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Short communication

Microbial evaluation of automated sorting systems in stone fruit packinghouses during peach packing

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ABSTRACT

Automated fruit sorting systems with individual fruit carriers are utilized in modern fruit packing facilities. This study evaluated the levels of naturally occurring microflora on the surfaces of peaches and fruit carriers during automated sorting operations at stone fruit packinghouses in California. The study also assessed the growth potential of *Salmonella enterica* and *Listeria monocytogenes* on fruit carriers under various environmental conditions. No difference of microbial loads was found on peaches (n = 420) before, during, and after fruit sorting at seven packinghouses. The average surface total microbial, coliform, and yeast and mold levels of peaches during sorting were 3.6, 2.7, and 1.9 log CFU/cm^2, respectively. Environmental swab testing indicated routine cleaning of fruit carriers (n = 192) reduced total microbes from 3.9 to 3.2 log CFU/cm^2 ($P = 0.003$) and coliforms from 1.5 to 0.9 log CFU/cm^2 ($P = 0.001$) on carriers' fruit contact surfaces. Laboratory exposures to temperature (22, 28, 34 or 40 °C) and humidity (65, 75, 85 or 95%) conditions significantly reduced inoculated *Salmonella* and *Listeria* on clean and commercially used (deposited with wax, fuzz, dirt, etc.) fruit carriers within 24 h ($P < 0.001$). The observed *Salmonella* reduction was greater on clean carriers ($P < 0.001$). On used carriers, *Salmonella* was persistent at 95% humidity and *Listeria* was persistent at 22 °C. The results showed the levels of surface microflora on peaches during fruit sorting, the reduction of microbial loads on fruit carriers due to packinghouses' cleaning, and the reduction, rather than growth, of *Salmonella* and *Listeria* under tested conditions on fruit carriers.

1. Introduction

A fruit packinghouse operation typically involves fruit receiving, washing, waxing, sorting, boxing, storage, and shipping steps (Crisosto and Valero, 2008; Yaptenco and Esguerra, 2012). Field fruit surfaces often retain 10^3–10^5 microbes/cm^2 when arriving at packing facilities (Narsaiah et al., 2012; Pao and Brown, 1998). Prior studies on citrus 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., 2000; Pao et al., 1999). However, a portion of the naturally occurring microflora will unavoidably enter and deposit along with detached fruit waxes over the subsequent sorting and packing lines. These microbes on packing equipment could potentially develop biofilms and sanitation issues (Allen et al., 2005; Kang et al., 2007). However, data regarding the influence of fruit sorting on the microflora associated with fruits (including stone fruit) and fruit contact surfaces is lacking.

In modern stone fruit packinghouses, an automated sorting system consists of electronic weight sensors, optical grader, automatic labeler, etc. for sorting and labeling prior to packing (Crisosto and Valero, 2008). Fruit carriers installed onto the sorting lines are small apparatuses (often made of rubber or plastic materials to minimize handling impact) for conveying washed and waxed fruit individually through the system (Londhe et al., 2013; Regier and Hiebert, 1993). This section of the packing line is usually kept dry to protect electrical components and is not compatible with washing by a large amount of water. There are hundreds, if not thousands, of individual carriers on one automated sorting system. The disassembling and reinstalling process of these carriers for daily cleaning would be exceedingly time and labor intensive. Thus, packinghouses typically clean carriers on the line.

Prior studies showed that the growth and survival of microorganisms on fruit surfaces could be influenced by storage temperature and humidity (Iturriaga et al., 2007; Pao et al., 2012). However, data is lacking regarding the influence of these environmental conditions

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toward microbes on the fruit contact surfaces of modern sorters. The purpose of this study is to survey the levels of naturally occurring microflora on peach and fruit carrier surfaces in automated stone fruit sorting systems. Furthermore, this study evaluated the growth potential of *S. enterica* and *L. monocytogenes* on the surfaces of individual fruit carriers under controlled temperature and humidity conditions.

