A REVIEW OF CHEMICAL DISINFECTION METHODS FOR MINIMALLY PROCESSED LEAFY VEGETABLES

by

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ABSTRACT

For the last decade in the U.S., consumers have demanded fresh, healthier convenience-type foods namely, fresh-cut vegetables. Globalization has played a major role in rapid growth of the fresh-cut industry sector. Thus, consumers may purchase their favorite seasonal vegetables in local grocery stores year-round. However, the convenience of year-round produce availability brings the potential of foodborne outbreaks. Thirty-two states reported 190 produce-associated outbreaks, 16,058 reported illnesses, 598 hospitalizations, and eight deaths from 1973 to 1997. Pathogenic bacteria contaminate raw agricultural commodities through various pathways such as irrigation with untreated water, use of noncomposted animal manure as fertilizer, and wash water systems. The increasing number of produce-related outbreaks has raised awareness to interventions that remove human pathogens on fresh produce. Washing solely with tap water cannot be relied upon to completely remove pathogens. Chlorinated water is the most frequently used sanitizer, however, reductions are less than 2.63-log CFU/g on leafy and salad vegetables. Such reductions, although significant, are not sufficient to assure the microbial safety of minimally processed vegetables. The efficacy of several other chemical agents such as chlorine dioxide, ozone, electrolyzed water, hydrogen peroxide, organic acids, and other commercial products have been evaluated as potential alternatives to chlorine.

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Special thanks go to my parents, Salomon Camacho and Aida L. Rivera, for their unconditional love, encouraging support, and endless prayers through all the years leading to this moment. Finally, thanks to the Almighty God who made all things possible.

DEDICATION

I have truly lived a blessed life filled with many loving and supportive people. This report, therefore, is dedicated to all the people who have brought so much into my life.

To my wonderful wife Olga, you have been my emotional, psychological, and intellectual support. You always expressed interest and pride in all my endeavors and without you this journey might not have been successful. With love and respect, I dedicate this report to you.

To my loving son Xavier, you have brought more into my life than you'll ever know. You are my most special gift from God. To the kind and gentle person that you are and the fabulous man you will become, I dedicate this report to you.

I. INTRODUCTION AND JUSTIFICATION

The fresh fruit and vegetable industry has witnessed exponential growth in the past decade. This trend is the result of a combination of many factors, such as globalization and increased demand by consumers for healthy, ready-to-eat products. Globalization has made it possible for the average consumer to purchase their favorite seasonal fresh fruits and vegetables year round in neighborhood retail outlets and foodservice operations. The fresh-cut segment of the produce industry, in turn, continues to fill the niche for value-added, conveniently packaged fruit and vegetables based on growing consumer demand. According to Glaser et al. (2001), fresh-cut salad items sold in mainstream supermarkets increased from 197 to 549 items between 1993 and 1999, respectively. This represents an increase in sales from $197 million in 1993 to $1.3 billion in 1999.

Inevitably, with the growing demand for fresh fruits and vegetables the Centers for Disease Control and Prevention (CDC) reported an increase in the frequency of produce associated foodborne disease outbreaks (Bean et al., 1997; Mead et al., 1999). Common foodborne pathogens associated with fresh produce include *Salmonella* spp., *Listeria monocytogenes*, *Shigella* spp., and *Escherichia coli* O157:H7 (Beuchat, 1996b; FDA, 2001a). Researchers have identified and listed the natural microbiological counts found in minimally processed fruits and vegetables, including whole and shredded lettuce (Nguyen-The and Carlin, 1994; Beuchat, 1996b). Beuchat (1996b) reported that aerobic mesophilic counts can range from 10^3 to 10^8 CFU/g in fresh produce. Recent

studies (Liao and Sapers, 2000; Takeuchi and Franks, 2000; Ukuku and Sapers, 2001) showed that the surface structure of lettuce can protect *E. coli* O157:H7 cells from disinfection by chlorine, the most widely used chemical disinfectant (Brackett, 1987; Beuchat and Ryu, 1997; WHO, 1998). Ackers et al. (1998) indicated that *E. coli* O157:H7 was confirmed as the agent in an outbreak involving lettuce. The potential for contamination increases as the fresh produce moves from farm to table (i.e. irrigation water, improperly composted manure, wash water systems, soiled equipment, unsanitary practices, etc.).

Noticeably, the attention given to minimally processed produce by academia, government, and industry has increased. The focus has been on the microbiological safety through interventions strategies aimed at eliminating or reducing microbial hazards (i.e. human pathogens), mainly by using chemical disinfectants. The addition of a chemical disinfectant to the wash water has proven to reduce the microbial load (Beuchat and Ryu, 1997; Sapers, 2001). Washing lettuce leaves with tap water alone was reported to reduce indigenous microflora by approximately 1 log CFU/g (Adams et al., 1989; Nguyen-The and Carlin, 1994). Consequently, the need for chemical sanitizers becomes evident to reduce microbial contamination in wash water systems. However, it is important to recognize that such reductions, although important, are not sufficient to assure microbiological safety of minimally processed fresh-cut vegetables (Sapers, 2001).

The aim of this report is to review 1) potential sources of contamination, 2) available literature on chemical disinfection methods applied to minimally

processed leafy vegetables, mainly lettuce, 3) evaluate the efficacy of these disinfectants, 4) analyze factors that limit their efficacy, 5) and compare the available disinfectant methods to chlorine. A glimpse into the fresh-cut produce industry, through the available literature, alerts us to the many challenges that remain to ensure public health is protected. This study also intends to serve as a reference guide for industry, academia, government, and military food safety auditors associated with fresh-cut minimally processed leafy vegetables.

II. LITERATURE REVIEW

A. Background Information

1. Industry Size and Consumption Trends

The fresh fruit and vegetable industry has witnessed a surge in the fresh-cut segment. The rapid growth is a result of a combination of many factors, including globalization and increase demand by consumers for healthy, convenient, ready-to-eat products.

The International Fresh-cut Produce Association (IFPA) defines fresh-cut produce as fruits or vegetables that have been trimmed, peeled, or cut into 100% usable product that is bagged or prepackaged to offer consumers high nutrition, convenience, and flavor while still maintaining freshness (IFPA, 2001).

The Produce Marketing Association (PMA) reported that the U.S. fresh produce industry reached $76 billion in sales for both the retail and foodservice operations in 1999 (Kaufman et al., 2000; PMA, 2000), up from $34.6 billion in 1987 (Kaufman and others, 2000). In 1994, fresh-cut retail sales single-handedly were $5.8 billion (Hodge, 1995), rapidly increased to $8.8 billion in 1998 (Rajkowski and Baldwin, 2003), and were estimated to reach $19 billion by 2003 (Greenleaf, 1999). In 1997, $1.1 billion worth of produce was sold directly to the consumer, $34 billion in retail stores, and $35.4 billion through foodservice establishments (Figure 1) (Dimitri et al., 2003).

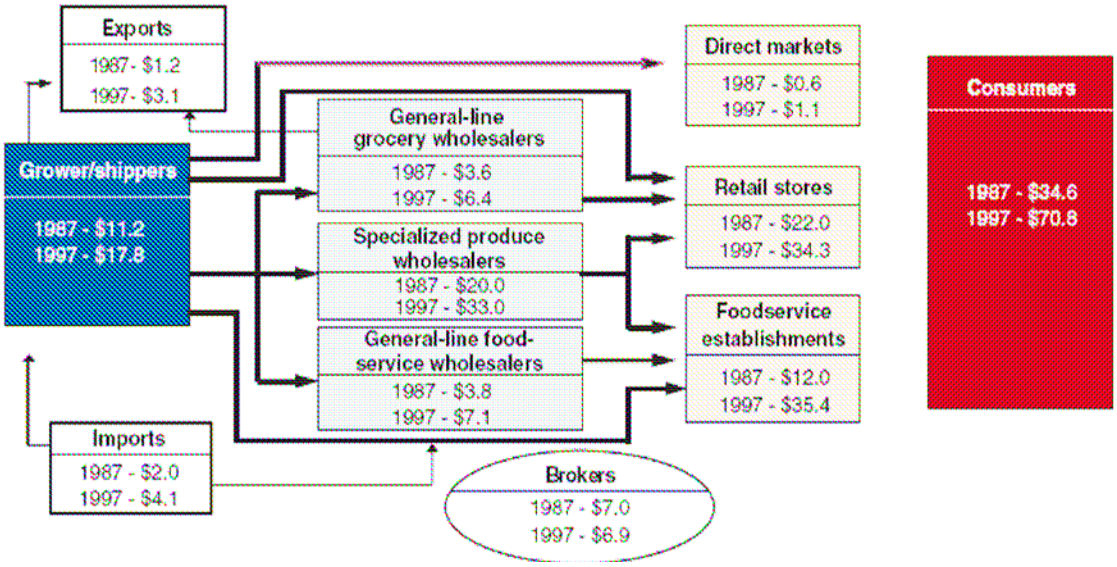

Figure 1. Fresh fruit and vegetable marketing channels in 1987 and 1999 (Taken from Dimitri and others, 2003).

In developed countries, the U.S. continues to dominate the international trade of fruits and vegetables, and is ranked number one as both importer and exporter, accounting for approximately 18% of the $40 billion in fresh produce world trade (Barbosa-Canovas et al., 2003).

The data shows that the American consumer is spending more on fresh produce and shifting their attention to value-added products, such as fresh-cut salads (Figure 2), which rose from 1 to 15% of total sales. Per capita consumption of fresh fruits and vegetables increased 6% between 1987 and 1995, and 8% between 1995 and 2000 (Table 1) (Dimitri et al., 2003). Leading consumption at the retail level were lettuce, tomatoes, and potatoes (Kaufman et al., 2000). As a consequence of the increased consumptions, so has consumer demand for variety, convenience, and quality (Figure 3) (Dimitri et al., 2003). Kaufman and others (2000) attributed the increase in consumption to:

> "First, Federal agencies, the private sector, and voluntary organizations stepped up efforts to improve the nutritional health of Americans through informed food choices. For example, to reduce the risk of cancer, the Food Guide Pyramid advises 5-9 daily servings of fruits and vegetables. The Produce for Better Health Foundation's 5-A-Day program has raised consumer awareness of produce's benefits. Improved quality, increased variety, and year-round availability via world trade have also boosted consumption of fresh fruits and vegetables." (Kaufman et al., 2000).

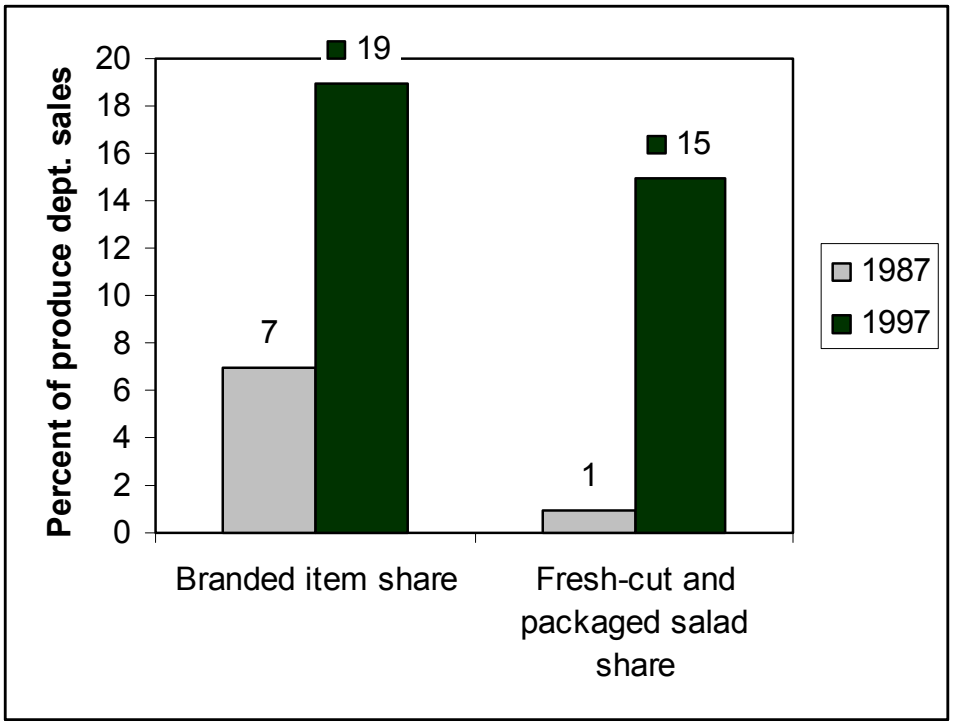

Figure 2. Percent of produce department sales of branded and packaged items in 1987 and 1997 (Taken from Dimitri et al., 2003).

Table 1. U.S. per capita consumption of fresh fruits and vegetables (Taken from Dimitri et al., 2003).

	Pounds of Consumption Per Capita		
	1987	**1995**	**2000**
Fresh fruits	121	125	130
Fresh vegetables	162	177	196
Total	283	302	326

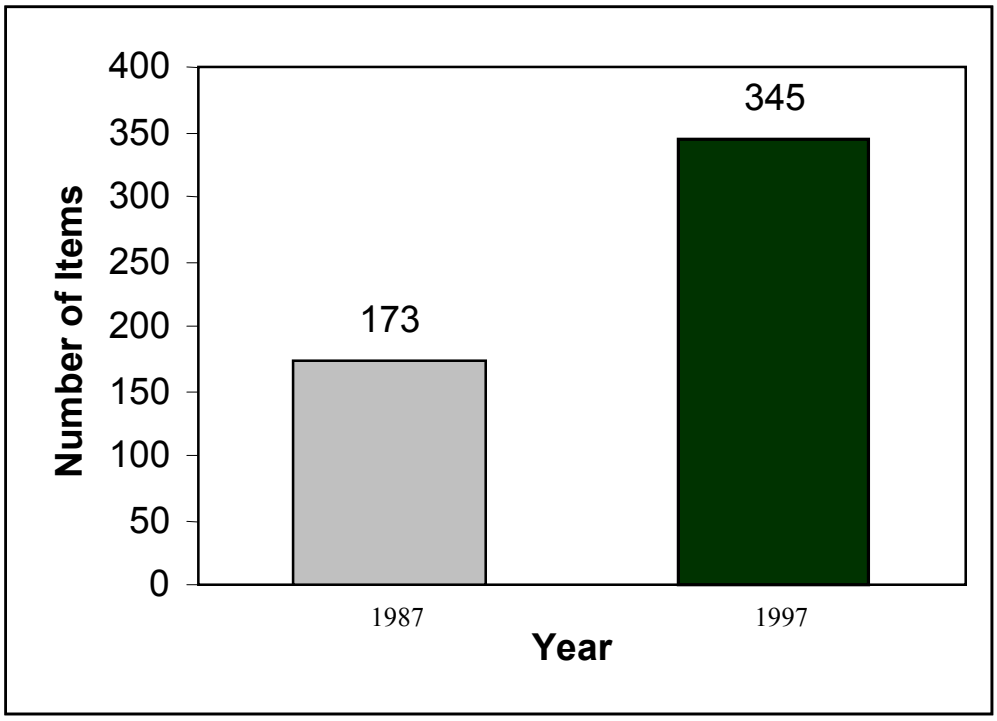

Figure 3. Number of fresh produce items carried by retail produce Departments (Taken from Dimitri et al., 2003).

