Efficacy of Integrated Treatment of UV light and Low-Dose Gamma Irradiation on Inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* on Grape Tomatoes

S. Mukhopadhyay, D. Ukuku, X. Fan, and V. K. Juneja

Abstract: The study evaluated the efficacy of integrated ultraviolet-C light (UVC) and low-dose gamma irradiation treatments to inactivate mixed strains of *Escherichia coli* O157:H7 and *Salmonella enterica* on inoculated whole grape tomatoes. A mixed bacterial cocktail composed of a 3-strain mixture of *E. coli* O157:H7 (C9490, E02128, and F00475) and a 3-serotype mixture of *S. enterica* (S. Montevideo G4639, S. Newport H1275, and S. Stanley H0558) was used based on their association with produce-related outbreaks. Spot inoculation (50 to 100 μL) on tomato surfaces was performed to achieve a population of approximately $10^7$–$8$ CFU/tomato. Inoculated tomatoes were subjected to UVC (253.7 nm) dose of 0.6 kJ/m² followed by 4 different low doses of gamma irradiations (0.1 kGy, 0.25 kGy, 0.5 kGy, 0.75 kGy). The fate of background microflora (mesophilic aerobic) including mold and yeast counts were also determined during storage at 5 °C over 21 d. Integrated treatment significantly ($P < 0.05$) reduced the population of target pathogens. Results indicate about 3.4 ± 0.3 and 3.0 ± 0.1 log CFU reduction of *E. coli* O157:H7 and *S. enterica*, respectively, per tomato with UVC (0.6 kJ/m²) and 0.25 kGy irradiation. More than a 4 log and higher reduction (>5 log) per fruit was accomplished by combined UVC treatment with 0.5 kGy and 0.75 kGy irradiation, respectively, for all tested pathogens. Furthermore, the combined treatment significantly ($P < 0.05$) reduced the native microflora compared to the control during storage. The data suggest efficacious treatment strategy for produce indicating 5 or higher log reduction which is consistent with the recommendations of the Natl. Advisory Committee on Microbiological Criteria for Foods.

Keywords: *E. coli* O157:H7, gamma irradiation, integrated treatment, *Salmonella*, tomato, UV light

Introduction

Consumption of fresh and fresh-cut (minimally processed) fruits and vegetables is highly recommended due to nutritional and health-related benefits. Due to this acknowledged merits, there has been a measurable increase in the consumption of fresh fruits and vegetables in the United States (USDA 2008). Tomatoes are the 2nd most popular vegetable, next to potatoes, and are consumed throughout the world. They are an excellent source of health-promoting components, including vitamins A, C, E, folic acid, flavonoids, potassium, and secondary metabolites such as β-carotene, lycopene, and phenolic compounds (Beecher 1998; Leonardi and others 2000; Sahlin and others 2004). Regular consumption of tomatoes has been associated with reduced risk of various types of cardiovascular and cancer diseases due to its high antioxidant capacity and micronutrient contents as flavonoids, lycopene, and carotenoids (Agarwal and Rao 2000; Lavello 2000). Unfortunately, with the increase in produce consumption the number of produce-related outbreaks of foodborne illnesses also increased (Lynch and others 2009), and the microbial safety of produce remains serious public-health concern in the United States and Europe. A recent report estimates 47.8 million episodes of food-related illnesses occur in the United States each year (CDC 2011), causing 127 839 hospitalizations and 3 030 deaths. About 23% of all foodborne illnesses from 1998 to 2007 were attributable to fresh produce (CSPI 2009). Tomatoes, like other fresh produce, come from multiple sources and countries depending on weather and the season, and are one of the major contributors to produce-related outbreaks. Grape tomatoes have been involved with several recalls due to *Salmonella* contamination (FDA 2011). Between 1996 to 2006, tomatoes caused 17.1% of all produce-related outbreaks (Gravani 2009). Multi-state outbreaks involving various *Salmonella* serotypes have been reported and one of the largest outbreaks related to *S. enterica* serovar Saintpaul was linked with consumption of tomatoes (CDC 2008).

The severity and frequency of the produce-related outbreak poses threat to further increases in per capita consumption due to lowered confidence in produce safety by consumers. Pathogens of primary concern appear to be *E. coli* O157:H7, nontyphoidal *Salmonella*, *L. monocytogenes*, and Norwalk-like viruses. In addition, foodborne outbreaks associated with consumption of produce can be very costly to growers and processors. These outbreaks have raised concerns over postharvest decontamination practices.