2. Materials and methods

2.1. Fruit surface evaluation

The study included seven commercial stone fruit packinghouses located in California's Central Valley during the 2015 peach season from June to August. At each packinghouse, ten newly waxed, peaches (medium-size fruit with a calculated average sphere of 172 cm^2) per sample were collected (using sterile gloves and bags) in duplicate before entering the automated sorting line (after waxing), during sorting on the conveying fruit carriers, and immediately after leaving the fruit sorting system. The samples (420 fruit overall) were kept under refrigeration before testing within 4 h at the Food Science Laboratory of California State University, Fresno. One liter of buffered peptone water was added to each 10-fruit sample before shaking on a rotatory shaker at 120 rpm for 20 min. Then 1 mL aliquots of this suspension were plated using Petrifilm Aerobic, Yeast and Mold, and Coliform Count Plates (3 M, St. Paul, Mn, U.S.A.) following manufacturer's instruction.

2.2. Contact surface evaluation

Environmental swabs (PUR-Blue dry swab, World Bioproducts, Bothell, Wa, U.S.A.) with phosphate buffer were used to sample 192 fruit carriers on automated sorting systems at eight participating stone fruit packinghouses in the Central Valley during packing in 2016 peach season from June to August. At each packinghouse, the fruit contact surfaces of 12 individual fruit carriers (~30 cm^2 of plastic and/or rubber surface per carrier) on an automated sorting line were swabbed during operation breaks once before and once after routine equipment cleaning. The swabs were then transported with neutralizing broth (PUR-Blue HiCap Neutralizing Broth, World Bioproducts) under refrigeration before testing within 8 h using 3 M Petrifilm Plates as described above.

2.3. Carrier inoculum

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* (ATCC7644, ATCC19115, ATCC43256, and ATCC51772) were maintained at 4 °C on tryptic soy agar (TSA) at CSU Fresno's Food Microbiology lab. The cultures were transferred to tryptic soy broth and incubated for ~23 h at 35 °C. The cultures were then centrifuged, resuspended, and pooled in sterilized, deionized tap water to obtain ~6.5 log CFU/ml inoculums.

2.4. Carrier treatment

Clean (non-used) fruit carriers that represent the typical sorter carriers used by the stone fruit packing industry in the Central Valley of California were obtained from two local equipment suppliers (Compac, Visalia, Ca, U.S.A. and Aweta Americas Inc., Fresno, Ca, U.S.A.). The clean carriers were sanitized in hot water (80 °C) for 1 min before cooled for inoculation. Used fruit carriers with deposits (containing fuzz, dirt, wax, etc.) were obtained from two stone fruit packinghouses (Abundant Harvest, Kingsburg, Ca, U.S.A. and Gerawan Farming, Kerman, Ca, U.S.A.) after packing shifts and kept in plastic bags under refrigeration until inoculation within 18 h. The carriers were immersed in 2.5-L *Salmonella* or *Listeria* inoculum for 15 min (to ensure thorough surface contact) before air-drying under a fan at room temperature (22 ± 2 °C) for 2 h to achieve surface contamination at 2.4 ± 0.1 log CFU/cm^2 as determined by swab tests that were performed after the drying time. Inoculated carriers were held at 22, 28, 34 or 40 °C under 65, 75, 85 or 95% humidity (to cover a broad range of possible packinghouse conditions) in environmental chambers (7000-10; Caron, Marietta, Oh, U.S.A.) for 1, 3, and 6 days before pathogen enumeration. Each experimental condition was tested with three replications.

2.5. Pathogen enumeration

The fruit contact surfaces of inoculated carriers were swabbed (30 cm^2/carrier). The swabs were vortexed with neutralizing broth before spread plating on TSA. The plates were incubated at 35 °C for 2 h (to recover injured cells) before overlaying with xylose-lysine-desoxychlolate agar (XLD) for *Salmonella* or modified Oxford medium with antibiotics (MOX; TermoFisher Scientific) for *Listeria* (Pao et al., 2009). The plates were further incubated at 35 °C before resembling black colonies were enumerated after 24 and 48 h of incubation for presumptive counts. Representative colonies were biochemically confirmed as *S. enterica* and *L. monocytogenes* using test strips (RapID One System, Remel, Lenexa, Ks, U.S.A.; MicroBact Listeria 12 L Kit, Basingstoke, U.K.).

2.6. Statistical analysis

Microbial counts were converted into logarithmic values for calculating means, standard errors (SE), and log reductions. Averaged fruit and fruit carrier data from each of the packinghouses were used to generate means for the overall packinghouse evaluation. Combined data of two carrier sets (each with three replications) were used for pathogen growth and survival evaluation. Data was analyzed with significant difference defined at $P \leq 0.05$ using paired *t*-test for fruit carrier survey, one-way ANOVA for peach fruit survey, and three-way ANOVA for the carrier inoculation study by SigmaPlot (Version 13, Systat Software, Inc., San Jose, Ca, U.S.A.).