2. Source of Contamination

Researchers have identified and listed the natural microbiological counts found in minimally processed fruits and vegetables, including whole and shredded lettuce (Nguyen-The and Carlin, 1994; Beuchat, 1996b). Beuchat (1996b) reported that aerobic mesophilic counts can range from 10^3 to 10^8 CFU/g, in fresh produce. Other studies reported that the total mesophilic counts present on lettuce or packaged salads were between 1.84 and 8.9 log CFU/g (Table 2). However, even within an individual product the populations of microorganisms are often not uniformly distributed. For example, Maxie (1978) isolated >10^4 CFU/g of mesophilic aerobic bacteria on the external lettuce leaves but only 32 CFU/g on the innermost leaves.

The widespread occurrence and use of uncomposted or improperly composted animal manure as fertilizer is a growing environmental concern, because it contaminates: water for drinking, irrigation, aquaculture and recreation; the hides, coats, and feathers of farm animals; and farm equipment and buildings. In the U.S., cattle, hogs, chickens and turkeys produce an estimated 1.36 billion tons of manure annually (EPA, 2000), with greater than 90% attributed to cattle.

Many of the most prominent foodborne pathogens in the U.S., including *Campylobacter jejuni, Salmonella* and *Escherichia coli O*157:H7, are carried by livestock and are principally transmitted to foods by fecal contamination. Microorganisms such as *Salmonella*, *Shigella*, and *E. coli* reside

Table 2. Total Mesophilic Counts Present on Leafy Vegetables and Packaged Salads (Adopted from Heard, 2002).

Fresh-cut Product	Total Mesophilic Count (Log cfu/g)	Reference
Mixed salad in school kitchens	1.8 – 3.0	Martínez-Tomé et al., 2000
Processed lettuce	2.5 – 6.2	Francis and O'Breirne, 1998
Green salads	<4.0 – 7.0	Fowler and Foster, 1976
Mixed green salad	<4.0 – 8.0	Fowler and Foster, 1976
Cabbage	4.1 – 7.1	Garg et al., 1990
Shredded lettuce	4.3	Delaquis et al., 1999
Cut lettuce	5.3	Priepka et al., 1976
Lettuce	5.4	Garg et al., 1990
Lettuce salads	7.2 – 6.2	Jayasekara, 1999
Prepackaged ready-to-serve salad	5.5 – 8.3	Lack et al., 1996
Packaged garden salad	5.3 – 8.9	Hagenmaier and Baker, 1998

CFU/g = Colony Forming Units/gram

in the intestinal tract of animals, including humans, and bacteria such as *L. monocytogenes* are normal inhabitants of many soils. A survey of cattle herds indicated that the prevalence of *E. coli* O157:H7 among feedlot animals was as high as 36.8% (Chapman et al., 1997). Wang and Doyle (1996) revealed that *E. coli* O157:H7 survived in bovine feces for 42 to 49 d at 37°C, for 49 to 56 d at 22°C, and for 63 to 70 d at 5°C.

Recent evidence of foodborne disease outbreaks associated with the consumption of fresh produce has prompted some to consider the role of contaminated irrigation and surface runoff waters. Irrigation water containing raw or improperly treated human sewage can be the source of many pathogens, with *Shigella* and the enteric viruses (hepatitis A virus, Norwalk-like viruses, rotaviruses) being perhaps the most significant (Beuchat, 1996b; Beuchat and Ryu, 1997). Irrigation water contaminated with animal fecal matter can also be a source of pathogens on fresh produce (Tauxe et al., 1997). The U.S. Department of Agriculture (USDA), in an attempt to decrease the risk of "manure-borne" pathogens, requires that at least 120 d elapse between noncomposted manure application and harvest of organic crops with edible portions exposed to soil particles (USDA, 2000). The proximity of domestic (or wild) animals to irrigation water may serve as a vehicle for *E. coli* O157:H7 to gain access to produce during preharvest operations (Wachtel et al., 2002). The researchers suggested that preharvest crop contamination via infected irrigation water can occur through lettuce plant roots. Solomon et al. (2002) reported that *E. coli* O157:H7 was transmitted to lettuce plants through spray and surface irrigation.

After harvest, contamination may occur as a result of using contaminated water or ice, improper handling by workers or consumers, transport containers, presence of wild or domestic animals in processing environment, cross contamination and improper storage or handling (Tauxe et al., 1997; FDA, 2001a). Garg et al. (1990) established that the shredders and slicers were a major source of contamination during the processing of lettuce and other vegetables. For example, the aerobic plate count of lettuce increased from 1.8 x 10^4 CFU/g to 140 x 10^4 CFU/g after shredding. Chen et al. (2001) reported that even if proper hand-washing methods are followed, microorganisms may still be present and can be transferred from washed hands to lettuce during chopping.

Figure 4 illustrates potential mechanisms by which pathogens may contaminate produce.

3. Pathogens of Concern and Associated Outbreaks

The potential for contamination increases as the fresh produce moves from farm to table (i.e. irrigation water, improperly composted manure, wash water systems, soiled equipment, unsanitary practices, etc.). The Centers for Disease Control and Prevention (CDC) reported that the mean number of produce related foodborne outbreaks more than doubled from 1973 to 1987 (4.3 per year) and again from 1988 to 1991 (9.75 per year) (Tauxe et al., 1997; Hurst, 2002). More recently, Sivapalasingam et al. (2004) detailed that from 1973 through 1997 in the U.S., 32 states reported 190 produce-associated outbreaks,

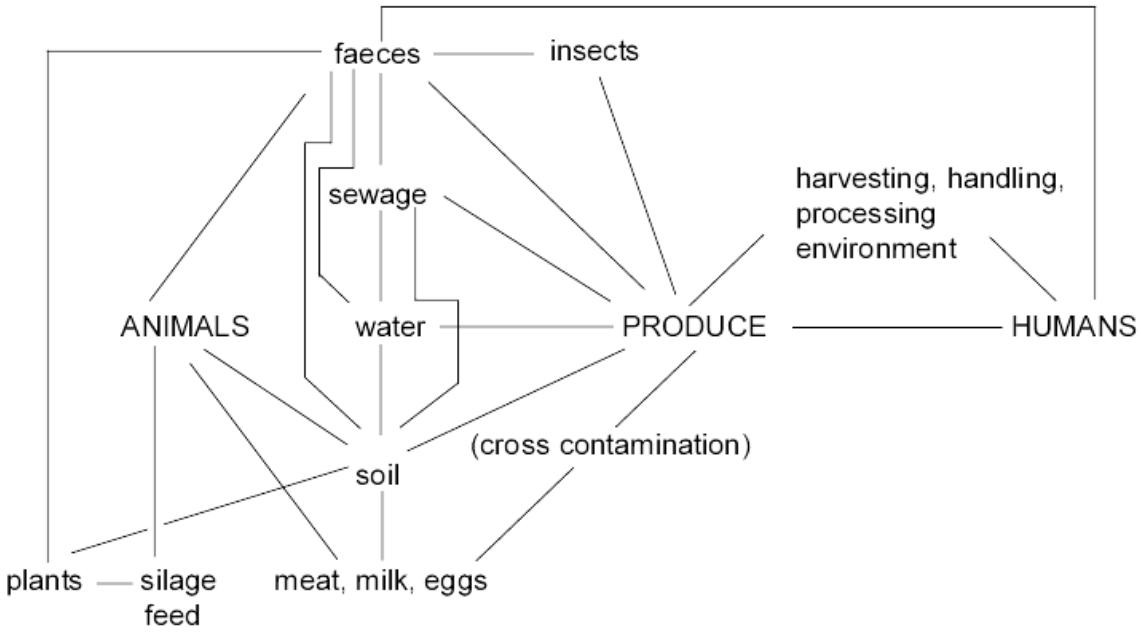

Figure 4. Mechanisms by which raw fruits and vegetables may become contaminated with pathogenic microorganisms (Taken from Beuchat, 1996b).

16,058 reported illnesses, 598 hospitalizations, and eight deaths. In addition, the researchers reported that among the 190 outbreaks, 25 were associated with lettuce causing 2,078 reported illnesses, 181 hospitalizations, and six deaths (Table 3).

Common foodborne pathogens associated with fresh produce include *Salmonella* spp., *Listeria monocytogenes*, *Shigella* spp., and *Escherichia coli* O157:H7 (Beuchat, 1996b; FDA, 2001a). A brief description of pathogens that have been isolated from minimally processed leafy vegetables and salads with emphasis on their association with foodborne outbreaks are given below.

a) *Listeria monocytogenes*

The genus *Listeria* contains six species, of which *Listeria monocytogenes* is considered to be of public health concern (Harris, 2002). *L. monocytogenes* is a Gram-positive, psychrotrophic, facultative, nonsporulating, and motile rod. The ability of this organism to grow and multiply in refrigerated and warm temperatures (1 and 50°C) is well documented (Ray, 1996; Harris, 2002). Optimum growth occurs between 35 to 37°C (Ray, 1996). *L. monocytogenes* is relatively resistant to freezing, drying, high salts (growth at 10%; survival at 20-30%), and pH < 5.0.

Symptoms, among people with normal health, appear within 1 to 7 d following ingestion and include mild flu-like symptoms with slight fever, abdominal cramps, and diarrhea. In contrast, symptoms among the elderly, pregnant women, unborn fetuses, and people with reduced immunity can be

Table 3. Reported lettuce associated outbreaks with known pathogens in the United States, from 1973 through 1997 (Taken from Sivapalasingam et al., 2004).

Year	Pathogen	No. of ill persons	Location of Outbreak	Lettuce type	Reference[a]
1981	Norwalk	92	Alabama	Lettuce	FOSS
1981	*Giardia*	61	New Jersey	Lettuce	FOSS
1985	*Shigella flexneri* 3	25	Texas	Lettuce	FOSS
1986	*Shigella sonnei*	347	Texas	Shredded lettuce	Davis et al., 1988
1988	Hepatitis A	202	Kentucky	Iceberg lettuce	Rosenblum et al., 1990
1990	Hepatitis A	130	Missouri	Lettuce	CDC, 1993
1993	*Salmonella* Heidelberg	18	Minnesota	Lettuce	FOSS
1994	*Salmonella* Thompson	16	Minnesota	Lettuce	FOSS
1994	*Salmonella* Braenderup	30	New York	Lettuce	FOSS
1995	*E. coli* O157:H7	92 (1 HUS[b])	Montana	Leaf lettuce	Acker et al., 1998
1995	Norovirus	76	Florida	Lettuce	FOSS
1995	*E. coli* O157:H7	11	Ohio	Iceberg lettuce	FOSS
1995	*E. coli* O157:H7	30	Maine	Iceberg lettuce	Martin et al., 1986
1996	*E. coli* O157:H7	61 (3 HUS)	Multiple states	Mesclun mix	Hillborn, et al 1999
1996	*E. coli* O157:H7	54	Michigan	Lettuce	FOSS
1997	*Cyclospora*	29	Florida	Mesclun mix	Herwaldt, 2000
1997	*Cyclospora*	12	Florida	Mesclun mix	Herwaldt, 2000

a For an outbreak reported only in the Foodborne Outbreak Surveillance System (FOSS), no reference is available.
b HUS, hemolytic uremic syndrome.

fatal. Several other symptoms are associated with this pathogen including septicemia and meningitis (Ray, 1996). Ingestion of contaminated food with as low as 100 to 1000 cells are sufficient to trigger symptoms particularly for those with reduced immunity (Ray, 1996).

L. monocytogenes is present in the intestinal tract of many animals, including humans. Hence the following sequence is set in motion, where the bacteria is found in the feces of the animals, on the land they occupy, in sewage, in soils in which raw sewage is applied, and on plants that grow in those soils (Van Renterghem et al,, 1991; Nguyen-The and Carlin, 1994; IFPA, 2001). In addition, *L. monocytogenes* exists in plant materials including shrubs and decaying vegetation (Welshimer and Donker-Voet, 1971; Beuchat, 1996a).

L. monocytogenes has been isolated from fresh-cut lettuce (Steinbruegg et al., 1988; Wong et al., 1990; and Francis et al., 1999). In addition, studies have showed that *L. monocytogenes* can grow and survive in lettuce (Steinbruegg et al., 1988; Beuchat and Brackett, 1990; Lin et al., 1996; Faber et al., 1998). In 1981, Canadian officials reported an outbreak of listeriosis which, was traced back to cabbage used by the regional producers to prepare coleslaw. The cabbage was suspected to be contaminated in the field with *L. monocytogenes* form uncomposted sheep manure. Tragically, 41 cases were identified resulting in 18 deaths (2 adults and 16 fetal or newborn) (Sewell and Faber, 2001; Harris, 2002).

b) Escherichia coli O157:H7

E. coli O157:H7 is a Gram-negative, motile, nonsporulating, rod-shaped, facultative anaerobic bacterium and produces a verotoxin (VTI). This pathogen grows rapidly at 30 to 42°C, poorly at 44 to 45°C, and does not grow at <10°C (Ray, 1996). *E. coli* O157:H7 was first recognized as a pathogen in 1982, when it was associated with two foodborne outbreaks of hemorrhagic colitis (Doyle et al., 1997).

E. coli O157:H7 causes hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). Symptoms normally occur within 3 to 9 d after ingestion and generally last for about 4 d.

