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Evaluation of the Microbiological Quality of Fresh Cilantro, Green Onions, and Hot Peppers from Different Types of Markets in Three U.S. States

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Abstract: The consumption of fresh produce and use of fresh herbs as flavoring agents in range of culinary preparation has increased in recent years due to consumer demand for a healthier lifestyle. Consumer preference for farmers' markets and locally owned grocery stores have also grown in the U.S. in recent years. Most consumers perceive locally available produce to be a safer choice, but limited data is available. We evaluated microbiological quality, including aerobic plate count (APC), generic *E. coli* and total coliforms (TC), and human pathogens (*Salmonella* spp., *E. coli* O157: H7, and *Shigella sonnei*/*Shigella* spp.), of cilantro (n = 132), green onions (n = 131), jalapeño peppers (n = 129) and serrano peppers (n = 126) purchased from national chains, farmers' markets and locally owned grocery stores in seven cities of the U.S. Of the 518 samples, enumerable populations of *E. coli* were found in one cilantro sample and three jalapeño samples, ranging from 1.18 to 2.42 $\log_{10}$ CFU/g. APC and TC ranged from 3.84 to 9.27 $\log_{10}$ CFU/g and from 0.84 to 5.84 $\log_{10}$ CFU/g, respectively. Overall, the APC of produce samples from national chains was lower than that from farmers' markets and locally owned grocery stores ($p < 0.05$). Cilantro had a significantly highest APC among tested produce types ($p < 0.05$). Risk factor analysis indicated that national chain had significant lower APC populations than farmer's market or local markets ($p < 0.05$) and cilantro had higher APC populations than the other three types of produce ($p < 0.05$). Risk factor analysis also showed that TC populations in green onions were significantly higher than those in serrano peppers ($p < 0.05$). No human pathogens (*Salmonella* spp., *E. coli* O157: H7, or *Shigella sonnei*/*Shigella* spp.) were detected in any of the tested produce samples. The high prevalence of TC and high APC counts highlight the importance of consumer vigilance and practice in handling fresh produce that is often consumed raw or used to garnish dishes.

Keywords: herb; coliform; aerobic plate count; foodborne illness; foodborne pathogen; food safety

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1. Introduction

The consumption of fresh produce has increased substantially in recent decades in the U.S. American consumers purchase fresh produce through different channels and have their own preferences and concerns for food safety, personal health and environmental sustainability. Consumer preference and concerns have led to the growing popularity for locally owned grocery stores and farmers' markets. One study reported that 56% of 1549 respondents preferred supermarkets as their primary fresh produce purchase locations, with 10% preferring supercenters and 25% choosing farmers' markets [1]. Convenience is the main reason that most people go to supermarkets, and freshness is the main reason that people choose farmers' markets or locally owned markets [1,2]. Cilantro, green onions and hot peppers (e.g., jalapeño peppers and serrano peppers) are among popular herbs and spices often garnished and consumed raw in many types of cuisines, and they have become a potential food safety concern [3]. Multiple studies have documented the microbiological quality of fresh produce sold at different types of markets [4–6]. Populations of fresh produce contamination varied among surveyed locations, but those studies have not provided comparisons of the prevalence of indicator organisms or pathogens among markets.

A wide range of fresh produce (particularly leafy greens and herbs) can become contaminated by human pathogens via different routes [7]. Contamination commonly occurs during production, harvesting, processing, and distribution processes [8]. Indeed, fresh produce is among the major food vehicles of foodborne illness outbreaks, exceeding traditional carriers, such as poultry, meat, and seafood [9]. Fresh produce accounted for 46% of total foodborne illnesses and 25% of deaths in U.S. outbreaks, according to data obtained from the Centers for Disease Control and Prevention (CDC) [10]. CDC data also implied that produce related outbreaks summed up around 30 to 60 each year and caused about 900 to 3000 illnesses per year from 1998 to 2016 [11]. *Salmonella*, *E. coli* O157: H7 and *Shigella* are the primary bacterial pathogens associated with foodborne illness outbreaks in the U.S. [12]. Cilantro, tomatoes and onions are ingredients frequently used in making salsa, and *Salmonella*, *Shigella* and *E. coli O157:H7* are among the pathogens commonly identified from the salsa-related foodborne outbreaks [13]. Red salsa, a side dish for ethnic cuisine, was the primary vehicle associated with 70 foodborne outbreaks in 1990–2006 in the U.S. [13]. A *Salmonella* outbreak was reported after the consumption of contaminated chopped cilantro in California in 2001 [14]. In April 2008, *Salmonella* serotype Saintpaul was identified from an outbreak with 1442 illnesses and salsa made from raw jalapeños served in restaurants of Texas was the food source of the outbreak [15].

