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Reduction of *Listeria monocytogenes* contamination on produce – A quantitative analysis of common liquid fresh produce wash compounds

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ABSTRACT

Contaminated produce has been identified as the cause of several listeriosis outbreaks in recent years. *Listeria monocytogenes* is widely distributed in the environment and complete prevention of produce contamination is therefore challenging. Mitigation options that reduce contamination on produce are valuable, especially for produce commodities that are commonly consumed fresh or minimally processed. We performed a systematic review and meta-analysis of the available peer-reviewed literature to evaluate the efficacy of liquid fresh produce wash compounds in reducing produce contamination with *L. monocytogenes*, and derive quantitative estimates of treatment efficacy for a variety of common liquid fresh produce wash compounds. Treatment efficacy differed considerably across produce commodities, with liquid fresh produce wash compounds generally showing considerably greater efficacy for some tested commodities than for other commodities. Most but not all of the evaluated liquid fresh produce wash compounds were significantly more effective in reducing *L. monocytogenes* contamination than water alone, with mean reductions in *L. monocytogenes* levels ranging from less than 1 $\log_{10}$ cfu to more than 5 $\log_{10}$ cfu. Liquid fresh produce wash compounds are therefore a possible tool for reducing contamination with *L. monocytogenes*, for certain produce commodities.

1. Introduction

In the United States, nearly 1600 foodborne illnesses are thought to be attributable to listeriosis every year, resulting in approximately 1500 hospitalizations and an estimated 255 deaths (Scallan et al., 2011). In recent years, produce commodities such as sprouts, celery, and cantaloupe melons have been identified as the source of listeriosis outbreaks (Anonymous, 2011; Cartwright et al., 2013; Gaul et al., 2013). *Listeria monocytogenes*, the causative agent of listeriosis, is a Gram-positive bacterium that can be widely distributed in the environment, including in soil and surface water used for agricultural purposes, wildlife feces, and pristine environments (Ivanek et al., 2009; Nightingale et al., 2004; Ryser, 1999; Strawn et al., 2013). During a recent survey of produce farm environments in the state of New York, *L. monocytogenes* was isolated from 15% of environmental samples, emphasizing the prevalence of this pathogen on produce fields (Strawn et al., 2013).

The prevalence and concentration of *L. monocytogenes* on produce commodities is difficult to assess because only limited surveys have been conducted to date. Various factors such as seasonality, climate, soil type or geographic region can impact the abundance of *L. monocytogenes* in the environment (Ivanek, Groehn, & Wiedmann, 2006) and may therefore impact the prevalence and concentration of *L. monocytogenes* on produce. Prevalence estimates reported in the literature for produce grown in the United States range from 0% to more than 35%, although the estimates are often based on small sample sizes which leads to imprecise estimates (Beuchat, 1996; Johnston et al., 2005; Johnston et al., 2006; Phillips & Harrison, 2005; Prazak, Murano, Mercado, & Acuff, 2002; Samadpour et al., 2004; Thunberg, Tran, Bennett, Matthews, & Belay, 2002).

Numerous chemical, physical, or biological control measures may be applied during produce processing and storage to reduce *L. monocytogenes* contamination (Parish et al., 2003). Some physical control measures including irradiation or pulsed UV light as well as certain biological means of control such as the use of bacteriophages or biocontrol agents have achieved reductions in experimental studies using *L. monocytogenes* (or other bacteria that may

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Table 1
Efficacy range of selected liquid fresh produce wash compounds (i.e., as aqueous solutions) used to reduce contamination with *L. monocytogenes* on agricultural commodities, including their regulatory considerations in the United States (list of regulatory considerations not necessarily comprehensive).

Fresh produce wash compound	Range of reductions in *L. monocytogenes* concentrations on produce reported in the available literature[a] (note that conditions in experimental studies do not equal conditions authorized for use in the U.S.)	Selected literature references (based on experimental studies; no distinction between 'raw agricultural commodities' and 'processed fresh produce')	Regulatory considerations in the United States regarding use on	
			Raw agricultural commodities	Processed fresh produce[c]
Water	~0–3 $\log_{10}$ cfu	(Akbas & Olmez, 2007; et al., 2002; Bari Bari, Sabina, Isobe, Uemura, & Isshiki, 2003; Bari, Nakuama, et al., 2005; Bari, Ukuku, et al., 2005; Beuchat, Adler, & Lang, 2004; Beuchat, Nail, Adler, & Clavero, 1998; Burnet, Iturriaga, Escartin, Pettigrew, & Beuchat, 2004; Deza, Araujo, & Garrido, 2003; Han et al., 2001; Hellstrom, Kervinen, Lyly, Ahvenainen-Rantala, & Korkeala, 2006; Kim et al., 2008; Lang, Harris, & Beuchat, 2004a, 2004b; Rodgers, Cash, Siddiq, & Ryser, 2004; Ruiz-Cruz, Acedo-Félix, Díaz-Cinco, Islas-Osuna, & González-Aguilar, 2007; Sagong et al., 2011; Stopforth, Mai, Kottapalli, & Samadpour, 2008; Su & D'Souza, 2012; Udompijitkul, Daeschel, & Zhao, 2007; Venkitanarayanan, Lin, Bailey, & Doyle, 2002; Wade et al., 2003; Walter, Nascimento, & Kuaye, 2009; Wei, Wolf, & Hammes, 2005; Yuk et al., 2006; Zhang & Farber, 1996)		Generally recognized as safe (GRAS)
Chlorine-containing compounds[b]	~0–6 $\log_{10}$ cfu	(Akbas & Olmez, 2007; Baert et al., 2009; Bari, Inatsu, Kawasaki, Nazuka, & Isshiki, 2002; Bari et al., 2003; Beuchat et al., 2004; Beuchat & Brackett, 1990; Beuchat et al., 1998; Burnett et al., 2004; Delaquis, Stewart, Cazaux, & Toivonen, 2002; Deza et al., 2003; Francis & O'Beirne, 2002; Hellstrom et al., 2006; Lang et al., 2004a, 2004b; Lee, Yun, Fellman, & Kang, 2002; Li, Brackett, Chen, & Beuchat, 2002; Rodgers et al., 2004; Ruiz-Cruz et al., 2007; Stopforth et al., 2008; Su & D'Souza, 2012; Szabo, Simons, Coventry, & Cole, 2003; Udompijitkul et al., 2007; Walter et al., 2009; Wei et al., 2005; Zhang & Farber, 1996)	Potassium hypochlorite is exempted from the requirement of a tolerance in or on all commodities (40 CFR 180.1300) Calcium hypochlorite is exempt from the requirement of a tolerance when used preharvest or postharvest in solution on all raw agricultural commodities and on grape when used as a fumigant postharvest by means of a chlorine generator pad (40 CFR 180.1054) Sodium hypochlorite is exempted from the requirement of a tolerance for residues and when used in accordance with good agricultural practices as a seed-soak treatment in the growing of the raw agricultural commodities vegetables, brassica, leafy , group 5 and radish, roots and radish, tops (40 CFR 180.1235; 40 CFR 180.1070)	Not all substances with chlorine are authorized as antimicrobials in the U.S. Calcium hypochlorite is authorized as food contact substance for use as an antimicrobial agent in water used to process fruits, vegetables, nuts, meat, and poultry and in pretreatment of water used in the manufacture of beverages subsequently bottled for drinking, if added at levels (as CaOCl2 or measured as free chlorine) not to exceed levels specified in the FCN (FCN No. 1023).[d]
Chlorine dioxide	~0–6 $\log_{10}$ cfu	(Han et al., 2001; Kim et al., 2008; Rodgers et al., 2004; Wu & Kim, 2007; Zhang & Farber, 1996)	Chlorine gas is exempted from the requirement of a tolerance when used preharvest or postharvest in solutions on all raw agricultural commodities (40 CFR 180.1095).	Under certain conditions, chlorine dioxide is authorized as an antimicrobial agent in water used to wash fruits and vegetables that are not raw agricultural commodities in an amount not to exceed 3 ppm residual chlorine and that will subsequently be rinsed with potable water or undergo blanching, cooking, or canning (21 CFR 173.300) Chorine dioxide is authorized as food contact substance for use as an antimicrobial in water used to wash fruits and vegetables that are not raw agricultural commodities (e.g., FCN Nos. 1011, 1123, 1137, 1158)[d] and for the fumigation of fruits and vegetables (i.e. FCN No. 949)[d] if used at concentrations not exceeding prescribed levels.

