THE EFFICACY OF CHITOSAN-BASED COATING IN REDUCING SURFACE ATTACHED *SALMONELLA* ON TOMATOES

By

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ABSTRACT OF THE THESIS

The Efficacy of Chitosan-based Coating in Reducing Surface Attached *Salmonella* on Tomatoes

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Recently, an increasing number of outbreaks related to produce consumption have been reported. Tomatoes are considered as one of the main produce consumed in the United States; approximately 14 million tons of tomatoes are produced every year. However, the microbial safety of raw tomatoes has been questioned following many outbreaks associated with eating tomatoes contaminated with human pathogens. The CDC reports that eating raw tomatoes caused at least 12 *Salmonella* multistate outbreaks from 1990-2009, and resulted in over 2,000 illnesses. Therefore, developing a novel and alternative method to improve the microbial safety and quality of raw tomatoes is necessary.

In the U.S., post-harvest tomatoes are often washed in chlorinated water (50-150 ppm) to remove debris, soil and prevent cross-contamination, with an additional effect of

lowering the microbial load on the tomatoes. However, previous studies showed that tomatoes washed with chlorinated water (320 ppm) failed to completely inactivate *Salmonella*. Chitosan is a non-toxic, bio-degradable and antimicrobial compound, which has been reported for its efficacy of extending the shelf-life and reducing the microbial decay of fresh produce. The objective of this study is to investigate the efficacy of chitosan-based coating against surface attached *Salmonella* and natural microbiota on fresh tomatoes.

All tomatoes were inoculated with *Salmonella*. After drying, tomatoes were washed in 100 ppm chlorinated water for 90 s. Tomatoes were then dipped in either 0.2% or 1.0% chitosan solution for 90 s. Controls were only dipped in tap water. Change in population of *Salmonella* and total aerobic microbes (Aerobic Plate Count, APC) were evaluated at pre-determined times. Results showed that the population of *Salmonella* and total aerobic microbes with the treatment of 1.0% chitosan solution following 100 ppm chlorinated wash step provided immediate inactivation on day 0, compared to control. On day 1 and day 5 post-treatment, the population of *Salmonella* and total aerobic microbes on tomatoes both increased during the five days of storage, except on tomatoes treated with 1.0% chitosan solution following 100 ppm chlorine wash step. Future research should consider evaluating up to 4.0% chitosan solutions for controlling microbial growth and extending the shelf-life of tomatoes.

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1. INTRODUCTION

Salmonella is a major foodborne pathogen found throughout the world. According to the CDC, *Salmonella* is estimated to cause about 1.2 million illnesses in the United States, and over 20,000 patients require hospitalizations every year (CDC, 2014). Salmonellosis is considered a "self-limiting" disease but can result in death in certain populations.

A growing number of outbreaks related to consuming fresh produce have been reported, and many of them were associated with *Salmonella* contamination. Based on the FDA data, from 1990 to 2009, there were at least 12 multistate *Salmonella* outbreaks related to consumption of contaminated raw tomatoes that resulted in approximately 2,000 illnesses (Valadez, Schneider, & Danyluk, 2012; U.S. Food and Drugs Administration, 2011). Therefore, to enhance the microbial safety and quality of raw tomatoes, alternative and novel methods are needed.

Chitosan is an edible, bio-degradable, non-toxic and antimicrobial compound, which is derived from chitin. Previous research suggested that chitosan edible coating effectively inhibited foodborne pathogens, yeast and mold, on raw fruits and vegetables. Moreover, chitosan coating may improve the quality of fresh produce, by reducing weight loss and maintaining tissue firmness during storage and transport.

In the U.S., tomatoes are often washed in chlorinated water (50 to 150 ppm) to remove debris and soil. Although the process is designed to prevent cross-contamination

between tomatoes and wash water, an additional effect may cause the reduction of microbial load on the tomatoes. However, previous studies indicated that washing tomatoes with water or water added with chlorine-derived compound may only result in 1-2 log reductions of microbial load (Gomez-Lopez, 2012). A previous study, which used up to 320 ppm chlorinated water didn't achieve complete inactivation of surface attached *Salmonella* on raw tomatoes (Zhuang, Beuchat,& Anhulo, 1995).

Currently, the efficacy of multiple wash steps with the combination of chlorine and chitosan in improving microbial safety and quality of fresh produce hasn't been fully explored. In this study, the efficacy of chitosan-based coating with different combination of either water or chlorinated water was evaluated for improving aspects of microbial safety and quality on fresh tomatoes.

2. HYPOTHESIS

The hypothesis of this study: chitosan can improve the microbial safety and extend the shelf-life of tomatoes.

The specific objectives are:

(1) Evaluate the antimicrobial activity of chitosan against *Salmonella* on tomatoes.

(2) Determine the influence of chitosan treatment on shelf-life and quality of tomatoes.

3. LITERATURE REVIEW

3.1 Tomato industry

Tomatoes are one of the most common produce in the supermarket, and they are also the most widely grown fresh fruit in the United States. In the U.S., the yearly production of fresh tomatoes is up to 14 million tons. California and Florida are the two largest commercial tomato producer in the U.S.; accounting for two-thirds to three-fourths of tomato production per year (U.S. Department of Agriculture, 2012). According to the United Nations International Trade Center, Mexico and Canada ranked as the top two countries for tomato production, and export tomatoes to the United States (Ramos-García et al., 2012).

Tomatoes are known for its various nutrition contents, such as lycopene, vitamin C, and E, potassium, digestible and non-digestible fibers, which are beneficial to the human body (Du et al., 2009). Tomatoes ranked as the first source of lycopene (75.1%) and the second source as vitamin C (12.0%) (García-Valverde, Navarro-González, García-Alonso, & Periago, 2011). Even though around 90% of tomatoes are processed into tomato-based products such as ketchup, soup and ready-to-eat food; the demand of eating fresh tomatoes has been gradually increased during the last two decades. In terms of the U.S. per capita consumption of fresh tomatoes, it has increased by 23% between 1993-1995 and 2003-2005 (Seale Jr, Zhang, & Traboulsi, 2013). According to latest data from FAO 2014, in the North America, per capita consumption of vegetables is around 120 kg per year; the tomato consumption is taking 40 kg out of total vegetable consumption (Garmin, 2014).

Tomatoes are considered as temperature sensitive produce, chilling injury can occur when the temperature is below 13℃ during long-term storage and transport (Biswas, East, Brecht, Hewett, & Heyes, 2012), or growing temperature during the cool night. The best temperature range for growing seasons is between 16°C to 32°C, temperature out of this range may cause some negative effects on the quality of tomatoes such as growth and maturation delay (Orzolek et al., 2006).