The microbiological quality of fresh produce is regularly monitored through the examination of indicator organisms and pathogens, among which the aerobic plate count (APC), total coliforms (TC) and generic *E. coli* are common indicator organisms. Absence of coliforms/*E.coli* is a good indicator of the microbiological safety of fresh produce and the environment in which the produce is grown and processed [16]. The overall purposes of this study were to (1) evaluate the microbial quality of selected fresh produce by measuring the populations of indicator organisms (APC, TC and generic *E. coli*) and human pathogens (*Salmonella* spp., *E. coli* O157: H7, and *Shigella sonnei*/spp.) in cilantro, jalapeño peppers, serrano peppers and green onions in national chains, locally owned grocery stores and farmers' markets; (2) analyze potential risk factors contributing to the contamination of fresh produce.

2. Materials and Methods

2.1. Sample Collection

Jalapeño peppers, serrano peppers, green onions and cilantro were purchased from seven cities in Alabama (Auburn, Birmingham, and Montgomery), Georgia (Atlanta, Columbus, and Lagrange) and Florida (Gainesville) during summer and winter over three years (August 2011 to February 2014). For each sampling location (city), three types of stores (one national chain, one locally owned store and one farmers' market) were sampled monthly during summer (AL, GA, FL) and winter (FL). This arrangement accommodated seasonal variations of the farmers' markets in three states. Produce samples in supermarkets and farmers' markets were sold loosely. However, produce was sold in packages in locally owned stores. Cilantro and green onions were sold in bundles, and hot peppers were sold in all sampling locations. One sample of each produce item (1.0 lb/450 g) was purchased, and samples were placed in separate coolers and kept refrigerated during transportation back to the laboratories of Auburn University (samples from AL and GA) and of the University of Florida (samples from FL) within two hours of purchase. Microbial enumeration and analysis were conducted within 24 h of collection, and each sample was tested in duplicate. Two laboratories implemented the same sampling procedures and analytical methods. Sample preparation was based on specific types and indicator organisms/human pathogens. Sample processing and analyses were conducted according to the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) [17].

2.2. Analyses of Indicator Organisms Aerobic Plate Count (APC), Total Coliforms (TC), and Generic E. coli

For each type of produce, 25 g of samples were homogenized in 225 mL 0.1% peptone water (BPW; BD, Franklin Lakes, NJ, USA) and stomached 2 min at 260 rpm in a Stomacher 400 (Seward Scientific, London, UK). After stomaching, 10-fold serial dilutions were made with sterile 0.1% PBW to 10^{-6} for aerobic plate count and 10^{-4} for *E. coli*/TC. After serial dilutions, 1 mL of each diluted sample was enumerated on aerobic count plate Petrifilm^{TM} and coliform/*E. coli* Petrifilm^{TM} plates (3M^{TM}, St. Paul, MN, USA) in duplicate, and incubated at 37 °C for 24 h. After overnight incubation, colonies on Petrifilm^{TM} plates were counted, the concentration calculated, and results were recorded as colony forming units (CFU). APC and TC plates that yielded more than 250 CFU and 150 CFU, respectively, were estimated to the nearest 250 and 150 CFU numbers multiply by the dilution factors, respectively. The average APC, *E. coli* and TC counts were calculated for each sample and results were recorded as $\log_{10}$ CFU/g.

2.3. Pathogen Analyses (Salmonella spp., E. coli O157:H7 and Shigella sonnei/Shigella spp.)

For *Salmonella* detection, 25 g samples were homogenized with 225 mL of sterile lactose broth (Bioxon, BD, Sparks, MD, USA) in a sterile stomacher bag using a Stomacher 400. After stomaching, samples were transferred to a secured capped sterile container, left to stand for 60 min at room temperature, and incubated for 24 h at 35 °C. The samples were mixed gently, and the pH was adjusted to neutral if necessary. After overnight incubation, 0.1 mL and 1 mL mixture were transferred to a 10 mL of Rappaport–Vassiliadis (RV) medium (Bioxon, BD) and tetrathionate (TT) broth (Bioxon, BD), respectively, mixed well, and incubated for 24 h at 42 °C in a circulating water bath and at 35 °C in an incubator, respectively. After overnight incubation, the TT broth and RV medium were streaked onto bismuth sulfite (BS) (Bioxon, BD) agar plates, xylose lysine desoxycholate (XLD) (Bioxon, BD) agar plates, and Hektoen enteric (HE) (Bioxon, BD) agar plates, in triplicate. All agar plates were incubated for 24 h at 35 °C and examined for presumptive colonies that could be *Salmonella*. Suspected colonies on XLD, HE, and BS agars were those that were black in color. After 24 h of incubation, two presumptive *Salmonella* colonies from selected agar plates were first analyzed by latex agglutination test (Remel, Lenexa, KS, USA) and further identified by API 20E Strips (BioMerieux, Durham, NC, USA).

