





Potravinarstvo Slovak Journal of Food Sciences vol. 14, 2020, p. 1027-1034 https://doi.org/10.5219/1408 Received: 3 June 2020. Accepted: 21 September 2020. Available online: 28 October 2020 at www.potravinarstvo.com © 2020 Potravinarstvo Slovak Journal of Food Sciences, License: CC BY 3.0 ISSN 1337-0960 (online)

TRANSFER EFFICACY OF *ESCHERICHIA COLI* 0157:H7 BETWEEN SURFACES OF GREEN MATURE TOMATOES AND COMMON FOOD PROCESSING MATERIALS

Oleksandr Tokarskyy, Mykhaylo Korda, Halyna Lukyantseva

ABSTRACT

OPEN 👩 ACCESS

The objectives of this study were: a) to evaluate E. coli O157:H7 survival on green mature tomatoes and squares of common food processing materials - stainless steel, plastic (HDPE), and vinyl conveyor belt (PVC) - post-drying, stored at 25 °C in the humidified environment for four days; b) to determine pathogen transfer rates (wet, 90 minutes, or 24-hours drying post-inoculation), from inoculated tomato surfaces to uninoculated steel, plastic, and vinyl conveyor belt squares and conversely. It was shown that E. coli O157:H7 did not survive well on the surface of tomatoes, resulting in a decline from 5.3 log₁₀ CFU.mL⁻¹ 90 minutes post-drying to 1.4 log₁₀ CFU.mL⁻¹ on day 4. Similarly, the pathogen did not survive well on the surface of food processing squares, with numbers declining over 4 days from 4.04, 4.44, and 4.19 CFU.mL⁻¹ of rinsate 90 minutes squares post-drying to 0.72, 0.50, 0.83 log₁₀ CFU.mL⁻¹, which is close to the detection limit, for the steel, vinyl belt, and plastic, respectively. Successful cross-contamination between tomatoes and food processing surfaces was achieved during wet transfer; while transfer after 90 minutes inoculum post-drying and 24 hours were less successful. This can be explained by both lack of liquid media with suspended bacteria for transfer and fast pathogen die-off after desiccation. Dry transfers, as shown by the percentage of "positive" for pathogen presence tomatoes and squares, as well as bacterial counts, were more successful from tomatoes to squares, but not conversely. Special concern raised vinyl conveyor belt, where the surface picked up the most pathogen cells from the surface of tomatoes, resulting in 100% positive during 90 minute-dry transfers, followed by plastic (66.7% positive) and steel (55.6% positive). To summarize, we presented data on the possibility of cross-contamination between mature green tomatoes and common food processing surfaces, which may be interesting for the processors for risk evaluation.

Keywords: tomatoes; steel; vinyl conveyor belt; plastic; E. coli O157:H7; survival; transfer; cross-contamination

INTRODUCTION

Several investigations concluded that consumption of contaminated raw tomatoes can lead to enteric pathogen outbreaks, especially Salmonella spp. (CDC, 2002; Cummings et al., 2001; Croby et al., 2005). There is significant consumption of raw tomatoes worldwide, for example, 42% of this commodity is eaten raw in the U.S., a total of 29.6 pounds of tomatoes consumed per person in 2016 alone (USDA-ERS, 2016). Beuchat and Ryu (1997) noted that the sources of enteric pathogens on the tomatoes can be irrigation water, wash water, handling by workers, or contaminated surfaces. Various groups of bacteria exist on the surface of green mature tomatoes as it was shown by Tokarskyy and Korda (2019a). This contamination can be removed by high-temperature treatment (Tokarskyy et al., 2009) or by gamma irradiation (Schilling et al., 2009), which are often undesirable, creating processed, but not a fresh product. Gram-negative enteric pathogens will grow in the tomato at room temperature only if introduced into the flesh through

abrasions, wounds, and stem scars (Wei et al., 1995; Zhuang, Beuchat and Angulo, 1995; Daş, Gürakan and Bayindirli, 2006; Shi et al., 2007). Regarding the fate of the pathogens on the healthy tomato skin, it is believed that counts of Gram-negative enteric bacteria will decline over time, depending on tomato storage temperature, resuspension medium, and humidity (Tokarskyy et al., 2018; Tokarskyy and Schneider, 2019). To the best of our knowledge, E. coli O157:H7 has not been implicated in tomato-related foodborne outbreaks, therefore, most research related to tomato safety was done with Salmonella. Hirai (1991) wrote that Salmonella spp. have always been regarded as having better survival rates after desiccation compared to Escherichia coli, therefore, data might be comparable at a first glance. For example, Lang, Harris and Beuchat (2004) showed that spot-inoculated tomatoes with Salmonella spp. or E. coli O157:H7 showed counts decline by 2.20 and 3.17 log, respectively, after twenty-four hours inoculum post-drying.