E. coli O157:H7 is common in the normal microflora of the intestinal tract of humans and other warm-blooded animals, including deer, horses, goats, sheep, cat, dogs, rabbits, and poultry, with prevalence rates of up to 5.2% (WHO, 1998; Fratamico et al., 2002). Houseflies can also serve as a vector of dissemination as they carry the pathogen in their intestine and other parts of their body. Since cattle appear to be a natural reservoir for the pathogen, with prevalence rates of 1.8 to 28% (Fratamico et al., 2002), contamination of raw fruits and vegetables may occur when cattle inadvertently enter fields, or improperly composted cow manure is applied as fertilizer (WHO, 1998). Wang et al. (1996) showed that *E. coli* O157:H7 can survive in bovine feces for 70 d. Therefore, the researchers concluded that regulations requiring the aging of bovine manure for 60 d before using it as a fertilizer was inadequate.

Although, the primary mode of transmission is undercooked ground beef,

studies have showed that this pathogen can survive and grow in salads (Adbul-Raouf et al., 1983; Lin et al., 1996). Internalization of the pathogen through stomata and cut surfaces has been reported in lettuce (Seo and Frank, 1999; Takeuchi and Frank, 2000). Lettuce was linked to outbreaks with *E. coli* O157:H7 and it has been suggested that leakage from cellular structure of the leaves may serve as nutrients for the pathogen (Beuchat, 1999). In 1995, an outbreak of *E. coli* O157:H7 infection among 70 people in Montana was associated with lettuce (Wang et al.,1996). The lettuce plants were grown downhill from a cattle pasture. The researchers speculated that the lettuce may have been contaminated with water used to irrigate the field.

c) Salmonella species

Salmonella is a member of the family Enterobacteriaceae which comprises a large and diverse group of Gram-negative rod-shaped bacteria. *Salmonella* are facultative anaerobic, nonlactose fermenting, nonspore forming, and most are motile. There are currently over 2400 serotypes. Complete inhibition of growth occurs at pH < 3.8 and > 9.0, temperature < 7°C, or water activity < 0.94 (Ray, 1996; Jay, 2000; Gray and Fedorka-Cray, 2002). Optimum growth occurs at pH near neutrality and temperatures between 35 and 37°C (Ray, 1996).

Salmonella gastroenteritis usually follows the ingestion of contaminated food or drinking water. Typically, gastroenteritis in humans begins 24-28 h after ingestion and normally consists of fever, chills, headache, nausea, and vomiting,

followed or concomitant with, abdominal cramps and diarrhea. These symptoms are usually accompanied by prostration, muscular weakness, faintness, and drowsiness. Ingestion of contaminated food with 10^5 - 10^6 cells is sufficient to trigger symptoms (Ray, 1996). The spectrum of disease ranges from loose stools to severe dysentery-like syndrome.

Salmonella grow readily in many foods, as well as water contaminated with feed or feces. The primary habitat of the bacteria is the intestinal tract of animals, humans, and on occasion insects (Ray, 1996; Jay; 2000). In addition, *Salmonella* has been isolated from soil, water, and sewage contaminated with fecal matter (Ray, 1996).

Salmonellae was isolated from fresh produce, many of which have were linked to outbreaks of salmonellosis (Hedburg and Olsterhol, 1993; WHO, 1998). The incidence (survival and growth) of this pathogen in fresh lettuce is well documented (Ercolani, 1976; Lin and others, 1996; Little and others, 1999; FDA, 2001c).

d) Shigella species

The genus *Shigella* belongs to the family Enterobacteriaceae as do the *Salmonella* and *Escherichia coli*. There are four serological subgroups under the genus *Shigella*: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. *Shigella* species are Gram-negative, facultative anaerobic, non-motile, rod-shaped bacteria. The strain can grow between 7 and 46°C, with optimum at 37°C (Ray, 1996; Jay, 2000).

The infective dose of this microorganism is as low as 200 cells, although, Lampel and Maurelli (2002) reported 10 cells are sufficient to cause disease. Most cases of shigellosis result from the ingestion of food or water contaminated with human feces. Shigellosis is characterized by an incubation period of 1 to 7 d and by signs and symptoms of diarrhea, abdominal pain, fever, and often vomiting. Stools may contain blood, mucus, or pus (Bryan, 1979).

Shigellae are transmitted by personal contact, flies, and water, as well as by food. Fresh produce can become tainted through the use of contaminated irrigation water, the use of raw sewage as fertilizer, insect transfer, or human contact (WHO, 1998).

Studies have showed that lettuce has been implicated as a vehicle for shigellosis (Davis et al., 1988; Frost et al., 1995). *Shigella* species can survive on shredded lettuce under refrigeration for up to 3 d without populations decreasing and can survive on raw fruits, including watermelon and raw papaya (Escartin et al., 1989; Satchell et al., 1990). In 1994, an outbreak of *Shigella sonnei* was traced back to infected lettuce in several European countries (Kapperud et al., 1995) and in 1995, another *Shigella* outbreak was traced back to lettuce in the United States (Tauxe et al., 1997).

4. Production / Processing Methods.

Nearly 100 percent of the lettuce consumed in the U.S. is produced domestically (Glaser et al., 2001). The vast majority of domestic production

takes place in just two States: California and Arizona (Glaser et al., 2001). Figure 5 illustrates a typical sequence of production for iceburg lettuce, which assures year round production. Fall planted lettuce may require as little as 65 days from the beginning of germination to harvest, while winter planted lettuce will require as long as 120 days (**http://ag.arizona.edu/pubs/crops/az1099/**).

The University of Arizona (1999), in a document titled, "Guidelines for Head Lettuce Production in Arizona," explains the process of harvesting lettuce. The following is a brief excerpt from the document:

> "In the field, harvesters cut the lettuce near the soil surface with a long knife then trim unwanted leaves usually leaving 4 to 5 wrapper leaves. After harvest, the lettuce is transported to a cooling shed and distribution center where it is stored at 35 to 36° F. Although lettuce storage life under these conditions is 16 to 20 d, almost all lettuce is shipped with 48 h to salad plants where they are sorted, washed with a dilute chlorine solution or fumigated with ozone, and then chopped for prepackaged or ready-made salads in sealed plastic bags."

A basic flow diagram for the production of minimally processed vegetables is depicted in Figure 6. The first step is the removal of the outer layer leaves or dirt. Wrapper leaves are usually removed at the field. This is followed by slicing or shredding based on customer needs. Then the lettuce is thoroughly washed with a disinfectant chemical and excess water is removed. Once dried, the lettuce is packaged in a modified atmosphere to slow down product respiration and extend shelf-life (Francis et al., 1999). The packaged lettuce is stored at refrigerated temperature to extend shelf-life and slow microbial growth.

Figure 5. Production sequence for iceburg lettuce in California and Arizona (Taken form Glaser et al., 2001)

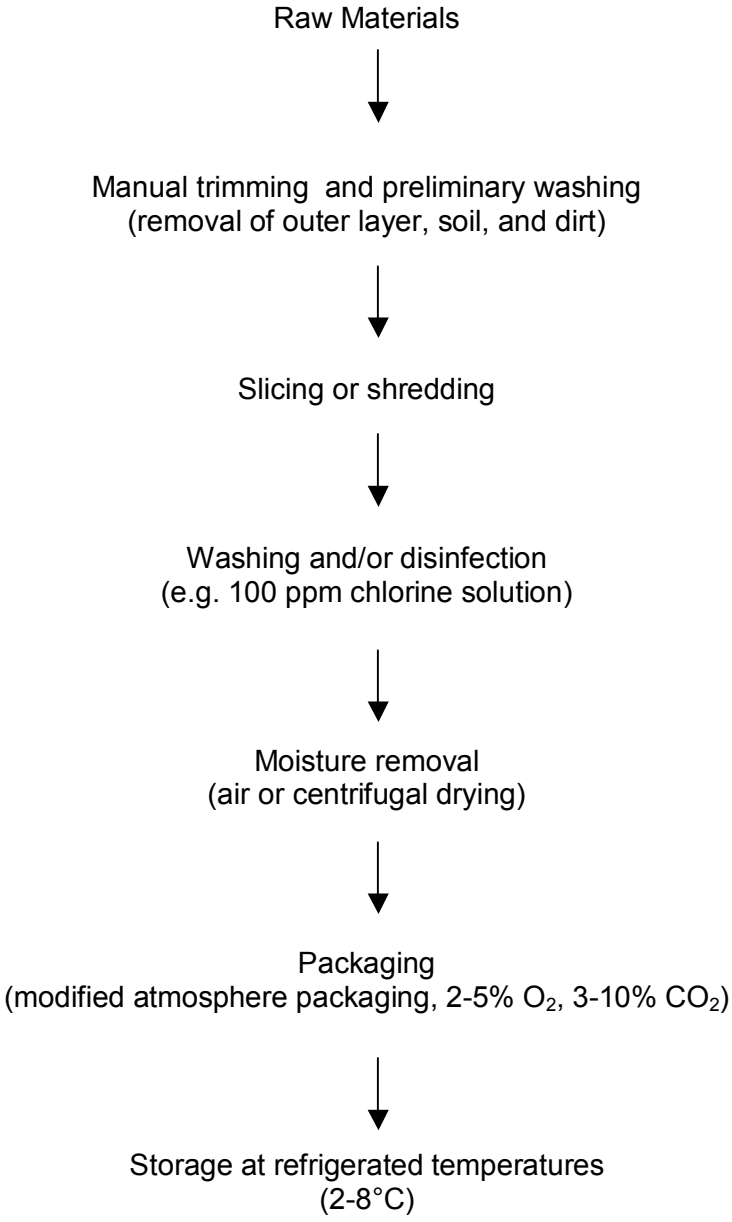

Figure 6. A flow diagram for the production of minimally processed vegetables (Taken from Francis, et al., 1999).

The critical step of washing with a chemical disinfectant has been extensively studied (Beuchat and Ryu, 1997; Sapers, 2001) and is discussed in the next section in detail.

B. Chemical Disinfection Methods

The simple step of thorough washing minimally processed salads reduces indigenous microflora and pathogens that may be present as a result of contamination at any point in the processing chain. Therefore, produce wash water systems are of great concern, in particular, if the water is recycled. Water recirculation can increase the potential for foodborne illness by distributing the source of contamination to product already in the wash water system and not previously contaminated or by contaminating newly introduced product.

Washing lettuce leaves with tap water alone was reported to reduce indigenous microflora by approximately 1 log CFU/g (Adams et al., 1989; Nguyen-The and Carlin, 1994). Similarly, Singh and others (2002), reported that deoinized water achieved close to 1 log and 0.22 CFU/g reductions in populations of *E. coli* O157:H7 inoculated on shredded lettuce after multiple washing and single wash, respectively. Product wash water, not treated with a chemical disinfectant, can become a source of microbial contamination if reused (IFPA, 2001), highlighting the need for chemical disinfectants in wash water systems.

The addition of a chemical disinfectant to the wash water further reduces the microbial load (Beuchat and Ryu, 1997; Sapers, 2001). The use of a chemical disinfectant in wash water provides a barrier to cross contamination of produce and is effective in removing disease-causing organisms from the surface minimally processed produce (FDA, 2001a). These chemical sanitizers can

therefore reduce microbial contamination of subsequent batches processed in the same recirculated wash water system. However, it is important to recognize that such reductions, although important, are not sufficient to assure microbiological safety of minimally processed fresh-cut vegetables (Sapers, 2001). Currently, sodium hypochlorite is the most commonly used chemical sanitizer in produce wash water (Brackett, 1987; Beuchat and Ryu, 1997; WHO, 1998). There is a growing number of alternative water sanitizing compounds which are used to reduce microbial populations in fresh-cut produce, including chlorine dioxide (Reina and others, 1995; Zhang and Faber, 1996), ozone (Kim and others, 1999), electrolyzed water (Park and others, 2001), hydrogen peroxide (Sapers, 2001), organic acids (Venkitanaraynana et al., 2002), peroxyacetic acid (Rogers and others, 2004), trisodium phosphate (Zhang and Faber, 1996), and radiation (Prakash and others, 2000). None of the previously mentioned chemical disinfectants completely eliminate pathogens from minimally processed produce when used at levels that cause no deterioration in quality (WHO, 1998).

1. Factors Affecting Efficacy of Disinfectants

Temperature:

Temperature plays an important role in the survival of pathogens on the surface of fruits and vegetables (Gawande and Bhagwat, 2002). There can be an exponential growth of bacteria when temperatures are increased and humid conditions are maintained (Splittstoesser, 1970). A cooler water temperature is

more effective at sanitizing processing equipment and surfaces of fresh fruits and vegetables (Beuchat, 2000). This is especially true when using chlorine, because maximum solubility in water is achieved at about 4°C (39°F) (Beuchat, 2000). Temperature plays a vital role in the prevention of infiltration and internalization of bacteria within the produce. Bacteria can infiltrate into the interior of produce when warm product is placed in colder water. As the freshly harvested product cools, the internal gas contracts, thereby creating a partial vacuum, which creates a transient differential in pressure that can result in diffusion of wash water and bacteria into the product via stem tissue, opens areas due to punctures, and damaged skin (Beuchat, 2000; Sapers, 2003; Ibarra-Sanchez, 2004). Zhaung and others (1995) showed that *Salmonella* Montevideo is filtered into the core of tomatoes when there is a temperature differential of 15 °C. Internalization of *E. coli* O157:H7 has been reported in lettuce (Seo and Frank, 1999; Takeuchi and Frank, 2000).

pH

The pH of chlorine based wash water systems is an important factor in the reduction and inactivation of bacteria. The lethal effects of chlorine were observed at pH range of 6.0-7.5 (Sapers, 2003). When bleach (sodium hypochlorite) is added to water, the pH increases. Raising the pH of the solution will result in lowering the amount of chlorine that is available as hypochlorous acid (HOCl). For example, if pH increases above 8 the hypochlorous acid splits to form hydrogen ions (H+) and hypochlorite (OCl-) ions. This hypochlorite ion

thus formed has weak bactericidal effects. The pH of the solution may be adjusted by the addition of organic or inorganic acids.

Microbial Attachment and Biofilms

Many researchers have reported that the location of microorganisms on produce surfaces affects their inactivation by chemical disinfectants (Cherry, 1999; Seo and Frank, 1999; and Sapers, 2003). Seo and Franks (1999) reported that bacteria tend to locate in pores, indentations or other natural irregularities when they attach to the surfaces of lettuce. They found that *Escherichia coli* O157:H7 was internalized in the stomata of lettuce leaves, therefore, escaping contact with disinfectant chemicals. In addition, the exposed cut-surface greatly increases the surface area for bacterial attachment, which in turn enhances their survival. Moreover, the exposed cut surface area introduces additional organic matter into the wash water, thereby decreasing sanitizer effectiveness (Rodgers et al., 2004).

