EFFECT OF WASHING PRACTICES ON THE MICROFLORA ON GEORGIA-GROWN CANTALOUPES

by

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(Under the Direction of Mark A. Harrison)

ABSTRACT

In recent years, there have been foodborne illness outbreaks associated with the consumption of cantaloupe. Cantaloupes can be contaminated with pathogens anywhere from the field to the packing line. Cantaloupes are handled and packed differently in the United States. Georgia-grown cantaloupes are brought to sheds, washed, and packed. The objective of this thesis was to compare the washing and packing practices of cantaloupes in Georgia. Sheds 1 and 4 utilized a chlorinated dump tank to wash melons in. Sheds 2 and 3 used heat and chlorine in the dump tanks. There was a significant ($p<0.05$) reduction in aerobic populations and *Escherichia coli* from the field to the dump tank for sheds 1 and 4. The water temperatures used at sheds 2 and 3 were not high enough to effectively reduce the microbial populations that were evaluated. Populations increased after the dump tank suggesting contamination after washing.

INDEX WORDS: Cantaloupe, food safety, water quality

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CHAPTER 1

INTRODUCTION

Foodborne diseases are a widespread and growing public problem, both in developed and developing countries. In industrialized countries, the percentage of people suffering from foodborne disease has been reported to be up to 30% according to the World Health Organization. In the United States, 76 million cases of foodborne diseases resulting in 325,000 hospitalizations and 5,000 deaths are estimated to occur each year (11). Most infections though go undiagnosed and unreported (8). Economic loss due to productivity loss, medical cost, and food recalls amounts to $6.9 billion in just the U.S. (9).

Today the risk of foodborne disease depends on the type of food, its production source, how it is prepared and handled, and the consuming host's resistance to the infectious agent. As these factors change, the epidemiology of foodborne diseases also necessarily changes. The relationship between cardiovascular disease and consumption of saturated fat has led many Americans to stop consuming the traditional meat and potato diet that accompanied the postwar boom of the 1950s. The new American diet emphasizes fruits, vegetables, and grains and deemphasizes meats and foods with a high fat content. The concept of a diet balanced between the four basic food groups has been replaced by a diet built on a food pyramid (6). The new MyPyramid published in 2005, replaces the old food pyramid and is a way to help consumers choose the foods and amounts that are right for them (10). Another national program entitled "5 a Day for Better Health," which began in 1991, raised attention to food choices and is sponsored by the National Cancer Institute and a nonprofit consumer education foundation, the Produce for Better Health Foundation. The objectives of this program are to help increase awareness of the importance of eating five to nine servings of raw or cooked fruits and vegetables per day and to provide consumers with information on how they can add these foods to their diets (7).

Another influence on our eating habits is the fact that, in general, people today tend to lead more hurried lives than in the past. In addition to the rapid rise of fast food restaurants consumers are demanding more take-home, ready to eat foods. Grocery stores are providing us with a variety of in-store prepared foods, including ready-to-eat prepackaged fresh fruits and vegetables (7).

Along with the increased amount of fresh produce consumed, there has been a corresponding rise in the number of reported cases of foodborne disease linked to produce. *Shigella* spp., *Salmonella*, enterotoxigenic and enterohemorrhagic *Escherichia coli*, *Campylobacter* spp, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium botulinum*, viruses and parasites such as *Giardia lamblia*, *Cyclospora cayetanensis*, and *Cryptosporidium parvum* are of a public health concern (2). There have been 14 reported outbreaks involving cantaloupe in the last 13 years. More than half of the outbreaks involved melons that were cut and not consumed quickly enough so pathogens were able to grow on the sugar-rich interior of the fruit (4).

Contamination of produce such as cantaloupes can occur anywhere along the farm-to-fork paths (3). Sources of contamination include irrigation water, runoff water from livestock farms adjacent to fields and orchards, manure, wash water, handling by workers, contact with contaminated surfaces, and feces of rodents and ruminants (5). Contamination of the skin on the cantaloupe can be a food safety problem. Even when the skin itself is not eaten, contamination can be spread to the edible part and the fruit can cross-contaminate other foods and food preparation areas (1). A concern for foodborne outbreaks from cantaloupes is to learn where the cantaloupes are being contaminated. Another concern is to see if treatments to the cantaloupes will reduce the microbial populations that may be found on the cantaloupes. The objective of

this thesis is to addresses microflora on Georgia-grown cantaloupes with respect to washing and packing practices. Information needs to be acquired so that recommendations can be given to farmers as to the best way to reduce microbial populations on cantaloupes. It is important to know where the cantaloupes are being contaminated on the packing line and if a water treatment will reduce the microbial numbers.

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CHAPTER 2

LITERATURE REVIEW

Produce Associated Foodborne Illness Outbreaks

Produce items have been associated with foodborne illness due to an increased per capita consumption of fresh and lightly processed produce in the United States (9). Fresh fruits and vegetables are grown in fields and orchards that are not sterile environments (61). Spoilage bacteria, yeasts, and molds dominate the microflora on raw fruits and vegetables, but pathogenic bacteria, parasites, and viruses have the potential to be found. *Shigella* spp., *Salmonella*, enterotoxigenic and enterohemorrhagic *Escherichia coli*, *Campylobacter* spp, *Listeria monocytoges*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium botulinum*, viruses and parasites such as *Giardia lamblia*, *Cyclospora cayetanensis*, and *Cryptosporidium parvum* are of public health concern (10). Many of these microorganisms carried from the field have the potential to attach and form biofilms on surfaces of fruits and vegetables, reducing possible removal through common procedures of hygiene and sanitation (8). *Salmonella* and *E. coli* O157:H7 outbreaks have been associated with a wide range of products such as lettuce, apple cider, alfalfa sprouts, bean sprouts, watermelon, radish sprouts, cabbage, celery, cucumbers, potatoes, radishes, tomatoes, and cantaloupe (50). Examples of recent foodborne illness outbreaks associated with produce can be found in Tables 2.1 and 2.2.

Foodborne Illness Associated with Cantaloupe Outbreaks

There have been 14 reported outbreaks involving cantaloupe in the last 13 years. Eleven of the 14 involved *Salmonella* from seven different species/serogroups, and one each involved *Escherichia coli* O157:H7, *Campylobacter jejuni*, and a viral agent. More than half of the outbreaks involved melons that were cut and not consumed quickly enough so pathogens were able to grow on the sugar-rich interior of the fruit (28). An outbreak of hemorrhagic colitis caused by *E. coli* O157:H7 occurred in Oregon in August of 1993 due to eating contaminated

cantaloupe (24). There were also 400 lab confirmed infections of *S*. Poona which occurred in 23 states and Canada. The outbreak occurred in June and July of 1991. In all the cases, people who became sick ate cantaloupe from salad bars or fruit salad (17). In 1997, 25 cases were confirmed with *S.* Saphra. All case studies reported eating cantaloupe and after an investigation found the cantaloupes were from a farm in Mexico (45). There were also three multistate outbreaks of *S.* Poona infections associated with eating cantaloupe from Mexico from 2000-2002. Forty-seven cases were confirmed between April and June of 2000, 50 cases from April to May in 2001, and 58 cases between March and May of 2002. All of the cantaloupe involved in the outbreaks were traced back to shippers and then to farms in Mexico. The U.S. Food and Drug Administration (FDA) conducted on-farm surveys and detained products. On October 28, 2002, FDA issued an import alert on cantaloupe from Mexico that detained all products offered at U.S. ports (21). Pathogens on contaminated melons can come from the field, irrigation water, animal or insect feces, workers, sewage, during washing, packaging or storage in a packing shed, and in final preparation at home or in a restaurant (31). Recommendations by the FDA to retail establishments that prepare or sell fresh cantaloupe are that melons should be washed before cutting, clean, sanitized utensils and surfaces should be used when cutting melons and they should be kept at or below 44.6°F (7°C). If the melons are displayed, such as with salad bars, they should only be kept out for 4 hours (31). Consumers purchasing cantaloupe should follow FDA recommendations to buy undamaged and unbruised fruits, wash fruits with cool tap water immediately before slicing, avoid using soaps or detergents, and scrub fruits with a clean produce brush (7).

Cantaloupe Characteristics

Cantaloupes (*Cucumis melo* L.var. *reticulatus* Naud.) are commonly called muskmelons and are members of the cucurbitaceae family which also include squash, pumpkins, cucumbers, watermelons, and gourds (48). The species is divided into 7 botanical variants which include cantaloupensis, reticulatous, indorous, flexuosus, conomon, chito and dudaim. Only reticulatous and indorous variants are commercially important in the United States. The indorous varieties are typically called honeydew (12). True cantaloupes (cantaloupensis) are not grown in the United States. True cantaloupes are rough, warty fruit grown in Europe, but in America cantaloupe is the generic name of all netted musk-scented melons. There are several varieties of cantaloupe including Athena, Burpee Hybrid, Ambrosia, Park's Whopper and Scoop II to name a few (44). Cantaloupes are annual plants that are long-running, non-climbing vines that prostrate the soil. Healthy plants have a canopy of large, soft-hairy leaves which are generally heart shaped and lobed (4). Cantaloupes are rich in sugars and other nutrients that can support significant pathogen growth. Melons contain the sugars fructose, glucose, and sucrose as percent soluble solids from 8 to 14% in cantaloupe (28). Cantaloupes are divided into eastern and western types. The eastern type is characterized by round shaped fruits with weight varying between 5 to 7 pounds. The eastern type has a large seed cavity and sutures which are green lines that divide the rind into several sections. The netting is a network of cork like marks that cover the rind (53). The western cantaloupe has uniformly netted rinds, orange flesh, lack any sutures, and weigh 3 to 4 pounds. This type normally has been grown in the western United States and shipped throughout the country (12). Due to the netting of cantaloupe attachment by microorganisms is problematic because the surface roughness will not allow complete recovery of microorganisms (57). These melons are hydrophobic due to the presence of waxes which

makes the cleaning and sanitizing process more difficult (8). Cantaloupes are also potentially dangerous because their acidity (pH 5.2 to 6.7), high water activity (0.97-0.99) (31), and high sugar concentrations (28) could allow for pathogen growth.

