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MICROBIAL PROFILES OF SELECT PRE-PACKAGED SALADS IN ODESSA TEXAS BEFORE AND AFTER THEIR BEST BUY DATES

Ugochukwu Anieto[1*], Pinidphon Prombutara[2], Jose Estrada[3], Yarelis Morales[3], Dominique Talavera[3], Karime Terrazas-Lujan[3], Kaitlyn Tittel[3]

[1]Department of Natural Science and Environmental Health, Mississippi Valley State University, 14000 HWY 82 WEST, ITTA BENA, MS 38941
[2]Chulalongkorn University, Bangkok, Thailand
[3]Science Department, Odessa College, Odessa Texas 79764

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KEYWORDS

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Salad

16S rRNA

Pathogenic

Pre- and post-expiration

Sterilization

ABSTRACT

A total of eight pre-packaged salads were purchased from eight different stores in Odessa Texas to analyze their microbial profiles based on shelf-life and sterilization regimens. The 3 sterilization regimens viz. (a) 25 g of salad in sterile water, (b) 25 g of salad in sterile water containing ten percent iodized salt and (c) 25 g of salad in sterile water maintained at 50 °C were adopted. Thereafter, one ml of each sample was inoculated into tryptic soy broth (TSB) and incubated at 37°C under anaerobic conditions for 24 hours. The DNA extractions and 16S rRNA gene sequences amplification and sequencing were subsequently performed. The same procedures were repeated under similar conditions after an average of ten days post-expiration dates of the salads with the replacement of the TSB with *Escherichia coli* (EC) Broth. Of a total of 48 DNA sequence results obtained and analyzed using the NCBI Blast software and RDP release 11 Sequence Analysis Tools, the pre-expiration salad samples revealed the presence of the Enterobacteriaceae group with the dominance of *Pantoea, Enterobacter, Tatumella, Klebsiella, Serratia, Erwinia* and *Yersinia* genera in descending order whereas the post-expiration samples showed the dominance of *Serratia, Yersinia, Klebsiella, Erwinia, Enterobacter, Pantoea* and *Tatumella* in descending order. Two pre-expiration samples indicated the possible presence

* Corresponding author
E-mail: ugochukwuanieto@my.unt.edu (Ugochukwu Anieto)

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of various *Salmonella species*. The results suggest that the microbial profiles of pre-packaged salad change with respect to aging and bearing potentially pathogenic bacteria more after expiration.

1 Introduction

Pre-packaged ready-to-eat vegetable salads and fruits is gaining popularity across the United States as evidenced by the assortment of brands that can be found in the different stores. This popularity can be attributed to the convenience it offers partly because it reduces the time spent on cutting different fruits and vegetables at different times before meals. In addition to this convenience, the price difference between cutting one's own vegetables and buying the pre-packaged options continues to shrink, making the pre-packaged option the preferred for many families especially those with young children and the working class. Whereas fresh produce can be considered vital components of a balanced and healthy diet, the risk of the transmission of foodborne pathogens cannot be overemphasized and cases of foodborne diseases outbreaks have been previously reported (Lynch et al., 2009). According to the Center for Diseases Control and Prevention (CDC), up to eight percent of foodborne diseases outbreak were linked to vegetables and fruits (Painter et al., 2013), one such example of fresh produce linked to foodborne outbreaks was the recent salad poisoning associated with *Escherichia coli* O157 in the United Kingdom which affected at least 109 persons (Hodgekiss, 2016). Recently, a business in Texas, United States, recalled 30,000 cases of fresh vegetable products from eight retail outlets after an examination returned positive results for *Listeria monocytogenes* (News Desk, 2016). Other notable incidents include cases where the parasite *Cyclospora* was linked to fresh produce in the State of Texas and sickened more than 90 persons and where cilantro and raspberries imported from the Puebla region of Mexico were contaminated with feces and resulted in 277 cases of illness (Merrill, 2015).

The microbiomes associated with different fruits and vegetables have been extensively reported by many researchers including Gombas et al. (2003), Uzeh et al. (2009), Taban et al. (2013), De Giusti et al. (2014), Gorni et al. (2015), Chau et al. (2017) etc. According to Gorni et al. (2015), beneficial and phytopathogenic microorganisms which could significantly influence the plant health and food quality are part of the profile. Similarly, Masuda et al. (2015), evaluated the dynamics of microbiological quality in lightly pickled NAPA cabbages during manufacture by analyzing the 16S rRNA composition. Other studies on the bacterial composition of fresh produce have also been reported (Abadias et al., 2006; Allen et al., 2013; Ottesen et al., 2013; Tango et al., 2014; Wijnands et al., 2014; Wood et al., 2015).