Chemical or antimicrobial sanitizer wash is usually the only process step practiced in produce industry to lower pathogenic and spoilage microorganisms (Ruiz-Cruz and others 2007;
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Allende and others (2008). A wide range of sanitizers has been investigated with varying degrees of success for killing *Salmonella* on tomatoes (Beuchat 1998a; Lang and others 2004). Chlorine-containing or peracetic acid-based sanitizers are most widely used for sanitization (Artes and Allende 2005). Chlorine wash was proven to be somewhat effective to inactivate pathogens and spoilage organisms merely 1 to 2 log cycles (Brackett 1999); tomatoes treated with 60 ppm chlorine were implicated in outbreaks (Wei and others 1995). Even at a much higher concentration of 320 ppm for 2 min, complete inactivation of *Salmonella* Montevideo cells on tomato surfaces was not achieved (Zheng and others 1995). Electrolyzed water on the other hand was effective in reducing *Salmonella enterica* serovar Enteritidis on tomatoes by *E. coli* and 2000 (Yau 2004).

Moreover, chlorination reactivates the organic load and is capable of forming harmful toxic compounds such as chloramines and trihalomethanes (Aieta and others 1984; Richard and others 1998) which raised safety concerns (Parish and others 2003; Allende and others 2004). Due to residual toxicity of chlorine and other sanitizers wash and their impact on produce quality and human health, alternatives strategies to chemical or antimicrobial wash have been considered.

Ultraviolet (UV) light and ionizing radiations have shown to be effective in inactivating foodborne pathogens on fresh fruits and vegetable surface. UV light, mainly UV-C, has long established applications in food due to its antimicrobial capacity. The germicidal effect of UV light is due to the formation of cyclobutane dimers which prevent DNA replication in microorganisms (Giese and Darby 2000). UV light has reduced *Salmonella* by 2.2 logs cycles and *E. coli* O157:H7 by 2.8 logs cycles on tomato and lettuce surface, respectively, at dose of 25 μJ/cm² (Yam and others 2004). Gamma radiation is capable to damage the sugar phosphate backbones or base pairs of DNA directly and hence inactivate the microorganisms. Gamma irradiation at 2 kGy has shown to eliminate 5 log of *S. Typhimurium* in pineapple (Shashidhar and others 2007). The reported D₀ value of gamma irradiation for *Salmonella* on tomato is 0.25 to 0.54 kGy (Schmidt and others 2006; Prakash and others 2007). The reported effect of UV-C and gamma irradiation on phenolic phytochemical contents (Luthria and others 2006) and antioxidant capacity (Thomas-Barberan and Espin 2001) of fresh produce is variable and mostly positive, although at a higher dose of irradiation some deterioration in quality (Fan 2012), mainly exhibiting loss of firmness of tomato flesh was reported (Prakash and others 2002; Schmidt and others 2006).

The required treatment intensities (concentration, dose, time) for a 5-log reduction per recommendation of the FDA guideline (NACMCF 1999), by any single treatment method are often quite high and can result in adverse effects on sensory properties and nutritional quality of fresh produce. Integrated treatments are capable of disrupting one or more of the homeostasis mechanisms in microorganisms at the cellular level and thus can limit pathogen growth and survival at a much lower intensities of individual treatments (Leistner 2002). UV-C light in combination with gamma irradiation may be used to control foodborne pathogens on produce. Although a higher dose of gamma irradiation may cause some quality degradation, such deterioration in quality can be minimized by using lower dose of irradiation. Thus, the objective of this research were to investigate the efficacy of sequential treatment of UV-C light followed by low-dose gamma irradiation for reduction of *Salmonella enterica* and *E. coli* O157:H7 on whole grape tomato surfaces and evaluate the effect of the combined treatment on the native microflora population of tomatoes during storage at 5 °C for 3 wk.

**Material and Methods**

**Strain, growth conditions, and inocula preparation**

A total of 3 strains each of *Salmonella* (S. Montevideo G4639, S. Newport H1275, and S. Stanley H0558) and *E. coli* O157:H7 (E02128, F00475, and C9490) were used in this study. Selection of these strains was based on their association, mainly with produce-related outbreaks. *E. coli* O157:H7 (E02128) was associated with a leafy green outbreak, *E. coli* O157:H7 (F00475) was isolated from a spinach outbreak, and *E. coli* O157:H7 (C9490) from an uncooked hamburger outbreak in the 1990s. These isolates were obtained from the USDA-ARS-ERRC culture collection. S. Montevideo G4639, which was isolated from a tomato-associated outbreak, was received from Dr. Larry Beuchat at the Univ. of Georgia. S. Newport H1275 and S. Stanley H0558 both were associated with alfalfa sprout-related outbreaks and these 2 isolates were obtained from Dr. Patricia Griffin, Center for Disease Control and Prevention, Atlanta, Ga. Bacterial strains were grown by 2 successive loop transfers of individual strains incubated at 37 °C for 24 h in 5 mL Tryptic Soy Broth (TSB, BBL, Becton, Dickinson DiFco, Sparks, Md., U.S.A.). A final transfer of 0.2 mL was made into 50 mL TSB with incubation at 37 °C for 18 h. The bacterial cells were harvested by centrifugation (5000 × g, 15 min) at 4 °C. The pelleted cells were washed twice in 0.1% (w/v) peptone water (PW, BBL/Difco) and was finally suspended in PW to a target level of 8 to 9 log CFU/mL. To enumerate the population densities in each cell suspension, appropriate dilutions (in 0.1% PW) were spiral plated (model D, Spiral Biotech, Bethesda, Md., U.S.A.), in duplicate, on tryptic soy agar (TSA; BD/Difco) plates. Equal volumes of each culture were combined in a separate sterile test tube to obtain a cocktail of 3 strains of *Salmonella* and *E. coli* O157:H7 (8 to 9 log CFU/mL) prior to inoculation of tomato.