(*continued on next page*)

Table 1 (*continued*)

Fresh produce wash compound	Range of reductions in *L. monocytogenes* concentrations on produce reported in the available literature[a] (note that conditions in experimental studies do not equal conditions authorized for use in the U.S.)	Selected literature references (based on experimental studies; no distinction between 'raw agricultural commodities' and 'processed fresh produce')	Regulatory considerations in the United States regarding use on	
			Raw agricultural commodities	Processed fresh produce[c]
Organic acids[b] (e.g., acetic acid, citric acid, lactic acid, etc.)	~0–6 $\log_{10}$ cfu	(Akbas & Olmez, 2007; Choi et al., 2012; Hellstrom et al., 2006; Kim, Kim, & Song, 2009a, 2009b; Lee et al., 2002; Park, et al., 2011; Rodgers et al., 2004; Ruiz-Cruz et al., 2007; Sagong et al., 2011; Szabo et al., 2003; Yuk, Yoo, Yoon, Marshall, & Oh, 2007; Yuk et al., 2006; Zhang & Farber, 1996)	Several organic acids are excluded from the requirement of a tolerance if used in accordance with good agricultural or manufacturing practices (FFDCA section 408; 40 CFR 180.950)	Not all substances with organic acids are authorized as antimicrobials in processed foods. Below are some selected examples: Citric acid is a direct food ingredient affirmed as generally recognized as safe (GRAS), and shall meet the identity, limitations, and specifications described in this authorization (21 CFR 184.1033). Lactic acid is a direct food ingredient affirmed as generally recognized as safe (GRAS) as an antimicrobial agent, and shall meet the identity, limitations, and specifications described in this authorization (21 CFR 184.1061). Acetic acid, as part of a mixture with peroxyacetic acid, and other substances, is authorized as food contact substance for use as antimicrobial in wash and chilling water used in the processing of fruits and vegetables

serve as surrogates in the absence of data for *L. monocytogenes*) , (Allende et al., 2007; Bari, Nakuama, et al., 2005; Bari, Ukuku, et al., 2005; Bialka & Demirci, 2008; Caillet, Millette, Turgis, Salamieri, & Lacroix, 2006; Critzer, Kelly-Wintenberg, South, & Golden, 2007; Dhokane, Hajare, Shashidhar, Sharma, & Bandekar, 2006; Fan, Niemira, & Prakash., 2008; Fan, Sokorai, Sommers, Niemira, & Mattheis, 2005; Kim et al., 2006; Leverentz et al., 2003; Leverentz, Conway, Janisiewicz, & Camp, 2004; Mahmoud, Bachman, & Linton, 2010; Niemira, 2003; Niemira, 2005; Randazzo, Pitino, Scifò, & Caggia, 2009; Schuenzel & Harrison, 2002; Trias, Badosa, Montesinos, & Bañeras, 2008; Waje et al., 2009; Yaun, Sumner, Eifert, & Marcy, 2004; Young Lee et al., 2006). Liquid fresh produce wash or rinse compounds (henceforth simply referred to as 'produce washes') with chemical compounds such as chlorine or chlorine dioxide are the traditional methods of reducing microbial populations on produce, and have therefore received the most attention to date (Parish et al., 2003).

