CENTER for PRODUCE SAFETY

CPS 2015 RFP
FINAL PROJECT REPORT (revised August 2017)

Project Title
Evaluation of sanitizing treatments for sizer carriers in stone fruit packinghouses

Project Period
January 1, 2016 – December 31, 2016 (extended to January 31, 2017)

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Objectives

1. Evaluating natural microbial loads on fruit contact surfaces of sizer carriers.

2. Evaluating the growth potentials of foodborne pathogens on fruit sizer carriers.

3. Evaluating potential CIP sanitizing treatments for fruit sizer carriers.

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FINAL REPORT

Abstract

This study evaluated the levels of naturally occurring microflora on fruit sizer carriers of eight commercial stone fruit packinghouses in California. Also, the influence of environmental conditions on the survival of experimentally inoculated *Salmonella enterica* and *Listeria monocytogenes* on sizer carriers was investigated. Environmental swabs of 192 carriers from packinghouses indicated that routine cleaning significantly reduced total microbial and coliform counts on carrier surfaces from 3.9±0.3 to 3.2±0.3 ($P = 0.002$) and 1.5±0.4 to 0.9±0.5 ($P = 0.001$), respectively. The survival of inoculated pathogens on sizer carriers was influenced by humidity (65, 75, 85 or 95%) and temperature (22, 28, 34 or 40°C) exposure. For example, *Salmonella* and *Listeria* declined to ≤0.0 log CFU/cm^2 from ~2 log CFU/cm^2 (initial contamination level) after one day of exposure to 65% humidity at 40°C, 75% humidity at 34°C, or 85% humidity at 40°C on carriers with or without wax deposits (containing fuzz, dirt, etc.) from commercial operations. However, at 95% humidity, *Salmonella* counts persisted over 6 days at 34°C on carriers with wax deposits. When carriers were inoculated with pathogens at ~6 log CFU/cm^2, a ≥3-log reduction was achieved in 4 h under 75 or 85% humidity at 34 or 40°C. A ≥5-log lethal effect was observed in 4 h under conditions of 75% humidity and 34°C for *Salmonella*, 65% humidity and 40°C for *Listeria*, and 75% humidity and 40°C for both *Salmonella* and *Listeria*. No significant growth ($P > 0.05$) of either pathogen was observed under all experimental conditions on sizer carrier surfaces. The results suggest that both sizer carrier sanitation and certain environmental conditions (e.g., humidity and temperature combinations) can be beneficial in minimizing microbial contamination of packing lines and food-contact surfaces.

Background

Field fruit surfaces often retain 10^3–10^5 microbes/cm^2 when fruits arrive at packing facilities (Narsaiah et al., 2012; Pao and Brown, 1998). These natural microflora are common in the fruit production environment and their presence on fresh fruit does not normally represent a public health issue. Prior studies by the PI's research group have revealed that fruit packing operations, such as washing and waxing, can help to reduce fruit surface microbial load (Pao and Davis, 1999; Pao et al., 1999, 2000). However, a portion of the natural microflora will unavoidably enter and deposit, along with detached fruit waxes, over the subsequent fruit sizing and/or packing lines. Microbes can transfer between processing equipment and, in some cases, develop biofilms and other food safety concerns (Allen et al. 2005; Kang et al., 2007; Pao and Davis, 2001). However, baseline microbial load data and data on the influence of packing operations on the microbial loads on fruit sizer carriers in stone fruit packinghouses are lacking.

In modern stone fruit packinghouses, fruit sizer carriers are small rollers/holders (often made of rubber or plastic materials and supported by plastic frames) for conveying washed and waxed fruit individually through optical sorters and automatic labelers for sizing, labeling, and packing (Crisosto and Valero, 2008; Regier and Hiebert, 1993). This section of the packing line typically is kept dry to protect electrical components and is not compatible to hosing with a large amount of water. Although sizer carriers can be manually removed for cleaning, they are not designed to be removed on a frequent basis. A single, larger sizer often has thousands of carriers; carrier removal and reinstalling processes are time consuming and labor intensive. Thus, the clean-in-

place process is commonly used. The sanitation operation for carriers varies among packing facilities, with some cleaning (and possibly also some sanitizing) in place every shift.

