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MICROBIOLOGICAL COMPARISON OF VISIBLY DIRTY AND VISIBLY CLEAN MATURE GREEN TOMATOES BEFORE AND AFTER TREATMENTS WITH DEIONIZED WATER OR CHLORINE IN MODEL OVERHEAD SPRAY BRUSH ROLLER SYSTEM

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ABSTRACT

The purpose of the current study was to compare natural microflora counts of mature green tomatoes as influenced by visual cleanness, and investigate ability of chlorine sanitizer to reduce different groups of natural microflora on the surface of tomatoes using overhead spray brush roller system. We hypothesized that natural microflora might not be equally affected, with vegetative Gram negative bacteria being more sensitive and soil-related Gram positive sporoforming bacilli and molds more resistant. Microflora from untreated visibly clean and visibly dirty tomatoes, as well as from visibly clean tomatoes after 30 seconds deionized water or 100 ppm chlorine treatments, was recovered and spread plated on Tryptic Soy agar, MacConkey agar, and acidified Potato Dextrose agar. Microflora from untreated and chlorine-treated tomatoes was non-specifically enriched and plated on agar with chlorine paper disc diffusion assay applied to check for inhibition zone differences. Interestingly, there was no significant difference in plate counts between visibly clean and dirty tomatoes (p >0.05). Chlorine was more effective than water alone to reduce microbial counts on tomatoes for all microbiological media tested. Based on similar relative reductions of microorganisms in each group, it was concluded that chlorine may have no preferential kill for investigated groups of microorganisms. High counts remaining after treatment with chlorine solution suggested possibility of resistant microbial biofilm formation on the surface of tomatoes.

Keywords: tomatoes; cleanness; natural microflora; overhead spray brush roller system

INTRODUCTION

Tomatoes are an important agricultural fruit, placing nine per production volume among most popular agricultural produce in the Ukraine. Referencing **FAOSTAT (2017)**, top ten tomato growing countries were China, India, Turkey, USA, Egypt, Iran, Italy, Spain, Mexico, and Brazil, with Ukraine present in the top twenty and producing as much as 2,267,460 tonnes in 2017 alone. The visual appearance of the tomato surface as "clean" may give a false feeling of safety of its consumption. However, two large groups of microorganisms, which are of concern on fresh produce, are spoilage and pathogenic, which may cause either spoilage or foodborne illnesses, are invisible to human eye **(Jay, 1998)**. Enteric Gram-negative pathogens, including *Salmonella* and *Escherichia coli* O157:H7, may be present on fresh tomatoes as contamination from environment and may persist on the surfaces **(Tokarskyy et al., 2018; Tokarskyy and Schneider, 2019)**. Common tomato spoilage microorganisms include Gram-negative rods (*Erwinia carotowora)*, Gram-positive sporeformers (*Bacillus* spp.), yeasts and molds **(Shi et al., 2009; Jay, 1998)**. Although novel methods to decontaminate surface of edible foodstuff are available **(Tokarskyy and Marshall, 2010)**, they remain expensive comparing to the use of low-cost alternatives, such as chlorine sanitizers **(Dychdala, 2001; Tokarskyy et al., 2015)**. One of the approaches to reduce microbial load and prevent cross-contamination on tomatoes before retail sale is through their washing with low concentration chlorine sanitizer **(Chang and Schneider, 2012; Gereffi, Sreedharan and Schneider, 2015)**. For example, one of the most common tomato processing system in the United States is a flume tank with 150 ppm free chlorine (pH 6.5 to 7.5) and a maximum of a 2 minute treatment **(Gereffi, Sreedharan and Schneider, 2015)**. **Gereffi, Sreedharan and Schneider (2015)** have shown that even 25 ppm of chlorine may be adequate to prevent cross-contamination of tomatoes with *Salmonella* if the concentration is properly maintained, chemical oxygen demand does not exceed 500 ppm, and tomatoes are treated for at least 120 seconds in a flume tank. Such tank may be terminally equipped with an additional overhead spray and brush roller system, where increased physical removal of bacteria with brushes in conjunction with antimicrobial efficacy of sanitizers may greatly improve decontamination step (Chang and

