Bacterial dynamics and the prevalence of foodborne pathogens associated with avocado fruit *Persea americana* Mill.

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ABSTRACT

The global demand for fresh produce, ready-to-eat food and the associated increase in foodborne disease outbreaks have motivated the industry to shift their focus to compliance, surveillance and preventative approaches to reduce the risk of product contamination. The microbiological quality and safety of avocado fruit from a major processing facility was evaluated over a two-year period (in and out of season). Hygiene indicator bacteria (*Escherichia coli* and coliforms) as well as Enterobacteriaceae were enumerated from 350 washed and unwashed avocado fruit *(Persea americana* Mill). The prevalence of foodborne pathogens (*Listeria* spp., including *L. monocytogenes* and *Salmonella* spp.) was also determined using the 3M™ Molecular Detection System. Presumptive positive *Listeria* and *Salmonella* isolate identities were confirmed using matrix assisted laser desorption ionization time-of-flight mass spectrometry. Isolates were characterised using virulence gene (*stx1*, *stx2* and *eae*) testing for *E. coli*, multilocus sequence typing for *E. coli* and *Salmonella* spp., as well as serotyping for *Salmonella* spp. Enterobacteriaceae was the dominant family isolated from the avocado fruit surface, representing between 72% and 92% of bacterial microbiome in season (June to Sept/October) and out of season fruit (December to February) during 2016 and 2017. The Enterobacteriaceae numbers on the in season avocado carpoplane decreased after fruit washing with oxiacid. In contrast the numbers in the fruit pulp increased after washing. The coliform numbers on both the carpoplane and pulp increased after fruit washing. The presence of *E. coli* decreased in both the carpoplane and fruit pulp following pre-washing of the fruit, while *E. coli* was isolated from 14.1% of the total number of samples. *Salmonella* spp. presence accounted for 2.4% of the total number of samples. *L. monocytogenes* was not detected in any of the avocado fruit samples during both sampling periods. Virulence genes (*stx1*, *stx2* and *eae*) were not detected in any of the *E. coli* isolates. The data indicated that 33.3% of *the E. coli* isolates belonged to ST1308 (n=8), 29.2% to ST7139 (n=7); 25% to ST1727 (n=6) and 4.2% (n=1) to ST5834; ST3580 and ST1146.

Keywords: *Persea americana*; avocado; microbial dynamics; *Salmonella* spp.; *Escherichia coli*

INTRODUCTION

Avocado is cultivated throughout the world with Mexico being the main producer (Affleck, 1986; Galindo-Tovar *et al.*, 2008). To put this in context, in 2014 Mexico produced an annual average of 1.5 million tonnes of avocado, representing 55% of the global volumes, but only export 38% of this to mainly the United States of America (USA) (Food and Agriculture Organization, 2017). South Africa is a small player in the global arena, with only 82 800 tonnes produced in 2016 (DAFF, 2017), with 51% of the total crop exported mainly to the European markets (FAO, 2017). Although the South African avocado industry is primarily export-oriented, the local demand has consistently increased over the last few decades (DAFF, 2015).

Similarly an increasing number of foodborne disease outbreaks linked to consumption of fresh produce contaminated with the potential foodborne pathogens have been reported (Beuchat, 2002; Gomba *et al.,* 2016; Kotzekidou, 2013). Several studies indicated that enteric pathogens have been detected on fresh produce (Beuchat, 2002; Lindow and Brandl, 2003). *Salmonella enterica*, *Escherichia coli* and *Pseudomonas syringe* have the ability to colonize a wide variety of fresh produce and is not limited to a specific plant type (Beuchat, 2002; Brandl and Mandrell, 2002; Lindow and Brandl, 2003).

Food safety has therefore been identified at domestic, regional and international levels as a public health priority.

In South Africa however, statistics are lacking due to incidences being underreported since most diarrhoeal illnesses are resolved within 24 to 48 hours without any medical intervention (Smith *et al*., 2007). A recent outbreak of *Listeria monocytogenes* in South Africa has resulted in more than 201 deaths and 1027 people affected (Situation report, 2018). This has shifted the attention to preventative approaches and improved surveillance systems. Since 2000, there have been 8 outbreaks associated with avocado fruit (CDC, 2016). It has been noted due to the number of outbreaks reported that *L. monocytogenes* are more frequently associated with avocado outbreaks. These pathogens are able to not only survive but also reproduce in fresh produce such as avocado stored at temperatures between 4 and 37 °C (Ma *et al*., 2016; Kirezieva *et al.,* 2015; Scallan *et al.,* 2011). Data from the Centers for Disease Control and Prevention (2014) indicates that *Salmonella* has a higher occurrence percentage with 91% in comparison to that of *E. coli* and *Listeria* spp. outbreaks with 7% and 2%, respectively.