Bacterial growth and survival on fruit surfaces can be influenced by both temperature and humidity. Iturriaga et al. (2007) found that *Salmonella* colonization on tomatoes was active during storage at 97% humidity under ambient temperatures. Furthermore, Pao et al. (2012) reported the risks associated with *Salmonella* population rebound on washed produce at 21 and 35°C in humid storage, which highlights the benefit of unbroken cold chain systems. In a study of walnut hull pieces, Blessington et al. (2014) found that storage at >40% relative humidity can promote *Salmonella* growth. The linkage between high humidity and pathogen population growth on fruit contact surfaces, however, has not been thoroughly explored.

The objectives of this study included 1) surveying the levels of naturally occurring microflora on the fruit-contact surfaces of sizer carriers in commercial stone fruit packinghouses; 2) determining the growth potential of *S. enterica* and *L. monocytogenes* on sizer carriers; and 3) investigating the potential of using humidity and temperature conditions to minimize the pathogens on sizer carriers.

Research Methods

Environmental sampling. Environmental swabs (PUR-Blue^{TM} dry swab, World Bioproducts, Bothell, WA) with phosphate buffer were used individually to sample 192 sizer carriers in eight stone fruit packinghouses in the Central Valley of California during packing operations in the fruit season (June to August 2016). In each packinghouse, 12 randomly selected carriers were swabbed on the fruit-contact surfaces (~30 cm^2) before and after routine equipment cleaning. The swabs were then transported on ice with neutralizing broth (PUR-Blue HiCap Neutralizing Broth, World Bioproducts) before plating within 8 h on petrifilm plates (3M Petrifilm, St. Paul, MN) according to manufacturer instructions.

Inoculum preparation. Four serotypes of H_2S positive *Salmonella enterica* (*S.* Enteritidis ATCC 13076, *S.* Montevideo ATCC 8387, *S.* Newport ATCC 6962, and *S.* Typhimurium ATCC 14028) and four strains of *Listeria monocytogenes* (ATCC 7644, ATCC 19115, ATCC 43256, and ATCC 51772) were maintained at 4°C on tryptic soy agar (TSA). The cultures were transferred to tryptic soy broth and incubated for 22–24 h at 35°C. The cultures were then centrifuged, re-suspended, and pooled in sterilized, deionized tap water to obtain ~8.0 and ~6.5 log CFU/ml inoculums for spot and immersion inoculations, respectively.

Carrier inoculation. Two brands of fruit sizer carriers were obtained from local equipment suppliers (Aweta Americas Inc., Fresno, CA; Compac, Visalia, CA). The same brands of carriers also were acquired after packing operations from stone fruit packinghouses (Abundant Harvest, Kingsburg, CA; Gerawan Farming, Kerman, CA) to include wax deposits (containing fuzz, dirt, etc). To determine pathogen growth potential, carriers and carriers with wax deposits were immersed in inoculum for 15 min before being air dried at room temperature (22 ± 2 °C) for 2 h, to achieve a surface contamination level of ~2.0 log CFU/cm^2 as determined by subsequent swab tests. To determine the potential lethality of environmental conditions, the fruit-contact surfaces (30 cm^2) of the sizer carriers were spot-inoculated with 1 ml of each inoculum in ~75 droplets

before being air dried at room temperature for 2 h, to achieve a surface contamination level of ~6.5 log CFU/cm^2.

Environmental treatment. For the pathogen growth study, inoculated carriers were held at 22, 28, 34 or 40°C under 65, 75, 85 or 95% humidity in environmental chambers (model 7000-10; Caron, Marietta, OH) for 1, 3 and 6 days before pathogen enumeration. For the pathogen lethality study, inoculated carriers were air dried for 2 h and then held at 34 or 40°C under 65, 75 or 85% humidity to monitor pathogen reduction for up to 2 days.