A review by Kramer, Schwebke, and Kampf (2006) suggested that lower temperatures, higher inocula, and the presence of protein, serum, or other organic matter favor the survival of bacteria on inanimate objects. Hirai (1991) showed that E. coli K12 resuspended in deionized water, inoculated on cotton lint and dried, died off upon desiccation (3.5 log reduction on day 1 and 4.5 log reduction on day 3), while resuspension in 2% Bovine Serum Albumin or 5% horse serum caused only ca. 2.0 log reduction on day 4. On the other hand, the humidity effect might be more complicated, as Møretrø et al. (2010) showed that shigatoxin-producing E. coli dried on plastic or steel had the highest inactivation rate at 85% RH, while survived the best at 98% and 70%. It can be argued that microorganisms in the dried up inoculum survive better at low humidity (no metabolic activity) compared to high humidity, where exhausted stationary culture, still metabolically active, slowly dies off. However, at low inoculation level and high organic matter high humidity might stimulate growth. Møretrø et al. (2010) showed that twelve Shiga-toxin producing E. coli strains, each analyzed separately, declined upon desiccation in Brain Heart Infusion broth (BHI) on the stainless steel (type 304) from 6 - 7 logs to 3 - 5 logs on day 1 and 2 - 3.5 logs on day 7. Follow-up studies comparing BHI and water, 12 °C and 20 °C, 70% RH, and 80% RH, showed a beneficial effect of BHI, 12 °C, and 70% RH for E. coli survival.

Mature green tomatoes contact with various food processing surfaces during processing steps, such as stainless steel (sorting tables), polyvinyl chloride surfaces (PVC, vinyl conveyor belts), high-density polyethylene (HDPE) parts of the various processing equipment. There is a possibility that a single tomato, highly contaminated with *E. coli* O157:H7, can transfer pathogen to other surfaces, while these surfaces, in turn, can contaminate hundreds of uncontaminated tomatoes. Therefore, the question of possible cross-contamination by *E. coli* O157:H7 between tomatoes and such food processing materials as stainless steel, HDPE, and PVC, remains open.

The first objective of the current study was to determine survival rates of *E. coli* O157:H7 on the surface of unwashed and undamaged green mature tomatoes, as well as on the surface of common packaging materials (plastic, HDPE; vinyl belt, PVC; and stainless steel) at summertime room temperature (25 °C) within four days of storage. The second objective of the study was to investigate transfer rates of *E. coli* O157:H7 from the inoculated surface of tomatoes to the surface of these common food processing materials and vice versa, as influenced by the timing of the transfer.

Scientific hypothesis

We hypothesize that there is a potential of survival of *E.coli* O157:H7 on the surface of tomatoes and common food processing surfaces, and there is a significant possibility of pathogen transfer between surfaces not only when surfaces are wet, but also when they are dry.

MATERIALS AND METHODOLOGY

Rifampin preparation

0.4 grams of rifampin (Fisher Scientific, BP26795) was dissolved in 40 mL methanol (HPLC grade, Fisher Scientific), filter-sterilized (0.2 micron nylon filter, Fisher Scientific) to prepare sterile 10,000 ppm stock solution, and stored refrigerated (4 °C) in the darkness for no longer than a month. Rifampin stock solution was added to the cooled autoclaved DifcoTM tryptic soy agar (TSA, Becton, Dickinson, and Co) or BactoTM tryptic soy broth (TSB, Becton, Dickinson, and Co.) to yield 100 ppm final rifampin concentration, such as 0.1 mL rif stock to 10 mL TSB tube, or 10 mL rif stock to 1,000 mL TSA medium.

Tomato and food processing materials preparation

Green mature tomatoes variety Florida 47, unwashed and unwaxed, were acquired from local packinghouse (DiMare Company, Ruskin, Florida, U.S.A). Stainless steel squares (7.6 x 7.6 cm) were purchased from a local welding shop. Vinyl belt squares (7.6 x 7.6 cm, PVC-120, white polyester, one-side coated with PVC) were purchased from WL Deckert Co, Inc (Milwaukee, WI, U.S.A.). Highdensity polyethylene (HDPE, plastic) sheets (0.16 cm thick) were purchased from US Plastic Corp (Lima, OH, U.S.A.) and manually cut into 7.6 x 7.6 cm squares. All squares were run through the Lancer dishwasher (Lancer USA, Longwood, FL, U.S.A.), and manually rinsed twice with deionized water before drying. Therefore, food processing surfaces were classified as used. Stainless steel and vinyl belt squares were also reused after autoclaving.

