Efficacy of selected chemicals and electron beam irradiation for destroying *Escherichia coli* 0157:H7 and *Salmonella* on cantaloupe skin

by

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CHAPTER 1. GENERAL INTRODUCTION

INTRODUCTION

Increased public awareness in health benefits of consuming fresh fruits and vegetables in recent years dramatically increased per capita consumption of fresh produce. This increased consumption resulted in increase of importation of fresh fruits and vegetables from countries with lower standards for growing and handling produce. Consequently, this increased consumption coupled with increased incidents of foodborne outbreaks in recent years.

Several outbreaks related to cantaloupes contaminated with *Salmonella* and *Escherichia coli* 0157:H7 occurred in recent years (CDC, 1991; Rise, 1990). The sources of these outbreaks were cantaloupes imported from Mexico. These outbreaks in total involved more than 800 cases in 40 states and even two deaths.

Most of the current antimicrobial sanitizers used to decontaminate fresh fruits and vegetables do not achieve complete elimination of bacterial microflora from the surface of the fresh produce, partly due to the inaccessibility to locations within structures and tissues that may harbor pathogens. Therefore, the use of more elaborate processes and sanitizer solutions or their combination might be needed for total elimination of bacteria from the surface of fresh produce.

Irradiation technology has shown to be effective in destroying pathogens from the surface of fruits and vegetables (Prakash, 2004; Niemira, 2003; Martins et al., 2004). Based on the research done in our laboratory low-dose irradiation can reduce the numbers *Salmonella* and *E. coli* 0157:H7 from the surface of cantaloupes (Mendonca, 2002). However,

the use of higher irradiation dosage required for destroying the pathogens results in reduction of the textural characteristics of the fruits and vegetables.

To our knowledge, there are no published reports on the combination of the sanitizing treatments and low-dose irradiation for destroying *Salmonella* and *E. coli* 0157:H7 from cantaloupe skin. Therefore, the objectives of our study were: 1) to formulate the sanitizer solution with increased accessibility to the surface of cantaloupes, and 2) to combine the effect of the sanitizer with low-dose irradiation to achieve maximum effect in destroying pathogens from the surface.

CHAPTER 2. LITERATURE REVIEW

Microbial contamination of fresh produce

During the growth in fields and orchards, fruits and vegetables become contaminated with microorganisms from many sources in the natural environment. Sources of contamination include but are not limited to contaminated irrigation water, soil, air-borne dust, inadequately composted manure, insects, wild and domestic animals and their feces. Therefore, fresh produce often has a bacterial load reaching 10^4-10^6 microorganisms per cm^2 by the time it arrives at the packinghouse (Beuchat, 1995). Procedures such as washing, cooling, handling in the food service facility, poor hygiene of field and processing workers, inadequate cleaning of processing equipment, and transportation could also contribute to the contamination or the cross-contamination of the fresh produce (Beuchat, 1995; Beuchat 1996; Brackett, 1992; Nguyen-The, et al.,1994).

Foodborne disease outbreaks caused by fresh produce

During the last decade consumers' year-round demand for fresh fruits and vegetables increased due to the increased public awareness of the health benefits linked to the consumption of fresh produce. This increased demand for fresh produce has contributed to a $36.2 billion increase in retail and food-service sales from 1987-1997 (Institute of Food Technologies, 2001). In order to support this year-round demand for fresh produce there has been an increase of importation of fruits and vegetables from foreign countries that may have lower standards for growing and handling produce.

Therefore, this increased consumption of fresh fruits and vegetables coupled with year-round availability of these products have increased the number of foodborne outbreaks caused in recent years. Even though, the total number of outbreaks did not dramatically change between periods of 1987 to 1992 and 1993 to 1997, the number of fresh produce-related outbreaks increased considerably (Bean, et al., 1997; Olsen, et al., 2000). *Escherichia coli* 0157-H7 and *Salmonella* were responsible for 61% of fresh produce outbreaks in years 1993-1997 (Olsen, et al., 2000).

Outbreaks associated with cantaloupes

Cantaloupe has been associated with six multistate outbreaks of foodborne disease and more than 800 cases of *Salmonella* infections between 1990 and 2001 in the United States and Canada (U.S. Food and Drug Administration, 2001). The first outbreak occurred in 1990 in thirty states and involved 245 individuals and two deaths (Ries et al., 1990). The second outbreak affected 23 states and Canada in 1991 and implicated more than 400 laboratory-confirmed cases (Centers for Disease Control, 1991). Two outbreaks occurring during 2000 to 2002 involved 89 people in 21 states and were associated with cantaloupes imported from Mexico. A 1997 outbreak in California sickened 24 individuals. Another outbreak occurred in Ontario, Canada and involved 22 cases. Several serotypes of *Salmonella enterica* caused these outbreaks including Chester, Poona, Saphra, and Oranienburg. These outbreaks led to the issuance of an import alert on cantaloupes imported from Mexico by the U.S. Food and Drug Administration and the Canadian Food Inspection Agency until importers could certify that Mexican cantaloupes were produced under more sanitary conditions (U.S. FDA, 2002: Canadian Food Inspection Agency, 2003). Additionally, in

1992 FDA released fresh produce handling recommendations for processors and consumers to reduce the chances of foodborne diseases from fresh produce (U.S. FDA, 2002). These recommendations suggest washing fruits and vegetables with the use of a clean produce brush under cold tap water immediately before cutting (Center for Food Safety and Applied Nutrition, 2001). Canadian Food Inspection Agency and Health Canada also published set of guidelines for consumers suggesting the washing of whole cantaloupes with hot water and produce brush, and avoiding the use of any bruised or damaged fruits (Canadian Food Inspection Agency, 2002).

Escherichia coli

History

Escherichia coli was first discovered by German physician Theodor Escherich in 1885 during his microbiological analysis of the gastrointestinal microflora of infants. He named one of the isolated microorganisms *Bacterium coli commune* (Sussman, 1985). It was found that *Bacterium coli* was one of the commonly isolated microorganisms from intestinal microflora of humans. Castellani and Chalmers in 1919 observed that *Bacterium coli* has different biochemical and morphologic properties for this genus and proposed to call the new genus *Escherichia* (Bettelheim, 1991). The genus was included in the family *Enterobacteriaceae* with its discovery in 1937. On the meeting of the Judicial Commission of the International Committee on Bacteriological Nomenclature in 1958, the name *Escherichia* was used to describe the type genus for the family and *Escherichia coli* to the type species (Judicial Commission, 1958). In 1971, with the fourteen state outbreak of *E. coli* in imported cheeses, *E. coli* has been recognized as a food-borne pathogen. Meatborne outbreaks

occurred in the United States during 1982-1993 confirmed the foodborne status of the pathogen (Jay, 2005).

Species and characteristics of type genus *Escherichia*

Presently there are five biochemically distinct species in the type genus *Escherichia*: *E.coli, E. blattae* (isolated from the hind gut of the cockroach *Blatta orientalis*), *E. fergusonii, E. hermannii* and *E. vulneris* (Farmer et al., 1985).*Escherichia coli* are defined as Gram-negative, non-sporing straight rods, with the size of bacterium being 1.1-1.5 × 2.0-6.0 μm. Often motile if peritrichate flagella present or non-motile. *Escherichia* species ferment glucose with the formation of acid or acid and gas. The pathogen is oxidase negative and catalase positive. *E. coli* is facultatively anaerobic with an optimum growth temperature of 37°C (Bell and Kyriakides, 1998). *Escherichia coli* are known as antigenically heterogeneous species due to the three types of antigens on the surface of the bacterial cell: O (outer membrane) antigens, H (flagella) antigens and K (extracellular envelope (capsule)) antigens.

Pathogenicity of the genus

There are wide ranges of diseases that are caused by *E. coli* depending on the expressed virulence factors. Possession of adhesions or colonization factors, haemolysin production, ability to invade epithelial cells of the small intestine and toxin production are some of the virulence factors possessed by pathogenic *E. coli* serotypes. Based on their virulence types these serotypes are currently divided into several groups: enteropathogenic (EPEC), enterotoxigenic (ETEC), Vero cytotoxigenic (VTEC), Enteroinvasive (EIEC), Enteroaggregative (EAggEC), Enterohemorhagic (EHEC) and diffusely adherent (DAEC).

Enetropathogenic *E. coli* (EPEC)

Strains of this particular group have several virulence factors but the A/E factor (attachment-effacement factor) considered one of the most essential. EPEC strains do not produce detectable quantities of Stxs. They have adherence factor plasmids that allow adherence to the intestinal mucosa. Formation of attachment-effacement (att-eff, A/E) lesions occurs following bacterial adherence to mucosa and begins with initial contact and continues with a help from plasmid-encoded bundle-forming pili. Cytoskeletal rearrangement with a subsequent tyrosine phosphorylation of Tir occurs after EPEC-secreted proteins (Esps) blocks phagocytosis. Tight attachment of Tir to the outer membrane protein intimin causes destruction of brush border microvilli of the intestinal wall and forms pedestals (Jay, 2005; Bell et al., 1998; Janda et al., 1998).

EPEC strains mainly cause diarrhea in infants and children under age of two. Infectious dose can vary from 10^6 to 10^{10} CFU (colony forming units). Onset of illness might take sometime between 17 to 72 h, with an average of 36 h and last from 6 h up to 3 days. In infants strains of this group cause severe diarrhea that may persist for more than 14 days. In adults symptoms include nausea, vomiting, abdominal cramps, headache, fewer, chills, and severe watery diarrhea with prominent amounts of mucus without blood (Janda et al., 1998).

Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* have four types of fimbrial colonization factors antigens (CFAs) I, II, III, and IV that help them to attach and colonize small intestine. Formation of

plasmid encoded CFAs do not occur under 20°C and are usually encoded with the same plasmid that encodes a heat-stable enterotoxin.

One or two enterotoxins are formed following attachment (Jay, 2005).