Pathogen enumeration. Sizer carriers were swabbed as described above and spread plated on TSA. The plates were incubated at 35°C for 2 h (for recovery of injured cells) before overlaying with xylose-lysine-desoxychlolate (XLD) agar for *Salmonella*, or with modified Oxford medium with antibiotics (MOX; ThermoFisher Scientific) for *Listeria* (Pao et al., 2009). Plates were further incubated at 35°C for 24 and 48 h, respectively, and then black colonies were enumerated for presumptive counts. Representative colonies were biochemically confirmed as *S. enterica* and *L. monocytogenes* using test strips (RapID™ One System, Remel, Lenexa, KS; MicroBact™ Listeria 12 L Kit, Basingstoke, UK).

Statistical analysis. Microbial counts were converted to logarithmic values for calculating means, standard errors (SE), and/or reductions. Averaged sizer carrier data (n = 12) from each of the eight packinghouses were evaluated to generate the means and SE for packinghouse microbial evaluations. Data were analyzed by paired t-test for packinghouse evaluations and three-way analysis of variance using statistical software (SigmaPlot 13.0, Systat Software, Inc., San Jose, CA) for inoculation studies, with significant difference defined at $P \leq 0.05$. For the inoculation study, three experimental replications were conducted per treatment.

Results

Sizer carrier surfaces, before cleaning, had average total microbial, yeast and mold, and coliform levels of 3.9±0.3 log CFU/cm^2, 3.2±0.4 log CFU/cm^2, and 1.5±0.4 log CFU/cm^2, respectively, during fruit packing at the eight commercial packinghouses evaluated (Figure 1). The routine cleaning procedures performed by the packinghouses significantly reduced the average total microbial (aerobes) and coliform counts on carrier surfaces to 3.2±0.3 log CFU/cm^2 ($P = 0.003$) and 0.9±0.5 log CFU/cm^2 ($P = 0.001$), respectively, but not yeast and mold counts ($P \geq 0.05$).

No growth of either *Salmonella* or *Listeria* was observed under all experimental humidity and temperature conditions on the new (unused) sizer carrier surfaces that had an initial inoculated pathogen level of ~2 log CFU/cm^2 (Table 1). Instead, the results show that both inoculated pathogens declined on the surfaces of both sizer carriers. *Salmonella* declined to ≤0.0 log CFU/cm^2 in 1 day under 75% humidity at all temperatures, and in 3 days under all experimental conditions except under 85% humidity at 40°C. Inoculated *Listeria* declined to ≤0.0 log CFU/cm^2 in 1 day under all experimental conditions, except under 95% humidity at 28 and 34°C. The recovery of *Salmonella* and *Listeria* was significantly reduced ($P \leq 0.001$) by the first day under all tested humidity (65–95%) and temperature (22–40°C) conditions for both tested sizer carriers.

No significant growth ($P > 0.05$) of either *Salmonella* or *Listeria* was observed under all experimental humidity and temperature conditions on the carriers with wax deposits that had an

initial inoculated pathogen level of ~2 log CFU/cm^2 (Table 2). The recovery of *Salmonella* and *Listeria* was significantly reduced ($P \leq 0.001$) by the first day under all tested humidity (65–95%) and temperature (22–40°C) conditions for both tested sizer carriers. *Salmonella* declined to ≤0.0 log CFU/cm^2 in 1 day under 65 or 85% humidity at 40°C or under 75% humidity at 34°C. In general, the survival of *Salmonella* was greater at 95% humidity than at all other tested humidities ($P \leq 0.001$). At 95% humidity and at all temperatures, inoculated *Salmonella* was recoverable for up to 6 days from at least one type of the sizer carrier. Inoculated *Listeria* declined to ≤0.0 log CFU/cm^2 within 1 day under 65 and 75% humidity at 28, 34 and 40°C. Temperature was a factor affecting the survival of *Listeria* on one type of the sizer carrier; pathogen survival, in general, was greater at 22°C than at all other temperatures ($P = 0.003$).