In the tomato industry, tomatoes are harvested at mature-green stage and then all tomatoes are sent to multiple wash steps with water or chlorinated water to remove soil or organic material on the surface (Figure 1). After drying, tomatoes are sent through waxing process and sorted by its size and color according to federal grading standard (Table 1) for field-grown tomatoes. Currently, tomatoes packers pack only 5X6, 6X6 and 6X7 into cartons with net weight of 25 pounds ("Commercial Tomato Production Handbook," 2012). After packaging, tomatoes are shipped to retail stores.

For tomato handling practice at retail stores or home, fresh tomatoes should be keep at room temperature rather than refrigerating. Refrigeration of tomatoes may result in development of fewer flavors. Fresh tomatoes must be rinsed with tap water before serving, and they must not be washed with detergent (Simonne, 2013).

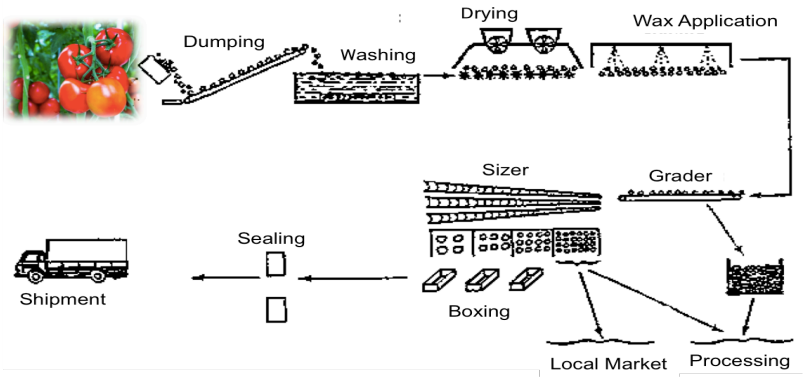


Figure 1. Tomato house operation.

(Source: FAO, 1986)

Table 1. USDA size classifications for field harvested tomatoes

Classification	Minimum Diameter[1]	Maximum Diameter[2]	Carton Size/ Arrangement[3]
Small	5.40 cm	5.79 cm	7X7
Medium	5.72 cm	6.43 cm	6X7
Large	6.35 cm	7.06 cm	6X6
Extra Large	7.00 cm	----	5X6

(Source: Commercial Tomato Production Handbook, 2012)

[1] will not pass through a round opening of the designated diameter when tomato is placed with the greatest transverse diameter across the opening.

[2] will pass through a round opening of the designated diameter in any position.

[3] Designates numbers of rows of tomatoes in top layer.

3.2 *Salmonella*

In the United States, *Salmonella* is one of the most prevalent and notorious foodborne pathogens, which has become a major concern of food safety for over 20 years. The CDC reported that *Salmonella* caused around 1,000,000 illnesses, up to 20,000 hospitalizations and 380 deaths from 2000-2008 (CDC, 2012). *Salmonella* was resulted in the second most foodborne illness behind norovirus. Jackson et al. (2013) reported that there is a great diversity of food associated with *Salmonella* contamination, which include poultry, eggs, juice, fruits and vegetables.

Salmonella spp., are rod-shape and facultative anaerobic gram-negative bacteria. There are only two species (*S. bongori* and *S. enterica)* of *Salmonella* but with over 2,500 serotypes confirmed. Over 2,400 serotypes are belonging to *Salmonella enterica* (Costa, Paixão, Tsolis, Bäumler, & Santos, 2012) . *Salmonella* can survive and even adapted to extreme conditions, the growing temperature and pH for *Salmonella* is between 4°C-54°C (optimum: 37°C) and 4.5-9.5 (optimum: 6.5-7.5), respectively. (Montiville, Matthews, & Kniel, 2012)

Salmonellosis, the disease caused by *Salmonella* infection, has the symptoms of diarrhea, abdominal pain and fever. The disease onset time is usually 12-36 h and the illness may last a week. *Salmonella* is generally regarded as a "self-limiting " disease. However, for people with compromised immune system such as young children and elderly people, salmonellosis may become a fetal disease (World Health Organization, 2013).

3.3 Tomato outbreaks

The consumption of fresh produce has increased dramatically because of the dietary trend and public health interest. Public health organizations from the United States and many other countries have encouraged consuming at least five daily servings of fresh fruits and vegetables, to lower the risk of developing chronic diseases (Abadias, Usall, Anguera, Solsona, & Viñas, 2008; Van Boxstael et al., 2013). However, a large number of outbreaks have been reported related to eating contaminated fresh produce (Beuchat, 2002). The CDC reported that most foodborne illness were associated with plant commodities, produce accounted for 46% of the total. Reports indicate that the produce is often contaminated with norovirus, *E.coli* and *Salmonella* (Gould et al., 2013; Painter et al., 2013). Improving the microbial safety by preventing the contamination of raw produce by foodborne pathogens is required necessary in the produce industry.

Recently, the safety of eating raw tomatoes has become a concern in the United States. From 1973-2010, a total of 15 multistate outbreaks associated to raw tomato was noted which resulted in 1,952 confirmed illnesses and 384 hospitalizations (Bennett, Littrell, Hill, Mahovic, & Behravesh, 2014). Tomatoes outbreaks during these years also caused large economic loss to the tomato industry and the collapse of consumer trust. In terms of tomato outbreaks, *Salmonella* is the leading foodborne pathogen; almost 90% of tomato confirmed outbreaks are related to *Salmonella* contamination (Table 2). *Salmonella enteria* serotypes were the leading cause (up to 8 serotypes); an outbreak in 2004 was related to multiple serotype contamination.

Table 2. Multistate outbreaks attributed to the consumption of raw tomatoes

Year	*Salmonella* serotype	No. of states	No. of ill	No. of hospitalized	Location of Contamination
1990	Javiana	4	176	18	Packinghouse
1993	Monrevideo	4	100	16	Packinghouse
1998	Baidon	8	86	16	Farm /Packinghouse
2000	Thompson	10	43	15	Farm /Packinghouse
2002	Newport	2	8	2	Farm /Packinghouse
2002	Newport	24	333	43	Farm
2004	Breanderup	16	125	25	Farm /Packinghouse
2004	Multiple *	5	429	129	Fresh-cut processing facility
2005	Newport	16	72	8	Farm
2005	Entertidis	8	77	7	Farm /Packinghouse
2005	Breanderup	8	82	29	Farm
2006	Newport	19	115	37	Farm /Packinghouse
2006	Typhimurium	21	190	24	Farm /Packinghouse
2007	Newport	18	65	11	Farm /Packinghouse
2010	Newport	9	51	4	Farm

*Javiana (89% of cases), Typhimurium (6%), Anatum (1%), Tompson (<1%), Muenchen(<1%).

