INTERVENTIONS FOR ENSURING FOOD SAFETY IN MANGOES DURING PHYTOSANITARY TREATMENTS

A Thesis

by

GRIHALAKSHMI KAKANI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2006

Major Subject: Food Science and Technology

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Approved by:

Chair of Committee,	Alejandro Castillo
Committee Members,	Gary Acuff
	Luis Cisneros-Zevallos
Chair of Food Science and Technology Faculty,	Rhonda Miller

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ABSTRACT

Interventions for Ensuring Food Safety in Mangoes during Phytosanitary Treatments.

(December 2006)

Grihalakshmi Kakani, B. Sc., Acharya NG Ranga Agricultural University;

M.A.B., Texas A&M University

Chair of Advisory Committee: Dr. Alejandro Castillo

Increased consumption combined with increasing risk to foodborne illnesses makes it necessary to identify potential sources of contamination in the food chain and apply intervention processes that prevent/minimize the risk of contamination. The current study investigated the effect of the decontamination treatments with chlorine and lactic acid on the survival of *Salmonella* on the rind and stem scar portions of inoculated mangoes. The presence of the pathogen in the treatment water, internalization and the effect of the treatments on the quality of the fruit were also determined.

For scar (hydrothermal), a 3.0 log reduction was obtained for control and additional reductions of approximately 2.2 and 1.3 log cycles were obtained with lactic acid and chlorine respectively. Data indicates reduction in pathogen population in cooling for all the treatments except two (Control – increase of 0.3 logs, LA-LA – increase of 0.3 logs).

A 0.5 log reduction was obtained for the control (initial - 4.4 $\log_{10}$ CFU/10 cm^2) and additional reductions of approximately 1.7 and 1.3 log cycles were obtained for treatments with lactic acid and chlorine respectively during hydrothermal treatment on

the rind. For cooling, lactic acid and chlorine gave an overall reduction of approximately 1.3 and 1.4 logs respectively compared to control.

Although *Salmonella* was not detected in the core stem tissue by direct plating method for most of the samples, it was detected after the enrichment method. The pathogen was detected on the rind, stem scar and the stem tissue for most of the samples for as long as 12 days. *Salmonella* was detected in treatment water with and without sanitizers after dipping mangoes. Lactic acid was found to be more effective in reducing pathogen population compared to chlorine in all the treatment combinations; however, the sensory aspects (color and texture) of the fruit were compromised.

DEDICATION

I dedicate this work to my parents, family and friends whose constant support and encouragement enabled me to complete the project successfully.

ACKNOWLEDGEMENTS

Many people both directly and indirectly were involved in the successful completion of this project. First, I would like to express my gratitude and sincere thanks to my supervisor Dr. Alejandro Castillo who gave me an opportunity to work with him on this project. He has been a constant source of inspiration and encouragement throughout my program. Second, I sincerely thank Drs. Gary R. Acuff and Luis Cisneros-Zevallos for being on my committee. I thank them for their constructive criticism, comments and valuable suggestions. I thank Ms. Lisa Lucia for her time and effort in helping me write the document. I express my sincere gratitude to all the faculty and lab members of Department of Food Microbiology, University of Guadalajara, Mexico, for providing excellent facilities to conduct my research project. This project would have been impossible without their cooperation, support and guidance.

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INTRODUCTION

Development of new technologies in packaging of fresh products, making it more convenient for people to consume together with increasing awareness of the health benefits has driven the sales ($76 billion) of fresh produce (Rodov 2004). The increase in consumption can be attributed to several factors; changes in dietary habits, increased emphasis on health benefits, (2005 Dietary Guidelines recommends 5 cups of fruits and vegetables per day) global distribution of food and expansion of commercial food services (Bender and others 1999). In addition, development of new technologies and trade agreements has favored imports from other countries to meet the ever-increasing domestic demand (Beuchat 1998). Imports accounted for 38.9% of US fresh fruit consumption, up from 24.2% in 1980 (Clemens 2004). It was reported that in 1996, 17% of all cantaloupes, 52% of all green onions, 36% of all cucumbers, 34% of all tomatoes and 66% of all mangoes sold in the US was from Mexico (Clemens 2004).

The increase in consumption however, has also introduced an increased risk of foodborne illness mainly due to pathogenic microorganisms. Approximately 76 million cases related to foodborne illness causing approximately 5000 deaths are estimated to occur each year (Mead and others 1999) in the US. Pathogens such as *Salmonella*, *Campylobacter jejuni, Shigella, Escherichia coli* O157:H7 and *Listeria monocytogenes* are implicated in many of the food related outbreaks. *Salmonella* is considered to be a serious and deadly pathogen since it has been implicated in approximately 48% of the

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produce-associated outbreaks (Sivapalasingam and others 2004).

Salmonella has been isolated from many types of raw fruits and vegetables (Beuchat 1996; Wells and Butterfield 1997). *Salmonella* is a facultative anaerobic gram-negative bacterium capable of multiplying and surviving in diverse ecosystems. Infection in humans is characterized by gastroenteritis, which manifests as diarrhea, vomiting, fever and in severe cases may cause death. Persons at risk include infants, the elderly, and the immuno-compromised individuals in whose case complications lead to meningitis, epticemia, and reiter syndrome (Pavia and Tauxe 1991).

Salmonella contamination has been linked to diverse fruits and vegetables, including tomatoes (Centers for Disease Control and Prevention (CDC) 2005; Cummings 2001), melons (Anderson and others 2002), mangoes (Sivapalasingam and others 2003; Beatty and others 2004), and unpasteurized orange juice (Cook and others 1996). It becomes essential to identify potential sources of contamination in the food chain (farm to the table) so as to reduce the risk of contamination/infection. Once the sources are identified it is important to apply/implement interventions/decontamination processes to prevent and/or minimize the risk of contamination.

The produce industry typically uses water and chlorine to clean/sanitize the produce (Li and others 2001). However, previous work with chlorine as a sanitizer in reducing microbial populations gave mixed results. Chlorine was found to be ineffective for produce with high organic matter (Beuchat and Ryu 1997). Lactic acid has been found to be a good alternative to decontaminate produce (Venkitanarayanan and others 2002). Research studies indicate lactic acid as a good disinfectant to decontaminate beef

carcasses (Castillo and others 2001). The use of organic acids for surface decontamination of raw produce has also been proved to be useful in reducing populations of microorganisms (Beuchat 1998).

The current study aims at investigating the effect of chemicals (chlorine and lactic acid) in reducing *Salmonella* populations on mangoes that are exported to the US.

OBJECTIVES

1. To determine the effect of adding sanitizers to water used for hydrothermal and cooling treatments in reducing populations of *Salmonella* on mangoes.
2. To investigate the internalization of *Salmonella* in the stem tissue of the fruit after cooling treatment.
3. To determine the survival of *Salmonella* in the stem scar, rind and the stem tissue of refrigerated mangoes – shelf life analysis to be performed for a period of 12 days.
4. To determine the effect of adding sanitizers in the treatment water in reducing populations of *Salmonella*.
5. To study the impact of the sanitizing treatments on the pH, color and texture of the fruit.

LITERATURE REVIEW

Incidence of Foodborne Outbreaks

Fresh produce has been known as a possible vehicle of human disease for at least a century. According to Beuchat (1998) an outbreak of typhoid fever caused by eating watercress (Warry) was reported back in 1903. Creel (1912) demonstrated that *Bacillus typhosa* (now *S.* Typhi) survives on lettuce and radishes for as long as 31 days. However, outbreaks of foodborne illness implicating fresh produce, in industrialized countries are not frequently documented. For example, only six of about 200 reported foodborne illness in 1996 in UK were associated with fresh produce (PHLS 1996).

The food supply in the US is considered to be one of the safest in the world, but yet there have been large and frequent outbreaks of foodborne illness documented in the last few years (Bender and others 1999; Crutchfield and Tanya 2000). The increased consumption of fresh produce has led to increased incidence of food related illness in the US (Beuchat 1996). Hedberg and others (1994) discussed some of the factors that are contributing to increased frequency of foodborne outbreaks. Factors include global distribution of produce, accidental introduction of pathogens into new geographical areas – e.g. introduction of *Cyclospora* in the US (CDC 1996; Herwaldt and others 1997), increased resistance or adaptation of pathogens to stress/environmental conditions, increased susceptibility to diseases due to compromised immune system among certain sections of the population, changes in dietary habits, and increasing international travel. Other factors such as contaminated irrigation water, poor personnel hygiene, poor

equipment sanitation and use of manure/compost for fertilization also contribute to microbial contamination and subsequent foodborne illness (Johnston and others 2005).

In spite of increasing documented outbreaks involving fresh produce, most of the producers, both domestic and foreign, were not involved in ensuring food safety (Calvin 2004). As a result, major outbreaks in the US associated with the consumption of fresh produce, both domestic and foreign, more than doubled between the periods of 1973-87 and 1988-91, from 4 per year to 10 (Tauxe and others 1997).

The heightened concern prompted FDA to initiate a survey of imported fresh produce (FDA 2001a). FDA began testing domestic and imported produce for three microbial pathogens – *Salmonella, Shigella* and *E. coli* O157:H7. Of the 1003 samples tested (1999), forty-four tested positive for *Salmonella* or *Shigella* (imported produce only). *E. coli* O157:H7 was not detected in the produce items. Of the several pathogens that are implicated with foodborne illness, *Salmonella* is considered to be the most deadly and most frequently reported pathogen causing approximately 15000 hospitalizations and 5000 deaths in the US (Mead and others 1999). *Salmonella* has been reported to cause approximately 95% of the total deaths related to foodborne illness in the US (Santos and others 2003).

A multistate outbreak of 86 cases associated with consumption of domestic raw tomatoes caused by *S.* Baildon was reported in 1999 (Cummings 2001). Subsequent investigation implicated two tomato grower/packer cooperatives in Florida. An outbreak involving 133 cases implicating *S.* Newport was reported in 1995 (Van Beneden and others 1999). The outbreak was reported to be associated with the consumption of alfalfa

sprouts. In the years 1990 and 1993 outbreaks of *S.* Javiana (Wood and others 1991) and *S.* Montevideo (CDC 1993) infections involving 170 and 100 cases respectively in Illinois, Michigan, Minnesota, and Wisconsin were linked to the consumption of fresh tomatoes. An outbreak in 1995 involving *S.* Hartford, *S.* Gaminara and *S.* Rubislaw occurred among 62 unrelated travelers in Orlando, Florida (CDC 1995). Subsequent investigation revealed that the illness was associated with the consumption of unpasteurized orange juice.

Attachment of Microorganisms to the Surface of Fruits and Vegetables

Microorganisms are ubiquitous and are capable of contaminating fruits and vegetables in several ways starting from the farm until the final consumption. Fruits and vegetables become contaminated during growth, harvesting, transporting, postharvest handling, processing, distribution and during final preparation before consumption (Beuchat 2002). The mechanism of attachment of bacteria on the surface has been a subject of intense research and is thought to be governed by several factors such as pH of the fruit, water activity and the medium in which the bacteria are grown (Iturriaga and others 2003). In addition, it is also suggested that the nature of waxy cuticle, presence of microflora also influence the presence and attachment of certain types of pathogens on the surface (Beuchat 2002). The explanation for the attachment of bacteria on the surface of fruits is still vague, and is an issue that warrants extensive research. However, based on previous research it is suggested that it is similar to the attachment of plant pathogenic bacteria (Iturriaga and others 2003). The presence of structures/substances on

the outersurface such as flagella, fimbriae and proteins is suggested to influence the bacterial attachment to the plant surfaces (Romantschuk 1992, Strom and Lory 1993).

Several studies reported that the microorganisms tend to attach to the surfaces of fruits and vegetables in pores, indentations and other natural irregularities on the intact surfaces where there are protected binding sites (Seo and Frank 1999). Burnett and others (2000) reported that *E. coli* O157:H7 preferentially attached to discontinuities and irregularities in waxy cuticle. The pathogen was detected in damaged tissue as deep as 70μm beneath the skin surface. A similar finding was reported by Liao and Sapers (2000) involving *S.* Chester. The microorganism was found to survive after washing when attached to cut surfaces of apple than on unbroken external surfaces.

Survival and Growth of Pathogens

The growth and survival of pathogens is influenced by several factors, which include type of the organism, type of produce and environmental conditions in the field, including storage conditions (FDA 2001b). Other intrinsic factors such as pH and extrinsic factors such as temperature also influence the survival of microorganisms on fruits and vegetables. It is thought that pathogens are not capable of surviving on the surface of fruits and vegetables due to several factors, which include antagonistic behavior of waxy cuticle, presence of other microflora, and inability of microorganisms to produce enzymes necessary to breakdown the protective barriers. However, previous studies indicate growth and survival of pathogens on the surface of fruits and vegetable. Growth of *E. coli* O157:H7 was reported on the surface of watermelon and cantaloupe rinds (Van Loosdrecht and others 1987). An FDA survey of imported produce revealed

that 7.3% of cantaloupes that were imported, tested positive for *Salmonella* and *Shigella* (FDA 2001a).