Cantaloupe Growth and Harvest

Cantaloupes are planted in late spring when the soil temperature is at least 68°F (20°C) which is good for germination (49). The melons are grown on raised beds covered with black plastic. The plastic helps to control weeds, increase soil temperature, conserve moisture, and protect melons from ground rot (33). Irrigation and fertilization help ensure optimum plant growth and yields (48). Cantaloupes require bees for pollination. Bees are brought into the field to increase earliness, yield, and quality. Each flower is only open one day, and while open several hundred pollen grains must be deposited in the stigma of the muskmelon flower so that a marketable size cantaloupe will be produced. This means each fruit needs 10-15 bees to visit. Since the highest quality fruit is produced near the crown, bees must be brought into the field as soon as the first perfect flowers appear. Introducing them later will delay harvest and reduce quality. At least one bee hive per acre is required, but up to 3 hives per acre will increase fruit size and earliness. Placing the hives within the field rather than around it will double bee visitations (5, 49). Cantaloupes mature in 35 to 55 days from full bloom, depending on the cultivar and environment and are harvested when the melons are at ¾ to full slip. Full slip is the stage of ripeness at which the melon comes away easily from the stem attachment and where the skin begins to take on a slightly yellow appearance under the netting. For distant shipping, less mature cantaloupes are picked at half slip where the pedicel remains attached to the fruit (33). The sugar content of cantaloupe does not increase after harvest so the highest quality fruit are picked at the level of maximum sugar content. U.S.D.A. Grade 1 melons must have a minimum

soluble solids level of 8% and a maximum level of 12-14% (33). Precooling (36 to 41°F (2-5°C) at 95% relative humidity) is necessary immediately after harvest to slow down respiration. If this is not done, the sugars will be depleted, decreasing quality and reducing the shelf life of the cantaloupe (49). Cooling can be done using cold air, cold water or ice (35). Harvest is handled differently throughout the United States (Fig. 2.1). In California, cantaloupes are hand picked and packaged in the field and then brought in for cold storage (32). In 2002, California harvested 54,900 acres of cantaloupes (58). In Georgia, cantaloupes are picked and brought to packing sheds to be washed and then packed (35). In 2002, Georgia harvested 5,700 acres of cantaloupe (58). Fields are generally picked on average 12 to 15 times to allow as many cantaloupes as possible to mature so they can be sent to market (41).

Previous Cantaloupe Microbiological Studies

There have been several previous microbiological studies on cantaloupe. Each study has its own uniqueness and different results. Castillo et al (16) sampled cantaloupe from Mexico and Texas from the field, after harvesting, after washing in chlorinated water, and after packing. In this study, 100 cm^2 of the cantaloupe surface was sampled using a sterile sponge moistened with 25 ml of 0.1% peptone water. Thirty-one samples (1.8%) were positive for *Salmonella*. Three and nine tenths percent from Texas and 27.7% from Mexico were positive for *E. coli*.

Ukuku et al (56) treated cantaloupe with hot water at temperatures of 70ºC (158°F) and 97ºC (206.6°F). Exposing cantaloupe at those temperatures for 30 s gave a 2.0 and 3.4 log cfu/cm^2 reduction of *Salmonella* respectively. When the temperature was increased to 97ºC for 60 s it caused a 4.4 log reduction. These results suggest that the longer the time and higher the temperature will give a greater reduction in microbes although quality of the melon may be altered.

Barak et al (7) washed cantaloupes with Butterfield's buffer with 1% Tween 80 added. The fruit was placed in plastic bags and the shaker method was used. The cantaloupes were washed with antimicrobial soap, scrubbed with a brush in tap water, and immersed in 150 ppm of sodium hypochlorite. More *Salmonella* cells were recovered by using the shaker when the Butterfield's buffer contained 1% Tween 80. In another study, Hammack et al (30) placed cantaloupe in sterile bags with 1:1.5 cantaloupe weight to preenrichment broth volume ratio. Four broths used for preenrichment were buffered peptone water (BPW), modified BPW, lactose (LAC) broth and Universal Preenrichment (UP) broth. The cantaloupes were shaken for 5 min at 100 rpm on a rotary shaker. This method was compared to a soak method, and they found that the soak method was more efficient for *Salmonella* recovery. The authors suggest that the development of biofilms did not allow the cells to detach from the melon during the shaking method. In another study, Bastos et al (8) found a 4 log cycle reduction after using of 1000 mg per liter of free available chlorine and Tween 80 in water at a pH of 6.5. The cantaloupes were sampled by homogenizing in a blender 25 g of rind samples for 2 minutes. There was a log reduction of 1.35-1.37 by using 1000 mg per liter of free chlorine. FDA has also surveyed imported cantaloupe for *Salmonella*, *E. coli*, and *Shigella*. Each sample was placed into a sterile plastic bag with 454 ml of Butterfield's phosphate buffer solution (1:1 dilution). The bags were shaken for 5 min using an orbital shaker at 100 rpm. Results from this study from 13 states, including Georgia, showed 0.5% confirmed positives from 164 samples. Out of the 164 samples from the 13 states there were 4 *Salmonella* positives and 1 *Shigella* positive.

Of all these experiments to date, no one has taken into account actual harvest and packing methods to see if their methods for recovering *Salmonella* and other microorganisms are feasible. Some factors that are important are the cost and time of adding a large quantity of chlorine to

wash water. Also heated water at such a high temperature such as 97ºC (206.6°F) may affect quality of the melon and could make it harder to sell in the market. Sampling methods including using a sterile sponge or homogenizing a small portion of the cantaloupe only takes in account one area of the melon. If microbes were evenly distributed all over the melon, then taking a small sample would be representative of the microbial population on the melon. If microbes are not evenly distributed, then sampling one area will not give an accurate depiction of the population found on the melon. The amount of rinsate to sample cantaloupes will also make a difference in results. One study using a 1:1.5 ratio would use a large quantity of rinsate to rinse melons. For a field study, it is not practical that huge quantities of rinsate can be taken to the field to wash melons in a 1:1.5 ratio.

Salmonella History and Taxonomy

In the early 19th century, pathologists in France first documented the association of human intestinal ulceration with a contagious agent; the disease was later identified as typhoid fever. After further investigation, the typhoid bacillus responsible for typhoid fever was isolated and characterized (23). In the United States, work by Salmon and Smith in 1885 led to the isolation of *Bacillus cholerae-suis* which is now known as *Salmonella enterica* serovar Choleraesuis. The genus name was coined by Lignières in 1900 to honor Dr. Salmon's work (3). *Salmonella* is a genus of the family Enterobacteriaceae. Members of this family are gram-negative, facultatively anaerobic, non-spore-forming, rod shaped bacteria. Motile forms have peritrichous flagella (3). The bacteria grow optimally at 37°C and catabolize D-glucose and other carbohydrates with the production of acid and gas. *Salmonella* are oxidase and catalase negative. They also use citrate as a sole carbon source, produce hydrogen peroxide, decarboxylate lysine and ornithine, and do not hydrolyze urea. Changes have been made in the

taxonomy of *Salmonella*. Microbiologists tend to treat the 2,324 serovars as though each was a species (37). The International Committee on Systematics of Prokaryotes divides the genus *Salmonella* into two species, *S. enterica* and *S. bongori* (23) each of which contains multiple serovars. The 2,324 serovars have been divided into 5 subspecies or groups, which most are classified in *S. enterica*. The major groups correspond to the following subspecies and are referred to by roman numerals: I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, *S. enterica* subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae*, and VI, *S. enterica* subsp. *indica* (23, 37). The biochemical identification of foodborne and clinical *Salmonella* isolates is generally coupled to serological confirmation involving the agglutination of bacterial surface antigens with *Salmonella*-specific antibodies. These antibodies include somatic (O) lipopolysaccharides (LPS) on the external surface of the bacterial outer membrane, flagellar (H) antigens associated with the peritrichous flagella and the capsular (Vi) antigen which only occurs with *Salmonella* serovars Typhi, Paratyphi C, and Dublin (23, 37).

***Salmonella* Occurrence**

Salmonellosis is the major bacterial foodborne disease in many countries (23) and is the most frequently reported cause of foodborne outbreaks of gastroenteritis in the United States (57). It is estimated that from 2 to 4 million cases of salmonellosis occur in the U.S. annually (60) with only 40,000 cases reported (62). The primary habitat of *Salmonella* spp. is the intestinal tract of animals such as birds, reptiles, farm animals, humans and insects. The organisms are excreted in feces and transported by insects or other animals to numerous places. The cycle continues as the organisms get into water and people or animals consume the contaminated water. These organisms can once again be shed. The augmentation of this cycle through shipping animal products internationally is part of the problem of the world-wide

distribution of salmonellosis. *Salmonella* has been found in food products commercially prepared and packaged with 17 of 247 being positive (37). Examples of foods contaminated are raw meats, poultry, eggs, milk, dairy products, fish, shrimp, frog legs, yeast, coconut, sauces, salad dresses, cake mixes, cream-filled desserts and toppings, dried gelatin, peanut butter, cocoa, chocolate (60), fruits, and vegetables (46). Poultry though is known to be the primary vehicle of transmission (42).

Salmonellosis Symptoms

To become infectious, *Salmonella* must penetrate and pass from the gut lumen into the epithelium of the small intestine where inflammation occurs. There is evidence that an enterotoxin may be produced, but it has not been proven (60). As few as 15-20 cells could be all that is needed depending on the age and health of the host and strain differences among the members of the genus (60). The incubation period ranges from 5 h to 5 d with symptoms beginning on average at 12-36 h after ingestion of a contaminated food. Symptoms include diarrhea, nausea, abdominal pain, mild fever and chills. Sometimes vomiting, prostration, anorexia, headache and malaise occur with symptoms usually lasting 2-5 d (3). Treatment of gastroenteritis may require only supportive therapy such as fluid and electrolyte replacement. Enteric fever can also be a symptom of salmonellosis. Incubation time ranges from 7 to 28 d and malaise, headache, high fever, abdominal pain, body aches and weakness occurs. Diarrhea or constipation normally is also associated. Long term problems can be associated with salmonellosis such as postenteritis reactive arthritis and Reiter's syndrome, which is characterized by painful joints, irritation of the eyes and painful urination. This can occur after 3 wk of infection (60).