Pathogen levels were significantly influenced by the treatment time ($P \leq 0.001$), with the most obvious decline within the first 4 h of the treatment (from hour 2 to 6; $P < 0.001$) (Figure 2). All humidity (65, 75 and 85%) and temperature (34 and 40°C) combinations led to a 3-log reduction in both inoculated pathogens in 4 h, except for *Salmonella* at 65% humidity and 34°C. Statistical analysis of the overall data for Figure 2 also indicates that the survival of *Salmonella* is greater under 65% humidity than under 75% humidity ($P \leq 0.013$). The combination of 75% humidity and 40°C was highly lethal to *Salmonella* and *Listeria* on the contact surfaces of sizer carriers, and a 5-log reduction occurred in 4 h. Furthermore, 5-log reductions in 4 h were also observed for *Salmonella* under 75% humidity and 34°C, and for *Listeria* under 65% humidity and 40°C. All conditions, except the combination of 65% humidity and 34°C, resulted >5-log reductions of *Salmonella* and *Listeria* within 2 days.

Outcomes and Accomplishments

The team recruited and trained four undergraduate students, one graduate student, and one visiting scholar to support the experimental efforts. Tasks involved in the laboratory procedures included media preparation, microbial inoculation, sample preparation, plate counting, pathogen isolation, and waste sterilization. Initial tests were performed to determine the adequate pathogen inoculum concentration to reach the desired artificial contamination of sizer carriers.

The team gained access to eight stone fruit packinghouses through the California Fresh Fruit Association (CFFA), an industry collaborator of the project. At each of the packinghouses, swab samples were taken from 12 sizer carriers before cleaning and from 12 samples after cleaning. Microbial counts (including total aerobes, yeasts and molds, and coliforms) were determined using petrifilms.

The team performed an inoculation study on two types of carriers to observe the growth potential of *Salmonella enterica* and *Listeria monocytogenes* on clean and used carriers under 16 different environmental (four temperature and four humidity) conditions. Pathogen counts were performed using agar overlay methods (TSA-XLD for *Salmonella* and TSA-MOX for *Listeria*). Pathogen-resembling colonies isolated were identified to species level for confirmation.

The experimental plan for Objective 3 (Evaluating potential CIP sanitizing treatments for fruit sizer carriers) was adjusted during the project based on observations that the inoculated foodborne pathogens (*Salmonella* and *Listeria*) were incapable of multiplying on the surface of the sizer carriers. The observation prompted us to identify potential temperature and humidity combinations as treatments for pathogen reduction on sizer carriers. This modification did not change the original goal and budget of Objective 3.

The team completed all experiments, conducted data analysis, and will communicate results and conclusion to the industry partners of this study both orally and in writing. A midterm report on "Evaluation of sanitizing treatments for sizer carriers in stone fruit packinghouses" was presented orally and via poster at the Center for Produce Safety Research Symposium in Seattle, WA, on June 29, 2016.

Summary of Findings and Recommendations

Packinghouse sanitation considers the environment of the facility, equipment design, packing practices, and personnel hygiene (Yaptenco and Esguerra, 2012). Many factors, including fruit condition, equipment cleanliness, washing system, wax and fungicide application may influence or contribute to the microbial loads of sizer carriers. The results of this study show that fruit carrier surfaces of sizers in stone fruit packinghouses are susceptible to general microbial attachment. The presence of microbes on the fruit-contact surfaces of sizer carriers is not surprising since field fruit has the ability to bring natural microflora into packing and processing facilities (Pao and Davis, 2001). For example, Pao and Brown (1998) reported average aerobic plate counts of ~4.0 log CFU/cm^2 and yeast and mold counts of 3.3 log CFU/cm^2 on citrus fruit surfaces before packinghouse fruit washing. Johnston et al. (2005) reported microbial increases in cantaloupes from field through packing, with ranges of 6.4 to 7.0 log CFU/g for aerobic plate count and 2.1 to 4.3 log CFU/g for coliforms. The presence of natural microflora on fresh produce and their direct contact surfaces is unavoidable and does not normally present a food safety issue.