Intrinsic factors such as pH also influence the survival and growth of bacteria. Some fruits (apples, oranges) are more acidic than others and do not support the growth of human pathogens. However, tomatoes are an exception since it was implicated in four multistate outbreaks of *Salmonella* infection (CDC 2002a; Cummings and others 2001; Hedberg and others 1999). Although acidic (pH 4.0), populations of *Salmonella* were unchanged in chopped tomatoes stored at 5 °C (Zhuang and others 1995).

Temperature plays a major role in the survival of the pathogens on the surface of fruits and vegetables (Gawande and Bhagwat 2002). A significant increase in populations of *S*. Montevideo on tomatoes stored at 20 °C was reported by Zhuang and others (1995). *E. coli* O157:H7 was reported to survive in broccoli, cucumber and green pepper when held at 4 °C (Richert and others 2000). While initial levels were maintained at 4 °C, growth was observed at 15 °C. Although chilled conditions do not favor growth of microorganisms, it cannot be ensured that the pathogens are completely inactivated at refrigerated temperature. For example, Parish and others (1997) reported longer periods of survival of *Salmonella* and *E. coli* O157:H7 in refrigerated fruit juices compared to those maintained at room temperature

The temperature of the wash water used during the handling of produce plays an important role in the safety of fresh produce. Bartz (1988) and Boyette and others (1995) showed that dip-washing tomatoes might result in the diffusion of water to the interior of the fruit. Processing conditions may promote opportunities for microorganisms to

infiltrate fruit. In a simulated study, a higher percentage of positive samples for *Salmonella* were reported in the core stem area of fresh mangoes after cooling (Penteado and others 2004). Bartz and Showalter (1981) demonstrated infiltration of bacteria into the stem tissue of warm tomatoes (26-40 °C) after a 10 min exposure in a cool aqueous bacterial suspension (20-22°C). A similar finding was reported by Zhuang and others (1995) involving tomatoes. A higher population of *S.* Montevideo was recovered in the core tissue when the temperature of the wash water was less than the temperature of the fruit (negative temperature differential). Buchanan and Edelson (1999) reported penetration of *E. coli* O157:H7 into the core of warm apples. *E. coli* O157:H7 was recovered in higher numbers in russet areas and floral tube of apples under a negative temperature differential (Burnett and others 2000). Based on these studies it is hypothesized that immersion of warm fruit in cool water will create a negative temperature differential causing contraction of gases in the fruit. The contraction of gases results in an inward hydrostatic potential as a result of which water is drawn into the fruit. Microorganisms present either on the surface or in the water gain entry into the internal structures along with the water (Buchanan and Edelson 1999, Zhuang and others 1995).

Infiltration of bacterial pathogens into the core tissues of fruit and vegetables and subsequent outbreak of foodborne illness (salmonellosis outbreak involving mangoes) upon consumption is a serious problem that is currently plaguing the produce industry. Current decontamination methods have been proved to be successful only in eliminating/reducing microbial population on the surface. So far none of the methods

have been proved to be efficient in eliminating bacterial populations in the core tissues. Hence, it is recommended to maintain adequate levels of sanitizers in the wash/treatment water to prevent/reduce the extent of infiltration into the internal structures (Sivapalasingam 2003).

Imported Produce and Foodborne Outbreaks

Two outbreaks (*E. coli* O157:H7 infection associated with California lettuce and cyclosporiasis involving Guatemalan raspberries) implicating domestic and imported produce highlighted the incidence of microbial contamination at the farm level (Tauxe 1997). The outbreaks had serious economic impacts on the produce industry and necessitated improved food safety programs to prevent or minimize the risk of microbial contamination at the grower and shipper level (Calvin 2003). Federal agencies (Food Safety Inspection Service (FSIS), FDA, Animal and Plant Health Inspection Service (APHIS)) are responsible for regulating and implementing the food safety programs both for the domestic and imported food in the US. The ultimate goal of these agencies is to ensure American consumers that food produced domestically or imported is safe for consumption (Calvin 2003). While the FSIS unit of United States Department of Agriculture (USDA) is responsible for products of animal origin (meat, poultry and eggs), FDA is responsible for produce related issues. In 1998, FDA published voluntary guidelines for both domestic and foreign producers, in response to the increasing microbial contamination being reported in fresh produce. These guidelines specifically outlined "good agricultural practices" (GAPs) for reducing microbial contamination (FDA 1998; Calvin 2003).

Raspberries and *Cyclospora*

A large outbreak of cyclosporiasis was reported in the spring and early summer of 1996 (Calvin and others 2002). This was considered as a very large outbreak since only three small outbreaks prior to 1996 were documented in US. The earlier reported cases (45) were confined only to few overseas travelers. After a thorough investigation, it was finally reported that simultaneous and continual contamination on multiple farms was the main reason for the outbreak (Herwaldt and others 1997).

Strawberries and Hepatitis A

A total of 213 cases of Hepatitis A were reported from schools in Michigan and Maine in 1997. An epidemiologic study conducted later revealed that the foodborne illness was associated with the consumption of frozen strawberries (Hutin and others 1999). Further trace back of the frozen strawberries led to a processing firm located in Baja California, Mexico. The firm shipped strawberries to US in 1996 (FAS 1998). The outbreak had serious economic implications on the Mexican industry.

Cantaloupe and *Salmonella*

Three multistate outbreaks implicating *S.* Poona associated with the Mexican cantaloupe were reported in the spring 2000-2002. A subsequent epidemiological study revealed an indistinguishable PFGE pattern for 2000 and 2002 outbreaks (CDC 2002b). However, the pattern for 2001 outbreak was unique. Forty-seven people became sick in March and April (2001) and by late May Cantaloupes imported from southern Mexico were implicated (Anderson and others 2002).

Two additional outbreaks associated with *Salmonella* involving cantaloupes were reported (first outbreak was associated with *S.* Poona and then *S.* Anatum) were reported

in spring 2001. Fifty people fell sick and two died from *S.* Poona (Anderson and others 2002; FDA 2001c). Fewer people were reported unwell in the *S.* Anatum outbreak. Trace back revealed that cantaloupes were imported from the same farm that was implicated in the 2000 outbreak

An outbreak of *Salmonella* Poona infections was reported in Canada and in 14 states of US in 2002. Fifty-eight cases were identified (Anderson and others 2002) and again cantaloupe imported from a Mexican farm was identified as the source of contamination. The importing firm issued a voluntary recall and FDA issued an import alert (FDA 2002).

Mangoes and *Salmonella*

In December 1999, thirteen states reported an outbreak of *S.* Newport infections that occurred during the previous month. There were seventy-eight confirmed cases, fifteen hospitalizations and eventually two died as a result of infection. An indistinguishable pulsed field gel electrophoresis pattern showed that all the seventy-eight people were infected with the same strain. Federal agencies subsequently traced the implicated mangoes to a Brazilian farm and hot water treatment was suspected as the possible point of contamination (Sivapalasingam and others 2003).

Hot water treatment of fresh mangoes is mandated by APHIS as a quarantine measure to prevent introduction of Mediterranean fruit fly into US. Mangoes exported to the US are dipped in unchlorinated hot water (46.7 °C) for 75-90 min and subsequently immersed in a cool water tank for 6-10 min (21.1 °C) before being packed and shipped

to the US. The hot water used for disinfestation treatment was not chlorinated however; the cool dip was chlorinated (100mg/L).

A laboratory investigation of the water and cloacal samples revealed presence of *Salmonella* and *E. coli* spp indicating that the water used for the disinfestation treatment was contaminated (Sivapalasingam and others 2003). Interestingly, mangoes grown on the same farm were also exported to Europe and no outbreak was reported. However, mangoes bound for the European market were not hydrothermally treated.

The cool dip is not mandated by APHIS however; the farm does it to prevent the deterioration of the quality of fruit. The negative temperature differential as a result of cool dip might have caused the infiltration of the pathogen into the core tissue of the fruit. To prevent this, federal agencies recommended having a lapse of at least 30-min between the hot and cool water dips (USDA/APHIS 2002).

The outbreak and subsequent investigation demonstrated that certain methods that are employed to prevent accidental introduction of unwanted pests and diseases can however, cause newer problems rendering the food unsafe for consumption. Hence, it becomes important to take into consideration various factors to identify potential problems before implementing the new technologies (Sivapalasingam and others 2003).

A second outbreak of *Salmonella* infection associated with the consumption of raw mangoes was reported in March 2001. The serotype implicated in this outbreak was *S.* Saintpaul. Mangoes implicated were imported from Peru. Although there was inadequate information for a complete trace back to the farm level, the investigators

suspected that contamination had occurred during the disinfestation treatment, as occurred with the outbreak of *S.* Newport (Beatty and others 2004).

Frequent documentation of foodborne illnesses associated with imported fresh produce can have serious implications for the exporting countries. According to Calvin (2003), "repeated outbreaks within an industry prompt several concerns. First, the industry fears that when people get sick, investigators may incorrectly focus on the product with a history of outbreak. Second, the produce industry is concerned that FDA might issue a consumer warning about eating the contaminated produce. Third, growers are concerned that if a problem looks like it affects more than a few growers, the FDA might initiate an import alert against all producers from a specific country as in the case of Guatemalan raspberries. Fourth, there is also a growing concern that an ongoing problem could hurt the reputation of other products from the same region. Fifth, FDA has the option of initiating tough mandatory regulations, something most growers would like to avoid".

Strategies for Mitigating the Problem

It is a well-established fact that fresh and fresh-cut produce becomes contaminated with pathogens and there is no process (heating) involved during processing of the produce that would effectively eliminate the pathogens (Schuenzel and Harrison 2002). Also, since most of these items are consumed raw unlike meat (cooked) it becomes imperative to implement decontamination techniques that are effective in reducing the microbial levels without adversely affecting the sensory aspects (Beuchat 1998).

There are several chemicals that are being used by the produce industry for decontamination; however, the effectiveness of these sanitizers is dependent on several factors like inoculation method (dip/spray), contact time, microbial load, pH and temperature of the solution (Materon 2003; Parish and others 2003). The current study focuses primarily on chlorine and lactic acid.

Chlorine

Chlorine is the most commonly used sanitizer by the fresh produce industry, typically applied at concentrations no greater than 200 ppm with a contact time of 1-2 min (Sapers 2003; Parish 2003). The lethal action of chlorine is due to hypochlorous acid (HOCl) that is in equilibrium with hypochlorite ion and it is highly dependent on the pH of the solution. The bactericidal activity is high when the pH is between 6.0 and 7.5. The effectiveness is reduced in the presence of soil, dust and organic material (Materon 2003).

Use of chlorine by the produce industry has been more of a tradition. It has been used for several decades although elimination of pathogens from the surface of fruits and vegetables is limited and unpredictable (Nguyen-the and Carlin 1994). The produce industry typically adds chlorine to the wash/treatment water that is used to wash the fresh produce. The inefficiency of chlorine is due to lack of accessibility to microorganisms located within pores, wounds, and resistance of bacteria within biofilms (Adams and others 1989; Sapers 2003).

Wei and others (1995) reported that *Salmonella* was protected by organic matter that was present in the dump tanks and on the surface of tomatoes. In the same study it

was demonstrated that treatment with chlorine at 100 ppm for 2 min did not significantly reduce bacterial population both at the stem scar (3.98 $\log_{10}$ CFU/g and the skin (3.25 $\log_{10}$ CFU/g). In another study by Weissinger and others (2000), it was demonstrated that application of chlorine at 200 ppm did not eliminate *S.* Baildon inoculated (0.86 $\log_{10}$ CFU/ml) onto diced tomatoes. Lettuce treated with 20 ppm chlorine at 20 or 50 °C did not significantly reduce populations of *E. coli* O157:H7 compared to treatment with water without chlorine (Li and others 2001)

Dipping Brussel sprouts inoculated with *L. monocytogenes* (10^6 CFU/g) in chlorine at 200 ppm reduced the bacterial population by approximately 2 logs (Brackett 1987). Dipping in water reduced the population by 1 log. A 1.3-1.7 $\log_{10}$ CFU/g reduction of *Listeria* was reported (Zhang and Farber 1996) on shredded lettuce treated with 200 ppm chlorine for 10 min. The reduction obtained on cabbage was less (0.9-1.2 $\log_{10}$ CFU/g). A significant reduction of *S.* Montevideo on the skin and core stem tissue (Zhuang and others 1995) was obtained by dipping tomatoes in a solution of chlorine at 60 or 110 ppm for 2 min.

Organic Acids

Several studies demonstrated the limited efficacy of chlorine in reducing microbial populations on the surface of fresh produce. Hence, alternatives such as use of organic acids have gained importance. The efficiency of organic acid washes (lactic, acetic, citric, peracetic, propionic) in reducing microbial populations on the surface of fruits and vegetables has been widely investigated (Nguyen-the and Carlin 1994).