Factors Affecting Growth, Death and/or Survival of *Salmonella*

Salmonella grow optimally at 37°C. They are resilient organisms and have been shown to grow in extreme conditions such as in < 5°C. The growth rate increases as the temperature increases until the optimum growth temperature of 37°C is met. The maximum growth temperature is 49.5°C. It is important to keep foods above this temperature to ensure that *Salmonella* will not grow. Freezing is another way to keep *Salmonella* from growing. A more rapid decrease in numbers is found in the freezing process with temperatures falling from 0 to -10°C than it is in the frozen stage of -17 to -20°C. Freezing is not a 100% guarantee of destruction. Some foods can provide protection to bacteria when they are being frozen (3). Most *Salmonella* can grow in foods in a water activity range of 0.945-0.999 (25). *Salmonella* growth is inhibited at an a_w less than 0.94 (37). *Salmonella* can survive for a year or more in foods having a low a_w (3). The pH for optimum growth is around neutrality (6.6-8.2) with lethal values typically being below 4.0 and above 9.0. A minimum growth pH of 4.05 has been recorded for some serotypes with HCl and citric acid, but depending on the acid used to lower the pH, the minimum may be as high as 5.5.

Types of Pathogenic *Escherichia coli*

There are six types of pathogenic *E. coli*. Enteropathogenic *E. coli* (EPEC) can cause severe diarrhea in infants, especially in developing countries. Enterotoxigenic *E. coli* (ETEC) is responsible for traveler's diarrhea. Enteroinvasive *E.coli* (EIEC) isolates can cause nonbloody diarrhea and dysentery similar to that caused by *Shigella* spp. Diffuse-adhering *E.coli* (DAEC) isolates are associated with diarrhea primarily in young children who are older than infants. Enteroaggregative *E.coli* (EAEC) is also associated with persistant diarrhea in children. Lastly

enterohemorrhagic *E.coli* (EHEC) causes hemorrhagic colitis, and serotype O157:H7 is the dominate serotype in this group that causes bloody diarrhea (26).

History and Taxonomy of *E. coli*

This organism which was originally called *Bacterium coli*, was isolated from the feces of infants by Escherich over a century ago. In 1920, the organism was renamed *Escherichia* and there was evidence that it could cause gastroenteritis with mortality in infants. In 1982, *E.coli* O157:H7 was identified as a foodborne pathogen (2). *E. coli* bacteria are members of the family Enterobacteriaceae (2). For the genus *E. coli*, over 200 O serotypes have been recognized (36). The organisms are gram-negative, catalase positive, oxidase-negative, facultatively anaerobic short rods. Most strains ferment lactose, although some are slow lactose fermenters and a few are anaerogenic. Strains of *E. coli* may be differentiated from one another serologically on the basis of somatic (O), flagellar (H), and capsular (K) antigens. Also fimbriae and related structures may be present and play a role in pathogenesis (2).

Occurrence of *E.coli* O157:H7

E.coli O157:H7 has caused major outbreaks of severe illness all over the world. At least 30 countries on six continents have reported illness in humans. Most outbreaks occur during the warmest months of the year. In the United States, around 86% of outbreaks and clusters reported happened from May to October. This may be due to an increased prevalence of the pathogen in cattle or other livestock or vehicles of transmission during the summer, greater human exposure to ground beef or other *E. coli* O157:H7 contaminated foods during the “cookout months”, and/or greater improper handling (temperature abuse and cross contamination) or incomplete cooking of products such as ground beef during the warm months. *E. coli* O157:H7 is found in the intestinal tract of animals and humans. Cattle are a reservoir of O157:H7, and undercooked

ground beef and unpasteurized milk are recognized vehicles. Besides cattle, other domestic animals and wildlife can carry *E. coli*., such as sheep, goats, deer, dogs, horses, swine, and cats. Humans can also shed *E. coli* O157:H7 in feces for up to weeks (26). Animals can spread O157:H7 to fields where fruits and vegetables can be contaminated. Since fruits and vegetables are not given a heat treatment, farmers must be aware of what animals access their fields.

Symptoms of *E. coli* O157:H7 Illness

E. coli O157:H7 infection often causes severe bloody diarrhea and abdominal cramps; sometimes the infection causes nonbloody diarrhea or no symptoms. Usually little or no fever is present, and the illness resolves in 5 to 10 days (19). Around 6% of patients progress to hemolytic uremic syndrome (HUS); half of these require dialysis, and 75% require transfusions of erythrocytes and/or platelets (26). HUS could set in one week after onset of diarrhea along with intravascular destruction of red blood cells, depressed platelet counts, lack of urine formation, swelling and acute renal failure (14). HUS is a clinical entity characterized by renal failure, microangiopathic hemolytic anemia, and thrombocytopenia (47). The infectious dose is unknown, but from a compilation of outbreak data, including the organism's ability to be passed person-to-person, the dose may be similar to that of *Shigella* spp. which is as few as 10 organisms (59).

Factors Affecting Growth, Death and/or Survival of *E. coli* O157:H7

E. coli O157:H7 can grow at temperatures ranging from 8°C to 44-45°C. The optimum temperature for growth though is 37°C. Del Rosario and Beuchat showed that *E. coli* O157:H7 could be detected on cantaloupe cubes that were held at 5˚C for 34 h (24). Pathogenic *E. coli* generally survive well in foods at refrigeration temperatures of about 3-7°C with up to a $10^{1.5}$ per gram reduction over 1-5 wk of storage. Little or no change was found in hamburger meat stored

at -20°C for 9 mo. Thermal inactivation studies have shown that *E. coli* O157:H7 is more sensitive to heat than typical *Salmonella,* therefore heat treatments that kill *Salmonella* will also kill *E. coli*. The effect of pH is dependent on the acid that is found. *E. coli* can grow at pH 4.5 in a medium adjusted with HCl but not in a medium adjusted with lactic acid. Pathogenic *E. coli* will not grow in fermented cheeses at pH < 5.4 (2). An outbreak involving apple cider showed that *E. coli* could live in a pH of 3.7-3.9 (36).

Coliforms/Fecal Coliforms

Coliforms were first isolated and studied by Escherich in 1885. Schardinger was the first to suggest the use of these organisms as an index of fecal pollution because they could be isolated and identified more readily than other enteric pathogens. A test for this organism to measure drinking water safety began in 1895 by Smith. This marked the beginning of the use of coliforms as an indicator of enteric pathogens in water, a practice that has been extended into foods (38). Coliforms are gram-negative, asporogenous rods that ferment lactose within 48 h to gas (CO_2) at 35.0˚C (40). Coliforms are represented by four genera of the family Enterobacteriaceae: *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella*.

The presence of coliforms indicates the possibility of poor sanitary condition and therefore, potential pathogenicity (34). Coliform testing can also give results for overall sanitation of plants and processing. The coliform test for the dairy industry is used for overall dairy farm and plant sanitation. Discovering the presence of *E. coli* on frozen vegetables may indicate a problem with processing. Coliforms, especially *E. coli* on fresh produce, indicates fecal contamination somewhere from the field to the consumers handling the food (38).

Coliforms grow well on a large number of media and foods. They have been reported to grow in temperatures as low as -2°C and as high as 50°C and over a pH range of 4.4-9.0.

Coliforms grow well on nutrient agar and produce visible colonies within 12-16 h at 37°C. They can grow to large numbers under the proper conditions on food. Coliforms are also able to grow on bile salts which inhibit the growth of gram-positive bacteria. Unlike most bacteria they have the capacity to ferment lactose with the production of gas, and this characteristic alone is enough to make presumptive determinations. The ease at which coliforms can be cultivated and differentiated because they have the capacity to ferment lactose to gas makes them good as indicators (38).

E. coli can be grown in a minimal medium containing only an organic carbon source as glucose, a source of nitrogen, and other minerals. Since *E. coli* is more of an indicator of fecal contamination, it is often desirable to determine its present in food or water. Fecal coliforms are defined by the production of acid and gas in EC (*E. coli*) broth between 44°C and 46°C. A test for fecal coliforms is typically a test for *E. coli* although some *Citrobacter* and *Klebsiella* fit the definition (38). Food samples positive for fecal coliforms have a higher probability of containing organisms of fecal origin than do coliforms that are made up of fecal and nonfecal organisms. Fecal coliforms can become established on equipment and utensils in the food processing environments and can then contaminate foods being processed (26).

Occurrence of Coliforms and Fecal Coliforms

The main habitat for fecal coliforms is the intestinal tract of most warm blooded animals. It is not hard to discover coliforms in the air and dust, on hands and in or around foods (38). Since coliforms do establish in the intestinal tract of humans and animals they can be used as indicators of fecal contamination (34). The presence of high numbers of coliforms and *E. coli* in foods could be alarming, but it would be impossible to rid fresh and frozen foods of these organisms (38).

Microbial Water Quality

Microbial water quality can be measured by the total number of microorganisms and fecal coliforms found in the water. Enumeration of total heterotrophic populations is commonly used as in indicator of overall microbiological quality (52). The heterotrophic plate count (HPC) is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment (22). There is no constant ratio of pathogens to heterotrophic bacteria and, therefore, the HPC test is not an effective means of indicating the presence of pathogens in water. To ensure absence of enteric pathogens, such as fecal coliforms, enumeration of appropriate indicator organisms is required (51). Tests for total coliforms, fecal coliforms, and *E. coli* are typically used for this purpose. Total coliform counts are used to monitor treated water supplies and to determine the adequacy of the treatment process. To avoid limitations of using total coliforms, monitoring fecal coliforms has been suggested. Since levels of fecal coliforms have been widely associated with *E. coli*, fecal coliform counts have been widely accepted for routine monitoring for water quality (52).