**Inoculation of whole grape tomato surfaces**

A total of 120 whole, fresh, unblemished (from the same lot) grape tomatoes (*Solanum lycopersicum*) were purchased from a local retailer (Wyndmoor, Pa., U.S.A.) the day before the experiment and stored at 4 °C. These tomatoes did not receive any sanitizing or washing before the experiment. The tomatoes were divided in 4 treatment groups and one control group with 20 tomatoes in each group. A spot inoculation method was used to inoculate tomatoes since it allows the application of a known amount of cells onto the surfaces, regardless of weight/size. About 100 μL of the mixed culture suspension was carefully spotted on the surface of tomatoes using an appropriate pipette. A circular area on the tomato skin was marked with indelible ink where the inoculums were spotted. This was helpful in guiding the inoculation spots for optimum exposure to radiation treatments. The inoculated tomatoes were placed on sterile Petri dishes and air-dried for 2 h at room temperature (22 °C) in a biosafety cabinet (NuaireTM, Plymouth, Minn., U.S.A.) to allow the bacteria to attach to the surfaces of tomatoes and to minimize the growth of cells during drying. After inoculation, tomatoes were subjected to integrated treatment using UV-C light followed by low-dose gamma irradiation as described below.

**UV-C treatment of inoculated tomatoes**

Inoculated grape tomatoes were treated with UV-C radiation by placing the inoculated surface under a UV light source.
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generated by 8 germicidal low-pressure mercury-vapor fluorescent lamps (model FG15T8, 15 W, Buylighting.com, Burnsville, Minn., U.S.A.) mounted into a casing (Ultra-Violet Products, San Gabriel, Calif., U.S.A.). The lamps emitted about 90% of their irradiation at 254 nm covering 1200 cm² cross-sectional area. UV-C intensity was determined prior to treatment by measuring the light intensity (mW/cm²/s) using a UV digital radiometer (UVP Inc., Upland, Calif., U.S.A.), several times during the experiments to ensure consistent output (6 mW/cm²). The applied dose (mJ/cm²) was calculated by multiplying the emitting UV light intensity with treatment time in seconds. All samples were treated equally for the same exposure time (10 s) to receive a UV-C dose of about 0.6 kJ/m².

Gamma irradiation of tomatoes

UV-C radiated (0.6 kJ/m²) tomatoes were separated into 4 irradiation treatment groups. Specific irradiation doses, 0.1, 0.25, 0.50, and 0.75 kGy, were generated using a self-contained cesium-137 gamma irradiation source (Lockheed Georgia Co., Marietta, Ga., U.S.A.) with an average dose rate of 0.076 kGy/min. The irradiation unit contained 23 137Cs pencils placed in an annular array around a 63.5-cm high and 22.9-cm wide (internal diameter) stainless steel cylindrical chamber. Actual dose was verified using alanine transfer dosimeters from the Natl. Inst. of Standards and Technology (Gaithersburg, Md., U.S.A.). Any variations in dose were minimized by introducing the samples within the uniform area of the irradiation, and by creating the same sample geometry during irradiation experimental trials. Dose uniformity in the chamber has been described previously (Shieh and others 1985). Dosimetry was performed on regular basis in accordance with standard practice (Intl. Organization for Standardization/American Society for Testing and Materials standards). Alamine dosimeters (5-mm dia.) were placed in a 1.2-mL cryogenic vials (Nalgene, Rochester, N.Y., U.S.A.), which were taped onto the samples prior to irradiation. At the completion of experiment, free-radical signals in the dosimeters were measured using an Analyzer (Bruker Esca EPR, Bruker Instruments, Rheinstetten, Germany). Irradiation doses (0.1, 0.25, 0.50, and 0.75 kGy) applied on UV-C treated tomatoes in the present study were lower compared to the maximum dose (1 kGy) permitted for fresh produce (Fan 2008). Actual doses verified using alanine dosimeters were accurate within 10% of targeted dose. Temperature of the samples during irradiation was maintained at 22 °C by injecting gas from liquid nitrogen into the irradiation chamber. At each examined dose, tomato samples were pulled from the exposure chamber for microbial enumeration to determine surviving cell populations.