Majority of the previously reported works focused mainly on whole and minimally processed fresh produce with little or no report on fresh pre-packaged salads sold in stores across the United States. In this study, we describe the microbiome of pre-packaged salads in Odessa Texas following treatments with distilled water at room temperature, distilled water and ten percent iodized salt and distilled water at 50 °C for 25 minutes respectively. The different treatment conditions employed were to mimic actual treatment conditions of fresh produce as is common in individual homes and to investigate if such treatments alter the microbiome profiles and numbers in the pre-packaged salads. Additionally, the microbiomes of the pre-packaged salads were analyzed after the expiration dates, to determine if there were changes in the profiles and how the results would influence the future consumption of pre-packaged salads before and after expiration by consumers.

2 Materials and Methods

2.1 Handling and Processing of Salad Samples

A total of eight pre-packaged salads were purchased from eight different stores in Odessa Texas. The salads were immediately transported to the laboratory and refrigerated to maintain the same conditions at which they were kept at the stores. All samples were analyzed within two hours of purchase.

Twenty-five grams each from the eight different samples were added to 250 mL of distilled water, 250 mL of distilled water with 10% iodized salt and 250 mL of distilled water at 50 °C respectively. The samples were vigorously agitated for not less than thirty seconds to mimic the washing conditions of fresh produce and thereafter incubated for twenty-five minutes at room temperature for 25 minutes except for the samples incubated at 50 °C for 25 minutes. One mL of each sample was added to 19 mL of Tryptic Soy Broth (Carolina) bringing the total volume to 20 mL and incubated under strict anaerobic conditions at 37 °C for 24 hours. Total sample size under the three described treatment conditions was 24 (8 X 3).

2.2 DNA Extraction, Gel Electrophoresis and Sequencing Analyses

The DNA extractions of the individual samples were performed using the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories) and as described by Jarvis et al. (2015). All DNA samples were preserved at -30 °C until further use. To analyze the

16S rRNA gene sequences of the isolated microbial DNA in the different samples, the 16S rRNA sequence primers (27F-AGAGTTTGATCMTGGCTCAG and 1492R-TACGGYTACCTTGTTACGACTT) were obtained from The Midland Certified Reagent Company (a Permian Basin company). Thereafter, DNA extractions were PCR amplified using the One-Taq Hot Start DNA Polymerase (New England Biolabs) and the amplicons analyzed ongel electrophoresis for size correctness (1500bp approx.). Subsequently, the amplicons were purified using the Monarch Nucleic Acid Purification Kit (New England Biolabs), the final elution performed with distilled water and the samples were sequenced by Eurofins Genomics.

After ten days, the eight pre-packaged salads with an average of nine days past their best before dates were again analyzed under similar conditions as previously described except that the medium used was changed to the *Escherichia coli* (EC) medium (Alpha Biosciences) and the DNA polymerase used changed to the Q5 High-Fidelity DNA Polymerase (New England Biolabs). The isolated DNA samples, a total of 24 were subsequently purified and sequenced as previously described.

2.3 Statistical Analyses

The R Program (3.3.2) "Sincere Pumpkin Patch" was employed to determine the differences in mean using the Wilcoxon signed-rank test and Kruskal-Wallis one-way analysis of variance. Differences were considered significant at $P \leq 0.05$.

3 Results

3.1. Sample Analyses

Of the total 48 DNA samples sequenced, four samples (three from the pre-expiration and one from the post-expiration) were excluded from the analyses because they returned a "no sequence similarity" on the NCBI blast search. The remaining 44 DNA sequences samples were analyzed using NCBI blast software (GenBank) and the RDP Release 11 Sequence Analysis Tools to determine all the possible microbial profiles represented before and after the expiration dates of the salad. No sequence trimmings or mending operations were performed; all the members represented in the microbial profiles were included as observed from the blast search.

3.2 Representative Genera

Figure 1 revealed the seven most represented microbial profiles from all the pre- and post-expiration samples (Figure 1). The genus *Serratia* had the highest representation at 34%, obtained mostly from the post-expiration samples where as the genus *Tatumella* had the lowest representation of the seven at six percent, mostly obtained from the pre-expiration samples.