Schneider, 2012). The primary purpose of chlorine sanitizer is to prevent cross-contamination and bacterial build-up **(Gil et al., 2009)**. Though chlorine is believed to have non-specific mode of action, bacterial spores have innate resistance at concentrations used in food industry **(Davidson and Harrison, 2002)**. Some of the naturally occurring bacteria found on tomatoes do include *Bacillus* spp., in addition to *Cyanobacterium* spp., *Erwinia* spp., *Enterobacter* spp., *Pantoea* spp., *Pseudomonas putida*, among others **(Shi et al., 2009)**. Chlorine is capable to reduce natural microbial contamination level on produce, but never eliminate it completely **(Allende et al., 2009; Rahman, Ding and Oh, 2010)**. **Chang (2011)** found that with initial population of natural microflora on tomato surfaces of 5.31 log units, 100 ppm chlorine significantly reduced more natural microflora than water with a 1.41 log units reduction after 30 seconds treatment (p <0.05), but never below detection limit. Increasing treatment time to 60 seconds did not significantly affect efficacy.

The purpose of the current study was to compare microbial loads of "visibly clean" and "visibly dirty" tomatoes, to evaluate influence of 100 ppm chlorine wash on different groups of natural microflora on tomato surfaces using overhead spray-brush roller system, as well as to evaluate resistance to chlorine of residual microflora in order to better understand surviving natural microorganisms after treatment.

Scientific hypothesis

We hypothesize that visibly dirty tomatoes will have significantly higher microbial counts on all microbiological media tested, comparing to visibly clean tomatoes. We hypothesize that 100 ppm chlorine treated tomatoes will have significantly lower microbial counts comparing to water treated and untreated tomatoes. We hypothesize that residual microflora, regrown from 100 ppm chlorine treated tomatoes, will be more resistant to chlorine in paper disc diffusion antimicrobial assay comparing to untreated tomatoes, with smaller inhibition zone diameter.

MATERIAL AND METHODOLOGY

Brush roller machine and chlorine preparation

Two rotating (180 rpm) nylon rollers (46 cm long and 12 cm diameter) sat alongside in a 46 cm by 34 cm box (Figure 1). Five tomatoes at a time were placed between two brush rollers and revolved in directions depending on their size and shape while being brushed by rollers. Simultaneously, three spray nozzles released a cone shaped spray (16 psi pressure) with a flow rate of ca. 21 mL.second^{-1} on the surface of rotating tomatoes. Treatment solution was fed to the nozzles using 20 L bucket, piping, and centrifugal pump.

Chlorine sanitizer was prepared by mixing 22 mL of 5.65 to 6.00% sodium hypochlorite (Thermo Fisher Scientific, Waltham, MA, USA) with ca. 10 L of deionized water. The sanitizer pH was adjusted to 6.50 ±0.05 with 1N HCl (Thermo Fisher Scientific, Waltham, MA, USA). Free chlorine concentration was measured using Hach DR/890 colorimeter, method 8091 (Hach Co., Loveland, CO, USA) by diluting treatment solution 1:100 in chlorine-free DI water to get to the required range of 0.98 to 1.02 ppm, corresponding to 100 ±2 ppm chlorine of undiluted solution.

The brush roller machine rotating brushes and pump were switched on and the system was flushed/rinsed with deionized water for 3 minutes. Following initial flushing, the cleanness of each brush was evaluated by swabbing it four times from one end to another with sterile cotton-tipped applicator (Thermo Fisher Scientific, Waltham, MA, USA) and swabbing the Tryptic Soy agar plate followed by confirmation of absence of microbial growth (32 °C, 48 hours). The first tomato treatment was deionized water (30 seconds), followed by chlorine treatment (30 seconds).