Results of this study (e.g., Figure 1) also show that, in general, the cleaning step for sizer carriers in Central California stone fruit packinghouses is capable of significantly reducing potential bacterial contamination introduced from stone fruit and/or handling operations. This bacterial reduction ability, if further validated and/or improved per individual packinghouse standard operation procedures, could be considered as a preventive control in practical operations for low levels of sporadic or accidental contamination of foodborne pathogens in packinghouses.

In risk assessment, all produce contact surfaces in packinghouse should be under review. For practical risk analysis it may be useful for packinghouse operators to characterize their environment, particularly near fruit handling areas, and see where they fall within the spectrum of conditions discussed in this study. Data from this study (Tables 1 and 2) revealed that the plastic and rubber surfaces of sizer carriers, with or without wax deposits from commercial packing operations, may not support the growth of *S. enterica* and *L. monocytogenes* in ambient conditions. Prior studies also demonstrated that foodborne pathogens such as *Salmonella* declined on the surfaces of plastic chopping boards (Gough and Dodd, 1998), rubber picker fingers for poultry processing (Arnold and Yates, 2009) and various tomato packing line materials (Allen et al., 2005). Similarly, Chaitiemwong et al. (2010) reported the decline of inoculated *L. monocytogenes* on a conveyor belt material.

Although no growth was found, *Salmonella* exhibited greater persistence on sizer carriers under 95% humidity. This finding corroborates previous observations related to greater survival and/or growth of surface attached foodborne pathogens under highly humid conditions (Iturriaga et al., 2007; Pao et al., 2012). The finding highlights the importance of good air ventilation and careful water usage to avoid excessive moisture accumulation in fruit packing areas.

Furthermore, this study demonstrated that moderate environmental conditions (such as 34 or 40°C in combination with 75 or 85% humidity) have the potential to minimize microbial contamination of packing lines. For example, the exposure of inoculated carriers to the combination of 75% humidity and 40°C resulted in >5-log reductions of both *Salmonella* and *Listeria* within 4 hours. This pathogen elimination phenomenon by natural conditions (without chemical intervention) merits further exploration for its practicality as a produce safety control in packinghouse operations. Currently, firms in the U.S. are required to inspect, maintain, and clean and sanitize, when necessary and appropriate, all food-contact surfaces of equipment and tools used in covered activities as frequently as reasonably necessary to protect against contamination of covered produce (FDA, 2013). Since this study shows rapid decline instead of growth for *Salmonella* and *Listeria* on sizer carriers under various experimental conditions, it would be interesting to either evaluate the necessity of having a sanitizing treatment after carrier cleaning or consider incorporating the temperature/humidity control as an appropriate sanitizing step.

In conclusion, this study confirms that cleaning is a beneficial step for reducing microbial contamination on sizer carriers in stone fruit packinghouse operations. Plastic or rubber surfaces of the carriers are unlikely to support the growth of *S. enterica* or *L. monocytogenes* under ambient conditions. Further evaluation on the influences of the ability of pathogens to adapt to environments and the presence of fruit debris on pathogen contamination of carriers would be interesting. We recommend additional research to explore the potential of applying moderate, yet lethal, temperature and humidity conditions to combat microbial contamination on produce- and food-contact surfaces.

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APPENDICES

Publications and Presentations

Pao, S. Evaluation of sanitizing treatments for sizer carriers in stone fruit packinghouses. Presented at Center for Produce Safety Research Symposium, 29 June 2016, Seattle, WA.

Budget Summary

Overall budget and spending ($)*

Items	**Budget limit**	**Spending**	**Unused**
Salary (Research Assistant)	31,200	5,630	25,570
Benefits (Research Assistant)	2,387	134	2,253
Other	2,800	20	2,780
Supplies	56,400	42,011	14,389
Travel	4,691	2,130	2,561
Indirect Costs	1,680	288	1,392
Total Program Expenditures	**99,158**	**50,213**	**48,945**

*****Reduced cost was primarily due to 1) the Graduate Assistant receiving a Scholarship Award that did not allow an additional stipend, and 2) extensive negative findings in pathogen growth studies, and 3) donation of research materials from the industry.