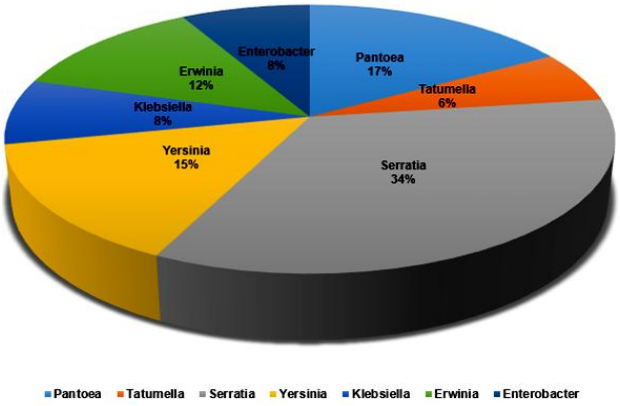


Figure 1: The seven most represented genera in the pre- and post-expiration salad samples.

In several high through put sequencing studies of fruits and vegetables used in fresh-cut product, the most abundant genus of bacteria identified includes *Erwinia* in ready-to-eat leaf vegetables and apples (Rastogi et al., 2012; Jackson et al., 2013, Masuda et al., 2015), it was therefore not surprising to have *Erwinia* well represented in the samples analyzed as it is a known plant inhabitant. Other related studies have reported the genera *Serratia, Pantoea* and *Klebsiella* as members of the fruits and vegetables communities (Nipa et al., 2011; Leff & Fierer 2013) and our findings here clearly support the previous observations as the above-mentioned genera were well represented both in the pre- and post-expiration analyses. A comparative analysis of the numerical fluctuations for the seven most represented genera was performed to determine the succession patterns with increasing storage time after the best before dates. Figure 2 below shows the

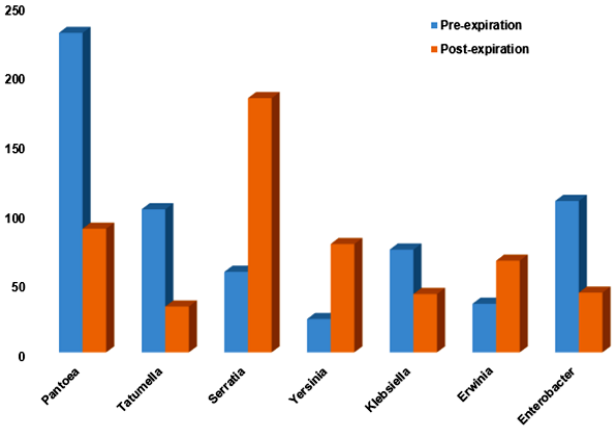


Figure 2: Comparison between the pre- and post-expiration salads samples

differences in population at pre- and post-expiration analyses From the figure 2, *Pantoea, Tatumella, Klebsiella* and *Enterobacter* genera decreased in numbers over time whereas

Serratia, *Yersinia* and *Erwinia* saw increases in numbers, with *Serratia* showing the highest increase. In a recent study, the genus *Yersinia* was identified as a food pathogen occurring in 12% of the salads analyzed (Söderqvist et al., 2016). The increase seen here therefore suggests spoilage to a level considered unsafe for human consumption even though the *Yersinia* isolates were not tested to determine if they were of the pathogenic strains. In another recent study by Dada & Olusola-Makinde (2015), *Serratia marcescens*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*etc. were identified as contaminants on different vegetables collected from retailers. Our results showed *Serratia* as a major pathogens in the contamination of the salads with increasing storage time and regardless of the treatment conditions applied.

The next step involved the analyses of all the possible genera found under the three different treatment conditions used in the pre- and post-expiration studies. A cut-off point of ten organisms per genus regardless of whether they occurred pre- or post-expiration was used to determine which organisms to be included under the study. Tables 1, 2 and 3 show the represented genera in the pre- and post-expiration studies and the percent net change across the three treatment conditions.