For *E. coli* O157: H7 detection, 25 g sample was weighed into a sterile stomacher bag with 225 mL of modified buffered peptone water (BPW; BD, Franklin Lakes, NJ, USA) with pyruvate (mBPWp) and stomached for 1 min (at 260 rpm). Samples were incubated at 37 °C static for 5 h, then 1 mL of acriflavin-cefsulodin-vancomycin (ACV) supplements was added to those samples. Then, samples were incubated at 42 °C static for 18–20 h in an incubator. Each overnight sample was diluted (1:10) in sterile 0.1% PBW, up to 10^{-2} dilutions. 10 µL of each dilution was streaked onto duplicate tellurite-cefixime sorbitol MacConkey (CT-SMAC) agar and Rainbow agar, and plates were incubated for 24 h at 37 °C. Typical colonies on CT-SMAC agar and Rainbow agar were colorless to neutral (sometimes with smoky centers) and black to black-blue colonies, respectively. After overnight incubation, presumptive colonies were tested for O157 and H7 antigens by latex agglutination (RIM Remel Kit, Thermo Scientific, Waltham, MA, USA) and further identified colonies with positive latex agglutination results by BioMerieu's API 20E strips.

For *Shigella sonnei* detection, 25 g sample was weighed into 225 mL of *Shigella* broth (Bioxon, BD) supplemented with 2.5 mL of novobiocin (0.5 µg/mL) (mTSBn; Remel, Lenexa, KS, USA). Samples were enumerated for 10 min with periodic shaking at room temperature, and incubated at 44 °C for 20 h in a forced air incubator with anaerobic gas generating sachets (Bioxon, BD) and anaerobic indicators. For *Shigella* spp. detection, same with

Shigella sonnei, but instead adding novobiocin (3 µg/mL) to the samples in *Shigella* broth and incubating at 42 °C. After incubation, samples were streaked onto the MacConkey (MAC) agar and plates were incubated for 24 h at 35 °C. Typical colonies on MAC agar were slightly pink and translucent, with or without rough edges. Suspect colonies on the agar were re-streaked onto glucose broth (Bioxon, BD), TSI agar slant (Bioxon, BD), lysine decarboxylase broth (Bioxon, BD), motility agar (Bioxon, BD) and Tryptone (tryptophane) broth (Bioxon, BD), and incubated at 35 °C for 48 h, but examined at 20 h.

2.4. Statistical Analyses

Statistical analysis was implemented in R 3.4.4 (http://www.r-project.org/, 16 March 2019), and a significance level was set at $\alpha = 0.05$. Range, mean, and standard deviations (SD) of APC and TC by store and produce types were calculated. One-way analysis of variance (ANOVA) and independent two-sample t-tests were used to find the differences among the populations of the indicator organisms for different locations, produce types and sampling time separately, and further differences between group means were tested with Tukey's honest significant difference (Tukey's HSD) test when necessary. Then, data were analyzed by the general linear model procedure to determine the effects of produce types, sampling time and sampling location on the APC and TC populations (APC/TC populations were dependent variables and sampling time, sampling location, as well as produce types were independent variables). In addition, a new variable was created to account for sampling time "cold" (December to March) and "warm" (May to September) months. The model structure was as below:

$$log(y_i) = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \beta_3 x_{i3} \quad (1)$$

where y_i were populations of indicator organisms on sample i, β_0 was the intercept, and β_1, β_2, β_3 were the slopes of independent variables fresh produce type, store type, and sampling time, which correspond to samples $x^{(1)}, \ldots, x^{(i)}$. National chain, serrano peppers and "warm" months were selected as reference attributes for the interpretation of risk factors.

3. Results

3.1. Populations of Indicator Organisms on Cilantro, Green Onions, Jalapeño Peppers, and Serrano Peppers

A total of 518 samples of cilantro (n = 132), green onions (n = 131), jalapeño peppers (n = 129), and serrano peppers (n = 126) were purchased from seven cities in three states in 2011–2014. Among the 518 total samples being collected, 518 were tested for APC, 397 were tested for TC and 398 were tested for generic *E. coli*. APC ranged from 3.84 to 9.27 $\log_{10}$ CFU/g and over 50% of hot peppers and approximately 90% of cilantro and green onions had APC populations at 6.00 to 8.99 $\log_{10}$ CFU/g (Table 1). The overall prevalence of TC was 83.1% (330 of 397) ranged from 0.84 to 5.84 $\log_{10}$ CFU/g, and over 50% of the samples had TC at 2.00 to 4.99 $\log_{10}$ CFU/g (Table 2). The populations of generic *E. coli* were 1% (4 of 398, including one cilantro sample and three jalapeño samples) and loads ranged from 1.18 to 2.42 $\log_{10}$ CFU/g (Table 2). Generic *E. coli* were not entered for univariate (ANOVA) and risk factor analyses (GLMs) due to a limited number of positive samples. No human pathogens (*Shigella sonnei/Shigella* spp., *Salmonella* spp., or *E. coli* O157: H7) were detected in any of the tested fresh produce samples.