Escherichia coli O157:H7 culture preparation

Two rifampin-resistant (200 ppm) strains of *Escherichia coli* O157:H7 (MDD20, MDD326) and two rifampinsensitive strains (MDD19 and MDD 327NA), were kindly provided by Dr. Michelle Danyluk's lab from the University of Florida. ATCC 35150 rifampin-sensitive strain was acquired from American Type Culture Collection (Manassas, WI, U.S.A.). All three rifampinsensitive strains were mutated to acquire rifampin resistance by transferring a pure culture from TSA plates (37 °C,

24 hours) to 10 mL TSB-rif 5ppm broth (37 °C, 24 hours), followed by sequential transfer of 0.1 mL aliquot to TBS containing 10, 20, and 40 ppm rifampin. Final turbid cultures (40 ppm rifampin) were streaked on TSA-rif200 plates (37 °C, 24 hours), and a single colony was transferred to TSB-rif200 broth to confirm growth. Five rif-resistant *E. coli* O157-H7 strains were maintained on TSA-rif80 ppm slants at 4 °C with bi-weekly transfers to fresh TSA-rif80 slants.

For each replication of the experiments, five strains were streaked on TSA-rif100 plates (37 °C, 24 hours), followed by three consecutive one-loopful transfers to 10 mL TSBrif100 tubes (37 °C, 12 hours, 12 hours, and 18 hours). A pathogenic cocktail (10 mL, 10⁹ CFU.mL⁻¹) was prepared by mixing 2 mL of each culture from the third broth. The cocktail was centrifuged (4,300 g, 10 minutes, Sorvall RC-5B centrifuge, DuPont Instruments) and washed once in 10 mL Dulbecco A phosphate-buffered saline (PBS, Oxoid, Hampshire, England), followed by final centrifugation (4,300 g, 10 minutes) and resuspension in 10 mL 0.1% peptone (Bacto peptone, Becton Dickinson and Co, Sparks, USA). Inoculum concentration was confirmed by serial dilutions in Buffered Peptone Water (BPW, Becton, Dickinson, and Co.) and pour plating using TSA-rif100.

Tomato inoculation and storage experiment

Fifteen mature green tomatoes were inoculated with 0.1 mL of the pathogenic cocktail as 10 spots of equal size around the blossom end each (10⁸ CFU.tomato⁻¹). One set of four tomatoes plus one tomato for immediate sampling was left uninoculated and served as negative controls. The procedure was carried out in a biosafety hood and tomatoes were allowed to dry for 90 minutes before moving into a 25 °C incubator. A shallow pan with water was placed inside the incubator to humidify the environment, while temperature and humidity were recorded for four days with 10-minute sampling intervals (Hobo® U12 data logger, Onset Computer Corp, Pocasset, MA). Sets of three inoculated and dried tomatoes with one negative control tomato were tested immediately after drying in the biosafety hood (day 0). Other tomatoes were sampled on day 1, day 2, day 3, and day 4 from a 25 °C incubator.

Food processing surfaces inoculation and storage experiment

Squares (7.6 cm by 7.6 cm) of the described earlier materials were spot inoculated with 0.03 mL of 10^9 CFU.mL⁻¹ five strains rif-resistant *E. coli* O157:H7 cocktail in 0.1% peptone. The inoculum was allowed to dry for 90 minutes in the biosafety hood before squares were moved into a 25 °C incubator. A shallow pan with water was placed in the incubator to humidify the environment and temperature/humidity were monitored for four days as described previously. Sets of three inoculated plus one negative control squares of each type were plated immediately after drying (day 0), as well as on day 1, day 2, day 3, and day 4.

Tomato and squares inoculation for the transfer studies

Two separate studies, involving transfers from tomatoes to food processing materials surfaces and vice versa, were performed. Mature green tomatoes were surface inoculated on a healthy circle-marked spot with a single 30 µL drop of pathogenic bacterial cocktail inside biosafety hood (3 x 10⁷ CFU.tomato⁻¹). Two sets of three steel, vinyl belt, or HDPE squares were firmly pressed against tomato surface for one second (one square per each tomato) either immediately (wet transfer), 90 minutes after the inoculum has dried up on the surface, or 24 hours after tomato inoculation. The first set of wet transfer was analyzed immediately (W, day 0), while the second set of squares was placed under the biosafety hood to allow transferred liquid to dry on squares for 90 minutes. The second set was then moved to a 25 °C incubator and analyzed 22.5 hours later (W, day 1). Similarly, one set of 90 minutes dry transfer squares (90 min dry, day 0) was analyzed immediately and another set was placed in a 25 °C incubator and tested for E. coli 22.5 hours later

(90 min dry, day 1). The last set of tomatoes was placed for an additional 22.5 hours incubation at 25 °C following 90 minutes drying period inside biosafety hood before two sets of steel, vinyl, and HDPE squares were pressed against inoculated spots and analyzed for pathogen transfer efficiency either immediately (24 h dry, day 0), or 24 hours later (24 h dry, day 1) after storage in the same incubator (25 °C). The shallow container filled with water was placed inside a 25 °C incubator for the duration of the humidify atmosphere study to the and temperature/humidity was monitored as described previously with Hobo® U12 data logger.

