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INFLUENCE OF SUSPENSION LIQUID TOTAL SOLIDS ON *E. COLI* O157:H7 SURVIVAL AND TRANSFER EFFICACY BETWEEN GREEN TOMATOES AND CARDBOARD

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ABSTRACT

The objectives of this study were: a) to determine *E. coli* O157:H7 survival on tomatoes and cardboard squares post-drying, stored at 25 ºC in humidified environment for four days, in buffered peptone water (BPW), and 0.1% diluted peptone (DP); b) to determine pathogen transfer rates (0, 1.5, or 24-hours drying post-inoculation), from inoculated tomato surfaces to uninoculated cardboard squares and conversely; and c) to evaluate SystemSure Plus ATP luminometer for recognizing contamination on visibly soiled (BPW) or visible clean (DP) cardboard. In tomato inoculation studies, *E. coli* O157:H7 survived better on the fruit when the inoculum was prepared using DP as compared to BPW. The 1.5-hours post drying counts of 5.34 and 5.76 log10 CFU.mL^{-1} in the rinsate substantially declined to 1.45 and 1.17 log10 CFU.mL^{-1} on day four, for DP and BPW, respectively. In cardboard inoculation studies, *E. coli* O157:H7 persisted for four days, with 1.5-hours post-drying counts and day four counts of 4.53 (DP) and 2.55 log10 CFU.mL^{-1} (BPW), contrary to 3.81 (DP) and 1.92 $\log_{10}$ CFU.mL^{-1} (BPW). Under the first impression, the slower die-off of *E. coli* O157:H7 on cardboard questions the possibility of reusing cardboard boxes due to the potential for cross-contamination. In wet transfer (0 hour drying) trials, both tomato-to-cardboard and cardboard-to-tomato yielded 100% positive transfers irrespective of diluent type. Dry transfer (1.5-hours drying interval post inoculation) from tomato-to-cardboard were 100% positive, but no positives were noted when inoculated, dried cardboard was contacted to tomatoes, irrespective of diluent. Results of transfers with BPW as the diluent showed 100% positive transfer from 24-hours dry tomatoes-to-cardboard, as inoculation spots on the tomatoes remained moist due to hygroscopic nature of solutes in BPW. Conversely, only a 40% positive transfer rate was observed under the same conditions with DP as diluent. No positive transfers were recorded from 24-hours dry cardboard-to-tomatoes, irrespective of diluent type. Though *E. coli* O157:H7 survived better on the surface of cardboard compared to the surface of tomatoes on day four, the dry transfers were more efficient from tomatoes-to-cardboard than conversely, possibly due to smooth and hydrophobic properties of the tomato, and rough and porous surface of the cardboard. ATP luciferase UltrasnapTM swab test showed 9/9 “pass” results for sterile liquid DP and BPW, while 9/9 “fail” results were observed with liquid peptone and BPW contaminated at ca. 9.0 log10 CFU.mL^{-1} *E. coli* O157:H7. Cardboard squares treated and dried, with sterile DP, showed 8/9 “pass” ATP luciferase results, and 1/9 “warning”, while cardboard squares with contaminated DP showed 9/9 “fail” result. Cardboard squares treated and dried, with sterile BPW, showed 7/9 “pass” ATP luciferase results, and 2/9 “warning”, while cardboard squares with contaminated BPW showed 9/9 “fail” result. Luminometer can simplify detection of microbial load, as well as organic residues, helping to check cardboard boxes for cleanness.

Keywords: tomatoes; cardboard; *E. coli* O157:H7; survival; transfer; cross-contamination; ATP luciferase test

INTRODUCTION

Tomatoes are important commodity, with the United States (US) an Ukraine among top-fifteen producers worldwide **(FAOSTAT, 2017)**. Morevover, the US is the fourth leading producer of tomatoes in the world, behind China, India, and Turkey **(FAOSTAT, 2017)**. Fresh tomatoes are produced in every state, with commercial scale production in 20 states. In addition, Florida has tomato production on ca. 30,000 – 40,000 acres, accounting for almost one-third of total US fresh tomato acreage **(FDACS, 2018)**. The food safety concerns associated with fresh tomatoes are related to absence of a terminal pathogen reduction step as tomatoes are often consumed fresh, not cooked **(Gurtler et al., 2018)**. Tomatoes are generally contaminated with various groups of microorganisms from the environment **(Tokarskyy and Korda, 2019)**. According to **Beuchat and Ryu (1997)**, enteric pathogens can contaminate tomatoes through

wildlife, irrigation water, handling by workers, wash water, or other contaminated surfaces. Fresh tomatoes prepared for a restaurant were implicated in a multistate outbreak of *Salmonella enterica* infection in 1999 **(Cummings et al., 2001)**. Other well-known outbreaks related to *Salmonella* contaminated Roma tomatoes occurred in the US and Canada in summer 2004 **(Croby et al., 2005)**. The common belief is that Gram-negative enteric pathogens will grow in the tomato pulp if introduced through a wound, cut surface, stem scar, or abrasions **(Wei et al., 1995; Zhuang, Beuchat and Angulo, 1995; Daş, Gürakan and Bayindirli, 2006; Shi et al., 2007; Beuchat and Mann, 2008; Bartz et al. 2015)**, however pathogens will die off if left on the undamaged or bruised skin of the fruit **(Lang, Harris and Beuchat, 2004; Allen et al., 2005; Tokarskyy et al., 2018)**. It is generally believed that *Salmonella* is more robust in surviving under harsh environmental conditions compared to *Escherichia coli* **(Hirai, 1991)**. **Lang, Harris and Beuchat (2004)** showed that *E. coli* O157:H7 spot-inoculated tomatoes showed counts decline by 3.17 log units, while *Salmonella* spp. declined only by 2.20 log units after 24 hours inoculum post-drying.