Physical Properties of the Dump Tank Water and Packing Sheds

Temperature

When produce is brought to packing sheds it is possible that it will be washed and/or water chilled before the packaging step. It is important to monitor the water for several reasons. Water temperature should be monitored if a sanitizer is being used. Some processors believe the hotter the water the better cleaning job they will do. This is not true because the efficiency of the sanitizing product, such as chlorine will be decreased if the temperature is too high (6). Temperature management of the produce after packing is also important. Temperature plays a

role in limiting water loss in storage and transit. It is also the primary means of lowering respiration rates of fruits and vegetables (31).

pH and Chlorine

Acids are substances that increase hydronium ion concentration when added to water and bases are ones that increase hydroxide ion concentration (39). The pH of wash and chill water is important when using sanitizers. The antimicrobial activity of chlorine compounds depend on the amount of hypochlorous acid (HOCl) present in the water. This form attacks the integrity of the cell membrane of microorganisms and is transferred across the cell membrane to begin the killing process. The activity of HOCl depends on pH, amount of organic and inorganic material present and to some extent water temperature. For wash water, a pH of greater than 7.5 will not have active chlorine and below a pH of 4.0 chlorine turns into gas. The pH of the water should be kept between 6.0 and 7.5 to ensure chlorine activity. Organic matter will also reduce the activity of chlorine so replacing or filtering the water is important to maintain wash water disinfectant levels. Chlorine levels to actively kill organisms should yield 1-2 ppm of free active chlorine. Levels of 200 ppm total chlorine should be used because organic and inorganic compounds will use up nearly all the chlorine and leave the 1-2 ppm needed for effectivness (6). Hand held portable devices can monitor pH and chlorine levels in wash and chill water in the processing sheds.

Oxygen Reduction Potential of the Dump Tank and Sprayer Water

Accurate monitoring and recording of disinfection procedures is an important part of postharvest food quality and safety. Oxygen-reduction potential (ORP) is measured in millivolts and is an approach to standardizing water disinfection parameters (6). Microorganisms affect the Eh (ORP) of their environments during growth just as they do pH. Aerobes can lower the Eh of

their environment where anaerobes do not. As aerobes grow oxygen in the water is depleted resulting in a lowering of the Eh. Growth is not slowed as much as might be expected because of their ability to make use of oxygen donating or hydrogen accepting substances. The results become that water is poorer in oxidizing and richer in reducing substances (38). Research has shown that an ORP value of 650 to 700 mV will kill spoilage bacteria and pathogens such as *E.coli* and *Salmonella* in as quickly as 10 sec in clean water (54). For very clean water, 1 to 2 ppm free chlorine will provide adequate control for microbes. This water quality will likely result in measurements of 650-700 mV ORP if the water pH is 6.5 to 7.0. Lowering the pH closer to 6.0 will raise the ORP because of the addition of more hypochlorous acid. Raising the pH to 8.0 will decrease the ORP because there is more hypochlorite ions present. Maintaining constant pH and adding more chlorine will top out the ORP level at 900-950mV which is generally found at 25 ppm of free chlorine.

Percent Relative Humidity of Packing Sheds

Relative humidity is a dimensionless ratio, expressed in percent, of the amount of atmospheric moisture present relative to the amount that would be present if the air were saturated with moisture. Since the latter amount is dependent on temperature, relative humidity is a function of both moisture content and temperature. As such, relative humidity by itself does not directly indicate the actual amount of atmospheric moisture present (13). The relative humidity directly influences the rate of water loss from produce at any point in the marketing chain. Water loss may result in wilting, shriveling, softening, browning, stem separation, or other defects (31).

Chemical Oxygen Demand of Dump Tank and Sprayer Water

Chemical oxygen demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The amount of oxidant consumed is expressed in terms of its oxygen equivalence (22). COD is a measurement of pollutants in natural and waste waters. Untreated wash and chill water is generally rich in organic matter. The organic matter will feed bacteria present in the water. The presence of excessive amounts of nutrients will result in an increase in concentration of bacteria. There are two main tests that can be performed to tell the oxygen level in water, COD and biological oxygen demand (BOD). COD is accepted because it is as accurate as and faster than the BOD method which requires 5 to 7 d to run (1).

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Fig.2.1 Postharvest handling system for cantaloupes

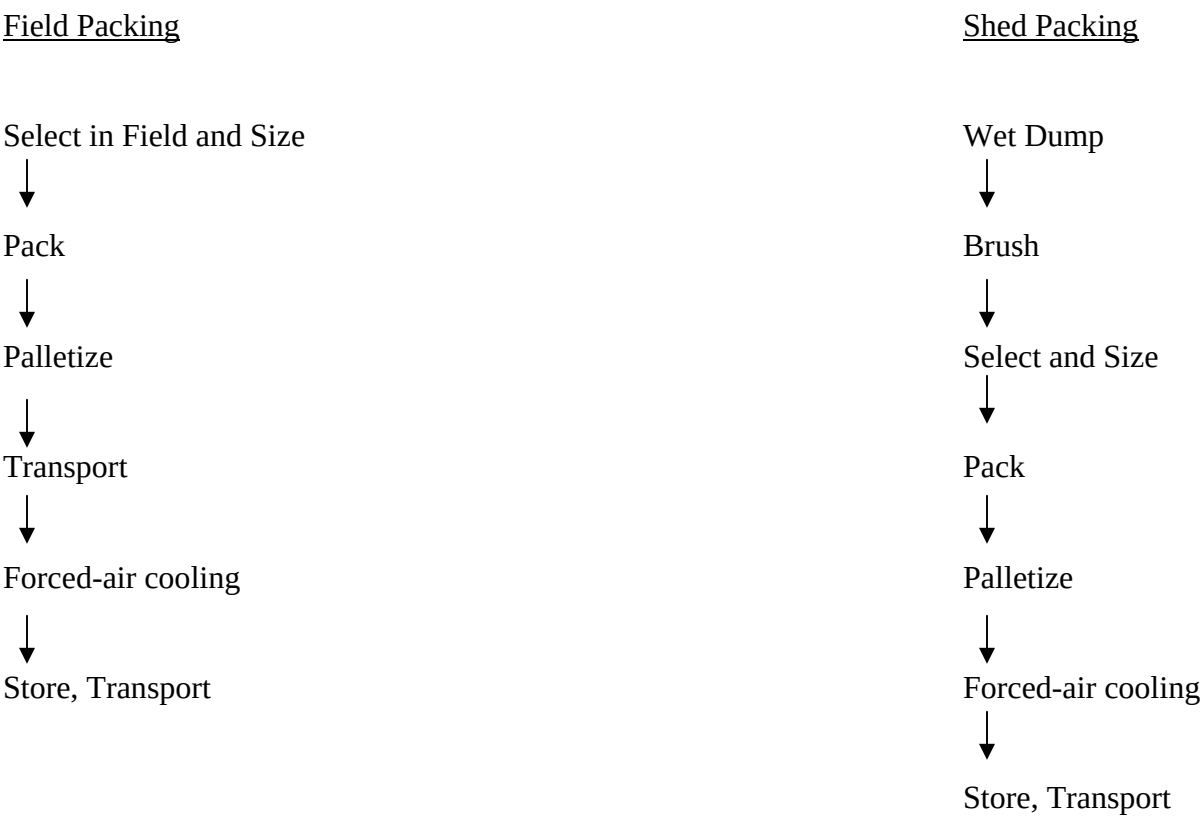

Table 2.1. Selected pathogenic *E. coli* O157:H7 foodborne illness outbreaks, location of outbreaks, source of outbreaks, and number of confirmed cases since 1993.

Year	Place	Source of Outbreak	Number of Cases	Source
1993	Oregon	Cantaloupe	9	24
1995	Idaho	Lettuce	21	20
1996	Washington	Apple	6	27
1997	Michigan and Virgina	Alfalfa	108	19
1998	Wisconsin	Catered event fruit Salad	47	29
1999	Oklahoma	Apple	7	27

Table 2.2. Selected pathogenic *Salmonella* foodborne illness outbreaks, location of outbreaks, source of outbreaks, and number of confirmed cases since 1989.

Year	Place	Source of Outbreak	Number of Cases	Source
1989-1990	Multistate U.S.	Cantaloupe	<245	17
1991	Michigan	Watermelon	26	11
1993	Multistate U.S.	Tomatoes	84	43
1995	Florida	Orange	62	18
1997	California	Cantaloupe	24	45
1998	California	Alfalfa	34	55
2000	Multistate U.S.	Citrus	14	15

CHAPTER 3

EFFECT OF WASHING PRACTICES ON THE MICROFLORA ON GEORGIA-GROWN CANTALOUPES[1]

[1] Akins, E.D., and M.A. Harrison. To be submitted to *Journal of Food Protection*.

ABSTRACT

In recent years, there have been foodborne illness outbreaks associated with the consumption of cantaloupe. Contamination of cantaloupes with microorganisms could occur anywhere from the field to the packing line. Cantaloupes are handled and packed differently in various regions of the United States. Typically, in Georgia they are brought to sheds, washed, and packed. The objective of this study was to enumerate aerobic bacteria, *Escherichia coli*, and coliforms on Georgia-grown cantaloupes from the field, after washing, and after packing. In addition, samples were also analyzed for the presence of *Salmonella* and *E. coli* O157:H7. Four Georgia growers with packing facilities using different variations in product handling were visited four times during the harvest season. For each visit, 20 cantaloupes were sampled after transport from the field, after washing and after packing. The washing methods varied among the facilities with 2 using chlorinated water and 2 using a combination of heat and chlorinated water. Aerobic populations on cantaloupes stayed approximately the same from the field to the packing stage. There was a significant ($p \leq 0.05$) reduction of coliforms from the field to the dump tank at shed 1. At shed 4 the numbers of microbes from the field stayed approximately the same as that of the dump tank microbial numbers. However, for the other 2 locations the coliform counts increased after the dump tank step due to not enough chlorine or heat. *E. coli* populations from the cantaloupes increased from the dump tank to the packing stage. Of the treatments utilized in the dump tanks the two sheds just using chlorine (sheds 1 and 4) showed significant ($p \leq 0.05$) reductions in aerobic populations and *E. coli* from the field to the dump tank. The water temperatures used at sheds 2 and 3 were not high enough to effectively reduce the microbial populations that were evaluated. Out of 870 samples only one was positive for *Salmonella* and no positives were found for *E. coli* O157:H7.

INTRODUCTION

Cantaloupes can be microbially contaminated anywhere from the field to the packing operation. Contamination can occur from wild or domestic animals in the fields, air, contact with contaminated soil, irrigation, wash and rinse water, contacting harvesting equipment or transport vehicles, by human handling, or during sorting and packing (5). Washing with unchlorinated or chlorinated water is a common practice used to clean and sanitize fruits and vegetables (11). Dirt, organic matter, and disease-causing pathogens can accumulate in process water during bin dumping, hydrocooling, and flume recirculation (10). Fruits and vegetables can have microbial populations of 10^4 to 10^6 microorganisms/g when they arrive at the packinghouse or processing plant. Surveys conducted by the United States Food and Drug Administration revealed that 7.3% of imported cantaloupes and 4.3% of domestically grown cantaloupes were contaminated with *Salmonella* or *Shigella* (14 and 15).