Table 1Representative general with ten or more organisms across the three treatment conditions (P = 0.5158)

S/N	Genus	Pre-expiration numbers (in water only)	Post-expiration numbers (in water only)	Percent increase or decrease
1	*Yersinia*	0	29	2900
2	*Hafnia*	0	15	1500
3	*Cronobacter*	0	10	1000
4	*Lonsdalea*	1	10	900
5	*Erwinia*	2	19	850
6	*Pectobacterium*	7	42	500
7	*Providencia*	9	27	200
8	*Rosenbergiella*	7	16	128.3
9	*Serratia*	20	53	62.3
10	*Buttiauxella*	29	26	-10.3
11	*Kluyvera*	20	14	-30
12	*Klebsiella*	32	21	-34.4
13	*Pantoea*	73	47	-35.6
14	*Cedecea*	12	7	-41.7
15	*Raoultella*	29	15	-48.3
16	*Leclercia*	13	6	-53.8
17	*Lelliotia*	11	5	-54.5
18	*Enterobacter*	47	19	-59.6
19	*Citrobacter*	33	13	-60.6
20	*Tatumella*	30	9	-70

Table 2 Representative general with ten or more organisms across the three treatment conditions. (P = 0.9108)

S/N	Genus	Pre-expiration numbers (in 10% iodized only)	Post-expiration numbers (in 10% iodized only)	Percent increase or decrease
1	*Morganella*	0	10	1000
2	*Hafnia*	4	24	450
3	*Yersinia*	5	20	300
4	*Lonsdalea*	3	11	266.7
5	*Serratia*	22	78	254.5
6	*Budvicia*	3	10	233.3
7	*Providencia*	16	46	187.5
8	*Pectobacterium*	25	59	136
9	*Erwinia*	15	26	73.3
10	*Rosenbergiella*	12	16	33.3
11	*Buttiauxella*	37	37	0
12	*Lelloitia*	10	7	-30
13	*Kluyvera*	13	9	-30.8
14	*Cedecea*	14	6	-57.1
15	*Leclercia*	12	5	-58.3
16	*Enterobacter*	41	14	-65.9
17	*Tatumella*	40	13	-67.5
18	*Pantoea*	90	28	-68.8
19	*Klebsiella*	21	5	-76.2
20	*Raoultella*	22	5	-77.3
21	*Citrobacter*	23	5	-78.3

Table 3 Representative general with ten or more organisms across the three treatment conditions (P = 0.5519)

S/N	Genus	Pre-expiration numbers (in 50 °c water only)	Post-expiration numbers (in 50 °c water only)	Percent increase or decrease
1	*Hafnia*	2	20	900
2	*Providencia*	6	26	333
3	*Serratia*	17	53	211.8
4	*Morganella*	4	12	200
5	*Pectobacterium*	14	40	185.7
6	*Yersinia*	11	30	57.9
7	*Buttiauxella*	14	22	57.1
8	*Erwinia*	16	19	18.8
9	*Rosenbergiella*	12	13	8.3
10	*Kluyvera*	11	9	-18.2
11	*Klebsiella*	21	17	-19.1
12	*Enterobacter*	24	14	-41.7
13	*Raoultella*	22	10	-54.5
14	*Pantoea*	67	30	-55.2
15	*Citrobacter*	21	9	-57.14
16	*Tatumella*	28	12	-57.14

Table 1 representing samples processed in water only showed a percent net change of 7500% in the bacterial populations analyzed whereas tables 2 and 3 representing samples in ten percent iodized salt and 50 °C water showed 2300% and 1600% net changes in bacterial populations respectively. These results strongly suggest that the different treatment conditions employed could have contributed to the decrease in bacterial populations with the 50 °C water treatment condition (heat shock) being the most effective and closely followed by the ten percent iodized salt treatment (osmotic pressure). Additionally, 20 and 21 genera made the ten organisms per genus cut-off for the 1st two treatment conditions whereas only 16 genera qualified for the 50 °C treatment conditions. To determine if any difference existed in the net change in actual numbers of the pre- and post-expiration bacterial populations analyzed under these different treatment conditions, a test for statistical significance using the Wilcoxon signed-rank test returned no significant differences in the pre- and post-expiration numbers for the water treatment only, 10 percent iodized salt treatment and the 50 °C treatment conditions (0.5158, 0.9108 and 0.5519 respectively, $p \leq 0.05$).

The figure 3 below shows the percent net differences under the three treatment conditions. The treatment in 50°C water produced the most effect on the microbial loads when compared to treatment in ordinary water. This is based on the observation that the samples when treated in 50°C presented less actual number of organisms in the included genera when compared to the water treatment samples only. An obvious significant difference exists in the percent net change of the bacterial populations included here and analyzed under the three treatment conditions used.