Several researchers showed that final resuspension diluent for the washed bacterial cells might influence their survival on the surface of tomatoes, with higher organic solids and protein favoring survival **(Wei et al., 1995; Guo et al., 2002)**. For example, **Wei et al. (1995)** showed rapid decline in *Salmonella* counts on the spot-inoculated tomato surface if deionized water was a diluent with counts declining from 5.5 $\log_{10}$ CFU.tomato^{-1} to below detection level in 3 days at room temperature, while pathogen suspended in tryptic soy broth showed minimal decline in numbers under the same conditions. Similarly, **Guo et al. (2002)** showed protective influence of soil favoring survival and growth of *Salmonella* on undamaged tomato surface compared to water alone causing rapid decline in counts. Conversely, the influence of humidity on *E. coli* O157:H7 and *Salmonella* survival in desiccated or humidified state might be more complicated **(Tokarskyy and Schneider, 2019)**. For example, **Møretrø et al. (2010)** showed that Shiga toxin-producing *E. coli* dried in brain heart infusion broth on plastic or steel had highest inactivation rate at 85% relative humidity (RH), while it survived best at 70% and even grew at 98%.

Raw tomatoes are transported to the distribution centers in various packaging, including cardboard, either waxed or unwaxed. The chemical nature of cardboard is a porous wood-derived material, which absorbs liquids, especially in unwaxed state. The question of possible cross-contamination by *E. coli* O157:H7 between tomatoes and unwaxed cardboard remains open.

The first objective of the current study was to determine survival rates of *E. coli* O157:H7, either in 0.1% diluted peptone (designated as low-solute liquid, DP) or buffered peptone water (designated as high-solute liquid, BPW), on the surface of unwashed and undamaged green mature tomatoes and cardboard squares stored at room temperature (25 ºC) in humidified environment within four days of storage. The second objective of the study was to estimate transfer rates of *E. coli* O157:H7 from inoculated surface of tomatoes to the surface of cardboard squares and conversely as influenced by the type of the diluent and timing of the transfer. The third objective of the study was to evaluate effectiveness of ATP luminescence SystemSure Plus luminometer to recognize contamination on heavily and visibly soiled (BPW) or loosely soiled and visible clean (DP) cardboard surfaces. All treatments were visually observed throughout experiments and appearance was subjectively noted, both in humidified 25 ºC incubator and non-humidified 25 ºC incubator.

Scientific hypothesis

We hypothesized that *E. coli* O157:H7 will survive better on the surface of porous cardboard than on the smooth surface of tomatoes, with protective properties of high-solute diluent used. We hypothesized that moisture and high solute would promote *E.coli* O157:H7 cross-contamination between cardboard and tomatoes. we hypothesized that ATP luciferase rapid test would be a helpful aid to identify dirty and contaminated cardboard.

MATERIALS AND METHODOLOGY

Rifampin preparation

Stock solution of rifampin (10,000 ppm) was prepared by dissolution of 0.4 g rifampin (Fisher Scientific, BP26795) in 40 mL HPLC grade methanol (Fisher Scientific) followed by filter sterilization (0.2 µm nylon filter, Fisher Scientific), and storage at 4 ºC in the dark. Antibiotic was added to cooled autoclaved media (Difco™ tryptic soy agar (TSA) or Bacto™ tryptic soy broth (TSB)) to yield 100 ppm final rifampin concentration.

Bacterial culture maintenance and preparation

Five rifampin (200 ppm) resistant *Escherichia coli* O157:H7 strains, MDD19 (alfalfa isolate), MDD20 (Odwalla juice isolate), MDD326 (cantaloupe isolate), MDD 327NA (spinach isolate), and ATCC 35150 (human feces) were used for this study. The first four strains were provided by Dr. M. D. Danyluk's lab (University of Florida, US), and the fifth strain was obtained from American Type Culture Collection (Manassas, WI). Rifampin-sensitive strains were adapted to 200 ppm rifampin as described previously **(Underthun et al., 2018)**. Cultures were maintained on TSA-rif80 ppm slants at 4 ºC with bi-weekly transfers to fresh TSA-rif80 slants.

E. coli O157:H7 strains were streaked on TSA-rif100 plates (37 ºC, 24 hours), and a single colony was transferred to 10 mL TSB-rif100 tube (37 ºC, 12 hours). Two more one loop transfers (ca. 10 µL) were done in 10 mL TSB-rif100 followed by 12 hours and 18 hours incubation at 37 ºC before cultures were ready for experiments. Two mL of each strain were mixed together (total 10 mL, 10^9 CFU.mL^{-1}) and centrifuged (4,300 g, 10 minutes, Sorvall RC-5B centrifuge, DuPont Instruments), followed by a single wash in 10 mL Dulbecco 'A' phosphate buffered saline (PBS, Oxoid, Hampshire, England), and final re-suspension in either 10 mL 0.1% Bacto™ peptone (DP, 0.1 g.L^{-1} of deionized water, Becton, Dickinson, and Co.) or 10 mL buffered peptone water (BPW, Becton, Dickinson, and Co.) using the same centrifugation procedure. Buffered peptone water contained 20 g.L^{-1} solutes, including enzymatic digest of protein (peptone) 10 g, sodium chloride 5 g, disodium phosphate 3.5 g, monopotassium phosphate 1.5 g, as

prepared by manufacturer's instructions. Inoculum concentrations were confirmed by pour plating using TSA-rif100 after serial dilutions in BPW.