Tables and Figures

Table 1. Survival of artificially inoculated foodborne pathogens at varied environmental conditions on new (clean) sizer carriers obtained from two sizer carrier suppliers.

Pathogen			*Salmonella enterica* (log CFU/cm^2)				*Listeria monocytogenes* (log CFU/cm^2)			
Humidity			65%	75%	85%	95%	65%	75%	85%	95%
Sizer Carrier A	Day 0		2.2±0.4	2.2±0.4	2.2±0.4	2.2±0.4	2.1±0.5	2.1±0.5	2.1±0.5	2.1±0.5
	Day 1	40 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		34 °C	0.0±0.0	0.0±0.0	0.8±0.7	0.4±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.2
		28 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.6±1.0
		22 °C	0.3±0.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Day 3	40 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		34 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		28 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		22 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Day 6	40 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		34 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		28 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		22 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Sizer Carrier B	Day 0		2.4±0.6	2.4±0.6	2.4±0.6	2.4±0.6	2.4±0.2	2.4±0.2	2.4±0.2	2.4±0.2
	Day 1	40 °C	0.0±0.0	0.0±0.0	0.1±0.2	0.6±1.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		34 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		28 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		22 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Day 3	40 °C	0.0±0.0	0.0±0.0	0.1±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		34 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		28 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		22 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Day 6	40 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		34 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		28 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		22 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

*Data represent the means and SD of ≥3 replications.

Table 2. Survival of artificially inoculated foodborne pathogens at varied environmental conditions on used (non-cleaned) sizer carriers obtained from two stone fruit packing operations

Pathogen			*Salmonella enterica* (log CFU/cm^2)				*Listeria monocytogenes* (log CFU/cm^2)			
Humidity			65%	75%	85%	95%	65%	75%	85%	95%
Sizer Carrier A	Day 0		2.2±0.8	2.2±0.8	2.2±0.8	2.2±0.8	2.2±0.8	2.2±0.8	2.2±0.8	2.2±0.8
	Day 1	40 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.5±0.9
		34 °C	0.1±0.2	0.0±0.0	0.3±0.6	2.1±0.2	0.0±0.0	0.0±0.0	0.3±0.6	0.2±0.3
		28 °C	0.2±0.8	0.1±0.2	0.8±1.4	0.9±0.6	0.0±0.0	0.0±0.0	0.1±0.2	0.0±0.0
		22 °C	0.4±0.3	0.9±1.1	0.4±0.6	0.0±0.0	1.2±0.1	0.9±0.5	1.3±0.9	0.0±0.0
	Day 3	40 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.7±1.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		34 °C	0.0±0.0	0.0±0.0	0.0±0.0	1.2±1.1	0.0±0.0	0.0±0.0	0.0±0.0	0.5±0.9
		28 °C	0.0±0.0	0.0±0.0	0.2±0.3	1.6±1.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		22 °C	0.6±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.8±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Day 6	40 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		34 °C	0.0±0.0	0.0±0.0	0.0±0.0	2.5±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.2
		28 °C	0.0±0.0	0.0±0.0	0.4±0.8	1.9±0.8	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		22 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.2	0.0±0.0	0.0±0.0	0.0±0.0	1.3±0.2
Sizer Carrier B	Day 0		1.9±1.0	1.9±1.0	1.9±1.0	1.9±1.0	1.7±1.0	1.7±1.0	1.7±1.0	1.7±1.0
	Day 1	40 °C	0.0±0.0	0.8±1.0	0.0±0.0	1.4±1.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		34 °C	0.1±0.2	0.0±0.0	0.6±0.2	0.9±1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		28 °C	0.4±0.6	0.3±0.6	0.2±0.4	0.8±0.1	0.0±0.0	0.0±0.0	0.1±0.2	0.0±0.0
		22 °C	0.0±0.0	0.4±0.7	0.3±0.6	0.4±0.8	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Day 3	40 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.5±0.9	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		34 °C	0.0±0.0	0.0±0.0	0.0±0.0	1.6±1.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		28 °C	0.1±0.2	0.2±0.3	0.0±0.0	1.1±0.9	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.2
		22 °C	0.5±0.6	0.1±0.2	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	Day 6	40 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.9±0.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		34 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.8±1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		28 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
		22 °C	0.0±0.0	0.0±0.0	0.7±1.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

*Data represent the means and SD of ≥3 replications.