Microbial biomes are a collection of microorganisms representing a complex, unique and complementary ecosystem that reflect the dynamic interactions of microorganisms in different habitats being influenced by environmental conditions such as temperature, humidity, pH, water activity, ultraviolet radiation and organic material (International Society for Microbial Ecology, 2013). Determining the microbiome of fresh produce will therefore contribute towards improved understanding of the persistence of human pathogens within the agricultural environment (Telias *et al.,* 2011; Ottesen *et al.,* 2013). The microbial ecology of fresh avocado fruit thus remains poorly understood (Ottesen *et al.,* 2013). According to our knowledge, we are not aware of any studies that described the natural microbiota of the avocado carpoplane.

The objective of the study was to quantify the total aerobic bacteria using coliforms and Enterobacteriaceae as indicator microbes of washed and unwashed avocado fruit samples and to determine the possible prevalence of *Listeria* spp. and *Salmonella* spp. using the 3M™ Molecular Detection System. The virulence genes and multilocus sequence types were determined for *E. coli* and *Salmonella* spp., respectively.

MATERIALS AND METHODS

Sampling site

Avocado fruit were collected from a Food Safety System Certification (FSSC) 22000 certified processing facility in Gauteng, South Africa.

Sample collection

Avocado fruit, originating from one of South Africa's largest avocado producers in the Mpumalanga Province, were collected at the point of receival and after washing at a Food Safety System Certification certified processing facility in Gauteng, South Africa. Avocado fruit were washed in a 10 000 ppm byosan sanitiser solution for 10 minutes and then placed into a dip tank for an additional 10 minutes containing 1 000 ppm oxiacid. After washing, fruit were transferred into a high-risk preparation area for further processing.

Ripe avocado fruit were collected weekly at receiving, and again after the oxiacid wash step at the processing facility. Samples were collected in and out of season during 2016 and 2017. In total, twenty avocado fruit (n=20) were sampled weekly using a random selection approach. Ten avocado fruit were sampled before washing of the fruit at the receiving point and an additional ten fruit were sampled after the oxiacid drenching. Sampled avocado fruit were placed in brown bags, labelled and kept below 5 °C during transportation to the Plant Pathology Laboratory of the University of Pretoria, South Africa, for laboratory analysis within 24 hours.

Samples were taken over a nine-week period in season (during avocado season June to Sept/Oct) and an eight-week period out of season (December to February). One sample consisted of two avocado fruit which were used for carpoplane processing and 25 grams of peeled avocado fruit for pulp processing. Twenty avocado fruit (n=20) were sampled weekly, in total 180 samples were collected in season and 160 samples out of season.

Microbiome sample processing

Avocado fruit surface

Individual avocado samples (two avocados each) were submerged in 500 mL of 0.25 x Ringer's solution containing 0.02% Tween 80 (Associated Chemical Enterprises, Johannesburg, South Africa). The samples were sonicated for 5 min using an ultrasonic bath (Eumax, Labotec, Johannesburg, South Africa). This process assists with the detachment of all microbes from the avocado fruit surfaces. After sonication, the liquid from the samples were filtered using a 0.2 mm sterile nitrocellulose membrane (Sartorius Stedim, Biotech, Germany). The filters were aseptically cut into smaller pieces, added to a 9 mL sterilized 0.1% peptone buffered water solution (Biolab Diagnostics, Johannesburg, South Africa). The tubes were vortexed vigorously to remove the organisms from the filter membrane. The samples were centrifuged (Hermle Z233 M-2, Lasec, Johannesburg, South Africa) for 7 minutes at 10 000 rpm.

DNA was extracted from the samples using Powersoil® DNA Isolation Kit following the manufacturer's instructions (Mo Bio Laboratories, Carlsbad, CA, USA); extractions were then subjected to 16S rDNA amplification using the universal primers F-27 (5'-AGAGTTTGGATCCTGGCTCAG -3') and R-1492 (5'-ACGGTTACCTTGTTACGACTT -3') (Weisburg *et al*., 1991). Amplicons were visualized in a 1% agarose gel stained with SYBR green dye (Fig. 1). A 1 kb molecular marker called Hyperladder I (Bioline, Celtic Molecular Diagnostics [Pty] Ltd, Cape Town, South Africa) was included for size estimation. The DNA concentration of each isolate was determined using the Qubit 2.0 Fluorometer (Life Technology, Johannesburg, South Africa).

