



Research Paper

Salmonella transfer potential between tomatoes and cartons used for distribution

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ABSTRACT

Corrugated fiberboard boxes (cartons) can be reused during fresh market tomato packing and repacking. The fate of *Salmonella* on the new, used, and dirty tomato packaging cartons, and *Salmonella* transfer between tomatoes and new, used, and dirty packaging cartons was assessed. Mature green tomatoes or blank cartons were spot inoculated with cocktail of rifampicin-resistant *Salmonella* strains before touching cartons/tomatoes at 0, 1, or 24 h postinoculation. Tomatoes were placed on new, used, and dirty carton squares (5 by 5 cm) for 0, 1, and 7 days of contact at 12°C and 25°C with a relative humidity value of 85%. Transfer coefficients (TCs) were calculated for all conditions. *Salmonella* populations decreased following inoculation by 2–3 log units during 24 h drying regardless of storage temperature; the presence of debris enhanced survival at 12°C. In general, the highest transfer rates occurred with wet inoculum. The highest *Salmonella* transfer was calculated for wet inoculated tomatoes with 7 days of contact time at 25°C (TC = 14.7). Increasing contact time decreased TCs for new cartons, but increased TCs for used and dirty cartons. Regardless of carton condition or storage temperature, a greater population of *Salmonella* was transferred from tomatoes to cartons than from cartons to tomatoes. *Salmonella* transfer between tomatoes and cartons is highly dependent on moisture, with increased levels of moisture increasing transfer, highlighting the importance of harvesting and packing dry tomatoes.

During tomato harvest in Florida, hand-picked mature green tomatoes are typically transferred to plastic bins from plastic picking buckets (Estabrook, 2011; Felkey et al., 2006) and transported to packinghouses. In the packinghouse, tomatoes are dumped from the plastic bins into a chlorinated dump tank prior to washing with overhead sprays on brush rollers, waxing, sorting, and packing into corrugated fiberboard boxes commonly known as cartons (composed of an inside liner and outside liner with fluting in between) (Sreedharan et al., 2014). Tomatoes will then begin to ripen in the cartons and may be transferred to repackers, where packed cartons are processed as described above, and sorted and repacked based on size or color, and may be packed into previously used tomato cartons.

Contaminated fresh tomatoes have been linked to a number of salmonellosis outbreaks in the U.S. as confirmed or suspected food vehicle and part of a complex food vehicle between 1990 and 2017 (Krug et al., 2020). A number of tomato-related multistate outbreaks have been traced back to farms located in the Southeastern states of the United States (U.S.) including Virginia and Florida (Centers for Disease Control and Prevention (CDC), 2007; Cummings et al., 2001;

Greene et al., 2008; Hedberg et al., 1999). Preharvest routes of contamination are the suspected source of contamination; however, confirmed identification of contamination source is not common and cross-contamination during packing has the potential to amplify the number of contaminated tomatoes from the field. Multistate outbreaks associated with the consumption of tomatoes suggest that these products are exposed to possible cross-contamination before, during, or after harvest (Lynch et al., 2006). In the tomato fields, agricultural waters, soil amendments, domesticated and wild animals, worker health and hygiene, and equipment and buildings are believed to be possible routes of contamination (Food and Administration, 2015). If tomatoes are damaged, low levels of *Salmonella* contamination have the potential to increase during postharvest processing and distribution (Lynch et al., 2006; Marvasi et al., 2013). The presence or proliferation of pathogens already on food contact surfaces can be sources of cross-contamination of tomatoes during postharvest applications as repacking and distribution.

Reuse of cartons from primary tomato packinghouses may occur in repack operations. In California, a carton box can be reused by the reg-

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istered growers or repackers after careful evaluation for any food safety risk and with adequate documentation (Suslow, 2004). The reuse of packaging boxes can significantly complicate the traceability of tomatoes during outbreak investigations. Reuse may also serve as a potential point of cross-contamination during packing and repacking operations (Watson et al., 2015). The risks of *Salmonella* contamination onto tomatoes from used cartons are not known. This information is needed to develop the best practices for fresh tomato carton reuse. The objectives of this study were to determine the fate of *Salmonella* on tomato cartons and *Salmonella* transfer coefficients (TCs) between (i) inoculated new, used, and dirty cartons and tomatoes; and (ii) inoculated tomatoes and new, used, and dirty cartons, under varying inoculation conditions, contact times, and temperatures.

Materials and methods

Tomatoes and cartons

Mature green, round, washed, and waxed tomatoes were purchased from a local supermarket in Florida. Tomatoes were stored at 4°C prior to use and left overnight at ambient temperature (18–23°C) prior to inoculation. Carton squares (5 cm by 5 cm) were prepared as new, clean used, and dirty. New cartons were obtained from a local supplier (Mulberry, FL) and used cartons from a local produce stand (Lake Alfred, FL). To standardize dirty used cartons, 250 g of tomato (locular cavity) was extracted and combined with 50 g of sandy soil and mixed in a food processor for 1 min. The tomato and soil slurry was spread on carton squares with a paintbrush and allowed to dry overnight dry.

Salmonella serotypes and inoculum preparation

Five rifampicin-resistant *Salmonella* strains were used including serotypes: Michigan (LJH 615; Cantaloupe outbreak), Montevideo (LJH0614; Almond survey), Newport (Tomato outbreak), Poona (LJH631; Cantaloupe outbreak), and Saintpaul (BAC 158; Orange juice outbreak). All strains were resistant to 80 µg/mL rifampicin following a stepwise procedure detailed in Parnell et al. (Parnell et al., 2005). Frozen stock cultures of each serotype were streaked onto tryptic soy agar with added rifampicin (TSAR: 80 µg/mL; Difco, BD, Sparks, MD) and incubation at 35 ± 2°C for 24 ± 2 h. Isolated colonies of each serotype were subcultured in 10 mL of tryptic soy broth with added rifampicin (TSBR: 80 µg/mL; Difco, BD, Sparks, MD) and incubated at 35 ± 2°C for 18 h. A loopful of (10 µL) growth was transferred into an additional tube of 10 mL TSBR for subsequent incubation at 35 ± 2°C for 18 h. Cells were pelleted by centrifugation at 3000 × g for 10 min. The supernatant was replaced with 10 mL of 0.1% peptone (Difco, BD, Sparks, MD), and the pellets were resuspended with a vortexer to wash cells. After washing twice, cells were resuspended in 5 mL of 0.1% peptone to obtain the inoculum (10⁹–10¹⁰ CFU/mL). Equal volumes of the five serotypes of *Salmonella* were mixed to obtain a cocktail for inoculation.