Tomato and cardboard squares preparation, inoculation, and storage

Field mature green and breaker stage round tomatoes (*Lycopersicum esculentum*, variety Florida 47) were acquired from local packinghouses in Florida, USA, before processing, being unwashed and unwaxed for the experiments. Tomatoes were dry rubbed with sterile nitrile gloves to remove visible surface contamination. Cardboard squares (ca. 8 by 8 cm) were cut from the lid portions of cardboard boxes in which the tomatoes were packed and were considered as "used."

Tomatoes were inoculated with 0.1 mL of *E. coli* cocktail, either in BPW or in DP, as 10 spots of equal size around blossom end (10^8 CFU.tomato^{-1}). Similarly, cardboard squares were spot inoculated in the center with 0.03 mL of cocktail, either in BPW or in DP (3×10^7 CFU.square^{-1}). The fruit or squares were allowed to dry in a biosafety hood for 90 minutes (1.5 hours) ensuring complete dryness before moving into 25 ºC incubator. A shallow pan with deionized water was placed in the incubator to humidify environment, while humidity and temperature were recorded at 10 minutes intervals for four days (Hobo® U12 data logger, Onset Computer Corp, Pocasset, MA). Sets of three inoculated and dried tomatoes or squares with one negative control were tested immediately after drying (day 0), and sampled on days 1, 2, 3, and 4 for each diluent type from the storage incubator.

On three different occasions, sets of tomatoes and squares were spotted with 30 µL of inoculated DP or inoculated BPW. The specimens were visually observed after 90 minutes drying period and 24 hours later after storage at 25 ºC in either high (shallow pan of water for humidification) or low humidity atmospheres with temperature and humidity in both incubators being monitored as described previously.

Tomato and squares inoculation for the transfer studies

Two separate studies involved pathogen transfers from tomatoes to cardboard and from cardboard to tomatoes. Mature green and breaker stage tomatoes were spot inoculated on undamaged sharpie circle-marked spot on a side of the fruit with 30 µL drop of *E. coli* O157:H7 cocktail, either in BPW or in DP (3×10^7 CFU.tomato^{-1}). Two sets of three cardboard 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 tomato surface (90 min dry), or 24 hours after tomato inoculation (24 h dry). 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 25 ºC incubator and analyzed after 24 hours (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 25 °C incubator and tested for pathogen presence 24 hours later (90 min dry, day 1). The last set of inoculated tomatoes was placed for an additional 24 hours incubation at 25 °C including 90 minutes drying period inside biosafety hood before two sets of cardboard 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 baking pan filled with deionized water was placed inside 25 °C incubator for the duration of the study to humidify atmosphere. Temperature and humidity were monitored as described previously. On each of three days, a negative control square was pressed against the marked surface of uninoculated tomato and analyzed as a negative control to ensure absence of rif-resistant microflora on tomatoes and squares. Transfers from cardboard to tomato surface were done as described previously, but in an opposite direction of inoculation and transfer.

Escherichia coli O157:H7 recovery from tomatoes and squares

A single tomato or square was transferred to a Stomacher® bag containing 20 mL BPW and subjected to vigorous manual shaking for 30 seconds, rubbing for 30 seconds, and final shaking for 30 seconds. The rinsate was either plated directly using spiral plater (WASP2 spiral plater, Don Whitley Scientific Limited, West Yorkshire England), or serially diluted in 9 mL BPW tubes before pour plating with TSA-rif100 medium. The plates were incubated for 48 hours at 37 °C before counting.

Cardboard squares cleanness evaluation by luminometer

The cleanness of uninoculated cardboard squares, as well as those spotted with either sterile DP and sterile BPW, or inoculated diluents, was accessed after 90 minutes drying period using Ultrasnap ATP test by swabbing 3.8 cm by 3.8 cm area including dried spot and measuring ATP activity in the swab following manufacturer's instructions (SystemSURE Plus luminometer, Hygiena, Camarillo, CA). A set of three cardboard squares were analyzed for each treatment. Liquid inocula and sterile diluents were analyzed as well by dipping three separate swabs sequentially in each liquid and proceeding as recommended by instructions.