A variety of factors can impact the efficacy of liquid produce washes including water temperature, contact time, and concentration of the active compound (Parish et al., 2003). Produce washes are generally less effective on damaged or cut produce than on intact produce. Contributing factors likely include limited accessibility of bacteria to washes in damaged areas, changes in produce surface characteristics that may favor bacterial adherence, and increased protein loads due to the liberation of proteins from the damaged produce cells; moreover, produce washes are likely ineffective if bacteria have been internalized into fruits or vegetables (Han, Linton, Nielsen, & Nelson, 2001; Sapers, 2001). Produce washes also appear more effective on certain produce commodities than on others (Parish et al., 2003). Efficacy is hampered if contamination occurs in inaccessible parts of the fruit or vegetable such as the stem scar (Sapers, 2001). The efficacy of a given produce wash may therefore differ considerably among produce commodities, application protocols, and exact application conditions (Table 1). Bacterial population densities after wash treatments and subsequent storage may be comparable to those observed on untreated produce, even though certain washes may exert residual treatment effects, and *L. monocytogenes* can grow on certain produce commodities during storage (Hoelzer, Pouillot, & Dennis, 2012).

The objectives of this study were to analyze data on the efficacy of different produce washes in reducing contamination with *L. monocytogenes*, and to derive quantitative estimates of treatment efficacy for a variety of produce commodities and various common produce washes. The study focused exclusively on reductions achieved by liquid produce washes immediately after treatment and did not consider potential growth during produce storage subsequent to treatment. It is important to note, regulatory considerations may limit the use conditions or prohibit the use of certain produce washes reviewed here (see for instance the Code of Federal Regulations (CFR) for regulatory considerations in the United States). The evaluations performed in this manuscript do not take into account the regulatory status of the produce washes, since the data were derived based on laboratory experiments. However, it is important to note that in the United States the Environmental Protection Agency (EPA) and U.S. Food and Drug Administration (FDA) have shared jurisdiction of these antimicrobial solutions, with each agency having regulatory authority for different uses of these antimicrobial solutions. Antimicrobial substances used to wash processed agricultural commodities are regulated by FDA as food additives under FFDCA §409 and regulated by EPA as pesticides under FIFRA. Antimicrobial substances used to wash raw agricultural commodities may fall under jurisdiction of the EPA as 'pesticide chemicals'. Some considerations regarding authorizations for use on produce in the United States are addressed in Table 1. The insights and quantitative estimates generated in this study will be

useful for risk assessments and will aid in decisions about how best to incorporate produce washes in the farm-to-fork continuum.

2. Material and methods

2.1. Literature review and data extraction

All literature searches were performed in January 2013 using the 'Google scholar' search engine and the search terms '*Listeria* produce wash', '*Listeria* produce water washing', '*Listeria* produce chlorine', '*Listeria* produce ozone', '*Listeria* produce peroxide', or '*Listeria* produce nisin pediocin'. These searches were complemented by cross-referencing in the evaluated studies. The abstracts (or full text articles if necessary to reach a final determination) of all studies detected using this method were evaluated to determine that i) the study evaluated disinfection on produce, ii) the study was conducted using *L. monocytogenes*, iii) the study evaluated liquid produce washes, iv) the study was a peer-reviewed, primary research study, and v) the study evaluated quantitative reductions in bacterial concentration. The full texts of all articles that meet these criteria were subsequently screened to determine whether the studies met the inclusion criteria: i) data presented in tabular form; ii) evaluation of wash efficacy; iii) efficacy evaluated immediately after treatment. For each study that met the inclusion criteria the following data were manually extracted (if available) and recorded in an Excel (Microsoft, Redmond, WA) spreadsheet: i) reference; ii) chemical compound; iii) concentration of the chemical compound; iv) produce commodity; v) contact time; vi) contact temperature; vii) inoculum concentration [$\log_{10}$ cfu] per gram or cm^2; viii) mean surviving concentration [$\log_{10}$ cfu]; ix) standard deviation of surviving concentration [$\log_{10}$ cfu]; x) mean reduction [$\log_{10}$ cfu]; xi) measurement unit; xii) weight of the analytical unit. Studies differed considerably in the method of wash application, with study protocols including applications as sprays, dips, and washes with varying amounts of manual or mechanical agitation. Due to the great variability in experimental conditions and the limited means of standardized comparison across studies a meaningful analysis of the impact of the application method was deemed impossible.

To obtain conservative estimates, the lower limit of detection was used for left censored observations (i.e., data points for which the exact numerical value is not known but the value is known to be below the lower limit of detection) of surviving *L. monocytogenes* concentrations. None of the observations was right censored by exceeding the upper limit of quantification. To simplify the analysis, combination treatments were classified by their primary active ingredient unless the combination treatment included non-chemical treatments (e.g., pulsed UV light), in which case the respective observations were excluded from the analysis. In cases where mean reductions in bacterial contamination were not provided in the primary research study, these were manually calculated. This data extraction strategy resulted in a dataset with 597 observations extracted from 30 publications. Data scarcity or sub-optimal reporting prevented in-depth evaluation of the impact of compound concentration, contact time, inoculum concentration, and of the variability in the surviving bacterial concentrations across experimental replicates, and in most studies produce washes were evaluated at temperatures around 20 °C–25 °C, thus preventing a meaningful analysis of treatment temperature effects.

2.2. Evaluation of the impact of produce commodity and chemical compound on mean reduction in L. monocytogenes concentration

To generate overall estimates of treatment efficacy for different produce washes and commodities, a fixed-effect general linear model (GLM) was identified as the most adequate model given the specific interest in the identified washes and produce commodities, and the limited number of studies available for most wash-commodity pairs (Borenstein, Hedges, Hiigins, & Rothstein, 2009; Ott & Longnecker, 2001). This model was fit to the complete data set using the Genmod procedure in SAS v. 9.3 (SAS Institute Inc, Cary, NC) with 'produce wash' and 'produce commodity' as predictors. This linear model was used to estimate Least Squares (LS) means for each wash and produce commodity. The absence or scarcity of data for certain wash-commodity combinations unfortunately precluded further in-depth analysis of this complete data set, including analysis of the potential impact of inoculum concentration.

To evaluate the potential impact of inoculum concentration, we created a reduced dataset with $n = 486$ observation (full dataset: $n = 597$ observations) that only included those observations for which clearly defined initial inoculum concentrations were available. This data set was used to build a second linear model with the predictors 'produce wash, 'produce commodity', and 'inoculum concentration'. This reduced dataset generated very similar LS mean estimates as the full data set, prompting us to utilize all available data for subsequent subgroup analyses, regardless of whether or not the initial inoculum concentration was known.