The varied surface topographies of fresh produce provide numerous sites for production of biofilms that are difficult to remove with chemical disinfectants (Cherry, 1999; Koseki and others, 2001; Yang and others, 2003). This is further exasperated when the time interval between contamination of the produce with human pathogens and washing is extended. Therefore, the time available for cellular attachment and biofilm formation depends on the adequacy and frequency of cleaning (Moore and others, 2000). Sapers et al. (2000), reported that apples inoculated with *E. coli* and held for various times before washing with

water indicated that 30 min after inoculation the bacteria was reduced by approximately 1 log CFU/g. Whereas, washing the apples after a 24 h wait the reduction was not significant because the bacteria had firmly attached to the apple. According to Yu and others (2001), the relative ineffectiveness of many sanitizers on strawberries is partially due to surface roughness, which provide an ideal site for bacteria to attach and form biofilms.

Han et al. (2000) reported that there was no significant growth of *E. coli* O157:H7 found on uninjured surfaces of green peppers after inoculation and incubation for 24 h at 37°C; whereas, significant growth and multiplication was found on injured surfaces, because cut or injured surfaces provide opportunity for bacterial attachment and growth. Figure 7 shows the level of microbial attachment on the surface of green peppers (Table 4) was mainly determined by the surface properties of the product (i.e. level of injury or uninjured).

Oxidation-Reduction Potential (ORP):

ORP measurements are based on displaying the response of a specialized electrode in a solution. Like pH electrodes, each ORP electrode has unique characteristics that cause variability in the signal the electrodes send to the meter. ORP refers to the oxidation reduction potential, a measure of the oxidizing properties of the sanitizer in water, which is determined by a sensor with a noble metal electrode, usually platinum, and a standard Ag/AgCl reference electrode. When an ORP sensor is placed in water containing a chemical sanitizer, such as chlorine or ozone, which is an oxidizer, it acts like a small

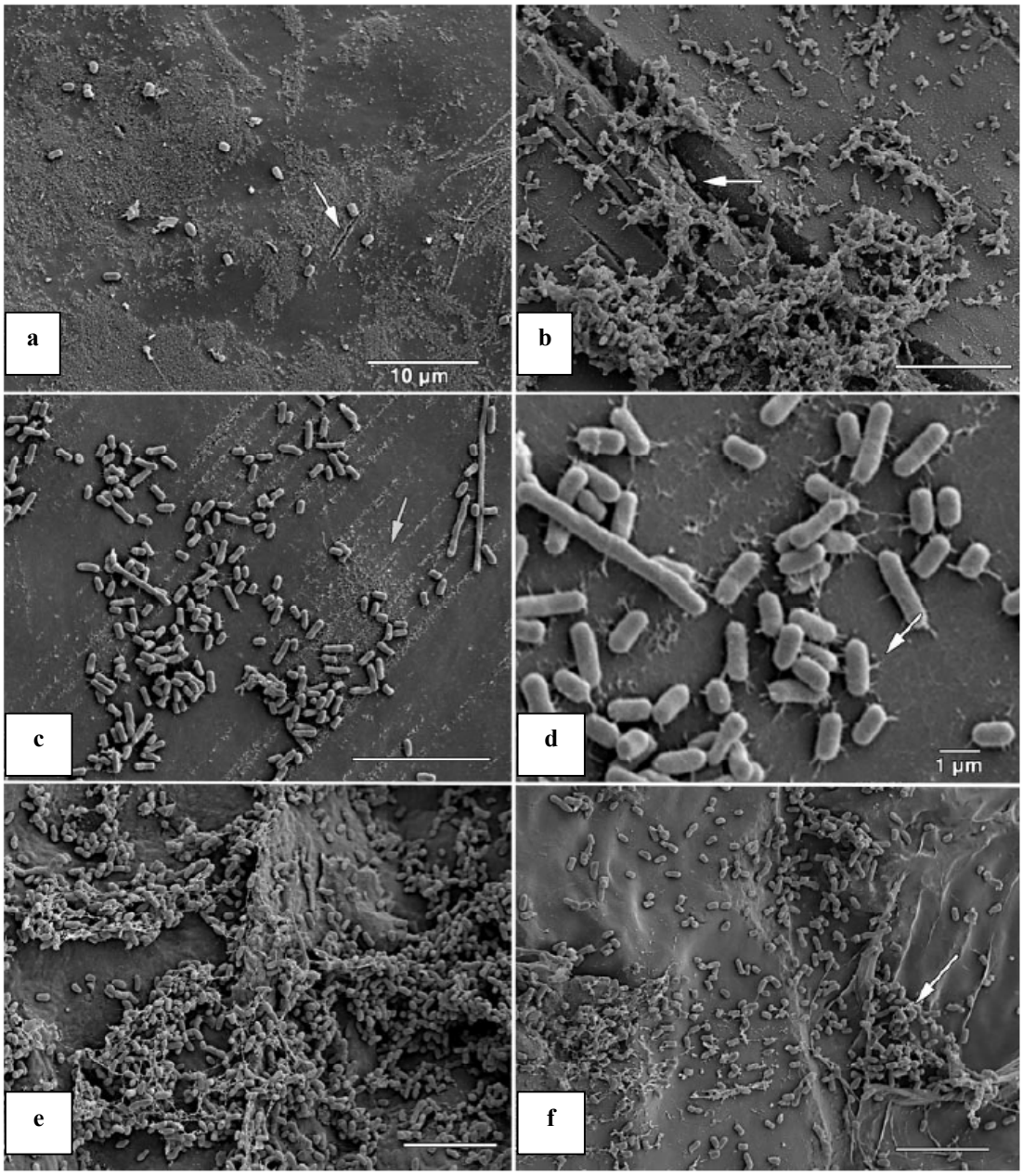

Figure 7. *Escherichia coli* O157: H7 cells attached to injured and uninjured green pepper surface. (a) *Escherichia coli* O157: H7 cells attached to uninjured surface of Sample A after inoculation, 2-h drying, and 24-h incubation at 37°C (63000). Bar=10 mm, SEM. (b). *Escherichia coli* O157: H7 cells growing and attached to injured surface of Sample B after inoculation, 2-h drying, and 24-h incubation at 37°C (62500). Bar = 10 mm, SEM. (c). *Escherichia coli* O157: H7 cells growing and attached to wax-layer-injured surface of Sample B (63000). Bar=10 mm, SEM.
(d). *Escherichia coli* O157: H7 cells with exocellular polymers on wax-layer-injured surface of Sample B (69000). Bar = 1 mm. (e) *Escherichia coli* O157: H7 cells growing and attached to injured surface of Sample C after inoculation, 2-h drying, and 12-h incubation at 37°C (62000). Bar = 10 mm, SEM. (f) *Escherichia coli* O157: H7 cells growing and attached to the injured surface of inoculated Sample D after water washing and 12-h incubation at 37°C (62000). Bar = 10 mm, SEM.
(Taken from Han et al., 2000).

Table 4. Preparation of samples (A-H) for SEM analysis and surface-plating colony enumerations (Taken from Han et al., 2000).

Samples	Green pepper surface	Incubation time after inoculation and 2-h drying (h)	Treatment	Incubation time after treatment (h)
A	Uninjured	24	No treatment	No incubation
B	Injured	24	No treatment	No incubation
C[a]	Injured	12	No treatment	No incubation
D	Injured	No incubation	Water washing	12
E	Injured	No incubation	0.62 mg 1^{-1} ClO_2	12
F	Injured	No incubation	1.24 mg 1^{-1} ClO_2	24
G	Injured	12	Water washing	No incubation
H	Uninjured	12	Water washing	No incubation

[a] Sample C was a control for sample D, E, F, G, and H.

battery and creates a small but measurable electric potential. The value of this potential varies with the type of sanitizer.

The ORP value grants a means to monitor the chemical efficacy of wash water treatments. The ORP value provides the operator with a rapid and single-value assessment of the wash water disinfection potential, which can prompt operators to better control the treatment by adding more hyphochlorite or adjusting the pH of the wash water. Suslow (2004) recommended a value of 640 mV in chlorine treatments.

2. Detergents

There are few reports on the efficacy of detergents or cleaners used on lettuce and/or fresh-cut vegetables perhaps because many are not approved for use in fresh-cut produce (Raiden, et al., 2003). Burnett et al. (2004), investigated a 0.5% (wt/vol) solution of FIT Professional Line Antimicrobial (FIT; Procter and Gamble Company, Cincinnati, Ohio) and reported only 1.51 log CFU per lettuce piece reduction of *L. monocytogenes*. Barak and his colleagues (2003) investigated the efficacy of Bac Down hand soap (Decon Laboratories, Inc. Bryn Mawr, PA) applied to the surface of cantaloupes. They reported approximately 1 log CFU/g reduction of *Salmonella enterica* serovar Poona or *Pantoea agglomerans*. Similar results were reported by Yu et al. (2001) when treating *E. coli* O157:H7 inoculated strawberries with 100 and 200 ppm solution of Tween 80 (polysorbate 80).

3. Chlorine

Chlorine is the most widely used sanitizer in reducing microbial load in fresh fruit and vegetable wash water; (WHO, 1998; IFPA, 2001). Chlorine is very reactive and combines with any oxidizable substrate to form secondary compounds, such as trihalomethanes (IFPA, 2001). For chlorine to disinfect produce the recommended usage level is 50-200 ppm, at a pH below 8.0, and with a contact time of 1 – 2 min (WHO 1998, FDA 2001a). The most common forms of free chlorine include liquid chlorine and hypochlorites. However, chlorine has a limited effect on reducing microorganisms on fresh fruit and vegetable surfaces (Beuchat, 2000; Sapers, 2001).

The inhibitory or antimicrobial activity of chlorine depends on the amount of hypochlorous acid (free chlorine) present in the water that comes into contact with the microbial cells. Hypochlorous acid is the form of available free chlorine that has the highest bactericidal activity against microorganisms commonly found in fresh fruits and vegetables (Sapers, 2003). Besides hypochlorous acid the bactericidal activity of chlorine is dependent on water pH, temperature, presence of organic matter, contact time, light, air, or metals (WHO, 1998, FDA 2001a, IFPA 2001).

The effects of pH on chlorine dissociation indicate that at pH 7.5 or greater the quantity of chlorine available as active hypochlorous acid (HOCL) is limited, rather, chlorine exists mainly as inactive hypochlorites (OCl^-). If the pH of the wash water decreases below 4.0, then chlorine gas may be formed which is a health hazard for employees (IFPA, 2001). Therefore, the pH of the water should

be maintained between 6.0 and 7.5 to ensure adequate and safe chlorine activity. The percentages of chlorine as HOCl at pH 6.0 and 8.0 are about 97% and 23%, respectively (WHO, 1998).

The effects of temperature on HOCl indicate that as the temperature of the water decreases; HOCl is in favor regardless of the pH. For example the proportion of chlorine as hypochlorous acid is slightly lower at 20°C than at 0°C, especially when the pH falls between 6 and 9 (Eifert and Sanglay, 2002). The maximum solubility of chlorine is achieved in water at approximately 4°C. However, the temperature of the water should be ideally at least 10°C higher than the fruit or vegetable to achieve a positive temperature differential, thereby minimizing the uptake of wash-water through stem tissue and open areas in the skin or leaves, whether due to mechanical assault or naturally present (e.g. lenticel and stomata) (WHO, 1998).

Beuchat (2000) described the reactions that occur when chlorine is added to water using the following reactions:

1) The addition of chlorine gas to wash water:

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$$

In this reaction, chlorine is hydrolyzed to produce hypochlorous acid (HOCl), hydrogen ion (H^+), and chlorine ions (Cl^-). The resulting hypochlorous acid is the primary reason for the antimicrobial properties of chlorine (Sapers, 2003).

2) The addition of liquid chlorine, such as, sodium hypochlorite (NaOCl) and calcium hypochlorite ($Ca(OCl)_2$) to the wash water can be expressed as follows:

$$NaOCl + H_2O \rightarrow NaOH + HOCl$$

$$Ca(OCl)_2 + 2H_2O \rightarrow Ca(OH)_2 + 2HOCl$$

In these reactions, both sodium and calcium hypochlorite are hydrolyzed to produce hypochlorous acid and sodium hydroxide (NaOH) and calcium hydroxide [$Ca(OH)_2$].

3) Thus, whether the addition of chlorine in a liquid or gas form hypochlorous acid is the most effective antimicrobial of all the chlorine residual fraction. The hypochlorous acid that is formed in the water may further dissociate to produce hydrogen ion (H+) and a hypochlorite ion (Cl-), as demonstrated in the following reaction:

$$HOCl \leftrightarrow H^+ + OCl^-$$

The dissociation of hypochlorous acid to hypochlorite and hydrogen ions is dependent on the pH of the wash water. Most of the HOCl will remain undissociated at pH < 7. The proportion of undissociated HOCl is greatest at pH > 5. If the pH falls < 4, the proportion of potentially hazardous chlorine gas increases. As the pH > 4.0 the ratio of HOCl to OCl^- decreases. At pH 8, the proportion of HOCl that remains undissociated will be less than 25%. Since OCl^-

is less germicidal than HOCl, a chlorine sanitizer solution with a pH range of 6.5 to 7.5 may have greater antimicrobial efficacy.

Beside pH and temperature, organic load can have a significant detrimental effect on chlorine efficacy (Li et al., 2001; Takeuchi and Frank, 2001). Taormina and Beuchat (1999) reported that free chlorine in a 200 ppm solution decreased to about 20 ppm (about 90% decrease), within 15 min after treating alfalfa seeds in varying foreign organic loads. In another study, Beuchat and others (2004a; 2004b) attributed the rapid decrease in chlorine concentration to the release of tissue juices from shredded lettuce, which increased the concentration of organic materials accessible for reaction with and neutralization of chlorine. In addition, chlorine is readily inactivated upon contact with organic matter, and the effectiveness depends on direct contact with cells. Takeuchi and Frank (2001) concluded that cells located 30 to 40 μm from the cut surface were the most protected from chlorine disinfection. Thus, disinfectants that can be inactivated by organic material are unlikely to be effective in eliminating viable *E. coli* O157:H7 cells that have penetrated into tissue.