Tomatoes preparation and treatment

Green mature unwashed round tomatoes (*Lycopersicum esculentum*) variety 602 were acquired from a single local packinghouse on three different days late May-early June in Florida, USA. Five tomatoes were selected as "visibly dirty" ("D") based on their appearance and presence of adhered soil, leaves, and dirt. Fifteen tomatoes were classified as "visibly clean" ("C") based on their appearance, for each round of experiments. These fifteen "C" tomatoes were rubbed each in three rounds with sterile nitrile gloves to "normalize" microbial flora among them.

Five of each "D" and "C" tomatoes were analyzed immediately untreated, while second set of five "C" tomatoes was treated with deionized water and third set – with 100 ppm chlorine wash.

Deionized water treatment was applied to five visibly clean tomatoes ("C-W") for 30 seconds by placing them simultaneously on the rollers, and pH of the liquid and absence of chlorine was verified using sample solution from nozzles as described previously. This set of clean and water-treated tomatoes was removed for microbiological analysis. Following deionized water treatment, the system was flushed for 1 min with prepared 100 ppm chlorine sanitizer (pH 6.5) and concentration of the chlorine and pH were verified using sample solution from the nozzles. The third set of five visibly clean tomatoes ("C-CHL") was placed on the rollers and treated with chlorine sanitizer for 30 seconds before microbiological analysis.

Microbiological analysis of tomatoes

Each tomato was transferred to 50 mL Bacto™ Tryptic Soy Broth (TSB, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) in a stomacher bag and was vigorously shaken for 20 seconds, rubbed for 20 seconds, and shaken again for 20 seconds. The rinsate was serially diluted in buffered peptone water and 0.1 mL aliquots were immediately spread plated on Tryptic Soy agar (TSA, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for Total Plate Count (TPC, 32 °C, 48 hours), MacConkey agar (MCA, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for Gram-negative bacterial counts (GNC, 37 °C, 48 hours), and acidified Potato Dextrose agar (aPDA, pH 3.5, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for Yeast and Mold Count (YM, 25 °C, 5 days). The countable agar plates contained preferred 25 to 250 CFU per plate range and conversion from CFU.mL^{-1} rinsate to CFU.tomato^{-1}

was done by multiplication factor 50. Therefore, the detection limit was 2.7 $\log_{10}$ CFU.tomato^{-1} (est.).

The TSB rinsates with tomatoes ("C" and "C-CHL") were further incubated for 10 hours at 32 °C to non-specifically enrich natural and residual microflora after tomato treatments for chlorine selective bactericidal activity evaluation.

To prepare paper discs soaked in chlorine, 6 mL of sodium hypochlorite (5.65 – 6%) was mixed with 41 mL of autoclaved deionized water and 3 mL 1N HCl, resulting in plates were incubated for 48 hours at 32 °C before inhibition zones were measured and pictures of the plates were taken **(Duran and Marshall, 2005)**.

Statistic analysis

The experiment was repeated three times and counts were analyzed for each microbiological medium (TSA, MCA, aPDA) using one-way ANOVA with a single factor of treatment ("D", "C", "C-W", "C-CHL"). Means were separated using Fisher LSD method if influence of the factor was significant (p <0.05). Chlorine inhibition zones for enriched microflora from "C" and "C-CHL" treated tomatoes around chlorine-soaked paper discs were measured with the ruler and data were analyzed using one-way ANOVA. Mean values of the inhibition zones were separated using Fisher LCD method. Statistical analysis of the obtained data was performed using commercial software Statistica ver. 10.0 (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

There was a significant influence of analyzed factor with variations as "C", "D", "C-W", and "C-CHL" on microbial counts of all three microbiological media plated (p <0.05).