Inoculation of cartons for transfer and *Salmonella* survival

Transfer was evaluated in both directions: inoculated tomato to carton and inoculated carton to tomato. Tomatoes or new, used, and dirty carton squares were spot (6 to 8 drops) inoculated with 100 µL of the five strain *Salmonella* cocktails to ca. 6 log CFU/item. Controls were inoculated with 0.1% peptone. Inoculated samples were dried for 0 (wet inoculum), 1, or 24 h at ambient temperature in a biosafety cabinet. Inoculated and dried (for 0, 1, and 24 h) tomatoes or cartons were placed in contact with uninoculated cartons or tomatoes for three-time intervals of 0 (touch), 1, and 7 days and stored at 12 and 25°C (n = 10 for each combination). Another set of inoculated cartons were stored under the same conditions without any contact to deter-

mine the survival of *Salmonella* at days 0, 1, and 7 (n = 6). Relative humidity and ambient temperature were measured with a portable datalogger during storage.

Enumeration and enrichment

At each sampling day, tomatoes or carton squares were placed into 530 mL Whirl-Pak bags with 20 mL of 0.1% peptone. Carton squares were macerated at high speed for 1 min. Tomatoes were shaken vigorously by hand for 15 s, rubbed for 30 s, and shaken for 15 s. Dilutions were plated onto TSAR (80 µg/mL). To increase the limit of detection, 1 mL from the lowest dilution was spread over four plates (0.25 mL/plate). Plates were incubated for 24 h at 35 ± 2°C. If population levels fell below the limit of detection (1.4 log CFU/surface), enrichments to determine *Salmonella* presence or absence on the surface of tomatoes were performed (Food and Administration, 2007). Briefly, 20 mL of double-strength lactose broth (Difco, BD, Sparks, MD) was added to stomacher bags containing tomatoes or carton squares treated with 20 mL of peptone to incubate at 35 ± 2°C. After 24 h incubation, 0.1 and 1 mL of enrichments were added to 9.9 mL of Rappaport-Vassiliadis (RV; Difco, BD, Sparks, MD) and nine mL of tetrathionate (TT; Difco, BD, Sparks, MD) broth, respectively. TT and RV broth tubes were incubated at 35 ± 2°C for 24 h and 42 ± 2°C for 48 h, respectively. Following incubation, a loopful (10 µL) of enrichment from each broth was streaked onto bismuth sulfite agar (BS), xylose lysine desoxycholate agar (XLD), and Hektoen enteric agar (HE) supplemented with rifampicin (XLDR: BSR: HER; 80 µg/mL; Difco, BD, Sparks, MD). All plates were incubated at 35 ± 2°C for 24 h. Presumptive *Salmonella* colonies were inoculated to lysine iron agar slants (LIA; Difco, BD), and triple sugar iron agar slants (TSI; Difco, BD) for another overnight incubation.

Calculation of transfer coefficients and statistical analysis

Average transfer coefficients were calculated by the following equation: TC = PG/PC where TC is the transfer coefficient, PG is the pathogen population enumerated from the previously uncontaminated surface in CFU/item surface, and PC is the pathogen population enumerated from the initial surface source of the pathogen in CFU/item surface. Prior research has shown that the logarithm of TC is normally distributed (Chen et al., 2001), so TCs were log transformed. Analysis of variance and Tukey's least square means significance test ($P \leq 0.05$) were performed on the transfer coefficients. JMP Pro 9.0 (SAS, Cary, NC, USA) was used to determine the statistical significance.

Results

Fate of *Salmonella* on tomato cartons

New, used, and dirty tomato cartons were inoculated with ca. 6 log CFU/carton *Salmonella* and stored at 12.3 ± 0.6 and 25.3 ± 0.4°C, for up to 7 days; relative humidity was 88.3 ± 3.4 and 89.9 ± 1.7%, respectively. Samples were enumerated as described above and are included in Figure 1. *Salmonella* populations declined between 1.9 and 3.4 log CFU/carton under all conditions on all cartons within 24 h. Following the initial decline, populations stabilized with no further decline. Within 24 h, population declines on the new and used cartons were up to log 1.3 CFU/carton greater than those on dirty cartons at both 12 and 25°C ($P \leq 0.05$). Following 7 days of storage at 12°C, *Salmonella* populations on dirty cartons are significantly higher than those on clean or used cartons ($P \leq 0.05$). No significant differences in *Salmonella* populations are present following 7 days of storage at 25°C ($P > 0.05$).