Statistic analysis

Escherichia coli O157:H7 survival on tomatoes (three replications) and on the squares (four replications) results were analyzed separately using two-factorial experimental design with independent factors of diluent (BPW or DP) and storage timing (90 minutes dry, day 1, 2, 3, and 4). If significant influence of factors were observed (p <0.05), the means were separated using Fisher LSD procedure. Transfer studies were repeated three times and counts data were analyzed using two-factorial experimental design with independent factors of diluent (BPW or DP) and transfer timing with storage (wet transfer, day 0; wet transfer, day 1; 90 min dry transfer, day 0; 90 min dry transfer, day 1; 24 h dry transfer, day 0; 24 h dry transfer, day 1). Similarly, means were separated using Fisher LSD procedure. Percent positive samples for each data point

were calculated for transfer studies as well. Relative air humidity in storage 25 °C incubators was shown as average values with standard deviations. ATP luciferase Ultrasnap™ swab test results (three replications) were expressed as average values of Relative Luminescence Units (RLU) as defined by manufacturer, with standard deviations, as well as ratio of pass/total, warning/total and fail/total per treatments. Statistical analysis was performed using commercially available software Statistica ver. 10.0 (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

A diluted peptone water (1 g peptone.L^{-1}, DP) represented a low solute inoculum, while buffered peptone water (Difco™, 10 g peptone, 5 g NaCl, 3.5 g disodium phosphate, and 1.5 g monopotassium phosphate per liter of deionized water, BPW) represented a high solute inoculum. Visual observation of 90 minutes dry inoculated squares and tomatoes followed by storage at 25 °C in either high humidity (RH = 72.5 ±3.0%) or low humidity (RH = 30.4 ±12.9%) confirmed differences between treatments. It was observed that 90 minutes dry inoculated spots appeared dry regardless of diluent. However, inoculated spots with BPW liquefied at high humidity but were dry at low humidity on tomatoes after 24 hours, while spots with DP remained dry in either environment. Spots remained dry on cardboard squares regardless of diluent or humidity; however, spots of DP were untraceable by naked eye, while BPW spots were visible. Therefore, the diluent in which the pathogens were resuspended and humidity where the tomato is stored, might cause moisture to be picked up by dried hygroscopic substances, as observed with BPW. Weather conditions in Florida, where approximately 45% of all tomatoes are grown, are known for high humidity **(FDACS, 2018)**. **Allen et al. (2005)** wrote that tomato packinghouse conditions in Florida late spring are 30 °C and 80% RH, while standard ripening room conditions are 20 °C and 90% RH. The recorded humidity conditions in 25 °C humidified incubator for all inoculation experiments are shown in Table 1.

For tomato surface survival studies, *E. coli* O157:H7 numbers declined from theoretical inoculation level of 6.75 and 6.73 $\log_{10}$ CFU.mL^{-1} rinsate to 5.34 and 5.76 $\log_{10}$ CFU.mL^{-1} upon 90 minutes drying time for DP and BPW, respectively. Both diluent types (BPW; DP) and storage factors, as well as their interaction, had a significant effect on *E. coli* O157:H7 recovery (p <0.05, Figure 1). Visual observation of inoculated spots of stored tomatoes (25 °C, humidified incubator) confirmed that spots with BPW liquified, while spots with peptone water were visibly dry. *E. coli* O157:H7 numbers significantly (p <0.05) declined by 2.4 and 4.9 $\log_{10}$ CFU.mL^{-1} from 90 minutes dry levels on day 2 for DP and BPW, respectively (Figure 1). Following next two days of storage, counts in BPW remained fairly stable (Figure 1). Overall, *E. coli* O157:H7 survived better in DP than in BPW in humidified 25 °C environment, but decline of 3.9 to 4.6 log on day 4 compared to day 0 dried tomato counts was observed in both cases. Similarly, **Lang, Harris and Beuchat (2004)** showed that *E. coli* O157:H7 counts in 5% horse serum on the dried spot-inoculated tomatoes decreased 1.07 $\log_{10}$ CFU.$tomato^{-1}$ after 1 hour drying and additional 2.10 $\log_{10}$ CFU.$tomato^{-1}$ 24 hours post-drying from initial 7.22 $\log_{10}$ CFU.$tomato^{-1}$. Studies by **Tokarskyy et al. (2018)** on survival of *E. coli* O157:H7 on the surface of undamaged raw tomatoes, inoculated at low levels, also showed substantial decline to 1.37 – 2.07 $\log_{10}$ CFU.$tomato^{-1}$ (day 1), 0.30 – 1.80 $\log_{10}$ CFU.$tomato^{-1}$ (day 3), and 0.04 – 0.33 $\log_{10}$ CFU.$tomato^{-1}$ (day 7) from inoculation level of 2.45 – 2.79 $\log_{10}$ CFU.$tomato^{-1}$ (day 0). To summarize, *E. coli* O157:H7 did not survive well on the intact surface of tomatoes.