The effect of lemon juice on microbial population was investigated by Escartin and others (1989). A significant reduction in populations of *S*. Typhi compared to control was demonstrated on papaya (pH 5.7) and jicama (pH 6.0). Castillo and Escartin (1994) showed that populations of *C. jejuni* were reduced on papaya (pH 5.6) and watermelon (pH 5.5) upon treatment with lemon juice. It was also demonstrated that lemon juice was more effective on papaya compared to watermelon. The authors suggested that the difference in action could be due to differences in buffer capacity of the fruits. Lactic acid was reported to be superior compared to chlorine in effectively reducing internalized salmonellae in tomatoes (Ibarra-Sánchez and others 2004).

Treatment with acetic acid (2%) or vinegar (40%) for 15 min was reported to reduce populations of *Yersinia enterocolitica* by 7-logs on parsley (Karapinar and Gonul 1992). Lettuce treated with a combination of lactic or acetic acid with chlorine significantly reduced population of *L. monocytogenes* (Zhang and Farber 1996). A solution of lactic acid (1.5%) with hydrogen peroxide (1.5%) eliminated populations of *Salmonella* and *E. coli* O157:H7 on apples, oranges and tomatoes without significantly affecting the quality of the fruits (Venkitanarayanan and others 2002). A similar result was reported by Lin and others (2002). A 4-log reduction for *E. coli* O157:H7 and *Salmonella* and a 3-log reduction for *L. monocytogenes* were obtained when lettuce was treated with a combination of lactic acid and hydrogen peroxide.

From the preceding observations it can be concluded that washing or rinsing fruits and vegetables with organic acids will bring reductions in microbial populations and may reduce the risk of illness upon consumption.

Quality Aspects

Mango is a favored fruit all over the world and is known for delicious taste, flavor, and a good source of minerals and vitamins (Kaswija and others 2005). Mango is a climacteric fruit and its cultivation is typically confined to tropical areas of the world (Lizada 1993; Mitra and Baldwin 1997). Due to its perishable nature, export of this fruit to other regions of the world is highly restricted and regulated. Quarantine treatments such as hot water dips have been developed and implemented by several importing countries to prevent introduction of Tephritid fruit flies (Couey 1989; Paull 1990).

However, negative effects of heat on the quality of the fruit especially color, and texture has been reported by several authors (Jacobi and Wong 1991; Jacobi and Wong 1992; Joyce and others 1993; Couey 1989). It has been suggested that several factors including species, stage of ripeness at the time of harvesting and exposure to various environmental conditions during growth and harvesting influence the ability of the fruit to tolerate extreme environmental stress such as heat (Shewfelt 1994).

Several studies reported that postharvest heat treatments delay/inhibit the ripening process of the fruit (Lurie 1998). Ripening is characterized by several physiological changes such as softening of the peel caused by the degradation of the cell wall, change in color (green to yellow/red), increase in soluble solids and increased ethylene production (Lurie 1998). “Lightness (L), ‘a*’ (Hue) and ‘b*’ (Chroma) are color terms that relate to color perception. For any measured color of lightness, L, the coordinates (a* and b*) locate the color on a rectangular-coordinate grid perpendicular to the L axis. The color of the grid origin (a*=0, b*=0) is achromatic (gray). On the

horizontal axis, positive a* indicates a hue of red-purple; negative a*, of bluish green. On the vertical axis, posititve b* indicates yellow and negative b* blue" (McGuire 1992).

A lower L value and a simultaneous increase in a* and b* values in both peel and the pulp indicates a higher degree of ripeness (indicated by yellow pigmentation). The yellow pigmentation is attributed to the synthesis of carotenoids during ripening process (Shewfelt 1993; Krishnamurthy and others 1971; Medlicott and others 1986). Several researchers have reported browning of tissues as a result of exposure of fruit to extreme temperatures. Browning is thought to be influenced by several factors such as cultivar, preharvest conditions, climate and stage of maturity at the time of harvesting (Shewfelt 1994). Texture is another important factor and plays an important role in consumer's evaluation of food product (Rodov 2004). Texture of the fruit is influenced to a large extent on the genetic makeup; however, other factors such as morphology, environmental factors during growth and postharvest handling of produce are also suggested to be affecting the overall textural quality of the fruit (Sams 1999; Harker and others 1997).

MATERIALS AND METHODS

Mangoes without any treatment were needed for this project. Since at the time of the study untreated mangoes were not available in the US or Canada, it was necessary to move to Mexico and obtain fresh raw mangoes. Therefore, the project was conducted in the Food Microbiology lab, Department of Biology and Pharmacy, University of Guadalajara, Mexico.

Bacterial Cultures

Rifampicin-resistant strains of *Salmonella enterica* serotypes Poona, Montevideo, Agona, Michigan, Newport, and Gaminara provided by Dr. Linda Harris from the University of California, Davis were used for the experiments.

Inoculum Preparation

The strains (Rif^{+}) were stored at -80 °C until further use. The microorganisms were resuscitated by two successive transfers to tryptic soy broth (TSB; Difco, Detroit, MI) and incubated at 35 °C for 18-24 h. The cells were then transferred onto tryptic soy agar (TSA; Difco, Detroit, MI) slants and stored at 4-5 °C until they were needed for the experiment. Prior to use, resistance to rifampicin was confirmed by streaking each organism onto TSA plates supplemented with 80 μg/ml rifampicin (Sigma, St Louis, MO) and incubated at 35 °C for 18-24 h. Characteristic colonies were then maintained on TSA slants and transferred twice to TSB and incubated at 35 °C for 18-24 h before inoculum preparation. The day before the actual experiment, each isolate was transferred into six glass bottles containing 250 ml of TSB and incubated at 35 °C for 18-24 h. Each bottle with an overnight growth had approximately 8-9 $\log_{10}$ CFU/ml. The cells were

then harvested by centrifugation (1610 x g) for 10 min at 4 °C. The cell pellets obtained were washed once in prepared 0.85% (w/v) saline solution. Finally, the pellets were resuspended individually in 0.1% sterile peptone (Difco) water. Each suspended strain contained in individual bottles (250 ml each for a total of 1500 ml) was transferred into a sterile plastic bucket containing 2500 ml of 0.1% peptone (Difco) water making a total of 4000 ml of suspension. The suspension containing a mixture of all strains (cocktail) served as the inoculum for the mangoes. The suspension was split into two portions (approximately 2000 ml of each). While one portion was used for inoculating mangoes intended for hydrothermal and cooling treatment (approximately 35), the other portion was stored (for approximately 170 min) at room temperature and was used for inoculating the second batch of mangoes intended for storage study (approximately 35). The density of cells both in the inoculum and on the mangoes was approximately the same for both the batches.

Collection of Mangoes

Fresh mangoes (*Mangifera indica*) belonging to 'Kent' variety were obtained from a local distributor in Guadalajara, Mexico. The mangoes were bought in three batches (630 in total) to the Food Microbiology Laboratory (University of Guadalajara) for further analysis. It was ensured that the mangoes were unwaxed, non-hydrothermally treated, free of any visual defects such as bruises, cuts or abrasions, and are of similar size and maturity (mature green). It was also ensured that mangoes obtained at different timings were of the same origin. A total of 70 mangoes were used for each treatment.

Inoculation of Mangoes

For each treatment approximately 70 mangoes (two batches, 35 in each batch) were inoculated by immersing each mango for 1 min in a bucket containing approximately 2 L of the inoculum suspension. To ensure even inoculation on the surface, the fruit was rotated manually. The inoculated mangoes were then placed in a plastic basket for about 20 min to drain at room temperature after which the fruits were subjected to hydrothermal treatment. The *Salmonella* population on the scar and the rind portion of the mangoes was approximately 5-6 $\log_{10}$ CFU/10 cm^2 and 3-4 $\log_{10}$ CFU/10 cm^2 respectively.

Preparation of Sanitizing Solutions

Industrial sodium hypochlorite containing 12-13% of active chlorine (hypochlorite) was used for the sanitizing treatments. The amount of free chlorine present in the solution was determined by a titration with thiosulfate. The pH of the solution was adjusted to 6.0 by addition of citric acid (20% w/v). The pH of the final solution was measured using an Orion Model 230A pH meter (Orion Research INC, Boston). L-lactic acid 88% (Purac, Lincolnshire, IL) was also used for the sanitizing treatments. These sanitizing solutions were added to the treatment water to achieve a final concentration of 200 ppm for chlorine and 2% for L-lactic acid.

Treatments

Eight treatments with sodium hypochlorite or L-lactic acid were performed to determine the survival of the microorganism (Table 1). A treatment where mangoes were inoculated and dipped in water only served as control.

Table 1 - Treatment Combinations

Hydrothermal (46.7 ºC)	Cooling (25 ºC)
Water (Control)	Water (Control)
L-lactic acid (2%)	Water
Chlorine (200 ppm)	Water
L-lactic acid (2%)	L-lactic acid (2%)
Chlorine (200 ppm)	Chlorine (200 ppm)
Water	Chlorine (200 ppm)
Water	L-lactic acid (2%)
L-lactic acid (2%) - 30 min lapse- water cooling	
Chlorine (200 ppm) - 30 min lapse- water cooling	

Procedure for Hydrothermal and Cooling Treatment

For each treatment, inoculated mangoes were placed in a plastic basket (59 x 38 x 20 cm) and immersed in a water bath (109 x 50 x 21.7 cm, Polyscience), maintained at a temperature of 46.7 °C. The level of the water was above the top of the basket. To ensure that mangoes were completely immersed in water, a plastic board was affixed to the top of the basket to prevent mangoes from floating. The temperature of the bath was monitored using a K-type thermocouple connected to a Traceable thermocouple (Control Company, Friendswood, TX) to ensure that the temperature was constant throughout the

treatment. Three mangoes were removed randomly at time intervals of 0, 30, 60, 90 and 120 min from the hot water bath for sampling at the stem scar and the rind regions, separately. At the end of 120 min, the basket with the remaining mangoes was removed from hot water and dipped in a cool bath (77 x 54 x 56 cm) maintained at a temperature of 25 °C (cooling treatment). The temperature of the bath was monitored using a thermocouple. At time intervals of 0, 5, 10, 15, and 20 min, 3 mangoes were randomly removed and sampled at the stem scar and the rind, separately (Fig 1).

Sampling

All mango samples were collected separately from the stem scar, rind and core stem tissue. The scar area was excised using a sterile stainless steel core borer of 10 cm^2 area. The borer was used to initially cut the stem area of the mango. Using a sterile scalpel and forceps the bored area was cut and sliced to approximately 1-2 mm deep. For rind samples, the entire rind was peeled using a sterile stainless steel knife. For core stem tissue, the internal tissue at the stem scar area was cored using a sterile stainless steel borer. The cored area was then removed by cutting an area of 10 cm^2 for approximately 1-2 mm deep using a sterile forceps and scalpel. The borer, scalpel and forceps were sterilized by dipping in 95% ethanol and flamed between samples.

Internalization of *Salmonella* in Stem Tissue

To investigate the internalization of *Salmonella* for each treatment, three mangoes at the end of cooling treatment were allowed to drain (until the liquid cease to drip) for approximately 20 min at room temperature. The mangoes then were dipped in

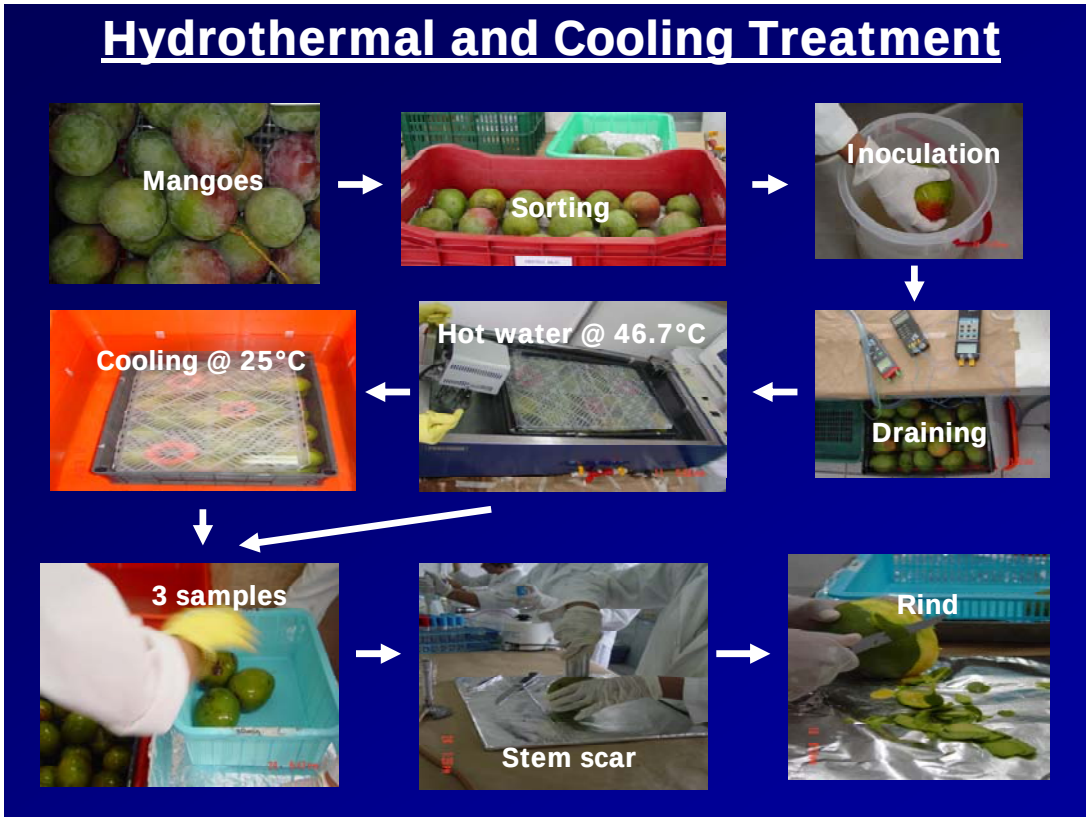

Figure 1 - Procedure for Hydrothermal and Cooling Treatments

1 L of 0.2% sodium hypochlorite solution for 1 min. To ensure even application of the chemical on the surface, the fruit was rotated manually. The treated fruit was drained for approximately 20 min at room temperature (until the liquid cease to drip) and subsequently cleaned with gauze cloth dampened by dipping in 100 ml of 70% ethanol for approximately 2-3 s and kept at room temperature until ethanol present on the surface evaporated (Penteado and others 2004; Fig 2). The above procedure was intended to prevent transfer of the pathogen from the surface to the internal tissue during sampling. An approximate weight (approximately 6 g) of the core stem tissue (uninoculated mangoes) was obtained for calculations.

