Evaluation of microbial loads and the effects of antimicrobial sprays in postharvest handling of California walnuts

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**Abstract**

Changes in aerobic plate count (APC) and *Escherichia coli*/coliform count (ECC) of inshell walnuts and walnut kernels were evaluated during commercial harvest and postharvest handling. APC and ECC for inshell walnuts collected from the tree were 6 and 4 log CFU/nut, respectively; counts increased by 1 log during harvest and hulling and decreased by 1 log during drying. Application of up to 200 ppm peracetic acid after hulling with or without a subsequent 2% lauric arginate spray reduced APC and ECC by less than 1 log CFU/nut; counts were not significantly different from the water control. A decrease in shell integrity was evident after drying: visible shell damage increased from 4 to 47% of walnuts after drying. Counts on kernels extracted from visibly intact walnuts from the tree were near the limit of detection (1.7 log CFU/nut). These counts increased by at least 1.4 log CFU/nut after hulling for both thin- and hard-shell cultivars. Microbial populations were 1.6–2.2 log CFU/nut higher for kernels from walnuts with broken shells than for kernels from walnuts with visibly intact shells before, but not after, drying. A better understanding of how microbial populations are affected by postharvest handling practices is important in the development of walnut-specific food safety programs.

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1. Introduction

Walnuts are generally considered a low risk for foodborne illness but recent events have led to an increased interest in the microbial safety of walnuts. An *Escherichia coli* O157:H7 outbreak was epidemiologically linked to the consumption of walnuts in 2011 (CFIA, 2011a; Health and Safety Watch, 2011). Walnuts have been recalled due to the detection of *Salmonella* (CDPH, 2010; FDA, 2010a, 2010b, 2010c, 2011), *E. coli O157:H7* (CFIA, 2011a, 2011b), and *Listeria monocytogenes* (FDA, 2009). A low prevalence of *Salmonella* (0.14%; 4 out of 2904 375-g samples) was detected in California-grown inshell walnuts collected throughout the state after the 2011, 2012, and 2013 harvests; *E. coli O157:H7* was not isolated from any of the same 2904 samples evaluated (Frelka, 2013).

English walnuts (*Juglans regia* L.) are drupes consisting of a fleshy green fruit, called the hull or husk, surrounding a hard shell encasing a seed that is commonly called the nut meat or kernel. When mature, the walnut hulls dehisce or split open, exposing the inshell nut. At maturity, walnuts are mechanically shaken from the tree. At this point the nuts may completely release from the hull or they may be fully or partially covered by the hull. The walnuts are then mechanically collected from the ground by sweeping into windrows, loaded into trailers, and transported to a huller-dehydrator (Kader and Thompson, 2002). At the huller-dehydrator, walnuts go through a number of operations to remove debris (sticks, leaves, rocks, etc.), including passage through a tank of water called the float or rock tank, which separates the floating walnuts from sinking rocks. The water in the float tank is not typically treated with antimicrobials and can contain high levels of organic material and aerobic plate counts of greater than 6 log CFU/ml (Blessington, 2011). Meyer and Vaughn (1969) correlated *E. coli*-contaminated water sampled during hulling of black walnuts (*Juglans nigra*) with a small sample of highly contaminated nuts, emphasizing the potential for cross-contamination at this step.

Immediately after the float tank, the remaining walnut hulls are removed by mechanical abrasion. Hulling is typically followed by a water rinse of the inshell nut, which is applied by spray bars over a conveyor or in a cylindrical open mesh “squirrel cage” that rotates the nuts while spraying with a pressurized water rinse to remove remaining dirt, debris, and adhering hull material. Then the nuts
are hand sorted to remove foreign material, and dried with forced air at temperatures between 32 and 43 °C to a final total moisture content (shells and kernels) of about 8% (Thompson et al., 1998). Walnuts are stored in-the-shell in bins or large silos for up to a year and are removed from storage and sorted, sized, and cracked as appropriate to yield inshell whole walnuts and kernels (halves and various sizes of pieces).