3. Results and discussion

3.1. Packinghouse microbial survey

Fig. 1 shows surface microbial loads of peaches before, during, and after sorting at seven packinghouses in California's Central Valley. The

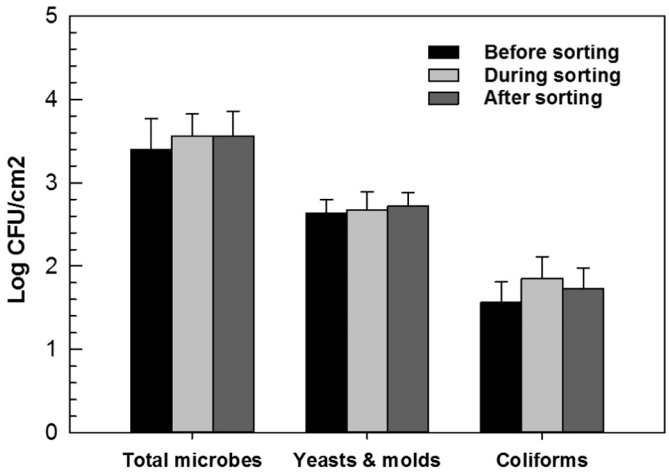

Fig. 1. Surface microbial loads of peaches before, during and after automated sorting at stone fruit packinghouses. Data represent the means and SE of seven packinghouse evaluations.

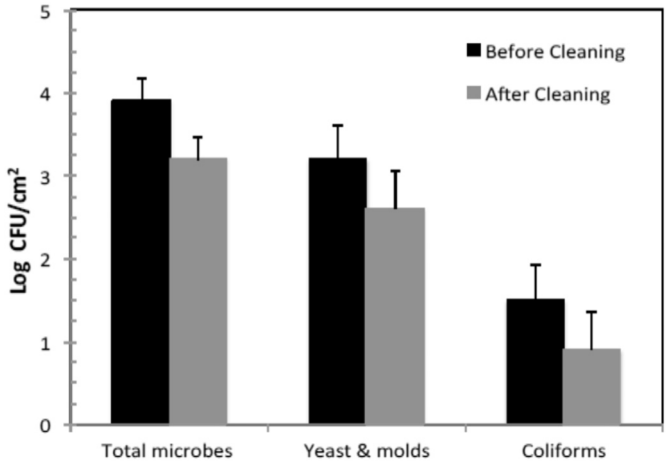

Fig. 2. Fruit contact surface microbial loads of sorter carriers before and after cleaning at stone fruit packinghouses. Data represent the means and SE of eight packinghouse evaluations.

average total microbial, yeast and mold, and coliform plate counts of peaches during fruit sorting were about 3.6 ± 0.3, 2.7 ± 0.2, and 1.9 ± 0.2 log CFU/cm^2, respectively. No difference was found among the surface microbial loads before, during or after the automated sorting step ($P > 0.05$). Fig. 2 reveals the fruit carrier surfaces had average total microbial, yeast and mold, and coliform levels respectively at 3.9 ± 0.3, 3.2 ± 0.4, and 1.5 ± 0.4 log CFU/cm^2 during stone fruit packing at eight commercial packinghouses in the Central Valley of California. The routine cleaning performed by the packinghouses apparently reduced the average total microbial and coliform counts on carrier surfaces respectively to 3.2 ± 0.3 ($P = 0.003$) and 0.9 ± 0.5 ($P = 0.001$), but not the counts of yeasts and molds ($P > 0.05$).

The presence of naturally occurring microflora on peach fruit and fruit carrier surfaces during fruit sorting is within expectation since field fruits are capable of bringing microorganisms to packing lines. Although data from prior research suggests that overall fruit packing operation could influence the surface microbial loads of packed fruit such as citrus, tomato, and cantaloupes (De et al., 2018; Johnston et al., 2005; Pao and Brown, 1998; Pao et al., 1999, 2000, 2009, 2012; Schneider et al., 2017); Yaptenco and Esguerra, 2012). This current study revealed for the first time that automatic fruit sorting systems used by the California's stone fruit industry on average neither increased nor decreased the overall fruit surface microbial loads.