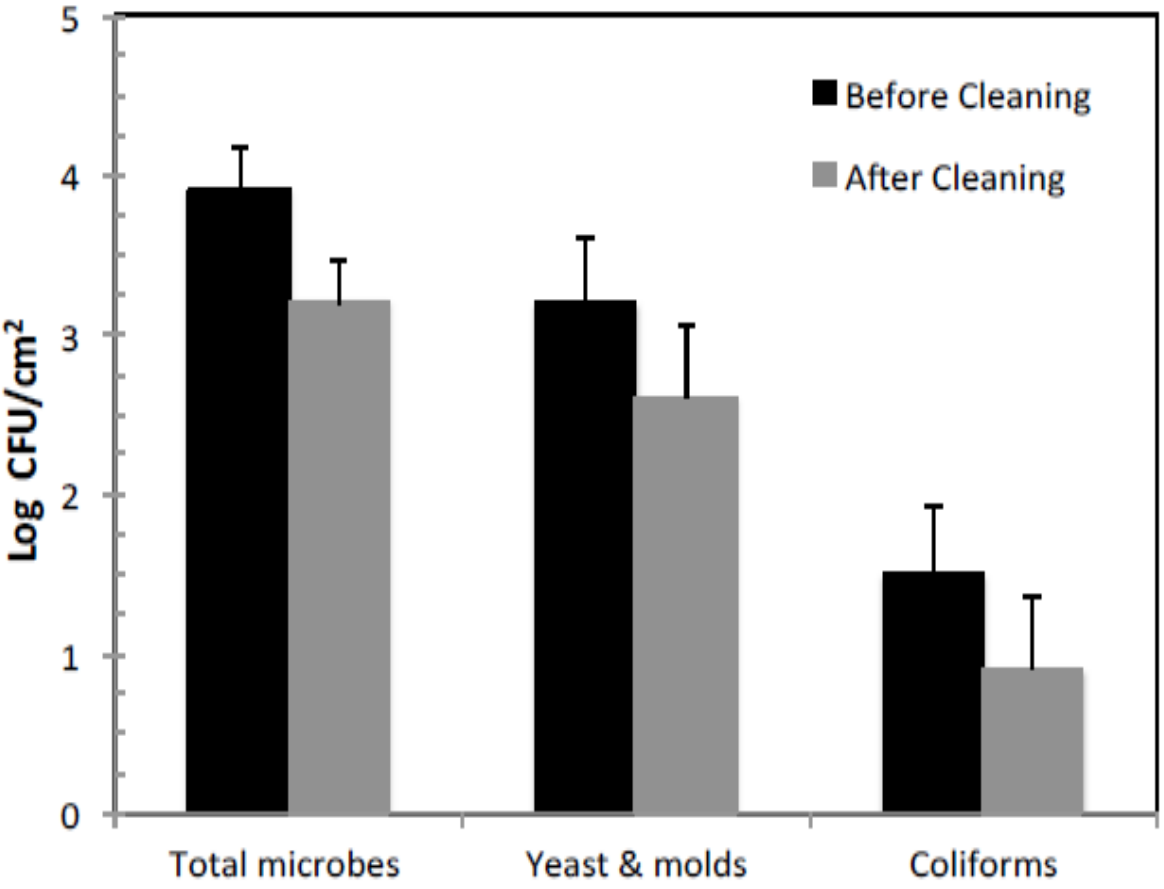

Figure 1. Microbial loads of sizer carriers before and after cleaning at stone fruit packinghouses. (Data represent the means and SE of eight packinghouse evaluations.)