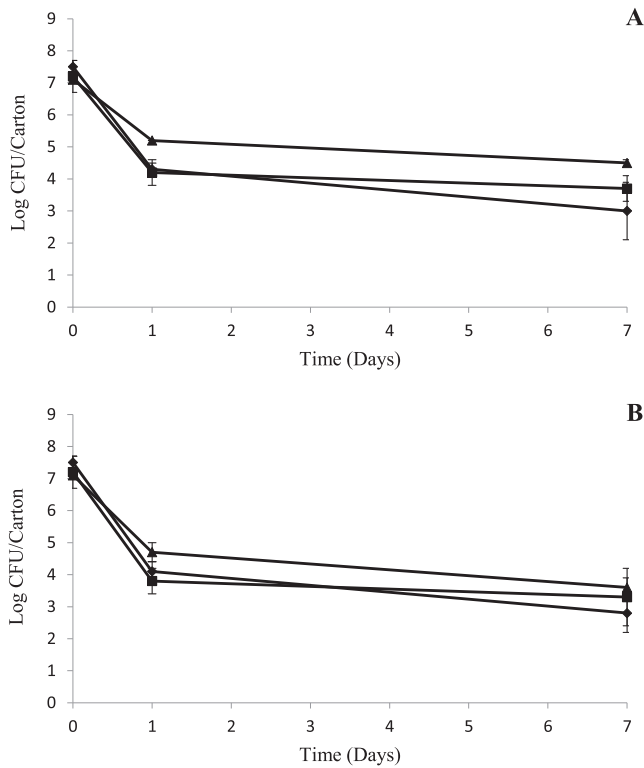


Figure 1. Survival of *Salmonella* on new (◆) used (■) and dirty (▲) cartons stored at 12°C (A) and 25°C (B) (n = 3).

Salmonella transfer from cartons to tomatoes

Salmonella transfer coefficients from cartons to tomatoes are shown for all tested conditions in Table 1. At 12°C, *Salmonella* transfer coefficients obtained from new and used cartons to tomatoes ranged from 0.01 to 0.47. Transfer from dirty cartons stayed below 0.03 except for wet inoculation with a touch contact (0.86). At 25°C, higher transfer coefficients from new and used cartons to tomatoes were calculated up to 1.00. Similar transfer coefficients were observed for dirty cartons

at both temperatures. Transfer from new and dirty cartons was not affected by storage temperature dramatically ($P > 0.05$) except for one case of new carton with 7 days of contact time after one-hour inoculum drying. In general, higher transfer coefficients at 25°C were observed for used and dirty cartons. At 25°C, *Salmonella* transfer coefficients from used cartons increased between 0.80 and 0.94 regardless of inoculation drying time ($P \leq 0.05$). The highest *Salmonella* transfer levels from dirty cartons to tomatoes (1.00 ± 0.00) were calculated for single touch (0-day contact time) and wet inoculation at 12 and 25°C ($P \leq 0.05$). Under all other tested conditions, *Salmonella* transfer from dirty cartons to tomatoes was lower than or close to those for new or used cartons.

The distribution of *Salmonella* log transfer coefficients from cartons to tomatoes are shown at 12°C in Figure 2 and at 25°C in Figure 3. In these figures, distribution shifting toward the left indicates lesser transfer; more transfer is noticed when distribution shifting toward the right. At 12°C, more transfer to tomatoes from new, used, and dirty cartons were observed for wet inoculation. The rates of *Salmonella* transfer to tomatoes from dirty cartons decreased after 1 or 24 h of inoculum drying compared to rates of wet inoculation at both temperatures based on distribution shifting. Higher levels of *Salmonella* were transferred from used cartons with 24 h inoculum drying than new cartons and dirty cartons for all contact time trials at 12 and 25°C ($P \leq 0.05$) with the exception of 1 h of contact time at 12°C ($P > 0.05$). Drying the inoculum (24 h) reduced *Salmonella* transfer from new and used carton to tomatoes at both temperatures except for used cartons with seven days of contact time.

Salmonella transfer coefficients from tomatoes to cartons

Salmonella transfer coefficients from tomatoes to cartons are shown for all tested conditions in Table 2. At 12°C, the highest and the lowest *Salmonella* transfer coefficients obtained from tomatoes to new, used, and dirty cartons ranged from 0.01 ± 0.00 to 2.39 ± 1.36 . At 25°C, similar transfer coefficients were observed for new and dirty cartons at both temperatures. Transfer from tomato to used cartons reached up to 14.75 ± 35.11 . The most prominent factor affecting the transfer coefficient of *Salmonella* from tomatoes to cartons was inoculum drying and contact time for new, used, and dirty cartons. The worst case for *Salmonella* transfer levels from tomatoes to used cartons was calculated for wet inoculum with seven days of contact time at 25°C, where

Table 1
Salmonella transfer coefficients from inoculated carton to tomato under tested conditions

Storage Temperature	Inoculum Drying	Contact Time (Day)	Transfer Coefficients (TCs) for Carton Conditions*		
			New	Used	Dirty
12°C	Wet	0	0.30 ± 0.34 ^{Bbc}	0.20 ± 0.03 ^{Bbc}	0.86 ± 0.24 ^{Aa}
		1	0.16 ± 0.17 ^{Abc}	0.23 ± 0.46 ^{Abc}	0.02 ± 0.01 ^{Ab}
		7	0.11 ± 0.03 ^{Bbc}	0.27 ± 0.00 ^{Abc}	0.03 ± 0.05 ^{Cb}
	One hour	0	0.47 ± 0.63 ^{Ab}	0.36 ± 0.35 ^{ABb}	0.00 ± 0.00 ^{Bb}
		1	0.01 ± 0.00 ^{Bc}	0.11 ± 0.13 ^{Abc}	0.00 ± 0.00 ^{Bb}
		7	0.23 ± 0.00 ^{Abc}	0.17 ± 0.17 ^{Abc}	0.00 ± 0.00 ^{Bb}
	24 hours	0	0.01 ± 0.00 ^{Bc}	0.06 ± 0.01 ^{Ac}	0.00 ± 0.00 ^{Bb}
		1	0.01 ± 0.00 ^{Ac}	0.02 ± 0.00 ^{Ac}	0.01 ± 0.00 ^{Ab}
		7	0.01 ± 0.00 ^{Bc}	0.18 ± 0.00 ^{Abc}	0.03 ± 0.00 ^{Bb}
25°C	Wet	0	0.30 ± 0.34 ^{Bbc}	0.20 ± 0.03 ^{Bbc}	0.86 ± 0.24 ^{Aa}
		1	0.17 ± 0.29 ^{Abc}	0.26 ± 0.00 ^{Abc}	0.13 ± 0.25 ^{Ab}
		7	0.07 ± 0.07 ^{Bc}	1.00 ± 0.00 ^{Aa}	0.08 ± 0.07 ^{Bb}
	One hour	0	0.47 ± 0.63 ^{Ab}	0.36 ± 0.35 ^{ABb}	0.00 ± 0.00 ^{Bb}
		1	0.07 ± 0.06 ^{Bbc}	0.28 ± 0.27 ^{Abc}	0.01 ± 0.03 ^{Bb}
		7	1.00 ± 0.00 ^{Aa}	1.00 ± 0.00 ^{Aa}	0.04 ± 0.00 ^{Bb}
	24 hours	0	0.01 ± 0.00 ^{Bc}	0.06 ± 0.01 ^{Ac}	0.00 ± 0.00 ^{Bb}
		1	0.01 ± 0.00 ^{Bc}	0.08 ± 0.00 ^{Abc}	0.00 ± 0.00 ^{Cb}
		7	0.01 ± 0.00 ^{Bc}	1.00 ± 0.00 ^{Aa}	0.03 ± 0.00 ^{Bb}