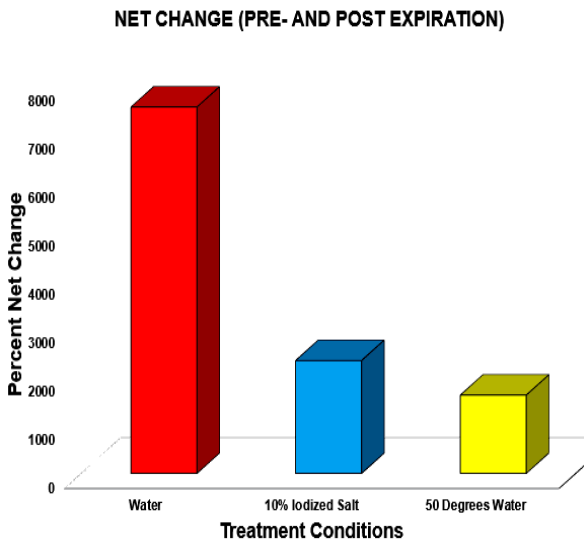


Figure 3: Percent net changes in microbial numbers of pre- and post-expiration salad samples

To determine if any difference existed in the microbial profiles of the genera represented across the three treatment conditions, all the represented genera and their net changes in the number of organisms were calculated, tabulated as seen in tables 4, 5 and 6 below and statistically analyzed using the Kruskal-Wallis one-way analysis of variance. Result obtained showed that there was no significant difference in the microbial profiles and the numbers obtained across the three treatment conditions used (0.9062, $p \leq 0.05$).`

Table 4 Net changes in microbiome profiles across the three treatment conditions. (P = 0.9062)

S/N	Water only treatment	Pre-expiration numbers	Post-expiration numbers	Net change
1	*Enterobacter*	47	19	-28
2	*Pantoea*	73	47	-26
3	*Tatumella*	30	9	-21
4	*Citrobacter*	33	13	-20
5	*Raoultella*	29	15	-14
6	*Klebsiella*	32	21	-11
7	*Leclercia*	13	6	-7
8	*Kluyvera*	20	14	-6
9	*Lelliotia*	11	5	-6
10	*Cedecea*	12	7	-5
11	*Yokenella*	6	2	-4
12	*Atlantibacter*	4	0	-4
13	*Buttiauxella*	29	26	-3
14	*Escherichia*	7	4	-3
15	*Salmonella*	2	0	-2
16	*Flavobacterium*	4	2	-2
17	*Trabulsiella*	2	0	-2
18	*Franconibacter*	1	0	-1
19	*Phaseolibacter*	2	1	-1
20	*Providencia*	9	8	-1
21	*Phytobacter*	0	1	-1
22	*Leminorella*	1	2	1
24	*Cosenzea*	0	1	1
25	*Samsonia*	0	1	1
26	*Pluralibacter*	2	4	2
27	*Siccibacter*	0	2	2
28	*Brennaria*	0	2	2
29	*Pragia*	1	4	3
30	*Pseudocitrobacter*	1	4	3
31	*Rahnella*	0	3	3
32	*Budvicia*	1	5	4
33	*Obesumbacterium*	0	4	4
34	*Dickeya*	0	4	4
35	*Edwardsiella*	0	4	4
36	*Moellarella*	0	5	5
37	*Xenorhadbus*	0	5	5
38	*Gibbsiella*	0	6	6
39	*Ewingella*	0	6	6
40	*Rouxiella*	0	6	6
41	*Lonsdalea*	1	10	9
42	*Rosenbergiella*	7	16	9
43	*Cronobacter*	0	10	10
44	*Hafnia*	0	15	15
45	*Erwinia*	2	19	17
46	*Yersinia*	0	29	29
47	*Serratia*	20	53	33
48	*Pectobacterium*	7	42	36

Table 5 Net changes in microbiome profiles across the three treatment conditions. (P = 0.9062)