ETEC strains of *E. coli* are leading causes of travelers' diarrhea among both children and adults. Usually more than 10^8 CFU are needed to cause illness and produce diarrhea that might last from 3 to 19 days. ETEC-induced symptoms are accompanied by low-grade fewer, abdominal cramps, malaise, nausea, and watery diarrhea. In severe cases of disease symptoms might include cholera-like extreme diarrhea with rice-water-like stools and could lead to dehydration (Janda et al., 1998; Bell et al., 1998).

Enteroaggregative *E. coli* (EAggEC)

Strains of this group are similar to EPEC strains but differ in one distinctive aggregative attachment behavior. These strains display a "stacked-brick" type adherence to HEp-2 cells. In addition, they carry a 60-MDa plasmid required to produce specific outer membrane protein (OMP) and fimbriae that help produce aggregation of the organism during attachment. Some strains produce a heat-stable enterotoxin (ST) designated as EAST1 (Jay, 2005).

Illness caused by the EAggEC strains has an extended duration of more than 14 days, particularly in infants. The disease is usually mild accompanied by fever, malaise, vomiting, abdominal pain, and bloody diarrhea (Janda et al., 1998; Bell et al., 1998).

Enteroinvasive *E. coli* (EIEC)

EIEC strains invade colonic epithelial cells and spread laterally cell to cell instead of just producing enterotoxins. Symptoms of the disease are very similar to dysentery caused by *Shigella* to which it is genetically related. 140-MDa Enteroinvasive plasmids (pINV) that resemble those in *Shigella Flexneri*, are responsible for the specific characteristics of this group (Jay, 2005). The onset of illness last 2 to 48 h with an average of 18 h. Commonly associated symptoms include fever, severe abdominal cramps, malaise, toxemia, and watery diarrhea (Jay, 2005; Janda et al., 1998; Bell et al., 1998).

Enterohemorrhagic *E. coli* (EHEC)

Strains of the EHEC group are different from other groups in their massive production of Shiga-like toxins and proliferation in large intestine environment. Attachment-effacement lesions produced by EHEC strains start with assembly of 60-MDa plasmid encoding fimbriae. Generally, invasion of HEp-2 or INT407 cells lines does not occur, even though attachment to culture cells happens with the assistance of the above stated fimbriae.

Onset of the disease usually lasts between 24 and 48 hours accompanied by abdominal pain and watery diarrhea. However, apart from diarrhea that ceases with the time, this infection progresses with increased severity of symptoms. Symptoms include vomiting, abdominal pain, nausea, bloody diarrhea, and chills. Hemorrhagic part of the infection most often last 2-4 days, and the full duration of gastrointestinal symptoms usually does not last more than 10 days.

There are several distinguishing features that differentiate *E. coli* 0157:H7 infection from others. First of all, it is possible to differentiate typical infection from the rest by the

presence of large amounts of blood in the stools of infected people. The number of bowel movements per person a day ranges from 3 to 30 (Cohen, 1991). Another distinguishing feature is prolonged incubation period of the disease that might last for 5 to 6 days. One more very important feature of *E. coli* 0157:H7 infection is the dose required to cause the disease. Less than 1,000 organisms could be enough to cause the infection which is very low compared to the standard infectious doses for some other pathogenic *E. coli* ranging from 10^6 to 10^{10} CFU. Lack of fever and fecal leukocytes or mucus in the stools of infected people also assists in distinguishing this particular infection. In adults excretion of the organism can continue for 4 to 9 days after infection; however, in children the duration of excretion might last for more than 21 days (Janda et al., 1998).

Following primary infection there is a very high probability of developing two very dangerous complications namely hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Among several of different forms of HUS, the "Classic" one is considered to be the most common cause of acute renal failure in children (Su, 1995). This Classic form is accompanied by three most important symptoms of the disease: microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure (Tarr, 1995; Su, 1995; Boyce, 1995). HUS develops in 2 to 14 days after the onset of diarrhea and accompanied by fever, abdominal cramps, vomiting, oligoanuria, edema, and pallor (Tarr, 1995; Su, 1995). The disease is rarely complicated by hemorrhagic cystitis, balanitis, and anal dilation (Karmali, 1989). The main concern in HUS cases is production of disease in parts of the body other than kidneys and can include pancreatic thrombotic microangiopathy, hepatomegaly, and cholelithiasis (Siegler, 1994). Coma, seizures, hemiparesis, and stroke

that are part of the neurologic sequelae develop approximately in quarter of children infected with HUS (Tarr, 1995).

Thrombotic thrombocytopenic purpura (TTP) usually prevalent in adults and in addition to symptoms associated with HUS, is accompanied by fever, prominent nerve-associated abnormalities, and milder damage to the kidneys (Ashkenazi, 1993).

Prevalence in fresh produce

A number of outbreaks of *E .coli* 0157:H7 have occurred in recent years in which consumption of fresh produce has been implicated. One of the largest outbreaks that occurred in Japan in 1996 has been traced to consumption of raw sprouts and involved more than 9,500 people (Gutierrez, 1996). The strain causing the infection has been identified as *E. coli* 0157:H7. This outbreak affected mostly children in more than 24 prefectures of the country. Radish sprouts present in lunches were believed to be a source of the microorganism. Two outbreaks occurred in the United States in period of June to July of 1997. There have been 60 reported cases of *E. coli* 0157:H7 infection in Michigan all traced to previously consumed alfalfa sprouts. The age of infected people ranged from 2 to 79 years old. Among affected people 54% required hospitalizations and 96% suffered bloody diarrhea. The outbreak involved two cases of HUS and one person suffered from TTP. Twenty four cases of infection that occurred at the same time in Virginia had the same bacterial isolates of those occurring in Michigan. The strain causing the infection was found to be *E. coli* 0157:H7 PT32 (Como-Sabetti et al., 1997).

Consumption of contaminated fresh apple cider led to an *E. coli* 0157:H7 outbreak in Massachusetts in 1991. The infection involved 23 people who developed haemorrhagic

colitis and hemolytic uraemic syndrome. An outbreak with similar syndromes occurred in Canada and involved 14 people. Even though medical workers were not able to isolate bacteria from the specimens, the cause was believed to be *E. coli* 0157:H7 due to the characteristic symptoms of the disease developed by the patients. Another two outbreaks occurred in 1996 in the USA. Commercial apple cider affected 66 people and resulted in one death in Western United States. Second outbreak occurred in Connecticut involving 14 people of which 7 required hospitalizations (CDC, 1996).

Salmonella

Brief history

The genus *Salmonella* has been established in 1900 in honor of Salmon who isolated the organism form pigs with hog cholera (LeMinor, 1984). Since then there have been numerous amounts of proposals for changes to the species designation.

Species of the genus

Recently *Salmonella* have been divided into two species: *S. enterica* and *S. bongori*, with the approximately 2,000 serovars being divided into five subspecies (Jay, 2005). The major group was divided into five subspecies: group II (*S. enterica* subsp. *salamae*); group IIIa (*S. enterica* subsp. *arizonae*); group IIIb (*S. enterica* subsp. *diarizonae*); group IV (*S.enterica* subsp. *houtenae*); and group VI (*S. enterica* subsp. *indica*). The group that was previously known as group V has been adjusted to species status as *S. bongori* (Jay, 2005). *Salmonella* has also been divided into distinct three groups based on epidemiological characteristics: the first group included strains affecting primarily humans such as *S.* Typhi, *S.*

Paratyphi A, and *S.* Paratyphi C; the second group has host-adapted serovars as *S.* Gallinarum, *S.* Dublin, *S.* Abortus-equi, *S.* Abortus-ovis, and *S.* Choleraesuis; and the last group included serovars with no host preferences.

Characteristics of *Salmonella*

The salmonellae are gram-negative, facultatively anaerobic, non-sporing wide rods. They are mostly found in the intestinal tract of animals and are able to reproduce in a range of warm- or cold-blooded organisms. The natural habitat of *Salmonella* is in a human and animal gastrointestinal tract but it could also survive for weeks in water environment and for years in soil. The range of temperature for growth of the organism is very wide and varies from 8 to 45°C.

Pathogenesis of the genus

All isolates of *Salmonella* serotypes are capable of causing the infections in humans. Pathogenicity of the disease could vary due to some factors such as strain virulence, initial dose, course of infection, and the immune system condition of the infected person.
After being ingested, the organism passes through stomach and localizes in small intestine by attachment to the terminal ileum. This process of attachment follows the previous bacterial adhesion to epithelial cells/M cells. Several researchers have shown that in some serotypes bacterial flagella and type 1 fimbriae might assist in the attachment process (Lockman et al., 1992). Rapid invasion of absorptive epithelial cells occurs following the attachment (Finlay et al., 1992). This process involves several different genetic loci. After invasion of the epithelial cells, the bacteria replicate within macrophages with subsequent formation of and

localization in large vacuoles. Host cell membrane ruffling, cytotoxic reaction, and apoptosis that follow permit infection, bacterial survival, and escape from the host's immune system (Janda et al., 1998).

The number of organisms enough to cause the infection varies greatly within the *Salmonella* genus and can be as low as 100-250 organisms and goes up to 10^8. Incubation period that might take 24 to 72 hours to develop the illness usually decreases with the increased numbers of bacteria (Guthrie, 1992; Blaser, 1982).

Salmonella infection is accompanied by four major syndromes that include gastroenteritis, enteric fever, septicemia with or without localized infection, and asymptomatic infection. Other symptoms include fever of short duration, mild to severe abdominal pain, transient bacteremia, and mildly to cholera-like diarrhea that is sometimes accompanied by blood. A small percent of the infected persons might develop irritable bowel syndrome characterized by diarrhea and urgency of bowel movements. Sequelae of *salmonella* infection can also develop incliding meningitis in 1.5 to 6% of newborns and children (Davis, 1981). The disease progresses very rapidly, with a big chance for sequelae even with the rapid and effective action taken to prevent the complications (Davis, 1981). Complete elimination of the organism from the cerebrospinal fluid is almost impossible and leads to relapses and treatment failures in 64% cases (Goldberg, 1988).