Nut kernels within an intact shell are thought to be protected from microbial contamination (Chipley and Heaton, 1971; Kajs et al., 1976; Meyer and Vaughn, 1969). However, microbial infiltration can occur even when nut shells are apparently intact (Beuchat and Heaton, 1975; Beuchat and Mann, 2010; Danyluk et al., 2008; Meyer and Vaughn, 1969). Shell breakage occurs to varying degrees during various harvest and processing steps thereby exposing the kernel within and leading to enhanced potential for contamination (Beuchat and Mann, 2010; King et al., 1970; Meyer and Vaughn, 1969).

The current study explores the impact of different postharvest handling and processing steps on changes in microbial loads of California walnuts from harvest through dehydration. Walnuts were sampled during two harvest seasons (2011 and 2012) to define microbial populations associated with walnuts during harvest, hulling, drying, and storage. Microbial loads on walnut shells and kernels were examined to determine whether harvest and/or processing operations provide opportunities for contamination of the nut meat. The microbial loads of walnut kernels from intact and broken shells were compared to assess the role of shell integrity on the potential for kernel contamination, and attempts were made to quantify shell breakage to identify operations that may increase shell breakage and, in turn, increase the potential for kernel contamination. Antimicrobial sprays were evaluated for efficacy in reducing microbial loads on inshell walnuts as a potential intervention step that could be incorporated into food safety programs at walnut hulling operations.

2. Materials and methods

2.1. Collaborating huller-dehydrators

Two different walnut huller-dehydrator facilities near Stockton, CA, owned by the same company, collaborated on this project. The facility sampled in 2011 (trial 1) was a small, pilot-scale operation (processing approx. 4500 kg/h) (Fig. 1), and the facility sampled in 2012 (trial 2) was a full-scale operation (processing approx. 45,000 kg/h). All samples were evaluated in an on-site laboratory located at the smaller huller-dehydrator facility.

2.2. Walnuts

Walnuts (J. regia L.) were collected from two commercial orchards within a 16-km radius of the laboratory and at the commercial huller-dehydrator facilities. On the day an orchard was being harvested, inshell walnuts that could be extracted easily from fruit with fully split hulls were aseptically removed directly from several trees within two adjacent rows of trees in the orchard. After the trees were shaken, walnuts that were free of hull were also collected from the ground between the same two rows of trees and again after the walnuts were swept to a center windrow. The same walnuts were followed to the huller-dehydrator after they were picked up and transported. Samples were aseptically collected with gloved hands covered in an inverted, new plastic bag. For each cultivar, corresponding samples were collected over a 3-day period: on day 1 from the trees before shaking and from the ground after shaking; on day 2 from the windrows; and on day 3 from the huller-dehydrator facility as described below.

Fig. 1. Flow diagram of commercial walnut hulling lines used in the present study. Gray arrows represent various conveyors. Locations denoted with “a” were sampled in trial 1 and those denoted with “b” were sampled in trial 2. The squirrel cage was present only in the system tested in trial 2.

In trial 1, inshell walnuts free of hull material were collected from the receiving pit, float tank, sort table, and dryer bins; walnut cultivars ‘Chandler’ and ‘Hartley’ were compared. In trial 2, samples were collected from the receiving pit, sort table, and dryer bins. To evaluate the impact of antimicrobial treatments the walnut cultivars ‘Chandler,’ ‘Howard,’ ‘Tulare,’ and ‘Vina’ were sampled but the effect of variety was not measured. The impact of shell thickness on kernel contamination was measured for ‘Chandler’ (thin shell) and ‘Hartley’ (thick shell) cultivars. Samples were retrieved from the receiving table, sort table, and dryer bins by using a sterile scoop (SterileWare, Bel-Art Products, Wayne, NJ). To retrieve walnuts from the float tank in trial 1 and after the squirrel cage in trial 2, a metal mesh strainer was used that allowed the water to drain from the sample. The strainer was sprayed with 70% ethanol and allowed to dry for 15 min between uses.

At each sampling point, enough walnuts (approximately 500–1000 g) were collected to half fill a zippered polyethylene bag (30.5 × 30.5 cm) (Bitran, Com-Pac International, Carbondale, IL). All samples were held on ice for no more than 4 h before sample preparation and plating.