Bacterial enumeration

For determination of the number of surviving bacteria in control (inoculated but untreated) and inoculated treated sample, sterile 0.1% peptone water (PW, Difco-Becton Dickinson) was combined with each sample in 1:2 ratio (wt: vol.) and pulmed in stomacher bags with a Stomacher 400 laboratory blender (Seward, Worthington, UK) for 2 min at 230 rpm to obtain a slurry. Decimal serial dilutions of the suspensions were then prepared in 0.1% PW, and appropriate dilutions were spread plated. Surviving bacterial populations on tomato surfaces were evaluated by plating 0.1 mL on nonselective TSA medium (BBL, Difco). After 5 h, TSA plates were overlaid with an appropriate selective medium (Juneja 1997) for each bacteria; Sorbitol MacConkey (SMAC, BD/Difco) agar for E. coli O157:H7, Xylose-lysine-tergitol 4 (XLT-4, BBL, BD/Difco) for S. enterica. The plates were incubated for 24 h at 37 °C and the colonies were counted and expressed as log CFU/tomato. The detection limit (LD) of the method was 1 log CFU/fruit.

D-value determination

The D10 values (gamma irradiation dose required for a 90% reduction of viable cells or 1 log CFU) were determined by plotting the log of surviving organisms per treatment dose using MS Excel Software (Microsoft Corp., Seattle, Wash., U.S.A.). The measured dose was within 10% of targeted dose. In calculating gamma irradiation D values, the contribution of UV-C intervention on log reduction was accounted for and separated from the total. The line of best fit for survivor plots was determined by linear regression analysis in which the D10 value is the negative reciprocal of the slope of the best straight line.

Treatment effect on the native microflora count of whole tomatoes during storage at 5 °C for 21 d

Whole grape tomatoes were used for indigenous microflora counts study. The untreated (control) tomatoes and the tomatoes treated with UV-C (0.6 kJ/m²) and gamma radiation (0.1 to 0.75 kGy) were packaged separately in a plastic container (ClearPAC®, Dart Container Corp., Mason, Mich., U.S.A.) with a lid perforated with 4 holes (0.6 mm dia.). The packaged tomatoes were then stored at 5 °C for analysis over 3 wk. Samples were withdrawn at 0, 7, 14, and 21 d of storage for microbiological analyses of mesophilic bacteria, and for yeast and mold. For each determination, 5 tomatoes, weighing approximately 59 ± 1 g, were placed in a Stomacher® bag with 149 mL of 0.1% PW and pulmed for 30 s in Stomacher (model 400, Dynatech Laboratories, Alexandria, Va., U.S.A.) set at 230 rpm. Decimal dilutions of the samples were made with 0.1% PW, and aliquots (0.1 mL) were plated in duplicate on a range of media. Plate Count Agar (PCA, BD/Difco) with incubation at 30 °C for 48 h was used for enumeration of mesophilic aerobic bacteria. Dichloran Rose Bengal Chlorotetracycline (DRBC, BD/Difco) agar with incubation at 25 °C for 5 d was used for enumeration of yeast and mold. DRBC plates were wrapped with aluminum foil to provide darkness and stored without turning the plates upside down. Experiments were conducted in triplicates (n = 3). Viable counts were expressed as log CFU/g.

Statistical analyses

All experiments were done in triplicate with duplicate samples analyzed at each sampling time. Data were subjected to the SAS statistical package (SAS/STAT 9.2 User's Guide, SAS Inst. Inc., Cary, N.C., U.S.A.) for analysis of variance (ANOVA) and the Bonferroni least significant difference (LSD) method (Miller 1981) to estimate significant differences (P < 0.05) between mean values of number of cells recovered after each treatment.

Results and Discussion

Inactivation of S. enterica and E. coli O157:H7 on whole grape tomatoes

The efficacy of various physical dose treatments combining UV light and gamma irradiation on reduction of S. enterica and E. coli O157:H7 on whole grape tomatoes at 22 °C are presented in Figure 1 and 2, respectively. The recovered initial population (mean value) of Salmonella spp. and E. coli from tomato surface was in the range of 7.8 ± 0.2 Log CFU per tomato fruit. Integrated treatments evaluated in this work resulted in the reduction of 2.1
to 5.9 logs CFU/tomato for the population of *S. enterica* and 2.4 to 6.2 log CFU/tomato in the population for *E. coli O157:H7*. Four different integrated treatment categories combining UV-C dose and gamma irradiation were evaluated. In all 4 different dose treatments (T1, T2, T3, and T4) tomatoes received the same UV-C light dose of 0.6 kJ/m² but variable low doses of gamma irradiation ranging from 0.1 to 0.75 kGy (Figure 1 and 2). As expected, the populations of tested pathogens were reduced linearly with increased treatment dose. At 0.6 kJ/m² UV-C and 0.25 kGy, gamma irradiation (treatment T2), approximately 3.4 and 3.0 log CFU reduction per tomato fruit were achieved for *E. coli O157:H7* and *S. enterica*, respectively, indicating about 12% greater (not significant, *P* > 0.05) resistance for *Salmonella* spp. at this lower experimental dose (T2) compared to *E. coli O157:H7*. The resistance of *S. enterica* was somewhat reduced at the higher treatment dose (T3) and approximately 4.9 and 4.6 log CFU reduction for *E. coli O157:H7* and *S. enterica* per tomato were achieved by combined treatment with UV-C (0.6 kJ/m²) and 0.5 kGy gamma irradiation. The populations of target pathogens on whole grape tomato were further decreased significantly (*P* < 0.05) to approximately 6 log CFU/tomato by treatment T4 combining UV-C (0.6 kJ/m²) and 0.75 kGy gamma irradiation.