Survival of *Salmonella* during Storage

The survival of *Salmonella* on the stem scar and the rind for each treatment was determined by analyzing the treated fruit during storage for a period of 12 days. The fruit was dried at room temperature after undergoing the cooling treatment and then stored in a refrigerator maintained at a temperature of 10 °C after ensuring no moisture remains on the surface. Hydrothermally treated fruit was used as control and was compared with chemically sanitized fruit. The stem scar, rind and core stem tissue were sampled separately on 0, 3, 6, 9, and 12 d of storage.

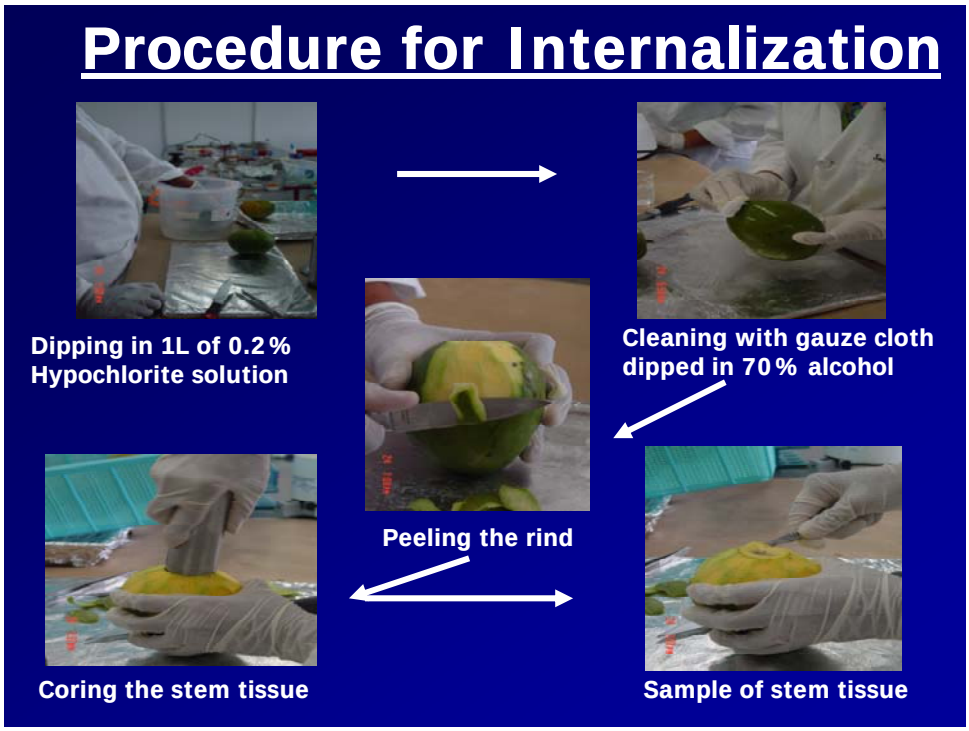

Figure 2 - Procedure for Determining the Presence of Pathogen in Stem Tissue

Transfer and Survival of *Salmonella* in Treatment Water

The presence of *Salmonella* in the treatment water was also determined. Water samples were collected from six sections (ensuring a representative sample) of the hot (46.7 ºC) and cool (25 ºC) bath at time intervals of 0, 30, 60, 90 and 120 min and 0, 5, 10, 15, 20 min respectively. Approximately 150 ml (25 ml from each section) of the sample was collected in a 250-ml sterile glass bottle using a transfer pipette. The sample was then analyzed for *Salmonella* both quantitatively and qualitatively.

Effect of Sanitizer Treatments on Fruit Quality

The impact of the sanitizing treatments on the quality of the fruit was investigated. The parameters analyzed included pH, color and texture. Approximately 35 uninoculated mangoes similar in size and level of maturity were subjected to hydrothermal treatment for 120 min at 46.7 °C followed by a cooling treatment for 20 min at 25 °C. The treated mangoes were then left to drain at room temperature for approximately 30 min and subsequently stored in a refrigerator maintained at a temperature of 10 °C. For each parameter analyzed, three mangoes were sampled on 0, 3, 6, 9, and 12 d of storage. Fruit that did not receive any treatment was used as control.

Microbiological Analysis

Stem scar, and rind samples were placed individually in separate sterile whirl-pak® bag (Nasco, Fort Atkinson, WI) containing 100 ml of 0.1% sterile peptone water. The contents of the bag were pummeled in a stomacher (Seward Scientific, London, England) for 1 min. Viable counts of the microorganism were obtained by spread plating 1 ml (divided over four plates), 0.1 ml of the homogenized sample from the whirl pak

bag and 0.1 ml of the serial ten-fold dilutions from the same homogenized sample onto the surface of previously dried plates of TSA supplemented with 80 μg/ml rifampicin (rif-TSA). The plates were then incubated at 35 °C for 18-24 h. For treatments with lactic acid, the pH of the sample in peptone water was adjusted to approximately 7.0 to recover injured cells.

To detect internalized salmonellae from cored area around stem scar, the excised samples were homogenized by placing in a sterile whirl-pak® bag containing 100 ml of rif-TSB for 1 min. Viable counts of the microorganism were obtained by spread plating 1 ml (divided over two plates) onto the surface of previously dried rif-TSA plates. The plates were then incubated at 35 °C for 18-24 h. To be able to detect *Salmonella* below the detection limit, the pre-enriched homogenized samples were incubated at 35 °C for 18-24 h and subsequently streaked onto rif-TSA plates and incubated at 35 °C for 18-24 h. The presence of *Salmonella* was reported as positive or negative.

The procedures described above were employed for analyzing the storage samples for stem scar; rind and core stem tissue respectively. For water samples, viable counts of the microorganism were obtained by spread plating 1 ml (divided over two plates), onto the surface of previously dried rif-TSA plates. The plates were then incubated at 35 °C for 18-24 h. To enable detection of *Salmonella* below the detection limit of the plate count method, samples were incubated at 35 °C for 18-24 h after addition of rifampicin. Samples were then streaked onto rif-TSA plates, incubated at 35 °C for 18-24 h to detect the presence of *Salmonella.*

Confirmation of the Isolates

For each day of analysis, 2-3 typical colonies of rif-resistant *Salmonella* were randomly chosen and streaked onto TSA slants, and incubated at 35 ºC for 18-24 h. *Salmonella* was then confirmed by biochemical tests on triple sugar iron agar (TSIA, Difco) and lysine iron agar (LIA, Difco) slants. Serological tests were not conducted since a cocktail of strains was used to evaluate the overall behavior of *Salmonella* to decontamination treatments.

Sensory Evaluation

The pH of mango rind and flesh was measured at three different regions (scar, cheeks and stem end) using a Markson Model 612 portable pH meter (Markson Science Inc., Phoenix, AZ). The measurement was conducted in triplicate. Color was measured using an Ultrascan XE Hunter Lab Colorimeter. Measurement was taken at four different areas (scar, bottom end, cheeks) and an average value for L, a*, and b* was recorded. The L, a*, b* values were recorded using illuminant 'C' as the standard with 2 ° observer angle and 2 mm slit width.

Texture of the fruit was measured using a texturometer (Stable Micro Systems; Texture Technologies Corp-NY). The instrument was calibrated prior to use on each day of analysis. Fruit was placed over a metal plate and punctured equatorially through the wall using a puncture probe with a diameter of 3 mm, and at a crosshead speed of 10 mm/min to a penetration depth of 10 mm. The parameters recorded were Area (Newton meter), Force (Newton), and Mean force (Newton) (Fig 3).

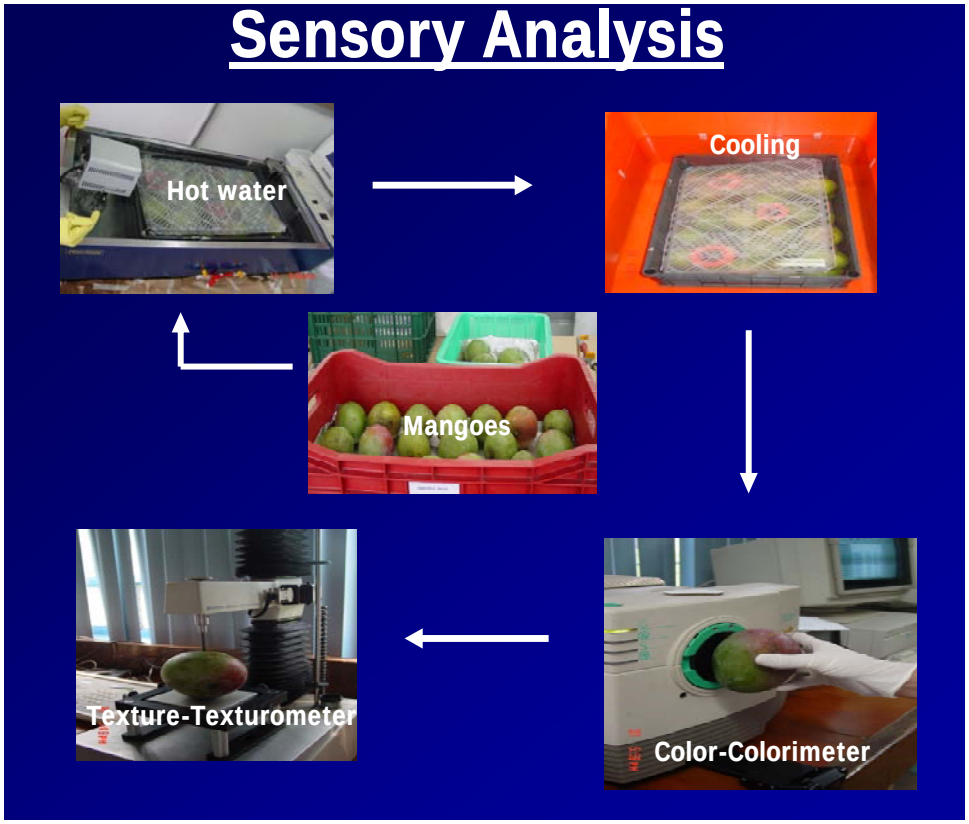

Figure 3 - Color and Texture Analysis

Statistical Analysis

All experiments were conducted in triplicate. Microbial counts were transformed logarithmically before statistical analysis and when the counts were lower than the detection limit (scar - 100 CFU/10 cm^2, rind – 3 CFU/10 cm^2, water – 1 CFU/ml, internalization - 17 CFU/g) a number half-way between 0 and the detection limit was used to facilitate the analysis only; however, data in the tables is reported as less than the detection limit. Mean populations obtained were compared by analysis of variance (ANOVA) using general linear model (GLM) in Statistical Package for Social Sciences (SPSS 12.0.1 for windows). Duncan's multiple range tests were used to identify significant differences in mean populations. Treatments where significant interaction between the factors (treatment and time) were identified; a further analysis was done by comparing the differences within a given treatment over various sampling times using least square means in Statistical Analysis Software (SAS Institute, Cary, NC). All the values are presented at a level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Reduction of Bacteria on the Scar of Mangoes: Hydrothermal Treatment

The population of *Salmonella* in the inoculum used for the study was approximately 7-8 $\log_{10}$ CFU/ml. The average populations recovered from the scar and rind portions of the mangoes after inoculation and before applying sanitizing treatments were approximately 5.9 and 4.4 $\log_{10}$ CFU/10 cm^2 respectively. The counts obtained on the scar and rind samples were significantly different. This was expected and the same was observed in a study where tomatoes were inoculated with *S*. Montevideo and *E. coli* O157:H7. The highest numbers of bacteria were recovered from the stem scar, blossom scar and surface scars (Lukasik and others 2001). Data on the populations recovered from the scar are presented in table 2. The control shows the populations of bacteria recovered from the scar of the inoculated mangoes at different time intervals during hydrothermal treatment.