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2.3. Antimicrobial treatments

Spray systems for antimicrobial treatments were installed in both hulling facilities. Four different peracetic acid (PAA) formulations were evaluated (Table 1); each differed in the relative concentrations of PAA and hydrogen peroxide ($\text{H}_2\text{O}_2$). In trial 1, BioSide HS 15% was applied at 100 ppm PAA and, in some cases, in combination with 2% CytoGuard LA (a lauric arginate ethyl ester (LAE) product); the LAE spray was applied as an additional spray immediately after the PAA spray. In trial 2, all PAA formulations were applied at both 100 and 200 ppm except for StorOx 2.0, which was only applied at 200 ppm.

PAA-containing products were applied with spray nozzles: 46500A-1-PP-VI, ProMax Clip Eyelet with QPTA6505 ProMax Quick VeelJet nozzles (Spraying Systems Co., Wheaton, IL) at a spray rate of 1.9 L/min/nozzle. In trial 1, a total of eight overhead nozzles (total flow rate = 15.2 L/min) was used over 2 m of an upward sloping metal mesh belt. In trial 2, a total of 18 overhead nozzles (total flow rate = 34.2 L/min) was used, with 13 nozzles distributed over a 2-m long mesh belt, followed by a shaker table leading to a 1-m long mesh belt with an additional five overhead nozzles. PAA dosage was controlled in trial 1 by a Dosatron water-powered dosing meter (Dosatron, Clearwater, FL), and in trial 2 by a ProMinent disinfection controller (ProMinent Fluid Controls, Inc., Pittsburgh, PA).

The LAE-containing product (CytoGuard LA, A&B Ingredients, Fairfield, NJ) was applied with a separate spray system that consisted of six Sanitary Pulsajet air atomizing spray nozzles (part number 1/4JCO-SS + SU13A-SS, Spraying Systems Co.) in two consecutive spray bars with three overhead nozzles on each. LAE spray bars were located behind the PAA spray bars and immediately before the sort table; this setup was designed to coat the walnuts with the LAE product as they tumbled off the first conveyor onto the second conveyor. Sprays were applied at 69 kPa (10 psi) liquid pressure and 140 kPa (20 psi) air pressure (flow = 2.46 L/h/nozzle = 3.26 ml/kg walnuts).

On each sampling day, water was applied through the spray system used for application of PAA but before any antimicrobial was added. Hulling equipment ran at normal speeds with walnuts for at least 30 min before the control (water sprayed) samples were collected. After switching from the water spray, antimicrobial sprays were applied for at least 15 min before test samples were collected, which allowed systems to become saturated with the PAA solutions. In all but one case, a single treatment (product and concentration combination) was tested on each sampling day. For the BioSide HS 15% in trial 2, the same product at two concentrations was applied in the same day, with water applied for least 3 h between the two treatments.

2.4. Quantifying walnut shell breakage

A scoring system was developed to quantify shell breakage in walnuts at various handling points. Samples of inshell walnuts were sorted by degree of shell breakage and classified into six categories of breakage levels from 0 to 5, with 0 representing no visible shell breakage and 5 representing the most severe breakage. A scoring guide was developed that included a description of shell breakage characteristics (Table 2). This scoring system was then used to determine the proportion of breakage for walnut samples collected in the field after tree shaking and at the receiving pit, sort table, and dryer bins. Samples were drawn by randomly filling a 9.5-L bucket with walnuts (approximately 200–300 nuts) at each sampling point.

2.5. Preparation of samples for analysis

Walnut samples were initially scored for shell breakage. Walnuts were manually divided with a sterile scoop (SterileWare) into two groups: those with visibly intact shells (breakage score 0 and 1) and those with visibly cracked, broken, or missing shells (breakage score 2–5). Microbial populations were determined for both inshell walnuts and for kernels that were aseptically removed from visibly intact or visibly cracked walnuts as described below.

Table 2
Inshell walnut breakage guide with levels 0–5 and descriptions and representative images for each breakage level.

<table>
<thead>
<tr>
<th>Breakage level</th>
<th>Examples</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>No visible breakage; suture appears tightly sealed (“intact”)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Very small cracks in the shell, kernel not visible; slight separation of suture</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Substantial cracks in the shell, no pieces of shell missing, kernel may be visible; 0–50% of suture open along circumference</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Small pieces of shell missing, kernel visible; 50% or more of suture open along circumference</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Up to 25% of shell missing; substantially loosened suture (gentle tugging will remove 25–50% of the shell)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>More than 25% of shell missing; kernel exposed; includes kernels which have completely lost the shell (loose kernels)</td>
</tr>
</tbody>
</table>

Table 1
Summary of antimicrobial products tested.