Inactivation mechanisms of UV-C and ionizing gamma radiation are quite different. The germicidal effect of UV-C dose is mainly due to thymine dimers formation which prevents DNA replication and hence causes a clonogenic death of cells (Giese and Darby 2000). Ionizing radiation, on the other hand, acts mainly through free radicals generated from the radiolysis of water in the food causing water molecules to lose an electron, thus producing highly reactive hydroxyl radicals (OH·), hydrogen ions, hydrated electrons, and hydrogen peroxide (H₂O₂) which breaks the bonds between nucleic acids causing damage to the cellular DNA. Some biomolecules in the cell membrane, such as lipids, enzymes, and proteins, are also affected by the free radicals causing damage to cell membranes (Simic 1983; Olson 1998). Sommers and others (2010) reported 2.6 to 3.1 logs CFU/g inactivation of *Salmonella* spp., *L. monocytogenes*, and *Staphylococcus aureus* on the surface of Roma tomatoes using a UV-C dose of 5 kJ/m², while Yaun and others (2004) obtained a reduction of 2.19 log CFU/tomato for *Salmonella* spp. on the tomato surface at UV-C dose of 0.24 kJ/m². In the present work, a UV-C dose of 0.6 kJ/m², inactivated 2.0 and 2.1 log cycles of *Salmonella* spp. and *E. coli O157:H7* strains, respectively, on the grape tomato surface (data not shown) which is comparable with reported values. Microorganisms vary in their resistance to irradiation. The relative resistances of different species can be compared by their D₁₀ values, where the D₁₀ value is the dose required (kGy) to achieve a 1-log10 (90%) reduction in viable numbers. The D₁₀ values of *E. coli* on fresh cut vegetables range from 0.12 to 0.20 kGy and for *Salmonella* spp., the D₁₀ values ranged from 0.16 to 0.54 kGy (Fan 2012). The D₁₀ values for *Salmonella* and *E. coli O157:H7* in tomato are scarce. The authors were unable to find any reports regarding gamma irradiation inactivation of pathogens on whole grape tomato surface. Table 1 provides the radiation sensitivity (D₁₀) of pathogens inoculated on whole grape tomato surface which has been subjected to integrated treatment of UV-C light followed by low-dose gamma radiation and compares the current findings with reported radiation sensitivity (D₁₀) data for these pathogens when treated with ionizing radiations alone. Schmidt and others (2006) reported that the *S. enterica* population on cut Roma tomato were reduced (*P* < 0.05) by 1.3 to 1.8 and 1.5 to 2.2 log CFU/g by irradiation with 0.7 and 0.95 kGy electron beam, respectively. Populations were also reduced in the range of 1.3 to 2.4, on stem scars during the treatment. As shown in Table 1, in this study, the radiation sensitivity or D₁₀-values of inoculated *E. coli* on grape tomato surface ranged from 0.17 to 0.19 kGy and the mean D₁₀ value was 0.18 ± 0.05 kGy while the D₁₀-values of inoculated *S. enterica* ranged from 0.19 to 0.24 kGy with a mean of 0.22 ± 0.03 kGy. These D₁₀ values are lower than those previously reported. The D₁₀ values obtained by Prakash and others (2007) for *Salmonella* on diced tomato treated with CaCl₂ were in the range of 0.26 to 0.39 kGy and that obtained by Schenidt and others (2006) on cut tomatoes were in the range of 0.29 to 0.54. In the present study, tomatoes were subjected to UV-C light prior to ionizing radiation. However, in the reported work, cut or diced tomatoes were treated with ionizing radiation alone. UV-C light inactivate microorganisms by producing cyclobutane pyrimidine dimers which...
prevents DNA replication at cellular level (Reardon and Sancar 2005). It is highly possible that a pre-UV-C light exposure causes microbial cells injury and makes cells vulnerable to ionizing irradiation treatments and thereby decreases the radiation sensitivity of the cells. Also, in the past reports, pathogens were allowed to grow on cut or diced tomatoes where the available nutrients from cut tomatoes flesh could have encouraged renewed cell survivability and hence the higher D_{10} values. The D_{10} values of inoculated \textit{Salmonella} obtained in this study is comparable with the values reported by various authors with the exception of Mahmoud (2010). The reason was probably due to use of a different irradiation source and treatment condition and dose rate. The D-values are also quite sensitive to strains and isolate types of pathogens and may vary with the maturity and cultivar of the produce (Fan 2012). The D_{10} value for \textit{Salmonella} spp. on grape tomato was about 20% higher (P > 0.05) than that for \textit{E. coli} O157:H7 (Table 1), indicating higher resistance for \textit{Salmonella} spp. to irradiation.