A hydrothermal treatment (control) alone without the sanitizers reduced the populations of *Salmonella* in the scar area by approximately 3 log cycles. Although results obtained were not consistent, in general, on average it was observed that additional reductions of approximately 2.2 and 1.3 log cycles were obtained by further sanitization with lactic acid and chlorine respectively. It has been suggested that the efficacy of the treatments on reduction/inactivation of *Salmonella* is dependent on the physiological state and location of organism and the time of treatment after inoculation (Ukuku and Sapers 2001). Treatment with lactic acid showed significant reduction of *Salmonella* on scar of the mango when compared to chlorine treatment.

Table 2 - Comparison of Effects of Sanitizing Treatments on the Populations of *Salmonella* during Hydrothermal Treatment on the Scar of Mangoes

	Time (min)				
Treatment	**0**	**30**	**60**	**90**	**120**
Control	6.0±0.2AX[ab]	3.8±0.6ABY	4.9±1.4AXY	3.7±1.1ABY	3.0±1.1BCY
LA-30-Water	3.3±1.6CXY	2.1±0.5BY	2.1±0.8BCY	3.4±0.4ABX	< 2.0±0.0[c]CY
Cl-30-Water	5.7±0.3ABX	4.1±0.5AY	3.8±0.1ABYZ	2.6±1.0BZ	3.1±0.8BYZ
LA-LA	4.8±0.7ABX	2.2±0.8BY	2.4±1.2BCY	2.1±0.6BY	< 2.0±0.2CY
Cl-Water	4.5±0.8BXY	2.6±1.0BY	3.2±1.1BY	4.3±0.6AXY	4.6±1.2AX
LA-Water	3.9±0.4BCX	< 2.0±0.0BY	< 2.0±0.0CY	2.3±0.7BY	2.0±0.4BCY
Water-Cl	5.1±0.4ABX	4.3±0.5AXY	3.8±0.8ABY	3.3±0.5ABY	4.0±0.6ABXY
Water-LA	5.1±0.4ABX	3.3±0.8ABY	3.1±0.3BY	3.6±0.5ABY	3.1±1.3BY
Cl-Cl	4.2±1.0BCX	2.7±0.8BY	3.1±0.1BXY	2.3±0.5BY	2.7±0.3BCY

[a] The microbial counts expressed are mean values of three replicates ± standard deviation in $\log_{10}$ CFU/10 cm^2

[b] Means within rows followed by same letter (X, Y, Z) are not significantly different ($P > 0.05$);
means within columns followed by same letter (A, B, C) are not significantly different ($P > 0.05$)

[c] Mean population below detectable limit (2.0 $\log_{10}$ CFU/10 cm^2)

Statistical analysis of the data indicated significant interaction between the factors, time and treatment. Results obtained indicate that hydrothermal treatments involving lactic acid (LA-30-Water, LA-Water, LA-LA) reduced the pathogen levels below or close to the detection limit (< 2.0 $\log_{10}$ CFU/10 cm^2) at 120 min. Also, the reduction was found to be considerably high (1.8 log cycles) immediately after 0 min. This is perhaps due to the lethal effect of lactic acid on the pathogen population during the holding time (time allowed to drain and sampling of the fruit; approximately 20 min). None of the treatments with chlorine (Cl-30-Water, Cl-Water, Cl-Cl) reduced the pathogen levels below the detection limit. In general, treatments where sanitizers were used had significant reductions in microbial populations between 0 and 30 min ($P < 0.05$). Although further reductions were achieved with increasing time interval, statistical analysis did not reveal any beyond 30 min ($P > 0.05$).

The results obtained are not unusual. Previous studies indicate that the treatment of produce with chlorine at concentrations < 200 mg/L may not be effective. Previous studies indicate that the effectiveness of chlorine as a disinfectant mainly depends on the produce surface (Han and others 2000), on inoculation method (Buchanan and others 1999). The inefficiency of chlorine as a sanitizer has already been explained. It has been suggested that hypochlorous acid enters the microorganism and reacts with the –SH group of proteins ultimately causing death of the microorganism (Leyer GJ and Johnson EA 1997**)**. Also, it is hypothesized that the nutrients that leach from the cut tissues of the produce reduces the amount of free chlorine that is actually available thereby reducing its efficiency (Parish and others 2003).

The experiments were conducted at room temperature in the presence of light and air thus rendering chlorine ineffective as a sanitizer when compared with lactic acid. Also, the concentration of chlorine decreased with increasing time interval. At 0 min, the concentration of chlorine in wash water was 200 ppm. The concentration decreased to 42 ppm by the end of 120 min. The factors listed above (pH and decrease in concentration) explain the higher populations of *Salmonella* recovered for treatments with chlorine. In general, it was also observed that chlorine was effective in reducing populations of *Salmonella* at 90 min when compared with lactic acid, which was effective at 120 min. It was also noticed that populations of *Salmonella* were higher by the end of 120 min for one treatment (Cl-Water) when compared at 0 min. Probable explanations for high populations in addition to the factors listed above include-higher initial population load of the sampled mangoes and/or contamination from water and/or transfer of bacterial cells between the samples (mangoes in water were in contact with each other). Studies also indicate that the removal of native microflora during washing and sanitizing allow pathogens to thrive on the surfaces of the fresh produce (Brackett 1992).

Reduction of Bacteria on the Scar of Mangoes: Cooling Treatment

On average, the population of *Salmonella* at the end of hydrothermal treatment was low for treatments involving lactic acid (approximately 1.8 $\log_{10}$ CFU/10 cm^2 which is less than the detectable limit (2.0 $\log_{10}$ CFU/10 cm^2)) when compared to treatments involving chlorine (approximately 3.5 $\log_{10}$ CFU/10 cm^2). Data is presented in table 3.

Table 3 - Comparison of Effects of Sanitizing Treatments on Population of *Salmonella* on Scar of Mangoes during Cooling Treatment

Treatment	Mean Log_{10} CFU/10 cm^2
Control	3.3 A[ab]
LA-30-Water	< 2.0[c] D
Cl-30-Water	2.8 AB
LA-LA	2.1 CD
Cl-water	3.3 A
LA-water	< 2.0 D
Water-Cl	3.1 AB
Water-LA	2.5 BC
Cl-Cl	2.5 BC

[a] The microbial counts expressed are mean values in log_{10} CFU/10 cm
[b] Means within column followed by same letter (A, B,C, D) are not significantly different ($P > 0.05$)
[c] Mean population below detectable limit (2.0 log_{10} CFU/10 cm^2)

Although a reduction in microbial population is evident over the time interval, statistical analysis did not reveal any ($P > 0.05$). Hence, only significant overall differences between the treatments were evaluated.

Control had a population of approximately 3 logs at the end of 120 min. Data indicates reduction in pathogen population in cooling for all the treatments except two (Control – increase of 0.3 logs, LA-LA – increase of 0.3 logs) when compared to population load at the end of hydrothermal treatment. The increase may be due to initial higher inoculum load on the tested samples (high variability in the acquired inoculum among individual test units), and/or contamination from the treatment water and/or inaccessibility and/or inefficiency of the sanitizer in effectively reducing the pathogen population. Only two treatments (LA-30-Water and LA-Water) reduced pathogen population below detectable limit. Treatments with chlorine did not cause appreciable reduction in the pathogen population when compared to control as expected. The results obtained also indicate that sanitizers were effective in reducing the pathogen population significantly during the cooling treatment (Water-LA, LA-LA, Water-Cl, and Cl-Cl) and lactic acid proved to be slightly more effective compared to chlorine. Overall, lactic acid and chlorine reduced the pathogen population by approximately 1 and 0.5 logs respectively when compared to control.

The reductions of the populations due to lactic acid may be attributed to the decrease in pH (2.0) of the mango to a level where *Salmonella* cannot survive. Recovery of sub-lethally injured cells is also affected by the presence of lactic acid that is transferred from the surface of the fruit to the diluent (Materon 2003). The lethality of

organic acids is thought to be primarily due to lowering of pH, membrane rupture, or lowering of cellular pH (Parish and others 2003). The results obtained reconfirm the fact that lactic acid at a concentration of 2% is superior to chlorinated water at a concentration of 200 mg/L in reducing populations of *Salmonella*.

An analysis of the data indicates that the control hydrothermal treatment had a higher reduction of the pathogen population (reduction by approximately 3 logs) on the stem scar when compared to the rind (reduction by approximately 0.5 logs). This is thought to be due to the lose attachment of the bacterial cells to the scar and also the mechanical action of the pump in the water bath might have caused the removal of the bacterial cells that are loosely attached making the cells more susceptible to heat when compared to the rind where the attachment of the cells is thought to be firmer. It is also suggested that the cuticle around the stem scar is occasionally ruptured or wounded at the time of harvesting making it more porous. As a result the bacterial cells that are attached to the scar area are more susceptible to hydrothermal treatment when compared to the rind where the cuticle is unbroken and hydrophobic. Consequently, the cells are protected against the harsh environments thereby causing a lower reduction of the pathogen population on the rind when compared to the scar. It is also suggested that certain strains of the pathogen might have been more susceptible to heat. The differential behavior of the strains with regards to the preferential attachment (scar or the rind) on the fruit, biofilm formation and resistance/susceptibility to heat are suggested to be other factors that might have influenced the removal of the bacterial cells from the surface of the fruit.

Reduction of Bacteria on the Rind of Mangoes: Hydrothermal Treatment

Data on the populations recovered from the rind for all the treatments are presented in table 4. On average, the initial population of *Salmonella* on the rind was approximately 4.4 $\log_{10}$ CFU/10 cm^2. A 0.5 log reduction was obtained for the control and additional reductions of approximately 1.7 and 1.3 log cycles were obtained for treatments with lactic acid and chlorine respectively. Statistical analysis revealed a significant interaction ($P < 0.05$) between the factors treatment and time. In general, it was observed that chlorine was effective in reducing populations of *Salmonella* at 90 min whereas for lactic acid the results were mixed (LA-LA and LA-Water were effective at 120 min; LA-30-Water and Water-LA were effective at 90 min). One treatment (LA-Water) reduced microbial populations to below detectable limit (< 0.5 $\log_{10}$ CFU/10 cm^2) at the end of 120 min whereas treatment with LA with 30-min lapse reduced the pathogen population to below detectable limit at the end of 90 min. Overall, treatments where sanitizers were used had significant reductions in microbial populations between 0 and 30 min ($P < 0.05$). Although, further reductions were achieved with increasing time interval, statistical analysis did not reveal any beyond 30 min ($P > 0.05$). Previous research reported that the attachment of pathogens in lettuce and apples occurs in cracks or cuticles and in broken trichomes, stomata and lenticels (Seo and Frank 1999; Takeuchi and Frank 2000). It is also hypothesized that microbial attachment to pores, lenticels and other structures on the surface is influenced to a large extent by the hydrophobic interactions between the waxy cuticle and the cells of microorganisms (Frank 2000).

Table 4 - Comparison of Effects of Sanitizing Treatments on the Populations of *Salmonella* during Hydrothermal Treatment on the Rind of Mangoes

	Time (min)				
Treatment	**0**	**30**	**60**	**90**	**120**
Control	3.4±0.2AX[ab]	3.0±0.2AX	3.0±0.3AX	2.4±0.7AX	2.9±0.4AX
LA-30-Water	2.8±0.1AX	1.8±0.5BXY	2.4±2.4ABX	< 0.5±0.0[c]BY	1.2±0.7BCX
Cl-30-Water	3.3±0.8AX	1.5±0.2BY	2.4±0.5ABXY	1.5±0.8ABY	1.4±0.3BCY
LA-LA	2.7±0.3AX	1.0±0.8BY	1.4±1.1BY	1.8±0.5ABXY	1.1±0.8BCY
Cl-Water	2.8±0.9AX	1.7±0.5BY	1.6±0.5BY	0.9±0.3BY	1.7±0.9BY
LA-Water	2.6±0.5AX	1.5±0.3BY	0.7±0.7BZ	1.4±0.5ABYZ	< 0.5±0.4CZ
Water-Cl	3.0±0.4AXY	3.1±0.9AX	2.4±0.3ABXY	2.0±0.3ABY	1.8±0.3ABY
Water-LA	2.6±0.3AX	1.9±0.2BXY	2.3±0.4ABXY	1.2±0.7BY	2.7±0.6ABX
Cl-Cl	3.2±1.2AX	1.3±0.7BY	0.8±0.5BY	0.5±0.3BY	0.9±1.0BCY

[a] The microbial counts expressed are mean values of three replicates ± standard deviation in $\log_{10}$ CFU/10 cm^2

[b] Means within rows followed by same letter (X, Y, Z) are not significantly different ($P > 0.05$);
means within columns followed by same letter (A, B,C) are not significantly different ($P > 0.05$).