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Concentration of active ingredient (% by weight)</th>
<th>PAA $^a$</th>
<th>$\text{H}_2\text{O}_2$ $^b$</th>
<th>LAE $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>StorOx 2.0</td>
<td>BioSafe Systems Co.</td>
<td>2.0</td>
<td>27.0</td>
<td>NA $^d$</td>
<td></td>
</tr>
<tr>
<td>SaniDate 5.0</td>
<td>BioSafe Systems Co.</td>
<td>5.3</td>
<td>23.0</td>
<td>NA $^d$</td>
<td></td>
</tr>
<tr>
<td>SaniDate 12.0</td>
<td>BioSafe Systems Co.</td>
<td>12.0</td>
<td>18.5</td>
<td>NA $^d$</td>
<td></td>
</tr>
<tr>
<td>BioSide HS 15%</td>
<td>EnviroTech Modesto, CA</td>
<td>15.0</td>
<td>22.0</td>
<td>NA $^d$</td>
<td></td>
</tr>
<tr>
<td>CytoGuard LA</td>
<td>A&amp;B Ingredients Fairfield, NJ</td>
<td>NA</td>
<td>NA</td>
<td>10.0</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ PAA, peracetic acid.
$^b$ $\text{H}_2\text{O}_2$, hydrogen peroxide.
$^c$ LAE, lauric arginate ethyl ester.
$^d$ NA, not applicable.
For analysis of inshell walnuts, five randomly selected walnuts were placed into a 710-ml (24-oz) Whirl-Pak bag (Nasco, Modesto, CA) with 50 ml of Dey-Engley (DE) neutralizing buffer (Difco brand, BD, Franklin Lakes, NJ) or 0.1% peptone, and the bags were alternately shaken and rubbed for 2 min. The DE was used to inactivate the residual test antimicrobials in treated samples. Either DE or 0.1% peptone was used as the wash buffer for the water controls. There was no significant difference in recovery of microorganisms from control walnuts between peptone and DE buffer (data not shown).

Before kernels were extracted for microbial analysis, the shells of intact or visibly cracked nuts were thoroughly wiped with an alcohol wipe (70% isopropyl; Bio-Pure, Dedham, MA) to reduce the potential for cross-contamination from the shell to the kernel during cracking. Walnuts were aseptically cracked using sterile nut crackers and the kernels were extracted aseptically using sterile forceps. The nut crackers and forceps were soaked in 70% ethanol and air dried between uses. Each kernel sample consisted of nutmeats extracted from three inshell walnuts. Kernels were placed into a 200-ml (7-oz) Whirl-Pak filtering bag with 30 ml of DE or 0.1% peptone, and then the samples were homogenized for 30 s at high speed with a Smasher blender-homogenizer (AES Chemunex, Combourg, France).

2.6. Microbial analysis

Ten-fold serial dilutions of the prepared samples were made in 9 ml of Butterfield's phosphate buffer (Hardy Diagnostics, Santa Maria, CA). Aerobic plate count (APC) was determined by plating appropriate dilutions onto tryptic soy agar (TSA; Difco brand, BD) supplemented with cycloheximide (cyclo) (Acros Organics, Geel, Belgium) at 50 mg/L to reduce mold growth. *E. coli* (coliform) (ECC) counts were determined on CHROMagar ECC plates (CHROMagar, Paris, France). Plates were incubated at 37 °C for 24 h, and colonies were counted. All colonies that were visible on TSA after 24 h were included in the APC count, and all pink (coliform) and blue (presumptive *E. coli*) colonies were counted on CHROMagar ECC.

2.7. Statistical analysis

Orchard samples (from tree to dehydrator) were obtained by tracking the walnuts collected in a single orchard from harvest through to hulling and dehydration; six random samples were analyzed from the bags of walnuts collected at each point. Three samples were analyzed during each antimicrobial application (*n = 6*). In trial 2, three samples were collected after a single treatment with BioSide HS 15% at 100 and 200 ppm (*n = 3*). All statistical analyses, including analysis of variance and t-tests, were performed with the JMP 10 software package (SAS Institute, Cary, NC). Differences between means were considered significant at *P < 0.05*.