APC populations differed significantly across fresh produce types. Cilantro, on average, had the highest APC populations ($p < 0.05$) followed by green onions, jalapeno peppers and serrano peppers (Table 3). The average APC populations of both locally owned stores and farmers' markets were significantly higher than those of the national chain grocery stores ($p < 0.05$; Table 4). No significant differences were found in average APC populations between "cold" and "warm" months (Table 5). No significant differences were found in TC populations regarding produce types, purchase vendors and sampling time (Tables 3–5).

Table 1. Aerobic plate count (APC) populations of four types of herbs from different sampling locations ***.

Produce Type	Store Type * (Number of Samples Tested)	APC Loads ** [Number of Sample (%)]					
		<5.00	5.00–5.99	6.00–6.99	7.00–7.99	8.00–8.99	>9.00
Cilantro (n = 132)	NC (n = 44)	1 (2.3)	2 (4.5)	13 (29.5)	21 (47.8)	6 (13.6)	1 (2.3)
	LS (n = 44)	2 (4.5)	1 (2.3)	9 (20.5)	25 (56.8)	7 (15.9)	0
	FM (n = 44)	0	3 (6.9)	11 (25.0)	17 (38.6)	11 (25.0)	2 (4.5)
Green onions (n = 131)	NC (n = 44)	3 (6.8)	3 (6.8)	25 (56.8)	13 (29.6)	0	0
	LS (n = 44)	0	3 (6.8)	16 (36.4)	19 (43.2)	6 (13.6)	0
	FM (n = 43)	2 (4.7)	4 (9.3)	14 (32.6)	22 (51.1)	1 (2.3)	0
Jalapeño peppers (n = 129)	NC (n = 44)	4 (9.1)	20 (45.4)	11 (25.0)	8 (18.2)	1 (2.3)	0
	LS (n = 44)	5 (11.4)	15 (34.1)	14 (31.7)	9 (20.5)	1 (2.3)	0
	FM (n = 41)	2 (4.9)	14 (34.1)	16 (39.0)	9 (22.0)	0	0
Serrano peppers (n = 126)	NC (n = 44)	2 (4.5)	17 (38.6)	15 (34.2)	10 (22.7)	0	0
	LS (n = 41)	2 (4.9)	9 (22.0)	14 (34.1)	15 (36.6)	1 (2.4)	0
	FM (n = 41)	4 (9.8)	10 (24.4)	15 (36.6)	6 (14.6)	6 (14.6)	0

* NC: National chain grocery store; LS: Locally owned grocery store; FM: Farmers' market. ** The unit is $\log_{10}$ CFU/g for each sample. *** Overall APC range is 3.84–9.27 $\log_{10}$ CFU/g.

Table 2. Total coliforms (TC) and generic *E. coli* [A] populations of four types of herbs from different sampling locations ***.

Produce Type	Store Type * (Number of Samples Tested)	TC Loads ** [Number of Sample (%)]					
		<1.00	1.00–1.99	2.00–2.99	3.00–3.99	4.00–4.99	>5.00
Cilantro (n = 102)	NC (n = 34)	9 (26.5)	8 (23.5)	6 (17.6)	8 (23.5)	2 (5.9)	1 (2.9)
	LS (n = 34)	6 (17.6)	7 (20.6)	8 (23.5)	8 (23.5)	3 (8.8)	2 (5.9)
	FM (n = 34)	5 (14.7)	7 (20.6)	9 (26.5)	5 (14.7)	5 (14.7)	3 (8.8)
Green onions (n = 101)	NC (n = 34)	2 (5.9)	3 (8.8)	9 (26.5)	13 (38.2)	6 (17.6)	1 (2.9)
	LS (n = 34)	4 (11.8)	6 (17.6)	12 (35.3)	9 (26.5)	3 (8.8)	0
	FM (n = 33)	8 (24.2)	2 (6.1)	10 (30.3)	9 (27.3)	3 (9.1)	1 (3.0)
Jalapeño peppers (n = 97)	NC (n = 34)	6 (17.6)	7 (20.6)	10 (29.4)	10 (29.4)	1 (2.9)	0
	LS (n = 33)	6 (18.2)	10 (30.3)	11 (33.3)	5 (15.2)	1 (3.0)	0
	FM (n = 30)	4 (13.3)	8 (26.7)	6 (20.0)	9 (30.0)	3 (10.0)	0
Serrano peppers (n = 97)	NC (n = 34)	9 (26.5)	7 (20.6)	7 (20.6)	9 (26.5)	2 (5.9)	0
	LS (n = 32)	7 (21.9)	5 (15.6)	8 (25.0)	8 (25.0)	4 (12.1)	0
	FM (n = 31)	6 (19.4)	7 (22.6)	8 (25.8)	7 (22.6)	2 (6.5)	1(3.2)