A 25 µl reaction mixture containing 5 µl genomic DNA, 0.3 µl of 16S F and 16S R primers, 12.5 µl Dream TAQ and 6.9 µl sterilised water was added to make up a final volume of 25 µl. The PCR amplification was carried out on a T100 Thermal cycler (Bio-Rad, South Africa) with amplification conditions described. Initial denaturation was at 95 °C for two minutes, followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 58 °C for 45 s and primer extension at 72 °C for 1 minute 30 s, followed by a final extension of 7 minutes at 72 °C. Agarose gel electrophoresis was performed on 1% agarose gel with TAE buffer containing 0.01% Ethidium Bromide in order to visualise the samples. The gel was run for two hours at 95 volts. Bacterial and fungal diversity assay bTEFAP®20k illumine and bioinformatics analysis to be performed on samples.

Hygiene indicator bacteria *(Escherichia coli*, coliform) and Enterobacteriaceae enumeration

Avocado fruit surface

Individual avocado samples (two avocados each) were submerged in 500 mL Ringer's solution containing 0.02% Tween 80 (Associated Chemical Enterprises, Johannesburg, South Africa). The samples were sonicated for 5 min using an ultrasonic bath (Eumax, Labotec, Johannesburg, South Africa). This process assists with the detachment of all microbes from the avocado fruit surfaces. Volume displacement (vd) was documented for each of the samples and converted to area (cm^2) using the following equation ($A = 4.84 [(vd) 1/3]^2$ (De Jager, 1999; Collignon and Korsten, 2010). After sonication, the liquid from the samples were filtered using a 2 µm sterile (Sartorius Stedim, Biotech, Germany). The filters were aseptically cut into smaller pieces, added to a 9 mL sterilized peptone buffered water solution (Biolab Diagnostics, Johannesburg). Tenfold serial dilutions were prepared from the samples in PBW and total bacterial counts, Enterobacteriaceae and hygiene indicator bacteria counts were determined using standard microbiological analysis. The samples in BPW were enriched by incubating the samples at 37 °C for 24 hrs. Bacterial counts were recorded and data was converted to log (x+1) cfu/g for avocado pulp samples and log cfu/cm^2 for avocado surface samples.

Avocado pulp

Twenty-five grams of the avocado pulp was weighed in a sterile stomacher bag, mixed with 225 mL of 3M™ Peptone Buffered Water (PBW) and homogenized for 30 seconds using a stomacher (400 Circulator, Steward Stomacher, England). Tenfold dilutions were prepared from homogenized samples in PBW and bacterial counts determined as described above. Bacterial counts were recorded and data was converted to log (x+1) cfu/g for avocado pulp samples and log cfu/cm^2 for avocado surface samples.

Selective enrichment for *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. detection

For enrichment purposes for detection of *E. coli* and *Salmonella* spp. the macerated avocado fruit surface and pulp samples in 3M buffered peptone water were incubated overnight at 37 °C. Subsequently 1 mL of the enrichment broth was pelleted by centrifugation at 10 000 x g for 10 minutes. A further 1 mL of each respective subsample was added to Listeria broth (3M), supplemented with modified Listeria broth supplement (3M), and incubated overnight at 37 °C. The homogenized samples in BPW were enriched by incubating the samples at 37 °C for 24 hrs.

Following enrichment, *E. coli* was isolated by streaking a loopful of each of the samples onto Eosin Methylene Blue (EMB) agar. *Salmonella* spp. were isolated by streaking a loopfull of the enriched samples onto Brilliance™ Salmonella agar (SBM) and Xylose Lysine Deoxycolate (XLD) agar. Listeria positive samples were streaked onto Oxford-Listeria selective medium for isolation of typical Listeria colonies. All media were from Oxoid Ltd, Basingstoke Hampshire, UK.