Zhang and Faber (1996) reported reduction of *Listeria monocytogenes* on shredded lettuce and cabbage. The researchers achieved a 1.3 and 1.7 log CFU/g reduction on lettuce and 0.9 and 1.2 log CFU/g reduction on cabbage after treatment with 200 ppm chlorine for 10 min at 4°C and 22°C, respectively. The study demonstrated that the bactericidal effect on *L. monocytogenes* was higher at 22°C than at 4°C, and was more effective on lettuce than on cabbage. Delaquis et al. (1999) reported similar results when treating shredded lettuce and

cabbage at 47 and 4°C for 3 min. Rodgers et al. (2004), reported that treatment with 100 ppm chlorine for 5 min reduced *L. monocytogenes* and *E. coli* O157:H7 to nondetectable levels on whole apples, whole lettuce, strawberries, and cantaloupe, whereas approximately 1 log CFU/g of *L. monocytogenes* and *E. coli* O157:H7 remained on sliced apples and shredded lettuce. The increased reduction on whole versus shredded lettuce can be attributed to the attachment of microorganisms to the cut edges of lettuce and increase organic materials in water due to the release of tissue juices in shredded lettuce.

Table 5 summarizes several studies using chlorine as the chemical disinfectant at varying treatments and efficacy at reducing populations of natural microflora and pathogens in whole or shredded lettuce. The results demonstrate that despite the chlorine concentrations, the maximum reduction was ≤ 2 logs in minimally processed vegetable products when treated for ≤ 3 min. These results create the need for research using alternative chemical disinfectants.

4. Chlorine Dioxide (ClO_2)

Chlorine dioxide is used as an antimicrobial for produce wash and is approved for use on uncut produce followed by potable water rinse (CFR, 2005a). ClO_2 is a yellow to red gas with 2.5 times the oxidizing potential of chlorine gas (Suslow, 1997). A maximum of 200 ppm ClO_2 is allowed for sanitation of processing equipment and 3 ppm is allowable for contact with whole produce. In addition, treatment of produce with ClO_2 must be followed by a

Table 5. Effects of chlorine treatments on minimally processed vegetables.

Disinfecting Treatment	Product	Microbial Reduction	Reference
200 ppm for 10 min at 4 and 22°C	Shredded lettuce and cabbage	1.3 and 1.7 log (lettuce) and 0.9 and 1.2 (cabbage) reductions in *Listeria monocytogenes* populations at 4 and 22°C, respectively	Zhang and Faber (1996)
200 ppm for 10 min	Lettuce leaves	1.79, 2.48, and 0.33 log reduction in *Salmonella*, *E. coli* O157:H7, and aerobic mesophilic populations, respectively	WHO (1998)
100 ppm for 3 min at 47(warm) and 4°C (chilled)	Shredded lettuce	3.0 and 1.0 log reduction in natural microflora using warm and chilled water, respectively	Delaquis et al. (1999)
100 ppm for 3 min at 25°C	Lettuce leaves	1.4 log reduction in APC (compared to untreated)	Kim et al. (1999)
200 ppm for 3 min at 25°C	Lettuce leaves	2.0 log reduction in APC (compared to untreated)	Kim et al. (1999)
200 ppm for 15 min	Lettuce leaves	2.63 log reduction in aeorobic mesophillic populations	Nascimento et al. (2003)
100 ppm for 10 min at 20°C	Shredded lettuce	0.9 log reduction in APC (compared to untreated)	Garcia et al. (2003)
200 ppm for 10 min at 20°C	Shredded lettuce	1.2 log reduction in APC (compared to untreated)	Garcia et al. (2003)
200 ppm for 5 min (dip method)	Iceburg lettuce leaves (inoculated for 2 h)	1.10 log reduction in *E. coli* O157:H7 populations (compared to untreated)	Lang et al. (2004)
200 ppm for 5 min (spot method)	Iceburg lettuce leaves (inoculated for 2 h)	1.42 log reduction in *E. coli* O157:H7 populations (compared to untreated)	Lang et al. (2004)
200 ppm for 5 min (spray method)	Iceburg lettuce leaves (inoculated for 2 h)	1.75 log reduction in *E. coli* O157:H7 populations (compared to untreated)	Lang et al. (2004)
200 ppm for 5 min (pH 6.8)	Water	4.5 and 1.8 log CFU/ml reduction of *Bacillus cereus* vegetative cells and spores, respectively	Beuchat et al. (2004b)
200 ppm for 5 min	Shredded lettuce	1.11 and 1.06 log reduction of *Listeria monocytogenes* in a five-strain inoculum mixture and a single strain inoculum, respectively	Burnett et al. (2004)

potable water rinse or blanching, cooking, or canning. Chlorine dioxide produces fewer potentially carcinogenic chlorinated reaction products than chlorine (Tsai et al., 1995). Because the sanitizer is explosive at concentrations above 10% active ingredient or at temperatures above 266°F (130°C); ClO_2 is shipped frozen or generated on site by combining either chlorine gas and sodium chlorite or sodium hypochlorite, hydrochloric acid, and sodium chlorite (Suslow, 1997).

Suslow (1997) states that the disinfecting power of ClO_2 is relatively constant within a pH of 6 to 10 and is effective against most microbes at concentrations of 3 to 5 ppm in clean water. However, the need for on-site generation, specialized worker safety programs, and closed injections systems for containment of concentrate leakage and fumes from volatilization makes ClO_2 relatively expensive for produce applications.

Chlorine dioxide in gaseous or aqueous form is among the sanitizers with demonstrated efficacy in killing vegetative cells and spores of foodborne pathogens and spoilage microorganisms (Reina et al., 1995; Han et al., 2001; and Beuchat et al., 2004b). Unlike chlorine, ClO_2 has the ability to break down phenolic compounds and remove phenolic tastes and odors in water, does not hydrolyze in water, is unaffected by pH changes between 6 to 10, and is capable of eliminating cyanides, sulfides, and mercaptans from wastewater (WHO, 1998; Beuchat et al., 2004b). In addition, ClO_2 does not react with nitrogen-containing compounds or ammonia to form dangerous chloramines, as does chlorine (White, 1972; WHO, 1998). Furthermore, ClO_2 is less reactive towards organic compounds, which makes its application as a sanitizer in the food industry of

greater significance than chlorine (Han et al., 2000). Another advantage of ClO_2 is that it produces fewer toxic, mutagenic byproducts (Rodgers et al., 2004; Richardson et al., 1998).

Han et al. (2000) proved that ClO_2 gas treatment (1.24 ppm) was an effective sanitation technique to achieve more than 5 log reductions of *E. coli* O157:H7 on green peppers. Rodgers et al. (2004) compared the effectiveness of chlorine dioxide at 3 and 5 ppm to inactivate *L. monocytogenes*, *E. coli* O157:H7, mesophilic bacteria, yeast, and molds from whole and shredded/sliced fresh produce. Their results confirmed conclusions by other researchers (Seo and Franks, 1999; Han et al., 2000; Sapers, 2003) that there is less reduction in the microbial population of shredded lettuce versus whole lettuce. Therefore, ClO_2 proved highly effective against *L. monocytogenes* and *E. coli* O157:H7 on surface inoculated whole produce, but not shredded or sliced produce (Rodger et al., 2004).

Zhang and Faber (1996) reported that a 10 min exposure of shredded lettuce to 5 ppm ClO_2 caused a maximum reduction of 1.1 and 0.8 log reduction of *L. monocytogenes* at 4 and 22°C, respectively. Based on these results, the researchers concluded that the efficacy of ClO_2 did not prove to be exceptionally effective against *L. monocytogenes*. Similar results were reported by Costilow et al. (1984) and Reina et al. (1995) when cucumbers inoculated with *L. monocytogenes* were treated with ClO_2.

Table 6 summarizes several studies using ClO_2 as the chemical disinfectant at varying treatments and efficacy at reducing populations of natural

microflora and pathogens on vegetables. The results demonstrate that despite the ClO_2 concentrations, the maximum reduction was ≤ 6.45 logs in minimally processed vegetable products when treated for 30 min. Unfortunately, all the treatment times in Table 6 are not practical in food applications.

5. Ozone (O_3)

In 1982, the U.S. Food and Drug Administration (FDA) affirmed ozone as generally recognized as safe (GRAS) with specific limitations, for use as a disinfectant in bottled water (FDA, 1982). In 1997, Ozone was approved by the U.S. Department of Agriculture (USDA) for reconditioning recycled poultry chilling water, and received GRAS status, by an expert panel. This assertion brought about a broader use of this gas in the food industry, particularly the minimally processed fresh produce segment (Graham, 1997; CFR 2005b). Ozone is permitted by the FDA for treatment of drinking water (CFR, 2005a). In 2001, the FDA approved the use of ozone on as an antimicrobial agent for the treatment, storage, and processing of foods in gas and aqueous phase in direct contact with foods, including raw and minimally processed fruits and vegetables (FDA, 2001b).

As with ClO_2, ozone has to be generated on site because of its instability. Ozone is highly unstable in water and decomposes to oxygen in a very short time. The half-life of ozone in distilled water at 20°C is generally considered to be 20 to 30 min (Khadre et al., 2001). The stability of ozone in aqueous

Table 6. Effects of chlorine dioxide treatments on minimally processed vegetables and salads

Disinfecting Treatment	Product	Microbial Reduction	Reference
5 ppm for 10 min, at 4°C and pH 7.4	Shredded lettuce	1.1 log reduction of *L. monocytogenes* (compared to untreated)	Zhang and Faber, 1996
1.24 ppm for 30 min at 22°C and 90-95% relative humidity	Surface injured green peppers	6.45 log reduction of *E. coli* O157:H7	Han et al., 2000
10 ppm for 10 min	Lettuce	1.55 to 1.93 log reduction of *E. coli* O157:H7 (compared to untreated)	Singh et al., 2002
4.3, 6.7, and 8.7 mg (gas) for 30 min at 22°C in a model gas cabinet	Lettuce leaves	3.4 log reduction of *E.coli* , a 4.3 log reduction of *S*. Typhimurium, and 5.0 log reduction of *L. monocytogenes*, respectively (compared to untreated)	Lee et al., 2004
4.3, 6.7, and 8.7 mg (gas) for 1 h at 22°C in a model gas cabinet	Lettuce leaves	4.4 log reduction of *E.coli* , a 5.3 log reduction of *S*. Typhimurium, and 5.2 log reduction of *L. monocytogenes*, respectively (compared to untreated)	Lee et al., 2004
4.3, 6.7, and 8.7 mg (gas) for 3 h at 22°C in a model gas cabinet	Lettuce leaves	6.9 log reduction of *E.coli* , a 5.4 log reduction of *S*. Typhimurium, and 5.4 log reduction of *L. monocytogenes*, respectively (compared to untreated)	Lee et al., 2004
5 ppm for 5 min	Lettuce	~ 5 log reduction *E.coli* O157:H7 and *L. monocytogenes*	Rodgers et al., 2004

solutions depends on the source of water. Water used in food processing or beverages generally contains readily oxidizable organic and inorganic substances. These substances may react rapidly with ozone, considerably decreasing the shelf-life.

Ozone results from the rearrangement of atoms when oxygen molecules are subjected to high-voltage electric discharge (Khadre et al., 2001). Ozone is a blue gas at ordinary temperature, but at the concentrations at which is normally produced the color is not noticeable. However, at -112°C, ozone condenses to a dark blue liquid (Guzel-Seydim et al., 2003). The oxidizing power of ozone is up to 3000 times faster than chlorine (EPRI, 1997). Unfortunately, this oxidizing power has the negative effect of causing deterioration and corrosion on metal and other types of surfaces. Ozone can react with contaminants directly as molecular ozone (O_3) or indirectly as ozone-derived free radicals such as OH and H_2O (Koseki et al., 2001). Ozone is readily detectable by human smell at 0.01 to 0.04 ppm; increased concentration to 1 ppm produces a pungent, disagreeable odor and irritation to the eyes and throat; and can be lethal to humans with prolonged exposure at concentrations above 4 ppm (Guzel-Seydim et al., 2003; Suslow, 1997).

Restiano et al. (1995) suggested that the bactericidal cell surface is the primary target of ozone activity. Khadre et al. (2001), described the inactivation of bacteria by ozone as a complex process because ozone attacks numerous cellular constituents including proteins, unsaturated lipids and respiratory enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in

spore coats and virus capsids. More recently, Guzel-Seydim and others (2004) confirmed previous assumptions and offered that ozone destroys microorganisms by the progressive oxidation of vital cellular components. According to Restaino and others (1995), pathogenic bacteria such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella* Typhimurium, and *Yersinia enterolitica,* are sensitive to treatment with 20 ppm ozone in water.

The studies summarized in Table 7 showed that 0.12 to 3.8 ppm aqueous ozone inactivated gram-positive bacteria by 1 to 7 $\log_{10}$ CFU/mL. Table 8 summarized studies in which gram-negative bacteria were treated with 0.004 to 6.5 ppm aqueous ozone resulting in 0.5 to 6.5 $\log_{10}$ CFU/mL reduction.