Survival of *E. coli* O157:H7 on the surface of cardboard squares is shown in Figure 2. As cardboard material was porous and absorbent, inoculated spot liquefaction due to moisture absorbance from the air was not observed in case of BPW. However, dried spots remained visible in case of BPW, but not DP. Both diluent types (BPW; DP) and storage factors, but not their interactions, had significant (p <0.05) effect on *E. coli* O157:H7 numbers (Figure 2). *E. coli* O157:H7 numbers significantly declined from 90 minutes post-drying counts of 4.53 and 3.81 $\log_{10}$ CFU.mL^{-1} rinsate to 2.55 and 1.92 $\log_{10}$ CFU.mL^{-1} rinsate upon 4 days of cardboard storage at 25 °C in humidified atmosphere (p <0.05). These results are comparable to *Salmonella* data by **Kusumaningrum et al. (2003)**, who showed that *S. Enteritidis* was recovered from inoculated dried steel squares for at least 4 days at contamination level of 10^5 CFU.cm^{-2}. It appeared that *E. coli* O157:H7 survived better on cardboard compared to plastic (HDPE), stainless steel, and vinyl belt (PVC), where counts on average declined below 1.0 log unit on the fourth day under the same conditions (unpublished data). It can be speculated that porous organic surface of cardboard might have protective effect on *E. coli* compared to impervious plastic, steel, and vinyl surfaces. Similarly, **Allen et al. (2005)** showed that *Salmonella* survived better on unfinished oak wood compared to stainless steel, vinyl belt, and sponge rollers at 20 °C and 60% RH. However, **Siroli et al. (2017)** showed a rapid decrease in *E. coli* populations from ca. 6.0 $\log_{10}$ CFU.cm^{-2} to ca. 1.5 and 2.5 $\log_{10}$ CFU.cm^{-2} after 24 hours on the surface of cardboard and plastic, respectively. Our results showed better survival of *E. coli* O157:H7 on the cardboard surface, though with substantial decline over 4-day period, possible due to the use of nutrient-rich medium as suspension medium for inoculum. To support our hypothesis, **Wei et al. (1995)** showed *Salmonella* counts fast decline on spot-inoculated surface with deionized water as a diluent (>5.0 $\log_{10}$ CFU.$tomato^{-1}$ in 3 days), while pathogen suspended in tryptic soy broth showed minimal decline within same conditions. Additionally, **Guo et al. (2002)** showed protective influence of soil supporting survival and growth of *Salmonella* compared to water as a diluent, which caused rapid decline in counts. To summarize, *E. coli* O157:H7 can survive on the surface of cardboard for longer than 4 days at room temperature, creating concerns about possible cross-contamination if cartons are reused (Figure 2).

Cross-contamination by *E. coli* O157:H7 between raw produce and common packaging materials, kitchen surfaces, is possible **(Buchholz et al., 2012; Jensen et al., 2013; Jensen el al., 2017, Jung et al., 2017)**, and only

harsh food-processing technologies, such as cooking and ionizing irradiation, can kill pathogenic bacteria in various foodstuff **(Tokarskyy et al., 2009; Schilling et al., 2009)**. Transfer rates studies of *E. coli* O157:H7 between surfaces involved fresh-cut produce and common kitchen surfaces **(Jensen et al., 2013)**, gloved hands and raw fruits and vegetables **(Jensen et al., 2017; Jung et al., 2017)**, as well as commercial pilot plant equipment and raw produce **(Buchholz et al., 2012)**. **Buchholz et al. (2012)** studied transfer possibility of *E. coli* O157:H7 from contaminated produce (iceberg and romaine lettuce) to the commercial processing equipment, followed by processing of uninoculated produce in the same contaminated equipment. The researchers found the highest transfers from inoculated lettuce to the commercial shredder and conveyor belt, with the processed uninoculated produce getting contaminated as well **(Buchholz et al., 2012)**.

Results of the transfer studies were expressed either as percent positive (where at least one *E. coli* O157:H7 CFU.mL^{-1} of rinsate was detected) or as counts, total $\log_{10}$ CFU.item^{-1} (either a cardboard square or a tomato), and are shown in Figure 3, Figure 4, Figure 5, and Figure 6. Samples yielding no counts were assigned a limit of detection count (1.3 $\log_{10}$ CFU.item^{-1}). Wet transfers (W) yielded 100% positive transfers on both day 0 and 1 irrespective of diluent type (Figure 3 and Figure 5). Similar results were shown by **Jensen et al. (2013)**, who investigated transfer rates of E. coli O157:H7 from fresh-cut produce to common kitchen surfaces (ceramic, stainless steel, glass, and plastic). They found the highest transfer rates (over 90%) in case of moist, freshly inoculated produce, and 1-hour dry produce had lower transfer rates, at ca. 0.01 to 5% from inoculated celery, carrots, and lettuce, to ca. 5% from inoculated watermelon. The authors also stressed that surface moisture and direction of transfer had the highest influence on transfer efficiency **(Jensen et al., 2013)**.

Dry transfers from tomatoes to squares appeared to be more efficient comparing to the opposite direction (Figure 3 and Figure 5) possibly due to smooth and hydrophobic properties of the tomato and rough surface of the cardboard. Dry transfers (90 min dry) were 100% positive from tomato to cardboard, and 0% positive from cardboard to tomato. Cardboard squares were easily deformed by the transfer procedure, shaping their surface as tomato was pressed against it. **Jensen et al. (2017)** studied cross-contamination by *E.coli* O157:H7 from gloved hands to carrots, celery, and cantaloupe, and vice versa, and also noted influence of surface type and structure on the transfer efficiency. From gloves, 30% of *E. coli* population was transferred to carrots, 10% to celery, and 1% to cantaloupe **(Jensen et al., 2017)**. Regarding reverse transfers, 1% was transferred from carrots and celery to gloves, and only 0.3% from cantaloupe **(Jensen et al., 2017)**. Results of transfers where the diluent was BPW showed 100% positive transfer from 24 hours dry tomatoes to squares on day 0, as spots on the tomatoes were moist, with residual bacterial concentration found on the squares after 24 hours storage as well. Regarding bacterial counts, influence of both factors (diluent type and transfer timing with storage), as well as their interaction, was significant in case of 'tomato to cardboard transfer' (p <0.05). However, only individual factors, but not their interaction, had significant effect on *E. coli* counts in case of 'cardboard to tomato' transfer (p <0.05). *E. coli* O157:H7 counts on contaminated items after transfer, either immediately after transfer or 24 hours later, are shown in Figure 4 and Figure 6.