* NC: National chain grocery store; LS: Local owned grocery store; FM: Farmers' market. ** The unit is $\log_{10}$ CFU/g for each sample. *** Overall TC range is 0.84–5.84 $\log_{10}$ CFU/g. [A] Generic *E. coli* was detected in 1 cilantro and 3 jalapeño pepper (1%, 4/398) samples; loads ranged from 1.18 to 2.42 $\log_{10}$ CFU/g.

Table 3. Comparisons of aerobic plate count (APC) and total coliforms (TC) populations of four types of produce *.

Produce	APC	TC
Cilantro	7.30 ± 0.94^{a}	2.38 ± 1.59^{a}
Green onions	6.88 ± 0.79^{b}	2.62 ± 1.39^{a}
Jalapeño peppers	6.19 ± 0.88^{c}	2.15 ± 1.33^{a}
Serrano peppers	6.45 ± 0.92^{c}	2.14 ± 1.42^{a}

[a–c] Mean values with different letters within each column are significantly different ($p < 0.05$, Tukey's HSD Test). * Values are $\log_{10}$ mean ± standard deviation.

Table 4. Comparisons of aerobic plate count (APC) and total coliforms (TC) populations of three sampling locations *.

Store Type	APC	TC
National Chain	6.52 ± 0.94 [a]	2.34 ± 1.49 [a]
Locally Owned Grocery Store	6.82 ± 0.97 [b]	2.23 ± 1.38 [a]
Farmers' Market	6.80 ± 1.01 [b]	2.41 ± 1.49 [a]

[a,b] Mean values with different letters within each column are significantly different ($p < 0.05$, Tukey's HSD Test).
* The unit is $\log_{10}$ CFU/g; values are $\log_{10}$ mean ± standard deviation.

Table 5. Comparisons of aerobic plate count (APC) and total coliforms (TC) populations of sampling time *.

Sampling Time	APC	TC
Warm months	6.77 ± 0.99 [a]	2.39 ± 1.44 [a]
Cold months	6.65 ± 0.97 [a]	2.24 ± 1.45 [a]

[a] Mean values with same letters within each column are not significantly different ($p > 0.05$, two sample *t*-test).
* The unit is $\log_{10}$ CFU/g; values are $\log_{10}$ mean ± standard deviation.

3.2. Risk Factors Associated with the Populations of Indicator Organisms in Fresh Produce

The results of GLMs testing the relationship between APC populations and its related factors were shown in Table 6. On average, APC in samples from farmers' markets and locally owned grocery stores were 0.28 and 0.31 $\log_{10}$ CFU/g, respectively, higher than those from the national chain. The average APC populations of cilantro and green onions were 0.85 and 0.42 $\log_{10}$ CFU/g, respectively, significantly higher than serrano peppers. The average APC populations of jalapeño peppers were 0.27 $\log_{10}$ CFU/g significantly lower than those of serrano peppers ($p < 0.05$). The average APC populations of samples collected in the "cold" months on average were 0.14 $\log_{10}$ CFU/g marginally lower than those collected in the "warm" months ($p < 0.1$).

Table 6. Linear regression estimates between risk factors and aerobic plate count (APC) populations of sampled produce.

Parameter	Estimate [a]	Standard Error	*p*-Value
Farmers' Market	0.28	0.09	0.0029 *
Locally Owned Store	0.31	0.09	0.0010 *
National Chain	0 (Ref.) [b]	-	-
Cilantro	0.85	0.11	<0.0001 *
Green onions	0.42	0.11	0.0001 *
Jalapeño peppers	−0.27	0.11	0.0143 *
Serrano peppers	0 (Ref.) [b]	-	-
Cold months	−0.14	0.33	0.0660
Warm months	0 (Ref.) [b]	-	-

[a] Values are $\log_{10}$ CFU/g. [b] Reference category. * $p < 0.05$.

Results of the association between TC populations and risk factors were shown in Table 7. Average TC populations on green onions were 0.49 $\log_{10}$ CFU/g significantly higher than those in serrano peppers ($p < 0.05$), while there are no significant differences between jalapeño peppers, cilantro and serrano peppers.

Table 7. Linear regression estimates between risk factors and total coliform (TC) populations of sampled produce.