The fruit carrier surfaces in stone fruit packinghouses are susceptible to microbial deposit as indicated by Fig. 2. Although there was no standardized cleaning protocol in California's stone fruit packing industry at the time of this study, the cleaning step for fruit carriers in its packinghouses, in general, was capable of reducing potential bacterial

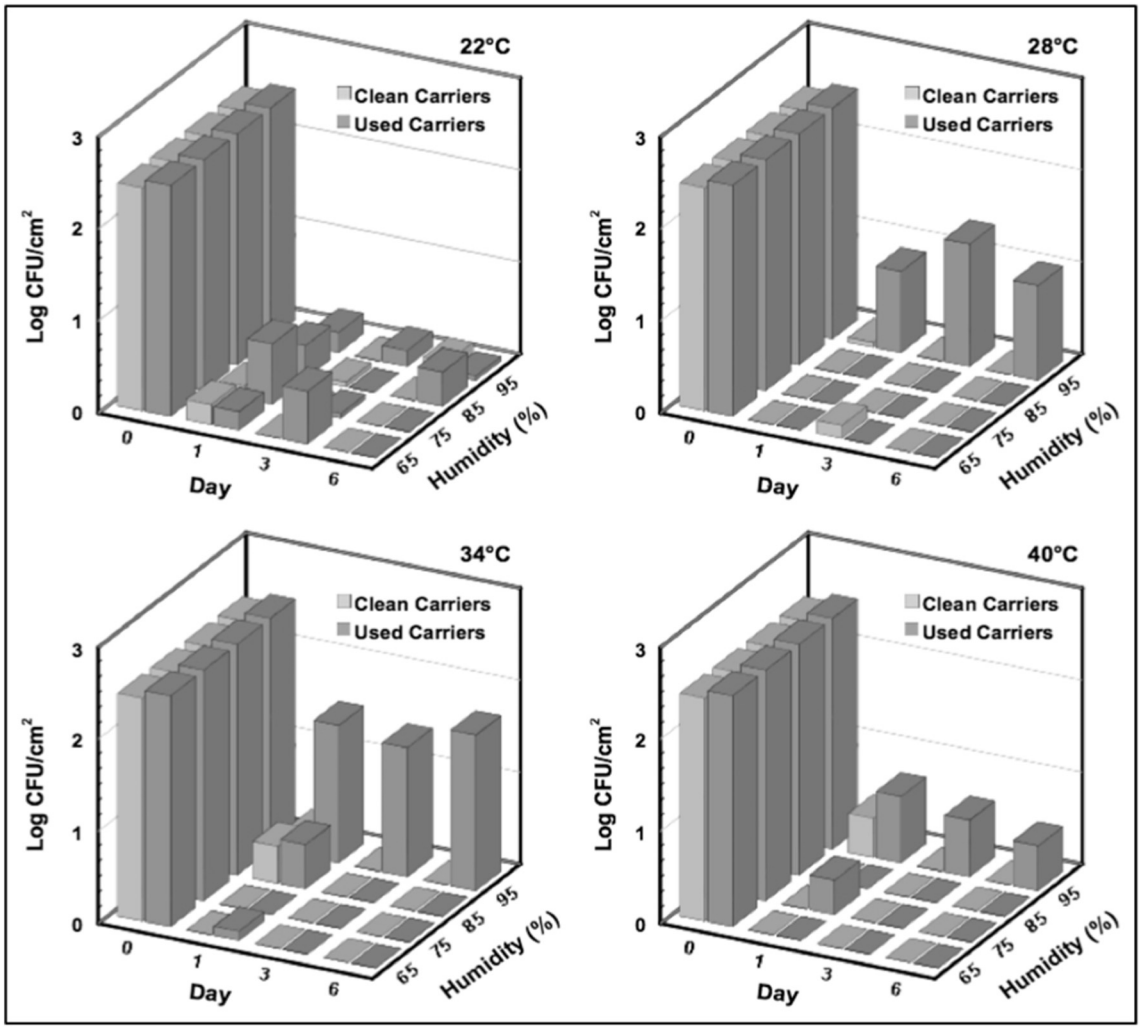

Fig. 3. Survival of inoculated *S. enterica* at varied environmental conditions on clean and used (with deposits from commercial packinghouse operations) fruit carriers.

