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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

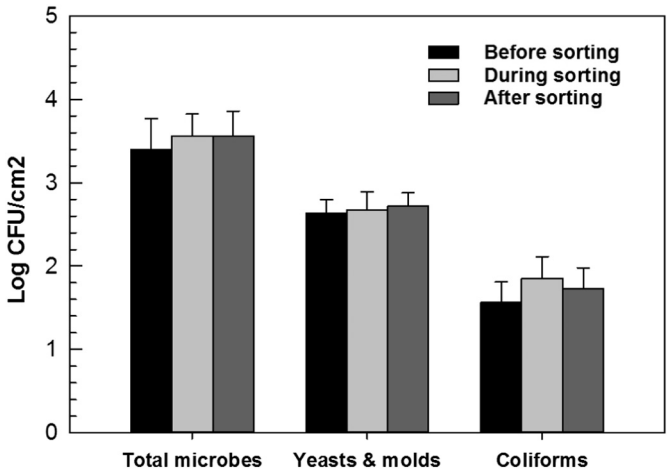

Fig. 1. Surface microbial loads of peaches before, during and after automated sorting at stone fruit packinghouses. Data represent the means and SE of seven packinghouse evaluations.

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