Waterborne microorganisms can be rapidly acquired and taken up on plant surfaces. Natural plant surface contours, natural openings, harvest and trimming wounds, and handling injuries can serve as points of entry for microbes (12). Only a 1 to 2 log unit reduction can be obtained by washing cantaloupes in water alone. Disinfection of water is a critical step to minimize the potential transmission of pathogens from a water source to produce, among produce within a lot, and between lots over time (12). Disinfection is the treatment of process water to inactivate or destroy pathogenic bacteria, fungi, viruses, cysts, and other microorganisms. The goal of disinfection is to prevent the transfer of these organisms from process water to produce and from one produce item to another during handling. Disinfection may employ chemicals such as chlorine, iodine, ozone, or it may use physical properties such as microfiltration and heat (10).

Studies have shown other treatments with wash water are an effective way of reducing microbial contamination. Heating the wash water to 97˚C resulted in a 3.4 log cfu/cm^2 reduction of *Salmonella* on cantaloupes that were in the water for 30 s. Population of mesophilic aerobes on cantaloupes that stayed in the water for 60 s were reduced by a 4.4 log reduction (13). A 4 log cycle reduction after using 1000 mg per l of free available chlorine and Tween 80 in water at a pH of 6.5 has also been shown (4).

Measurements of environmental parameters are important to help understand presence and fate of microorganisms in water. Controlling water temperature is important if sanitizers are used in cantaloupe dump tank wash water. The efficiency of some cleaning products, such as chlorine, will be decreased if the temperature is too high (3). The chlorine and pH relationship is also important in determing the effectiveness in killing microbes. The antimicrobial activity of chlorine compounds depends on the amount of hypochlorous acid (HOCl) present in the water. The activity of the HOCl depends on the pH, amount of organic and inorganic material present, and to some extent water temperature. Water with a pH of greater than 7.5 will not have active chlorine and below a pH of 4.0 chlorine volatizes. It is important to keep the pH of the water between 6.0 and 7.5 to ensure maximum activity. Chlorine levels should be monitored to ensure that free chlorine levels do not drop below 2 ppm (3).

The oxygen reduction potential of the water is important to monitor because it will show the activity level of the chlorine in the water. Research has shown that an ORP value of 650 to 700 mV will kill spoilage bacteria and pathogens as quickly as 10 sec in clean water (12). Clean water will likely be at a pH of 6.5 to 7.0 if the ORP value is 650 to 700 mV. Fresh-cut operators are recommended to not operate at an ORP value above 800 mV since high concentrations of wound exudates can be released into the water at levels above this (3).

Chemical oxygen demand (COD) is another measurement of water pollutants. Untreated wash water is generally high in organic matter which will feed the bacteria that may be present in the water. Understanding the oxygen level of the water will help processing operators to adjust their water treatments to ensure that microorganisms will not live and contaminate produce that may come in contact with it (1).

Investigating whether cantaloupes are being contaminated in the field or after harvest is important to understand. California field packages cantaloupes grown there. Georgia cantaloupes are brought to packing sheds to be packaged (7) Since handling practices differ in the two regions of the United States, it is important to determine microbial-related issues for each region. The objective of this study was to sample Georgia-grown cantaloupes for the enumeration of aerobic bacteria, coliforms/*E. coli*, and to isolate for the presence of *Salmonella* and *E. coli* O157:H7 on cantaloupes and to determine the amount of heterotrophic bacteria, coliforms, and *E. coli* found in the dump tank water used in the cantaloupe processing lines.

MATERIALS AND METHODS

Field Sampling

Cantaloupes were sampled from 4 south Georgia farms and packing sheds (sheds 1-4) at weekly intervals for four weeks. After harvesting the cantaloupes they were brought to packing sheds, washed, sorted, and hand packed into shipping boxes. Sheds 1 and 4 utilized dump tanks (approx. 25,000 gal) where trailers from the field were backed into the dump tank and the cantaloupes were floated off. These dump tanks contained chlorinated water. At sheds 2 and 3 cantaloupes were side-dumped off trailers into smaller dump tanks (approx. 5,000 gal) that contained heated water and chlorine. The water was heated at shed 2 to up to 57.2˚C and at shed 3 up to 36.7˚C. The cantaloupes stayed in the dump tank anywhere from 1 min to 5 min depending on being first or last off the trailer. After leaving the dump tank, the cantaloupes by way of conveyor belts, were taken through brushes and sprayers and sized for packing.

During each trip, cantaloupes were sampled in three places on the packing line. Twenty cantaloupes were randomly selected off the trailer from the field aseptically using sterile bags. Twenty cantaloupes were also randomly chosen after the melons came out of the dump tank and 20 more were randomly collected after packing in shipping boxes. Of the 20 cantaloupes collected at each location, 10 were collected in the morning approximately 2-3 h after start up and the other 10 early in the afternoon for all sheds for 4 weeks. Once the cantaloupes were placed in bags, 200 ml of 0.1% peptone water (Bacto peptone, Difco Labs, Division of Becton Dickinson and Co., Sparks, MD) was added and the melons were rinsed for 1 min with vigorous shaking. After rinsing, the cantaloupes were removed from the bags and the bags were placed in coolers until analyzed within 4 h in the lab.

Microbiological Analysis of cantaloupe

The 0.1% peptone water used to rinse the cantaloupes samples were analyzed for aerobic plate counts (APC). Serial dilutions were prepared and 1 ml of the dilutions were pipetted onto Aerobic Plate Count Petrifilm (3M Microbiology Products. St. Paul Minn.) in duplicates. APC petrifilms were incubated at 37°C. After 48 h, colony forming units (cfu) on the petrifilm were counted as per manufacturer's instructions. The peptone water was also analyzed for coliforms and *E. coli*. One ml of portions of rinsate or the serial dilutions prepared for the APC counts were pipetted onto coliform/*E. coli* Petrifilm (3M Mirobiology Products) in duplicates. The coliform/*E. coli* petrifilm was incubated at 35°C for 48 h. After incubation, colony forming units on the petrifilm plates were counted as per manufacturer's instructions.

To determine the presence or absence of *Salmonella* and *E. coli* O157:H7, 1 ml of each rinsate was transferred into 9 ml portions of lactose broth (Difco) and modified tryptic soy broth (modified TSB; 10.0g casamino acids, 1.5 g bile salts No. 3, 6.0 g dibasic, anhydrous sodium phosphate and 1.35 g potassium phosphate per liter of TSB; Difco) for *Salmonella* and *E. coli* O157:H7 enrichment, respectively. Both enrichment broths were incubated at 37°C for 24 h. Subcultures were also made from lactose broth into selenite cystine (Difco) and Rappaport-Vassiliadis R10 broths (RV; Difco) that were incubated at 37 and 42°C, respectively, for 24 h. After incubation of the broths, portions were streak plated onto bismuth sulfite agar (BSA; Difco), brilliant green agar (BGA; Difco) and XLT-4 agar (Difco) for possible *Salmonella* isolates. Plates were incubated at 35°C for 24 h and examined for the presence of presumptive colonies. Presumptive positives were inoculated onto triple sugar iron agar slants (TSI; Difco) and lysine iron agar slants (LIA; Difco). After incubation at 35°C for 48 h, slants were checked for *Salmonella* positives. Portions of the modified TSB cultures were streak plated onto sorbitol

MacConkey agar (SMAC; Oxoid; Basingstoke, Hampshire, England) plates. Plates were incubated at 37°C for 24 h and examined for the presence of representative colonies. Presumptive *Salmonella* spp. and *E. coli* O157:H7 isolates were identified using latex tests (Oxoid) as per manufacturer's instructions.

Microbiological analysis of dump tank water

Water for heterotrophic plate counts (HPC) and *E. coli*/coliform counts was collected aseptically using a 500 ml scoop. Two samples, once in the morning and once in the afternoon, were collected from the dump tank and sprayer during each trip to the sheds for four weeks. One-hundred and twenty ml of water collected from the dump tank and sprayers were put into sterile cups containing a sodium thiosulfate tablet to inactivate any chlorine present in the water. IDEXX's SimPlate (IDEXX Laboratories, Inc., Westbrook, Maine) for HPC method was used for the quantification of heterotrophic plate counts in the water as per manufacturer's instructions (2). IDEXX's Colisure® Test Kit (IDEXX, Inc.) was used to detect total coliforms and *E. coli* in the sample water as per manufacturer's directions.

Physical properties of dump tank water

Measurements for the following tests were taken directly from the dump tank and sprayer water once in the morning and afternoon at each shed for four weeks. Free and total chlorine measurements were taken as per the manufacturer's instructions with a hand held chlorine meter (model number HI 95711, Hanna Instuments, Woonscoket, RI). The oxygen reduction potential (ORP) was measured using a hand held QuiKcheK™ ORP pocket meter (Model 108, Thermo Orion, Beverly, MA). The pH of the water was measured using a QuiKcheK™ pocket pH meter (Model 106, Thermo Orion). The temperature of the water was taken with a hand held

QuiKcheK™ pocket temperature meter (Model 110, Thermo Orion). The chemical oxygen demand was measured as per manufacturer's directions using a COD meter (Thermo Orion).

Statistical analysis

Data gathered from the aerobic plate counts, *E. coli* counts, and coliform counts collected from the field, dump tank, and packing stages of the cantaloupe processing line for 4 farms for a total of 4 trips was entered into SAS software (Statistical Analysis Systems Institute; Cary, NC) for statistical modeling. General linear models using SAS software was used to correlate the data for the APC numbers. The data for *E. coli* was analyzed using logistic regression analysis using SAS software. Coliform data was analyzed by the analysis of variance, general linear model, using SAS software.