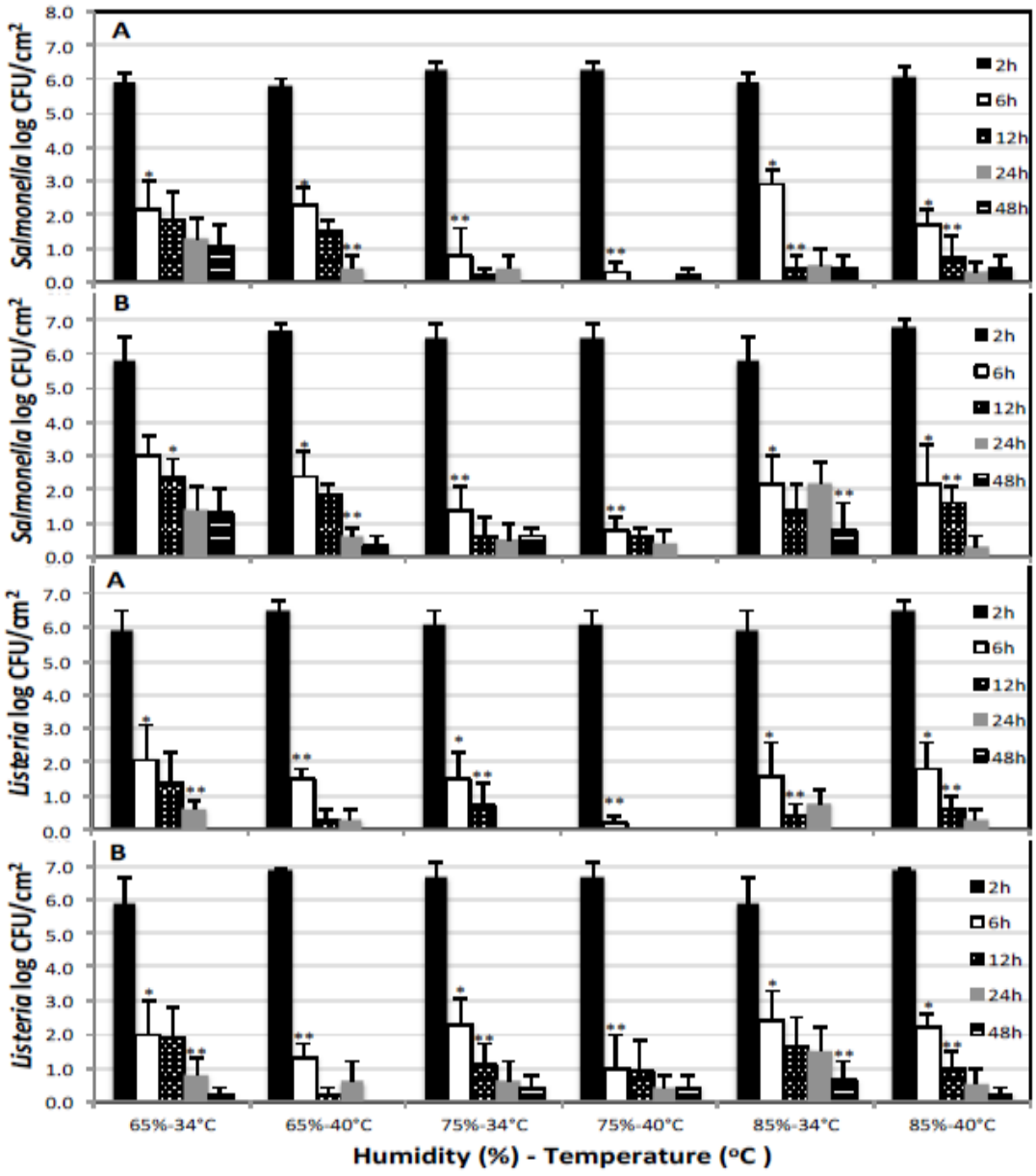

Figure 2. The influence of humidity and temperature conditions on the survival of inoculated foodborne pathogens on two brands (A and B) of sizer carriers. Bars represent the averages of log values ± SE of ≥ 3 replications. Symbols "*" and "**" indicate a 3-log and 5-log reduction, respectively, from the initial contamination measured at hour two.