[c] Mean population below detectable limit (0.5 $\log_{10}$ CFU/10 cm^2)

Other studies implicated that the populations of the cells in the inoculum influences the level of attachment of microorganisms (Liao and Sapers 2000). Attempts were made to understand the mechanisms involved in the attachment of bacterial cells to the surface of fruits and vegetables. Some of these are discussed below. The attachment of bacterial cells onto the surface of fruits is suggested to be influenced by several factors, which include temperature, duration of contact time, motility, surface charge, hydrophobicity of bacterial cells, presence of surface structures such as flagella and fimbriae, outermembrane proteins and production of extracellular polysaccharides (Hood and Zottola 1997; Vandevivere and Kirchman 1993; Ukuku and Fett 2002). An interesting phenomenon was suggested by Iturriaga and others (2003). The attachment of bacterial cells is supposed to proceed in two steps, the first being a "reversible phase" (Marshall and others 1971) which involves Van der Waal forces whereby the cells get close to the surface followed by an "irreversible attachment" (Marshall and others 1971) whereby the cells are bound to the surface by a combination of "physical and chemical forces". Also, it was suggested that attachment was more rapid and strong on surfaces that are course textured and hydrophobic. Perhaps, the smooth texture of mango fruit did not favor the attachment of the bacterial cells, which explains lower populations on the rind. "Two mechanisms of adhesion of cells to the fruit surfaces are suggested. The adhesion might be nonspecific and determined entirely by general physicochemical properties of the cell and the produce surface, or retention of cells is favored by physical entrapment of cells in depressions and discontinuities in the cuticle" (Iturriaga and others 2003).

It is suggested that the waxy cuticle present on the surface of fruits and vegetables acts as a physical barrier preventing the entry of foodborne pathogens (Burnett and Beuchat 2001; Kenney and Beuchat 2002). This function is suggested to be due to the chemical nature of cuticle, a highly polymerized hydrophobic material consisting of fatty acids (Richards and Beuchat 2004).

Numerous workers have shown that bacterial cells in the stationary phase are more tolerant to stresses such as heat, pH and osmolarity than logarithmic phase cells (Foster and Spector 1995; Fang and others 1996; Turner and others 2000). This difference in the behavior of cells in different phases is attributed to presence of sigma factor, σ^S that is encoded by *rpoS* and is supposed to be active in the stationary phase of many bacteria. These factors direct RNA polymerase to transcribe genes that encode proteins responsible for protecting cells from a variety of environmental stresses (Loewen and Hengge-Aronis 1994; Chen and others 1996).

Another mechanism explaining the resistance of bacteria to extreme stresses has been suggested by Taylor-Robinson and others (2003). Data obtained from a study with *S*. Typhimurium indicated that the behavior of the cells in the stationary phase was cyclic, whereby at certain times they exhibited the properties/features of logarithmic phase, where the cells were susceptible to heat alternated by a phase where the cells were tolerant to heat stress. A cell-cell interaction between the old and young cells resulting in a change in the phenotype of young cells has also been suggested in the same study. Increased mutations in the stationary phase rendering the bacteria resistant to stresses was reported by Foster (2000)

Reduction of Bacteria on the Rind of Mangoes: Cooling Treatment

The microbial load on the rind after hydrothermal treatment was approximately 2.9 $\log_{10}$ CFU/10 cm^2 for control. Treatments with sanitizers during hydrothermal treatment overall had lower populations of *Salmonella* compared to control (LA – approximately 1.4 logs and chlorine-approximately 1.5 logs) at the end of 120 min.

Statistical analysis did not show any significant correlation between treatment and time and also, only significant differences were observed between the treatments. So, an average overall effect of treatments is presented in table 5. An analysis of the data shows that all the treatment combinations with sanitizers had significant lower populations of *Salmonella* compared to control. In general, on average, it was observed that treatments with lactic acid and chlorine gave an overall reduction of approximately 1.3 and 1.4 logs respectively compared to control. Although it appears that chlorine was more effective compared to lactic acid, an accurate conclusion is that the effectiveness of chlorine and lactic acid were comparable. None of the treatment combinations reduced the pathogen population below detection limit (0.5 $\log_{10}$ CFU/10 cm^2). However, two treatments (LA-Water and Cl-Cl) reduced pathogen populations close to detection limit. Also, it was observed that lactic acid in cool bath was not effective in reducing pathogen population when compared to lactic acid in hydrothermal treatment. This is consistent with previous work where reduced antimicrobial activity of lactic acid at low temperatures (5 °C) has been reported (Ibarra-Sánchez and others 2004).

Numerous studies have documented the inefficiency of a variety of sanitizers to completely eliminate and/or inactivate *Salmonella* inoculated on produce (Sapers and

Sites 2003; Annous and others 2004, 2005). It has been suggested that increased contact time of bacteria to the surface of fruit favors formation of protective biofilms by the microorganisms (Ukuku and Sapers 2001). This explains the recovery of higher populations of *Salmonella* on the surface of mangoes with increasing time. In addition to protecting the bacteria from sanitizers, biofilms are also thought to provide an environment (embedding of bacterial cells in extracellular polysaccharides) whereby the ability of bacteria to survive unfavorable conditions are enhanced. (Fett and Cooke 2003; Annous and others 2004). Further investigation on the composition of extracellular matrix was conducted by Solano and others (2002). It has been suggested that the matrix is made up of thin aggregate fimbriae and cellulose and it is also thought that cellulose enhances resistance of *Salmonella* to chlorine.

Although more extensive bactericidal activity was observed when lactic acid was used, the sensory quality of mangoes was compromised. Severe browning of the skin was observed during the treatment. Similar observations were made in a study involving lettuce leaves (Lin C-M and others 2002). Based on previous studies it is suggested that the combined effect of heat with lactic acid enhanced the enzymatic activities of the browning enzymes (Phenylalanine ammonia-lyase) released from the injured cells in mangoes. The enzyme further initiates several phenolic compounds that are oxidized by polyphenol oxidase resulting in formation of brown-colored compounds causing discoloration (Loaiza-Velarde and Saltveit 2001; Ke and Saltveit 1989).

Table 5 - Comparison of Effects of Sanitizing Treatments on the Populations of *Salmonella* during Cooling Treatment on the Rind of Mangoes

Treatment	Mean Log_{10} CFU/10 cm^2
Control	2.7 A[ab]
LA-30-water	1.6 BC
Cl-30-water	1.2 CDE
LA-LA	1.1 DEF
Cl-water	1.1 DEF
LA-water	0.8 EF
Water-Cl	1.9 B
Water-LA	1.4 BCD
Cl-Cl	0.7 F

[a] The microbial counts expressed are mean values in log_{10} CFU/10 cm^2

[b] Means within column followed by same letter (A, B, C, D, E, F) are not significantly different ($P > 0.05$)

Reduction/Survival of Bacteria in the Core Stem Tissue of the Mangoes

Salmonella and *E. coli* O157:H7 were recovered from the core tissue of tomatoes after treatments with chlorine and lactic acid (Ibarra-Sánchez and others 2004). It has also been suggested that water containing inoculum gains entry into the fruit through the stem scar and promotes growth/protection of the pathogen/microorganism in the tissue (Buchanan and others 1999; Seo and Frank 1999). Ukuku and others (2004) demonstrated that the population load on the stem scar affects the infiltration of the pathogen into the stem tissue.

Table 6 shows the efficacy of sanitizing treatments in reducing populations of *Salmonella* in the stem scar tissue. Seven treatments including control had populations below or close to below detection limit (1.2 $\log_{10}$ CFU/g). Two treatments (Cl-30-Water and Cl-Water) produced counts significantly different from the other treatments ($P < 0.05$). The observed high populations of *Salmonella* for these treatments may be due to higher inoculum in the stem scar and/or presence of abrasions/cuts in the stem area that might have aided in the penetration of the pathogen during inoculation/treatment. Cells could have penetrated through the cut edges and might have been unaffected by the sanitizers. Statistical analysis did not reveal any significant effect of time in the internalization of pathogen; hence, an overall average effect of each treatment was taken into consideration in determining the efficacy of the sanitizers.

The pathogen was detected in the stem core tissue for all the treatments after the enrichment method for a time period of 12 days (table 7). The presence is thought to be due to recovery of sublethally injured cells and no growth is suspected during the

refrigerated storage. A high rate of pathogen internalization was observed for immature (80%) and ripened (87%) mangoes at the stem end (Penteado and others 2004). The present study is consistent with other work showing that warm fruit submerged in cool water permits pathogens to internalize (Zhuang and others1995).

Based on the investigation of the salmonellosis outbreak involving mangoes it was suggested that the cool dip after the hydrothermal treatment was causing the infiltration of the pathogen into the tissue of the stem area due to temperature differences between the fruit and the wash water. To overcome this problem, it was suggested by USDA/APHIS to have a time lapse of at least 30 min between the hot and cool dips. Two treatments (LA-30-Water and Cl-30-Water) were conducted to determine the effect of time lapse (30 min) between the hydrothermal and cooling treatment in the internalization of the pathogen. Although the presence of *Salmonella* was not evident by direct plating method for most of the samples, the pathogen was detected after the enrichment method indicating that the time lapse did not significantly reduce the temperature differential that existed between the wash water and the produce. It was observed that the temperature of the fruit at the end of 30 min lapse was significantly high (approximately 40 °C) than the wash-water temperature (approximately 25 ºC). The negative temperature differential might have caused the infiltration of the pathogen into the core stem tissue during cooling treatment.

Table 6 - Comparison of Effects of Sanitizing Treatments on the Internalization of *Salmonella* after Cooling Treatment in the Stem Tissue of Mangoes

Treatment	Mean Log_{10} CFU/g
Control	<1.2B[abc]
LA-30-water	<1.2 B
Cl-30-water	2.0 A
LA-LA	<1.2 B
Cl-water	1.6 A
LA-water	1.2 B
Water-Cl	<1.2 B
Water-LA	<1.2 B
Cl-Cl	<1.2 B

[a]The microbial counts expressed are mean values in log_{10} CFU/g
[b] Means within column followed by same letter (A, B) are not significantly different ($P > 0.05$)
[c] Mean population below detectable limit (1.2 log_{10} CFU/g)

Table 7 - Presence of *Salmonella* in the Core Stem Tissue after Enrichment Method

	Time (min)				
Treatment	**0**	**5**	**10**	**15**	**20**
Control	1.5[ab] (+)	1.7 (+)	1.8 (+)	1.5 (+)	1.8 (+)
LA-30-Water	<0.0 [c](-)	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)
Cl-30-Water	<0.0 (-)	<0.0(-)	<0.0 (+)	<0.0 (+)	<0.0 (+)
Water-LA	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)
LA-LA	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)
Water-Cl	<0.0 (-)	<0.0 (-)	<0.0(-)	<0.0 (-)	<0.0(-)
LA-Water	<0.0 (-)	<0.0 (+)	<0.0 (+)	<0.0 (-)	<0.0(-)
Cl-Water	<0.0 (-)	<0.0 (-)	<0.0(-)	<0.0 (-)	<0.0 (+)
Cl-Cl	<0.0 (-)	0.0 (+)	<0.0 (-)	<0.0 (-)	<0.0 (-)

[a] No. of *Salmonella*-positive samples/total no. of samples tested after enrichment

Reduction of Bacteria on the Scar and Rind of the Mangoes during Storage

In general, cold storage for 12 d reduced the populations of *Salmonella* compared to day 0, although there were differences in levels of reductions between the treatments. The effect of various treatments on the populations of *Salmonella* on the scar and rind are presented in tables 8 and 9.

For stem scar, the storage time was found to be an insignificant factor statistically; hence, the average overall effect of treatments was taken into consideration for comparison. On average, the initial population on the scar after inoculation was approximately 5.9 $\log_{10}$ CFU/10 cm^2. A reduction in population of *Salmonella* by approximately 2.4 logs was obtained for control and further significant reductions of approximately 1.5 and 1 log cycles were obtained with lactic acid and chlorine respectively on day 0 after the cooling treatment. Although time was an insignificant factor, generally, it was observed that all the treatments except three (control, Cl-30-Water, Cl-Water) reduced populations to below (LA-LA) or close to detection limit (2.0 $\log_{10}$ CFU/10 cm^2) by day 12. Lactic acid again proved to be superior to chlorine in reducing pathogen population.