3. Results and discussion

3.1. Microbial loads on walnuts through harvest, hulling, and drying

Walnuts were sampled during two harvest seasons (2011, trial 1; 2012, trial 2) to quantify microbial populations during harvest, hulling, drying, and storage. The change in microbial loads on nut samples from the tree through hulling and drying was determined for visibly intact inshell ‘Chandler’ walnuts in trial 1. APC and ECC on inshell walnuts were 6 and 4 log CFU/nut, respectively, when collected directly from the tree before shaking (Fig. 2), which is comparable to previously reported results (Blessington, 2011). These levels generally increased by approximately 1 log CFU/nut immediately prior to the huller and sorting, and then decreased by approximately the same amount to 6 and 5 log CFU/nut, respectively, during drying. (Nuts were sampled after drying was complete, approximately 24 h after entering the dryer).

Walnuts develop inside an intact moist, green hull that is thought to protect the shell and kernel from microbial contamination (Meyer and Vaughn, 1969). Depending on maturity at harvest, the hull may remain firm, moist, and green, or it may have begun to decompose, or dry out and dehisce or split open (Blessington et al., 2014; Olson et al., 1998). Both green and brown hulls can support microbial growth especially when crushed to release cell nutrients (Blessington et al., 2014). Although freshly crushed green hulls do have antimicrobial properties, these have been observed to diminish after a 3-day period as the polyphenols oxidize (Blessington et al., 2014).

Inshell walnuts in the current study were sampled from fruit with fully split hulls. Walnuts collected directly from the tree may have been contaminated with microorganisms from the decomposing hull or from other orchard sources such as insects, animals, or aerosols. The observed increase in microbial loads after the walnuts were harvested is likely due to a combination of exposure to orchard soil and cross-contamination among nuts and from equipment during harvest and storing. The low temperature used in drying walnuts is necessary to prevent excessive oxidation of the unsaturated fat in the walnuts (Kader and Thompson, 2002). Typical drying temperatures do not exceed 43 °C. The low drying temperatures are likely a contributing factor in the minimal reductions observed after drying.

Microbial levels were also determined for kernels extracted from corresponding walnuts with visibly intact shells and, in some cases, from walnuts with visibly broken shells. Although inshell walnuts collected from the tree had APC and ECC of more than 4 log CFU/nut, the kernels aseptically extracted from these walnuts had counts that were orders of magnitude lower. APC and ECC on kernels extracted from walnuts with intact shells were 2.1 and 1.7 log CFU/nut, respectively (limit of detection [LOD] was 50 CFU/nut or 1.7 log CFU/nut). In most cases, there were fewer than 10 colonies per sample at the lowest dilution, demonstrating that it was possible to extract kernels with very low-level contamination and suggesting that the shell provides reasonable protection to the kernel from microbial contamination while the nut is on the tree.

Microbial loads on the kernels increased after the walnuts were harvested even when the shell was visibly intact. Kernel APC and ECC levels increased by 2 and 1 log CFU/nut, respectively, for walnuts with intact shells at huller receipt (after shaking, sweeping, transport, and unloading) (Fig. 2). Thereafter, levels were not significantly different (APC) or increased by about 1 log (ECC) during hulling, sorting, and drying. In contrast, APC and ECC on kernels extracted from broken shells at the sort table were 6.3 and 5.9 log CFU/nut, respectively, which was significantly higher than the corresponding levels on kernels from intact shells (Fig. 2). However, after drying, the levels of APC (5.3 or 5.6 log CFU/nut for intact and broken shells, respectively) and ECC (4.2 log CFU/nut for both) on kernels were not significantly different.