RESULTS

Aerobic plate counts enumerated from cantaloupes

Statistically the aerobic plate counts for cantaloupes sampled in the morning were not different ($p > 0.05$) from cantaloupes sampled from the afternoon. Total microbial aerobic populations for all 4 trips were averaged and are as follows: shed 1 stayed approximately the same from the field (log cfu/ml 6.88) to the packing step (log 6.7 cfu/ml). Shed 2 cantaloupes had aerobic plate counts of log 6.83 cfu/ml out of the field, log 6.89 cfu/ml from the dump tank, and log 7.00 cfu/ml at the end of the packing line. Shed 3 APCs ranged from log cfu/ml 6.92 from the field to log cfu/ml 7.15 after packing. Shed 4 had aerobic populations of log 6.83 cfu/ml out of the field, log 6.65 cfu/ml coming from the dump tank, and log 6.83 cfu/ml after packaging (Table 3.1). Microbial populations on cantaloupes sampled from the packing step from sheds 1 and 4 using chlorinated treatments were < 0.5 log lower than sheds utilizing heat and chlorine (sheds 2 and 3). However, aerobic populations after packing for sheds 1 and 4 were

approximately the same as that on the prewashed cantaloupes. Sites 1 and 4 showed a slight but significant decrease ($p \leq 0.05$) in microbial populations from the field to the dump tank. Shed 3 had a significantly larger ($p \leq 0.05$) microbial population coming out of the field compared to microbial populations enumerated after packaging. As seen with shed 2, exposing cantaloupe to water temperatures between 25°C and 57°C for 5 -10 min did not result in a significant reduction in microbial population sizes. For sheds 2 and 3, aerobic populations were slightly higher after packing than from initial harvest from the field.

***Escherichia coli* counts and coliform counts enumerated from cantaloupe samples**

E. coli and coliforms were enumerated from the cantaloupes to determine potential fecal contamination. Eighty-three percent of the cantaloupes sampled for *E. coli* resulted in detecting <15 cfu of *E. coli*/ml. The results for *E. coli* were analyzed by comparing a proportion of the samples that were <15 cfu/ml to samples having >15 cfu/ml. There was a significant difference ($p \leq 0.05$) between sheds 1 and 4 compared with sheds 2 and 3 for *E. coli* when each shed was compared individually (Table 3.2). There were also significant differences ($p \leq 0.05$) between the field, dump tank and packing steps at all four sheds (Table 3.2). Numbers of *E. coli* increased from the field to the dump tank and then again from the dump tank to the packing step for sheds 2 and 3. Shed 1 the counts decreased after the field and stayed roughly the same after the dump tank. Shed 4 counts decreased from the field to the dump tank, but increased after packing. For coliform counts, similar trends were found as that of *E. coli*.

Sheds 1 and 4 were significantly different ($p \leq 0.05$) than sheds 2 and 3 for coliform counts (Table 3.3). Coliform counts from shed 1 cantaloupes were shifted from log 1.05 cfu/ml out of the field to log 0.73 cfu/ml from the dumptank to log 1.57 cfu/ml after packing. Coliform counts on cantaloupes from shed 2 increased from log 1.27 cfu/ml out of the field to log 2.09

cfu/ml after washing in the dump tank to log 2.80 cfu/ml after packing. Shed 3 cantaloupes had less than 1 log /ml of coliforms coming out of the field but log 4.00 cfu/ml after packing. Cantaloupes from shed 4 also had less than 1 log coliforms/ml coming out of the field, were approximately the same after the dump tank but increased to log cfu/ml 2.38 after packing.

Isolation of *E. coli* O157:H7 and *Salmonella*

Cantaloupes were enriched for the isolation of *E. coli* O157:H7 and *Salmonella*. *E. coli* O157:H7 was not found on the cantaloupe samples. *Salmonella* was detected on one cantaloupe.

Heterotrophic plate counts enumerated from dump tank and sprayer water

Heterotrophic plate counts were enumerated from the dump tank and the sprayer at each packing shed for each sampling time. Microbial populations varied from shed to shed and for each trip (Tables 3.4 and 3.5). HPC from the dump tanks were higher than the counts from the sprayers. Microbial populations ranged from 15 MPN/ml of water from the dump tank at shed 4 to >355,000 MPN/ml from shed 2. HPC from sprayers ranged from < 2 MPN/ml of sprayer water at shed 4 to >114,600 cfu/ml of sprayer water at shed 2. There were increases in the HPC numbers as the day progressed from the morning samples to the afternoon samples of the dump tank water at shed 2. Shed 2's sprayer heterotrophs increased during the day for 3 out of the 4 sample times. The HPC counts for the morning samples versus the afternoon samples increased in both the dump tank and sprayer water for trips 1 and 3. There was a decrease in HPC for trip 4. Shed 4 HPC were consistent in the dump tank and sprayer water for all 4 trips Shed 3 did not use sprayers. At shed 3, the heterotroph count in the dump tank water decreased considerably between the morning and afternoon sampling during the first trip, but remained relatively consistent for trips 2 and 3. The fourth trip samples for this shed were not taken due to the fact the shed was not in operation.

***E. coli*/ Coliform counts enumerated from dump tank and sprayer water**

E. coli populations ranged from < 1.0 MPN/ml to >2,419.6 MPN/ml of dump tank water from all the sheds (Table 3.8). Except for one sampling the sprayer water contained < 1 *E. coli* per ml of water (Table 3.9). Shed 4 had the lowest *E. coli* population out of all the sheds for both the dump tank and sprayer water with the highest counts from shed 2.

Coliform counts were also greater in the dump tank water compared to the sprayer water. Coliform populations ranged from <1.0 MPN/ml to > 2,419.6 MPN/ml from the dump tank and sprayer water (Tables 3.6 and 3.7). With the exception of one sampling, shed 4 had the lowest coliform population overall out of the 4 sheds. Shed 1 had an increase in coliform counts for the first trip for the dump tank and sprayer water. Sheds 2 and 3's dump tank water was contaminated the greatest with coliform populations. Shed 2 also showed an increase in coliforms in the sprayer water 2 out 4 trips.

Physical properties of the dump tank and sprayer water

Free and total chlorine levels from the dump tank and sprayers were measured at each shed (Tables 3.10-3.13). Free chlorine ranged from undetectable to 34.3 ppm. The total chlorine from the dump tank and sprayers ranged of undetectable to 50.0 ppm. ORP was also measured and ranged from 151mV to 724mV (Tables 3.14 and 3.15). The ORP numbers increased as the *E. coli* numbers decreased (Table 3.16). The pH and COD of the dump tank and sprayer water are shown in Tables 3.17 and 3.18 and 3.19 and 3.20 respectively. The COD range in ml/l of the dump tank and sprayer water was from undetectable to 531. The dump tank and sprayer water temperatures were also measured and are recorded in Tables 3.21 and 3.22 respectively.

DISCUSSION

Cantaloupes can be contaminated with microorganisms at many different places as they travel from the field to the packing shed (5). Microbial contamination of cantaloupes could also come from the dump tank or sprayer waters that are used to remove any microbes coming from the field. Since cantaloupes are consumed raw or minimally processed they pose an increased food safety risk if contaminated with foodborne pathogens (9). This project addressed the microbial contamination as cantaloupes are brought from the field, washed in dump tanks, and packed and also looked at possible contamination of cantaloupes as a result of the dump tank and sprayer waters.

Chlorine can be an effective way to reduce microbial populations on produce (3). As cantaloupes were received at the packinghouse, aerobic bacteria and coliform numbers on the cantaloupes decreased after the dump tank for step for two of the four packing sheds (sheds 1 and 4). For chlorine to be effective as an antimicrobial there needs to be at least 2 ppm of free chlorine available (3), as was the case with sheds 1 and 4. When looking at the *E. coli,* populations on the cantaloupe surfaces decreased after the dump tank step for shed 4 but stayed approximately the same for shed 1. The coliform counts increased for sheds 1 and 4 after the dump tank. The *E. coli* counts for shed 1 decreased after the dump tank step and increased for shed 4. Contamination of cantaloupes after the dump tank is a problem that should be addressed since the chlorine is effective in reducing microorganisms. Contamination after the dump tank negates the chlorines effectiveness. Studies looking at conveyor belt sanitation and worker sanitation should be preformed to see why the microbial numbers are increasing.

For sheds 1 and 4 the sprayers after the dump tank used clean water and OxiDate, respectively. OxiDate is a broad-spectrum bactericide/fungicide that was formulated to help

fruit, nut and vegetable growers (6). The OxiDate used in the sprayer water gave consistently low microbial population numbers. The one high coliform number found from shed 4 suggests that the OxiDate may have needed to be added. The fresh water used in the sprayer in shed 1 would not reduce any microbial populations that may be found in the water.

Sheds 1 and 4 had lower numbers of heterotrophs, *E. coli*, and coliforms in the dump tank water. The chlorine added to the water would have killed any microbes coming from the shed's water supply. The water supply for both of these sheds came from a pond that is feed by a deep well. During the course of the day though, the numbers varied, in some cases increasing and in some cases decreasing at both sheds.

When the physical properties of the dump tank water was measured it was found that when the ORP levels were high, the *E. coli* counts were low as with sheds 1 and 4, with the exception of one trip for shed 4. The outlier shows a high ORP value but also a high *E. coli* population (Table 3.16). Microbes can lower the Eh of their environment (8). An effective ORP value for killing or inhibiting microorganisms is between 650 to 700 mV. Microorganisms can also affect the pH of the dump tank water (3). For wash water, a pH of greater than 7.5 will not have active chlorine and a pH below 4.0 the chlorine turns into gas. Sheds 1 and 4 pH values were high enough to start causing the chlorine to be inactive, which would allow microbial survival. The higher pH values explain why at times sheds 1 and 4 had just enough free chlorine available for effectiveness. Low microbial populations can also be explained when the COD is analyzed. Untreated wash and chill water is generally rich in organic matter which will feed any microbes found. Sheds 1 and 4 had low COD values in the dump tank. Enough free chlorine, a high ORP value, a low COD value, and a pH range just outside of normal, as in the case of sheds

1 and 4, shows the physical properties of the wash water helped explain the reduction of microbial populations found.

Shed 2's heated water treatment was not as effective as the chlorine used at sheds 1 and 4. Studies have shown that at high enough temperatures microbes on cantaloupe surfaces will be killed. One study showed a 4.4 log reduction of *Salmonella* on cantaloupes when treating the cantaloupes to 97°C water for 60 s (13). Shed 2's equipment took a lengthy amount of time to heat the water and was very hard to regulate and maintain a constant temperature once up to 2500 cantaloupes at ambient temperature (approximately 32°C) per load were added to the water. To be effective the water would have to be increased to a higher temperature than desired and would have to be monitored during the processing hours to insure a lethal treatment was applied. Increasing water temperature would also increase energy costs thus increasing processing expenses.