The 24h 3M BPW and 3M *Listeria* specific enrichment broths were additionally used to determine the presence/absence of *E. coli* O157 (including H7), *Salmonella* spp. and *Listeria* spp. using the respective 3M Molecular Detection System kits according to the manufacture's specifications: 3M Molecular Detection Assay *Salmonella* (AOAC RI Certificate 031208, April 2012), 3M Molecular Detection Assay *E. coli* O157 (including H7) (AOAC RI Certificate 071202, July 2012) and 3M Molecular Detection Assay *Listeria* (AOAC RI Certificate 081203, August 2012).

Confirmation of presumptive *Escherichia coli*, *Salmonella, Listeria* isolate identities using Matrix Assisted Ionisation Time of Flight

Purified presumptive positive isolates from the selective chromogenic media were transferred in duplicate directly to the MALDI-TOF steel polished target plate (Bruker, Bremen, Germany) and subsequently analysed using the Bruker MicroFlex LT MALDI-TOF in conjunction with Bruker Biotyper Automation Software and library. Duplicate score values (SV) were recorded: a SV score higher than 1.9 indicated species identification, a score of between 1.7 – 1.9 indicated genus identification, and a score below 1.7 indicated no identification. An isolate was considered correctly identified by MALDI-TOF mass spectrometry if the score was above 2, a score of above 1.9 indicated species identification and a score above 1.7 indicated genus identification.

Bacterial characterization

DNA was extracted from each of the bacterial isolates using Quick-gDNA™ MiniPrep DNA isolation kit following the manufacturer's instructions. The presence of enterohaemorrhagic *E. coli* virulence factors (*stx1*, *stx2* and *eae* genes) were determined according to Omar and Barnard (2010). Multilocus sequence typing (MLST) was conducted based on the Achtman 7 gene scheme for *E. coli* (Wirth *et al.*, 2006) and for *Salmonella* (Achtman *et al*., 2013).

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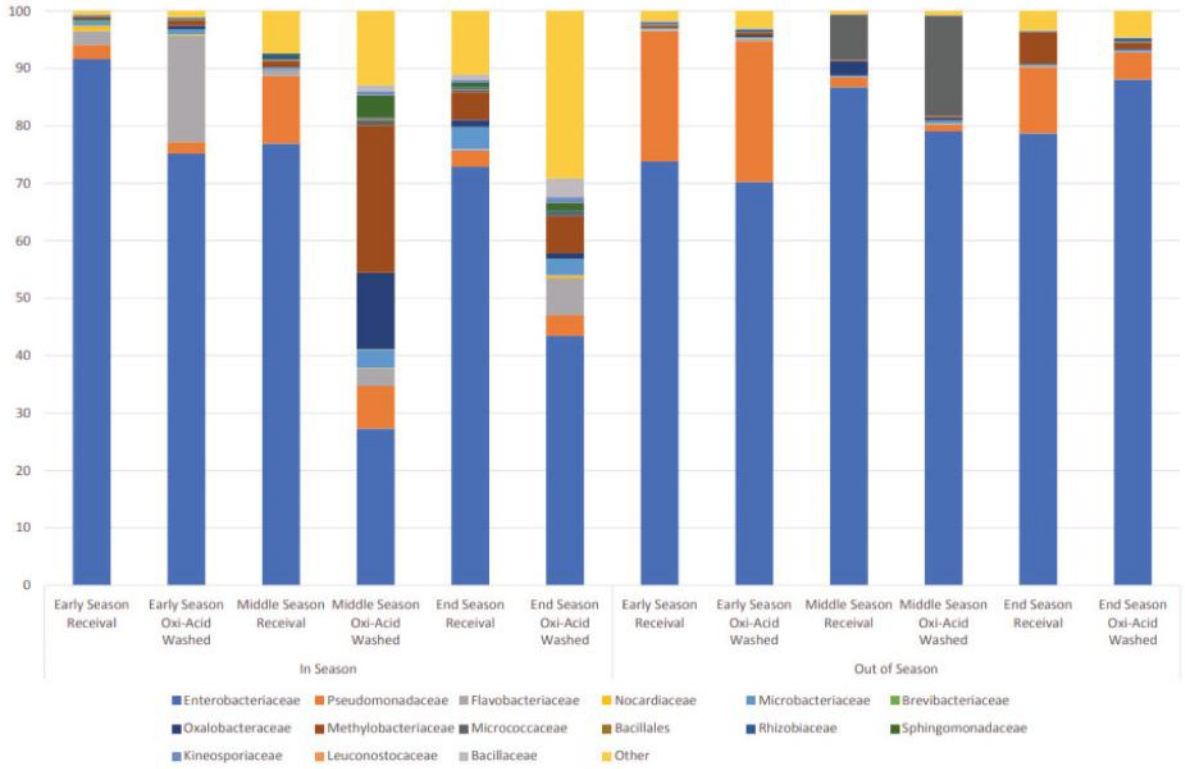

Figure 1: Microbial dynamics associated with the avocado fruit carpoplane in season and out of season.