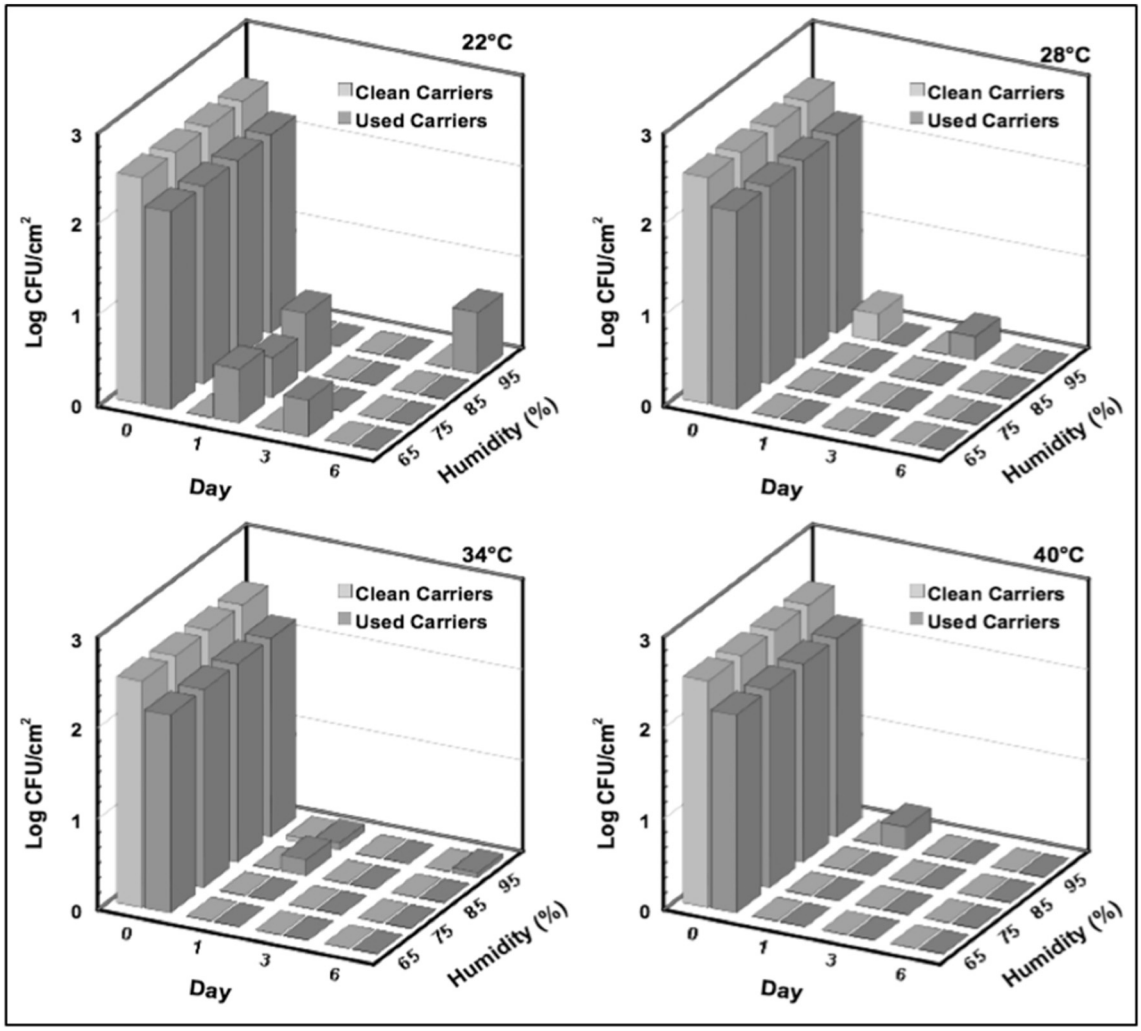

Fig. 4. Survival of inoculated *L. monocytogenes* at varied environmental conditions on clean and used (with deposits from commercial packinghouse operations) fruit carriers.

contamination introduced from stone fruit and its handling operations. This observed reduction, if further strengthened and/or validated per individual packinghouses' standard operation procedures, potentially could be considered as a preventive control measure in practical operations for low levels of sporadic or accidental microbial cross-contamination during sorting.