For 11 different produce washes as well as water, data on mean reductions in *L. monocytogenes* concentration were available on apples and lettuce, leading to a reduced dataset with 294 observations. This data set was used to evaluate whether chemical produce washes were more effective in reducing *L. monocytogenes* concentrations on produce than washes with water alone, and to evaluate whether efficacy differed by commodity. A general linear model was fit to this reduced dataset. 'Produce commodity', 'produce wash', and 'reference', as well as the interaction term between 'produce commodity' and 'produce wash' were initially evaluated as potential predictors in the model, with the best-fitting model identified using the deviance statistic, likelihood ratio test (i.e., for nested models) and Aikaike Information Criterion (AIC, for non-nested models). Goodness of fit for the final model, containing the predictors 'produce commodity' and 'produce wash', was evaluated visually by inspection of residuals, Cook's D and DFBETAS (as described in the program documentation for SAS v. 9.3, SAS Institute Inc, Cary, NC). LS means and linear contrasts were estimated to determine the impact of produce type and wash. To minimize the number of comparisons, all produce washes were compared in efficacy to water only. All comparisons were pre-specified and adjustments for multiple comparisons were not used.

2.3. Estimation of mean reductions in L. monocytogenes concentration on produce

Because wash efficacy clearly differed by produce commodity, mean reductions in *L. monocytogenes* concentration were estimated separately for produce commodities with sufficient numbers of available observations for multiple washes to permit such analysis (i.e., at least 20 observations including at least 2 different washes). A separate analysis of produce wash efficacy was deemed possible for 6 commodities, but not for the remaining produce commodities due either to a low total number of available observations or because only a single wash type was evaluated. For each produce commodity, a separate general linear model with the predictor 'produce wash' was fit to the data as described above, and LS means and linear contrasts were estimated as described above. Goodness of fit was evaluated for each model as described above.

3. Results

Our literature search identified 30 peer-reviewed articles that met the inclusion criteria (see Table 2 for references).

Table 2
Efficacy of various sanitizers (i.e., as aqueous solution) in reducing contamination with *L. monocytogenes* on different produce commodities (based on either the full data set with $n = 597$ observations that did not consider inoculum concentration or based on a reduced data set with $n = 486$ observations that was restricted to observations for which inoculum concentrations were available and that considered inoculum concentration).[a]

Sanitizer	Number of observations (studies)	Mean reduction [$\log_{10}$ cfu] (Std. error)		Commodity	Number of observations (studies)	Mean reduction [$\log_{10}$ cfu] (Std. error)	
		Full data	Reduced data			Full data	Reduced data
Electrolyzed water	7 (4)	2.00 (0.41)	2.23 (0.35)	Apples	67 (4)	2.66 (0.16)	2.82 (0.14)
FIT	2 (1)	1.06 (0.73)	0.78 (0.63)	Blueberries	40 (1)	0.47 (0.26)	n/a
Acetic acid	22 (2)	1.34 (0.25)	1.35 (0.22)	Broccoli	28 (1)	2.46 (0.29)	2.43 (0.25)
Ascorbic acid	6 (1)	1.08 (0.44)	1.15 (0.37)	Cabbage	28 (1)	3.01 (0.29)	2.94 (0.25)
Calcinated calcium	4 (1)	5.03 (0.54)	4.88 (0.47)	Cantaloupe	7 (1)	4.07 (0.40)	3.63 (0.35)
Chlorine	75 (16)	1.71 (0.16)	1.93 (0.14)	Carrots	8 (1)	0.81 (0.39)	1.15 (0.34)
Chlorine dioxide	70 (7)	3.32 (0.20)	3.28 (0.19)	Green peppers	8 (1)	1.71 (0.38)	1.31 (0.34)
Chlorous acid	3 (2)	3.73 (0.63)	3.91 (0.54)	Leafy greens	10 (1)	2.52 (0.36)	2.31 (0.31)
Citric acid	43 (4)	1.68 (0.20)	1.65 (0.21)	Lettuce	237 (13)	2.17 (0.11)	2.17 (0.10)
Fumaric acid	7 (2)	3.41 (0.42)	3.24 (0.37)	Oranges	1 (1)	3.38 (1.06)	3.58 (0.91)
Hydrogen peroxide	11 (2)	3.01 (0.32)	2.92 (0.28)	Parsley	12 (1)	2.97 (0.32)	2.44 (0.29)
Lactic acid	43 (4)	1.89 (0.20)	1.74 (0.21)	Spinach	16 (1)	1.47 (0.31)	1.84 (0.28)
Malic acid	68 (3)	1.99 (0.18)	1.46 (0.19)	Sprouts	77 (6)	1.57 (0.17)	1.50 (0.15)
Nisin	42 (1)	2.50 (0.25)	2.74 (0.22)	Strawberries	10 (2)	3.59 (0.34)	3.24 (0.29)
Ozone	19 (2)	2.74 (0.27)	2.90 (0.23)	Tomatoes	48 (6)	3.73 (0.18)	3.61(0.16)
Neutral Electrolyzed Water	1 (1)	5.23 (1.03)	5.04 (0.89)				
Pediocin	36 (1)	1.86 (0.26)	2.10 (0.22)				
Peracetic acid	16 (3)	2.53 (0.28)	2.87 (0.24)				
Proprionic acid	16 (1)	1.02 (0.28)	1.00 (0.25)				
Trisodium phosphate	16 (2)	3.39 (0.28)	4.44 (0.27)				
Water	90 (22)	0.70 (0.14)	0.88 (0.12)				

[a] Data extracted from 30 peer-reviewed articles (Akbas & Olmez, 2007; Bari et al., 2002; Bari et al., 2003; Bari, Nakuama, et al., 2005; Bari, Ukuku, et al., 2005; Beuchat et al., 2004; Beuchat & Brackett, 1990; Beuchat et al., 1998; Burnett et al., 2004; Choi et al., 2012; Deza et al., 2003; Han et al., 2001; Hellstrom et al., 2006; Jin & Lee, 2007; Kim et al., 2008; Kim et al., 2009a; Kim et al., 2009b; Lang et al., 2004a; Lang et al., 2004b; Lee et al., 2002; Lin, Moon, Doyle, &, McWatters, 2002; Park, Hung, Doyle, Ezeike, & Kim, 2001; Park et al., 2011; Rodgers et al., 2004; Sagong et al., 2011; Stopforth et al., 2008; Su & D'Souza, 2012; Udompijitkul et al., 2007; Venkitanarayanan et al., 2002; Wade, et al., 2003; Wu & Kim, 2007).