Statistical analysis

Statistical analysis was performed on log CFU/cm^2 avocado surface counts and log CFU/g avocado pulp counts. Data were subjected to an appropriate analysis of variance (ANOVA).

RESULTS

Microbiome analysis

The Enterobacteriaceae was the dominant family isolated from the avocado fruit surface, ranging between 72% - 92% of bacterial families identified in the microbiome in season (June to Sept/October) and out of season fruit (December to February) during 2016 and 2017 (Fig.1).

Bacterial analysis

Avocado fruit surface

The results obtained for coliforms and Enterobacteriaceae from the unwashed and washed fruit are presented in Tables 1 and 2, respectively. The mean coliform count from unwashed fruit out of season was significantly higher compared to unwashed samples in season (p<0.0008). Similar to unwashed samples, the mean coliform count on washed samples out of season was significantly higher compared to washed samples in season (p<0.0008). The mean Enterobacteriaceae count on both washed and unwashed fruit surfaces out of season was significantly higher compared to samples in season (p<0.0030). Coliforms were enumerated from 100% of the samples in and out of season, however it was not possible to enumerate Enterobacteriaceae for all the samples in season.

Avocado pulp

The results obtained for coliforms and Enterobacteriaceae on unwashed and washed fruit pulp were summarized in Tables 3 and 4, respectively. Mean coliform counts on unwashed fruit pulp samples out of season were significantly higher when compared to unwashed samples in season (p<0.0001). Similar to unwashed samples, the mean coliform count on washed samples out of season are significantly higher when compared to washed samples in season (p<0.0001). The mean Enterobacteriaceae count on both washed and unwashed fruit pulp in season were significantly higher compared to samples out of season (p<0.0030).

Prevalence of *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. (including *L. monocytogenes*) on avocado fruit surfaces and in the fruit pulp

E. coli were isolated during both seasons, with 4.1% (n=7) isolated for unwashed fruit surfaces and 4.7% (n=8) isolated from washed fruit surfaces. No *Salmonella* spp. were isolated from the fruit carpoplane during the season, however out of season *Salmonella* spp. were isolated from unwashed fruit surfaces from 1.3% avocado fruit samples (n=1). No *Listeria* spp. were isolated from fruit surfaces during both seasons.

E. coli were only isolated from in season fruit pulp with 3.5% (n=6) accounted for *E. coli* on unwashed fruit and 1.8% (n=3) from washed fruit. No *E. coli* were isolated from pulp out of season. A 1.8% (n=3) of *Salmonella* spp. were isolated from pulp during the season. No *Listeria* spp. were isolated from pulp during both seasons.

Table 1: Total coliforms and Enterobacteriaceae counts on unwashed avocado carpoplane over both seasons.

Avocado carpoplane – unwashed samples						
Week	No of samples (% coliforms)	Total coliforms (log CFU/ml)		No of samples (% Enterobacteriaceae)	Total Enterobacteriaceae (log CFU/ml)	
		Min-Max	Mean[a]		Min-Max	Mean[a]
In season						
1	5 (100)	TNTC[b]		5 (100)	3.85-5.26	
2	5 (100)	4.15-5.14		5 (100)	3.98-5.38	
3	5 (100)	1.88-3.83		5 (100)	3.41-5.40	
4	5 (100)	3.56-5.05		5 (100)	3.95-4.16	
5	5 (100)	1.89-3.87		3 (60)	2.71-5.90	
6	5 (100)	3.67-4.03		5 (100)	3.90-4.51	
7	5 (100)	2.43-3.48		2 (40)	1.89-2.43	
8	5 (100)	3.55-3.99		3 (60)	2.66-3.70	
9	5 (100)	2.60-4.10		4 (80)	2.74-4.15	
Total	45		3.5806 $_{B}$	37		3.29566667 $_{B}$
Out of season						
1	5 (100)	4.15-5.16		5 (100)	3.59-4.19	
2	5 (100)	1.89-3.80		5 (100)	3.73-4.18	
3	5 (100)	3.83-5.26		5 (100)	3.75-3.87	
4	5 (100)	4.98-5.28		5 (100)	4.19-4.51	
5	5 (100)	4.16-4.25		5 (100)	3.76-4.45	
6	5 (100)	3.31-3.80		5 (100)	3.32-3.66	
7	5 (100)	3.60-3.67		5 (100)	3.48-4.36	
8	5 (100)	3.52-4.09		5 (100)	3.92-4.22	
Total	40		4.098425 $_{A}$	40		3.97455 $_{A}$