The water heated for the dump tank at shed 2 came from a deep well. The heat treatment did not result in a decrease in microbial populations. Trace amounts of chlorine were found in the dump tank water. There was not enough free chlorine available to reduce the microbes. The ORP values were low, which suggests that the chlorine level in the dump tank was also low. Shed 2 had high COD values which reiterates the higher microbial populations that were found.

There was free chlorine in the water coming from the sprayers used at shed 2. The water temperature of the sprayer was not hot enough to gas off the chlorine found in the water. The ORP values were also in range to inhibit microorganisms. The sprayers applied a mist of chlorinated water to the tops of the cantaloupes and were not able to coat the entire surface of the melons. If the sprayer water could reach all areas of the melons and the contact time was longer then the chlorine may have helped reduce the microbial numbers found at shed 2.

Shed 3's microbial counts increased after cantaloupes left the dump tank and were packaged. Shed 3 utilized chlorine and hot water for the dump tank treatments of the cantaloupe. When enough heat, approximately 82°C, is used in combination with chlorine the chlorine gases off and is no longer effective as an antimicrobial (3). Shed 3's temperatures may have been high enough to gas off some of the chlorine added to the dump tank. The water temperature at shed 3 reached up to 57˚C and was not successful in reducing microbial numbers on the cantaloupe surfaces. After looking at the chlorine readings there was enough free chlorine for 3 trips at this shed to be effective in reducing microbial numbers. The chlorine levels though, decreased as the day progressed which suggests that the chlorine level was not maintained consistently during the day. There is no reason to use both treatments in the dump tank. Chlorine alone would help reduce the microbial populations as seen with sheds 1 and 4.

The ORP measurements for the morning sampling at shed 3 were within a range that suggests there should have been lower microbial counts. The afternoon cantaloupe samples lowered the ORP readings. There was a buildup of microbes in the dump tank water by the second sample taken in the afternoon. Another measurement to explain the increase in microbial populations of shed 3 comes from the COD readings. For the 2 readings taken from the dump tank one was a lower COD reading than the other. The higher value corresponds to the higher ORP reading. The pH of the dump tank water was slightly higher than the recommended 6.5 to 7.5 range. This pH range would have affected the amount of free chlorine available for inhibiting microorganisms. The sprayers for shed 3 were not operating during sampling visits.

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Table 3.1. Average mesophilic aerobic populations (log CFU/ml of rinse) on cantaloupe surfaces sampled from the field, dump tank, and packing stages at four different packing sheds in Georgia (n=4).

	Field	Dump tank	Packing
Shed 1	6.88	6.76	6.76
Shed 2	6.83	6.89	7.00
Shed 3	6.92	6.91	7.15
Shed 4	6.83	6.65	6.83

Table 3.2. Proportion of cantaloupe samples positive for *E. coli* (> 15 cfu/ml) to those that were negative (<15cfu/ml).

	Field	Dump tank	Packing
Shed 1	$_{x}0.114_{b}$	$_{y}0.129_{b}$	$_{z}0.072_{b}$
Shed 2	$_{x}0.125_{a}$	$_{y}0.200_{a}$	$_{z}0.329_{a}$
Shed 3	$_{x}0.000_{a}$	$_{y}0.133_{a}$	$_{z}0.583_{a}$
Shed 4	$_{x}0.128_{b}$	$_{y}0.088_{b}$	$_{z}0.138_{b}$

$_{x\,y\,z}$ Letters in the same row that differ indicates significant differences ($p \leq 0.05$).
$_{a\,b}$ Letters in the same column that differ indicates significant differences ($p \leq 0.05$).

Table 3.3. Frequency of distribution of coliform bacteria (log cfu/ml of rinse) on cantaloupe surfaces sampled from the field, dump tank, and packing stages at four different packing sheds in Georgia.

	Field						Dump tank						Packing					
	<1	1-2	2-3	3-4	4-5	5-6	<1	1-2	2-3	3-4	4-5	5-6	<1	1-2	2-3	3-4	4-5	5-6
Shed 1	44	10	10	3	3	0	50	9	10	1	0	0	32	17	4	12	5	0
Shed 2	42	18	8	8	3	1	11	32	15	16	5	1	6	14	24	11	22	2
Shed 3	43	5	7	2	3	0	25	12	17	4	2	0	0	0	5	21	33	1
Shed 4	56	8	7	5	1	3	54	8	9	3	6	0	23	12	21	4	9	11

Table 3.4 Most probable number heterotrophic plate counts per ml of dump tank water used in 4 cantaloupe packing sheds over the course of two sampling trips. Trips 3 and 4 are continued on the following page.

	Dump tank							
	Trip 1				Trip 2			
	A.M.	95% confidence limit	P.M.	95% confidence limit	A.M.	95% confidence limit	P.M.	95% confidence limit
Shed 1	209	159-273	2,990	2,290-3,900	>738	>476->1146	–[b]	-
Shed 2	50,700	37,100-69,500	>73,800	>47,600->114,600	623	432-899	>738	>476->1,146
Shed 3	>73,800	>47,600->114,600	342	-	>738	>476->1,146	>738	>476->1,146
Shed 4	324	248-425	156	117-207	>738	>476->1,146	231	177-302

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.4 cont. Most probable number heterotrophic plate counts per ml of dump tank water used in 4 cantaloupe packing sheds over the course of two sampling trips.

	Dump tank							
	Trip 3				Trip 4			
	A.M.	95% confidence level	P.M.	95% confidence level	A.M.	95% confidence level	P.M.	95% confidence level
Shed 1	19	10 36	355	270-466	90	64-126	30	18-51
Shed 2	>7,380	>4,760->11,460	>7,380	>4,760->11,460	55,500	39,800-77,500	355,000	270,000-466,000
Shed 3	1,000	730-1,390	>7,380	>4,760->11,460	–[b]	-	-	-
Shed 4	26	15-45	56	38-84	17	8 -33	15	7-30

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.5. Most probable number heterotrophic plate counts per ml of sprayer water used in 4 cantaloupe packing sheds over the course of two sampling trips. Trips 3 and 4 are continued on the following page.

	Sprayer							
	Trip 1				Trip 2			
	A.M.	95% confidence level	P.M.	95% confidence level	A.M.	95% confidence level	P.M.	95% confidence level
Shed 1	43	27-56	>73,800	>47,600- >114,600	555	398-775	–[b]	-
Shed 2	>73,800	>47,600- >114,600	41,400	31,100-55,100	30	18-51	>738	>476->1146
Shed 3	-	-	-	-	-	-	-	-
Shed 4	28	16-48	38	23-61	53	35-80	2	0.3-14

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.5. cont. Most probable number heterotrophic plate counts per ml of sprayer water used in 4 cantaloupe packing sheds over the course of two sampling trips.

	Sprayer							
	Trip 3				Trip 4			
	A.M.	95% confidence level	P.M.	95% confidence level	A.M.	95% confidence level	P.M.	95% confidence level
Shed 1	507	371-695	>738	>476->1,146	1,000	730-1,390	26	15-45
Shed 2	10	4 25	30	18-51	12	6-27	26	15-45
Shed 3	–[b]	-	-	-	-	-	-	-
Shed 4	<2	<0.3-<14	6	2-9	2	0.3-14	<2	<0.3-<14

[a] A.M./P.M.=morning/afternoon

–[b] measurement not take

Table 3.6. Most probable number of coliforms per ml of dump tank water used in 4 cantaloupe packing sheds over the course of two sampling trips. Trips 3 and 4 are continued on the following page.

	Dump tank							
	Trip 1				Trip 2			
	A.M.	95% confidence level	P.M.	95% confidence level	A.M.	95% confidence level	P.M.	95% confidence level
Shed 1	201.4	135.7-284.0	>2419.6	1439.5-infinite	2	0.3-5.6	–[b]	-
Shed 2	461.1	292.7-687.9	>2419.6	1439.5-infinite	>2419.6	1439.5-infinite	>2419.6	1439.5-infinite
Shed 3	>2419.6	1439.5-infinite	>2419.6	1439.5-infinite	>2419.6	1439.5-infinite	>2419.6	1439.5-infinite
Shed 4	2	0.3-5.6	<1	0.0-3.7	95.8	72-125	<1	0.0-3.7

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.6. cont. Most probable number of coliforms per ml of dump tank water used in 4 cantaloupe packing sheds over the course of two sampling trips.

	Dump tank							
	Trip 3				Trip 4			
	A.M.	95% confidence level	P.M.	95% confidence level	A.M.	95% confidence level	P.M.	95% confidence level
Shed 1	<1	0.0-3.7	18.7	11.6-28.2	7.3	2.9-13.9	<1	0.0-3.7
Shed 2	>2419.6	1439.5-infinite	>2419.6	1439.5-infinite	>2419.6	1439.5-infinite	>2419.6	1439.5-infinite
Shed 3	1553.1	1016.2-2353.1	>2419.6	1439.5-infinite	–[b]	-	-	-
Shed 4	<1	0.0-3.7	<1	0.0-3.7	3.1	0.7-8.9	3.1	0.7-8.9

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.7. Most probable number of coliforms per ml of sprayer water used in 4 cantaloupe packing sheds over the course of two sampling trips. Trips 3 and 4 are continued on the following page.

	Sprayer							
	Trip 1				Trip 2			
	A.M.	95% confidence level	P.M.	95% confidence level	A.M.	95% confidence level	P.M.	95% confidence level
Shed 1	<1	0.0-3.7	>2419.6	1439.5-infinite	62.4	45.7-82.3	–[b]	-
Shed 2	2	0.3-5.6	<1	0.0-3.7	4.1	1.2-9.0	201.4	135.7-284.0
Shed 3	-	-	-	-	-	-	-	-
Shed 4	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7	1732.9	1167.7-2709.5

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.7. cont. Most probable number of coliforms per ml of sprayer water used in 4 cantaloupe packing sheds over the course of two sampling trips.