Parameter	Estimate [a]	Standard Error	*p*-Value
Farmers' Market	0.07	0.18	0.6846
Locally Owned Store	−0.10	0.18	0.5687
National Chain	0 (Ref.) [b]	-	-
Cilantro	0.24	0.20	0.2318
Green onions	0.49	0.21	0.0174 *
Jalapeño peppers	0.02	0.21	0.9280
Serrano peppers	0 (Ref.) [b]	-	-
Cold months	−0.16	0.19	0.2724
Warm months	0 (Ref.) [b]	-	-

[a] Values are $\log_{10}$ CFU/g. [b] Reference category. * $p < 0.05$.

4. Discussion

Indicator organisms, such as total coliforms (TC) and *E. coli*, are commonly used and analyzed to demonstrate the hygienic qualities of food. The findings of the current study indicated high populations of TC and low populations of generic *E. coli*. In total, four samples had an enumerable population of generic *E. coli*. This result was consistent with another study also reporting a low prevalence of *E. coli* in minimally processed fresh produce in Brazil [18]. The presence of generic *E. coli* generally indicates direct or indirect fecal contamination [19]. In the current study, only four samples (1%) were detected with generic *E. coli* with loads ranging from 1.18 to 2.42 $\log_{10}$ CFU/g. According to the Commission of the European Communities' (EC) "ready-to-eat fresh produce" safety guidelines, the *E. coli* populations on the majority of tested fresh produce samples in the present study were satisfactory ($\leq$2 $\log_{10}$ CFU/g) and less than 1% were acceptable ($\leq$3 $\log_{10}$ CFU/g) [20].

TC prevalence in the tested fresh produce was 80.4%, 86.1%, 83.5%, and 77.3% (results not shown) for cilantro, green onions, jalapeños, and serrano peppers, respectively, and were not significantly different among produce types in this study (Table 3). The mean populations of tested fresh produce in our study were around 2 $\log_{10}$ CFU/g. Moreover, TC populations were not significantly different between market locations or produce types in the current study. This result agreed with the finding from a survey which found that produce from conventional farms had either lower or similar populations of coliforms compared to produce from organic farms [21].

Cilantro had the highest APC populations among all the four types of fresh produce in the present study. Nevertheless, the factors that are associated with this trend have been explored extensively in other studies. Indicator organism counts are often associated with the morphology of produce leaves; rough surfaces make it easier for dirt and bacteria to adhere [22]. In our selected samples of produce, hot peppers have smooth surfaces, while cilantro and green onions have relatively rough surfaces, which may explain the high loads of indicator organisms on cilantro and green onions. Additionally, the fresh produce was without packaging, except for the cilantro sold in locally owned stores, and a CDC report showed an increase in APC populations of cilantro during the packing process [23]. Furthermore, APC populations vary significantly among different types of fresh produce and within different processing environments, thus it is difficult to formulate a complete set of regulations for standard operations [24]. In addition, we found fresh produce from national chain had significantly lower populations of APC than from the local stores or from the farmers' markets. Although APC is not an indicator of fecal contamination, this still should raise attention on how different stores may need to have quality control of their fresh produce. Different practices during harvest, packing and shipping for various stores, as well as good agricultural practices (GAPs) and standard operating procedures (SOPs), are carried out after arriving at the stores, and those processes may potentially contribute to the different microbial loads on the fresh produce. Supermarkets are more likely to have the supply chain of the large-scale conventional farming, while farmers' markets may have produce harvested from organic farms which apply animal manure which poses a potential risk of enteric microbial contamination [25].

Our study indicated that sampling time ("cold" and "warm" months) did not significantly affect populations of indicator organisms (APC and TC) in tested fresh produce. Additionally, we did not have an adequate number of positive samples of generic *E. coli* to demonstrate the effect of seasonal factor on generic *E. coli* populations in fresh produce, although all four positive samples were detected in "warm" months (data not shown), while in a microbial risk assessment study, results showed no significant difference in *E. coli* populations of leafy greens at harvest between spring and winter after accounting for temperature, rain, and length of growth [26]. In addition, the intactness of produce affects the behaviors of the pathogens. Damaged produce tissues provide a growth substrate for foodborne pathogens and spoilage microorganisms [27].

We did not detect *Salmonella* spp., *E. coli* O157:H7 or *Shigella sonnei*/*Shigella* spp. in the selected samples in the current study, which agreed with FDA's ready-to-eat foods guidelines that no human pathogen can be detected in ready-to-eat food samples [24]. Although no test pathogens in fresh produce were detected in our study, studies have shown that the opportunity for produce to be contaminated with foodborne pathogens are different among various types of produce regarding their intrinsic characters and external environment [28]. Additionally, the chance of detecting foodborne pathogens supposes to affect results and may be related to facility settings and the freshness of produce. Although previous studies have shown that *Salmonella* can adapt to adverse conditions, even with sanitation measures in place, one multi-state study provided strong evidence from environmental samples that *Salmonella* are rarely detected in retail grocery produce [29].