Surprisingly, there was no significant difference in total plate count (6.01 ±0.50 vs. 6.33 ±0.52 $\log_{10}$ CFU.tomato^{-1}), Gram-negative counts (5.71 ±0.65 vs. 5.80 ±0.60 $\log_{10}$ CFU.tomato^{-1}), and yeast and mold counts (4.42 ±0.54 vs. 4.56 ±0.41 $\log_{10}$ CFU.tomato^{-1}) between clean and dirty tomatoes, respectively (p > 0.05, Figure 2). This can be attributed to smaller sample sizes, comparing to other studies **(Schneider et al., 2017; De et al., 2018)**. Water alone decreased TPC by 0.40 $\log_{10}$ CFU.tomato^{-1}, GNC by 0.48 $\log_{10}$ CFU.tomato^{-1}, and YM by 1.24 $\log_{10}$ CFU.tomato^{-1}on water-treated tomatoes (Figure 2).

Chlorine wash was more effective with corresponding average reductions of 1.12, 1.19, and 1.66 $\log_{10}$ CFU.tomato^{-1} for TPC, GNC, and YM, respectively (Figure 2, Table 1). Interestingly, the highest reduction was observed in YM counts, while **Dychdala (2001)** noted that higher chlorine, 135 to 500 ppm, is required to inactivate molds. Based on water alone data, it can be concluded that yeasts and molds might have been simply washed off tomatoes without kill step. Similarly, **Schneider et al. (2017)**, while analyzing pre- and post-processed tomatoes from Florida, New Jersey and Maryland packinghouses in spring, have found that average microbial TPC per untreated tomato was 6.25 $\log_{10}$ CFU.tomato^{-1} and 5.31 $\log_{10}$ CFU.tomato^{-1} for chlorine water flume tank treated tomatoes, corresponding to 0.94 $\log_{10}$ CFU.tomato^{-1} reduction. Considering large number of analyzed samples, overall range for TPC for tomatoes collected year-round was 2.3 to 12.1 $\log_{10}$ CFU.tomato^{-1} with median of 6.9 $\log_{10}$ CFU.tomato^{-1} **(Schneider et al., 2017)**.

MacConkey agar is selective and differential medium for bacteria, formulated to selectively isolate Gram-negative and enteric bacilli. Therefore, it may be argued that GNC is a subset of TPC, and though similar relative log reductions of microorganisms in each group of these two groups were found, absolute reductions in counts as CFU.tomato^{-1} suggest that chlorine may have had an "all kill" approach, reducing not only Gram-negative bacteria counts, but also Gram positive (Table 1). Similarly, **Schneider et al. (2017)**, while analyzing larger sets of pre- and post-processed tomatoes, have found total coliforms counts on CHROMagar™-ECC to be 5.13 $\log_{10}$ CFU.tomato^{-1} and 4.70 $\log_{10}$ CFU.tomato^{-1} for untreated and chlorine flume tank treated tomatoes, respectively.

This observation, together with no significant difference between inhibition zones by chlorine for untreated and chlorine-treated tomato residual microflora (p >0.05), 18.4 ±1.7 and 19.9 ±2.3 mm, respectively, suggested that chlorine may have had no preferential kill, but rather a shotgun approach (Figure 3).

However, concentrated circle patterns were observed on disc diffusion plates, suggesting that certain microorganisms on the tomato surface might be indeed more sensitive to chlorine (Figure 4).

High counts remaining after treatment with chlorine suggested resistant biofilm formation on the surface of tomatoes. Another suggested explanation by **Fatica and Schneider (2009)** is that natural microflora is hiding in crevices and pockets of the hydrophobic, waxy cuticles of the produce, where aqueous chlorine sanitizer cannot enter.

Table 1 Average values of $\log_{10}$ and absolute reductions in microbial population counts on TSA, MCA, and aPDA of water-treated (C-W) and chlorine-treated (C-CHL) tomatoes comparing to visually clean tomatoes (C) used for overhead spray brush roller experiments.