* Capital letters in rows indicate a significant difference in TCs between new, used, and dirty cartons. Lowercase letters in columns indicate a significant difference in TCs within each temperature, inoculum drying, and contact time ($P < 0.05$).

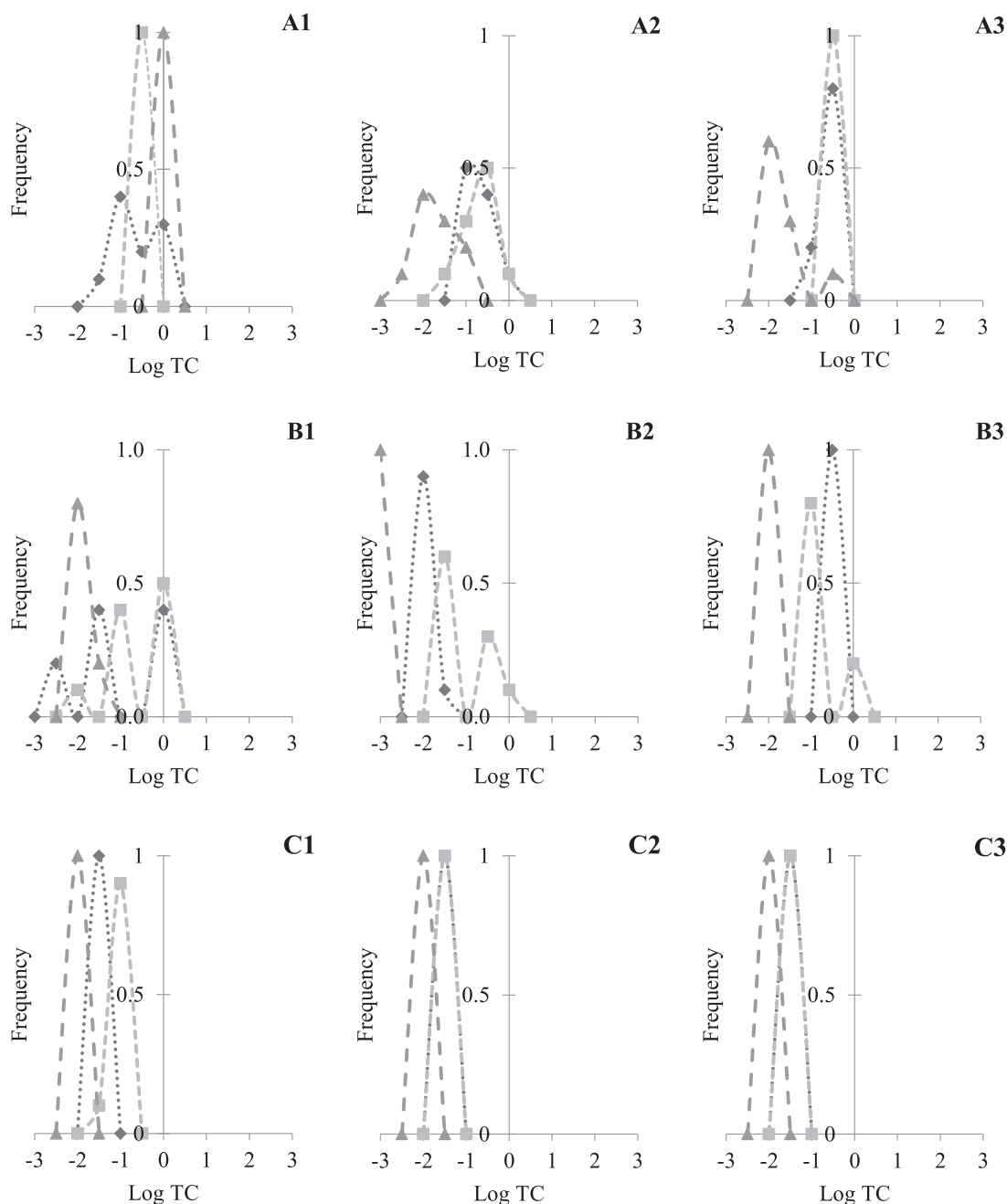


Figure 2. Distributions of transfer coefficients (TCs) from inoculated and wet (A), 1 h dried (B) and 24 h dried (C) new (◆) used (■) and dirty (▲) cartons to tomatoes in contact after inoculation (1), one (2) and seven (3) days of storage at 12°C (n = 10).

there was the equivalent of 1470% (16 times) higher transfer than from a new carton ($P \leq 0.05$).