In case of successful dry transfers from tomato to cardboard, certain *E. coli* O157:H7 population remained viable on the next day after transfer (Figure 4). Dry transfers from cardboard to tomatoes were unsuccessful and bacterial counts were expressed at detection limit for statistical purposes (Figure 6).

The surface of used uninoculated cardboard squares passed Ultrasnap ATP swab test, as well as surface spotted with sterile DP or sterile BPW followed by 90 minutes drying period (Table 2). Squares inoculated with bacterial suspension in either DP or BPW followed by drying, failed ATP test (Table 2), however, DP inoculated spots appeared visibly clean compared to spots in BPW. Luminometer measures ATP activity, a universal energy molecule for all living cells, transferred to the swab from the surface. Food residues containing remnants of cells, as well as microbial contamination, may harbor ATP in significant quantities. Autoclaving does not destroy ATP **(Ceresa and Ball 2005)**. Though designed to measure organic residue/cleanness, and to a lesser extent, microbial contamination, the ATP test showed that uninoculated used cardboard squares passed cleanness test both if uninoculated or spotted with sterile diluents (with an exception of few "warnings"), while reported contamination when *E. coli* O157:H7 inoculum was used.,

Similarly, **Chen and Godwin (2006)** confirmed that microbial ATP bioluminescence assay can provide quick and convenient test to assess microbial contamination in refrigerators. Significant correlation coefficient between microbial ATP and psychrotrophic plate count PPC (r = 0.851) was slightly higher than that between microbial ATP and aerobic plate count APC (r = 0.823), which indicated a potential discrepancy in the populations of psychrotrophic and mesophilic bacteria on the refrigerator surface; nevertheless, microbial ATP assay appeared to have a potential as a reliable indication of the average of APC and PPC (r = 0.895) **(Chen and Godwin, 2006)**. However, a study performed by **Larson et al. (2003)** of comparing results between colony-forming units counts as natural microbiota on hands and kitchen table from one side, and ATP monitor readings from the other side, showed no significant correlation between the two. The authors noted a precaution of using ATP monitor test instead of aerobic plate counts for evaluation of microbial contamination **(Larson et al., 2003)**. A mini-review by **Shama and Malik (2013)** summarized observations: though significant correlations were shown between microbial numbers and ATP levels under certain conditions (but not within healthcare settings), intracellular ATP levels unfortunately vary between microbial taxa and also depend on environmental conditions. They warned that rapid ATP assays cannot be used instead of microbial pathogen culturing methods, but can be used to estimate effectiveness of cleaning and evaluate overall bacterial load **(Shama and Malik, 2013)**.

Table 1 Relative air humidity with standard deviations (%RH ±st.dev) in the incubators with stored tomatoes and cardboard during survival and transfer studies at 25 °C.

Experiment	Diluent	Trial 1	Trial 2	Trial 3	Trial 4
Survival on tomatoes	DP	58.8 ±3.6	59.4 ±3.8	59.5 ±4.1	NA
	BPW	65.2 ±6.2	64.8 ±6.4	65.3 ±6.0	NA
Survival on cardboard	DP	67.4 ±2.2	70.8 ±2.0	71.6 ±2.1	73.2 ±1.9
	BPW	72.5 ±2.1	72.7 ±2.0	73.0 ±1.8	72.5 ±1.9
T2C transfer & C2T transfer	DP	69.0 ±5.9	67.8 ±6.8	58.7 ±10.8	NA
	BPW	66.6 ±9.9	67.8 ±4.5	70.7 ±3.8	NA

Note: T2C – tomato to cardboard transfer; C2T – cardboard to tomato transfer.

Table 2 Cleanness of the media (sterile and *E. coli* O157:H7 inocula) and inoculated dried cardboard squares as assessed by ATP luciferase Ultrasnap™ swab test.

Liquid/ Squares	Diluent	Avg RLU ± st dev	Pass	Warning	Fail
Liquid	BPW, sterile	5.4 ±1.4	9/9	0/9	0/9
	BPW, 9.0 $\log_{10}$ CFU.mL^{-1}	5223.8 ±949.4	0/9	0/9	9/9
	DP, sterile	0.0 ±0.0	9/9	0/9	0/9
	DP, 9.0 $\log_{10}$ CFU.mL^{-1}	6848.1 ±434.5	0/9	0/9	9/9
Squares	Negative control square	2.9 ±2.9	9/9	0/9	0/9
	BPW, sterile	5.3 ±7.0	7/9	2/9	0/9
	BPW, 7.5 $\log_{10}$ CFU.square^{-1}	4033.7 ±2049.0	0/9	0/9	9/9
	DP, sterile	6.8 ±8.7	8/9	1/9	0/9
	DP, 7.5 $\log_{10}$ CFU.square^{-1}	1486.4 ±1451.7	0/9	0/9	9/9