S/N	10% iodized salt	Pre-expiration numbers	Post-expiration numbers	Net change
1	*Pantoea*	90	28	-62
2	*Tatumella*	40	13	-27
3	*Enterobacter*	41	14	-27
4	*Citrobacter*	23	5	-18
5	*Raoultella*	22	15	-17
6	*Klebsiella*	21	5	-16
7	*Cedecea*	14	6	-8
8	*Leclercia*	12	5	-7
9	*Kluyvera*	13	9	-4
10	*Yokenella*	4	0	-4
11	*Phaseolibacter*	4	0	-4
12	*Flavobacterium*	6	3	-3
13	*Siccibacter*	6	3	-3
14	*Lelliotia*	10	7	-3
15	*Pluralibacter*	3	1	-2
16	*Rahnella*	1	0	-1
17	*Leminorella*	2	1	-1
18	*Brenneria*	1	0	-1
19	*Escherichia*	7	7	0
20	*Buttiauxella*	37	37	0
21	*Atlantibacter*	1	1	0
22	*Cosenzaea*	0	1	1
23	*Cronobacter*	0	1	1
24	*Franconibacter*	0	1	1
25	*Pragia*	4	5	1
26	*Proteus*	1	3	2
27	*Moellerella*	1	3	2
28	*Providencia*	16	19	3
29	*Samsonia*	0	3	3
30	*Rosenbergiella*	12	16	4
31	*Ewingella*	1	6	5
32	*Obesumbacterium*	1	6	5
33	*Rouxiella*	1	6	5
34	*Dickeya*	0	6	6
35	*Budvicia*	3	10	7
36	*Lonsdalea*	3	11	8
37	*Morganella*	0	10	10
38	*Erwinia*	15	26	11
39	*Yersinia*	5	20	15
40	*Hafnia*	4	24	20
41	*Pectobacterium*	25	59	34
42	*Serratia*	22	78	56

Table 6 Net changes in microbiome profiles across the three treatment conditions. (P = 0.9062)

S/N	50 °c water	Pre-expiration numbers	Post-expiration numbers	Net change
1	*Pantoea*	67	30	-37
2	*Tatumella*	28	12	-16
3	*Citrobacter*	21	9	-12
4	*Raoultella*	22	10	-12
5	*Enterobacter*	24	14	-14
6	*Leclercia*	6	0	-6
7	*Cedecea*	8	2	-6
8	*Phaseolibacter*	4	0	-4
9	*Klebsiella*	21	17	-4
10	*Escherichia*	1	5	-4
11	*Brenneria*	4	0	-4
12	*Xenorhabdus*	5	2	-3
13	*Salmonella*	0	3	-3
14	*Kluyvera*	11	9	-2
16	*Cronobacter*	4	2	-2
16	*Pluralibacter*	2	0	-2
17	*Enterobacillus*	2	0	-2
18	*Lelloitia*	6	4	-2
19	*Yokenella*	4	2	-2
20	*Flavobacterium*	4	2	-2
21	*Leclercia*	2	0	-2
22	*Siccibacter*	4	3	-1
23	*Franconibacter*	1	0	-1
24	*Pluralibacter*	1	0	-1
25	*Pragia*	2	3	1
26	*Rosenbergiella*	12	13	1
27	*Samsonia*	0	1	1
28	*Leminorella*	0	1	1
29	*Phytobacter*	0	1	1
30	*Gibbsiella*	1	3	2
31	*Trabulsiella*	0	2	2
32	*Photorhabdus*	0	2	2
33	*Erwinia*	16	19	3
34	*Rahnella*	1	4	3
35	*Rouxiella*	1	4	3
36	*Dickeya*	0	3	3
37	*Moellarella*	0	3	3
38	*Obesumbacterium*	1	5	4
39	*Ewingella*	0	4	4
40	*Budvicia*	0	5	5
41	*Lonsdalea*	1	8	7
42	*Morganella*	4	12	8

Table 6 Net changes in microbiome profiles across the three treatment conditions. (P = 0.9062)

S/N	50 °c water	Pre-expiration numbers	Post-expiration numbers	Net change
43	*Buttiauxella*	14	22	8
44	*Proteus*	0	8	8
45	*Yersinia*	19	30	11
46	*Hafnia*	2	20	18
47	*Providencia*	6	26	20
48	*Pectobacterium*	14	40	26
49	*Serratia*	17	53	36

Of the three treatment conditions employed, four genera increased the most in numbers while another four genera saw the largest reduction in numbers after the post-expiration analyses. This was determined by taking the average in the net changes in numbers of the genera across the three treatment conditions. Figure 4 below depicts net changes seen in the eight genera representing the most increases and the most decreases in actual numbers of organisms.