Kim and others (1999) explained that the decontamination of produce by ozone depended, among other factors, on the number and kind of contaminating microorganisms, physiology of vegetables, reactor design, water quality, temperature, and pH. In their study, when ozonated water, without turbulence, was used on lettuce treatment, minimal elimination of contaminants was observed (no data provided). However, bubbling ozone (1.3 ppm) in water-lettuce mixture for 3 min inactivated 1.2 and 1.8 log CFU/g mesophilic and psychrotrophic microorganisms, respectively. Hence, bubbles and agitation likely enhanced the efficacy of ozone by breaking cell clusters (Rodgers et al., 2004). When the duration of the treatment was extended to 5 min, populations of mesophilic and psychtrotrophic microorganisms were reported to decrease 3.9

Table 7 – Inactivation on gram-positive bacteria by ozone in ozone demand water (Taken from Khadre et al., 2001)

	Treatment Conditions					
Bacterium	**Ozone (µg/mL)**	**Time (min)**	**pH**	**Temp. (°C)**	**Log_{10}-units decreased**	**References**
Bacillus megaterium	0.19	5	NR	28	> 2.0	Broadwater and others, 1973
B. cereus	0.12	5	NR	28	> 2.0	Broadwater and others, 1973
Leuconostoc Mesenteroides	0.3 to 3.8	0.5	5.9	25	1.3 to ~7	Kim and Yousef, 2000
Listeria monocytogenes	0.2 to 1.8	0.5	5.9	25	0.7 to ~7	Kim and Yousef, 2000
L. monocytogenes	0.1[a]	10	7.2	25	60 to 70%[b]	Lee and others, 1998
Mycobacterium fortuitum	0.23 to 0.26	1.67	7.0	24	1.0	Farooq and Akhlaque, 1983
S. aureus	NR	0.25	7.0	25	> 2.0	Burleson and others, 1975

[a]Phosphate buffer
[b]Per cent injured cells
NR, not reported

Table 8 – Inactivation on gram-negative bacteria by ozone in water (Taken from Khadre et al., 2001)

	Treatment Conditions					
Bacterium	**Ozone (µg/mL)**	**Time (min)**	**pH**	**Temp. (°C)**	**Log reduction**	**References**
Escherichia coli	0.065[a]	0.5	NR	NR	3.5	Katzenelson and others 1974
E. coli	0.004 to 0.8[b]	0.5 to 2.0	6.9	NR	0.5 to 6.5	Finch and others 1988
E. coli	0.19[a]	5	28	NR	> 2.0	Broadwater and others 1973
E. coli	0.23 to 0.26[a]	1.67	7.0	24	4.0	Farooq and Akhlaque 1983
E. coli	0.53[b]	0.1	6.8	1	2.0	Fetner and Ingols 1956
E. coli O157:H7	0.3 to 1.0[a]	< 0.5	5.9	25	1.3 to 3.8	Kim and Yousef 2000
Legionella peumophila	0.32[a]	20	7.0	24	> 4.5	Edelstein and others 1982
L. peumophila	0.47	20	7.0	24	> 5.0	Edelstein and others 1982
L. peumophila	0.21	5	NR	NR	> 2.0	Domingue and others 1988
Salmonella enteritidis	0.5 to 6.5	0.5	NR	25	0.6 to ~4	Dave 1999
S. typhimurium	0.23 to 0.26[a]	1.67	7.0	24	4.3	Farooq and Akhlaque 1983
Pseudomonas fluorescens	0.2 to 1.2[a]	< 0.5	5.9	25	0.9 to 5	Kim and Yousef 2000

[a] O_3 demand-free water
[b] Phosphate buffer
NR, not reported

and 4.6 log CFU/g, respectively. Unfortunately, this longer exposure time is likely to be impractical in food applications. Moreover, in the same study, ozone treatment (~3 to 10 ppm) was ineffective in reducing *Pseudomonas fluorescens* inoculated (24 h prior to treatment) on lettuce, resulting in <1 log reduction. Koseki et al. (2001) that the number of aerobic organisms on lettuce decreased only 1.5 log following a 10-min exposure at 5 ppm ozone. More recently, Garcia and others (2003) conducted a study to determine the effectiveness of ozone (2.5, 5.0, and 7.5 ppm) on the microbiological attributes of shredded lettuce and reported a 0.6 to 0.8 log reduction in aerobic plate count after a 10 min treatment.

Table 9 summarizes several studies using ozone as the chemical disinfectant at varying treatments and its efficacy at reducing populations of natural microflora and pathogens on vegetables. The results demonstrate that despite the ozone concentrations, the maximum reduction at 3 min was ≤ 1.8 logs in minimally processed vegetable products when agitated (bubbling). Higher reductions were achieved with longer treatment times, however, they are impractical in food applications.

Table 9. Effects of ozone treatments on minimally processed vegetables and salads

Disinfecting Treatment	Product	Microbial Reduction	Ref.
1.3 ppm for 3 min (bubbling)	Lettuce	1.2 and 1.8 log reduction of mesophilic and psychrotrophic microorganisms, respectively.	Kim et al., 1999
1.3 ppm for 5 min (bubbling)	Lettuce	3.9 and 4.6 log reduction of mesophilic and psychrotrophic microorganisms, respectively.	Kim et al., 1999
10 ppm for 1 min (bubbling)	Lettuce	<1 log reduction of *Pseudomonas fluorescens*	Kim et al., 1999
5 ppm for 10 min	Lettuce	1.5 log reduction	Koseki et al., 2001
2.5, 5.0, 7.5 ppm, stirred for 10 min at ~20ºC	Iceburg lettuce	0.6 to 0.8 log reduction in APC (compared to untreated)	Garcia, et al., 2003
3 ppm for 5 min (bubbling)	Lettuce	4 to 5 log reduction of mesophilic bacteria	Rodgers et al., 2004

6. Electrolyzed Oxidizing (EO) Water

The use of EO water is a special case of chlorination (Izumi, 1999; Sapers, 2001) that appears promising as a non-thermal process for microbial inactivation but differs from commonly used chlorine treatments in that the inactivating agent is generated directly in the water. Izumi (1999), Venkitanarayanan et al. (1999), and Fabrizio et al. (2003) attribute EO water as a new concept developed in Japan. However, Stevenson et al. (2004) credited Russia for the development of this new technology citing an earlier study (Shtannikov et al. 1977) than the researchers that credited Japan.

Electrolytic oxidizing water is approved for direct and indirect food contact applications (CFR, 2004a; CFR, 2004b; CFR, 2005a). The EPA has given EO water approval (CFR, 2004c) for washing raw foods that are to be consumed without processing. The alkaline water is classified as an A1 cleaning compound and used in clean-in-place (CIP) applications or as the primary cleaner on food processing equipment. The acidic water is classified as a D2 which requires no fresh water rinsing after sanitizing (http://www.roxwater.net/page1.html).

Electrolytic water is created by electrochemical disassociation of salt water solution between anode and cathode electrodes separated by a diaphragm (see Figure 13). This process splits salt water into two separate streams, acidic (anode) and alkaline (cathode) water. EO water from the anode stream possesses at least 3 antimicrobial properties that include low pH (ca. 2.5), high oxidation-reduction potential (ORP; ca. > 1,100 mV), and chlorine-based

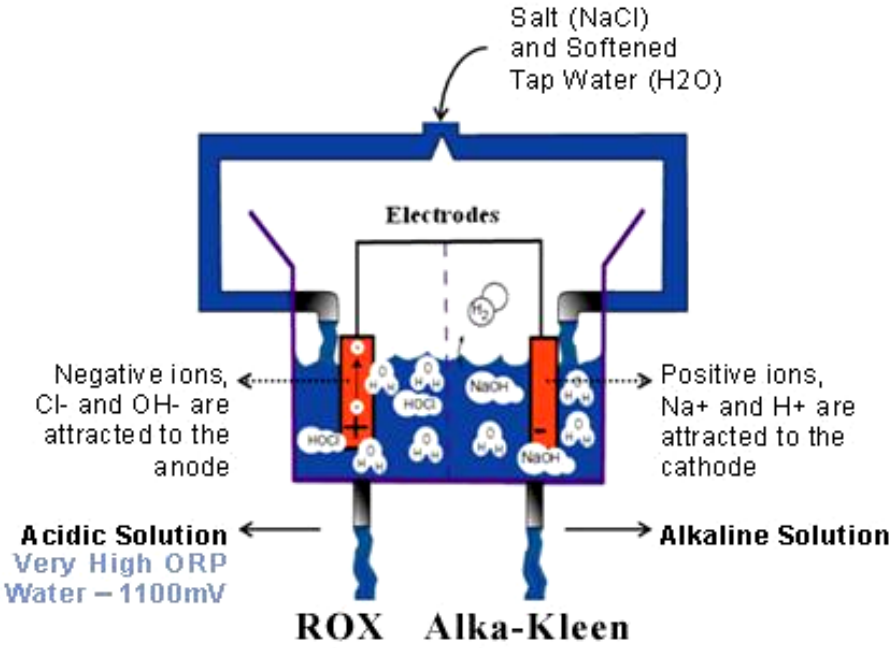

Figure 8. Process of EO water production using the ROX model water electrolyzer (Hoshizaki Electric Company Ltd.).
Taken from: http://www.roxwater.net/page1.html

reactants (Park et al., 2001). The concentration of the chlorine reactants (usually 10 to 90 ppm) is influenced by the amperage of EO water generator. Electrolyzed oxidizing water contains a mixture of inorganic oxidants such as HClO, OCl^-, Cl_2, and O_3, which are effective disinfectants (Yang et al., 2003).

Electrolyzed oxidizing water has been used for inactivation of a wide variety of pathogenic and spoilage microorganisms including *E. coli* O157:H7, *Salmonella enteritidis* serovar typhimurium, *L. monocytogenes,* and *Campylobacter jejuni* (Park et al., 1999; Venkitanarayanan et al., 1999b; Kim et al., 2000a; Park et al., 2002; Fabrizio and Cutter, 2003). Several studies have shown that EO water is capable of reducing pathogens and/or spoilage microorganisms associated with fresh fruits and vegetables (Izumi, 1999; Kim et al., 2000b; Park et al., 2001), attached to cutting boards (Venkitanarayanan et al., 1999a), and poultry (Park et al., 2002).

The bactericidal activity of EO water is quantitatively correlated to the free chlorine in the form of hypochlorous acid (HOCl) that exists in the solution. On the basis of chemical and spectroscopic data, Nakagawara and others (1998) concluded that the major component of acidic electrolyzed water is Cl_2/HOCl in chemical equilibrium at given pH values. The researchers showed that the population of HOCl, ClO^-, and Cl_2 at different pH values can be observed at 728, 715, and 540 cm^{-1}, respectively. The pH profiles in Figure 14, clearly indicate that the major components are Cl_2 (pH < 3), HOCl (pH range 4 – 7), and ClO^- (pH> 8.5). Some studies have suggested that the very high ORP level of the

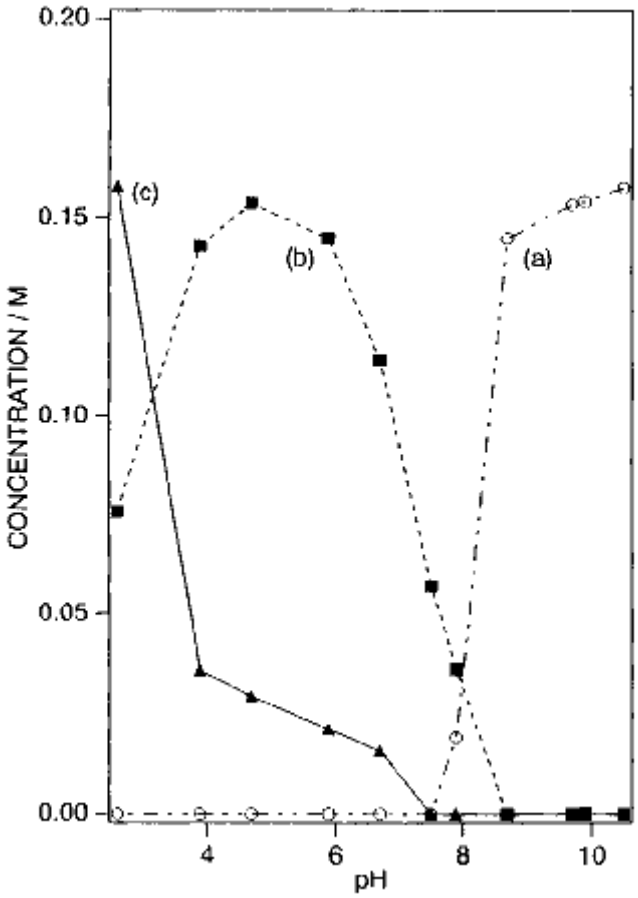

Figure 9. The pH profiles of the concentrations of (a) ClO, (b) HClO, and (c) Cl_2 in sodium hypochlorite solution upon acidification of HCl.

Taken from Nakagawara and others, 1998.

anode water (> 1,100 mV) is a greater indicator of bactericidal activity than pH (Kim et al., 2000b; Stevenson et al., 2004). Thus, the concentration of HOCl is correlated to high oxidation-reduction potential (ORP) levels.

In a study by Izumi (1999), the effects of EO water was evaluated on several fresh-cut vegetables, including some of which are normally found in fresh-cut salads (i.e. spinach, sliced carrots, and sliced cucumbers). The researcher found that mesophilic aerobic microorganisms were reduced by 0.6 to 2.6 logs CFU/g when treated with electrolytic water containing 15, 30, and 50 ppm available chlorine. The electrolyzed water containing 50 ppm chlorine had the strongest bactericidal effect. Furthermore, the researchers noted that the effectiveness of electrolyzed water was the greatest with spinach leaves which had the maximum surface area/unit weight of tissue among the tested fresh-cut vegetables. The assertion that chemical sanitizers effectiveness is influenced by the type and style of fresh-cut vegetables was also confirmed by Zhang and Faber (1996).

The efficacy of EO water for inactivating *E. coli* O157:H7, *L. monocytogenes*, and *S. enteritidis* was evaluated by Venkitanarayanan and others (1999a, 1999b). The first study (1999a) exhibited the effect of EO water on the inactivation of *E. coli* O157:H7 and *L. monocytogenes* on the surface of plastic cutting boards. They reported a reduction of > 5.0 log CFU/100 cm^2 and nondetectable levels for *E. coli* O157:H7 and *L. monocytogenes* populations on cutting boards, respectively. Their second study (1999b), focused on the efficacy of EO water for inactivating *E. coli* O157:H7, *S. enteritidis*, and *L.*

monocytogenes incubated at different times and temperatures. They reported that *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes* were more rapidly inactivated at temperatures >23°C. After 1 min exposure to EO water at 45°C, *E. coli* O157:H7 was killed completely (a reduction of approximately 8.0 log CFU/ml). *S. enteritidis* and *L. monocytogenes* were reduced by approximately 7.0 log CFU/ml under the same treatment.

A study by Koseki and Itoh (2001), demonstrated that washing lettuce with alkaline EO water for 1 min followed by decontamination with acidic EO water for 1 min, reduced total aerobic bacteria, coliform bacteria, *Bacillus cereus*, and psychrotrophic bacteria by 1.7, 1.6, 1.0, and 1.1 logs CFU/g, respectively. Using the same treatment on cabbage, the research reported that total aerobic bacteria, coliform bacteria, *B. cereus*, and psychrotrophic bacteria were reduced by 1.5, 1.5, 1.5, and 1.0 logs CFU/g, respectively. By contrast, when lettuce was soaked in acid EO water for 10 min the viable aerobic bacteria was reduced by 2 log CFU/g (Koseki et al., 2001). The researchers concluded that the alkaline EO water increased the decontamination of microorganisms on the surface of lettuce leaves, which explains why the lettuce was decontaminated within a shorter time when washed with alkaline EO water (1 min) followed by a treatment with acidic EO water (1 min). Koseki et al. (2004) reported similar results.