[a] Means followed by the same letter are not significantly different (Coliforms: $p<0.0008$; Enterobacteriaceae: $p<0.0030$)
[b] TNTC, too numerous to count

Table 2: Total coliforms and Enterobacteriaceae counts on washed avocado carpoplane over both seasons.

Avocado carpoplane – washed samples						
Week	No of samples (% coliforms)	Total coliforms (log CFU/ml)		No of samples (% Enterobacteriaceae)	Total Enterobacteriaceae (log CFU/ml)	
		Min-Max	Mean[a]		Min-Max	Mean[a]
In season						
1	5 (100)	TNTC[b]		5 (100)	6.22-7.14	
2	4 (80)	4.18-5.23		3 (60)	3.46-4.49	
3	5 (100)	2.75-4.24		3 (60)	3.37-4.40	
4	5 (100)	3.86-5.09		3 (60)	3.20-3.72	
5	5 (100)	2.37-3.68		3 (60)	3.55-4.37	
6	5 (100)	2.43-3.80		3 (60)	2.67-4.35	
7	2 (40)	2.82-2.83		2 (40)	2.90-3.89	
8	5 (100)	2.36-4.07		4 (80)	3.74-5.50	
9	4 (80)	1.90-3.54		3 (60)	2.08-3.06	
Total	40		3.0503375 $_{B}$	29		2.70545556 $_{B}$
Out of season						
1	5 (100)	3.85-4.31		5 (100)	3.38-4.24	
2	5 (100)	3.74-3.82		5 (100)	3.90-4.45	
3	5 (100)	4.03-4.19		5 (100)	3.47-3.70	
4	5 (100)	3.91-4.15		5 (100)	3.94-4.43	
5	5 (100)	3.62-4.03		5 (100)	4.339-4.60	
6	5 (100)	4.16-4.25		5 (100)	3.75-4.53	
7	5 (100)	3.82-3.95		5 (100)	4.14-4.44	
8	5 (100)	3.94-4.23		5 (100)	4.02-4.06	
Total	40		4.00605 $_{A}$	40		4.107175 $_{A}$

[a] Means followed by the same letter are not significantly different (Coliforms: $p<0.0008$; Enterobacteriaceae: $p<0.0030$)
[b] TNTC, too numerous to count

Table 3: Total coliforms and Enterobacteriaceae counts on unwashed avocado pulp over both seasons.

Avocado pulp – unwashed						
Week	No of samples (% coliforms)	Total coliforms (log CFU/g)		No of samples (% Enterobacteriaceae)	Total Enterobacteriaceae (log CFU/g)	
		Min-Max	Mean[a]		Min-Max	Mean[a]
In season						
1	5 (100)	1.28-1.58		4 (80)	1.54-2.23	
2	2 (40)	0.78-2.93		4 (80)	2.59-2.65	
3	4 (80)	0.41-1.41		5 (100)	2.41-2.89	
4	0 (0)	-		5 (100)	1.54-2.71	
5	1 (20)	0.3		0 (0)	-	
6	5 (100)	0.15-0.34		5 (100)	2.78-3.19	
7	5 (100)	0.51-0.92		5 (100)	1.97-2.55	
8	3 (60)	0.15-0.34		5 (100)	1.90-2.40	
9	5 (100)	0.20-1.57		5 (100)	2.63-2.75	
Total	30		0.535151 $_{B}$	33		2.07848178 $_{A}$
Out of season						
1	5 (100)	1.23-1.84		5 (100)	0.70-0.90	
2	5 (100)	1.71-2.18		4 (80)	0.85-1.43	
3	3 (60)	0.41-1.18		3 (60)	1.66-2.08	
4	5 (100)	0.15-1.37		2 (40)	2.11-2.13	
5	3 (60)	0.87-1.20		3 (60)	1.94-2.17	
6	5 (100)	0.88-1.43		5 (100)	1.43-1.92	
7	5 (100)	0.97-1.18		5 (100)	1.43-2.18	
8	3 (60)	1.22-1.86		5 (100)	0.95-1.81	
Total	34		1.114705 $_{A}$	32		1.29597925 $_{B}$

[a] Means followed by the same letter are not significantly different (Coliforms: $p<0.0001$; Enterobacteriaceae: $p<0.0030$)

Table 4: Total coliforms and Enterobacteriaceae counts on washed avocado pulp over both seasons.