Sequential treatment of UV light and low-dose (0.5 to 0.75 kGy) gamma irradiation was more effective against test pathogens compared to other methods of treatments at specific concentrations. Water wash was reported to remove 1 to 2 logs of pathogens like \textit{E. Enteritidis}, \textit{L. monocytogenes}, and \textit{E. coli} O157:H7 on tomatoes (Venkitanarayanan and others 2002), whereas water immersion with agitation was reported to reduce the population of inoculated \textit{E. coli} by 1.0 log (Sapers and Jones 2006). In addition to its little or no effect on killing microorganisms, tap water may actually contribute to cross contamination (Nguyen-the and Carlin 1994).

Antimicrobial sanitizers wash is the sole process step followed by the produce industry to minimize the level of microorganisms. Commonly used sanitizers include chlorinated water, hydrogen peroxide, ozonated water, organic acids, phosphates, and detergents such as sodium lauryl sulfate (SLS), Tween 80, or sodium dodecyl sulfate (SDS), and so on. However, none has been shown to eliminate bacterial populations by more than 2 log CFU/g (Beuchat and others 1998b). Effectiveness of free chlorine or chlorinated water was dependent on the chlorine concentration, contact time, pathogen type, and location on the tomato skin or stem scar. At 200 ppm free chlorine concentration, reduction in inoculated \textit{Salmonella} spp. was 1.0 log for 40 s contact time (Weissinger and others 2000) and 3 to 4 log CFU/tomato for 40 min (Beuchat and others 2001). The reduction for inoculated \textit{E. coli} O157:H7 on tomato surface treated with 200 ppm free Cl₂ for 3 min was 1.5 log (Beuchat and others 1998b). The reported log reduction level obtained at the tomato stem scar (0.6 logs) site was lower than at the skin surface (1.7 logs) site (Wei and others 1995). Sapers and Jones (2006) reported 2.6 log CFU/g reductions for inoculated \textit{Salmonella} and \textit{E. coli} population on tomato with 5% H₂O₂ treatment and at 60 °C, whereas 1% H₂O₂ treatment at 20 °C for 20 min reduced population of \textit{Salmonella} and \textit{E. coli} by about 1.4 logs. Washing with SDS and Tween 80 produced similar log reductions as a simple water wash for \textit{Salmonella} on tomato surface and, therefore, was ineffective in removing pathogens from tomato surface (Raiden and others 2003).

Pathogen internalization may occur in the field or during postharvest processing through the root system, wounding, stem scar, or natural openings such as the stomata as reported in some studies (Ryser and others 2009). Although antimicrobial wash proven to be effective to some extent in killing the pathogens on the surface of produce, they are ineffective for internalized pathogens. Chlorine wash at 200 ppm failed to eliminate \textit{E. coli} O157:H7 in internalized lettuce tissue and other vegetables (Nthenge and others 2007; Niemira 2008). In such situations, irradiation may be the only viable option. In this study, the potential of integrated treatment combining UV-C light and low-dose ionizing radiation for decontamination of inoculated pathogens such as \textit{E. coli} O157:H7 and \textit{S. enterica} on fresh tomato surface has been demonstrated. The log reduction obtained by the integrated treatment was substantially higher than the reduction obtained with various antimicrobial wash. Reduction of inoculated pathogens in excess of 5 log was achieved on whole grape tomato by combining UV-C (0.6 kJ/m²) with 0.75 kGy gamma irradiation. The cumulative reductions may be sufficient to render a safe product at the retail level. The current U.S. Food and Drug Administration (FDA) regulations prohibit the application of radiation doses in excess of 1 kGy to fresh fruits and vegetables to prevent quality deteriorations. The irradiation doses applied in this study are well within the permissible limit. Data obtained from this work indicate 5 or higher log reduction, suggesting an efficacious treatment.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Produce media</th>
<th>Treatment</th>
<th>D_{10} value, kGy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampin-resistant Salmonella</td>
<td>Fresh-cut Roma tomatoes</td>
<td>Electron beam irradiation at doses 0.7 and 0.95 kGy</td>
<td>0.29 to 0.54</td>
<td>Schmidt and others (2006)</td>
</tr>
<tr>
<td>Montevideo and Salmonella Agona</td>
<td>Diced table ripe hothouse tomatoes dipped in 1% calcium chloride</td>
<td>Electron beam irradiation at doses 0.3 to 0.9 kGy</td>
<td></td>
<td>Prakash and others (2007)</td>
</tr>
<tr>
<td>Nalidixic acid-resistant strains of S. Hartford, S. Montevideo, or a mixture of 5 strains of S. Hartford, S. Michigan, S. Montevideo, S. Poona, and S. Gaminara</td>
<td>Whole fresh Roma tomatoes</td>
<td>X-ray irradiation at doses 0.1 to 1.5 kGy</td>
<td>0.56</td>
<td>Mahmoud (2010)</td>
</tr>
<tr>
<td>Three strain mixture of S. Montevideo, S. Javiana, and S. Typhimurium</td>
<td>Whole fresh Roma tomatoes</td>
<td>X-ray irradiation at doses 0.1 to 1.5 kGy</td>
<td>0.39</td>
<td>Mahmoud (2010)</td>
</tr>
<tr>
<td>Three strain mixture of E. coli O157:H7 (C7927, EDL933, and 2049)</td>
<td>Whole fresh grape tomatoes</td>
<td>UV-C (0.6 kJ/m²) followed by low-dose (0.1 to 0.75 kGy) gamma irradiation</td>
<td>0.22</td>
<td>Present work</td>
</tr>
<tr>
<td>Three strain mixture of S. Montevideo G4639, S. Newport H1275, and S. Stanley H8058</td>
<td>Whole fresh grape tomatoes</td>
<td>UV-C (0.6 kJ/m²) followed by low-dose (0.1 to 0.75 kGy) gamma irradiation</td>
<td>0.18</td>
<td>Present work</td>
</tr>
</tbody>
</table>
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Table 2—Changes in the mean total aerobic mesophilic bacterial population, PCA (log CFU/g) of control, and treated grape tomatoes during storage at 5 °C for 21 d.  