The contamination of fresh produce can occur at multiple points: during pre-harvest, post-harvest, or the handling processes of the production chain [30]. Each step, from production to consumption, may affect microbial populations of produce and influence quality [23]. Therefore, to ensure the food safety of fresh produce, it is critical to reduce contamination in the field, as well as cross-contamination during the pre- and post-harvest handling process. In addition, the FDA released specific guidelines for culinary herbs [31] and green onions [32] on how these produce can be handled properly for growers, packers and shippers. Our findings in the study provide useful information of microbiological quality in fresh produce.

5. Conclusions

Overall, leafy fresh produce, like cilantro and green onions, had significantly higher populations of APC than jalapeño peppers and serrano peppers. APC populations in farmer's markets and local markets were significantly higher than that of national chains. Risk factor analysis indicated that TC populations in green onions had a significantly higher population than that of serrano peppers. Although human pathogens (*Salmonella* spp., *E. coli* O157: H7, or *Shigella sonnei*/*Shigella* spp.) were not detected in any of the tested fresh produce, high populations of APC and TC potentially reflect poor hygienic quality of the fresh produce. It may pose a food safety and health concern to consumers and susceptible populations when this produce is consumed raw or used as garnish in prepared dishes. By properly handling, washing, preparing, and storing fresh produce, consumers can enhance the flavor of their food dishes and prevent or reduce the risk of foodborne illnesses.

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References

1. Keeling Bond, J.J.; Thilmany, D.D.; Bond, C.A. Direct Marketing of Fresh Produce: Understanding Consumer Purchasing Decisions. *Choices* **2006**, *21*, 229–235.
2. Thompson, G.D.; Kidwell, J. Explaining the Choice of Organic Produce: Cosmetic Defects, Prices, and Consumer Preferences. *Am. J. Agric. Econ.* **1998**, *80*, 277–287. [CrossRef]
3. Lee, J.H.; Hwang, J.; Mustapha, A. Popular Ethnic Foods in the United States: A Historical and Safety Perspective. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13*, 2–17. [CrossRef]
4. Denis, N.; Zhang, H.; Leroux, A.; Trudel, R.; Bietlot, H. Prevalence and trends of bacterial contamination in fresh fruits and vegetables sold at retail in Canada. *Food Control* **2016**, *67*, 225–234. [CrossRef]
5. Levy, D.J.; Beck, N.K.; Kossik, A.L.; Patti, T.; Meschke, J.S.; Calicchia, M.; Hellberg, R.S. Microbial safety and quality of fresh herbs from Los Angeles, Orange County and Seattle farmers' markets. *J. Sci. Food Agric.* **2015**, *95*, 2641–2645. [CrossRef]
6. Scheinberg, J.A.; Dudley, E.G.; Campbell, J.; Roberts, B.; DiMarzio, M.; DebRoy, C. Prevalence and Phylogenetic Characterization of Escherichia coli and Hygiene Indicator Bacteria Isolated from Leafy Green Produce, Beef, and Pork Obtained from Farmers' Markets in Pennsylvania. *J. Food Prot.* **2017**, 237. [CrossRef] [PubMed]
7. Heiman, K.E.; Mody, R.K.; Johnson, S.D.; Griffin, P.M.; Gould, L.H. Escherichia coli O157 Outbreaks in the United States, 2003–2012. *Emerg. Infect. Dis.* **2015**, *21*, 1293–1301. [CrossRef]
8. Murray, K.; Wu, F.; Shi, J.; Xue, S.J.; Warriner, K. Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions. *Food Qual. Saf.* **2017**, *1*, 289–301. [CrossRef]
9. CDC. List of Selected Multistate Foodborne Outbreak Investigations. Available online: https://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html (accessed on 10 October 2019).
10. Painter, J.A.; Hoekstra, R.M.; Ayers, T.; Tauxe, R.V.; Braden, C.R.; Angulo, F.J.; Griffin, P.M. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerg. Infect. Dis.* **2013**, *19*, 407–415. [CrossRef]
11. Johnson, R. *Foodborne Illnesses and Outbreaks from Fresh Produce*; Congressional Research Service: Washington, DC, USA, 2019.
12. Iwu, C.D.; Okoh, A.I. Preharvest Transmission Routes of Fresh Produce Associated Bacterial Pathogens with Outbreak Potentials: A Review. *Int. J. Environ. Res. Public Health* **2019**, *16*, 4407. [CrossRef]
13. Franco, W.; Simonne, A.H. Mexican Food Safety Trends: Examining the CDC Data in the United States from 1990 to 2006. *Food Prot. Trends* **2009**, *29*, 204–210.
14. Campbell, J.V.; Mohle-Boetani, J.; Reporter, R.; Abbott, S.; Farrar, J.; Brandl, M.; Mandrell, R.; Werner, S.B. An outbreak of Salmonella serotype Thompson associated with fresh cilantro. *J. Infect. Dis.* **2001**, *183*, 984–987. [CrossRef]
15. CDC. Outbreak of Salmonella serotype Saintpaul infections associated with multiple raw produce items—United States, 2008. *MMWR Morb. Mortal. Wkly. Rep.* **2008**, *57*, 929–934.
16. Teplitski, M.; Butler, J.D. Indicator Organisms: What Every Floridian May Want to Know about Microbiological Water Quality. EDIS 2008. Available online: https://journals.flvc.org/edis/article/view/117252 (accessed on 5 March 2018).
17. FDA. Bacteriological Analytical Manual (BAM). Available online: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm (accessed on 2 January 2013).
18. Teixeira, M.C.A.; Barreto, M.L.; Melo, C.; Silva, L.R.; Moraes, L.R.S.; Alcântara-Neves, N.M. A serological study of Cryptosporidium transmission in a periurban area of a Brazilian Northeastern city. *Trop. Med. Int. Health* **2007**, *12*, 1096–1104. [CrossRef]
19. HKCFS. *Microbiological Guidelines for Food (For Ready-to-Eat Food in General and Specific Food Items)*; Centre for Food Safety: Hong Kong, China, 2014.
20. Brandl, M.T. Fitness of Human Enteric Pathogens on Plants and Implications for Food Safety. *Annu. Rev. Phytopathol.* **2006**, *44*, 367–392. [CrossRef] [PubMed]
21. Mukherjee, A.; Speh, D.; Jones, A.T.; Buesing, K.M.; Diez-Gonzalez, F. Longitudinal microbiological survey of fresh produce grown by farmers in the upper midwest. *J. Food Prot.* **2006**, *69*, 1928–1936. [CrossRef]
22. Cardamone, C.; Aleo, A.; Mammina, C.; Oliveri, G.; Di Noto, A. Assessment of the microbiological quality of fresh produce on sale in Sicily, Italy: Preliminary results. *J. Biol. Res. Thessalon.* **2015**, *22*, 1–6. [CrossRef] [PubMed]