Yang and others (2003), reported similar results (2 log CFU/g reduction) when fresh-cut lettuce was treated with 300 ppm EO water at pH 7.0 and 30°C for 5 min. Park and others (2001) reported similar results. Swem and others (2002), reported lower *E. coli* O157:H7 reductions (3.0 log CFU/g) under the

same treatment. However, Swem and others (2002) treated the sample at 30 min after inoculation, whereas, Yang and others (2003) treated the inoculated lettuce after 24 h of storage at 7°C. Using scanning microscopy, bacterial film, a sticky and threadlike substance surrounding bacterial cells, was observed on the inoculated lettuce sample after 24 h storage. Therefore, biofilm formation affects the efficacy of EO water. Park et al (2001) reported 2.65 log10 CFU per lettuce leaf reduction of *L. monocytogenes* inoculated on whole lettuce leaves after 3 min treatments. Yang and others (2003) reported a 2.0 and 2.1 log10 cfu/g when fresh-cut romaine lettuce was treated with 300 ppm EO water at pH 7 and 30°C of *Salmonella* Typhimurium and *L. monocytogenes*, respectively.

Table 10 summarizes several studies using EO water as the chemical disinfectant at varying treatments and its efficacy at reducing populations of natural microflora and pathogens on vegetables. The results demonstrate that despite the concentrations, the maximum reduction was 2.65 logs in minimally processed vegetable products when treated for 3 min. Higher reductions were achieved when treated for 5 min, however, this extended treatment period is impractical in food applications. Thus, EO water proved to be equally effective to chlorine.

Table 10. Effects of EO Water Treatments on Minimally Processed Vegetables and Salads

Disinfecting Treatment	Product	Microbial Reduction	Reference
EO water 20 ppm at 3 min at pH 6.8	Fresh–cut vegetables	0.6 to 2.6 log reduction of total microbial count	Izumi, 1999
EO water 45 ppm for 3 min at 22ºC	Lettuce	2.41 and 2.65 log reduction of *E. coli* O157:H7 and *L. monocytogenes*, respectively	Park et al., 2001
Alkaline EO water (pH 11.3, ORP, -870 mV for 1 min) followed by acidic EO water (pH 2.5, ORP 1,140 mV, 40 ppm for 1 min)	Lettuce	1.7, 1.6, 1.0, and 1.1 log reduction of total aerobic bacteria, coliform bacteria, *B. cereus*, and psychrotrophic bacteria, respectively.	Koseki and Itoh, 2001
Alkaline EO water (pH 11.3, ORP, -870 mv for 1 min) followed by acidic EO water (pH 2.5, ORP 1,140 mV, 40 ppm for 1 min)	Cabbage	1.5, 1.5, 1.5, and 1.0 log reduction of total aerobic bacteria, coliform bacteria, *B. cereus*, and psychrotrophic bacteria, respectively.	Koseki and Itoh, 2001
Acidic EO water (pH 2.6, ORP 1140 mV, 30 ppm) for 10 min	Lettuce	2.0 log reduction of viable aerobes	Koseki et al., 2001
Alkaline EO water for 1 min follwed by Acidic EO water for 1 min	Lettuce	2.0 log reduction of viable aerobes	Koseki et al., 2001
EO water (300 ppm, pH 7, 30ºC) for 5 min	Lettuce	~2.0 log reduction of *S.* Thyphimurium, *E. coli* O157:H7, and *L. monocytogenes*.	Yang et al., 2003
Alkaline EO water at 20ºC for 5 min followed by acidic EO water for 5 min at 20ºC	Cut lettuce	~1.8 log reduction of *E. coli* O157:H7 and *Salmonella*.	Koseki et al., 2004
Acidic EO water for 5 min at 50ºC	Cut lettuce	3 log reduction of *E. coli* O157:H7 and *Salmonella*.	Koseki et al., 2004

7. Other Chemical and non-chemical Disinfection Methods

In addition to the chemical disinfectants there are a number of other sanitizing agents (chemical and non-chemical) that are approved that have been evaluated in laboratory-scale investigations for fresh-cut vegetables. A brief discussion follows.

Hydrogen peroxide is currently classified as GRAS for use in food products but has not yet been approved as an antimicrobial wash for produce (Sapers, 2001). Acceptable uses include that of bleaching agent, oxidizing and reducing agent, and antimicrobial. Specific regulations on the use of H_2O_2 in foods are found in CFR, Title 21 Part 184.1366 (CFR, 2005b). Lin and others (2002) reported that the treatment of lettuce with 2% H_2O_2 at 50ºC reduced *E. coli* O157:H7 and *L. monocytogenes* by ≤ 4 and 3 logs.

In 1986, the FDA approved the use of peroxyacetic acid as a food-grade sanitizer at concentrations not to exceed 100 ppm (Rodgers et al., 2004). Moreover, unlike chlorine and ozone, peroxyacetic acid is noncorrosive, unaffected by changes in temperatures, and remains effective in the presence of organic matter (Rodgers et al., 2004). Peroxyacetic acid is a strong oxidizer formed from hydrogen peroxide and acetic acid. Beuchat et al. (2004a) reported approximately 1 log CFU/g reduction of *L. monocytogenes* inoculated on shredded lettuce and Romaine lettuce pieces when treated with Tsunami 100 (80 ppm) at 3 to 4ºC for 15 s.

Removal of pathogenic and spoilage bacteria from minimally processed leafy vegetables by organic acids has been studied. Acids are either naturally

present as constituents of the food or are added to the product through formulation. The undissociated form of the acid is responsible for the antimicrobial activity, which is highly dependable on pH. Nascimento et al. (2003) compared the results of sodium hypochlorite with seven different sanitizing solutions (vinegar at 6, 25, and 50%; acetic acid at 2 and 4 %; peroxyacetic acid at 80 ppm; and sodium dichloroisocyanurate at 200 ppm). The statistical analysis of the results demonstrated that the effectiveness levels of all the sanitizing agents tested were equivalent to or higher than that for sodium hypochlorite at 200 ppm. The best results were achieved with 4% acetic acid, which reduced the initial aerobic mesophilic population by 3.93 $\log_{10}$ CFU/g and reduced the mold and yeast population by 3.58 $\log_{10}$ CFU/g. Nascimento et al. (2003) concluded that the results of the study demonstrated the effectiveness of acetic acid and vinegar as alternative sanitizing agents for the disinfection of fresh produce.

Antimicrobial activities of essential oils and their components are well documented (Kim et al., 1995; Hammer et al., 1999; Ceylan and Fung, 2004). Essential oils increase the permeability of cytoplasmic membrane and lead to the loss of cellular constituents (Sikkema et al., 1994). Wan et al. (1998) reported washing lettuce with 0.1% (v/v) and 1.0% (v/v) suspensions of basil essential oil resulted in 2.0 and 2.3 log reduction of viable bacteria on fresh cut lettuce, respectively.

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound with the potential application in disinfection of fresh-cut leafy vegetables.

Cetylpyridinium chloride is in certain commercial mouthwashes to prevent dental plaque (Frost and Harris, 1994). Wang and others (2001) reported reductions of 2.4 to 3.2 log CFU/g for *S*. Typhimurium and 1.0 to 1.6 log CFU/g for *E. coli* following a 5 min immersion treatment of vegetables using 0.1% to 0.5% CPC. Yang and others (2003) reported that 0.3% CPC reduced *S*. Typhimurium and *E. coli* O157:H7 by 0.96 and 1.21 log CFU/g, respectively, at a spray pressure of 0.7 kg/cm^2. When spray pressure increased from 0.7 to 2.1 kg/cm^2, *S*. Typhimurium and *E. coli* O157:H7 were further reduced by 1.5 and 0.5 log CFU/g, respectively. However, approximately 300 ppm of CPC persisted on the lettuce after 2- min water rinse.

In the U.S., the FDA has authorized the use of irradiation for pork, poultry, red meats, fruits, vegetables, herbs, spices, grains, seeds for sprouting, and shell eggs. Current U.S. regulations limit the use of irradiation for fresh fruits and vegetables up to 1 kGy and specifically for disinfestations and inhibition of produce growth and maturation (CFR, 2005c). However, dry or dehydrated vegetables derived spices, seasonings, flavorings and coloring agents may be irradiated to 30.0 kGy (CFR, 2005c). Foods treated with ionizing radiation must be labeled with the Radura symbol (CFR, 2005c) or with the statement *Treated by irradiation* or *Treated with radiation*. Most of the research on irradiation of produce concentrated on determining dose response while preserving the product quality. In a study by Farkas and others (1997), *L. monocytogenes* and spoilage bacteria were reduced by approximately 4 and 5 logs on pre-cut bell peppers and carrot cubes, respectively, when treated with 1.0 kGy. Hagenmaier

and Baker (1997) significantly reduced the normal microflora and moderately increased respiration on commercially prepared fresh-cut lettuce by treating the produce with a radiation dose of 0.19 kGy. The study demonstrated that eight days after irradiation, the unirradiated lettuce had 2.2 x 10^5 CFU/g, while the irradiated lettuce had 2.9 x 10^2 cfu/g. Prakash et al. (2000) treated cut romaine lettuce with irradiation at 0.35 kGy decreasing the aerobic plate counts by 1.5 logs and yeast and molds by 1 log; these differences were maintained through 22 d storage at 4°C.

Table 11 summarizes several chemical disinfectants at varying treatments and efficacy at reducing populations of natural microflora and pathogens on vegetables. The results demonstrate that although higher reductions were achieved with acetic acids and CTP, the treatment required 5 min. Unfortunately, this extended treatment period is impractical in food applications. Irradiation, demonstrated significant reductions on a variety of vegetables inoculated with *L. monocytogens*, however, the cost of treatment may outweigh the benefits.

Currently, new washing technologies using chemical sanitizing agents, vacuum infiltration, vapor-phase treatments, surface pasteurization, bacteriophage control, high hydrostatic pressure, ultraviolet light, and pulse electric are needed to overcome failures of conventional methods by targeted treatment of microbial attachment or internalization sites are being developed (FDA, 2001a, Sapers, 2001; Greer, 2005).

Table 11. Effects of Several Treatments on Minimally Processed Vegetables and Salads

Disinfecting Treatment	Product	Microbial Reduction	Reference
2% H_2O_2 at 50ºC	Lettuce	< 4 and 3 log reduction of *E. coli* O157:H7 and *L. monocytogenes*, respectively	Lin and others, 2002
Peroxyacetic acid (Tsunami 100), 80 ppm at 3 to 4ºC for 15 s	Shredded lettuce and romaine lettuce pieces	~ 1 log reduction of *L. monocytogenes*	Beuchat and others, 2004a
Acetic acid (2%) for 15 min	Lettuce leaves	3.37 and >2.25 log reduction in aeorobic mesophillic and total coliform populations, respectively (compared to untreated)	Nascimento et al. (2003)
Acetic acid (4%) for 15 min	Lettuce leaves	3.91 and >2.25 log reduction in aeorobic mesophillicand total coliform populations, respectively (compared to untreated)	Nascimento et al. (2003)
Peracetic acid (80 ppm) for 15 min	Lettuce leaves	1.85 and 1.44 log reduction in aeorobic mesophillic and total coliform populations, respectively (compared to untreated)	Nascimento et al. (2003)
Vinegar (6%) for 15 min	Lettuce leaves	1.83 and 1.58 log reduction in aeorobic mesophillic and total coliform populations, respectively (compared to untreated)	Nascimento et al. (2003)
Vinegar (25%) for 15 min	Lettuce leaves	2.42 and >1.99 log reduction in aeorobic mesophillic and total coliform populations, respectively (compared to untreated)	Nascimento et al. (2003)
Vinegar (50%) for 15 min	Lettuce leaves	2.89 and >2.21 log reduction in aeorobic mesophillic and total coliform populations, respectively (compared to untreated)	Nascimento et al. (2003)
Acetic acid (2%) for 15 min	Lettuce leaves	3.37 and >2.25 log reduction in aeorobic mesophillic and total coliform populations, respectively (compared to untreated)	Nascimento et al. (2003)

Acetic acid (4%) for 15 min	Lettuce leaves	3.91 and >2.25 log reduction in aeorobic mesophillicand total coliform populations, respectively (compared to untreated)	Nascimento et al. (2003)
Peracetic acid (80 ppm) for 15 min	Lettuce leaves	1.85 and 1.44 log reduction in aeorobic mesophillic and total coliform populations, respectively (compared to untreated)	Nascimento et al. (2003)
Vinegar (6%) for 15 min	Lettuce leaves	1.83 and 1.58 log reduction in aeorobic mesophillic and total coliform populations, respectively (compared to untreated)	Nascimento et al. (2003)
CTP (100 and 200 ppm) for 5 min	Fresh produce	4.8 and 5.1 log reduction of *E. coli* O157:H7 and *L. monocytogenes*, respectively.	Rodgers et al., 2004
Basil essential oil (0.1 and 1.0% (v/v))	Fresh-cut lettuce	2.0 and 2.3 log reduction of viable bacteria, respectively.	Wan et al., 1998
CPC (0.1 to 0.5%) for 5 min	Vegetables	2.4 to 3.2 log reduction of *S.* Typhimurium and 1.0 to 1.6 log reduction for *E. coli*	Wang et al., 2001
CPC (0.3%), spray pressure at 0.7 kg/cm^2	Lettuce	0.96 and 1.21 log reduction of *S.* Typhimurium and *E. coli* O157:H7, respectively	Yang et al., 2003
CPC (0.3%), spray pressure at 2.1kg/cm^2	Lettuce	1.5 and 0.5 log reduction of *S.* Typhimurium and *E. coli* O157:H7, respectively	Yang et al., 2003
Gamma irradiation (1 kGy)	Pre-cut bell peppers	4 log reduction of *L. monocytogenes*	Farkas et al., 1997
Gamma irradiation (1 kGy)	Carrot cubes	5 log reduction of *L. monocytogenes*	Farkas et al., 1997
Gamma irradiation (0.35 kGy)	Cut romaine lettuce	1.5 log reduction of aerobic plate count	Prakash et al., 2000
Irradiation (1 kGy)	Broccoli, mung beans, cabagge, and tomato	4.14 to 5.25 log reduction of *L. monocytogenes*	Bari et al., 2005

8. Hurdle Technology and Synergistic Effects of Chemical Disinfectants Used in Combination:

According to Leistner (1994), in food preserved by hurdle technology, the possibility exists that different hurdles in a food will not just have an additive effect on stability, but could act synergistically. A synergist effect, achieved when the combination of two compounds is more effective than each compound alone, could work if the hurdle in a food hits different targets (e.g., cell membrane, DNA, enzyme systems, pH, a_w, Eh) within the microbial cell, and thus disturbs the homeostasis of the microorganisms present in several aspects. The physiological responses of microorganisms during food preservation such as homeostasis, metabolic exhaustion, and stress reaction are the basis for the application of hurdle technology. Therefore, deliberately disturbing several homeostasis mechanisms simultaneously by using multiple hurdles in the preservation of a particular food should be an advantage, because microbial stability could be achieved with a combination of gentle hurdles. Since different hurdles have different spectra of antimicrobial action, the combined hurdles could attack microorganisms in different ways and may increase synergistically the effectiveness of preservation. In practical terms, the use of different preservatives in small amounts may be more effective than only one preservative in a large amount. The reason for the efficacy is that different preservatives might hit different targets within the bacterial cell, and thus act synergistically (Leistner, 1994).