For the rind, on average, the initial population after inoculation was approximately 4.4 $\log_{10}$ CFU/10 cm^2. Statistically, no significant differences were evident between the treatments, however, time was found to be a significant factor, hence, overall reduction in populations of *Salmonella* is compared across the storage period (12 d). A reduction of approximately 0.8 logs was obtained by day 3 as compared to day 0. A further reduction of approximately 0.5-0.7 logs was obtained by day 12 when

compared to day 3. A reduction in population of *Salmonella* by approximately 1.5 logs was obtained by day 12 when compared to day 0. Overall, reduction in population was evident throughout the storage period for samples treated with sanitizers when compared to control. Although treatment was found to be an insignificant factor, in general, it was observed that all the treatments reduced populations of *Salmonella* below detection limit (0.5 $\log_{10}$ CFU/10 cm^2 including control (data not shown) by day 12.

The survival of *Salmonella* over the storage period is of microbiological significance. As a possible explanation, sublethal injury should be considered while examining the results of microbial populations of treated samples over the storage period. Although, the sanitizing agents inactivated most of the inoculated bacteria, the surviving bacteria may have been deeply attached to the surface of the mangoes and perhaps few sublethally injured cells might have resuscitated during storage. All the samples in the study were subjected to a storage temperature of 10 ºC, and due to the mesophilic nature of *Salmonella,* it might have been difficult for the cells to multiply. It is suspected that unfavorable conditions; a temperature of 10 ºC, a low pH of approximately 4.8, might have contributed to the failure of recovery of injured cells and/or multiplication of the bacterial cells in the storage. The results obtained are in confirmation with previous research. Survival of *Salmonella* and *E. coli* O157:H7 was reported in low pH conditions (Parish 1998; Buchanan and others 1999).

Table 8 - Comparison of Effects of Sanitizing Treatments on Survival of *Salmonella* on the Scar of Mangoes during Storage

Treatment	Mean Log_{10} CFU/10 cm^2
Control	3.5 A[ab]
LA-30-water	2.0 C
Cl-30-water	2.8 B
LA-LA	< 2.0 [c]C
Cl-water	2.5 BC
LA-water	2.2 C
Water-Cl	2.0 C
Water-LA	2.2 BC
Cl-Cl	2.1 C

[a] The microbial counts expressed are mean values in log_{10} CFU/10 cm^2

[b] Means within column followed by same letter (A, B, C) are not significantly different ($P > 0.05$)

[c] Mean population below detectable limit (2.0 log_{10} CFU/10 cm^2)

Table 9 - Effect of Storage Time on the Survival of *Salmonella* on the Rind of Mangoes

Time (days)	Mean Log_{10} CFU/10 cm^2
0	2.0 A[ab]
3	1.2 B
6	0.7 C
9	0.5 C
12	0.5 C

[a] The microbial counts expressed are mean values in log_{10} CFU/10 cm^2

[b] Means within column followed by same letter (A, B, C) are not significantly different ($P > 0.05$)

Reduction/Presence of *Salmonella* in the Treatment Water: Hydrothermal and Cooling

Salmonella was detected in treatment water with and without sanitizers after dipping mangoes, which indicates that some of the cells were removed from mangoes during hydrothermal and cooling treatments. Data on the presence of *Salmonella* in the treatment water (hydrothermal and cooling) is presented in tables 10 and 11 respectively.

The bacterial load in water during hydrothermal treatment without sanitizers was approximately 3.7 logs at 0 min and decreased by approximately 1.2 logs at the end of 30 min. Further decrease (approximately 0.2-0.3 logs) with increasing time was evident. During cooling treatment the population was approximately constant (1.6 logs). The decrease in population is suggested to be due to inactivation of the cells by heat and/or attachment of cells to mangoes. The pathogen levels were below detectable limit (1 CFU/ml) with sanitizers both in the hydrothermal and cooling treatments. Although *Salmonella* was not detected in most of the samples after incubation at 35 ºC (positive for three samples in hydrothermal and seven in cooling), it cannot be assumed that the pathogen was completely eliminated in the treatment water. Several factors (sampling of treatment water, failure of recovery of sublethally injured cells in the selective medium, attachment of bacterial cells to the mangoes) could have attributed to the absence of pathogen in the treatment water. Results obtained also agree with the previous findings in which sanitizing agents were found to be more effective against microorganisms present in water than those attached to the produce surfaces (Sapers 2003).

Table 10 - Survival/Presence of *Salmonella* in Water during Hydrothermal Treatment

	Time (min)				
Treatment	**0**	**30**	**60**	**90**	**120**
Control	3.7 (+)[ab]	2.5 (+)	2.2 (+)	2.4 (+)	2.4 (+)
LA-30-Water	<0.0[c] (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)
Cl-30-Water	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)
Water-LA	2.7 (+)	1.7 (+)	1.6 (+)	1.5 (+)	1.4 (+)
LA-LA	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (+)	0.0 (+)
Water-Cl	2.4 (+)	2.1 (+)	2.0 (+)	2.4 (+)	2.1 (+)
LA-Water	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)
Cl-Water	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)
Cl-Cl	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (+)	<0.0 (-)

[a] Microbial counts expressed as $\log_{10}$ CFU/ml
[b] *Salmonella* positive/negative sample after enrichment
[c] Population below detectable limit (1 CFU/ml)

Table 11 - Survival/Presence of *Salmonella* in Water during Cooling Treatment

Treatment	Time (min)				
	0	**5**	**10**	**15**	**20**
Control	1.5[ab] (+)	1.7 (+)	1.8 (+)	1.5 (+)	1.8 (+)
LA-30-Water	<0.0 [c](-)	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)
Cl-30-Water	<0.0 (-)	<0.0(-)	<0.0 (+)	<0.0 (+)	<0.0 (+)
Water-LA	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)
LA-LA	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)	<0.0 (-)
Water-Cl	<0.0 (-)	<0.0 (-)	<0.0(-)	<0.0 (-)	<0.0(-)
LA-Water	<0.0 (-)	<0.0 (+)	<0.0 (+)	<0.0 (-)	<0.0(-)
Cl-Water	<0.0 (-)	<0.0 (-)	<0.0(-)	<0.0 (-)	<0.0 (+)
Cl-Cl	<0.0 (-)	0.0 (+)	<0.0 (-)	<0.0 (-)	<0.0 (-)

[a] Microbial counts expressed as $\log_{10}$ CFU/ml
[b] *Salmonella* positive/negative sample after enrichment
[c] Population below detectable limit (1 CFU/ml)

Effect of Sanitizing Treatments on the pH (Rind and Pulp) of Mangoes

The pH data for the rind and pulp for the various treatment combinations are presented in tables 12 and 13. An initial pH value for the rind of untreated mango was approximately 5.1. The pH values of rind for controls (untreated mangoes) and samples treated with water remained close to initial values during the treatment process, whereas pH values of samples treated with lactic acid in cool bath (LA-LA and Water-LA) were the lowest (approximately 2.9) on day 0 ($P < 0.05$). This is expected. However, treatments involving lactic acid only in the hydrothermal treatment (LA-30-Water and LA-Water) did not significantly lower the pH (approximately 5.4) of the samples. The subsequent dipping of mangoes in cool bath probably negated the lactic acid effect. The pH value of samples treated with chlorine was approximately 4.8 except for two treatments (Cl-Water and Water-Cl) where the pH was significantly high (6.4, $P < 0.05$). There was a gradual increase in pH (approximately 0.3 units) of the samples for all the treatment combinations exception being Cl-Water and Water-Cl (decrease by approximately 1.3 units) during 12 days of storage.

The pH of pulp was low, average of 4.7 as compared to rind on day 0. A slight but significant reduction in pH of samples (approximately 0.3 units, $P < 0.05$) on day 12 compared to day 0 was observed during the storage period. No appreciable change in pH was observed between day 3 and day 12 for all treatment combinations. The results obtained are consistent with the treatment and/or storage conditions. Treated mangoes were stored in a refrigerator maintained at a temperature of 10 ºC. The heat treatment combined with low storage temperature had a significant impact in inhibiting the

ripening of the mangoes. Mango chemical composition has been the subject of several studies. Many of these studies have shown that the changes in chemical composition during mango ripening are strongly dependent on cultivar, climate, stage of maturity at harvest, and conditions of postharvest storage. The studies of sugar and organic acid compositions (Selvaraj and others 1989; Castrillo and others 1992) have indicated that total sugars increase and acidity decreases as ripening proceeds. Although not investigated, it is suspected that there is no appreciable decrease in organic acid content (pH 4.7) of the pulp and perhaps there is no simultaneous increase in total soluble solids-hydrolysis of starch to sugars (characteristics that define ripening). It is also observed that acid content is high in the pulp as compared to the rind, which probably explains the high pH values of rind. Previous research also confirms the results obtained. Nyanjage (2001) reported that mango fruits immersed in hot water at 46.5 ºC for 90 min and stored at 13 ºC had either constant or declining levels of soluble solids. However, a low pH value for untreated mangoes is difficult to explain but may be attributed to variations in the stage of fruit ripeness. It is also hypothesized that cultivar, climate, level of maturity at the time of harvest and other postharvest changes influence the response of the fruit to the treatments and/or storage conditions.

Table 12 - Comparison of Effects of Sanitizing Treatments on pH value of the Rind of Mango Fruit

	Time (days)				
Treatment	**0**	**3**	**6**	**9**	**12**
Cl-Cl	4.7±0.15BY[ab]	4.6±0.41BY	4.8±0.3BY	5.2±0.17ABXY	5.5±0.46ABX
Cl-30-water	4.8±0.50BY	5.0±0.26ABY	5.0±0.29BY	5.4±0.20ABXY	5.8±0.45AX
Cl-water	6.5±0.51AX	5.5±0.32AY	5.3±1.17ABY	5.5±0.25ABY	5.2±0.36ABY
LA-LA	2.7±0.15CZ	4.0±0.15CY	4.0±0.17CY	5.1±0.31ABX	4.9±0.42BX
LA-30-water	4.8±0.17BXY	4.5±0.42BCY	5.0±0.25BXY	5.4±0.29ABX	5.0±0.31BXY
LA-water	6.0±0.85AX	4.8±0.35BY	5.2±0.15ABY	5.5±0.47ABXY	5.2±0.26ABY
Untreated	5.2±0.58BXY	4.6±0.21BY	4.9±0.29BY	5.1±0.58ABY	5.7±0.58AX
Water-Cl	6.4±0.67AX	4.7±0.23BZ	4.9±0.49BZ	5.7±0.21AY	5.0±0.21BZ
Water-LA	3.1±0.29CZ	4.3±0.29BCY	3.9±0.42CY	5.0±0.26BX	4.7±0.23BXY
Water-water	4.9±0.26BY	4.9±0.50ABY	5.8±0.55AX	5.3±0.21ABY	5.4±0.47ABXY

[a] Mean pH values ± standard deviation
[b] Means within rows followed by same letter (X, Y, Z) are not significantly different ($P > 0.05$);
means within columns followed by same letter (A, B, C) are not significantly different ($P > 0.05$)

Table 13 - Comparison of Effects of Sanitizing Treatments on pH value of the Pulp of Mango Fruit

	Time (days)				
Treatment	**0**	**3**	**6**	**9**	**12**
Cl-Cl	4.7±0.2BX[ab]	4.2±0.15ABY	4.5±0.23BCXY	4.4±0.15ABY	4.7±0.06AX
Cl-30-Water	4.6±0.1BYZ	4.3±0.21ABZ	5.0±0.29AX	4.7±0.12AY	4.5±0.12ABYZ
Cl-Water	4.9±0.3BX	4.4±0.12AY	4.3±0.30BCY	4.4±0.15ABY	4.3±0.1BY
LA-LA	4.1±0.17CY	4.4±0.06AXY	4.5±0.06BCX	4.3±0.1BXY	4.3±0.12BXY
LA-30-Water	4.7±0.34BX	4.5±0.06AX	4.6±0.32BX	4.4±0.1ABX	4.5±0.17ABX
LA-Water	4.5±0.17BX	4.1±0.17BY	4.3±0.31BCXY	4.4±0.06ABXY	4.2±0.12BXY
Untreated	4.8±0.1BX	4.3±0.0ABY	4.4±0.06BCY	4.5±0.46ABXY	4.5±0.17ABXY
Water-Cl	5.2±0.08AX	4.4±0.19AY	4.2±0.26CY	4.5±0.22ABY	4.3±0.28BY
Water-LA	4.5±0.2BX	4.5±0.06AX	4.3±0.17BCX	4.3±0.15BX	4.4±0.21ABX
Water-Water	5.0±0.1ABX	4.5±0.15AY	4.5±0.12BCY	4.6±0.15AY	4.7±0.1AXY

[a] Mean pH values ± standard deviation
[b] Means within rows followed by same letter (X, Y, Z) are not significantly different ($P > 0.05$);
means within columns followed by same letter (A, B, C) are not significantly different ($P > 0.05$)

Effect of Sanitizing Treatments on the Texture of Mango Fruit

Mangoes treated with lactic acid had adverse affects on the quality of the fruit (skin browning upon treatment and hardening of the skin during storage at 10 ºC). Unpeeled mango rather than the peeled fruit was subjected to textural analysis and hence, the data presented do not reflect the visual differences observed. Mangoes treated with lactic acid had significant adverse affects on the quality of the fruit (skin browning during treatment and hardening of the skin during storage at 10 ºC). However, treatments with chlorine when compared with control (untreated mango) did not show any significant differences ($P > 0.05$) in texture. Statistical analysis of the data revealed no interaction between the factors (treatment and storage time) hence, overall average effects of the factors are presented. Differences between the treatments and storage time are presented in tables 14 and 15.