23. Johnston, L.; Jaykus, L.; Moll, D.; Martinez, M.; Anciso, J.; Mora, B.; Moe, C. A field study of the microbiological quality of fresh produce. *J. Food Prot.* **2005**, *68*, 1840–1847. [CrossRef] [PubMed]
24. USDA. Microbiological Standards and Guidelines. Available online: https://www.nal.usda.gov/fsrio/microbiological-standards-and-guidelines (accessed on 5 March 2018).
25. Maffei, D.F.; Batalha, E.Y.; Landgraf, M.; Schaffner, D.W.; Franco, B. Microbiology of organic and conventionally grown fresh produce. *Braz. J. Microbiol.* **2016**, *47*, 99–105. [CrossRef] [PubMed]
26. Allende, A.; Truchado, P.; Lindqvist, R.; Jacxsens, L. Quantitative microbial exposure modelling as a tool to evaluate the impact of contamination level of surface irrigation water and seasonality on fecal hygiene indicator *E. coli* in leafy green production. *Food Microbiol.* **2018**, *75*, 82–89. [CrossRef]
27. Francis, G.A.; Gallone, A.; Nychas, G.J.; Sofos, J.N.; Colelli, G.; Amodio, M.L.; Spano, G. Factors Affecting Quality and Safety of Fresh-Cut Produce. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 595–610. [CrossRef] [PubMed]
28. Alegbeleye, O.O.; Singleton, I.; Sant'Ana, A.S. Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. *Food Microbiol.* **2018**, *73*, 177–208. [CrossRef] [PubMed]
29. Burnett, J.; Wu, S.T.; den Bakker, H.C.; Cook, P.W.; Veenhuizen, D.R.; Hammons, S.R.; Singh, M.; Oliver, H.F. Listeria monocytogenes is prevalent in retail produce environments but Salmonella enterica is rare. *Food Control* **2020**, 113. [CrossRef]
30. Kljujev, I.; Raicevic, V.; Vujovic, B.; Rothballer, M.; Schmid, M. Salmonella as an endophytic colonizer of plants—A risk for health safety vegetable production. *Microb. Pathog.* **2018**, *115*, 199–207. [CrossRef] [PubMed]
31. FDA. *Commodity Specific Food Safety Guidelines for the Production, Harvest, Post-Harvest, and Processing Unit Operations of Fresh Culinary Herbs*; Food and Drug Administration: Silver Spring, MD, USA, 2013.
32. FDA. *Commodity Specific Food Safety Guidelines for the Production, Harvest, Post-Harvest, and Valued-Added Unit Operations of Green Onions*; Food and Drug Administration: Silver Spring, MD, USA, 2010.