The distribution of *Salmonella* log transfer coefficients from tomatoes to cartons are shown at 12°C in Figure 4 and at 25°C in Figure 5. In general, temperature rise along with the increase in contact time decreased the transfer of *Salmonella* from tomatoes to new and used cartons. Inoculum drying reduced *Salmonella* transfer coefficients from tomatoes to dirty cartons compared to wet and 1 hour of dry inoculation regardless of contact time and temperature. Longer inoculum drying and contact time increased the transfer coefficient of *Salmonella* from tomatoes to used cartons ($P \leq 0.05$). The highest *Salmonella* transfer levels from tomatoes to cartons were observed for used carton with all tested inoculum drying and seven days of contact time at both

temperatures ($P \leq 0.05$). Overall, the worst case for *Salmonella* transfer for new, used, and dirty cartons was under wet conditions (0 h, 1 h drying inoculum) with various degrees of contamination.

Discussion

Potential contamination sources for tomatoes vary during growth, harvesting, processing, and packaging. During production, environmental factors such as microbiological water quality used for agricultural purposes, animal intrusion, and biological soil amendments may cause the transfer of pathogens onto fruits (Food and Administration, 2015). Laboratory simulated and field trials have identified several

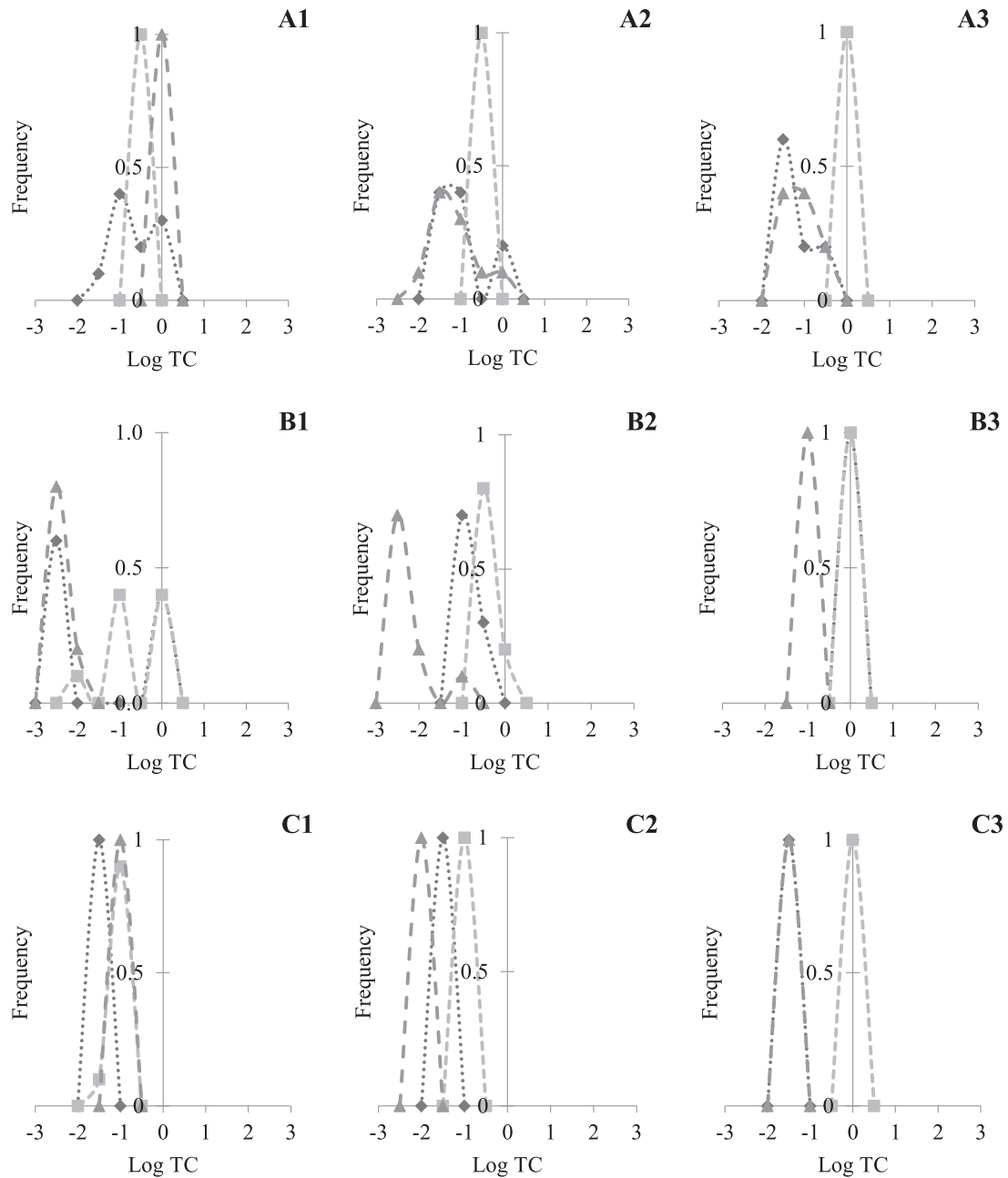


Figure 3. Distributions of transfer coefficients (TCs) from inoculated and wet (A), 1 h dried (B) and 24 h dried (C) new (◆) used (■) and dirty (▲) cartons to tomatoes in contact after inoculation (1), one (2) and seven (3) days of storage at 25°C (n = 10).

factors that could impact the contamination of tomatoes during harvest such cross-contamination via equipment, or handling by farm workers (Taormina et al., 2009). *Salmonella* can be transferred to tomatoes by workers with gloves used during harvesting and cloth used for removal of debris in the fields (Brar and Danyluk, 2013; Sreedharan et al., 2014). In the packing houses, tomatoes can be contaminated via water in dump tanks and flume systems when wash water management is poor (Harris et al., 2003; Tomás-Callejas et al., 2012). The food safety risks related to the reuse of boxes, beyond the obvious traceability issues, remain unexplored. In this study, *Salmonella* population transfer from box to fruit and from fruit to box varied between new, used, and dirty tomato cartons, indicating cross-contamination risks may increase under some conditions when cartons

are dirty or reused (Fig. S1 and Fig. S2). The greatest variability in *Salmonella* transfer is seen following a 1 h drying time in all conditions. This may be due to variable moisture remaining in the inoculum and may be representative of variable moisture during tomato packing. The round surface of tomatoes on the flat surface of cartons can be another reason for nonuniform results of *Salmonella* transfer.

Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* serotypes have been reported as hours to weeks on carton packaging materials under various storage conditions and inoculum levels (Siroli et al., 2017; Tokarsky and Korda, 2019; Watson et al., 2015; Zwilling et al., 2022); the total time of pathogen survival is relative to the methodology used (inoculation concentration and enumeration limit of detection). In this study, *Salmonella* survival on used

Table 2
Salmonella transfer coefficients from inoculated tomato to carton under tested conditions

Storage Temperature	Inoculum Drying	Contact Time (Day)	Transfer Coefficients (TCs) for Carton Conditions*			
			New	Used	Dirty	
12°C	Wet	0	1.38 ± 0.88 ^{Aa}	0.30 ± 0.34 ^{Bb}	0.62 ± 0.29 ^{Bbcd}	
		1	1.11 ± 2.13 ^{Aa}	0.16 ± 0.27 ^{Ab}	1.46 ± 1.83 ^{Aab}	
		7	0.85 ± 0.90 ^{Ba}	0.11 ± 0.03 ^{Bb}	2.39 ± 1.18 ^{Aa}	
	One hour	0	0.20 ± 0.14 ^{Ba}	0.12 ± 0.05 ^{Bb}	0.50 ± 0.21 ^{Abcd}	
		1	0.83 ± 0.94 ^{Aa}	0.79 ± 0.34 ^{Ab}	0.31 ± 0.10 ^{Ac}	
		7	0.67 ± 0.54 ^{Ba}	2.39 ± 1.36 ^{Aab}	0.98 ± 0.92 ^{Bbcd}	
	24 hours	0	0.01 ± 0.01 ^{Ba}	0.74 ± 0.17 ^{Ab}	0.22 ± 0.66 ^{Bcd}	
		1	0.01 ± 0.00 ^{Ba}	1.75 ± 0.66 ^{Aab}	0.09 ± 0.09 ^{Bd}	
		7	0.23 ± 0.00 ^{Ba}	1.86 ± 1.51 ^{Aab}	0.11 ± 0.13 ^{Bd}	
	25°C	Wet	0	1.38 ± 0.88 ^{Aa}	0.30 ± 0.34 ^{Bb}	0.62 ± 0.29 ^{Bbcd}
			1	0.21 ± 0.19 ^{Ba}	0.17 ± 0.27 ^{Bb}	0.79 ± 0.44 ^{Abcd}
			7	0.93 ± 2.31 ^{Aa}	14.75 ± 35.11 ^{Aa}	1.18 ± 0.75 ^{Abc}
One hour		0	0.20 ± 0.14 ^{Ba}	0.12 ± 0.05 ^{Bb}	0.50 ± 0.21 ^{Abcd}	
		1	0.09 ± 0.07 ^{Ba}	0.73 ± 0.80 ^{Ab}	0.85 ± 0.45 ^{Abcd}	
		7	0.31 ± 0.12 ^{Ba}	2.54 ± 2.58 ^{Aab}	0.31 ± 0.39 ^{Bcd}	
24 hours		0	0.01 ± 0.01 ^{Ba}	0.74 ± 0.17 ^{Ab}	0.22 ± 0.66 ^{Bcd}	
		1	0.24 ± 0.00 ^{Ba}	0.81 ± 0.34 ^{Ab}	0.00 ± 0.01 ^{Cd}	
		7	0.37 ± 0.00 ^{Ba}	1.89 ± 1.04 ^{Aab}	0.01 ± 0.00 ^{Bd}	

* Capital letters in rows indicate a significant difference in TCs between new, used, and dirty cartons. Lowercase letters in columns indicate a significant difference in TCs within each temperature, inoculum drying, and contact time ($P < 0.05$).

tomato cartons is increased in the presence of dirt and debris and under cool, moist conditions. While no growth of *Salmonella* on tomato cartons was observed, populations inoculated onto dirty cartons and stored at 12°C for 7 days were significantly higher than those on new or used cartons, and those at all conditions stored at 25°C. The presence of debris on tomato cartons coupled with storage at temperatures below ambient increases the survival of *Salmonella* on tomato cartons. Similarly, Sreedharan et al. (Sreedharan et al., 2014) reported that *Salmonella* survival on both clean and dirty cloths used for removal of dirt, debris, and stem during harvest was higher at 15°C than cloths held at 25°C and 35°C.

Under no conditions was the transfer of *Salmonella* to/from tomatoes from new cartons greater than that from used or dirty cartons similar to results obtained for *Salmonella* transfer to/from tomatoes from reusable and single-use gloves and from the clean and dirty cloth used to remove dirt, debris, and/or stems during harvesting (Brar and Danyluk, 2013; Sreedharan et al., 2014). Under a number of different scenarios, transfer from dirty cartons was less than from clean or used cartons, consistent with results of studies looking at the transfer to and from clean and dirty gloves and cloth used in tomato harvest and packing (Brar and Danyluk, 2013; Sreedharan et al., 2014). While the presence of debris on tomato cartons may increase the survival of *Salmonella* on these surfaces, the reuse of dirty cartons would not significantly increase the risk of *Salmonella* transfer to tomatoes. An explanation of lower *Salmonella* transfer may be simply that *Salmonella* gets trapped in the dirt matrix reducing its chance of transfer. This result should not be considered a scientific justification by the industry to use of dirty boxes. Dirty cartons in this study were standardized by using predetermined quantities of debris painted onto used cartons and allowed to dry overnight following experimentation evaluating soil, stem and leaf residuals, internal tomato debris, and tomato wax. In practice, the composition of debris will change depending on various factors before and after harvest and may impact both *Salmonella* survival time and ability to transfer.