Prevalence of *Salmonella* in fresh produce

Until the last decade, the biggest percentage of food-borne outbreaks was caused by *Salmonella* serotypes coming from food stuffs of animal origin such as meat, dairy and eggs. However, due to the increased consumption of fresh fruits and vegetables in recent years the

frequency of *Salmonella* outbreaks associated with consumption of fresh produce had increased (Beuchat, 2002). The total number of laboratory confirmed *Salmonella* cases accounts for more than 40,000 incidents and results in approximately 500 deaths annually (CDC, 1998).

In the United States outbreaks caused by *Salmonella* serotypes were traced back to a large variety of following fruits and vegetables and minimally processed products such as: *S. Miami* and *S. Oranienburg* in watermelons (Gaylor, 1995; CDC, 1991); *S. Chester, S. Oranienburg, S. Poona,* and *S. Saphra* in cantaloupes (CFSAN, 2001; CDC, 1991; Mohle-Boetani et al., 1999); *S. Javiana* and *S. Montevideo* in tomatoes (Hedberg, 1994; Wood, 1991); *S. Typhimurium* in apple cider (Parish, 1997); *S. Hartford, S. Gaminara* and *S. Rubislaw* in orange juice (CDC, 1995); and *S. Stanley, S. Montevideo* and *S. Mbandaka* in alfalfa sprouts (Mahon et al., 1997; National Advisory Committee on Microbiological Criteria for Foods,1999). In addition to this list of fruits and vegetables implicated in salmonellosis outbreaks in the US there are other types of fresh produce that have been implicated in outbreaks of Salmonella in other countries such as: artichokes, beet leaves, cauliflower, chili, lettuce and cabbage in Spain (Garcia-Villanova Ruiz et al., 1987); bean sprouts in Sweden and Thailand (Anderson et al., 1989; Jerngklinchan et al., 1993); endive, fennel, and lettuce in Netherlands, and cilantro in Surinam (Tamminga et al., 1978).

In recent years, number of salmonellosis outbreaks was also traced back to consumption of unpasteurized "fresh- squeezed" orange juice. One of these outbreaks was caused by *Salmonella Hartford* and occurred in the state of Florida in 1995 (Parish, 1998). Another outbreak traced back to orange juice has implicated in ~ 300 cases and occurred in

the 15 states of the US and two provinces of Canada. The serovar responsible for this outbreak was *Salmonella Muenchen*.

Several food-borne outbreaks related to consumption of *Salmonella* contaminated cantaloupes have occurred in the US in 1990 to 2001. The first outbreak was caused by *S. enterica Chester* and has implicated in 245 cases occurring in 30 states of United States (CFSAN, 2001). The second outbreak occurred in the 23 states of US and Canada, and involved more than 400 laboratory confirmed cases of salmonellosis infection caused by *S. Poona* (Francis et al., 1991). One of the outbreaks was caused by *S. Saphra* in 1997 and involved 24 illnesses in California (Mohle- Boetani et al., 1999). *Salmonella* serotype *Oranienburg* was responsible for 22 cases in the capital of Canada in 1998 (CFSAN, 2001). Two outbreaks caused by *S. Poona* in 2000-2001 implicated in total of 89 illnesses and even two deaths (CFSAN, 2001).

Citric acid

Citric acid is a weak organic acid that is naturally present in citrus fruits. Another name of the citric acid is tricarboxylic acid. Molecular formula for the acid is $C_6H_8O_7$ and it has molecular weight of 192.14. The melting point of citric acid is 153°C. Solubility of the chemical varies with the temperature. It has good natural preservative characteristics and is therefore widely used in food and beverage industry. Citric acid (CA) naturally occurs in a large variety of fruits and vegetables, but it is mostly concentrated in citrus fruits such as lemon, lime, orange, and grapefruit.

The acidity of citric acid results from the three carboxy groups (-COOH) which can lose a proton in solution and form citrate ions. The reactions of these citrate ions with a variety of

metal ions can result in formation of citrates. One of the important citrates in food industry is calcium citrate or "sour salt" that is used for preservation and flavoring purposes.

Mode of action

The inhibitory action of citric acid is believed to be due to the several processes that take place inside the affected cell: membrane disruption, inhibition of essential metabolic reactions, stress on intracellular pH homeostasis (Salmond, 1984), and the accumulation of toxic anions (Brul, 1999). Organic acids in general have optimal inhibitory activity at low pH that allows free diffusion of uncharged molecule through plasma membrane of the cell. Following entry to the cell molecule starts dissociating and consequently releases charged anions and protons that do not cross membrane (Brull, 1999). In addition, acid molecules continue diffusing into the cell until equilibrium between outside and inside of the cell is reached, thus entrapping more anions and protons inside the cell (Booth, 1989). The accumulation of protons in the microbial cell results in acidification of the cytoplasm and subsequent inhibition of metabolic activities (Salmond, 1984).

Use of citric acid on fresh produce

Lopes investigated the effect of several combinations of sanitizers involving organic acids and surfactants on bacterial reduction. He used a combination of 19.3 mM of citric acid and 1.0 mM sodium lauryl sulfate; 161.5 mM of acetic acid combined with 0.67 mM dioctyl sodium sulfosuccinate; and a combination of 94.76 mM of lactic acid with 1.25 mM of sodium capryl lactylate. The sanitizers were tested against gram-negative and gram-positive bacteria such as *Salmonella typhimirium*, *Listeria monocytogenes*, *Escherichia coli* 0157:H7,

Pseudomonas aeruginosa, *Aeromonas hydrophyla*, and *Staphylococcus aureus*. All three sanitizers showed 99.999% or 5-log reduction in all bacterial population in 30 sec. These sanitizers also exhibit lethal activity on antibiotic-resistant bacteria such as *S. typhimirium* and *L. monocytogenes* (Lopes, 1998). Another study employing citric acid combined with sodium lauryl sulfate and ethylenediaminetetraacetic acid (EDTA) investigated the effect of the sanitizer on reduction of *S. typhimirium, L. monocytogenes, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* Sanitizing treatment of 0.6% resulted in >5 logs reduction in bacteria tested. The same treatment at concentrations of 0.6 to 1.75% was used for sanitizing Formica surface inoculated with *Staphylococcus aureus* in the presence and absence of organic material. Sanitizers at concentrations of 1.2% and higher produced significantly higher bacterial reduction compared to control in the absence of organic material. However, the presence of organic material seemed to decrease the antimicrobial properties of the sanitizer (Restaino, 1994).

In another study researchers evaluated inactivation of *Escherichia coli* 0157:H7 on apple slices after immersion into acid-containing treatments. Treatments contained 1.7% citric, 2.8% ascorbic, 50% commercial lemon juice, and 50% commercial lemon juice with preservatives. Apple slices were either stored at ambient temperature or dried for 6 h following the treatment. Initial bacterial reduction ranged from 0.9 to 1.3 logs CFU/g. Drying of apples at 62.8°C for 6 h decreased the numbers of bacteria on acid-pretreated slices by 6.7-7.3 logs CFU/g compared to 2.5-3.1 logs CFU/g in control treatments. Based on the results it was concluded that dipping of apples alone was not effective in destroying *E. coli*, but combination of dipping and drying processes significantly reduce the numbers of bacteria (Derrickson-Tharrington, 2005).

Hydrogen peroxide

Louis-Jacques Thenard first discovered hydrogen peroxide in 1818. It was first produced in United Kingdom by burning barium salt (Ba), to produce barium peroxide (BaO_2), which was subsequently dissolved in water to form hydrogen peroxide. At that time chemical was primarily used in medicine due to its instability. Development of electrochemical process of obtain of concentrated hydrogen peroxide with increased stability widened the area of use for this chemical.

Hydrogen peroxide is a chemical compound containing peroxide ion (O_2^{2-}). The structure formula of hydrogen peroxide (HP) is H-O-O-H. HP has very strong oxidizing potential. Its oxidizing power is higher that that of chlorine (Cl_2), chlorine dioxide (ClO_2) and potassium permanganate ($KMnO_4$). In fact its oxidizing power is rated just below that of ozone. HP solidifies at low temperatures. Pure HP is very dangerous: it might spontaneously ignite if comes in contact with flammable materials (wood, paper, oil or cotton); it can explode if mixed with organic materials (alcohol, acetone, ketones, aldehydes, and glycerol). It can also cause massive explosion in reaction with iron, copper, chromium, lead, silver, manganese, sodium, potassium, magnesium, nickel, gold, platinum, metalloids, metal oxides or metal salts. HP is not very stable: it slowly degrades into water and oxygen. Factors such as impurities (organic or inorganic), increase in temperature, and exposure to light accelerate this process.

HP is generally recognized as safe (GRAS) for use in foods as an oxidizing and reducing agent, antimicrobial agent and as a bleaching agent. It is also used in the formulation of sanitizing solutions: a concentration of 0.001-0.1% is inhibitory to bacteria and fungi, and 0.1% and higher provides bactericidal and fungicidal activity (Cords and Dychdala, 1993).

Antibacterial effect increases with increase in concentration. The food and Drug Administration approved its use in cheese production, preparation of modified whey and a thermophile-free starch.

Mode of action

Hydrogen peroxide acts as an antimicrobial by production of powerful reaction products, such as singlet oxygen or superoxide (O_2•). Superoxide oxygen is considered extremely reactive form of oxygen and is toxic to microorganisms. It can be produced chemically or biochemically or by reactions of specific enzyme systems. These systems found in milk, saliva, honey, white blood cells, and other living cells sustain a metabolic balance between hydrogen peroxide production and catalase generation (Cords and Dychdala, 1993). Reduction of hydrogen peroxide to water by glutathione peroxidase occurs in case of unbalance. Activation of peroxidase and consequent formation of toxic and fatal superoxide oxygen occurs following ingestion of microbial cell by phagocyte (Cords and Dychdala, 1993).