(Source: Benneet, Littrell, Hill, Mahovic, & Behravesh, 2014)

Based on outbreak data of the past 20 years, tomatoes are confirmed as one of the major food vehicles for carrying *Salmonella* (Hedberg et al., 1999; Gupta et al., 2007). However, there is still lacking a promising explanation about how tomatoes become contaminated with *Salmonella*. Based on the current research, tomato contamination can occur any time during different processing steps, which include the farm field to the table.

Contaminated irrigation water was one of the reasons for tomato outbreaks. Green et al. (2007) reported that the multistate tomato outbreaks due to *S.* Newport contamination in 2005 and 2002, in both outbreaks, *S.* Newport from pond water in the tomato growing region matched the outbreaks isolates. This example demonstrates the potential risk of irrigation water that causes the reoccurrence of tomato outbreaks.

Fecal contamination from animals also serves as pre-harvest source of tomato contamination. As agricultural business becomes more intense and with demand of fresh produce rising (Lynch, Tauxe, & Hedberg, 2009), an increasing number of farmers are conducting produce and livestock businesses in the same region, raising the probability of fecal contamination. Also, the feces from wild birds are known to carry *Salmonella* and *Campylobacter*, which is even harder to control and sometimes being overlooked (Tizard, 2004; Keener, Bashor, Curtis, Sheldon, & Kathariou, 2004).

Environmental factors such as irrigation water and fecal contamination are regarded as the main sources of tomato outbreaks before harvesting. Since most produce

is usually consumed raw, lacking a thermal process to inactivate harmful microorganisms before serving. Burnett and Beuchat (2000) indicated that washing produce by water only removes a portion of microorganism on the surface, and only delays the growth of spoilage and pathogenic organisms. With an addition of disinfectant to the wash water, 10-100 fold microbial reductions can sometimes be reached (Beuchat, 1998). The wash step in the post-harvest tomato industry is the significant part, which influences the safety and shelf-life of raw tomatoes.

Dump tank or flume system is often used in produce industry for washing. Currently, in the tomato industry, harvested tomato are sent to the first wash step with tap water to remove the debris and soil on the tomato surface and then going through additional wash step with disinfectant. The purpose of adding disinfectant in to water is to (1) reduce the population of spoilage and potential pathogenic microorganisms on the surface (2) prevent the cross-contamination in the wash water. Currently, there are several chemical compound that are commercially available, but the chlorine-based compounds are reported to inactivate or inhibit a larger range of microbes compared to other commercially approved sanitizer (Wirtanen & Salo, 2003), in addition, the low-cost and easy to achieve processing properties of chlorine-based chemicals make it as one of the most common sanitizer used in produce industry. Based on the FDA regulation, the suggested concentration of chlorinated wash water was 50-200 mg/L of total chlorine at pH between 6.0-7.5 with 1-2 min of contact time to sanitize produce (FDA, 2014).

During the washing process pathogen internalization can be a concern. Microorganisms could penetrate into the tomato via stem scar while the temperature of wash water is lower than contaminated tomatoes. Hanning et al. (2009) reported that when the warm tomatoes were immersed into cold wash water contaminated with *Salmonella*, the *Salmonella* was rapidly taken into tomatoes via openings such as stem scar and wounds on the surface. Therefore, monitoring the wash water temperature and chlorine concentration are the two vital factors that need to be controlled in tomato industry (Rushing, Angulo, & Beuchat, 1996).

Even though the GAPs and GMPs regulations were practiced from pre-harvest to post-harvest handling process for preventing the tomato contamination; salmonellosis related to eating raw tomato are still increasing from 1990 until now. Cummings indicated that once tomatoes are contaminated, *Salmonella* on tomatoes might be difficult to be removed since the cell may be shielded by the growth cracks on the surface or stem scar (Cummings et al., 2001). Zhuang et al. (1995) indicated that dipping contaminated tomatoes into 60 and 110 ppm chlorinated water for 2 min resulted in significant reduction of *Salmonella*, nevertheless, dipping solution up to 320 ppm chlorinated failed to reach the completely inactivation (Jin & Gurtler, 2012; Zhuang et al, 1995). Jin and Gurtler (2012) also reported that the survived *Salmonella* on the tomato after sanitizing process is able to recover and population up to 10^7 CFU/g with proper temperature during storage time (Hanning et al., 2009; Iturriaga, Tamplin, & Escartin, 2007; Jin & Gurtler, 2012; Zhuang et al, 1995).

Based on the limitations mentioned above, an alternative way is needed to improve the microbial safety and quality of tomatoes. In this study, chitosan wash step was added to the tomato wash process as part of multiple hurdle approach to investigate the quality change and safety of fresh tomatoes.

3.4 Chitosan

Chitosan (β-(1,4)N-acetyl-D-glucosamine) is one of the most abundant polysaccharides in the world which shares a similar chemical structure with cellulose (Figure 2). Chitosan is mainly derived from chitin, which is extracted from the exoskeleton of crustaceans and cell wall of fungi via multiple chemical steps (Devlieghere, Vermeulen, & Debevere, 2004; Kumar, 2000).

Chitosan has the properties of low-cost, easy-to-produce and abundant, and it is economically feasible for industry use. The interest in chitosan increased because of serious environmental problems regarding the disposal of marine. Biochemists started to develop a method to generate a low-lost and bioactive by-product from the tremendous crustacean waste (Hayes, Carney, Slater, & Brück, 2008; Kim & Mendis, 2006), chitin and chitosan were two of the main by-products. So far, chitosan has been produced commercially in many countries such as India, Korea, and Japan and used in various applications.

The non-toxic, bio-degradable, bio-compatible, and bio-functional properties of chitosan, have been applied in many industrial fields including biochemistry, material

science, drugs, pharmaceuticals and gene therapy (Harish Prashanth & Tharanathan, 2007) (Figure 3). In the food industry, numerous studies demonstrated that chitosan-based product could be used as packaging material, food preservative, food additive and edible coating, because of its antimicrobial and film-forming ability. According to Kong et al. (2010), chitosan fit the properties of the ideal antimicrobial compound which can be used in food: (1) can be synthesized easily (2) should not be toxic (3) has a broad range against pathogenic and spoilage microorganism in short contact time with food (Kenawy, Worley, & Broughton, 2007; Kong, Chen, Xing, & Park, 2010).

