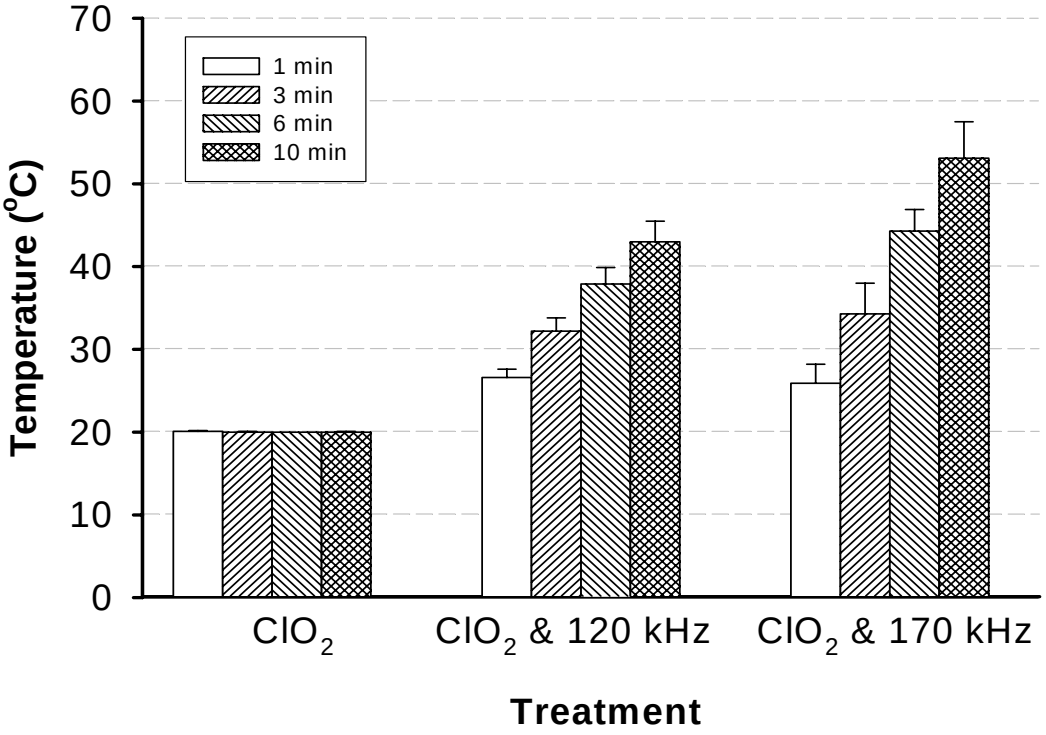

Figure 49-Temperature monitoring of ClO2, ClO2 and ultrasound 120 kHz, or ClO2 and ultrasound 170 kHz at different times of 1, 3, 6, and 10 minutes in lettuce. Data are shown as means of temperature ± SD.