On each of three days, a set of negative control squares (one of each) was touched to the marked surface of uninoculated tomatoes and analyzed as a negative control to ensure the absence of rif-resistant microflora on tomatoes and squares.

Transfers from squares to tomato surfaces were done as described previously but in the opposite direction of inoculation and transfer.

Escherichia coli O157:H7 recovery from tomatoes and squares

To recover pathogen, a single tomato or a square was transferred to 20 mL BPW in a stomacher bag and vigorously manually shaken for 30 seconds, rubbed for 30 seconds, and shake again for 30 seconds. The rinsate was plated directly or serially diluted in 9 mL BPW tubes before plating using the pour plate method and TSA-rif100 medium. The plates were incubated for 24 hours at 37 °C before counting.

Statistic analysis

Escherichia coli O157:H7 survival on tomatoes (three replications) and the squares (four replications) results were analyzed separately using one-way ANOVA (factor "day": day 0; day 1; day 2; day 3; day 4) with means separated using Fisher LSD procedure. Transfer studies were repeated three times and count data were analyzed using one-way ANOVA and treatment factor (W day 0; W day 1; 90 min dry day 0; 90 min dry day 1; 24 h dry day 0; 24 h dry day 1) labeled by each material (steel, belt, plastic). Samples yielding no counts were assigned a limit of detection count. Percent positive samples were calculated for transfer studies as well, for each treatment for each square, combining data from three replications. Statistical analysis was done using commercially available software Statistica ver.10.0 (StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

For tomato surface survival studies, *E. coli* O157:H7 numbers declined 1.4 log units from theoretical inoculation level of 6.8 log units per mL of rinsate to 5.3 logs upon 90-minute drying, and continued to decline significantly and rapidly during storage at 25 °C (p < 0.05), resulting in final counts of 1.5 logs on day 4 (Figure 2). Similarly, **Lang, Harris and Beuchat (2004)** showed that *E. coli* O157:H7 counts in 5% horse serum on the dried spotinoculated tomatoes decreased 1.07 logs after 1-hour drying and 3.17 logs 24 hours post-drying from initial 7.22 log₁₀ CFU.tomato⁻¹.



Figure 1 Recovery of *E. coli* O157:H7 from squares either immediately after drying (d0), or after storage for four days (d0-d4) at 25 °C. Note: Counts expressed as log10 CFU.mL⁻¹ recovered from 20 mL rinsate. Inoculated level calculated theoretically based on stationary culture and is shown for reference. The same letters within the same material (steel, belt (PVC), or plastic (HDPE)) mean no significant difference (p > 0.05). *Average air relative humidity ±SD: replication No 1 = 67.4 ±2.2%; replication No 2 = 70.8 ±2.0%, replication No 3 = 71.6 ±2.1%, replication No 4 = 73.2 ±1.9%.



Figure 2 Recovery of *E. coli* O157:H7 from inoculated tomatoes either immediately after drying (90 min dry), or after storage for four days (d1-d4) at 25 °C. Note: Counts expressed as log_{10} CFU.mL⁻¹ recovered from 20 mL rinsate. Inoculated level calculated theoretically based on stationary culture concentration and is shown for reference. The same letters mean no significant difference (p > 0.05). *Average air relative humidity ±SD: replication No 1 = 58.8 ±3.6%, replication No 2 = 59.4 ±3.8%, replication No 3 = 59.5 ±4.1%.

Table 1 Percentage of squares and tomatoes yielding at least 1 CFU.mL⁻¹ of *E. coli* O157:H7 in rinsate after inoculated tomatoes touched squares or inculated squares touched tomatoes. Cross-contaminated items were checked for *E. coli* either immediately after the transfer (d0), or stored 24 h after the transfer at 25 °C (d1).

	W	W	W	90m	90m	90m	24h	24h	24h	W	W	W	90m	90m	90m	24h	24h
	Р	В	S	Р	В	S	Р	В	S	Р	В	S	Р	В	S	Р	В
	dO	d0	d0	d0	d0	d0	d0	d0	d0	d1	d1	d1	d1	d1	d1	d1	d1
S2T	100	100	100	11.1	44.4	11.1	0	0	0	88.9	100	88.9	11.1	0	0	0	0
T2S	100	100	100	66.7	100	55.6	11.1	33.1	0	100	100	100	0	100	0	11.1	22.2
Note:	Note: W – wet; 90 m – 90min dry; 24 h – 24 h dry. P – plastic; B – belt; and S – steel. *T2S – Tomatoes to Squares																
transf	er: \$2'	T – Sa	uares f	o Toma	atoes tre	nsfer	-	-								-	