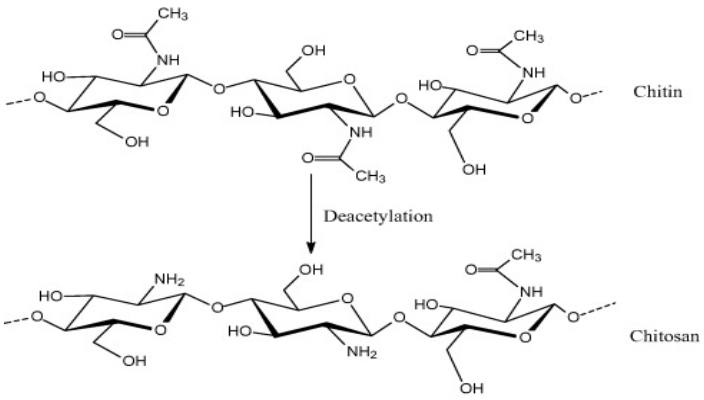


Figure 2. The structure of chitin and chitosan.

(Source: Pham et al., 2011)

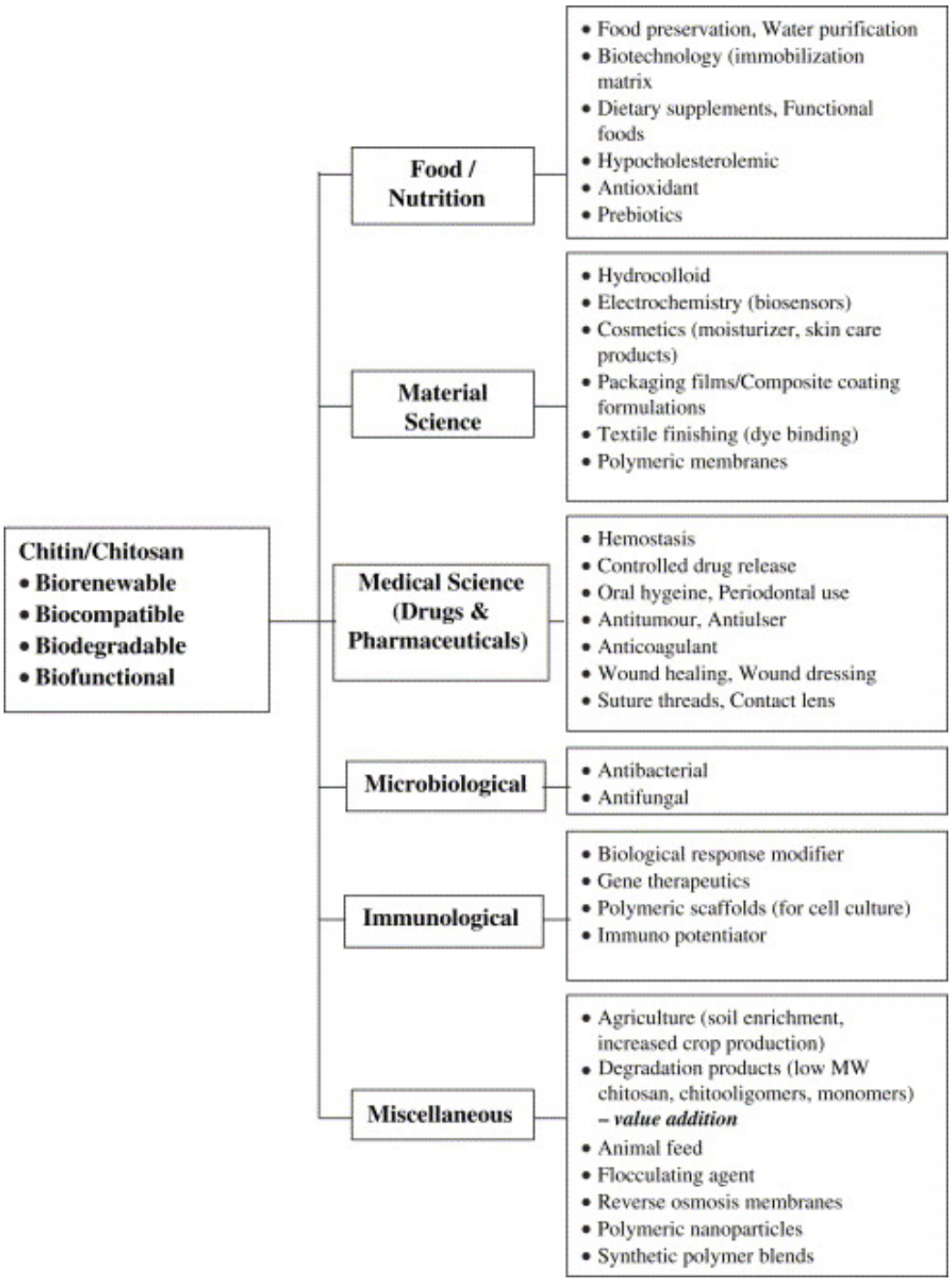


Figure 3. Application of chitin/chitosan.

(Source: Harish Prashanth & Tharanathan, 2007)

Antimicrobial ability and film-forming properties are the two main characteristics, which made chitosan a potential source as a food additive to extend the shelf-life and improve the safety of food products. Previous studies have used chitosan in various of food products (meat, poultry, seafood and juice) against different kinds of pathogenic, spoilage microorganisms, or only for shelf-life analysis (No, Meyers, Prinyawiwatkul, & Xu, 2007). Recent, research on using chitosan-based coating technology to improve the safety and quality of fresh produce have been discussed following a large number of produce outbreaks.

Several studies have discussed the antimicrobial activity of chitosan, which may have the potential for reducing the occurrence of produce outbreaks. Currently, an increasing number of studies address the use of chitosan against foodborne pathogens: commodities include, broccoli (Alvarez, Ponce, & del R Moreira, 2013), carrots (Durango, Soares, & Andrade, 2006), cantaloupes (Chen, Jin, Gurtler, Geveke, & Fan, 2012) and tomatoes (Jin & Gurtler, 2012), these studies were focused on the foodborne pathogens, *E. coli* O157:H7 and *Salmonella,* which are often associated with produce contamination. The antifungal activity of chitosan against spoilage microorganisms on produce was also reported (Garrido Assis & de Britto, 2011; Guo et al., 2006).