3.1. Produce wash efficacy as a function of commodity and wash

The estimated efficacy of produce washes in reducing concentrations of *L. monocytogenes* on produce was highly variable in our simplified model (Table 2) and clearly indicated differences in efficacy among washes and commodities. Mean reductions achieved by different washes ranged from approximately 1 $\log_{10}$ cfu to more than 5 $\log_{10}$ cfu. Notably, inclusion of an interaction effect between 'produce commodity' and 'produce wash' did not significantly impact model predictions, possibly because the washes were generally not evaluated on all produce commodities, thus resulting in a highly unbalanced data set and prompting us to subsequently analyze more balanced data sets restricted to washes evaluated on all produce commodities of interest.

The fit of the GLM for two produce commodities (i.e., lettuce and apples) and 11 washes (Table 3) was adequate as determined by inspection of residuals, Cook's distances, and DFBETA, with only a very small number of standardized residuals exceeding an absolute value of 2 and only 5 observations with Cook's distances exceeding a value of 0.05 (data not shown). As shown in Table 3, with the exception of propionic acid, all evaluated washes proved significantly ($p < 0.05$) more effective at reducing contamination on produce than washing with water alone. However, for some washes, such as ozone, relatively few observations were available (Table 3), raising potential concerns about the robustness of these estimates. Mean reductions in *L. monocytogenes* concentration were significantly ($p < 0.05$) greater on apples than on lettuce, confirming differences in efficacy among produce commodities and prompting separate analyses for produce commodities with sufficient observations available to merit such analysis.

3.2. Efficacy of produce washes in reducing L. monocytogenes from apples, lettuce, tomatoes, sprouts, broccoli, and cabbage

Estimates of the mean reduction in *L. monocytogenes* concentration on apples, lettuce, tomatoes, sprouts, broccoli, and cabbage achieved by commonly used produce washes are displayed in Table 4, based on separate generalized linear models. Washing with water resulted in variable reductions in *L. monocytogenes* concentrations on lettuce (LS mean: 0.48 $\log_{10}$ cfu, 95% CI: 0.11–0.85 $\log_{10}$ cfu), apples (LS mean: 0.54 $\log_{10}$ cfu, 95% CI: 0.00–1.09 $\log_{10}$ cfu), sprouts (LS mean: 0.43 $\log_{10}$ cfu, 95% CI: 0.19–0.67 $\log_{10}$ cfu), broccoli (LS mean: 0.58 $\log_{10}$ cfu, 95% CI: 0.00–1.59 $\log_{10}$ cfu), tomatoes (LS mean: 1.67 $\log_{10}$ cfu, 95% CI: 1.22–2.12 $\log_{10}$ cfu), and cabbage (LS mean: 0.95 $\log_{10}$ cfu, 95% CI: 0.32–1.57 $\log_{10}$ cfu).

On lettuce, ozone and electrolyzed water exceeded a mean inactivation of 4 $\log_{10}$ cfu but in both cases data were scarce (Table 4), potentially limiting the precision of these estimates. Chlorine dioxide, hydrogen peroxide, citric acid, lactic acid, malic acid, peracetic acid, and trisodium phosphate also performed significantly ($p > 0.05$) better than water (Table 4). FIT (a commercial compound containing citric acid and oleic acid, among other ingredients), acetic acid, ascorbic acid and proprionic acid, on the contrary, were not significantly ($p < 0.05$) more effective than water (Table 4). However, for most of these washes the number of available observations was again relatively limited.

On apples, mean reductions achieved by ozone, peracetic acid, trisodium phosphate, chlorine dioxide, and hydrogen peroxide exceeded 4 $\log_{10}$ cfu, while chlorine achieved mean reductions of 1 $\log_{10}$ cfu which proved not significantly different from the efficacy of water. For some of the washes, however, data were scarce. Observations on sprouts (i.e., sprouts several days after sprouting) were available for 8 washes, of which all but chlorine appeared significantly more efficient than washes with water alone (Table 4). Again, for some washes only small numbers of observations were available (Table 4). For tomatoes, only data for 5 produce washes were available, of which all but electrolyzed water proved significantly more effective than washing with water alone (Table 4). However, the numbers of observations on which these data point are based are very limited (Table 4). Nisin and pediocin performed

Table 3
Efficacy of different sanitizers (i.e., as aqueous solution) in reducing contamination with *L. monocytogenes* on different produce commodities ($n = 294$ observations total, $n = 67$ for apples and $n = 227$ for lettuce; only includes sanitizers with data available for both commodities).[a]

Sanitizer	Impact of sanitizer				Impact of produce commodity			
	Number of observations (studies)	Mean reduction [$\log_{10}$ cfu] (Std. error)	Statistical test to evaluate enhanced efficacy of sanitizer compared to water (i.e., linear contrast)		Commodity	Mean reduction [$\log_{10}$ cfu] (Std. error)	Statistical test to evaluate enhanced efficacy on apples compared to lettuce (i.e., linear contrast)	
			Chi Square value	*p*-value			Chi Square	*p*-value
Acetic acid	22 (2)	1.31 (0.22)	5.48	0.02	Apples	2.45 (0.14)	8.43	<0.01
Chlorine	33 (7)	1.33 (0.19)	7.18	<0.01	Lettuce	2.01 (0.09)	n/a	
Chlorine dioxide	11 (2)	3.67 (0.32)	63.12	<0.01				
Citric acid	43 (4)	1.64 (0.17)	17.40	<0.01				
Hydrogen peroxide	9 (2)	2.77 (0.35)	28.35	<0.01				
Lactic acid	42 (3)	1.83 (0.17)	23.89	<0.01				
Malic acid	52 (3)	1.96 (0.15)	31.84	<0.01				
Ozone	4 (1)	4.98 (0.52)	55.51	<0.01				
Peracetic acid	14 (3)	2.35 (0.29)	25.75	<0.01				
Propionic acid	16 (1)	1.00 (0.26)	1.27	0.26				
Trisodium phosphate	12 (2)	3.31 (0.30)	52.91	<0.01				
Water	36 (10)	0.64 (0.18)	n/a					

[a] See Table 2 for source references.

significantly ($p < 0.05$) better than water on broccoli and cabbage but all data originated from the same research study potentially raising questions about the generalizability of results and study repeatability in other laboratories (Table 4).