Another mechanism also uses the peroxidase system. In this system chloride ion in the cell reacts with hydrogen peroxide and consequently forms toxic hypochlorite ion (Klebanoff, 1968). This reaction follows by reaction of hypochlorite with hydrogen peroxide and formation of lethal superoxide ion:

$$Cl^- + H_2O_2 \rightarrow OCl^- + H_2O$$

$$H_2O_2 + OCl^- \rightarrow O_2 + OH + HCl$$

In a number of reactions occurring in the series of O_2 reductions by a single electron formation of reactive substances takes place followed by hydrolysis (Fridovich, 1975):

$O_2 + e^- \rightarrow O_2^-$ superoxide

$O_2 + e^- + 2H^+ \rightarrow H_2O_2$ hydrogen peroxide

$H_2O_2 + e^- + H^+ \rightarrow H_2O_2 + OH$ hydroxyl radical

$OH + e^- + H^+ \rightarrow H_2O$ water

Oxidative destruction of lipids and other biochemical components occurs under the influence of superoxide oxygen. Hydrogen peroxide produced by the following reaction can be produced in small amounts by most anaerobic organisms. Hydroxyl free radical is the most potent oxidizing agent and has the capability of causing severe cellular damage (Brock, et al., 1984). The presence of nontoxic metallic ions, such as iron, copper, and manganese usually enhances the bactericidal activity of hydrogen peroxide.

Another theory of hydrogen peroxide mode of action was proposed by Gould and Dring in 1962. They believed that hydrogen peroxide was responsible for oxidation of sulfhydryl groups and double bonds of proteins, lipids, and surface membranes. A different idea was described by Russel (1982). He suggested that hydrogen peroxide exhibits sporicidal activity by removing protein from the coat of the bacterial spore (Cords and Dychdala, 1993).

Use on fresh produce

Use of hydrogen peroxide in various forms such as liquid wash or as a vapor treatment on vegetables has been investigated by several researchers. USDA conducted research focused on reduction of *Botrytis cinerea* spores on table grapes with the use of both wash and vapor forms of hydrogen peroxide. Treatment of grapes with 30-35% H_2O_2 for 10

min at 40°C resulted in significant reduction in number of spores (Forney et al., 1991). In the following study, vapor treatment of grapes at concentration of 0.27 mg/L reduced the number of spores without compromising the quality of treated grapes (Rij and Forney, 1995). In 1996, Simmons treated cantaloupes with vapor concentration of 3 mg/L of air for 60 min. This resulted in reduction of microbial count and prevented decay for 4 weeks at 2°C (Sapers, 1998). Later, Sapers investigated the effect of H_2O_2 wash on mushrooms by dipping them in 5% H_2O_2 for 30 sec. In order to prevent browning following H_2O_2 treatment mushrooms were dipped in the erythorbate solution. Wash treatment with H_2O_2 suppressed blotching inducing bacteria.

Use of H_2O_2 as a method to extend the shelf-life of the vegetables has been investigated by Sapers and Simmons (1998). They dipped cut cantaloupe cubes for 2 min in 5% solution of H_2O_2 and rinsed them next in water to avoid residue. This treatment was shown to be effective in delaying the onset of spoilage. The same researchers investigated the dip of whole vegetables such as cucumbers, green bell peppers, and zucchini in 5 or 10% H_2O_2 for time not exceeding 2 min. This resulted in effective delay of soft rot formation (Sapers, 1998).

D. Ukuku investigated the effect of 5% H_2O_2 at 70° C on whole cantaloupes inoculated with *Salmonella* cocktail. Dipping of cantaloupes for 1 min resulted in significant reduction of aerobic mesophilic bacteria, yeast and mold and 3.8 log CFU/cm^2 reduction in *Salmonella*. Fresh cut pieces obtained from treated with H_2O_2 cantaloupes were *Salmonella* negative when plated onto agar (Ukuku, 2004). In another study conducted by Ukuku et al. (2005), *E. coli* inoculated cantaloupes treated with 2.5% H_2O_2 were compared to treatment containing 1% H_2O_2, nisin (25 mg/ml), 1% sodium lactate, and 0.5% citric acid (HPLNC). That study

was conducted using whole cantaloupes and honeydew melons. The HPLNC treatment of whole cantaloupes and honeydew melons resulted in reduction of *E. coli* counts by more then 4 and 3 $logs_{10}$ CFU/cm^2 respectively. H_2O_2 2.5% treatment reduced bacteria by approximately 3 $logs_{10}$ CFU/cm^2 on both types of cantaloupe. Populations of mesophilic aerobes on whole cantaloupes were reduced by 4 $logs_{10}$ CFU/cm^2 in H_2O_2 wash and more then 5 $logs_{10}$ CFU/cm^2 in HPLNC wash (Ukuku, 2005).

Sodium Lauryl Sulfate

CH_3-$(CH_2)_{11}$-O-$SO_3^-Na^+$

Sodium lauryl sulfate (SLS) is an anionic surfactant and characterized by structural balance between a hydrophobic residue and a negatively charged hydrophilic group (Cords and Dychdala, 1993). SLS act as a wetting agent by reducing the surface tension of aqueous solution, therefore, enhancing the spread of water over product surface. Despite the fact that SLS was approved for use on food contact surfaces in 1950, it has been successfully used in the dairy, beverage, and food processing industries only in the last three decades. It has been incorporated into the variety of chemical sanitizer solutions designed for equipment, utensils, and other surfaces of the eating and drinking establishments, and the processing plants. FDA has approved its use as an emulsifier in egg whites, as a whipping agent in the marshmallow preparation, and as an additive in dry beverages and fruit juice drinks (FDA, 1978).

Anionic surfactants are widely used in combination with variety of acids as sanitizing treatment. This combination is known as acid-anionic surfactant and exhibits excellent bactericidal activity against Gram-positive and Gram-negative bacteria (Dychdala, 1983). In general acid anionic sanitizing agents have a number of advantages compared to most

chemical sanitizing agents currently used by the industry, such as aldehydes, hypochlorites, peroxygen, and quaternary compounds. These beneficial differences are absence of covalent reactions, chemical stability, convenience of use, freedom from organoleptic properties, environmental safety, and biodegradability (Lopes, 1998). The optimum pH for antimicrobial action of acid-anionic surfactants ranges from 1.9 to 3.0 with reduction of bactericidal potency with increase of pH of the solution. Organic materials such as 0.05% skim milk or 0.05% peptone and water hardness exceeding 1000 ppm (as $CaCO_3$) do not readily decrease in bactericidal potency of acid-anionic sanitizer, but more time is needed to achieve bacterial reduction. Temperature increase increases bactericidal activity with an opposite effect with decrease of temperature (Dychdala, 1983).

Mode of action

There is still not an established theory of SLS mechanism of action, but several hypotheses have been proposed: general denaturation of proteins, inactivation of essential enzymes necessary to cell membranes, and disruption of cell membranes, resulting in alterations in permeability.

In 1941, Baker showed that anionic or ionic surfactants damage the cell by disorganization of the cell membrane. Later, several researchers reported that anionic surfactants actually dissolve the vegetative cell membrane that resulted in the leakage of the enzymes, essential ions, coenzymes, and cell intermediates. However there is insufficient information was insufficient to draw conclusions on whether the disruption of the membrane is the main mode of action of anionic surfactants. Consequent studies have suggested that effect of anionic

surfactant is actually due to combination of two processes: ability to change the permeability and protein denaturation of the cell (Dychdala, 1983).

Use of sodium lauryl sulfate as sanitizer

In one study researchers investigated the efficacy of 0.1% SLS, 0.1 % Tween 80, and water at 22 and 40°C as a rinse solution on reduction of *Salmonella* population on the surface of strawberries, tomatoes and green leaf lettuce. The study enumerated the numbers of survivors in rinse solutions and in fruits homogenate. Initial populations of *Salmonella* on strawberries, tomatoes, and leaf lettuce were 5, 4, and 5.5 logs CFU/ml respectively. Bacterial population in all rinse solutions except SLS ranged from 3.1 to 4.3 logs CFU/ml. Enumerated bacterial numbers in strawberry SLS rinsate were 2 log and <1 log CFU/ml for 22 and 40°C rinsates, respectively. Homogenate of the strawberries was also analyzed for the number of survivors and for 22°C was 3.5 log CFU/ml and <1 log CFU/ml at 40°C. Based on the results of the study, the authors concluded that SLS containing solution might have lethal or sublethal effect on *Salmonella*, and this effect increased with increasing temperature (Raiden, 2003).

The effectiveness of the three sanitizers containing anionic surfactants and organic acids against *Listeria monocytogenes*, *E.coli* 0157:H7, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Staphylococus aureus*, nalidixic acid resistant *Salmonella Typhimirium*, and several antibiotic resistant strains of *L. monocytogenes* has been investigated by Lopes (1998). Sanitizing agents used in the study were AP, DO, and DL and were designed by Microcide, INC. The concentrations of the anionic surfactants in the final dilution of the sanitizers were 0.1 mM of SLS, 0.67 mM dioctyl sodium sulfosuccinate, and 1.25 mM

sodium capryl lactylate respectively. The formulation of the sanitizing solutions also included different amounts of three organic acids. The AP sanitizer contained 19.3 mM of citric acid, sanitizer DO had 161.5 mM of glacial acetic acid, and DL solution contained 94.76 mM of lactic acid in the final dilution for use of the sanitizers. All three sanitizers exhibit 99.999% (5-log) reduction in the bacterial population in 30 sec of exposure time. Sanitizers were equally effective in reduction of bacterial populations in both gram-positive and gram-negative bacteria: *A. hydrophila*, *E.coli* 0157:H7, *L. monocytogenes*, *P. aeruginosa*, and *S. Typhimirium.* All sanitizers expressed lethal activities (>99.999 %) against antibiotic-resistant bacteria irrespective of its type (Lopes, 1998).