A comparison between treatments showed that only one treatment (LA-Water) was found to be significantly different ($P < 0.05$) from other treatments. While analyzing the effect of storage time, it was observed that there was significant difference between day 0 and day 9 (perhaps, hardening of the skin during storage). Although not evident from the data, a visual observation of the fruit treated with lactic acid had intense browning on day 0. The intensity of the brown color decreased during the storage period. Also, the rind of the fruit treated with lactic acid hardened (evident from the difficulty in peeling) during the storage period. The fruit quality responses observed in this work (browning and hardening of the skin) is consistent with previous work. However, the quality response of the fruit in the current study is attributed to the combined effect of

both the chemical (lactic acid) and hot water. No significant adverse impact on the quality of the fruit was observed with treatments involving chlorine.

Although the exact mechanism involved in the browning and hardening of the rind in mangoes is unknown at this point in time it is thought that a mechanism similar to that explained in the browning of avocado fruit might be involved. It is suggested that browning is a result of damage caused to the chloroplast membranes causing leakage of browning enzymes (Polyphenol oxidase) from the skin cells. The released enzymes further react with the substrates released from the vacuole that are damaged by the heat and cause browning of the skin (Woolf and Laing 1996). Another study demonstrated that storage temperature and not the hot water treatment influences the production and changes in the phenolic compounds responsible for the ripening of the fruit (Talcott and others 2005). Significant inhibition of ethylene synthesis, carotenoid concentrations, and cell wall degradation resulting in delayed ripening has been reported by several studies (Lurie and Klein 1990).

Table 14 - Comparison of Effect of Sanitizing Treatments on the Texture of Mango Fruit

Treatment	Area (Nm)
LA-water	0.38A[ab]
Water-Cl	0.28B
Cl-water	0.28B
Cl-30-water	0.28B
Untreated	0.27B
Water-water	0.27B
LA-LA	0.26B
Cl-Cl	0.26B
LA-30-water	0.25B
Water-LA	0.22B

[a]The values expressed are mean values in Newton-Meter
[b] Means within column followed by same letter (A, B) are not significantly different ($P > 0.05$)

Table 15 - Comparison of Effect of Storage Time on the Texture of Mango Fruit

Time (days)	Area (Nm)
0	0.27ABC[ab]
3	0.29AB
6	0.3A
9	0.25BC
12	0.24C

[a] The values expressed are mean values in Newton-Meter
[b] Means within column followed by same letter (A, B, C) are not significantly different ($P > 0.05$)

Effect of Sanitizing Treatments on Color of Mango Fruit

The parameter color measures the degree of ripeness in fruit**.** Parameters L, a* and b* were measured to evaluate the color of mango fruit. The parameter 'L' is a measure of fruit brightness, whereas a*(hue) measures the actual color of fruit (a positive value indicates red whereas a negative value indicates green color) and b*(chroma) measures the vividness or saturation of color (a positive value indicates yellow and a negative value indicates blue). The parameter L had significant interaction between treatment and storage time. On an average brightness (L) of the fruit decreased by approximately 6-8 units during storage period for six treatment combinations including control. Four treatments involving lactic acid (LA-LA, LA-30-Water, LA-Water, Water-LA) had low values of L (darkening effect) when compared with other treatments on day 0 ($P < 0.05$). However, significant increase in L values (approximately 10 units) was observed for treatments with lactic acid during storage period. Also, it is noticed that treatments involving chlorine in hydrothermal treatment (Cl-Cl, Cl-30-Water, Cl-Water) had significant reductions in L values when compared with control between day 0 and day 12; however, no significant difference was evident between day 3, day 6 and day 12.

A statistical analysis of the hue values revealed that only treatment was a significant factor ($P < 0.05$) and hence only an overall average effect of treatment was taken into consideration for analysis. An analysis of the data shows that the treatments involving lactic acid except for one treatment (Water-LA) had positive values when compared with the treatments involving chlorine. The negative values for treatments

involving chlorine indicates that the color of the fruit was intense green and the values were significantly different ($P < 0.05$) when compared to the other treatments. The positive values for treatments involving lactic acid was due to intense browning (red) of the skin and within lactic acid treatments one treatment (LA-LA) was found to be significantly different when compared to the others. Control fruit (untreated) had a positive value, however, the positive value is towards the lower end of the scale, and hence, the color of the fruit is still considered to be green.

Chroma measures the saturation/vividness/intensity of color. Chroma indicates the intensity of the color and statistical analysis of the data shows that both treatment and time were significant factors affecting the chroma values of the fruit. However, there was no significant interaction between the factors, hence, only an overall average effect of treatment and time are presented in tables 18 and 19. A further analysis of the data shows that in general, treatments with lactic acid had significantly low values (less intense green) when compared to the treatments with chlorine and the intensity of the green color decreased significantly during the storage period of 12 days. Although not evident visually, the decrease in the intensity was by approximately 4 units on day 6 compared to day 0 and a further decrease by approximately 2 units was evident on day 12 when compared to day 6. An overall decrease by approximately 6 units was evident by day 12 when compared to day 0. Data obtained for L (table 16), hue (table 17) and chroma (tables 18 and 19) after statistical analysis are presented.

The results obtained are consistent with previous research. Civello and others (1997) reported that a/b values of strawberry fruits that were heat treated were

significantly low compared to control fruits. Another study demonstrated that strawberry fruit had low brightness value during postharvest (Collins and Perkins-Veazie 1993). Browning of the skin due to heat treatments has been reported by several other authors (Kerbel and others 1987; Klein and Lurie 1992; Woolf and Laing 1996). Change in color from green to yellow/red indicates ripeness of the fruit and this is reflected in several ways such as increased respiration, higher ethylene production, carotenoid synthesis, softening of the skin due to cell wall degradation, increasing soluble solids content and decreasing acidity (Lurie 1998; Paull 1990). Several studies demonstrated that hydrothermal treatments inhibit/retard enzymes such as polygalacturonase (Yoshida and others 1984) and α- and β-galactosidase (Sozzi and others 1996) that cause cell wall degradation, thus inhibiting the ripening process in fruit (Klein and Lurie 1991; Lurie 1998). The inhibition of ethylene synthesis is suggested to involve a complex mechanism of gene expression and protein synthesis. It is hypothesized that hydrothermal treatments cause an increased production of heat shock proteins with a simultaneous decrease in proteins that cause senescence in fruit (Picton and Grierson 1988; Lurie and others 1996).

Table 16 - Comparison of Effect of Sanitizing Treatments on L Value of the Mango Fruit

	Time (days)				
Treatment	**0**	**3**	**6**	**9**	**12**
Cl-Cl	46.1±2.6AX[ab]	38.7±2.30AY	42.4±0.96AXY	41.8±1.90AY	39.2±2.69AY
Cl-30-Water	48.3±2.42AX	41.0±2.45AY	41.3±1.76ABY	39.8±0.37BY	38.8±2.20AY
Water-Water	46.3±2.14AX	37.7±0.63AY	37.7±1.72BY	39.8±0.69ABY	38.6±0.69AY
LA-LA	29.0±2.9CY	37.8±1.45AX	38.9±1.71ABX	37.2±1.31BX	39.4±1.68AX
LA-30-Water	30.8±2.69CY	38.2±1.11AX	39.3±0.60ABX	38.0±1.21ABX	39.1±1.82AX
LA-water	29.5±6.71CY	37.5±1.66AX	39.5±1.72ABX	38.3±0.93ABX	39.5±1.42AX
Untreated	48.1±4.82AX	37.3±2.50AY	39.0±4.63ABY	37.7±1.15BY	37.6±2.36AX
Water-Cl	39.4±1.60BX	37.6±3.62AX	38.5±0.96ABX	38.2±1.53ABX	37.9±5.38AX
Water-LA	28.5±1.19CY	37.5±1.87AX	39.5±0.28ABX	38.3±1.96ABX	39.5±2.03AX
Cl-water	41.0±4.82BX	39.3±2.50AX	40.9±4.63ABX	37.2±1.15ABX	38.7±2.36AX

[a] Mean L values ± standard deviation of three replicates
[b] Means within rows followed by same letter (X, Y) are not significantly different ($P > 0.05$);
means within columns followed by same letter (A, B, C) are not significantly different ($P > 0.05$)

Table 17 - Comparison of Effect of Sanitizing Treatments on Hue Value of the Mango Fruit

Treatment	Mean
LA-LA	18.80A[ab]
LA-30-Water	14.30AB
LA-Water	12.39AB
Water-Water	9.68AB
Untreated	3.44AB
Cl-Cl	-27.32ABC
Water-LA	-42.05BC
Cl-30-Water	-53.64C
Cl-Water	-63.61C
Water-Cl	-63.78C

[a] The values expressed are mean values of hue
[b]Means within column followed by same letter (A, B, C) are not significantly different ($P > 0.05$)

Table 18 - Comparison of Effect of Sanitizing Treatments on Chroma Value of Mango Fruit

Treatment	Mean
Cl-Water	14.8A[ab]
Untreated	14.6A
Water-Cl	14.4A
LA-Water	14.3A
Cl-30-Water	14.1A
Water-LA	14.0AB
Cl-Cl	12.5BC
LA-LA	12.4BC
Water-Water	11.9C
LA-30-Water	11.3C

[a] The values expressed are mean values of chroma
[b]Means within column followed by same letter (A, B, C) are not significantly different ($P > 0.05$)

Table 19 - Comparison of Effect of Storage Time on Chroma of Mango Fruit

Time (days)	Area (Nm)
0	17.1A[ab]
3	12.8BC
6	13.2B
9	12.4BC
12	11.8C

[a] The values expressed are mean values of chroma
[b] Means within column followed by same letter (A, B, C) are not significantly different ($P > 0.05$)

CONCLUSIONS

The study demonstrated the fact that lactic acid is more efficient compared to chlorine in reducing/eliminating *Salmonella* populations on the surface of the mangoes. Also, it was observed that the extent of infiltration is reduced or inhibited with treatments involving lactic acid (few positive samples after enrichment method). This is suggested to be due to elimination of microbial populations on the surface thereby; reducing the number of cells penetrating the core tissue. However, recovery of pathogen for almost all the treatments demonstrates that *Salmonella* is capable of surviving hydrothermal treatments and has the potential for internalizing and surviving long periods of time.

Resistance of *Salmonella* to chlorine has been suggested to be "acquired" (Davidson and Harrison 2002) and is affected by several factors including catalase production (Mokgatla and others 2002), exposure of cells to lower concentrations of sanitizer and/or exposure of cells to dissociated form of acid rather than the undissociated form (Pickett and Murano 1996). Lactic acid is superior in reducing microbial populations compared to chlorine; however, the current study demonstrated that application at a concentration of 2% has negative impact on the quality of fruit. Perhaps, further studies at lower concentrations or in combinations with other sanitizers such as hydrogen peroxide (Venkitanarayanan and others 2002) might prove it to be efficient both microbiologically and qualitatively. Although chlorine has been proved inefficient when compared to lactic acid in reducing microbial populations its use both in the hot and cool dips is recommended. It is suggested that application of chlorine to

wash water at high temperatures (perhaps 50°/60° C) and for short duration (perhaps 30-40 min) might result in appreciable reduction of microbial population present on the surface of mangoes. However, this needs further investigation.

Also, the cool dip is not recommended by APHIS, however, the industry adopts it for quality purposes (Sivapalasingam and others 2003). Investigation by federal agencies subsequent to the outbreak revealed that this practice perhaps caused the infiltration of pathogen into the stem tissue. Alternative methods such as air-cooling need to be considered and adopted rather than hydrocooling to prevent the problem. Irradiation has been suggested as an alternative for hydrothermal treatment; however, its use so far has been limited. The reasons for its limited use needs to be investigated.

Foodborne outbreaks involving mangoes have not been documented prior to 1999 and this is considered as one of the primary reasons for limited research in this area. Two outbreaks (1999 and 2001) highlighted the need for extensive research in identifying potential sources of contamination while implementing the new techniques. The current research is successful partially in suggesting few alternatives; however, further studies are needed to have an in depth understanding of the several complicated mechanisms involved. Further research might help in developing new efficient technologies that might prove beneficial both to the grower and the importing country.

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VITA

Name:	Grihalakshmi Kakani
Permanent Address:	1100, Hensel Drive # X2E, CS, TX-77840
Marital Status:	Single
Place of Birth:	INDIA
Education:	Acharya NG Ranga Agricultural University Hyderabad, INDIA B. Sc., Agricultural Sciences, 1994 M.A.B, Texas A&M University, 2003
Professional Societies:	Institute of Food Technologists