4. Discussion

A variety of studies have evaluated the efficacy of different produce washes and rinses in reducing contamination with *L. monocytogenes* from produce, with often highly variable results. While a great variety of washes can achieve greater reductions in *L. monocytogenes* concentration than water alone, efficacy clearly differs by produce commodity as well as washes and experimental conditions. Notably, in the studies synthesized here, mean reductions in *L. monocytogenes* concentration varied from less than 1 $\log_{10}$ cfu to more than 5 $\log_{10}$ cfu. These estimates may be slightly biased towards lower treatment efficacy values by the fact that we substituted the lower limit of detection for censored data below the limit of detection, but a considerable impact of this bias is unlikely because only a small number of observations (i.e., <5% of the data) were left-censored. In addition, the included studies differed considerably in the *L. monocytogenes* inoculum concentrations used but estimates generated based on models that did or did not consider inoculum concentrations were highly comparable, again indicating that censored data likely only had a limited impact in this study.

A considerable number of published studies on the efficacy of produce washes in reducing *L. monocytogenes* contamination on produce were excluded from our study because they met our exclusion criteria or failed to meet our inclusion criteria. Despite relatively stringent criteria, we were able to extract 597 data points from 30 articles, providing data on 21 washes and 15 different produce commodities. In the included studies, experimental conditions were often incompletely described, and limited information on important experimental details such as wash concentration, inoculum concentration or variability across individual replicates limited our data analysis. Improvements in study design, analysis and reporting including more rigorously standardized wash application and microbiological analysis methods, more detailed description of experimental conditions, optimization of study conditions to minimize censored observations, and, if possible, reporting of raw data (e.g., through a dedicated database) would clearly aid future meta-analysis efforts and would potentially allow for the quantification of factors that could not be addressed explicitly in this study, such as impact of mechanical agitation.

4.1. Various produce washes achieve greater reductions in *L. monocytogenes* concentration on produce than water alone

Chlorine, typically in form of hyphochlorites or liquid chlorine, is among the most commonly used washes for produce treatments because of its microbicidal activity against a broad range of pathogens and its low cost (Hirneisen et al., 2010; Parish et al., 2003). In commercial operations, chlorine is primarily used as a spray or wash water additive to reduce microbial contamination on produce and to prevent cross-contamination (Hirneisen et al., 2010). However, our analysis indicated that chlorine only reduce contamination with *L. monocytogenes* by on average 1–3 $\log_{10}$ cfu depending on the produce commodity. These findings are in good agreement with various previous reports that have questioned the efficacy of chlorine-based produce washes (Burnett & Beuchat, 2001; Pao, Long, Kim, & Kelsey, 2012; Parish et al., 2003; Rico, Martín-Diana, Barat, & Barry-Ryan, 2007). Notably, our analysis also suggested that the relative efficacy of chlorine may be partially commodity-specific. The efficacy of chlorine – based washes is strongly inhibited by organic matter (Hirneisen et al., 2010). Because of the high organic load, free chlorine is rapidly inactivated during use, requiring close monitoring of free chlorine concentrations (Hirneisen et al., 2010). Importantly, the demand for free chlorine appears to differ across produce commodities (Hirneisen et al., 2010). This may explain, in part, the differences in efficacy observed in our analysis. Chlorine is also inactivated by high temperatures, light, metal ions, and hard water, its efficacy is affected by pH changes, and only certain chlorine-containing compounds are authorized for use in the United States (see Table 1), complicating its use in commercial operations (Hirneisen et al., 2010; Parish et al., 2003; Pfunter, 2011).

The efficacy of some of the other washes analyzed in this study may be higher than that of chlorine, at least on certain commodities. However, the number of available observations for our analysis differed considerably across washes and commodities, potentially impacting the robustness of certain efficacy estimates generated in this study, and the compounds or experimental conditions may not be authorized for use on produce destined for

Table 4
Efficacy of produces sanitizers (i.e., as aqueous solution) in reducing concentrations of *L. monocytogenes* on different produce commodities.[a]