Restaino (1994) examined the antimicrobial properties of 0.6 to 1.75% BOAAS (buffered organic acid anionic sanitizer) on the reduction of *Staphylococcus aureus* (*S. aureus*) population on Formica surface. BOAAS was compared with six sanitizers such as organic chlorine, two iodophors, peoxyacetic acid, acid anionic, and quaternary ammonium compound. The efficacy of sanitizer was investigated in presence and absence of organic material. The effect of the same concentrations of BOASS was also evaluated on the survival of *Salmonella typhimirium*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa.* Formulation of BOAAS contained citric acid, chelating agent (ethlenediaminetetraacetic acid- EDTA), and SLS in a buffered solution. Population of *S. aureus* was not significantly reduced by traditional sanitizers in the presence of organic material, but ≥1.2% BOAAS achieved significant reduction. When compared with water in a period of 60 min, only BOAAS showed significant bacterial reduction. Presence of organic material (0.5% protein) was shown to reduce the effectiveness of all sanitizing treatments. BOAAS treatment of ≥0.6% was significantly more effective against *S. aureus* in 60 min exposure compared to

organic chlorine solution. BOAAS at 0.6% have also shown to be very effective in reduction of *S. aureus, S. typhimirium, L. monocytogenes*, and *Pseudomonas aeruginosa* populations by reducing them by >5 logs (Restaino et al., 1994).

British researchers investigated the bactericidal activity of chemical combination of different organic acids (0.5% and 1%) combined with various chemicals against *Salmonella typhimirium* attached into chicken breast skin. Chemicals incorporated into combinations were 125 ppm SLS, 2% ethanol, 100 ppm sorbitan monolaurate (SPAN 20), and 15% dimethyl sulfoxide (DMSO). List of organic acids used in the study included acetic, citric, malic, and tartaric acids of 0.5% and 1%. The addition of SLS or DMSO chemicals to lactic acid increased activity of the sanitizing solution; on the other hand, addition of SLS and DMSO to all other 0.5% acids did not increase activity and even decreased it in some cases. Addition of ethanol to 0.5% acids did not exhibit any effect. Maximum effect was achieved in combination of SLS and 1% organic acids (Tamblin, Conner, 1997).

Irradiation

Irradiation is the process involving the application of ionizing radiation, including alpha particles, beta rays or electrons, and x-rays generated by machines or gamma rays from radioisotopes. Three types of radiation source can be used for food irradiation according to the Codex Alimentarius General Standard (FAO/WHO, 1984):

(a) Gamma radiation from radionuclides such as ^{60}Co or ^{137}Cs [8];

(b) Machine sources of electron beams with energies of up to 10 MeV,

(c) Machine sources of X-rays with electron energies of up to 5 MeV.

γ-rays and X-rays have greater penetrating capability and can be used for irradiation of relatively thick materials. If deep penetration is not required, the process involves a large amount of product, and or use of rapid conveyor speed is desirable, then electron beam may provides a lower cost per unit of product due to its high energies (unknown, 2002).

Gamma Radiation

The γ-rays are generated by ^{60}Co or ^{137}Cs. The γ-rays used in food industry are usually obtained form large ^{60}Co radionuclide sources. Radioisotopes sources emit mostly monoenergetic photons. Irradiation facilities that utilize gamma irradiation use an elevating device with radioactive material assembled on top. This device stored in the pool of water to prevent emission of radioactive rays and elevated to the surface only for irradiation of the material.

Electron Beams

High energy electrons employed by this type of irradiation are accelerated to the speed of light by a linear accelerator. The source of electrons is electricity and does not use the radioisotopes. Electron beam radiation allows shortening of exposure time due to the capability to achieve very high dose rates of 10^3-10^5 Gy/ sec, which are high compared to gamma radiation that allows only 0.01-1 Gy/sec (Jaczynski, 2003). Unlike gamma irradiation, energy emitted by electron beams is controlled and occurs at couple of stages. First, emitted electrons have a narrow spectral energy limits. Further control occurs with the use of bending magnets on the beam handling apparatus. Ability to turn the accelerating machine on and off exhibits an additional advantage for use in commercial establishments.

An international group of microbiologists in 1964 suggested the following terminology for radiation treatment of foods:

Radurization. This type of irradiation uses doses such as 0.5 to 2.5 kGy for reduction of viable spoilage microorganisms and mostly used for the treatment of fresh meats, poultry, seafood, fruits, vegetables, and cereal grains.

Radicidation. Standard irradiation level of this process ranges from 2.5 to 10 kGy. This dose is enough to reduce the number of viable specific non-spore forming pathogens (excluding viruses) to level undetectable by standard methods. This method is very similar to heat pasteurization of food, and is hence also called radiopasteurization. Refrigerated storage of irradiated products following treatment is important in preventing the possible growth of the bacteria.

Radappertization. Level of irradiation used in this type of radiation is 30-40 kGy. Application of such high levels of radiation are equal to process of sterilization or "commercial sterility" of the product. However, this method is not recommended for use in foods (Mendonca, 2002).

Units of irradiation

Irradiation dose applied to a food is the most important factor of the irradiation process (Mendonca, 2002). Traditionally, irradiation dose was measured in 'rad' that is equal to 0.01 Gy or 100 ergs of energy absorbed per gram of irradiated material, but this unit has been discontinued.

The currently unit of measurement of absorbed dose is the gray (Gy) and it is equal to the absorption of 1 joule of energy per kilogram of irradiated material. One kilogray (kGy) is equivalent to 100,000 rads.

Mechanism of antimicrobial action

The major effects of ionizing radiation are ionization, dissociation, and excitation. Passage of ionizing radiation through food matter leads to the
loss of energy leading to the absorption of this energy by the food called primary radiolysis effect. This absorption of energy leads to the random formation of energetic electrons throughout the matter consequently leading to formation of energetic molecular ions. The ions may undergo several possible processes, such as electron capture and dissociation, rapid rearrangement of ion-molecule reactions, and ion dissociation with time, which depends on the complexity of the molecular ions. These processes further lead to chemical changes in matter (Patil, 2004; Morehouse and Komolprasert, 2004). Free radicals produced as a result of these primary effects of ionization lead to secondary effects. Highly unstable unpaired electrons of free radicals react with each other and with other molecules in order to stabilize themselves. Collision energy occurring between ionizing radiation and the matter leads to expulsion of an electron from an atomic orbit to produce ion pairs, and thus results in atomic level changes (Mendonca, 2002).

The antimicrobial mechanism of action of irradiation involves two different effects on the cell components (DNA and cytoplasmic membrane): direct and indirect. The process of electron removal from the DNA injures vital genetic material and is considered as direct effect. Indirect action involves the radiolysis of water in the cell and its surroundings. It leads

to alteration of molecule of water and consequent production of highly reactive hydrogen and hydroxyl radicals. Theses radicals have ability to alter bases, can cause oxidation, reduction, and even destruction of carbon-carbon bonds of cell components, including DNA. Radiation can cause equally single-strand and double-strand breakage in the DNA of microorganism (Mendonca, 2002).

Irradiation of fresh produce

Primary benefit of irradiation is the increase of the shelf-life for the products with limited shelf-life due to microbial action. Radiation at dose levels of less than 2 kGy could extend the shelf-life by 2-12 days for fresh-cut products such as diced bell peppers and radish (Farkas, 1997), romaine lettuce (Prakash, 2000), iceberg lettuce (Fan, 2002; Goularte et al., 2004; Niemira, 2002), diced tomatoes (Prakash, 2002), celery (Prakash, 2000), shredded carrots (Chervin, 1994), prepacked soup greens (Langerak, 1978), fresh sweet corn (Deak, 1987), watercress (Martins et al., 2004) and endive (Niemira, 2003).

Irradiation also has been used as an ideal quarantine treatment to control several types of fruit flies (Oriental, Mediterranean, melon) due to its effectiveness against most insect and mite pests at dose levels that do not affect the quality of most commodities (Follet P.A, 2004). As a quarantine treatment it has been mostly used for tropical fruits such as citrus, mango, papaya, pineapple, avocado, bananas, lytchees, etc. USDA-APHIS for quarantine treatment suggested the use of minimum absorbed doses ranging from 150 Gy to 250 Gy for different types of fruit flies (APHIS, 1996).

Irradiation at the levels optimal for the shelf-life extension is also effective against vegetative pathogens found in the fresh produce. There are concerns that the complete

elimination of background microflora by irradiation might lead to the proliferation of pathogens; however these concerns have been proven baseless.

In the experiment done by Prakash et al. (2004), cilantro irradiated with 0.5 kGy had showed reduction in *E. coli* counts by over 4 logs, 3.7 logs reduction for yeast and mold counts, and 3.3 logs in total aerobic counts.

Martins et al. (2004), evaluated the effect of irradiation on reduction of *Salmonella* spp. on minimally processed watercress. In their study they used irradiation doses of 0.0, 0.2, 0.5, 0.7, 1.0, 1.2, and 1.5 kGy. With the use of 1.7 kGy they achieve reduction in *Salmonella* population by 4 logs (Martins et al., 2004). In another study, those same authors showed that irradiation of watercress inoculated with *Salmonella* achieves from 0.29 to 0.43 kGy in radiation D_{10} values (Martins et al., 2004). Irradiation effect on the survival of *E. coli* 0157:H7 on four different types of lettuce has been investigated by Niemira. Lettuce types used in the study included Boston, Iceberg, Green leaf and Red leaf. The radiation D-values for the pathogen differed slightly in different types of lettuce: Red leaf and Green leaf expressed significantly greater sensitivity (D-value = 0.12 kGy) than in the Iceberg and Boston lettuce (D-value = 0.14 kGy) (Niemira, 2002). In the study involving endive inoculated with *Listeria monocytogenes* and *Listeria innocua* (nonpathogenic surrogate bacterium), it was found that radiation sensitivities of the two strains were similar (0.21 kGy). But the use of 0.84 kGy, that was equivalent to 99.99% reduction, suppressed the numbers of *Listeria monocytogenes* during the refrigerated storage (Niemira, 2003).

Effect of irradiation on fresh produce

Fruits and vegetables that have been irradiated demonstrate dose level dependent responses similar to stressed and wounded plant tissues. These responses include increased respiration, ethylene production, physical and chemical reactions such as enzymatic browning, lipid oxidation, loss of cellular integrity, and increased moisture loss. All above stated processes are interrelated and lead one to another. For example, increased ethylene levels stimulate respiration leading to increased oxygen uptake and release of carbon dioxide. Therefore, they have major impact on the plant tissue deterioration process (Prakash, Foley, 2004).