Similar results were obtained by **Tokarskyy and Korda** (2019b) for surface inoculated tomatoes with *E. coli* 0157:H7 and stored for four days in a high humidity incubator at 25 °C. They noted better survival of pathogen if final inoculum was prepared in less hygroscopic 0.1% diluted peptone water, compared to buffered peptone water, where pathogen declined from 5.4 log₁₀ CFU.mL⁻¹ in 90 min dry tomatoes to 1.4 log₁₀ CFU.mL⁻¹ on those stored for four days at 25 °C (Tokarskyy and Korda, 2019b). The decrease in numbers of viable *E. coli* 0157:H7 on the surface of bruised and unbruised tomatoes at 20 °C was even more drastic when a low contamination level was used (4.0 log₁₀ CFU.tomato⁻¹), where counts dropped to below detection level in just three days (Tokarskyy et al., 2018).

To summarize, *E. coli* O157:H7 did not survive well on the intact surface of tomatoes at 25 °C.

Survival of E. coli O157:H7 on the surface of common food processing materials is shown in Figure 1. The counts declined from theoretical inoculation level of 6.1 log₁₀ CFU.mL⁻¹ as calculated per mL of the rinsate to 4.0, 4.4, and 4.2 log₁₀ CFU.mL⁻¹ for steel, belt, and plastic, respectively, upon drying (p < 0.05). After four days incubation period at 25 °C, the counts on average felt below 1.0 log₁₀ CFU.mL⁻¹ for all squares. These data are similar to Kusumaningrum et al. (2003), who showed that Salmonella enteritidis was recovered from inoculated steel squares after drying for at least 4 days at a high contamination level (10⁵ CFU.cm⁻²), while at a moderate level (10³ CFU.cm⁻²) and low level (10 CFU.cm⁻²) inoculation counts went below detection limit within 24 hours and 1 hour, respectively. Møretrø et al. (2010) showed that STEC E. coli inoculated on stainless steel in water or BHI and dried declined by ca 1.6 and 3.6 logs, respectively, at 20 °C after 24 hours post-drying. The authors noted that results for polyoxymethene copolymer were not significantly different from stainless steel (Møretrø et al., 2010). It might be noted that food-grade (type 304) stainless steel was used for the study, as different metal alloys might impact survival. As was shown by Jiang and Doyle (1999), E. coli O157:H7 inoculated in 0.1% peptone and dried on glass and coins at 4.7 log₁₀ CFU.coin⁻¹ declined at room temperature to below detection level on day 4, 7, 9, 11, and 11 for glass, pennies, nickels, dimes, and quarters, respectively. Contrary, Tokarskyy and Korda (2019b) showed better survival of E.coli O157:H7 on the surface of unwaxed cardboard, with counts declining from 4.5 to only 2.5 log₁₀ CFU.mL⁻¹ after 4-day storage at 25 °C, what can be attributed to the porous and water-absorbing nature of cardboard surface.

To summarize, little to no potential of *E. coli* O157:H7 survival was shown in the current study for common impervious food processing surfaces.

Several research groups used different approaches and techniques to measure transfer rates of enteric pathogens from inanimate surfaces to produce and conversely (Buchholz et al., 2012; Soares et al., 2012; Brar and Danyluk, 2013; Jensen et al., 2013; Todd-Searle et al., 2020). Going further, Buchholz et al. (2012) even used pilot pant settings for transfer studies and showed that *E. coli* 0157:H7 continuously cross-contaminated lettuce through flume tank, conveyor tank, and shredder,

considering that all surfaces remained wet through the processing line and sanitizer was not applied. **Brar and Danyluk (2013)** studied Salmonella transfer from contaminated plastic gloves to tomatoes and conversely, after 24 hours inoculum drying on the surfaces. They did not find any difference between transfers from dirty and from clean reusable gloves (**Brar and Danyluk, 2013**).