To further explore the impact of shell integrity on contamination of walnut kernels we compared cultivars ‘Hartley’ (hard shell) and ‘Chandler’ (thin shell) in trial 2. In general, APC and ECC on inshell walnuts collected from the tree in trial 2 were at least 1 log higher than observed in trial 1; counts for walnuts collected at the sort table were similar in both trials. In trial 2 the microbial levels on the shells of both ‘Hartley’ and ‘Chandler’ walnuts ranged from 5.4 to 7.9 log CFU/nut (APC) and 4.7 to 7.1 log CFU/nut (ECC). Although there was a significant difference in microbial counts between the two varieties in some cases and at some sample locations within a variety, there was no apparent trend in the data (Fig. 3).
Before hulling, the microbial levels of kernels extracted from visibly intact ‘Hartley’ and ‘Chandler’ walnuts were similar, ranging from 1.6 to 2.7 log CFU/nut (APC) and 1.3 to 2.1 log CFU/nut (ECC) (Fig. 3). APC and ECC were not significantly different among samples collected from the tree through to huller receipt (Fig. 3). APC and ECC levels on the kernels increased significantly after hulling for both walnut cultivars: on ‘Hartley’ kernels the populations increased to 3.6 and 3.1 log CFU/nut, respectively, and on ‘Chandler’ kernels the populations increased to 4.1 and 3.4 log CFU/nut, respectively. There was no significant difference between the two cultivars for either APC or ECC levels on kernels at any collection point.

Salmonella can infiltrate intact, inshell almonds in an aqueous suspension (Danyluk et al., 2008), demonstrating that the nutsheels do not provide an impervious barrier to microorganisms. Meyer and Vaughn (1969) showed that dye was able to infiltrate through apparently intact shells of black walnuts (primarily through the suture) to the kernel when nuts were briefly submerged in an aqueous environment; contamination of these walnuts with E. coli also increased with decreasing shell integrity. Likewise, water uptake (and Salmonella) into pecans increased as the amount of shell damage increased and when pecans were warmer than the water temperature (Beuchat and Mann, 2010). Pecans are conditioned to soften the shell prior to cracking by spraying with water or soaking in chlorinated water for up to 24 h. In contrast, walnuts typically pass through the float tank in a matter of seconds; thus temperature differential is less likely to play a role in infiltration into walnuts. It is possible that ingress of water (and microorganisms) for visibly intact walnuts occurred through undetectable breaks in the walnut shell or along the suture or at the stem end.

When shells are compromised, the opportunity for contamination of the kernel increases. In the current study, significantly higher microbial levels were observed on walnut kernels extracted...
from walnuts with visibly broken or missing shells. An increase in the microbial loads of nut kernels when the shell was broken or otherwise compromised has also been observed in almonds (King et al., 1970), black walnuts (Meyer and Vaughn, 1969), and pecans (Beuchat and Mann, 2010). The point at which breakage occurs will influence the potential source of microorganisms—from direct exposure to orchard soil, from float tank water, or from huller equipment.

3.2. Shell breakage during processing

Walnuts are exposed to substantial physical forces from harvest to drying. To determine the degree of shell breakage between harvesting and drying, walnut shell breakage levels were determined for inshell ‘Chandler,’ ‘Tulare,’ and ‘Vina’ walnuts sampled from the orchard floor, at huller receipt, after hulling and sorting, and after drying. ‘Chandler’ and ‘Tulare’ are thin-shell varieties, which are typically cracked and sold as kernels, and ‘Vina’ is a hard-shell variety primarily sold inshell. Walnut shells of all cultivars had lower levels of breakage when sampled at points before drying (orchard, receiving pit, and sort table) than after drying (Fig. 4). Before drying, 96–99% of ‘Chandler’ walnuts were visibly intact (breakage level 0). After drying, 23% of ‘Chandler’ walnuts were intact, 47% had minor breakage, and 30% had more significant damage (level 2–5). At receipt, 97–99% of ‘Tulare’ and ‘Vina’ walnuts were intact; after drying, 68% of ‘Tulare’ walnuts were intact, 13% had minor breakage, and 20% had more significant damage; 70% of ‘Vina’ walnuts were intact, 15% had minor breakage, and 14% had more significant damage.

Visible shell breakage increased dramatically in walnuts between receipt at the huller and collection after drying (Fig. 5), likely the result of pressure stresses on the shell as the nuts move over and through various pieces of equipment. Greater than 90% of the walnuts sampled in this study had visibly intact shells upon arrival at the huller and through the hulling process, regardless of variety or shell thickness. After drying, the proportion and degree of
breakage increased significantly, most substantially in ‘Chandler’ variety walnuts. This observation is consistent with Meyer and Vaughan (1969) who noted that dried samples of black walnuts had a higher proportion of shell breakage than wet (undried) samples. Dried shells are more brittle than undried shells, which potentially increases the opportunity for additional breakage during post-drying transport, especially for thin-shell varieties. While there is some potential for mechanical damage as the drying bins are loaded, it also is possible that invisible shell fractures present before drying become more apparent as the shell contracts during drying.