Failure to adequately address the food safety risks related to the reuse of cartons during packing may allow box-to-fruit cross-contamination during packing. *Salmonella* can survive on tomato fruit and plant surfaces or tissues (Guo et al., 2001) and proliferate when

there is a damage or spoilage on the fruit (Gurtler et al., 2018; Ma et al., 2010). The removal of bacteria with washing practices is practically impossible after attachment to plant surface (Castillo et al., 2014). Sanitizer applications during repacking are likely not be sufficient to eliminate *Salmonella* on tomato surfaces if tomatoes are contaminated.

The highest transfer of *Salmonella* from tomato to carton and vice versa is observed under moist or dry conditions with 7 days of contact time. Under these conditions, where more than 100% transfer was seen ($TC \geq 1.0$), the contact between the tomato and the carton may be providing a microclimate where the *Salmonella* survive better than on a carton alone and may be growing. *Salmonella* growth was not observed on any of the cartons alone, nor has it previously been seen on intact whole green tomatoes (Ma et al., 2010) implying that the interplay of the tomato and carton, when moisture was present, had an impact on the fate of *Salmonella*. This highlights the importance of the current industry practice of ensuring tomatoes are dry prior to placing them into cartons.

Tomato Good Agricultural Practices (T-GAPs) and Tomato Best Management Practices (T-BMPs) are required to enhance the safety of fresh tomatoes produced, packed, repacked, distributed, and sold in Florida (Administrative and code and Florida Administrative Register (FAC&FAR), 2008). Previously, the transfer potential of *Salmonella* from gloves and cloths used for the removal of dirt, debris, and stem during harvest were studied to support safe tomato harvesting (Brar and Danyluk, 2013; Sreedharan et al., 2014). Within T-GAPs, specific metrics are given to ensure the cleanliness of any container used to hold tomatoes and to prevent the use of final packaging containers such as cartons during harvesting or packing in the field. (Administrative and code and Florida Administrative Register (FAC&FAR), 2008). No such metrics exist for the use or the reuse of cartons during final packing and repacking operations. The lack of specific metrics results in a reliance on individual company standard operating procedures (SOPs), specific for their procedures and location. This study highlights the influence of moisture in transfer, and the importance of keeping the cartons and tomatoes dry for safe production. While the transfer potential of *Salmonella* between tomatoes and cartons was identified for new, used, and dirty cartons under var-

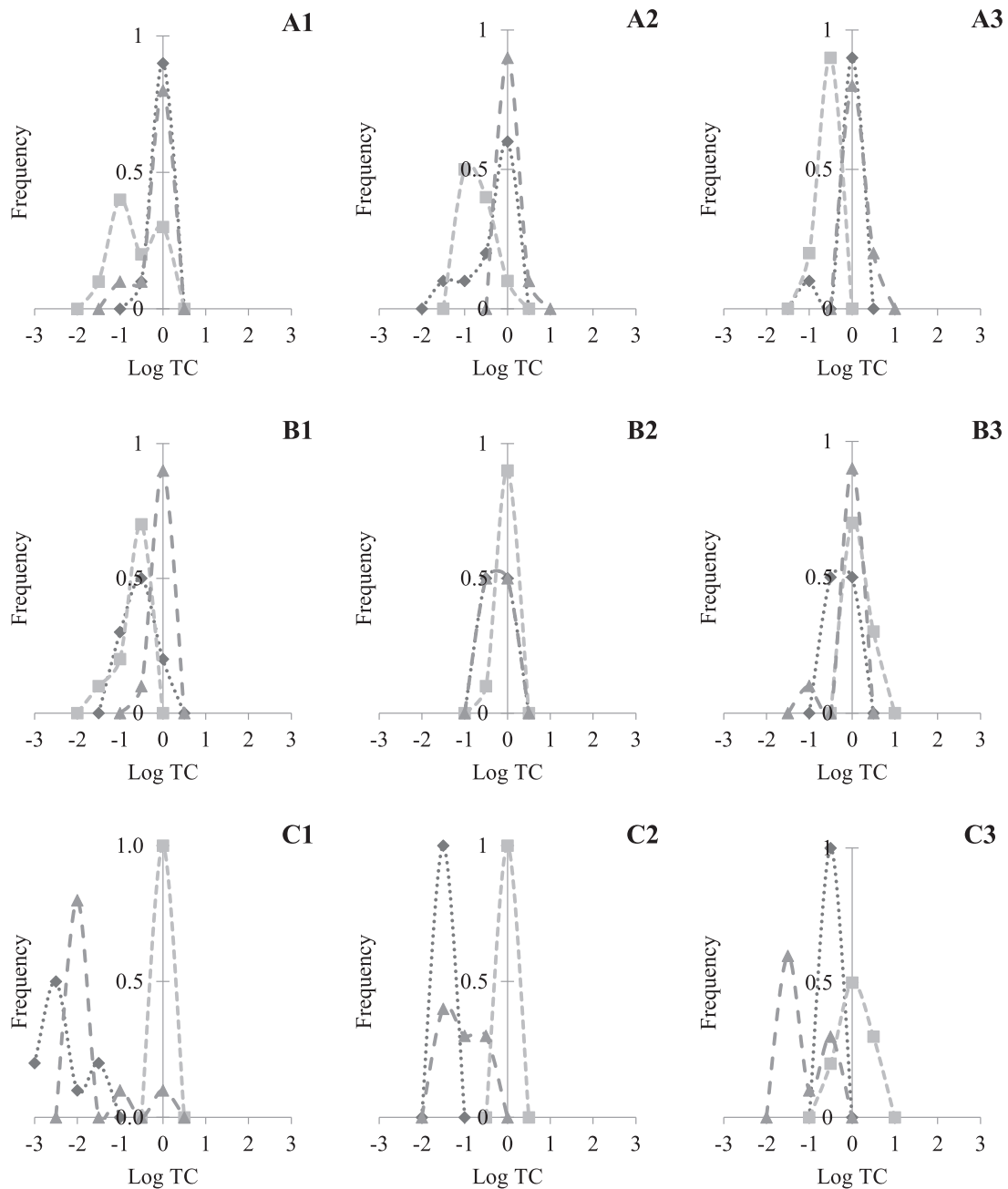


Figure 4. Distributions of transfer coefficients (TCs) from inoculated and wet (A), 1 h dried (B) and 24 h dried (C) tomatoes to new (◆) used (■) and dirty (▲) cartons in contact after inoculation (1), one (2) and seven (3) days of storage at 12°C (n = 10).

ious conditions, when evaluating the risk of reusing tomato cartons, its important to note that only those tomatoes in the contaminated box would be affected, which will result in a lower number of contaminated tomatoes, than if a field were contaminated during production or harvest, or if a risk was amplified in a packinghouse.