Results of our transfer studies were expressed either as percent positive (where at least one typical E. coli O157:H7 CFU per 1 mL of rinsate was detected) or as counts, total log₁₀ CFU.item⁻¹ (either a food processing surface square or a tomato), and are shown in Table 1 and Figure 3 and Figure 4. Samples yielding no counts were assigned a limit of detection count. Overall, wet transfers (W) yielded the most consistent data, with 100% transfers being positive on both day 0 and day 1 (Table 1). Dry transfers (90 min dry and 24 h dry) appeared to be less efficient, partially because of lack of wetness with liquid carrying bacteria and having adhesive properties, and possibly due to the pathogen number decline during drying and storage. Burnett, Chen, and Beuchat (2000) hypothesized that E. coli O157:H7 may firmly attach to the surfaces of fruits as evidenced by confocal scanning laser microscopy and may evade decontamination and detachment, which could be one of the explanations why dry transfer was not efficient in our study. However, dry transfers from tomatoes to squares appeared to be more efficient than vice versa (Table 1, Figure 3 and Figure 4), possibly due to the hydrophobic properties of the tomato peel surface. Interestingly, the vinyl belt picked up the most pathogen cells from the surface of tomatoes, resulting in 100% positive during 90 min dry transfers, followed by plastic (66.7% positive) and steel (55.6% positive). Subjectively, the vinyl belt appeared to have a "sticky" surface. Similarly, dry transfers at 24 h storage were the most efficient from tomatoes to the vinyl belt, followed by plastic (Figure 3). Similar results were shown by Tokarskyy and Korda (2019b), who showed that wet transfer of E.coli O157:H7 was more efficient between tomatoes and cardboard comparing to dried surfaces, as well as transfer from tomatoes to cardboard was more efficient than vice versa. Todd-Searle et al. (2020) showed that the transfer of Salmonella between tomatoes and plastic mulch or soil was dependent on the dryness of the inoculum, contact time, and contact surface. They also noted that transfer from plastic mulch was greater than from soil, possibly due to the surface characteristics, while wet and 1-hour dry transfers were more efficient than 24hours dry transfers (Todd-Searle et al., 2020). Soares et al. (2012) showed that Salmonella spp. easily transferred from wet poultry skin to the cutting surfaces made of wood, stainless steel, and plastic (100% positive for contamination), and then from those contaminated surfaces to the red tomatoes (also 100% tomatoes became contaminated), unless cutting surfaces were washed with soap followed by surface sanitation. Jensen et al. (2013) noted that freshly inoculated lettuce or celery transferred more bacteria (ca. 2 % to ca. 25 % of the inoculum) comparing with freshly inoculated carrots or watermelon (ca. 1% to 8%) to the surfaces made of ceramic, stainless steel, glass, and plastic. Such high transfer rates were probably due to the residual moisture left after fresh inoculum application.



Figure 3 Total *E. coli* O157:H7 counts per square after pathogen transfer from tomato compared to total inoculated \log_{10} CFU.tomato⁻¹ (Inoc). Note: Tomatoes (W – wet; 90 m – 90 min dry; 24h – 24 h dry) touched squares (P, plastic; B, belt; and S, steel) which were analyzed either immediately (d0) or 24 hours later (d1). Detection limit 1.3 log per item. The same letters mean no significant difference (p > 0.05). Tomato inoculation level calculated theoretically based on inoculum concentration and is shown for reference. T2S – Tomatoes to Squares transfer. *Average air relative humidity ±SD: replication No 1 = 61.1 ±9.0%, replication No 2 = 68.3 ±8.1%, replication No 3 = 66.2 ±6.2%.



Figure 4 Total *E. coli* O157:H7 counts per tomato after transfer from inoculated squares. Note: Squares (W – wet; 90 m – 90 min dry; 24 h – 24 h dry) of different types (P, plastic; B, belt; and S, steel) touched tomatoes which were analyzed either immediately (d0) or 24 hours later (d1). Detection limit:1.3 log10 CFU per item. The same letters mean no significant difference (p >0.05). Square inoculation level (Inoc) calculated theoretically based on inoculum concentration and is shown for reference. S2T – Squares To Tomatoes transfer. *Average air relative humidity ±SD: replication No 1 = $62.9 \pm 6.7\%$, replication No 2 = $70.2 \pm 7.0\%$, replication No 3 = $70.4 \pm 6.3\%$.

However, after one hour of drying time, the transfer rate from inoculated celery, carrot, and lettuce decreased significantly to less than 0.01 to ca. 5% and to less than 1% to ca. 5% for watermelon (Jensen et al., 2013). The authors concluded that the surface moisture and the direction of the transfer had the greatest influence on microbial transfer rates (Jensen et al., 2013). To support this statement, Todd-Searle et al. (2020) also emphasized that tomatoes should be harvested dry, not wet, to avoid cross-contamination.

To summarize, the dry transfer is limited and is more efficient from the tomatoes to the packaging squares, and less efficient from packaging squares to tomatoes. Speaking of risks involved, the vinyl belt appeared to be the most affected. The study has limits in accuracy, as chances of getting 1 CFU.plate⁻¹ are not statistically profound.

CONCLUSION

Pathogen transfers are of great concern if the surface is wet, but less of a concern if the surface is dry. Because pathogens do not survive well under the conditions tested, prolonged storage reduces the chances of crosscontamination. Dry transfers from tomatoes to food contact surfaces are more efficient compared to transfers from food contact surfaces to tomatoes. This could be due to the hydrophobic nature of the tomato surface. The results suggest that the vinyl belt (PVC) might represent a higher risk. Overall, we partially failed our hypothesis, showing that there is a low possibility of pathogen transfer if surfaces are dry after prolonged tomato storage under proposed model conditions.