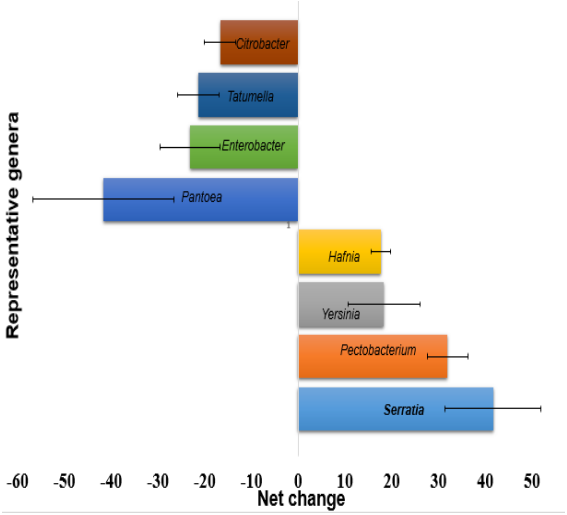


Figure 4: Average net changes for the 8 most increased and decreased genera

Whereas the genus *Citrobacter* decreased the most, *Serratia* gained the most in population after the post-expiration analyses. Of the genera that gained population in post-expiration, only the genus *Pectobacterium* is a plant pathogen whereas the rest can cause diseases in immune-compromised individuals, with the *Hafnia alvei* recently implicated in causing an infection in open fractures (Litrenta & Oetgen, 2017). All other genera have been identified by many researchers as part of the microbiomes of vegetables.

A survey of the all genera across the three treatment plans, revealed the genus that appeared only once within a treatment condition in a pre- and post-expiration analyses as seen in the tables 7, 8 and 9.

Table 7 Genus appearing only once across the three treatment conditions

S/n	Pre-expiration genera in water only	Post-expiration genera in water only
1	*Franconibacter*	*Cosenzea*
2	*Brenneria*	*Edwardsiella*
3	*Trabusiella*	*Brenneria*
4	*Phytobacter*	*Samsonia*
5	*Pseudocitrobacter*	
6	*Pragia*	
7	*Budvicia*	
8	*Lonsdalea*	

Table 8 Genus appearing only once across the three treatment conditions

S/n	Pre-expiration genera in 10% iodized salt	Post-expiration genera in 10% iodized salt
1	*Hafnia*	*Klebsiella*
2	*Rouxiella*	*Citrobacter*
3	*Ewingella*	*Pluralibacter*
4	*Proteus*	*Atlantibacter*
5	*Obesumbacterium*	*Franconibacter*
6	*Moellerella*	*Leminorella*
7	*Rahnella*	*Cronobacter*
8	*Brenneria*	*Consenzea*
9	*Atlantibacter*	
10	*Lonsdalea*	

Table 9 Genus appearing only once across the three treatment conditions

S/n	Pre-expiration genera in 50 °c water	Post-expiration genera in 50 °c water
1	*Salmonella*	*Xenorhabdus*
2	*Xenorhabdus*	*Photorhabdus*
3	*Franconibacter*	*Trabusiella*
4	*Rahnella*	*Phytobacter*
5	*Rouxiella*	*Leminorella*
6	*Gibbsiella*	*Samsonia*
7	*Hafnia*	
8	*Obesumbacterium*	
9	*Lonsdalea*	

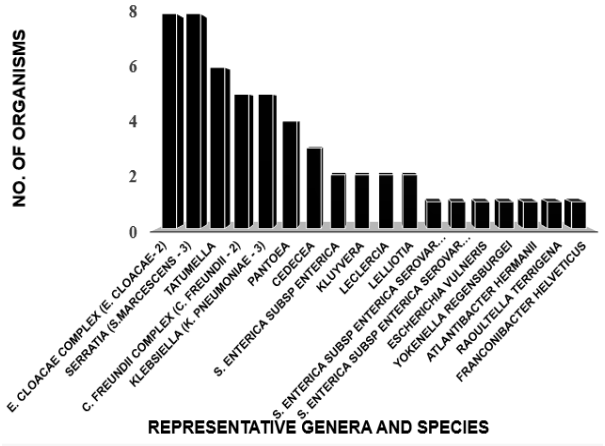
From the tables presented above, *Edward siellatarda* and *Photorhabdus temperata* subsp. *thracensis* were the only species that appeared once across the three treatment conditions, the former being a well-known source of fish poisoning (Xu & Zhang 2014; Hirai et al., 2015; Jnaneshwara et al., 2016; Habtamu &

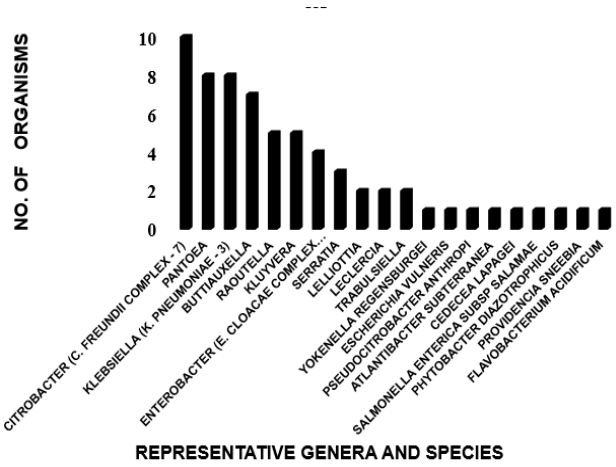
Kedebe, 2017) and the latter has extensive uses as a bioinsecticide which explains it's possible presence in the samples.