Salmonella transfer between tomato cartons is highly dependent on moisture, with increased levels of moisture increasing transfer. To decrease risks associated with *Salmonella* transfer during tomato harvesting and packing, the presence of moisture on tomatoes and cartons should be minimized. The presence of debris on used tomato cartons, especially when stored at cooler temperatures, increases the survival

of *Salmonella*; however, *Salmonella* can survive on tomato packing cartons for at least seven days even in the absence of debris. Reused tomato cartons, beyond being an issue for traceability, may be a source of contamination for the subsequent tomatoes packed in these cartons. Under no conditions was the transfer of *Salmonella* from new tomato cartons greater than that from used or dirty cartons. Under some conditions, dirty cartons transferred less *Salmonella* than new and used cartons; the presence of debris on used cartons does not significantly increase the risk of *Salmonella* transfer between cartons and tomatoes; however, this should not be used as an endorsement encouraging the use dirty boxes.

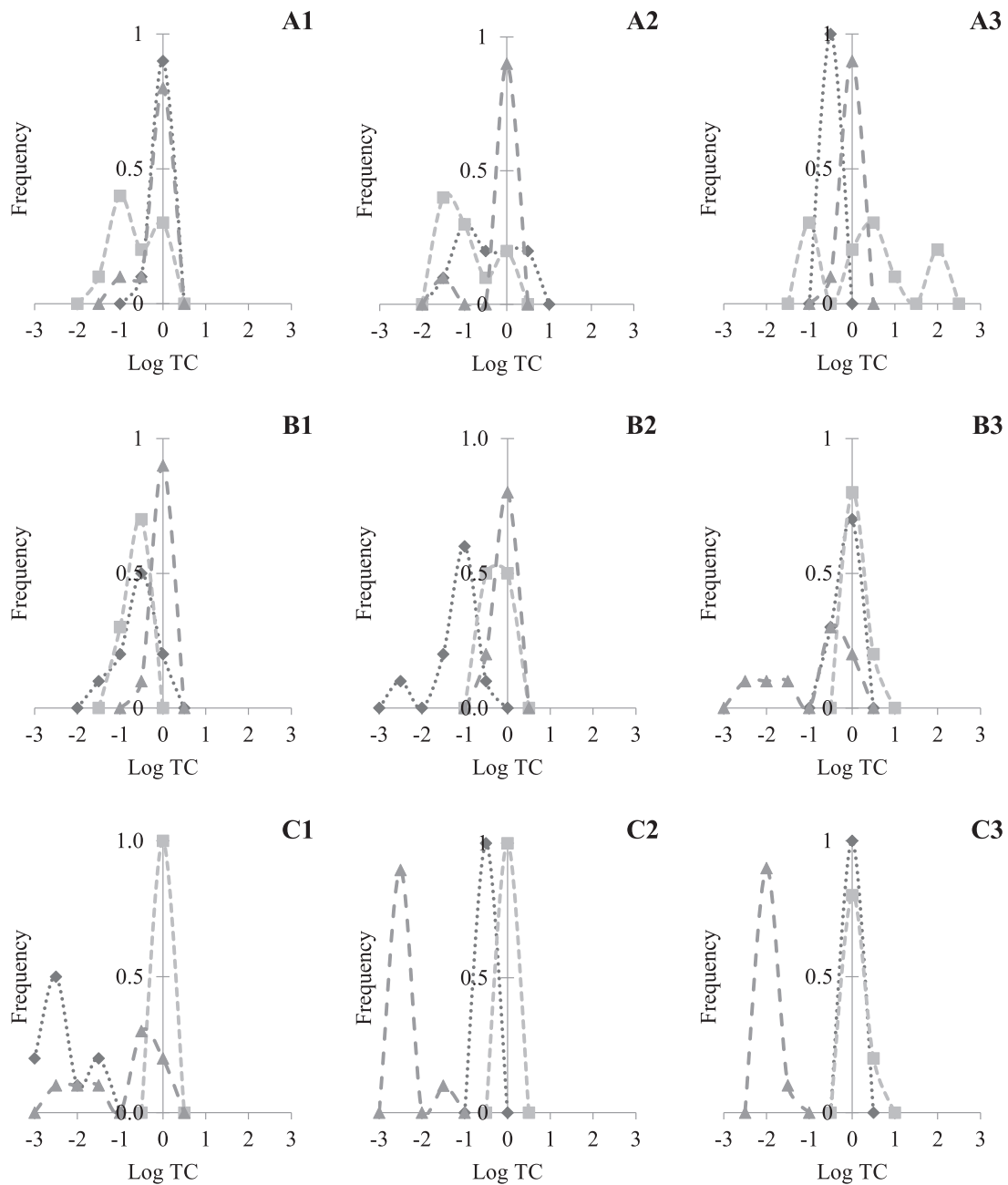


Figure 5. Distributions of transfer coefficients (TCs) from inoculated and wet (A), 1 h dried (B) and 24 h dried (C) tomatoes to new (◆) used (■) and dirty (▲) cartons in contact after inoculation (1), one (2) and seven (3) days of storage at 25°C (n = 10).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jfp.2022.11.008>.

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