The exact mechanism of antimicrobial of chitosan is still controversial. The early studies usually regarded chitosan as bactericidal compound, which means the properties of inactivating bacteria. However, recently studies tend to consider chitosan as bacteriostatic compound, which means chitosan can inhibit the growth of bacteria but

doesn't inactivate the bacteria (Goy, Britto, & Assis, 2009). Two main mechanisms are often discussed in the literature: (1) The polycationic structure of chitosan, which results in strong electrostatic activity with negative charge bacteria membranes, osmosis imbalance and leakage of intracellular electrolytes, then inhibit the microbial growth. The positive charge of chitosan is related to degree of deacetylation (DD), the higher DD rate (>90%) of chitosan indicate the stronger antimicrobial activity (Kong et al., 2008). In addition, the studies also reported that the antimicrobial activity of chitosan is pH dependent. Since the pKa of chitosan molecule is around 6.3-6.5, the protonation effect of chitosan, which make it become polycationic, can only be displayed when in the solution with pH below 6.3. (2) Another mechanism is using chitosan to block the RNA transcription process from DNA via penetration, which results in the inhibition of protein synthesis and shut down the metabolic activity of bacteria. Fei Liu et al. (2001) indicated that the molecular weight of chitosan has to be lower than 5000 Da in order to penetrate into the bacteria cell.

Chitosan not only has the antimicrobial activity that may improve the safety of produce; chitosan is also known for its film-forming activity beneficial to extend the shelf-life and maintain product quality. El Ghaouth et al. (1992) mentioned that the major loss of postharvest produce is due to fungal infection and physical injuries during the storage and transport. The chitosan edible-film has the ability of adjusting the carbon dioxide and oxygen which could reduce the respiration rate, then lower the ripen process (Elsabee & Abdou, 2013; No, Meyers, Prinyawiwatkul, & Xu, 2007). During the various hurdle technologies of extending the shelf-life of produce, edible-coating is one of the

ways that has been investigated most (Jianglian, 2013). According to previous studies, the efficacy of chitosan edible-coating associated with shelf-life has been studied with a broad range of fruit and vegetables which included blueberries (Yang et al., 2014), mangoes (Chien, Sheu, & Yang, 2007), strawberries(Han, Zhao, Leonard, & Traber, 2004),asparagus (Qiu, Jiang, Ren, Huang, & Wang, 2013) and pears (Ochoa-Velasco & Guerrero-Beltrán, 2014).

Even though the efficacy of chitosan to control foodborne pathogens on fresh produce has been discussed, there is limited research related to fresh tomatoes. Jin & Gurtler (2012) tested the efficacy of chitosan by applying the solution on the stem scar only; Zhuang et al. (1995) discussed the antimicrobial activity as a function of temperature and chlorine on fresh tomatoes. There are also several studies which evaluate the storage life of chitosan coated fresh tomatoes (El Ghaouth, Ponnampalam, Castaigne, & Arul, 1992; Benhabiles et al., 2013). In this study, the efficacy of chitosan in reducing surface attached *Salmonella* and quality of fresh tomatoes will be evaluated.

4. MATERIALS AND METHODS

4.1 Tomatoes

Tomatoes (*Solanum lycopersicum*) were purchased from the local farmer's market and damaged ones were eliminated in advance. The weight of each tomato ranged from 160-300 g. Tomatoes were stored at 4°C and were used within 48 h of purchase. Based on tomato color classification from USDA (U.S. Department of Agriculture, 1997), "Pink" and "Turning" were used in this study (Figure 4).

4.2 Preparation of inoculum

A cocktail of *Salmonella* (*S.* Typhimurium, *S.* Newport, *S.* Breaderup and *S.* Javiana) was used. Strains were clinical isolates from previous tomato-related outbreaks. The *Salmonella* strains were cultured individually in Tryptic Soy Broth (Difico, Sparks, MD) for 21 h at 37°C, and the cultures were mixed together, then suspended in sterilized tap water to achieve desired concentration (~10^8 CFU/ml).

4.3 Preparation of chitosan

K-1 chitosan (High Molecular Weight, Mw = 300 kDa, 95% Degree of Deacetylation, South Korea), which derived from shrimp shells, was provided by Dulcette Technologies LLC, NY. Chitosan solution was prepared by adding 8 g of chitosan powder into 4 L of sterilized tap water to make 0.2% dipping solution; for 1% chitosan solution, 40 g of chitosan powder was dispensed in 4 L of sterilized tap water.

4.4 Preparation of chlorinated wash water

Chlorinated wash water was prepared by adding sodium hypochlorite into sterilized tap water to achieve the target concentration of 100 ppm of free chlorine. The chlorine concentration was checked using a chlorine test paper (Lamotte, Chedtertown, MD) and Chlorometer Duo (Palintest, Erlanger, KY).

4.5 Treatment

All tomatoes were dipped into the inoculum for 60 s and dried for 60 min in a biological safety cabinet, to remove extra moisture on the surface and achieve the desired surface attached *Salmonella* of 10^4~10^5 CFU/g. After inoculation, tomatoes were subjected to one of six treatments (Table 2).

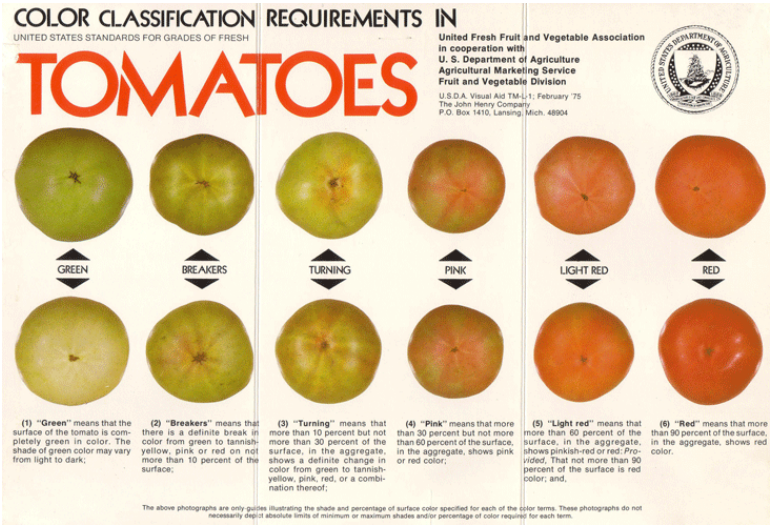


Figure 4. USDA color classification for tomato grading.