Microbial population	Ave log reduction, $\log_{10}$ CFU.tomato^{-1}		Ave absolute reductions, CFU.tomato^{-1}	
	C-W	C-CHL	C-W	C-CHL
TPC/TSA	0.40	1.12	608,095	939,583
GNC/MCA	0.48	1.19	342,430	480,962
YM/aPDA	1.24	1.66	24,815	25,773

Figure 1 Overhead spray brush roller system used in the experiments, manufactured by Agri Machinery Inc. (Orlando, Fla., USA).

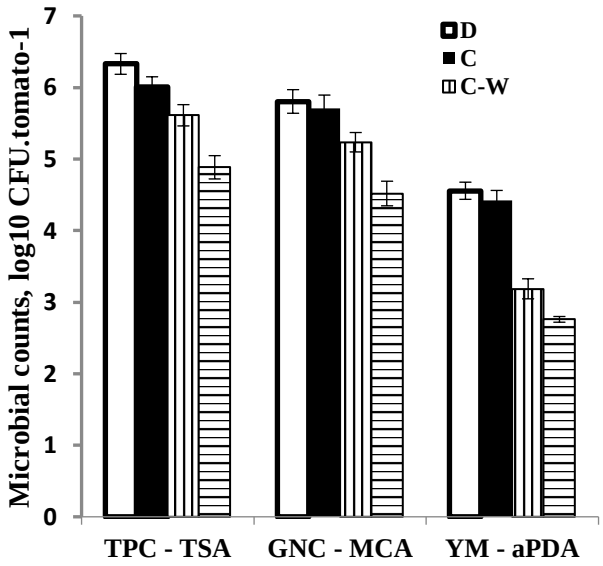

Figure 2 Microbial counts of visibly dirty (D), visibly clean (C), visibly clean treated with water (C-W), and visibly clean treated with chlorine (C-CHL) tomatoes on Tryptic Soy agar (TPC-TSA), MacConkey agar (GNC-MCA), and acidified Potato Dextrose agar (YM-aPDA). Counts are expressed as $\log_{10}$ CFU.tomato^{-1}. Note: Error bars reflect standard errors of mean. Means within the same microbiological medium with the same letters are not significantly different (p >0.05).

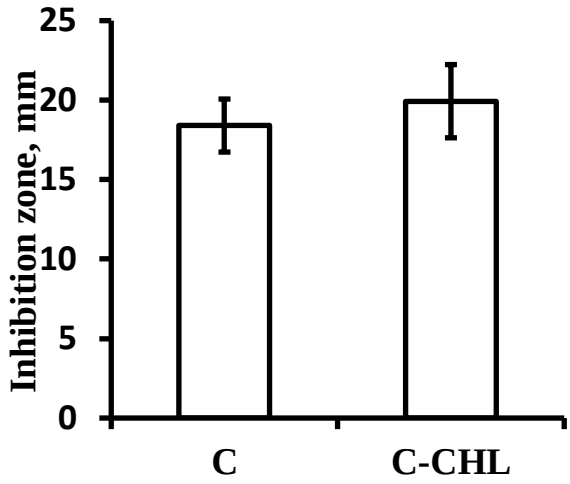

Figure 3 Inhibition zones of 7,200 ppm soaked paper discs on non-selective enrichments of natural microflora and residual microflora of untreated and chlorine-treated tomatoes. Note: Error bars reflect standard deviation. Same letters mean non-significant difference (p >0.05).