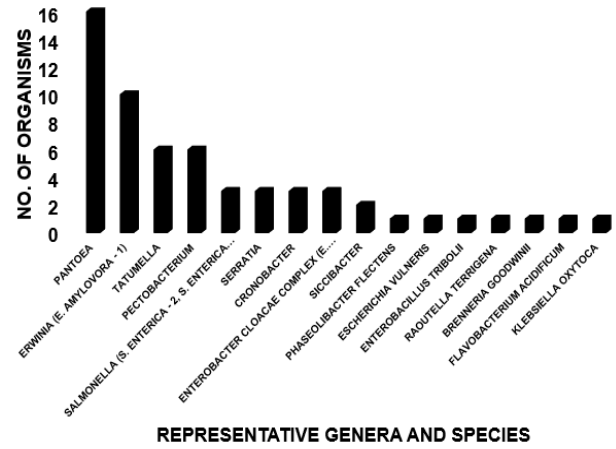
The figures 5, 6 and 7 show the samples with possible presence of *Salmonella* (two treated in water only and one treated in 50 °C water). The possible species include *S. enteric* subsp. *enteric* serovar typhi str. Ty2, *S. enteric* subsp. *enteric* serovar typhimurium,*S. enterica* subsp. *salamae* and *S. bongori.* In several studies conducted with vegetables, the occurrence of *Salmonella* has been reported. For instance, Koukkidis et al. (2017) found that exposure to salad leaf juice could contribute to the persistence of *Salmonella* on salad leaves and primed it for causing an infection in the consumer and just recently*Salmonella* outbreak was connected to hazelnuts in the state of Oregon (Beach, 2017). The possible *Salmonella* detections in this study were seen in two pre-expiration samples and did not appear in the post-expiration samples. *Salmonella* is not a common occurrence in vegetables and these occurrences is an indictment on the quality of the two salads samples in which they occurred.

4 Discussion and Conclusion

From the results obtained, it is obvious that pre-packaged salads continue to be a potential source of food poisoning more so, after their best before dates. Majority of the genera that were associated with our samples have been previously identified as members of the plant communities, with a few being pathogenic. Cases of *Serratia* and *Yersinia* seen in increasing numbers after the best before dates were clear indications of the likely pattern of microbial succession in pre-packaged salads. It is not clear as to why these genera increased substantially after the best before dates but it can be safe to assume that the degradation of the vegetables together with accumulation of fluids in the bags could have provided the right environment for them to thrive. Similarly, the previously dominant organisms observed during the pre-expiration analyses, could have reduced in numbers because of changing environmental conditions. The use of ten percent iodized salt and 50°C water appeared to have had some effect on the number of organisms represented in each genus as seen in the lower percentages in net changes for their pre- and post-expiration counts. These conditions however, did not appear to have significantly resulted in any shift in the microbiome profiles when compared to the water only treatments. Given a recent report in the State of Florida where a bat carcass was found in a pre-packaged salad (Bowman 2017), it is unwise to assume that pre-packaged salads are generally wholesome and it is advisable to discard them immediately after their best before dates to avoid possible food poisoning. This present study focused on the microbial profiles seen in salads before and after expiration and compared the profiles to the different treatment conditions that could be employed in the washing of fruits and vegetables. The data used were raw and did not undergo any further bioinformatic







Figures 5, 6 and 7: Microbiomes of the salad samples using water treatment (5 and 6) and 50 °C water (7) showing the possible presence of *Salmonella*

trimmings to narrow down the genera that could best represent the members of the communities in question. It is not known if there could have been any observable differences in the microbiomes had the cultures been incubated aerobically as against the anaerobic method used. Furthermore, the use of tryptic soy broth and subsequent changes in the medium using the EC medium did not seem to have resulted in any significant changes in the microbial profiles seen. More detailed analyses would be relied upon in the future to arrive at a conclusive evidence of the presence of potential pathogens.

Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise

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