	Sprayer							
	Trip 3				Trip 4			
	A.M.	95% confidence level	P.M.	95% confidence level	A.M.	95% confidence level	P.M.	95% confidence level
Shed 1	5.2	2.3-11.9	13.2	7.1-22.0	33.6	24.6-44.4	–[b]	-
Shed 2	<1	0.0-3.7	15.3	8.5-25.1	<1	0.0-3.7	2	0.3-5.6
Shed 3	-	-	-	-	-	-	-	-
Shed 4	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.8. Most probable number of *E. coli* counts per ml of dump tank water used for 4cantaloupe packing sheds over the course of two sampling trips. Trips 3 and 4 are continued on the following page.

	Dump tank							
	Trip 1				Trip 2			
	A.M.	95% confidence level	P.M.	95% confidence level	A.M.	95% confidence level	P.M.	95% confidence level
Shed 1	<1	0.0-3.7	435.2	276.2-650.0	<1	0.0-3.7	–[b]	-
Shed 2	193.5	145.6-251.4	>2419.6	1439.5-infinite	222.4	158.5-303.3	35.4	25.2-47.8
Shed 3	7.2	3.0-13.7	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7
Shed 4	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.8. cont. Most probable number of *E. coli* counts per ml of dump tank water used for 4 cantaloupe packing sheds over the course of two sampling trips.

	Dump tank							
	Trip 3				Trip 4			
	A.M.	95% confidence level	P.M.	95% confidence level	A.M.	95% confidence level	P.M.	95% confidence level
Shed 1	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7
Shed 2	>2419.6	1439.5-infinite	>2419.6	1439.5-infinite	4.1	1.7-9.5	139.6	99.5-190.0
Shed 3	<1	0.0-3.7	<1	0.0-3.7	–[b]	-	-	-
Shed 4	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.9. Most probable number of *E. coli* counts per ml of sprayer water used for 4 cantaloupe packing sheds over the course of two sampling trips. Trips 3 and 4 are continued on following page.

	Sprayer							
	Trip 1				Trip 2			
	A.M.	95% confidence level	P.M.	95% confidence level	A.M.	95% confidence level	P.M.	95% confidence level
Shed 1	<1	0.0-3.7	387.3	245.9-567.0	<1	0.0-3.7	–[b]	-
Shed 2	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7
Shed 3	-	-	-	-	-	-	-	-
Shed 4	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.9.cont. Most probable number of *E. coli* counts per ml of sprayer water used for 4 cantaloupe packing sheds over the course of two sampling trips.

	Sprayer							
	Trip 3				Trip 4			
	A.M.	95% confidence level	P.M.	95% confidence level	A.M.	95% confidence level	P.M.	95% confidence level
Shed 1	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7	–[b]	-
Shed 2	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7
Shed 3	-	-	-	-	-	-	-	-
Shed 4	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7	<1	0.0-3.7

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.10. Free chlorine (ppm) readings from the dump tank water used during cantaloupe processing that was measured at four south Georgia packing sheds for 4 trips.

	Dump tank							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M. [a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	3.0	u[b]	3.5	-[c]	28.1	3.1	11.9	12.5
Shed 2	u	u	0.14	-	u	0.1	0.1	0.03
Shed 3	3.0	0.0	5.0	5.0	25.3	0.1	-	-
Shed 4	20.0	20.0	1.61	5.0	37.0	u	35.0	22.3

[a] A.M./P.M.=morning/afternoon
u[b] undetectable
-[c] measurement not taken

Table 3.11. Free chlorine (ppm) readings from the sprayer water used during cantaloupe processing that was measured at four south Georgia packing sheds for 4 trips.

	Sprayer							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M. [a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	3.0	u[b]	u	-[c]	u	u	u	-
Shed 2	3.0	3.0	4.1	-	2.45	2.28	3.2	2.9
Shed 3	-	-	-	-	-	-	-	-
Shed 4	-	-	-	4.9	10.7	11.3	34.3	25.4

[a] A.M./P.M.=morning/afternoon

u[b] undetectable

-[c] measurement not taken

Table 3.12. Total chlorine (ppm) readings measured from dump tank water at 4 sheds from south Georgia for 4 trips.

	Dump tank							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M.[a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	3.0	1.0	3.9	-[b]	26.4	3.6	12.5	14.1
Shed 2	u[c]	u	1.29	-	0.4	0.4	2.8	2.5
Shed 3	3.0	3.0	5.0	5.0	41	2.3	-	-
Shed 4	50.0	50.0	5.0	4.1	36.0	u	42.0	24.4

[a] A.M./P.M.=morning/afternoon

-[b] measurement not taken

u[c] undetectable

Table 3.13. Total chlorine (ppm) readings measured from sprayer water at 4 sheds from south Georgia for 4 trips.

	Sprayer							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M.[a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	3.0	u[b]	u	-[c]	u	u	u	-
Shed 2	6.0	6.0	4.5	-	2.65	2.51	3.2	3.1
Shed 3	-	-	-	-	-	-	-	-
Shed 4	-	-	-	5.0	20.7	17.4	34.1	27.9

[a] A.M./P.M.=morning/afternoon

u[b] undetectable

-[c] measurement not taken

Table 3.14. Oxygen reduction potential measurements in mV for dump tank water for 4 trips to cantaloupe packing sheds in south Georgia.

	Dump tank							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M.[a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	–[b]	-	660	-	685	678	685	693
Shed 2	-	-	270	310	151	193	310	176
Shed 3	-	-	685	385	683	295	-	-
Shed 4	-	-	680	669	704	696	690	694

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.15. Oxygen reduction potential measurements in mV for sprayer water for 4 trips to cantaloupe packing sheds in south Georgia.

	Sprayer							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M.[a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	–[b]	-	303	-	420	410	212	-
Shed 2	-	-	594	239	556	550	656	656
Shed 3	-	-	-	-	-	-	-	-
Shed 4	-	-	-	-	708	692	724	714

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.16. Oxygen reduction potential (ORP) (mV) values compared to the morning and afternoon samples of cantaloupes from south Georgia farms with *E. coli* counts >15 mpn/ml.

			ORP	
	Trip	Number of Cantaloupes with *E. coli* counts > 15 mpn/ml	A.M.	P.M.
Shed 1	1	3	-[a]	-
Shed 1	2	2	660	660
Shed 1	3	3	685	678
Shed 1	4	1	685	693
Shed 2	1	3	-	-
Shed 2	2	4	270	310
Shed 2	3	5	151	193
Shed 2	4	4	310	176
Shed 3	1	1	-	-
Shed 3	2	2	685	385
Shed 3	3	5	683	295
Shed 3	4		-	-
Shed 4	1	0	-	-
Shed 4	2	6	680	669
Shed 4	3	0	704	696
Shed 4	4	1	690	694

-[a] measurement not taken

Table 3.17. pH reading from dump tank water from 4 trips to Georgia cantaloupe packing sheds.

	Dump tank							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M.[a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	7.9	8.4	8.0	–[b]	8.7	8.6	8.1	8.0
Shed 2	7.5	7.4	8.0	8	7.7	7.6	7.7	7.6
Shed 3	7.7	8.1	8.7	8.3	8.7	8.2	-	-
Shed 4	8.5	8.3	8.0	8.1	8.6	8.7	8.1	8.0

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.18. pH reading from the sprayer water from 4 trips to Georgia cantaloupe packing sheds.

	Sprayer							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M.[a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	–[b]	-	7.6	-	8.3	8.2	7.8	-
Shed 2	7.3	7.2	8.1	8.2	7.6	7.6	7.7	7.7
Shed 3	7.7	-	-	-	-	-	-	-
Shed 4	-	-	-	8.3	-	-	8.0	7.9

[a] A.M./P.M.=morning/afternoon

–[b] measurement not taken

Table 3.19. Chemical oxygen demand in mg/l for 4 trips from the dump tank water measured at four cantaloupe packing sheds.

	Dump tank							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M.[a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	-[b]	-	88	-	3	15	u[c]	u
Shed 2	-	-	170	131	57	81	291	291
Shed 3	-	-	531	98	129	u	-	-
Shed 4	-	-	80	184	9.0	30.0	u	u

[a] A.M./P.M.=morning/afternoon

-[b] measurement not taken

u[c] undetectable

Table 3.20. Chemical oxygen demand in mg/l for 4 trips from the sprayer water measured at four cantaloupe packing sheds.

	Sprayer							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M.[a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	-[b]	-	u[c]	-	u	4	u	u
Shed 2	-	-	17.0	u	36	15	u	u
Shed 3	-	-	-	-	-	-	-	-
Shed 4	-	-	34	71	7.0	u	u	5.0

[a] A.M./P.M.=morning/afternoon

-[b] measurement not taken

u[c] undetectable

Table 3.21. Water temperatures (˚C) of the dump tank measured twice a day for 4 cantaloupe packing sheds.

	Dump tank							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M. [a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	25.4	26.2	37.7	-[b]	24.3	26.8	23.9	n/a
Shed 2	57.2	45.6	42.2	46.4	41.3	45.0	24.6	43.6
Shed 3	32.5	41.2	26.5	31.2	36.7	35.7	-	-
Shed 4	26.2	26.8	27.4	27.7	26.3	27.5	25.0	26.3

[a] A.M./P.M.=morning/afternoon

-[b] measurement not taken

Table 3.22. Water temperatures (˚C) of the sprayers measured twice a day for 4 cantaloupe packing sheds.

	Sprayer							
	Trip 1		Trip 2		Trip 3		Trip 4	
	A.M.[a]	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Shed 1	-[b]	-	24.4	-	23.1	24.0	-	-
Shed 2	27.4	22.2	23.2	-	22.9	23.2	23.0	23.0
Shed 3	-	-	-	-	-	-	-	-
Shed 4	-	-	-	-	24.9	24.8	25.7	25.2

[a] A.M./P.M.=morning/afternoon

-[b] measurement not taken

CHAPTER 4

CONCLUSION

Sheds 2 and 3 statistically ($p \leq 0.05$) had higher microbial populations when compared with sheds 1 and 4 counts that were taken at all steps of the packing line. This indicates that chlorine alone was more effective than the heat treatment used in this study. The chlorine though, does not affect the increase in numbers after leaving the dump tank. Sanitary conditions after the cantaloupes leave the dump tank should be monitored to prevent additional microbial contamination. Conveyor belts should be washed and sanitized and workers should follow proper hand washing techniques. Further studies should look at sanitary conditions of conveyor belts and worker sanitation to understand exactly why cantaloupe microbial populations increase after leaving the dump tank.