Status of food irradiation and its application in the United States

In the US, the Food Additives Amendment to the Federal Food, Drug, and Cosmetic Act (FD&C Act) of 1958 placed food irradiation under food additive regulations. This is the reason why presently FDA regulates food irradiation as a food additive and not a food process (Morehouse and Komolprasert, 2004).

In 1981, a joint Food and Agriculture Organization (FAO)/ International Atomic Energy Agency (IAEA)/WHO Expert Committee on food irradiation found that food given an overall average of up to 10.0 kGy were unconditionally safe. Currently, approximately 40 countries have approved irradiation of one or more food products and about 30 of them are using food irradiation for commercial purposes (Jay, 2005).

In the U.S. U.S Food and Drug Administration approved irradiation of spices and vegetable seasonings with the dose of up to 10 kGy (U.S. *Federal Register*, July 15, 1983). In 1985, in order to prevent *Trichinella spiralis* in pork the FDA permitted irradiation of pork up to 1.0

kGy. For control of fruit flies and mites FDA approval was granted for irradiation of Hawaiian tropical fruits intended for transport to mainland. Irradiation sterilization of frozen, packaged meats was approved in 1995 for NASA's space flight program. This approval followed by approval of industry petition for irradiation of nonfrozen and frozen red meats with doses not exceeding 4.5 kGy and 7.0 kGy respectively.

It is important to note that organizations such as WHO, American Medical Association (A.M.A., 1993), American Dietetic Association (A.D.A., 1996), Institute of Food Technologists, and the U.S. Council for Agricultural Science and Technology all have a positive attitude for processing of food by irradiation for safety (Farkas, 1998).

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CHAPTER 3. EFFICACY OF SELECTED CHEMICALS AND ELECTON BEAM IRRADIATION FOR DESTROYING *ESCHERICHIA COLI* 0157:H7 AND *SALMONELLA* ON CANTALOUPE SKIN

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ABSTRACT

This study evaluated the efficacy of citric acid (1, 2, or 3% CA) alone or combined with hydrogen peroxide (5% HP), sodium lauryl sulfate (1% SLS), and irradiation for destroying *Escherichia coli* 0157:H7 and *Salmonella* spp. on the outer rind surface of cantaloupe. Cantaloupe rinds were cut into 2.5 cm^2 pieces and inoculated with either a five strain mixture of nalidixic acid resistant *Salmonella* spp. or *E. coli* to give ~ 10^8 CFU/ rind. Inoculated rinds were dipped for 2.0 min in 500 ml of water (control) or sanitizer solutions at 23°C. Samples treated with combinations of HP + SLS alone or combined with 1% CA were irradiated at 0.0, 0.5, and 1.0 kGy. Non-irradiated and irradiated cantaloupe rinds were homogenized in buffered peptone water (100 ml). Samples of homogenate were serially diluted and plated into Bismuth Sulfite agar and MacConkey Sorbitol agar, both containing nalidixic acid. All inoculated agar plates were incubated at 35°C for 48 h. *Salmonella* and *E. coli* survivors were enumerated following incubation and expressed as log CFU/ rind. Compared to the water dipped control, exposure of rinds to 5% HP reduced populations of *Salmonella* and *E. coli* by 2.7 and 2.83 log CFU/rind, respectively. A combination of 5% HP and 1% SLS decreased populations of *Salmonella* and *E. coli* by 3.37 and 2.98 log. Further reductions were achieved by combining 5% HP and 1% CA with 1% SLS; populations of *Salmonella* and *E. coli* decreased by 3.9 and 4.0 log, respectively. Combination of 0.5 kGy

irradiation with a chemical solution of HP, SLS and CA reduced the numbers by 4.5 and 5.48 log, for *Salmonella* and *E. coli* respectively. Irradiation of 1.0 kGy in combination with HP, SLS and CA reduced the population of *Salmonella* by 5.67 log CFU/rind and by more than 7 log CFU/rind for *E. coli.* Based on these results, the combined use of acidified HP with an anionic surfactant (SLS) and irradiation has good potential for improving the microbial safety of cantaloupe melons. Further research is needed to determine the effects of these interventions on quality characteristics of melons.

INTRODUCTION

Salmonella and *Escherichia coli* 0157-H7 are recognized as important foodborne pathogens. *Salmonella* serotypes cause approximately 1.5 million cases of foodborne gastroenteritis annually in the United States resulting in approximately 15,000 hospitalizations and 500 deaths (1). There are also nearly 73,500 cases of illnesses and 60 deaths associated with *E. coli* 0157:H7 that occur annually in the United States (2). A well-published *E. coli* outbreak during 1992-1993 involving undercooked ground beef was responsible for more than 700 cases of human infection and four deaths in the western United States (13). A part form ground beef fresh fruits and vegetables such as seed sprouts (19, 22), watermelon (15, 9), apple cider (23), orange juice (10), tomatoes (16), cantaloupes (7, 8, 21) have been found implicated in foodborne illness from *Salmonella* outbreaks.

During their growth in fields and orchards fruits and vegetables become contaminated with microorganisms from many sources in the natural environment. For example, some fruits such as cantaloupe melons are frequently in contact with soil, insects or animals during growth or harvesting (5, 3). On arrival at the packing house most fruits and vegetables carry

microbial populations ranging from 10^4 to 10^6 colony forming units (CFU) per gram (5, 6). Since many fruits and vegetables are eaten raw there is a growing concern for the microbial safety of fresh and fresh-cut produce in salad bars and supermarkets (Hurst and Schuler, 1992).

There have been several cases of foodborne illnesses and several multistate outbreaks (28, 8, 21, and 7) linked to consumption of cantaloupes contaminated with *Salmonella*. The first outbreak involving *Salmonella Chester* in 1990 occurred on the territory of 30 states and involved 245 people and even two deaths. Another outbreak occurred in the US and Canada and was related to consumption of cantaloupes contaminated with *Salmonella* Poona complicated in > 400 laboratory confirmed cases. Recent outbreaks in 2000 to 2002 affected 89 people on territory of 21 states (28, 8, 11).

Foodborne disease outbreaks linked to consumption of fresh produce contaminated with *Salmonella* and *E. coli* O157:H7 have focused much research on the development and testing of sanitizers for fresh fruits and vegetables. Although sanitizing agents such as peroxyacetic acid, ozone, chlorine dioxide, and hydrogen peroxide are actively being evaluated for destroying pathogens on fresh produce, chlorine is still the most widely used sanitizer in the fresh produce industry.

Concentrations of chlorine commonly used to sanitize fresh fruits and vegetables range from 50 to 200 ppm. Higher concentrations up to 2,000 and 20,000 ppm are permitted for use in sanitizing fresh produce and alfalfa seeds, respectively (4). The application of 100 to 2,000 ppm chlorine to fresh produce can reduce microbial populations by approximately 2 to 4 log (4, 12, 29, 31, 32, 33) with the 1 log reduction attributable to the washing effect of water. A concern over the use of chlorine is that it can react with organic matter to form

various organochlorine compounds, which may be carcinogenic (27). Also, the antimicrobial effect of chlorine is reduced by organic matter.

Most sanitizers are water-based chemicals that would be repelled from the natural wax plant surface, thus preventing an effective wetting of fresh produce surfaces to destroy organisms. Therefore, there is a need for sanitizers with high wetting capabilities that would aid in enhancing contact of the sanitizer with the waxy layer on the surface of the fruits and vegetables. Enhancing contact of sanitizers with the surface of fresh produce may result in improved destruction of microbial contaminants.

Antimicrobial activity of the sanitizer solutions might be decreased by inaccessibility of microbial cells in natural crevices and gaps on the waxy surface of the fruits and vegetables (20). Therefore, more elaborate processes might be needed to decrease microbial load on the surface of fresh produce. One of the effective methods in destruction of bacteria is the application of low-dose irradiation at levels that do not change organoleptic properties of fresh fruits and vegetables. Combination of low dose irradiation and chemical sanitizers with increased wetting ability might be a good strategy for eliminating pathogens from fresh produce. Accordingly, the main objective of this study was to evaluate the antibacterial efficacy of various combinations of citric acid, hydrogen peroxide, and a surfactant (sodium lauryl sulfate), against *Salmonella* and *E. coli* O157:H7 on the outer rind surface of cantaloupe. An additional objective was to determine the survival of *Salmonella* and *E. coli* O157:H7 on the outer rind surface of cantaloupe following surface-treatment with chemical sanitizers and electron beam irradiation.

MATERIALS AND METHODS

Bacterial strains

A five-strain mixture of nalidixic acid resistant *Salmonella enterica* (*S.* Typhimirium ATCC 14028, *S.* Graminara, *S.* Hartford, *S.* Enteritidis, and *S.* Heidelberg) or *Escherichia coli* 0157:H7 (ATCC 43895, ATCC 43894, ATCC 35150, C 467 and 93-062) was used in the present study. *S.* Typhimirium and *E. coli* 0157:H7 (43894, 43895, and 35150) were obtained from the American Type Culture Collection (ATCC). *E. coli* C 467 was obtained from Dr. Charles Kaspar at the University of Wisconsin, Madison, WI. All other strains were obtained from the culture collection of the Department of Food Science and Human Nutrition at Iowa State University.

Growth conditions and preparation of inoculum

Each culture was maintained as frozen (-70°C) stock in brain heart infusion broth (BHI, Difco Laboratories, Boston Dickinson, Sparks, MD) supplemented with 10% glycerol until used. Prior to each experiment, individual stock cultures were transferred twice in 10 ml of tryptic soy broth (Difco) supplemented with 50 µl of nalidixic acid (Fisher Scientific, Fair Lawn, NJ) (TSBN) and incubated at 35°C for 20 h. Equal amounts of each culture were combined to prepare a five-strain mixture of *Salmonella* or *E. coli* 0157:H7. Cells from each mixture were harvested by centrifugation (10,000 x g, 10 min, 4°C) in a Sorvall Super T21 centrifuge (DuPont Instruments, Willmington, DE) and washed once via centrifugation in 30 ml of sterile 0.1% (w/v) peptone water (Difco). Pelleted washed cells of *Salmonella* serotypes or *E. coli* 0157:H7were suspended in 30 ml of fresh 0.1% peptone water (Difco) and used for inoculating cantaloupe rind.