3.2. Carrier inoculation study

Data in Fig. 3 shows no growth of *Salmonella* under all combinations of the tested temperature and humidity conditions on fruit carrier surfaces. Instead, the inoculated *Salmonella* declined on both clean and commercially used fruit carriers within 24 h ($P < 0.001$). A ≥ 2.0 log *Salmonella* reduction was observed by day one on all clean carriers and on used carriers that were exposed to 85% at all temperatures and 65–75% humidity at ≥28 °C. The observed *Salmonella* reduction was greater on clean carriers than that of the used carriers ($P < 0.001$). For example, *Salmonella* was more persistent on used carriers at 95% humidity, failing to reach a ≥ 2.0 log reduction at 28 and 34 °C in 6 days. On used carriers, *Salmonella* also was more persistent at 95% humidity than other tested humidity conditions ($P < 0.001$).

Similarly, a decline, instead of growth, of *Listeria* was found on inoculated fruit carriers (Fig. 4). *Listeria* on fruit carriers reduced significantly on the first day under all experimental conditions ($P < 0.001$). A 2-log cycle reduction was found in 24 h on both clean and used carriers under all experimental conditions except for the used carriers at 22 °C at 65–85% humidity or 40 °C at 95% humidity. On used cleaners, *Listeria* was more persistent at 22 °C ($P < 0.01$). Under this temperature, no reduction reached 2-log cycles at 75–85% humidity until day three or at 65% humidity until day six. The environmental conditions that resulted in ≥2.0 log reductions for both pathogens on either clean or used carriers include the humidity range from 65 to 85% at temperature zone from 28 to 40 °C. In risk assessment, all produce contact surfaces in packinghouses should be under hazard evaluation (Food and Drug Administration, 2015). The findings of this study on automated sorting systems suggest that the fruit contact surfaces of individual carriers (with or without wax deposits from commercial packing operations) likely cannot support the growth of *S. enterica* and *L. monocytogenes* in the tested fruit packing conditions. Under certain temperature and humidity conditions, *S. enterica* and *L. monocytogenes* may decline naturally o-n fruit carriers without additional antimicrobial treatments. Prior research also showed that *Salmonella* declined naturally on the surfaces of plastic chopping boards, rubber picker fingers for poultry processing, and certain tomato packing line materials (Allen et al., 2005; Arnold and Yates, 2009; Gough and Dodd, 1998). Similarly, *L. monocytogenes* declined considerably on a conveyor belt material for food processing without antimicrobial additives especially at warmer temperatures (Chaitiemwong et al., 2010). Prior studies have well established the necessity of using antimicrobial chemicals in produce packing to prevent outbreaks of plant and human

pathogens (Crisosto and Valero, 2008; Food and Drug Administration, 2015; Pao et al., 2009). The reported natural declines of human pathogens under certain environmental conditions, when considered in conjunction with conventional antimicrobials, might open new research possibilities in the overall management of produce safety.

As long as the carrier surface is at equilibrium with ≤95% relative humidity, we do not expect any significant growth of *Salmonella* or *Listeria*. Although no growth was found, *Salmonella* and *Listeria* exhibited a greater persistence on used fruit carriers respectively at relatively high humidity (95%) and low temperature (22 °C) in this study. This finding corroborates with previous observations related to greater survival and/or growth of surface-attached *Salmonella* under highly humid conditions (Iturriaga et al., 2007; Pao et al., 2012) and the psychrotrophic nature of *Listeria*. Also, it is well understood that the presence of food debris could lessen microbial reduction on food contact surfaces (Chaitiemwong et al., 2010). Since both nutrients and water promote microbial proliferation, the importance of fruit carrier cleaning to prevent deposit buildup and careful water usage to avoid excessive moisture accumulation in fruit packing areas cannot be overstated. Although not tested, we speculate that damaged or wet fruit also can introduce nutrients and moisture to a sorting line.

4. Conclusion

This study showed the levels of naturally occurring surface microflora on peaches and fruit carriers at the automated sorting step in California's stone fruit packinghouses. No significant impact of automated sorting on the overall surface microbial loads of peaches was found. However, it affirms that cleaning is a beneficial step for reducing microbial loads on fruit carriers in stone fruit packinghouse operations. *S. enterica* and *L. monocytogenes* survive better respectively at 95% humidity (within tested range of 65–95% humidity) and 22 °C temperature (within tested range 22–40 °C) on fruit carriers used for automated sorting. The natural declines of foodborne pathogens under the observed environmental conditions on fruit contact surfaces, if evaluated further, might be useful in the overall packinghouse sanitation.

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