(Source: USDA, 1997)

Table 3. Treatment scheme for tomatoes*

	Procedure of Treatment		
	Tap water	Chlorinated Water	Chitosan Solution
Control	➔		
Chlorine		➔100 ppm	
Chlorine 0.2% CS		➔100 ppm	➔0.2%
Chlorine 1.0% CS		➔100 ppm	➔1.0%
Water 0.2% CS	➔		➔0.2%
Water 1.0% CS	➔		➔1.0%

*All tomatoes were stored at 15°C for up to 5 days of storage.

4.6 Microbial analysis

The population of *Salmonella* and total aerobic microbes were tested immediately after treatment and on day 1/day 5 post-treatment. After treatment, the tomato was placed in sterilized bag and specific amount of TSB was added into bags to cover the top of tomato. The volume of TSB was 1.0 times the weight of tomato. For example, a tomato weighing 200 g is placed in 200 ml of TSB. Tomato was dipped in TSB for 1 h, to allow surface attached bacteria transfer to TSB (Bacteriological Analytical Manual, FDA, 2014). After that, the 1.0 ml of TSB was taken out and plated onto Tryptic Soy Agar (Difico, Sparks, MD) and XLT4 agar (Difico, Sparks, MD) to evaluate the population of total aerobic microbes and *Salmonella,* respectively. All plates were incubated at 37°C for 21 h.

4.7 Weight loss analysis

The change in weight of each tomato was evaluated daily during the storage period. Each treatment contains eight tomatoes for analysis. The average weight loss was represented by percentage (%).

4.8 Statistical analysis

The experiments were conducted two or three times with 3 samples per treatment being analyzed. For weight loss test, eight tomatoes were used per treatment. The average of bacteria population and weight loss rate were compared by statistical program (Statistical Analysis Systems, Cary, NC) for variance analysis (ANOVA) and Duncan's multiple range tests.

5. RESULTS

5.1 Population of total aerobic microbes on tomatoes

The population of total aerobic bacteria on pre-treated tomatoes was 5.12, 3.50 and 3.74 log CFU/g, respectively. After inoculating with *Salmonella,* the average total aerobic microbe (which include *Salmonella*) population was 5.12 log CFU/g. The population of total aerobic microbes after inoculation and following treatments is shown on Figure 5.

On day 0, immediately following treatment, a 3.16 log CFU/g reduction compared to control (water only) in the population of total aerobic microbes occurred on tomatoes treated with chlorine plus 1.0% chitosan. The population of microbes on controls was reduced by 1.25 log CFU/g. There was a significant difference ($P < 0.05$) observed between control and tomatoes treated with chlorine plus either 1.0% or 0.2% chitosan solution. No significance difference was observed in the population of total aerobic microbes for tomatoes washed in chlorinated water and exposed to either 1.0% or 0.2% chitosan.

After 24 h post-treatment (day1), the populations of total aerobic microbes increased ~1 log CFU/g with an average population of 4.15 log CFU/g for the six treatments. The population of total aerobic microbes on tomatoes treated with chlorine plus 1.0% chitosan had a relatively low microbial load (3.31 log CFU/g) compared to other treatments. However, no significant differences ($P > 0.05$) were observed on day1.

The population of total aerobic microbes on day 5 post-treatment increased to ~5 log CFU/g, close to the initial pre-treatment population (5.12 log CFU/g), except for tomatoes treated with chlorine plus 1.0% chitosan. Total aerobic microbe population after chlorine plus 1.0% chitosan treatment was 3.41 log CFU/g, which was relatively low, compared to populations on tomatoes receiving the other treatments. A significant difference (P <0.05) in total aerobic microbes was observed between chlorine plus 1.0% chitosan treatment and control. Overall, for each of the three experiments, from day 0 to day 5, a similar trend in shift in population of total aerobic microbes occurred.

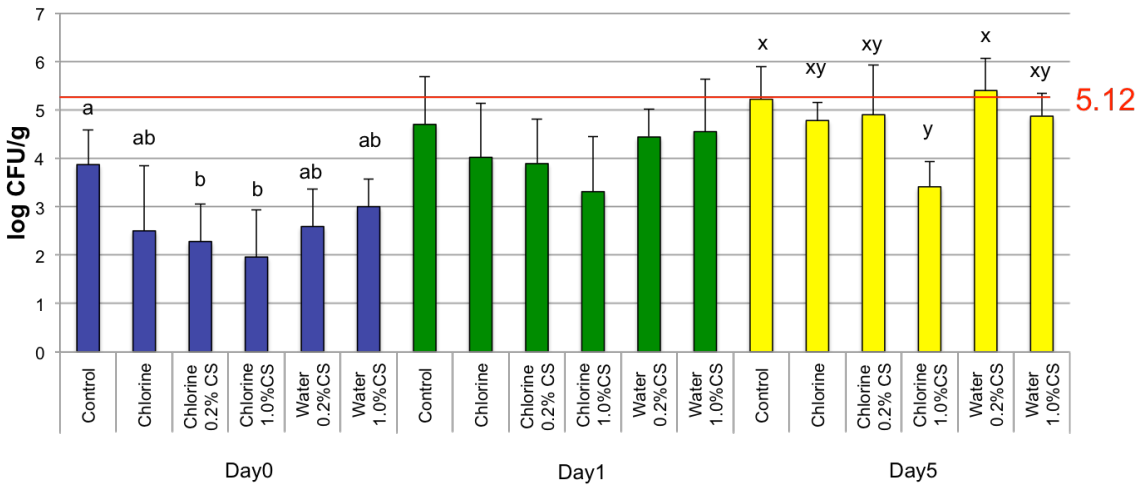


Figure 5. Survival of total aerobic microbes on tomato surface during five days of storage at 15°C.

The average population of commensal flora prior to inoculation was 4.12 log CFU/g; following inoculation with *Salmonella*, the population was 5.12 log CFU/g.

*Different letters on the bar graph indicate that the means are significantly difference (P <0.05). The error bars indicate the standard deviations from the means of 3 replicate experiments.