The thickness of the walnut shell differs among cultivars and also can be influenced by growing conditions or maturity at harvest. In general, thin-shell varieties, which have a shell that is easy to crack and remove, are used for kernels; hard-shell varieties, which mostly have a thicker and stronger shell, are sold in-the-shell. There was no significant difference between the microbial loads of walnut kernels from intact shells of ‘Chandler’ (thin-shell variety) and ‘Hartley’ (hard-shell variety) walnuts during harvest. However, microbial levels on ‘Chandler’ walnut kernels were significantly higher after hulling; it is possible that the higher level of shell breakage post-drying led to higher contamination rates. ‘Chandler’ walnuts account for over 40% of the total walnut acreage in California (USDA, 2014). Food safety has not been a consideration in traditional breeding programs, but the selection of varieties that are more resistant to shell breakage may offer opportunities to decrease microbial contamination of kernels. Additional evaluation of and modifications to the harvest and hulling equipment that would reduce shell breakage could provide further protection.

3.3. Effect of antimicrobials on walnut microbial loads

Given the increase in microbial counts during hulling there was interest in evaluating the application of antimicrobials at the huller as a potential means to control these populations. Peracetic acid–based sanitizers are an equilibrium mixture of PAA with H2O2 and acetic acid and various stabilizers depending on formulation. PAA is less impacted by organic load than chlorine-based sanitizers (Olmez and Kretzschmar, 2009), and the breakdown products are water and acetic acid, which reduces wastewater disposal issues since no toxic by-products are formed (Kitis, 2004).

In addition to PAA, LAE was also evaluated after consultation with sanitizer supply companies. LAE is a cationic surfactant synthesized by esterifying arginine, a positively-charged amino acid, with lauric acid, a long-chain fatty acid (Rodríguez et al., 2004). LAE has been shown previously to work synergistically with other antimicrobial agents, such as carvacrol, lactates, and diacetates, in packaged, processed, and raw meat products (Luchansky et al., 2005; Martin et al., 2009; Oladunjoye et al., 2013), but its effectiveness in produce had not been evaluated.

PAA sprays applied after the float tank were evaluated as a means to reduce microbial loads on walnut shells and kernels. Application of PAA did not significantly reduce native microbial populations on walnut shells under laboratory conditions (data not shown) or under commercial conditions.

In trial 1 the effect of a single formulation of PAA (i.e., BioSide HS 15% at 100 ppm PAA), alone and in combination with LAE, on the microbial loads of walnut shells and of kernels was evaluated. No significant difference in microbial loads was found on inshell walnuts (Fig. 6) or on kernels from either intact or broken shells, between the water-sprayed control samples and either of the antimicrobial treatments. Aerobic plate counts were 3.9 ± 0.17 log CFU/nut for kernels extracted from intact walnuts collected immediately after the float tank and 4.7 ± 0.48, 4.2 ± 0.23, or 4.6 ± 0.36 for intact walnuts collected from the sort table after being sprayed with water, 100 ppm PAA, or 100 ppm PAA +2% LAE, respectively. E. coli/coliform counts were 2.9 ± 0.33 log CFU/nut for kernels extracted from intact walnuts collected immediately after the float tank and 3.7 ± 0.77, 4.1 ± 0.26, or 3.7 ± 0.33 for intact walnuts collected from the sort table after being sprayed with water, 100 ppm PAA, or 100 ppm PAA +2% LAE, respectively.

In trial 2 the effect of different PAA products at different concentrations on microbial levels on walnut shells was compared. The highest average reduction in microbial levels observed on walnut shells was 0.9 log CFU/nut and was achieved with StorOx 2.0 at 200 ppm (Fig. 6). Greater reductions in APC and ECC were observed
when PAA formulations were applied at 200 ppm than at 100 ppm, with the exception of BioSide HS 15%, which had similar reductions at both 100 and 200 ppm. Compared with water, only StorOx 2.0 at 200 ppm resulted in a significantly higher reduction in both APC and ECC on inshell walnuts; treatment with SaniDate 5.0 at 200 ppm had a significantly higher reduction in ECC only.