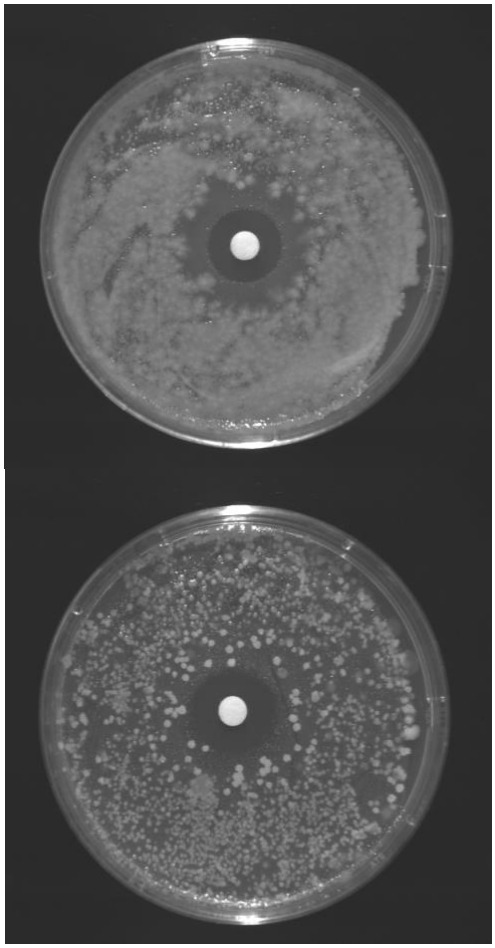

Figure 4 Examples of paper disc diffusion assays (7,200 ppm chlorine) on non-selective residual microflora enrichments of chlorine treated tomatoes. Note: Circles of bacterial populations with different sensitivities are shown with arrows.

CONCLUSION

To summarize, cleanness may attribute to lower counts on the surface of tomatoes, irrespective of microbial group analyzed, though microbial counts were not significantly different. Larger sets of tomatoes are needed to fortify this statement. Although 100 ppm chlorine treatment reduced all microbial counts significantly better than water alone, it failed to bring them below detection levels, suggesting strong interaction such as biofilm formation, between natural microflora and tomatoes. Comparing reductions of microorganisms in each group, it was concluded that chlorine may have no preferential kill but rather a shotgun approach.

REFERENCES

Allende, A., McEvoy, J., Tao, Y., Luo, Y. 2009. Antimicrobial effect of acidified sodium chlorite, sodium chlorite, sodium hypochlorite, and citric acid on *Escherichia coli* O157:H7 and natural microflora of fresh-cut cilantro. *Food control*, vol. 20, no. 3, p. 230-234. https://doi.org/10.1016/j.foodcont.2008.05.009

Chang, A. S. 2011. *Evaluation of overhead spray-applied sanitizers for the reduction of Salmonella on tomato surfaces*: master of science thesis. Gainesville, FL, USA : University of Florida. https://doi.org/10.1111/j.1750-3841.2011.02486.x

Chang, A. S., Schneider, K. R. 2012. Evaluation of overhead spray-applied sanitizers for the reduction of *Salmonella* on tomato surfaces. *Journal of food science*, vol. 71, no. 1, p. M65-M69. https://doi.org/10.1111/j.1750-3841.2011.02486.x

Davidson, P. M., Harrison, M. A. 2002. Resistance and adaptation to food antimicrobials, sanitizers, and other process controls. *Food technology*, vol. 56, no. 11, p. 69-78.

De, J., Li, Y., Sreedharan, A., Goodrich Schneider, R. Gutierrez, A., Jubair, M., Danyluk, M. D., Schneider, K. R. 2018. A three-year survey of Florida packinghouses to determine microbial loads on pre- and post-processed tomatoes. *Food Control*, vol. 86, p. 383-388. https://doi.org/10.1016/j.foodcont.2017.11.036

Duran, G. M., Marshall, D. L. 2005. Ready-to-eat shrimp as an international vehicle of antibiotic-resistant bacteria. *Journal of food protection*, vol. 68, no. 11, p. 2395-2401. https://doi.org/10.4315/0362-028X-68.11.2395

Dychdala, G. R. 2001. Chlorine and chlorine compounds. In: Block S. S. *Disinfection, sterilization, and preservation.* 5th ed. Philadelphia, PA : Lippincott Williams and Wilkins. p 135-158. ISBN-13: 978-0683307405.