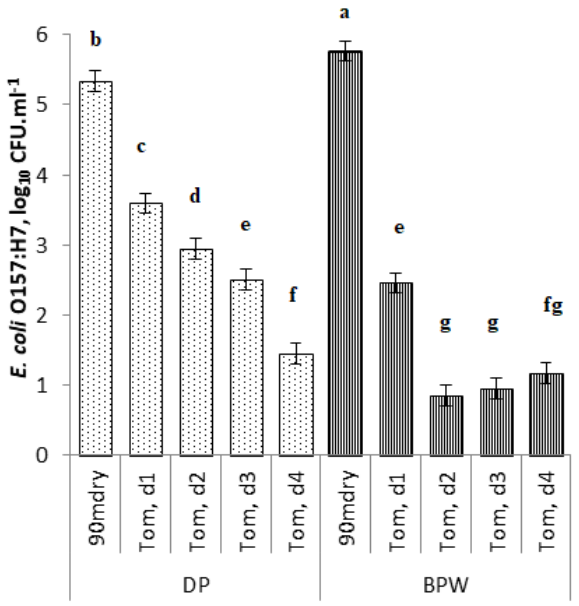

Figure 1 Recovery of *E. coli* O157:H7 (DP or BPW diluent) from inoculated tomatoes either immediately after drying (90 min dry), or after storage for four days (d1-d4) at 25 °C. Note: Counts expressed as $\log_{10}$ CFU.mL^{-1} recovered from 20 mL rinsate. Means with the same letters are not significantly different (p >0.05).

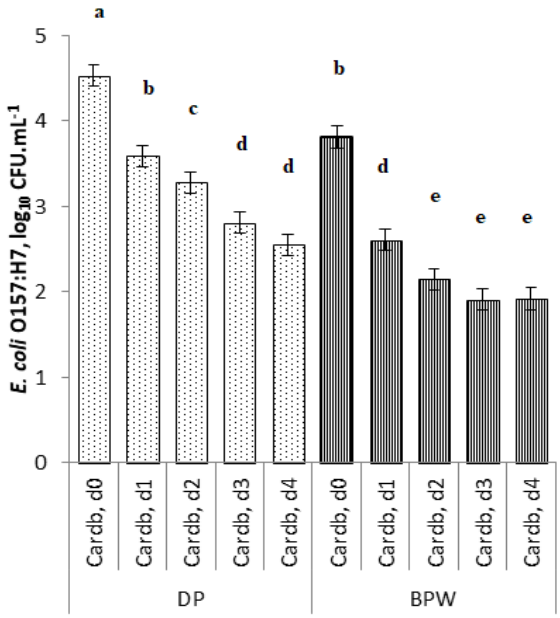

Figure 2 Recovery of *E. coli* O157:H7 (DP or BPW diluent) from inoculated cardboard squares either immediately after drying (90 min dry), or after storage for four days (d1-d4) at 25 °C. Note: Counts expressed as $\log_{10}$ CFU.mL^{-1} recovered from 20 mL rinsate. Means with the same letters are not significantly different (p >0.05).

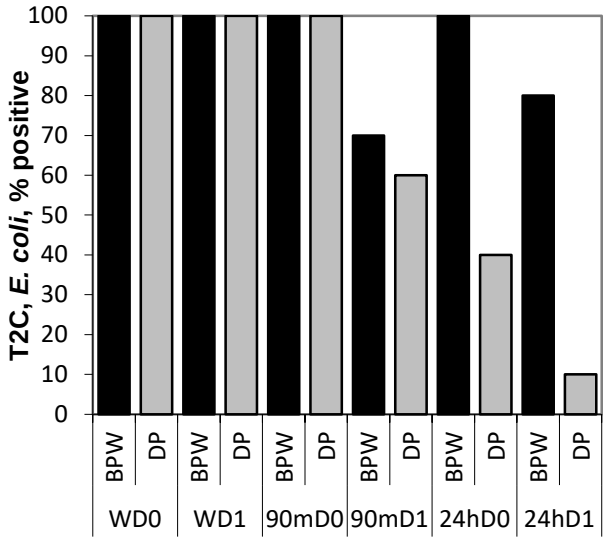

Figure 3 Percentage of squares yielding at least 1 cfu.mL^{-1} of *E. coli* O157:H7 in rinsate after inoculated tomatoes (w – wet; 90m – 90 minutes dry; 24h – 24 hours dry) touched cardboard squares. Note: Squares sampled for *E. coli* either immediately after the transfer (D0) or stored 24 hours after the transfer at 25 ºC (D1). T2C – Tomatoes to Cardboard transfer.