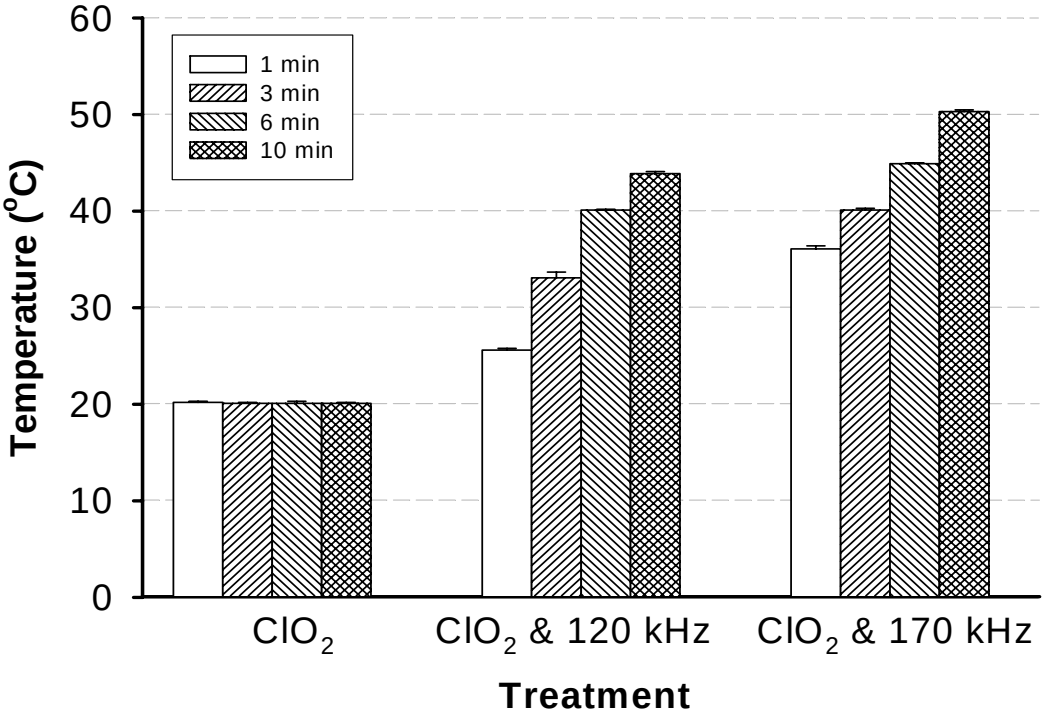

Figure 50-Temperature monitoring of ClO_2, ClO_2 and ultrasound 120 kHz, or ClO_2 and ultrasound 170 kHz at different times of 1, 3, 6, and 10 minutes in apples. Data are shown as means of temperature ± SD.

CONCLUSION

In this study, the effectiveness of ClO_2 at 5, 10, 20, and 40 ppm combined with ultrasonication at 120 and 170 kHz in decontaminating *E. coli* O157:H7 and *Salmonella* inoculated onto lettuce, chicken breasts, and apples have been evaluated at 1, 3, 6, 10 min.

The effectiveness of removing and inactivating *E. coli* O157:H7 or *Salmonella* on lettuce mainly depended on the ClO_2 concentration, not on the treatment times and ultrasonication. Various ClO_2 concentrations showed significantly different bactericidal effects, the higher the ClO_2 concentration the higher the log reduction. With a high inoculum of *Salmonella* on lettuce, the log reduction for the ClO_2 treatment combined with ultrasonicaton was significantly higher than that for the ClO_2 treatment alone. However, this did not occur for the low inoculum level for *Salmonella* or for the high or low inoculum level for *E. coli* O157:H7.

The effectiveness of removing and inactivating *E. coli* O157:H7 or *Salmonella* on chicken breasts was mainly dependant on the treatment time, and not on ClO_2 concentration and not ultrasonication. The longer treatment time had a significantly higher log reduction for both the high and low inocula levels for the bacteria, especially for *Salmonella*. The log reduction for *E. coli* O157:H7 for the ClO_2 treatment was significantly higher than for the ClO_2 treatment combined with ultrasonication, which was

similar for both the low and high inocula levels for *Salmonella*. Combined treatment with 170 kHz ultrasonication resulted in lower log reductions than that with 120 kHz ultrasonication. This is an unexpected result, and needs further investigation.

In comparing the log reduction for water treatments of chicken breasts and lettuce, the decontamination of lettuce was significantly more effective than for chicken breasts. This might suggest that it was due to the different surface structures of lettuce and chicken breasts. Bacteria attached or entrapped on the chicken breasts surface were more difficult to remove or inactivate by liquid disinfectant; while, the bacteria on the lettuce surface were easily removed and inactivated by liquid disinfectant.

On the high and low inocula of *E. coli* O157:H7 inoculated chicken breasts or lettuce, the decontamination efficiencies were varied with the same treatments. In the high inoculum of *E. coli* O157:H7 chicken breasts, the log reduction was significantly higher than that in low inoculum samples, but the lettuce resulted in the opposite effect. In the high and low inoculum of *Salmonella* chicken breasts or lettuce, the decontamination efficiencies were similar under the same treatments. In investigating the role of ultrasonication combined with chlorine dioxide treatments in decontamination of pathogens, results showed the opposite between chicken breasts and lettuce. Ultrasonication decreased the effectiveness of ClO_2 treatments in decontamination of chicken breasts, but it increased the decontamination effectiveness on lettuce.

In this experiment, the effectiveness of removing and inactivating *Salmonella* on apples was mainly dependant on the ClO_2 concentration and ultrasonication, it was not based on the treatment time; for *E. coli* O157:H7, it mainly depended on the ClO_2 concentration, not treatment time and ultrasound. A significant difference was shown

between ClO_2 treatments combined with ultrasonication and ClO_2 treatments alone for *Salmonella* attached to apples, but this result didn't appear in *E. coli* O157:H7.

The ClO_2 residual and temperature change during the treatment of ClO_2 and ultrasonication were also monitored. In chicken breasts, ClO_2 residuals dropped dramatically with longer treatment times, while, ClO_2 residuals only dropped a little in lettuce and apples. ClO_2 residuals dropped more dramatically in combination treatments than in ClO_2 only treatments. However, no significant differences were found between these two treatments on lettuce and apples. These results indicated that the ultrasonication could accelerate the reaction of ClO_2 with chicken components but not with lettuce and apples, or the decomposition of chlorine dioxide. Among these three samples, the temperature changes were similar to each other under the same treatments. Temperature went up to almost 60°C after the application of ultrasonication, and it elevated more dramatically under the treatment of ClO_2 combined with ultrasonication than with ClO_2 treatment only.

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