Sanitizer	Number of observations (studies)	Mean reduction in *L. monocytogenes* concentration [$\log_{10}$ cfu] (Std. error)	Statistical test to evaluate enhanced efficacy of sanitizer compared to water (i.e., linear contrast)	
			Chi Square value	*p*-value
Lettuce ($n = 237$ observations)				
Electrolyzed water	2 (1)	4.17 (0.73)	22.54	<0.01
FIT	2 (1)	0.80 (0.73)	0.17	0.68
Acetic acid	14 (2)	1.08 (0.28)	3.11	0.08
Ascorbic acid	6 (1)	0.82 (0.42)	0.51	0.47
Chlorine	25 (7)	1.28 (0.21)	7.95	<0.01
Chlorine dioxide	7 (2)	2.95 (0.40)	29.95	<0.01
Citric acid	35 (4)	1.45 (0.18)	13.47	<0.01
Hydrogen peroxide	8 (1)	2.42 (0.37)	20.93	<0.01
Lactic acid	34 (3)	1.73 (0.18)	21.95	<0.01
Malic acid	44 (3)	1.81 (0.16)	27.48	<0.01
Ozone	2 (1)	4.95 (0.75)	32.40	<0.01
Peracetic acid	12 (3)	1.78 (0.31)	12.97	<0.01
Propionic acid	8 (1)	0.65 (0.37)	0.16	0.69
Trisodium phosphate	8 (2)	2.44 (0.37)	21.38	<0.01
Water	30 (8)	0.48 (0.19)	n/a	
Apples ($n = 67$ observations)				
Acetic acid	8 (1)	1.55 (0.24)	7.12	<0.01
Chlorine	8 (1)	0.96 (0.24)	1.18	0.26
Chlorine dioxide	4 (1)	4.80 (0.34)	58.34	<0.01
Citric acid	8 (1)	1.76 (0.24)	10.12	<0.01
Hydrogen peroxide	1 (1)	4.07 (0.69)	19.64	<0.01
Lactic acid	8 (1)	1.52 (0.24)	6.74	<0.01
Malic acid	8 (1)	1.77 (0.24)	10.35	<0.01
Ozone (liquid)	2 (1)	5.00 (0.48)	44.79	<0.01
Peracetic acid	2 (1)	4.70 (0.48)	40.41	<0.01
Propionic acid	8 (1)	1.35 (0.24)	4.70	0.03
Trisodium phosphate	4 (1)	4.83 (0.34)	58.80	<0.01
Water	6 (2)	0.54 (0.28)	n/a	
Tomatoes ($n = 48$ observations)				
Electrolyzed water	2 (1)	1.44 (0.70)	0.09	0.76
Neutral Electrolyzed Water	1 (1)	6.5 (0.99)	18.53	<0.01
Calcinated calcium	4 (1)	6.33 (0.50)	44.15	<0.01
Chlorine	22 (5)	3.36 (0.21)	22.63	<0.01
Hydrogen peroxide	1 (1)	6.11 (0.99)	16.03	<0.01
Water	18 (5)	1.67 (0.23)	n/a	
Sprouts ($n = 77$ observations)				
Chlorine	1 (1)	1.02 (0.49)	1.38	0.24
Chlorous acid	1 (1)	4.49 (0.49)	47.34	<0.01
Chlorine dioxide	11 (3)	2.35 (0.15)	65.51	<0.01
Fumaric acid	7 (2)	2.54 (0.18)	60.92	<0.01
Lactic acid	1 (1)	1.98 (0.49)	9.01	<0.01
Nisin	14 (1)	1.33 (0.13)	22.53	<0.01
Ozone	13 (1)	1.05 (0.14)	11.02	<0.01
Pediocin	12 (1)	1.26 (0.14)	18.25	<0.01
Water	17 (4)	0.43 (0.12)	n/a	
Broccoli ($n = 28$ observations)				
Nisin	14 (1)	2.64 (0.20)	11.26	<0.01
Pediocin	12 (1)	1.75 (0.21)	4.05	0.04
Water	2 (1)	0.58 (0.52)	n/a	
Cabbage ($n = 28$ observations)				
Nisin	14 (1)	3.23 (0.12)	26.89	<0.01
Pediocin	12 (1)	2.28 (0.13)	12.09	<0.01
Water	2 (1)	0.95 (0.32)	n/a	

[a] See Table 2 for source references.

human consumption. Therefore, the provided estimates should be interpreted carefully, taking into account the amount of data and range of experimental conditions on which the estimates were based as well as all relevant regulatory considerations. These limitations notwithstanding, certain washes show promise as effective treatments for reduction of *L. monocytogenes* contamination on produce.

Chlorine dioxide is another commonly used produce wash (Hirneisen et al., 2010; Parish et al., 2003). Compared to chlorine, chlorine dioxide is characterized by increased pH stability, fewer toxicity concerns, and generally increased efficacy (Hirneisen et al., 2010). Organic matter does not inactivate chlorine dioxide nearly as dramatically as chlorine, and chlorine dioxide can be used over a wide range of temperatures (Hirneisen et al., 2010). Similar to chlorine, chlorine dioxide is primarily used in commercial operations as a spray or wash water additive to reduce microbial contamination on produce and to prevent cross-contamination during washing (Hirneisen et al., 2010). In our analysis chlorine

dioxide reduced contamination with *L. monocytogenes* by on average 2.5–5 $\log_{10}$ cfu. However, practical considerations may limit the application of chlorine dioxide: for example, chlorine dioxide solutions have to be generated at the time of use because the solutions are highly unstable and concentrated chlorine dioxide is explosive (Hirneisen et al., 2010).

Organic acids are used in some commercial food operations as washes (Parish et al., 2003). In addition, fruits and vegetables often contain relatively high concentrations of naturally occurring organic acids (Parish et al., 2003), resulting in innate antilisterial properties of certain produce commodities (Hoelzer et al., 2012). The antimicrobial mechanism of action for organic acids is believed to be primarily attributable to pH changes and disruption of cell membrane functions (Parish et al., 2003; Rico et al., 2007). A variety of organic acids such as acetic, ascorbic, lactic, malic, and propionic acid were evaluated here with regard to their efficacy in reducing contamination with *L. monocytogenes* on produce. These organic acids and their salts act as antioxidants and can prevent browning of fresh-cut produce (Rico et al., 2007); however, not all of them are authorized to be used as antimicrobial substances in processed foods in the U.S and premarket approval is required, unless the use is GRAS. We found variable efficacy among these organic acids, and for certain organic acids and produce commodities, including for example ascorbic and proprionic acid on lettuce, efficacy was not significantly different from that of water. These results are in good agreement with previous reports that mentioned variable and often limited efficacy of organic acids against foodborne pathogens (Parish et al., 2003).

Trisodium phosphate, a monovalent inorganic phosphate salt, is a strong alkaline cleanser with broad activity against a wide array of pathogens (EFSA, 2008; Weiner et al., 2001). According to (EFSA, 2008), the primary mode of action is believed to be associated with its surfactant and detergent properties (i.e., removing surface tension and improving mixing between hydrophobic surfaces or substances and water) and, at higher concentrations, protein denaturation in response to the very alkaline pH, although the mechanism is still incompletely understood. In our analysis, trisodium phosphate appeared to be highly effective on apples and lettuce with mean reductions in the range of 2.5–5 $\log_{10}$ cfu, while lack of data precluded an analysis for other produce commodities. These data, though limited, suggest trisodium phosphate may be an alternative to chlorine.