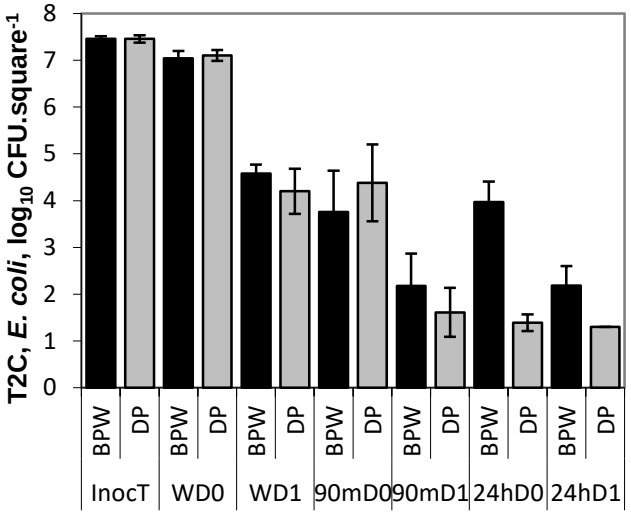

Figure 4 Total *E. coli* O157:H7 counts per square after pathogen transfer from tomato (w – wet; 90m – 90 minutes dry; 24h – 24 hours dry) compared to total inoculated log$_{10}$ CFU.tomato^{-1} (InocT). Note: Squares sampled for *E. coli* either immediately after the transfer (D0) or stored 24 hours after the transfer at 25 ºC (D1). Detection limit 1.3 log$_{10}$ CFU.square^{-1}. Tomato inoculation level (InocT) calculated theoretically based on stationary culture concentration and is shown for reference. Means with the same letters are not significantly different (p >0.05).

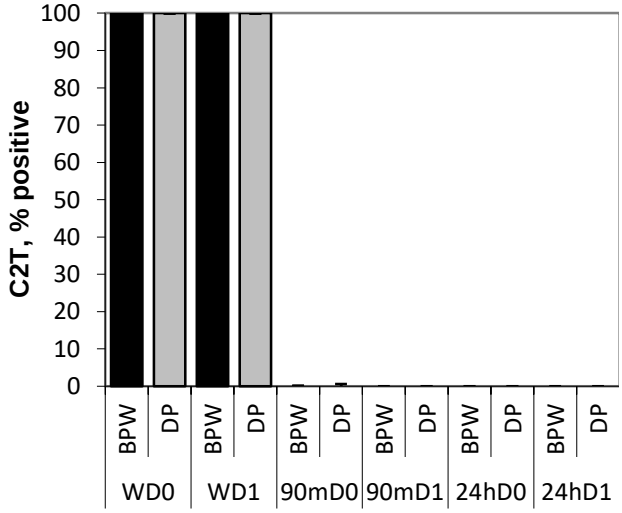

Figure 5 Percentage of tomatoes yielding at least 1cfu.mL^{-1} of *E. coli* O157:H7 in rinsate after inoculated squares (w – wet; 90m – 90 minutes dry; 24h – 24 hours dry) touched tomatoes. Note: Tomatoes were sampled for *E. coli* either immediately after the transfer (D0) or stored 24 hours after the transfer at 25 ºC (D1). C2T – Cardboard to Tomatoes transfer.

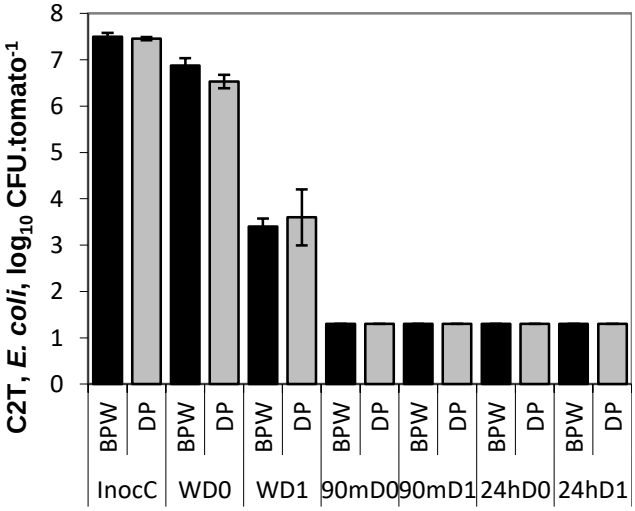

Figure 6 Total *E. coli* O157:H7 counts per tomato after pathogen transfer from square (w – wet; 90m – 90 minutes dry; 24h – 24 hours dry) compared to total inoculated log $_{10}$ CFU.square^{-1} (InocC). Note: Tomatoes were sampled for *E. coli* either immediately after the transfer (D0) or stored 24 hours after the transfer at 25 ºC (D1). Detection limit 1.3 log$_{10}$ CFU.tomato^{-1}. Cardboard square inoculation level (InocC) calculated theoretically based on stationary culture concentration and is shown for reference. Means with the same letters are not significantly different (p >0.05).

CONCLUSION

E. coli O157:H7 survived better on porous cardboard surfaces than on smooth tomato surfaces in humidified atmosphere. Bacterial cells survived for longer than 4 days on cardboard surfaces, questioning possibility of cardboard boxes reuse. Moreover, survival on smooth tomato peel was influenced by diluent type with BPW negatively impairing survival. The observed phenomenon was possible related to hygroscopic nature of solutes present in BPW, where dried inoculated spots liquefied during storage and possibly created environment of high osmotic pressure. Pathogen transfers are of great concern if the surface is wet, but less of a concern if the surface is dry. Though *E. coli* O157:H7 survived better on the surface of cardboard compared to the surface of tomatoes, the transfers were more efficient from tomatoes to cardboard than from cardboard to tomatoes. High humidity storage might cause decrease in bacterial counts of stationary phase cells inoculated in high solids/high salt diluent, therefore, choice of diluent of inoculation studies should be carefully decided. Rapid ATP measuring devices can simplify estimation of overall microbial load, and to some extent, present organic residues, questioning efficiency of surface sanitizing or checking cardboard boxes for cleanness and overall microbial contamination.

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