<table>
<thead>
<tr>
<th>Storage time, days</th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
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<tbody>
<tr>
<td>0</td>
<td>4.73 ± 0.2ABac</td>
<td>4.0 ± 0.3ABb</td>
<td>3.45 ± 0.1Ac</td>
<td>3.27 ± 0.2Ac</td>
<td>3.15 ± 0.2Ac</td>
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<tr>
<td>7</td>
<td>4.45 ± 0.2Aa</td>
<td>3.79 ± 0.2ABa</td>
<td>3.48 ± 0.1Ab</td>
<td>3.81 ± 0.2Ab</td>
<td>2.62 ± 0.2Ac</td>
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<tr>
<td>14</td>
<td>4.92 ± 0.2ABac</td>
<td>3.99 ± 0.2ABb</td>
<td>3.32 ± 0.1Ac</td>
<td>2.07 ± 0.2Ad</td>
<td>3.30 ± 0.2Ac</td>
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<tr>
<td>21</td>
<td>5.36 ± 0.1Ac</td>
<td>4.42 ± 0.2Ab</td>
<td>1.39 ± 0.1Ac</td>
<td>0.86 ± 0.2Ad</td>
<td>2.89 ± 0.1Ac</td>
</tr>
</tbody>
</table>

*Treatments: T1, 0.6 kJ/m² UV-C dose plus 0.1 kGy gamma irradiation; T2, 0.6 kJ/m² UV-C dose plus 0.2 kGy gamma irradiation; T3, 0.6 kJ/m² UV-C dose plus 0.5 kGy gamma irradiation; and T4, 0.6 kJ/m² UV-C dose plus 0.75 kGy gamma irradiation.

Mean values with different lowercase letters in same row are significantly different (P < 0.05).

Table 3—Changes in the mean yeast and mold counts (log CFU/g) of control and treated grape tomatoes during storage at 5 °C for 21 d.  

<table>
<thead>
<tr>
<th>Storage time, days</th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.95 ± 0.2Cc</td>
<td>2.78 ± 0.2Cc</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>3.55 ± 0.1Bc</td>
<td>3.68 ± 0.2ABc</td>
<td>ND</td>
<td>0.86 ± 0.2Ab</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>3.79 ± 0.2ABb</td>
<td>3.45 ± 0.2Ba</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>21</td>
<td>3.95 ± 0.2Aa</td>
<td>3.86 ± 0.2Ab</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Treatments: T1, 0.6 kJ/m² UV-C dose plus 0.1 kGy gamma irradiation; T2, 0.6 kJ/m² UV-C dose plus 0.2 kGy gamma irradiation; T3, 0.6 kJ/m² UV-C dose plus 0.5 kGy gamma irradiation; and T4, 0.6 kJ/m² UV-C dose plus 0.75 kGy gamma irradiation.

Mean values with different lowercase letters in same row are significantly different (P < 0.05).

Effect of integrated treatment on the microbiological quality of grape tomatoes during storage at 5 °C for 21 d

Shelf life of produce is quite short and that is one of the major concerns for produce industry. Decontamination techniques such as irradiation are known to affect the microbiological and physical qualities of produce (Howard and others 1995; Hagenmaier and Baker 1997). Many studies have shown that both UV and gamma irradiation significantly extend the shelf life of fruits and vegetables by hindering the ripening process and inactivating spoilage microorganisms (Arvanitoyannis and others 2009). Due to its delicate tissue structure, tomatoes are very susceptible to injury and microbial invasion. Prakash and others (2002) reported the extension of microbial shelf life of tomatoes due to gamma irradiation.