Sample preparation

Cantaloupe melons free from bruises were purchased from a local grocery store and stored at 5°C for no more than 3 days before being used in the experiment. Whole cantaloupes were washed with soap water (Neutrad, Decon Labs, King of Prussia, PA) using soft brush to remove dirt and rinsed with distilled water. Washed cantaloupes were placed on a clean paper towels and air-dried at 23 ± 1°C for 1 h in a laminar flow-hood. A ball point pen and flexible plastic ruler were used to delineate parallel lines 2.5 cm apart on the outer rind surface of the cantaloupes. Using sterile knives and cutting boards dried the melons were cut into 2.5 cm thick slices. Subsequently, strips of rind were thinly removed from the slices and cut into 2.5 cm^2 pieces.

Preparation of sanitizer solution

Sixteen sanitizing treatment were prepared by mixing chemicals in sterile distilled water, and evaluated for their effectiveness in killing *E. coli* 0157:H7 and *Salmonella*. Sanitizers tested were 200 ppm sodium hypochlorite (v/v; Clorox, Clorox Co., Oakland, CA) titrated with 5% citric acid (w/v; Science Lab. Com, Kingwood, TX) to give a pH value of 6.4; hydrogen peroxide (v/v; Fisher Scientific, Fair Lawn, NJ) (5% HP); citric acid (Science Lab. Com, Kingwood, TX) (1, 2, and 3% w/v CA); sodium lauryl sulfate (Fisher Scientific, Fair Lawn, NJ) (1% w/v SLS); a combination of SLS with H_2O_2 (1% SLS+ 5% HP); combinations of 5% HP with 1, 2, or 3% CA; combinations of 1% SLS with 1, 2, or 3% CA; combinations of 5% HP, 1% SLS and 1, 2, or 3% CA (table 1). Five hundred ml of each treatment solution were dispensed into separate 600-ml beakers.

Inoculation of cantaloupe rinds

Two separate sets of cantaloupe rinds were each inoculated with *Salmonella* or *E. coli* 0157:H7 on the day before surface treatment with sanitizers. Each rind (2.5 cm^2) was spot-inoculated with 0.05 ml (50 µl) of cell suspension by dispensing 5-6 drops of the inoculum on the outer surface of the cantaloupe rind to give a final cell concentration of approximately 10^7 CFU/rind. Inoculated rind samples were placed in plastic containers and air-dried at ambient temperature (23 ± 1 ^{O}C) for 0.5 h in laminar flow-hood with the blower running. After drying, samples were stored overnight in a walk-in refrigerator at 4.4°C.

Sanitizing treatment application

Sanitizing of the inoculated rinds was performed by dipping separate sets of duplicate rind samples in each sanitizer solution (23 ± 1°C) for two minutes. Non-treated inoculated rinds (no dip) and inoculated rinds dipped for 2.0 minutes in sterile distilled water served as controls.

Irradiation treatment and dosimetry

Control rinds and rinds treated with chemical solutions containing HP and SLS or HP, SLS, and 1% CA were placed in sterile stomacher bags and irradiated at the Iowa State University Linear Accelerator Facility equipped with a MeV CIRCE III Linear Accelerator (MeV Industries S.A., Jouyen-Josas, France). Duplicate cantaloupe rinds (4°C) were irradiated at three target doses (0.0, 0.5, and 1.0 kGy) using electron beam irradiation at an energy level of

10 MeV and an average dose rate of 70.4 kGy/min. Each target dose represented an arithmetic average of doses measured at the top and bottom surfaces of the rinds.

The absorbed doses were determined by positioning 5mm (diameter) by 5 mm (length) alanine pellets (Bruker Analytische Messtechink, Rheinstetten, Germany) on top and bottom of the bags containing the rind samples. Measurement of the absorbed doses was conducted immediately after irradiation by inserting the pellets in a Bruker EMS 104 EPR Analyzer (Bruker Analytische Messtechnik, Rheinstetten, Germany), which uses electronic paramagnetic resonance to conduct the analysis.

Microbiological analysis

Portions (100-ml) of sterile 2% (w/v) buffered peptone water (BPW; Difco) were added to the stomacher bags containing the non-irradiated or irradiated cantaloupe rinds. Samples were vigorously rubbed by hand for 30 sec and then pummeled for 1 min at regular speed using a Seward stomacher 400 Lab blender (Seward Ltd., London, England). Serial dilutions of the slurry were prepared in BPW (Difco) and 0.1-ml aliquots of appropriate dilutions were surface plated, in duplicate, on MacConkey Sorbitol Agar (Difco) supplemented with 50 µl of nalidixic acid (Fisher) (SMAN) and Bismuth Sulfite Agar (Difco) supplemented with 50 µl of nalidixic acid (Fisher) (BSAN) for *E. coli* 0157:H7 and *Salmonella*, respectively. All inoculated agar plates were incubated aerobically at 35°C and *E. coli* 0157:H7 and *Salmonella* colonies were counted at 48 h. Counts of *E. coli* 0157:H7 and *Salmonella* survivors were expressed as log colony forming units (CFU) per rind.

Data analysis

All experiments were replicated three times, and for each experiment two samples per treatment were tested. Analysis of variance (ANOVA) was performed with the General Linear Models procedure of the Statistical Analysis System software program (SAS Institute Inc., Cary, NC) (30). Differences were considered statistically significant at $P < 0.05$ unless otherwise stated. Differences among variables were tested for significance using Tukey's honestly significant different multiple comparison test. Mean values and standard error of the means (SEM) were reported.

RESULTS

Table 2 shows the numbers of survivors of *Salmonella* in log reduction of initial numbers of this pathogen on cantaloupe rinds following immersion for 2.0 min in water (control) or chemical solutions. Initial numbers of the pathogen attached to the outer surface of each rind surfaces were ~ 10^8 CFU. Viable numbers of the pathogen decreased by approximately 0.76 log after inoculated rinds were dried and held at 4.4 ^{O}C for 18 h. Immersion of rinds in distilled water resulted in 0.42 log reduction in *Salmonella*. Chlorine (200 ppm) reduced numbers of the pathogen by 1.04 log ($P<0.05$). Treatment of rinds with SLS or CA (1, 2, or 3%) did not significantly reduce initial counts ($P > 0.05$). In contrast, significant reductions in survivors were observed when CA and SLS were used in combination ($P < 0.05$). All treatments involving HP alone or combined with SLS and/or CA produced significant reductions in numbers of *Salmonella* ($P <0.05$). Combination of HP, SLS and three concentrations of CA achieved reductions ranging from 3.66 to 3.90 log CFU/rind; however,

there were no significant differences among these treatments (P >0.05). Similar results were obtained for *E. coli* O157:H7 (Table 3).

Table 4 shows the effect of selected chemical solutions and irradiation on the survival of Salmonella on cantaloupe skin. Initial population of *Salmonella* on cantaloupe skin not treated with water or chemical sanitizers was 7.24 log CFU/ rind. Treatment of rinds with 0.5 kGy resulted in 2.84- and 2.65 log reduction of Salmonella in dry and water control, respectively. However, a combination of chemical treatment and 0.5 kGy reduced the bacterial numbers by 4.11 (HP + SLS) and 4.5 (1%CA + HP + SLS) log. Further reduction in population of bacteria was achieved by the application of 1.0 kGy. Numbers of bacteria on dry and water controls were reduced by 4.18 and 3.63 log, respectively. Combining irradiation of 1.0 kGy and chemical dipping achieved maximum reduction of 4.54 and 5.67 log for samples treated with HP + SLS and 1%CA + HP + SLS, respectively.

The effect of chemical treatments and irradiation on reduction of *E. coli* O157:H7 on cantaloupe rind is shown in table 5. Initial population of bacteria on untreated cantaloupe skin was 7.38 log CFU/ rind. Treatment of rinds with HP + SLS and 1%CA + HP + SLS reduced numbers of the pathogen by 2.23 and 3.21 log respectively. Irradiating of cantaloupe rinds with 0.5 kGy reduced the bacterial population by ~ 3 logs on dry and water-treated samples. Irradiation (0.5 kGy) produced reductions of 4.41 and 5.48 log, respectively, on rinds that were treated with HP + SLS and 1%CA + HP + SLS. Higher reductions in numbers of the pathogen on rinds were observed for HP + SLS (6.70 log) and 1%CA + HP + SLS (7.26 log) when used in combination with 1.0 kGy.

DISCUSSION

The reduction in initial numbers of both pathogens 18 h after drying may have resulted from death of a portion of the cell population during drying of the inoculum on rinds (Park and Beuchat, 1999). Because water does not destroy bacteria the observed decrease on numbers of the pathogen on water-dipped rinds is certainly due to physical removal of bacterial cells from rinds. Although washing fresh produce with water alone may give some reduction in numbers of pathogens, water can spread pathogens from contaminated produce to non-contaminated produce.

Results of the present study regarding SLS are consistent with results reported by Raiden et al. (2003) in which sanitizers incorporating 0.1% SLS at 22°C did not reduce the numbers of *Salmonella* more than water (25). However, when SLS was combined with citric acid, this combination produced significant ($P<0.05$) reduction in *Salmonella* population. In an in vitro study conducted by Lopes (1998), the combination of 1.00 mM of SLS and 19.3 mM of CA reduced the numbers of *E. coli* 0157:H7 and *Salmonella typhymirium* by 5 logs CFU/ml within 30 sec of exposure (18). Restaino et al. (1994) demonstrated that combination of SLS (60 ppm), CA (5640 ppm) and EDTA (240 ppm- chelating agent) reduced the population of *S. typhymirium* by >5 log CFU/ml in a period of 30 s (26). These results indicate that SLS when used alone does not aid in detaching bacteria from the surface of fruits or vegetables. However, the combination of SLS with citric acid increases potency of the sanitizer. It is likely that SLS, due to its surfactant properties, might be increasing effectiveness of CA when they are used in combination. In other words, by "wetting" SLS actually permits greater interaction of CA with the surface of the rind and thus facilitates greater contact of the antimicrobial with the attached cells. In addition, anionic surfactants including SLS are

known to exhibit increased antimicrobial effectiveness at pH range of 2.0 and 3.0 (14). Hence, use of CA resulting in pH decrease enhances the effectiveness of the sanitizer. Addition of HP to the combination of SLS and CA may further potentiate this antimicrobial effect by displaying oxidative action lethal to bacteria.