Hagenmaier and Baker (1997) found that refrigerated cut iceburg lettuce irradiated at 0.2 kGy after a chlorine wash and modified atmosphere packaging (MAP) had only 3.2 x 10^2 CFU/g 8 d after irradiation. At the same time the control had 1.99 x 10^5 CFU/g. Thus, irradiation in combination with chlorine can significantly reduce microbial levels. Foley and others (2002) reported that chlorination plus irradiation (0.55 kGy) resulted in a 5.4 log reduction of *E. coli* O157:H7 on shredded iceburg lettuce with little significant effect on quality.

A mixture of 1.5% lactic acid and 1.5% H_2O_2 on apples, oranges and tomatoes reduced counts of *Salmonella* and *E. coli* O157:H7 by >5 log per fruit without damage to the sensory quality of the fruit (Venkitanaraynana et al., 2002). The combination of acids with other chemical sanitizers provided more hurdles for bacteria to clear, thus increasing the chances of a lethal effect or at least an inhibition of growth.

Garcia et al. (2003), conducted a study to determine the effectiveness of ozone in combination with chlorine on the microbiological and sensory attributes of lettuce as well as the quality of water used for processing commercial lettuce. In their study, iceburg lettuce was inoculated with 8.0 log CFU/g microorganisms isolated from spoiling lettuce, treated with combinations of chlorine and ozone, and analyzed microbiologically. They reported that chlorine, ozone, and chlorine-ozone reduced aerobic plate count by 1.4, 1.1, and 2.5 log, respectively. The use of combination of ozone and chlorine resulted in better microbial reduction. The unintentional benefit is that using a reduced chlorine treatment (by adding

ozone) may reduce the formation of trihalomethane compounds, which are carcinogenic.

Table 12 summarizes several studies using a combined mixture of two or more chemical and non-chemical disinfectant and efficacy at reducing populations of natural microflora and pathogens on vegetables. The results demonstrate that combining chlorine and irradiation or lactic acid and hydrogen peroxide had greater reduction than using chlorine alone.

Table 12. Effects of Combination Treatments on Minimally Processed Vegetables and Salads

Disinfecting Treatment	Product	Microbial Reduction	Reference
Chlorine wash, irradiation (0.2 kGy) and MAP.	Fresh-cut iceburg lettuce	3.2 x 10^2 CFU/g 8 d after irradiation	Hagenmair and Baker, 1997
Chlorination plus irradiation (0.55 kGy)	Shredded iceburg lettuce	5.4 log reduction of *E. coli* O157:H7	Foley et al., 2002
Mixture of 1.5% lactic acid and 1.5% H_2O_2	Apples, oranges, and tomatoes	> 5 log reduction of *Salmonella* and *E. coli* O157:H7	Venkitanaraynana et al., 2002
Combined 7.5 ppm ozone and 150 ppm chlorine	Shredded lettuce	1.4 log reduction in APC (compared to untreated)	Garcia et al. (2003)
Mixture of ClO_2 (85 ppm) and Fit powder product (0.5%), pH 3.5 for 5 min.	Water	>5.3 and >6.0 log CFU/ml reduction of *Bacillus cereus* vegetative cells and spores, respectively (compared to untreated)	Beuchat et al. (2004b)

C. Packaging Technology

Minimally processed vegetables are living entities that continue to change after harvest. Plant tissue incur damage during processing (e.g. trimming and shredding), which trigger certain metabolic reactions. Within minutes of processing, the rate of respiration and ethylene production increase rapidly (Brecht, 1995). Both the elevated respiration and ethylene production will result in decreased shelf-life by using up energy reserves and by accelerating ripening, softening, and senescence, respectively (Brecht, 1995). Consequently, inhibition of respiration plays an important role in extending the shelf life of fresh produce.

The basic principle behind modified atmosphere packaging (MAP) is that a modified atmosphere can be created passively by correctly using permeable packaging materials, or actively, by using a special gas mixture combined with such materials. The purpose of both is to create an optimal gas balance inside the package, where the respiration activity of a product is as low as possible while ensuring the oxygen concentration and carbon dioxide levels are not detrimental to the product. For lettuce, Koseki and Itoh (2002) reported an O_2 concentration of 5% and a CO_2 concentration of 0%.

The survival and growth of microorganisms is affected by the O_2 and CO_2 concentration in MAP produce (IFPA, 2001). In general, Gram-negative microorganisms are more sensitive to CO_2. Modified atmosphere packaging containing elevated levels of CO_2 (70-100%) inhibits the growth of *L. monocytogenes* in a variety of products (meat products, cottage cheese, turkey

roll slices), whereas 100% N_2 allows multiplication of the pathogen (Phillips, 1996). Fresh-cut and whole fruits and vegetables, in particular, do not usually tolerate CO_2 concentrations above 15%, well below the inhibitory level for *L. monocytogenes*. The CO_2 rich atmosphere, however, can select for lactic acid bacteria, which have been shown to be inhibitory towards *L. monocytogenes* (Francis and O'Beirne, 1998). Unfortunately, elevated CO_2 suppresses spoilage bacteria, mostly of the genus *Pseudomonas*, thus creating opportunities for slower growing pathogens to reproduce (IFPA, 2001). Therefore, raising questions as to whether spoilage occurs before or after toxin production by *Clostridium botulinum*, which is known to grow in elevated gaseous CO_2 concentrations typical of MAP. The persistence of spoilage microflora is assured by using packaging materials that do not lead to an environment supportive of only anaerobic and facultative microorganisms.

D. Government Oversight for Minimally Processed Fresh-cut vegetables

Prior to 1906, there were no food laws in this country. In 1906, the federal government passed the Pure Food and Drug Act and the Meat Inspection Act and gave the USDA the power of supervision of the laws. In 1938, the federal government passed the Food, Drug, and Cosmetic Act (FD&C Act). This law prevents the manufacture and shipment of unsafe or spoiled food and food ingredients. The Act assures consumers that foods are pure and wholesome, safe to eat, and were produced under sanitary conditions. The FD&C Act,

prohibits distribution in the U.S., or importation, of articles that are adulterated or misbranded.

Preventing contamination of fruits and vegetables is the responsibility of a broad range of government agencies. The FDA, which is part of the U.S. Department of Health and Human Services, is delegated the power to administer and enforce the Act. Through its Center for Food Safety and Applied Nutrition (CFSAN) and the Office of Regulatory Affairs (ORA), the FDA regulates both domestic and imported foods, except meat and poultry and processed eggs and has primary responsibility for enforcing food safety laws including food import and export regulations. The USDA, through the Agricultural Marketing Services (AMS) and Animal and Plant Health Inspection Service (APHIS) may play a role in assuring food safety by establishing the safety of imported fruits and vegetables. The duties of the U.S. Environmental Protection Agency (EPA) include regulating pesticides and assuring that drinking water meets standards for health. Through the Office of Pesticide Programs (OPP), EPA determines the safety of new pesticide products, sets tolerance levels for pesticide residues in foods, which FDA then enforces, and publishes directions for the safe use of pesticides.

Systems that assure the safety and wholesomeness of fruits and vegetables during growing, harvesting, postharvest handling, and fresh-cut processing fall into three prevention program categories:

- Good Agricultural Practices (GAPs);

• Good Manufacturing Practices (GMPs);

• Hazard Analysis Critical Control Points (HACCP).

Growing, Harvesting, and Postharvesting Handling:

For food safety, the FDA has published GAP guidelines to reduce or eliminate pathogen contamination in the field or packinghouse operations. The GAP guidelines are generic in nature because of the wide variety of fruit and vegetable commodities. Currently, compliance with the FDA's GAPs is not mandatory. However, with the growing food safety awareness, farms that do not implement GAPs may eventually have difficulties continuing in the marketplace because many customers from the foodservice and retail markets are demanding evidence of these programs from their suppliers. Intervention strategies to reduce pathogens in the produce industry depends begin in the field.

In 1998, to assist industries in preventing contamination of produce before it reaches the consumer the FDA and USDA released a "Guidance for industry - *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables"* (*The Guide*). Later, Cornell University released "*Food Safety Begins on the Farm – a Grower's Guide*." These voluntary programs spell out what producers, packers, and distributors of fresh produce should do to decrease the risk of produce contamination.

When addressing the issue of water quality, organic fertilizers, and their potential for microbial contamination on produce, compliance with following federal laws administered by the Environmental Protection Agency is necessary:

- Solid Waste Disposal Act of 1965;
- Coastal Zone Management Act of 1972;
- Safe Drinking Water Act of 1974;
- Clean Water Act of 1977;
- Organic Foods Production Act of 1990;

Fresh-cut Processing:

The FDA has promulgated GMP regulations that apply to all food processing facilities, including fresh-cut operations. Currently, the use of HACCP is voluntary, but is widely used in the food processing industry as a successful component of a comprehensive food safety program.

Good Manufacturing Practices are FDA regulations directed at food processors and are located in the U.S. Code of Federal Regulations 21(CFR) Part 110.1 to 110.99. GMPs cover all aspects of a processing environment from the design of a sanitary facility to rules forbidding jewelry on workers. Unlike GAPs, GMPs are rules that are clearly defined and easy to apply because a processing environment has easily defined boundaries and the processing activities can be contained and controlled. In addition, the industry has published "Food Safety Guidelines for the Fresh-cut Produce Industry" through International Fresh-cut Produce Association (IFPA, 2001). The IFPA guidelines incorporate GMPs as well as other food safety standards such as a model HACCP plan, sanitary facility design, and proper use of antimicrobials in safeguarding fresh-cut produce.

Although HACCP is not mandatory, the fresh-cut processing industry has embraced the plan as a useful tool for implementing food safety practices in the production environment. HACCP is well suited to identify hazards, monitor production for adherence to operational standards, and develop an effective record keeping system in a fresh-cut produce facility. With close attention to prerequisite programs, a processor can implement HACCP to round out their food safety program.

The following documents provide detailed information on guidelines and regulatory requirements pertaining to fresh-cut minimally processed vegetables:

- FDA. 1999. Guidance for industry: Reducing microbial food safety hazards for sprouted seeds and guidance for industry: Sampling and microbial testing of spent irrigation water during sprout production. Fed. Reg. 64: 57893-57902.

- FDA/CFSAN. 1998. Guide to minimize microbial food safety hazards for fresh fruits and vegetables. U.S. Department of Health and Human Services, Food and Drug Administration/Center for Food Safety and Applied Nutrition. Washington, D.C. http://www.foodsafety.gov/~dms/prodguid.html.

- FDA/CFSAN. 2001. Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial

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- USDA/FSIS. 2001. FSIS Guidance for Water, Ice, and Solution Reuse. U.S. Dept. of Agriculture/Food Safety and Inspection Service. Washington, D.C. http://www.fsis.usda.gov/oppde/rdad/frpubs/sanupdate1.pdf

- USDA/NASS. 2001. Fruit and vegetable agricultural practices – 1999, June. U.S. Department of Agriculture/National Agricultural Statistics Service, Washington, D.C. http://www.usda.gov/nass/pubs/rpts106.htm

III. CONCLUSION

Unfortunately, the “fresh” nature of minimally processed vegetables prevents the use of traditional processing such as cooking/heating as a means to reduce or eliminate pathogens. Therefore, most of the emphasis at preventing microbial contamination must be at the farm level rather than relying on corrective actions once contamination has occurred. However, given the large number of possible sources of pathogens contamination prior to harvest the government has developed science based farming guidelines, known as GAP, to control microbial contamination in an effort to improve the safety of fresh-cut produce. In the farm-to-table approach to food safety, GAP can provide ingredients with improved microbiological safety.

Once the minimally processed vegetables reach the processing facilities GMPs set the basic standards for facility sanitation and hazard control. Additional safeguards can be introduced through HACCP. HACCP establishes the critical control points and manages the application of control methods, ensuring that the process is effective. Finally, microbiological criteria and testing may be used, if necessary, to further verify that the process safety objectives are met.

A variety of mitigation regimes and sanitizers are available to reduce the microbial populations of fresh-cut vegetables. Chlorine is the sanitizer of choice. Although chlorine’s effectiveness is limited by the inability to reach into tissue crevices and rapid inactivation from contact with organic matter in wash water systems. Other factors that limit the efficacy of sanitizers are produce

topography, water quality, organic matter, contact time, internalization of microorganisms, microbial attachment, and formation of resistant biofilm.

New washing technologies using chemical sanitizing agents, vacuum infiltration, vapor-phase treatments, surface pasteurization, or bacteriophage control are needed to overcome failures of conventional methods by targeted treatment of microbial attachment or internalization sites. While it is unlikely that a single strategy will be successful in eliminating contamination from fresh-cut vegetables, using a combined mixture of two or more chemical and/or non-chemical disinfectant in conjunction with sound regulatory policies may reduce the risk of foodborne illness. Unfortunately, the delicate nature of fresh-cut minimally processed leafy vegetables makes optimizing decontamination efficacy, while ensuring physical product quality, somewhat of a challenge.

Consumers are advised to thoroughly wash fresh-cut vegetables immediately before consuming with running tap water (<10°F warmer than the produce) or with 50 to 200 ppm chlorine for at least 2-3 minutes. In addition, consumers should limit the amount of time fresh-cut vegetables remains outside refrigerate temperatures. Finally, it is critical to avoid cross contamination of fresh-cut vegetables via contaminated equipment and poor personal hygiene during food preparation.

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V. APPENDIX:

Additional information on fresh-cut produce related topics are available from these sources:

- *FDA Advises Consumers about Fresh Produce*
  http://vm.cfsan.fda.gov/~lrd/tpproduc.html

- Food Marketing Institute. *FMI Introduces Best Practices Guide for Fresh-Cut Produce.*
  http://www.fmi.org/media/mediatext.cfm?id=583

- *Food Safety Begins on the Farm; A Grower's Guide*
  http://www.gaps.cornell.edu/pubs/Farm_Boo.pdf

- *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*
  http://www.cfsan.fda.gov/~dms/prodglan.html