5.2 Population of *Salmonella* on tomatoes

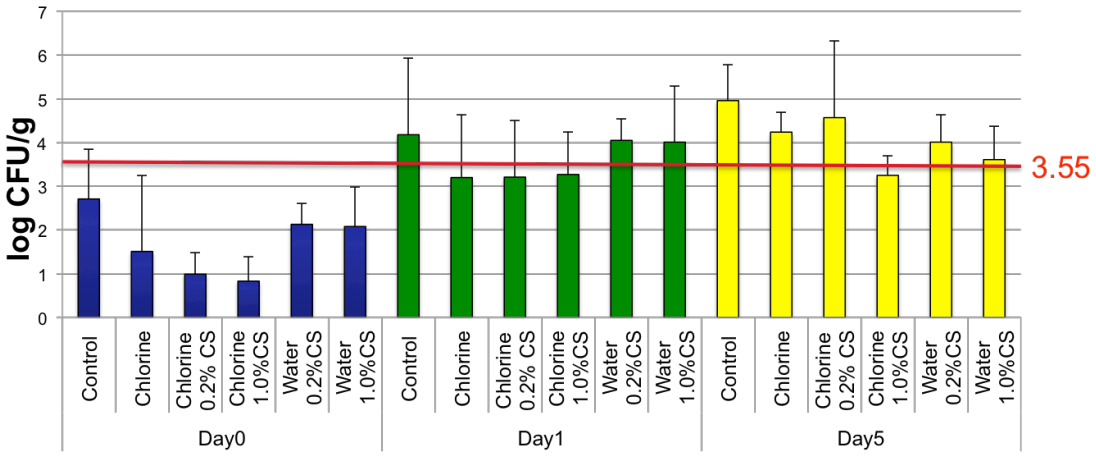
The populations of surface attached *Salmonella* are shown in Figure 6a and 6b. The initial population of *Salmonella* on tomatoes after inoculation was 3.55 and 5.41 log CFU/g, respectively. The populations of commensal microbes varied for each experimental batch of tomatoes, subsequently influencing initial surface attached populations of *Salmonella* between the experiments. Therefore, the *Salmonella* data were not combined prior to statistical analysis.

On day 0, the greatest reduction (~3 log CFU/g) in population of *Salmonella* occurred on tomatoes treated with chlorine plus 1.0% chitosan (Figure 6a). No significant differences ($P > 0.05$) were observed between each treatment. In the second experiment (Figure 6b), the population of *Salmonella* on tomatoes were reduced up to 4 log CFU/g following treatment with chlorine plus either 1.0% or 0.2% chitosan. A 2 log CFU/g reduction in *Salmonella* occurred on control tomatoes. Significant differences ($P < 0.05$) were observed between control tomatoes and treatments with chlorine alone or chlorine plus either 1.0% or 0.2% chitosan.

By day1 post-treatment, the *Salmonella* population increased by ~2 log CFU/g across all six treatments; *Salmonella* populations were greatest on control tomatoes (Figure 6a). Treatment of tomatoes with chitosan (1.0% or 0.2% chitosan) following chlorinated water wash was most effective in controlling outgrowth of *Salmonella.* Although no significant differences ($P > 0.05$) occurred, results suggest a benefit to the use of chitosan.

By day 5 post-treatment, *Salmonella* populations increased for all treatments (Figure 6a and 6b). For both experiments, the increase in *Salmonella* populations was least on tomatoes treated with chlorine plus 1.0% chitosan treatment. In the second experiment (Figure 6b), significance difference ($P < 0.05$) in *Salmonella* population of chlorine plus 1.0% chitosan treatment compared to control tomatoes. Both experiments (Figure 6a and 6b) show similar trends in outgrowth of *Salmonella* on tomatoes for each sampling point post-treatment.

(a)



(b)

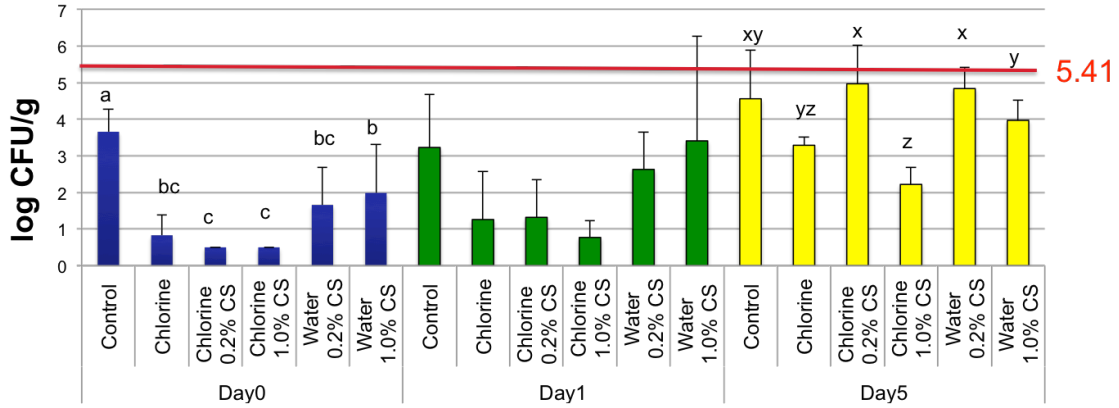


Figure 6. Survival of *Salmonella* on tomato surface during 5 days of storage at 15°C.

(a) The population of *Salmonella* inoculated on tomato was 3.55 log CFU/g.

(b) The population of *Salmonella* inoculated on tomato was 5.41 log CFU/g.

**Different letters on the bar graph indicate that the means are significantly different (P <0.05). The error bars indicate the standard deviations from the means of 3 replicate samples.

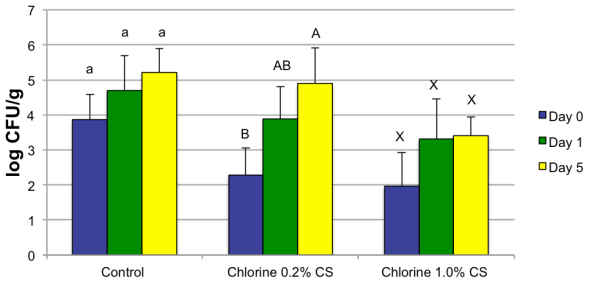
5.3 The growth trend of bacterial population

The shift in growth of total aerobic microbes and *Salmonella* population treated with water (control), chlorine plus either 0.2% or 1.0% chitosan solution during 5 days of storage is shown in Figure 7a, 7b, and 7c. The different patterns of growth trend of bacterial population were found. Populations of total aerobic microbes and *Salmonella* treated with water (control) steadily increased during 5 days of storage (Figure 7a, 7b and 7c).

For total aerobic microbes (Figure 7a), the populations on tomatoes treated with chlorine plus 0.2% chitosan solution increased during the storage periods. A significant difference (P <0.05) in bacterial population occurred between day 0 and day 5, whereas the bacterial populations on tomatoes treated with 1.0% chitosan solution following chlorine wash step showed only 0.1 log CFU/g difference between day 1 and day 5. No significance difference (P >0.05) was observed in the bacterial population of tomatoes treated with chlorine plus 1.0% chitosan from day 0 to day5.