REFERENCES

Beuchat, R. Y., Ryu, J. H., 1997. Produce handling and processing practices. *Emerging Infectious Disease*, vol. 3, p. 439-465. <u>https://doi.org/10.3201/eid0304.970407</u>

Brar, P. K., Danyluk, M. D. 2013. *Salmonella* transfer potential during hand harvesting of tomatoes under laboratory conditions. *Journal of Food Protection*, vol. 76, no. 8, p. 1342-1349. <u>https://doi.org/10.4315/0362-028X.JFP-13-048</u>

Buchholz, A. L., Davidson, G. R., Marks, B. P., Todd, E. C. D., Ryser, E. T. 2012. Transfer of *Escherichia coli* O157:H7 from equipment surfaces to fresh-cut leafy greens during processing in a model pilot-plant production line with sanitizer-free water. *Journal of Food Protection*, vol. 75, no. 11, p. 1920-1929. <u>https://doi.org/10.4315/0362-028X.JFP-11-558</u>

Burnett, S. L., Chen, J., Beuchat, L. R. 2000. Attachment of *Escherichia coli* O157:H7 to the surfaces and internal structures of apples as detected by confocal scanning laser microscopy. *Applied and Environmental Microbiology*, vol. 66, no. 11, p. 4679-4687. https://doi.org/10.1128/aem.66.11.4679-4687.2000

CDC. 2002. Centers for Disease Control and Prevention. 2002. Outbreak of *Salmonella* serotype Javiana infections - Orlando, Florida, June 2002. *Morbidity and Mortality Weekly Report*, vol. 51, p. 683-684.

Croby, R., Lanni, V., Kistler, V., Dato, V., Weltman, A., Yozviak, C., Waller, K., Nalluswami, K., Moll, M., Lockett, J., Montgomery, S., Lyuch, M., Braden, C., Gupta, S. K., Dubois, A. 2005. Outbreaks of *Salmonella* infections associated with eating Roma tomatoes-United States and Canada, 2004. Morbidity and Mortality Weekly Report, vol. 54, p. 325-328.

Cummings, K., Barrett, E., Mohle-Boetani, J. C., Brooks, J. T., Farrar, J, Hunt, T., Fiore, A., Komatsu, K., Werner, S. B., Slutsker, L. 2001. A multistate outbreak of *Salmonella enterica* serotype Baildon associated with domestic raw tomatoes. *Emerging Infectious Disease*, vol. 7, no.6, p. 1046-1048. https://doi.org/10.3201/eid0706.010625

Daş, E., Gürakan, G. C., Bayindirli, A. 2006. Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of *Salmonella* Enteritidis on cherry tomatoes. *Food Microbiology*, vol. 23, no. 5, p. 430-438. https://doi.org/10.1016/j.fm.2005.08.002

Hirai, Y. 1991. Survival of bacteria under dry conditions; from a viewpoint of nosocomial infection. *Journal of Hospital Infection*, vol. 19, no. 3, p. 191-200. https://doi.org/10.1016/0195-6701(91)90223-U

Jensen, D. A., Friedrich, L. M., Harris, L. J., Danyluk, M. D., Schaffner, D. W. 2013. Quantifying transfer rates of Salmonella and *Escherichia coli* O157:H7 between fresh-cut produce and common kitchen surfaces. *Journal of Food Protection*, vol. 76, no. 9, p. 1530-1538. https://doi.org/10.4315/0362-028X.JFP-13-098

Jiang, X., Doyle, M. P. 1999. Fate of *Escherichia coli* O157:H7 and *Salmonella enteritidis* on currency. *Journal of Food Protection*, vol. 62, no. 7, p. 805-807. https://doi.org/10.4315/0362-028x-62.7.805

Kramer, A., Schwebke, I., Kampf, G. 2006. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infectious Diseases*, vol. 6, 8 p. https://doi.org/10.1186/1471-2334-6-130

Kusumaningrum, H. D., Riboldi, G., Hazeleger, W. C., Beumer, R. R. 2003. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal of Food Microbiology*, vol. 85, no. 3, p. 227-236. <u>https://doi.org/10.1016/S0168-1605(02)00540-8</u>

Lang, M. M., Harris, L. J., Beuchat, L. R. 2004. Evaluation of inoculation method and inoculum drying time for their effects on survival and efficiency of recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inoculated on the surface of tomatoes. *Journal of Food Protection*, vol. 67, no. 4, p. 732-741. https://doi.org/10.4315/0362-028X-67.4.732