Fatica, M. K., Schneider, K. R. 2009. The use of chlorination and alternative sanitizers in the produce industry. *CAB Reviews: perspectives in agriculture, veterinary science, nutrition and natural resources*, vol. 4, no. 52, p. 1-10. https://doi.org/10.1079/PAVSNNR20094052

FAOSTAT. 2017. Food and Agriculture Organization of the United Nations. Commodities by country. Countries by commodity. Available at: http://www.fao.org

Gereffi, S., Sreedharan, A., Schneider, K. R. 2015. Control of Salmonella cross-contamination between green round tomatoes in a model flume system. *Journal of food protection*, vol. 78, no. 7, p. 1280-1287. https://doi.org/10.4315/0362-028X.JFP-14-524

Gil, M. I., Selma, M. V., López-Gálvez, F., Allende, A. 2009. Fresh-cut product sanitation and wash water disinfection: problems and solutions. *International journal of food microbiology*, vol. 134, no. 1-2, p. 37-45. https://doi.org/10.1016/j.ijfoodmicro.2009.05.021

Jay, J. M. 1998. *Modern food microbiology.* 5th ed. GAITHERSBURG, MD, USA : Aspen Publishers, 790 p. ISBN: 978-1-4899-4407-8.

Rahman, S. M. E., Ding, T., Oh, D. H. 2010. Inactivation effect of newly developed low concentration electrolyzed water and other sanitizers against microorganisms on spinach. *Food control*, vol. 21, no. 10, p. 1383-1387. https://doi.org/10.1016/j.foodcont.2010.03.011

Schneider, K. R., De, J., Li, Y., Sreedharan, A., Goodrich Schneider, R., Danyluk, M. D., Pahl, D. M., Walsh, C. S., Todd-Searle, J., Schaffner, D. W., Kline, W., Buchanan, R. L. 2017. Microbial evaluation of pre- and post-processed tomatoes from Florida, New Jersey and Maryland packinghouses. *Food control*, vol 73, p. 511-517. https://doi.org/10.1016/j.foodcont.2016.08.048

Shi, X., Wu, Z., Namvar, A., Kostrzynska, M., Dunfield, K., Warriner, K. 2009. Microbial population profiles of the microflora associated with pre- and postharvest tomatoes contaminated with *Salmonella* Typhimurium or *Salmonella* Montevideo. *Journal of applied microbiology*, vol. 107, no. 1, p. 329-338. https://doi.org/10.1111/j.1365-2672.2009.04211.x

Tokarskyy, O., De, J., Fatica, M. K., Brecht, J., Schneider, K. R. 2018. Survival of *Escherichia coli* O157:H7 and *Salmonella* on bruised and unbruised tomatoes from three ripeness stages at two temperatures. *Journal of food protection*, vol. 81, no. 12, p. 2028-2034. https://doi.org/10.4315/0362-028X.JFP-18-220

Tokarskyy, O., Schneider, K. R. 2019. Influence of temperature, humidity, and diluent type on survival of Salmonella spp. on the surface of raw tomatoes. *Potravinarstvo Slovak journal of food sciences*, vol.13, no 1., p. 325-330. https://doi.org/10.5219/1121

Tokarskyy, O., Marshall, D. L. 2010. Novell technologies for microbial spoilage prevention. In: Toldra, F. *Handbook of meat processing*. Hoboken, NJ : Wiley-Blackwell Publishing, p. 263-286. ISBN 978-0-813-82182-5. https://doi.org/10.1002/9780813820897.ch14

Tokarskyy, O., Schneider, K. R., Berry, A., Sargent, S. A., Sreedharan, A. 2015. Sanitizer applicability in a laboratory model strawberry hydrocooling system. *Postharvest biology and technology*, vol. 101, p. 103-106. https://doi.org/10.1016/j.postharvbio.2014.12.004

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