The *Salmonella* population for each experiment (Figure 7b and 7c) increased steadily on tomatoes treated with chlorine plus 0.2% chitosan from day 0 to day 5. However, the *Salmonella* populations on tomatoes treated with chlorine plus 1.0% chitosan showed a limited increase from day1 and day 5.

(a)



(b) (c)

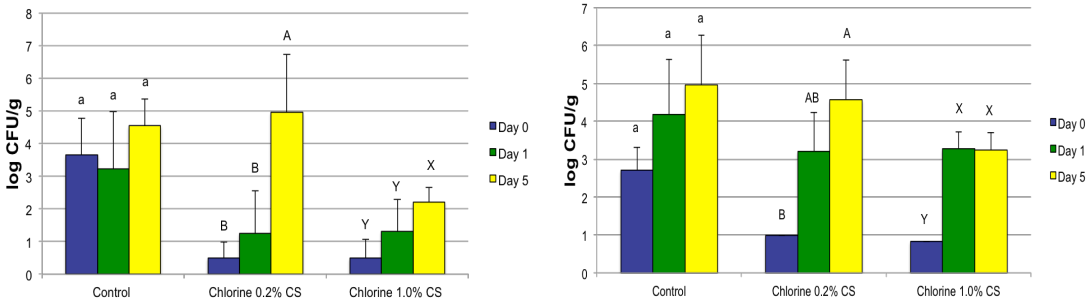


Figure 7. The growth trend of total aerobic microbes and *Salmonella* during 5 days of storage at 15°C.

(a) The growth trend of total aerobic microbes.

(b) The growth trend of *Salmonella* (with 3.55 CFU/g surface attached *Salmonella* after inoculation).

(c) The growth trend of *Salmonella* (with 5.41 CFU/g surface attached *Salmonella* after inoculation).

*Different letters on the bar graph indicate that the means are significantly different (P <0.05). The bacterial populations were compared by each treatment during 5 days of storage, individually.

5.4 Weight loss

Change in tomato weight during storage is shown in Figure 8. The tomatoes were stored 8 days at 15°C and 85-90% RH condition. No significance difference (P >0.05) was observed between treatments. Weight loss was greatest for tomatoes treated with water plus 1.0% chitosan.

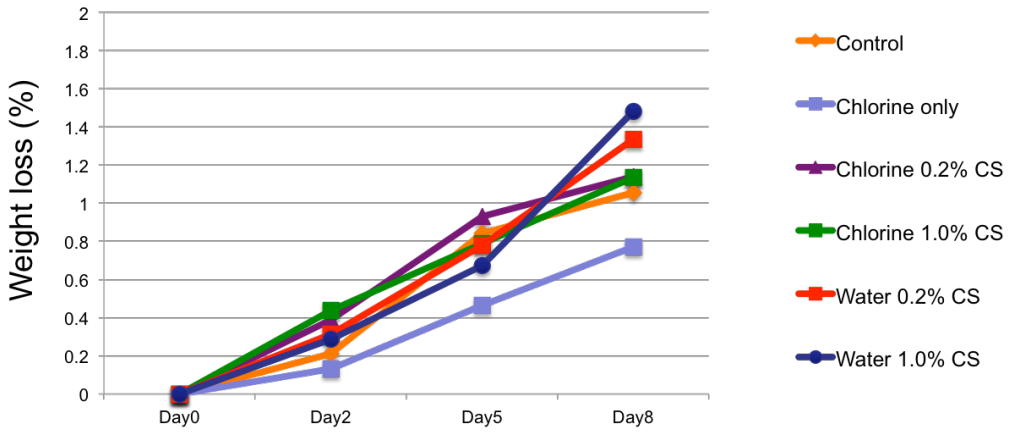


Figure 8. The percentage of tomatoes weight loss during 8 days of storage.

6. DISCUSSION

The trend of eating raw produce as part of a healthy diet may be associated with the increased consumption of raw tomatoes. Improving the microbial safety and quality of raw tomatoes is important to the industry when it comes to preserving the market. The majority of tomato outbreaks associated with *Salmonella* can be traced to contamination occurring at the farm or packaging house. *Salmonella* have been found in production field, soil and animal manure; providing a means for tomato contamination. Chlorine-based compounds are often used by the produce industry due to low costs and ease of use. Washing tomatoes in chlorinated water may significantly reduce the levels of *Salmonella* on tomatoes, but will not eliminate the pathogen or prevent outgrowth during storage.

In the present study, a chitosan treatment was added following the chlorine wash step to improve the microbial safety and quality of the tomatoes. Chitosan is known for its antimicrobial and edible film forming ability for improving the safety and quality of fresh produce. The results indicate that immediate after treatment, chlorine plus 0.2% or 1.0% chitosan treatments achieved the greatest reduction in microbial population. Treatment with chlorine plus 1.0% chitosan provided continuous suppression of microbial growth during the five days of storage. Treatments with water plus either 1.0% or 0.2% chitosan failed to limit the microbial growth during the 5 days of storage. The synergistic effect of chitosan and chorine may be associated with initial stress or injury and then failure of cells to recover in the presence of chitosan.

The quality of tomatoes used in each study also affected the growth of bacterial population during 5 days of storage and the level of initial surface attached *Salmonella* after inoculation. For the first experiment, the background microbiota (no treatment and no inoculation) was 5.04 log CFU/g; the surface attached *Salmonella* was 3.55 CFU/g. However, for the second experiment, the background microbiota was 3.5 log CFU/g and following inoculation of the surface attached *Salmonella* was 5.41 log CFU/g. Based on this information, we inferred that the background microflora may impact the attachment of *Salmonella* to the tomato.

Previous studies indicated that the film-forming activity of chitosan on the surface of produce may limit respiratory rate, reduce weight loss and extend the shelf life. In the present study, weight loss was monitored to determine whether the chitosan coating could reduce the weight loss of fresh tomatoes during storage. Under conditions of the present study, dipping in chitosan did not reduce weight loss. Polysaccharide, protein and lipids are the three main types of edible film. Pascall et al. (2010) reported that the polysaccharide-based film has a good barrier for the oxygen but relatively low moisture barrier. To overcome these limitations, researchers evaluated edible films that combined chitosan with acid or wax. A decided negative aspect of using combination edible films is the higher costs, which may lower industry acceptance.

To summarize, dipping tomatoes in a 1.0% chitosan solution immediately after the chlorine wash step results in additional inactivation of *Salmonella* and total aerobic microbes on the surface of tomatoes. This study demonstrated the efficacy of chitosan-

based solutions and potential beneficial impact to the tomato industry through improving the microbial safety and quality of tomatoes.

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