The observed minimal reductions in microbial loads on walnuts after application of PAA may be the result of a number of contributing factors. The topography of walnut shells and kernels is complex and may have impacted coverage. Contact times were approximately 13 s along the conveyance system where the PAA was applied, limiting exposure. The contact time was extended as much as possible given the configuration of the collaborating hullers and the desired product throughput (the larger of which was fairly representative of the industry as a whole). Additionally, initial microbial loads were inconsistent among samples, decreasing our ability to demonstrate smaller before and after treatment differences with the number of samples evaluated.

The reported effectiveness of PAA to reduce microbial loads on whole produce surfaces varies with the study. Reductions of 2.4 log CFU/g were observed for inoculated Salmonella on inshell pecans that were submerged for 2 min in 40 ppm PAA (Beuchat et al., 2012) under laboratory conditions; a commercial application was not assessed. Wisniewsky et al. (2000) were able to achieve a 5-log reduction in inoculated E. coli O157:H7 on the surface of whole apples at 160–1280 ppm of PAA with an exposure time of at least 5 min. PAA at 500 ppm and 60 s exposure times reduced inoculated L. monocytogenes by 1.7 log CFU/g in cut lettuce.

Fig. 5. Comparison of ‘Chandler,’ ‘Tulare,’ and ‘Vina’ variety walnuts for shell breakage (according to the guide in Table 2) distribution for walnuts collected at the huller receiving pit and after drying, with the degree of breakage from none visible (lightest gray, level 0) to over one quarter missing shell and exposed kernels (black, level 5) (trial 2).
Similar reductions were seen in the natural microbiota of the lettuce, with reductions of 1.8, 2.3, and 1.4 log CFU/g in APC, yeasts and molds, and total coliforms, respectively, after 15 min of exposure to 80 ppm PAA (Beuchat et al., 2004). The current study did not find significant reductions with PAA concentrations up to 200 ppm. The concentrations used in the current study were based on recommendations from the firms supplying the chemicals and the anticipated increase in the maximum permitted usage level for PAA in fresh produce. At this time, the upper current limit for PAA use in fruit and vegetable processing is 80 ppm (CFR, 2014).

Antimicrobials often are applied to produce during postharvest handling to prevent cross-contamination rather than to impact overall microbial loads (Gil et al., 2009); the impact of PAA application on overall huller sanitation and reduction of cross-contamination was not evaluated in this study. Use of PAA in walnut hullers is also limited by the potential impact on kernel quality. The potential for PAA to enhance oxidation and off-flavor development in the kernel would need to be evaluated.

During application of the PAA sprays, a fine mist of the sanitizer was generated, which was a noted irritant for the employees working in close proximity to the spray bars. Thus measures would need to be taken to ensure employee safety and comfort before

![Fig. 6. Reductions in aerobic plate counts (A) and E. coli/colliform counts (B) on walnut shells after treatment with water or antimicrobial product containing PAA at 100 or 200 ppm (trial 2, unless otherwise noted). The black circle indicates the mean of each treatment, the center line indicates the median value, the top and bottom of the box represent the upper and lower quartiles (meaning 50% of the data points fall within the box), and the upper and lower whiskers represent the maximum and minimum values. Treatments marked with an asterisk (*) had significantly higher average reductions than water, n = 6 (except for BioSide HS 15%, trial 2: n = 3 for both concentrations).](image-url)
installation of this type of system in a walnut heller. Walnut hullers are each uniquely configured and the added cost of the equipment, water, and sanitizer would also need to be taken into account when evaluating the potential benefits of installing an antimicrobial spray system.

Despite the opportunity for microbial contamination of walnuts during hulling, foodborne pathogens are infrequently isolated from walnut kernels (Frelka, 2013). Understanding the routes of contamination will help to identify where contamination risks exist and will allow for identification and evaluation of appropriate intervention methods. Although not addressed in this study, the use of culture-independent methods to assess microbial communities on walnuts collected from the tree through to the dehydrated product might aid in identifying more specific sources of contamination. In the current study, antimicrobial sprays were not effective at reducing microbial populations on walnut shells, but the application of these sprays to control microbial loads on hulling equipment may aid in reducing the potential for cross-contamination. All these factors should be considered by walnut handlers and used to supplement existing individualized food safety programs.

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References

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