In the present study the antibacterial effectiveness of chemical treatments combined with irradiation (0.5 or 1.0 kGy) against the pathogens on cantaloupe rinds is not surprising. The use of irradiation with foodgrade chemical sanitizers achieves desirable higher reductions in bacterial numbers, as was shown by previous studies (24). The use of multiple interventions in the present study is consistent with the concept of hurdle technology used in food preservation in which antimicrobial interventions may be more effective in multiple rather than in single form by creating simultaneous and variable injuries in bacterial cells (17). In this regard, bacterial maintenance of homeostasis while repairing multiple injuries requires the activation of several different cellular mechanisms. The activation of cellular mechanisms for repair of a variety of injuries can be energetically demanding for sub-lethally injured cells and result in loss of viability in bacterial cells (17). Based on the results of the present study, the combined application of chemical and physical interventions for controlling pathogens seems to have good potential for improving the microbial safety of cantaloupe. Further research is needed to optimize treatment combinations to effect destruction of pathogens while maintaining quality attributes of cantaloupe melons.

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CHAPTER 4. GENERAL CONCLUSIONS.

All hydrogen peroxide containing chemical sanitizers performed better than water and chlorine (200 ppm) for reducing numbers of *E. coli* 0157:H7and *Salmonella* on cantaloupe skin. It can be concluded that chemical sanitizers used in the present study are more effective than water and chlorine, which are both currently used in industry.

The combination of hydrogen peroxide (5%) with sodium lauryl sulfate (1%) and a combination of hydrogen peroxide (5%), citric acid (1%) and sodium lauryl sulfate (1%) were the most effective sanitizing solutions tested. Hydrogen peroxide (5%) + citric acid (1%) + sodium lauryl sulfate (1%) was most effective in reduction of *E. coli* 0157:H7 and *Salmonella* by reducing the numbers of bacteria by 3.9 and 4.0 log CFU/ rind, respectively. These results of the study led to the selection of the hydrogen peroxide (5%) + sodium lauryl sulfate (1%) and hydrogen peroxide (5%) + citric acid (1%) + sodium lauryl sulfate (1%) for further combination with low-dose irradiation.

Application of irradiation of 0.5 and 1.0 kGy following the chemical treatments reduced bacterial population of *E. coli* 0157:H7 and *Salmonella* greater than the use of chemical treatments alone, with the maximum effect achieved by the use of irradiation of 1.0 kGy following chemical treatment. Therefore, these combinations of sanitizing solutions and low-dose irradiation might be used by the industry as an effective method for destroying pathogens on the surface of the cantaloupes.

Further research is needed to optimize treatment combinations to effect destruction of pathogens while maintaining quality attributes of cantaloupe melons.

TABLE 1. Description, code and pH of chemicals used for treating cantaloupe rinds

Treatment code	Description	pH
Control	Dry control, no treatment	N/A
Water	Distilled water	5.80
Chlor	200 ppm sodium hypochlorite	6.50
HP	5% (v/v) hydrogen peroxide	4.47
SLS	1% (w/v) sodium lauryl sulfate	5.30
1% CA	1% (w/v) citric acid	2.08
2% CA	2% (w/v) citric acid	1.90
3% CA	1% (w/v) citric acid	1.79
HP+ SLS	1% (w/v) citric acid+ 5% hydrogen peroxide	5.47
1% CA+ SLS	1% (w/v) citric acid+ 1% sodium lauryl sulfate	2.06
2% CA+ SLS	2% (w/v) citric acid+ 1% sodium lauryl sulfate	1.85
3% CA+ SLS	3% (w/v) citric acid+ 1% sodium lauryl sulfate	1.72
1% CA+ HP	1% (w/v) citric acid+ 5% hydrogen peroxide	1.87
2% CA+ HP	2% (w/v) citric acid+ 5% hydrogen peroxide	1.68
3% CA+ HP	3% (w/v) citric acid+ 5% hydrogen peroxide	1.59
1% CA+ HP+ 1%SLS	1% (w/v) citric acid+ 5% hydrogen peroxide+ sodium lauryl sulfate	1.93
2% CA+ HP+ 1%SLS	2% (w/v) citric acid+ 5% hydrogen peroxide+ sodium lauryl sulfate	1.72
3% CA+ HP+ 1%SLS	3% (w/v) citric acid+ 5% hydrogen peroxide+ sodium lauryl sulfate	1.66

TABLE 2. Population of *Salmonella* spp on the outer rind surface of cantaloupe following immersion (2 min) in water (control) or selected chemical solutions.

Treatment Code	Log CFU/ rind (± SD)[a]	Log Reduction[b]
Dry control	7.24 (± 0.34) A[c]	N/A
Water	6.82 (± 0.35) A	0.42
Chlor (200 ppm)	5.78 (± 0.68) B	1.04
5% HP	4.05 (± 0.41) C	2.77
1% SLS	6.42 (± 0.50) A	0.40
1% CA	6.62 (± 0.24) A	0.20
2% CA	6.60 (± 0.47) A	0.22
3% CA	6.51 (± 0.14) A	0.31
5% HP + 1% SLS	3.45 (± 1.43) C	3.37
1% CA + 1% SLS	5.78 (± 0.28) B	1.04
2% CA + 1% SLS	5.88 (± 0.31) B	0.94
3% CA + 1% SLS	5.79 (± 0.13) B	1.03
1% CA + 5% HP	3.93 (± 0.22) C	2.89
2% CA + 5% HP	3.95 (± 1.11) C	2.87
3% CA + 5% HP	4.01 (± 0.37) C	2.81
1% CA + 5% HP + 1% SLS	2.92 (± 1.20) D	3.90
2% CA + 5% HP + 1% SLS	3.16 (± 0.93) CD	3.66
3% CA + 5% HP + 1% SLS	3.00 (± 1.17) CD	3.82

[a] Means (standard deviation) of three independent replications of the experiment
[b] Log reduction based on numbers of *Salmonella* recovered from dry control (for water) or from water-dipped samples (for chemical treatments)
[c] Mean values followed by different letters are significantly different ($P < 0.05$)

TABLE 3. Populations of *Escherichia coli* 0157-H7 on the outer rind surface of cantaloupe following immersion (2.0 min) in water (control) or selected chemical solutions.

Treatment code	Log CFU/ rind (± SD) [a]	Log Reduction [b]
Dry control	7.18 (± 0.20) A[c]	N/A
Water	6.79 (± 0.44) A	0.39
Chlor (200 ppm)	5.64 (± 0.53) B	1.15
5% HP	3.96 (± 0.78) C	2.83
1% SLS	6.37 (± 0.17) A	0.42
1% CA	6.58 (± 0.37) A	0.21
2% CA	6.50 (± 0.24) A	0.29
3% CA	6.33 (± 0.35) A	0.46
5% HP + 1% SLS	3.81 (± 0.27) C	2.98
1% CA + 1% SLS	5.67 (± 0.12) B	1.12
2% CA + 1% SLS	5.44 (± 0.32) B	1.35
3% CA + 1% SLS	5.76 (± 0.09) B	1.03
1% CA + 5% HP	3.85 (± 0.42) C	2.94
2% CA + 5% HP	3.89 (± 0.90) C	2.90
3% CA + 5% HP	3.94 (± 0.88) C	2.85
1% CA + 5% HP + 1% SLS	2.79 (± 0.12) D	4.00
2% CA + 5% HP + 1% SLS	2.88 (± 0.46) CD	3.91
3% CA + 5% HP + 1% SLS	2.81 (± 0.43) CD	3.98

[a] Means (standard deviation) of three independent replications of the experiment
[b] Log reduction based on numbers of *E. coli 0157:H7* recovered from dry control for water) or from water-dipped samples (for chemical treatments)
[c] Mean values followed by different letters are significantly different ($P < 0.05$)

TABLE 4. Effect of selected chemicals and electron beam irradiation on the survival of *Salmonella* on cantaloupe skin

Treatment	Irradiation dose		
	0.0 kGy	0.5 kGy	1.0 kGy
Dry (no dip)	7.24 (± 0.13)[a] A[b]	4.40 (± 0.15) B	3.06 (± 0.26) C
Water	6.90 (± 0.32) A	4.25 (± 0.16) B	3.27 (± 0.09) C
5% HP + SLS	4.63 (± 0.32) B	2.79 (± 0.24) C	2.36 (± 0.60) C
1% CA + HP + SLS	4.38 (± 0.13) B	2.40 (± 0.37) C	1.23 (± 0.17) D

[a] Means (standard deviation) of three independent replications of the experiment
[b] Log reductions in mean values followed by different letters are significantly different ($P < 0.05$)

TABLE 5. Effect of selected chemicals and electron beam irradiation on the survival of *E. coli* 0157:H7 on cantaloupe skin

Treatment	Irradiation dose		
	0.0 kGy	0.5 kGy	1.0 kGy
Dry (no dip)	7.38 (± 0.08)[a] A	4.28 (± 0.24) B	2.38 (±0.42) C
Water	7.26 (± 0.21) A	4.27 (± 0.30) B	2.52 (± 0.24) C
5% HP + SLS	5.03 (± 0.05) B	2.85 (± 0.59) C	0.56 (± 0) D
1% CA + HP + SLS	4.05 (± 0.10) B	1.78 (± 0.68) C	N/D (± 0) D

[a] Means (standard deviation) of three independent replications of the experiment

[b] Log reductions in mean values followed by different letters are significantly different ($P < 0.05$)