The effect of integrated treatment of UV-C light and low-dose gamma irradiation on the population of aerobic mesophilic bacteria and yeast and mold on whole grape tomatoes was evaluated over time during storage at 5 °C for 21 d. Table 2 shows the changes in the mean total aerobic mesophilic microorganism populations (log CFU/g unit) of control and treated grape tomatoes. The initial mesophilic aerobic bacterial count was 4.7 logs CFU/g, which is in agreement with the result obtained by Prakash and others (2002), who reported the total aerobic microorganism population on untreated tomato was 4.4 logs CFU/g. Treatments (T1) with 0.1 kGy significantly (P < 0.05) reduced the initial populations of mesophilic bacteria on UV treated grape tomato by approximately 15%, whereas 0.75 kGy treatment (T4) caused a higher reduction (1.6 log CFU/g) of initial population from 4.73 log CFU/g to 3.15 log CFU/g. Schmidt and others (2006) also observed a similar reduction and reported 1.3-log reduction of lactic acid bacteria on tomato cubes after irradiation with 0.7 kGy electron beam. The effectiveness of integrated treatment on yeast and mold population (log CFU/g unit) of control and treated grape tomatoes during storage at 5 °C for 21 d is given in Table 3. The initial count of yeast and mold on the untreated control was 2.95 log CFU/g. Schmidt and others (2006) reported 2.3 and 1.6 log CFU/g yeasts and molds counts, respectively, on untreated Roma tomato cubes. This initial count is in line with our finding although some variation is expected due to difference in the tomatoes type and sample preparation method. Treatment with 0.1 kGy reduced the initial yeast and mold population on UV treated tomato, but not significantly (P > 0.05) from 3.0 to 2.8 log CFU/g. Meanwhile treatments (T2, T3, and T4) using higher doses significantly (P < 0.05) reduced the initial populations of yeast and mold to less than the detectable limit (<3 CFU/g), as shown in Table 3. The background microflora on untreated and treated grape tomatoes increased gradually during storage, but due to the effect of treatments, treated tomatoes hold microbial populations significantly lower (P < 0.05) compared to untreated ones. In control sample, the mesophilic population increased significantly from 4.7 log CFU/g to 5.4 log CFU/g by 21 d whereas the populations were decreased significantly for treated (T2 and T3) samples to a level of 1.4 and 0.9 log CFU/g, respectively, over the same time period. Also, for the control sample, the yeast and mold populations increased 2.8 log CFU/g to 4.0 log CFU/g at the end of 3 wk. Treatments using 0.6 kJ/m² UV-C with 0.25, 0.5, and 0.75 kGy gamma irradiation (treatments T2, T3, and T4) maintained the population of yeast and mold under detection limit (<3 CFU/g) for 0, 7, 14, and 21 d, respectively. Fresh picked tomato has a shelf life of about 10 to 14 d (Prakash and Foley 2004) and postharvest spoilage and rotting are mainly due to molds and yeasts (Wang and others 2008). Control of spoilage microorganisms can play an important role in the improvement of quality of tomato. Microbial counts that influence changes in sensory quality factors of minimally processed vegetables resulting in rejection of
the product are in most cases >7 to 8 log CFU/g (Ragert and others 2007). In the present work, aerobic mesophilic microbial count for treated tomatoes remained below 4 log CFU/g and the population exhibited a declining trend with time during storage at 5 °C except for treatment T1 (Table 2) and also the mean yeast and mold populations fell below the detection limit (Table 3). On the basis of this fact, the integrated treatment using UV-C (0.6 kJ/m²) and gamma irradiation (0.25 to 0.75 kGy) prolonged the shelf life of grape tomatoes beyond its normal limit. Similar to findings in the present work, Howard and others (1995) reported those aerobic mesophilic microfloras were reduced during storage of tomatoes that had been irradiated (1.0 kGy).

Conclusion
In this study, the potential of an integrated treatment combining UV-C light and low-dose ionizing radiation for decontamination of inoculated pathogens, such as E. coli O157:H7 and S. enterica, on fresh tomato surface has been demonstrated. The study indicated that the integrated treatment was effective against the tested pathogenic bacteria and inherent microflora on tomatoes. The log reduction obtained by the integrated treatment was substantially higher than the reduction obtained with various antimicrobial washes. Reduction of inoculated pathogens in excess of 5 log was achieved on whole grape tomato by combining UV-C (0.6 kJ/m²) with 0.75 kGy gamma irradiation. The cumulative reductions may be sufficient to render a safe product at the retail level. The current FDA regulations prohibit the application of radiation doses in excess of 1 kGy to fresh fruits and vegetables to prevent quality deterioration. The doses applied in this study are well within the FDA permissible limit. The effects of this combined treatment on tomato firmness (texture), lycopene content, and color were minimal (data not shown). Results obtained from this work suggests that efficacious integrated treatment strategy achieved 5 or higher log reduction, which is consistent with the recommendations of the Nat. Advisory Committee on Microbiological Criteria for Foods (NACMCF 1999).

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References
Effects of integrated treatments on pathogens...