Peroxyacetic acid is occasionally used to reduce microbial contamination on produce (Parish et al., 2003). Peroxyacetic acid is less affected by organic residues than chlorine, and remains effective over a broader pH range even though it is generally more expensive (Parish et al., 2003). The mode of action is believed to be primarily attributable to the strong oxidizing function, which disrupts cell membranes and inhibits protein synthesis (Australian Government, 2010; Pfunter, 2011). In our study, peroxyacetic acid was highly effective in reducing contamination with *L. monocytogenes* on lettuce and apples. Lack of data precluded an analysis for other produce commodities. Peroxygen compounds may be corrosive to metals, are relatively expensive, and are fairly unstable, complicating their use in commercial operations (Australian Government, 2010).

The efficacy of hydrogen peroxide in reducing microbial contamination on produce has received considerable attention (Parish et al., 2003). Similar to peroxyactic acid, the mechanism of action for hydrogen peroxide is thought to be primarily attributable to oxidization (Rico et al., 2007). In our study, hydrogen peroxide was effective on lettuce, apples and tomatoes, with no data available for other produce commodities. However, other studies for different pathogens have indicated that efficacy may differ considerably by produce commodity (Parish et al., 2003). Similar to peroxyacetic acid, hydrogen peroxide can be corrosive to metals, is relatively expensive, fairly unstable, and can be hazardous to use in concentrated form. In addition, negative impacts on the organoleptic properties of certain produce commodities, manifested for instance as browning or bleaching, have been reported (Parish et al., 2003; Rico et al., 2007).

Taken together, our analysis emphasizes that a variety of produce washes may be effective at reducing contamination with *L. monocytogenes* on produce. However, more data on the prevalence and concentration of *L. monocytogenes* contamination on different produce commodities, and on the efficacy of these treatments under real-world industrial conditions, are needed before the usefulness of different produce washes can be evaluated comprehensively.

4.2. Produce washes are more effective on certain produce commodities such as apples than on other produce commodities such as lettuce

It has been long recognized that the efficacy of produce washes differs among commodities, likely at least partially as a function of surface structure and accessibility of the bacteria to the chemical compound (Han et al., 2001; Parish et al., 2003; Sapers, 2001). In addition, produce washes often appear to be considerably less effective at reducing contamination on damaged or cut produce than on intact produce (Han et al., 2001; Parish et al., 2003; Sapers, 2001). Here, we generated quantitative estimates that are specific to a given wash-commodity pair. Consistent with previous reports we detected considerable differences of several $\log_{10}$ cfu in the efficacy of produce washes across commodities. Even though the available data did not permit an explicit evaluation of the impact of surface structure, our findings are compatible with the hypothesis of surface structure as one of the primary determinants of produce wash efficacy because efficacy on produce with smoother surface structure such as apples generally was higher than on produce with rough surface structure such as lettuce. These findings emphasize that commodity type influences produce wash efficacy.

Among the commodities with sufficient available data to permit a separate subgroup analysis are sprouts. Sprouts have been the vehicle of numerous foodborne illness outbreaks with pathogens such as *L. monocytogenes*, *Salmonella* and *E. coli* (Buchholz et al., 2011; Hoelzer et al., 2012; Mohle-Boetani et al., 2001; Taormina, Beuchat, & Slutsker, 1999; Thomas, Palumbo, Farrar, Farver, & Cliver, 2003). Contaminated seeds represent a potential source of sprout contamination, together with other contamination routes such as cross-contamination during or after sprouting. Notably, in our analysis several produce washes such as chlorine, ozone, nisin, or pediocin only led to modest reductions in *L. monocytogenes* concentrations on sprouts, but for some washes the available data were scarce, thus potentially limiting the precision of the estimates. The quantitative estimates presented here emphasize the difficulty of reducing contamination on sprouts and reinforce the importance of strategies that minimize the risk of spouts or seeds for sprouting becoming contaminated (Thomas et al., 2003).

4.3. Selection of produce washes should be based on evaluation of the strengths and limitations of the given wash for a given commodity, and a consideration of the available alternatives

Our analysis focused on pathogen reduction efficacy of a given produce wash treatment. However, negative side effects are associated with many produce washes, including potential concerns about toxicity, environmental impacts and worker safety, economic costs and negative impacts on the organoleptic properties and shelf life of the produce (Parish et al., 2003). Moreover, regulatory

considerations limit the use of certain produce washes in commercial operations (see CFR for regulatory considerations in the U.S.). In addition, certain produce commodities , such as many berries, cannot be washed without prohibitively negative consequences on quality (Parish et al., 2003). Alternatives to liquid produce washes include treatments with gaseous compounds such as ozone or chlorine dioxide, irradiation, or pulsed UV light (Parish, et al., 2003). Such treatments may be more feasible or effective for certain produce commodities, and the selection of a treatment should be based on careful consideration of the strengths and limitations of all available options. Moreover, individual treatments may generate synergistic, additive, or antagonistic effects if combined with other prevention or control measures due to the simultaneous or subsequent effect of multiple factors (Parish et al., 2003). Each produce wash should therefore ideally be seen as one component of an integrated food safety system that stretches from farm to fork.

5. Conclusion

In conclusion, produce washes can be effective in reducing contamination of produce with *L. monocytogenes*, but the efficacy depends on the wash and the produce commodity. Not all produce washes may be viable options for all produce commodities because of limitations related to efficacy, economic viability, practical feasibility, governmental regulations, or because of negative impacts on produce quality. The selection of measures to reduce contamination on produce therefore should be based on a comprehensive evaluation of all possible alternatives, must carefully consider regulatory and business requirements, and should ideally be adapted to the specific needs associated with the produce commodity and specific production system. Our analysis demonstrated that produce wash efficacy may be insufficient to remove high concentrations of *L. monocytogenes* on produce. Therefore, produce washes should only be regarded as one of many interventions in an integrated food system aimed at preventing and controlling produce contamination pre- and post-harvest. Importantly, a better understanding of *L. monocytogenes* contamination patterns on produce, including contamination sources as well as prevalence and concentrations on produce, would aid in the evaluation of benefits and drawbacks of the different alternatives under real-world conditions. Additional data would allow for a more comprehensive evaluation of compound and commodity effects.

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