Møretrø, T., Heir, E., Mo, K. R., Habimana, O., Abdelgani, A., Langsrud, S. 2010. Factors affecting survival of Shigatoxin-producing *Escherichia coli* on abiotic surfaces. *International Journal of Food Microbiology*, vol. 138, no. 1-2, p. 71-77.

https://doi.org/10.1016/j.ijfoodmicro.2010.01.002

Schilling, M. W., Yoon, Y., Tokarskyy, O., Pham, A. J., Williams, R. C., Marshall, D. L. 2009. Effects of ionizing irradiation and hydrostatic pressure on *Escherichia coli* O157:H7 inactivation, chemical composition, and sensory acceptability of ground beef patties. *Meat Science*, vol. 81, no. 4, p. 705-710.

https://doi.org/10.1016/j.meatsci.2008.10.023 Shi, X., Namvar, A., Kostrzynska, M., Hora, R., Warriner, K. 2007. Persistence and growth of different *Salmonella* serovars on pre- and postharvest tomatoes. *Journal of Food Protection*, vol. 70, no. 12, p. 2725-2731.

https://doi.org/10.4315/0362-028X-70.12.2725 Soares, V. M., Pereira, J. G., Viana, C., Izidoro, T. B., dos Santos Bersot, L., de Almeida Nogueira Pinto, J. P. 2012. Transfer of *Salmonella* Enteritidis to four types of surfaces after cleaning procedures and cross-contamination to tomatoes. *Food Microbiology*, vol. 30, no. 2, p. 453-456. https://doi.org/10.1016/j.fm.2011.12.028

Todd-Searle, J., Friedrich, L. M., Oni, R. A., Shenge, K., LeJeune, J. T., Micallef, S. A., Danyluk, M. D., Schaffner, D. W. 2020. Quantification of *Salmonella* enterica transfer between tomatoes, soil, and plastic mulch. *International Journal of Food Microbiology*, vol. 316, 9 p. https://doi.org/10.1016/j.ijfoodmicro.2019.108480

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. 2028-2033. https://doi.org/10.4315/0362-028X.JFP-18-220

Tokarskyy, O., Korda, M. 2019a. 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. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 13, no. 1, p. 779-783. https://doi.org/10.5219/1178

Tokarskyy, O., Korda, M. 2019b. Influence of suspension liquid total solids on *E. coli* O157:H7 survival and transfer efficacy between green tomatoes and cardboard. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 13, no. 1, p. 941-949. <u>https://doi.org/10.5219/1210</u>

Tokarskyy, O., Marshall, D. L., Schilling, M. W., Willeford, K. O. 2009. Comparison of methods to verify end point cooking temperature of channel catfish (*ictalurus punctatus*) fillets. *Journal of Muscle Foods*, vol. 20, no. 3., p. 325-340. https://doi.org/10.1111/j.1745-4573.2009.00151.x

Tokarskyy, O., Schneider, K. 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

USDA-ERS. 2016. United States Department of Agriculture, Economic Research Service. Loss-adjusted food availability data. Available at: https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=58340

Wei, C. I., Huang, T. S., Kim, J. M., Lin, W. F., Tamplin, M. L., Bartz, J. A. 1995. Growth and survival of *Salmonella montevideo* on tomatoes and disinfection with chlorinated water. *Journal of Food Protection*, vol. 58, no. 8, p. 829-838. https://doi.org/10.4315/0362-028X-58.8.829

Zhuang, R. Y., Beuchat, L. R., Angulo, F. J. 1995. Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied and Environmental Microbiology*, vol. 61, no. 6, p. 2127-2131. https://doi.org/10.1128/AEM.61.6.2127-2131.1995

Acknowledgments:

The authors are grateful to Dr. Keith R. Schneider and his lab at the Department of Food Science and Human Nutrition, University of Florida, USA, for resources in his lab to conduct these experiments.

Contact address:

*Oleksandr Tokarskyy, Ternopil National Medical University, International Students' Faculty, Department of Medical Biochemistry, Maidan Voli 1, 46001, Ternopil, Ukraine, Tel: +380964102536,

E-mail: otokarsky@tdmu.edu.ua

ORCID: https://orcid.org/0000-0001-6279-1803

Mykhaylo Korda, Ternopil National Medical University, Department of Medical Biochemistry, Maidan Voli 1, 46001, Ternopil, Ukraine, Tel: +380352524492,

E-mail: korda@tdmu.edu.ua

ORCID: <u>http://orcid.org/0000-0002-6066-5165</u>

Halyna Lukyantseva, National University of Ukraine on Physical Education and Sport, Biomedical Disciplines Department, Fizkultury street 1, 03150, Kyiv-150, Ukraine, Tel: +380975777765,

E-mail: lukjantseva@gmail.com

ORCID: https://orcid.org/0000-0002-8054-0108

Corresponding author: *