Avocado pulp – washed						
Week	No of samples (% coliforms)	Total coliforms (log CFU/g)		No of samples (% Enterobacteriaceae)	Total Enterobacteriaceae (log CFU/g)	
		Min-Max	Mean[a]		Min-Max	Mean[a]
In season						
1	5 (100)	0.45-3.64		5 (100)	1.32-3.74	
2	4 (80)	0.90-1.20		4 (80)	2.40-2.63	
3	4 (80)	0.45-0.68		5 (100)	2.09-3.13	
4	4 (80)	0.51-0.79		5 (100)	2.50-2.83	
5	0 (0)	-		3 (60)	2.26-3.07	
6	3 (60)	0.38-1.38		5 (100)	2.63-3.17	
7	5 (100)	0.08-0.64		5 (100)	1.76-2.56	
8	3 (60)	0.38-3.36		5 (100)	1.90-2.38	
9	4 (80)	0.41-1.56		3 (60)	1.43-1.99	
Total	32		0.7012 $_{B}$	40		2.20170844 $_{A}$
Out of season						
1	5 (100)	1.88-2.88		5 (100)	1.32-2.57	
2	5 (100)	1.74-2.21		5 (100)	0.95-2.21	
3	5 (100)	0.41-2.23		5 (100)	1.84-2.11	
4	5 (100)	1.22-1.60		3 (60)	1.93-2.22	
5	5 (100)	0.48-1.30		5 (100)	1.46-2.22	
6	5 (100)	1.05-0.57		5 (100)	1.91-2.28	
7	5 (100)	1.22-1.49		5 (100)	1.18-2.19	
8	5 (100)	1.27-1.68		5 (100)	1.81-2.08	
Total	40		1.4859 $_{A}$	38		1.8132125 $_{B}$

[a] Means followed by the same letter are not significantly different (Coliforms: $p<0.0001$; Enterobacteriaceae: $p<0.0030$)

Virulence genes in *E. coli* isolates

None of the enterohaemorrhagic *E. coli* virulence genes (*stx1*, *stx2* and *eae*) were detected in any of the *E. coli* isolates.

Multilocus sequence typing

The *E. coli* isolates used for virulence gene testing were used for MLST characterization to determine which ST type each isolate belonged to. The data indicated that 33.3% belonged to ST1308 (n=8), 29.2% to ST7139 (n=7); 25% to ST1727 (n=6) and 4.2% (n=1) to ST5834; ST3580 and ST1146.

DISCUSSION

The recent *Listeria* foodborne disease outbreak in South Africa emphasized the need for determining the microbiological quality and safety of fresh produce, including ready-to-eat products in the whole supply chain. The increase in foodborne disease outbreaks globally, and the USA compulsory inspection of import fruit consignments, created a need to better understand the avocado bacterial microflora and assess the prevalence of foodborne pathogens on the avocado carpoplane and fruit pulp. In this study, the Enterobacteriaceae numbers on the avocado fruit carpoplane decreased, while it increased in the pulp after washing of in season fruit. An increase in the coliform numbers on both the carpoplane and pulp following pre-processing fruit washing was observed. These results indicated that the wash water might potentially contribute to the increased microbial load. Similarly, Duvenage *et al*. (2016) reported an increase in bacterial populations on the pear carpoplane following drenching. In contrast, the presence of *E. coli* decreased in both the carpoplane and fruit pulp following pre-washing of the fruit, with *E. coli* isolated from 14.1% of total number of samples. A study on ready-to-eat salads, containing avocado, also reported contamination with faecal coliforms and *E. coli* (Castro-Rosas *et al.,* 2013). *Salmonella* spp. presence accounted for 2.4% of the total number of samples. No *L. monocytogenes* were isolated from the avocado fruit samples, which is in agreement with the results reported by Strydom *et al.* (2016). They concluded that regular in-house monitoring of the listerial population in avocado processing facilities and implementing risk management strategies based on these results can prevent contamination and associated food borne disease outbreaks.

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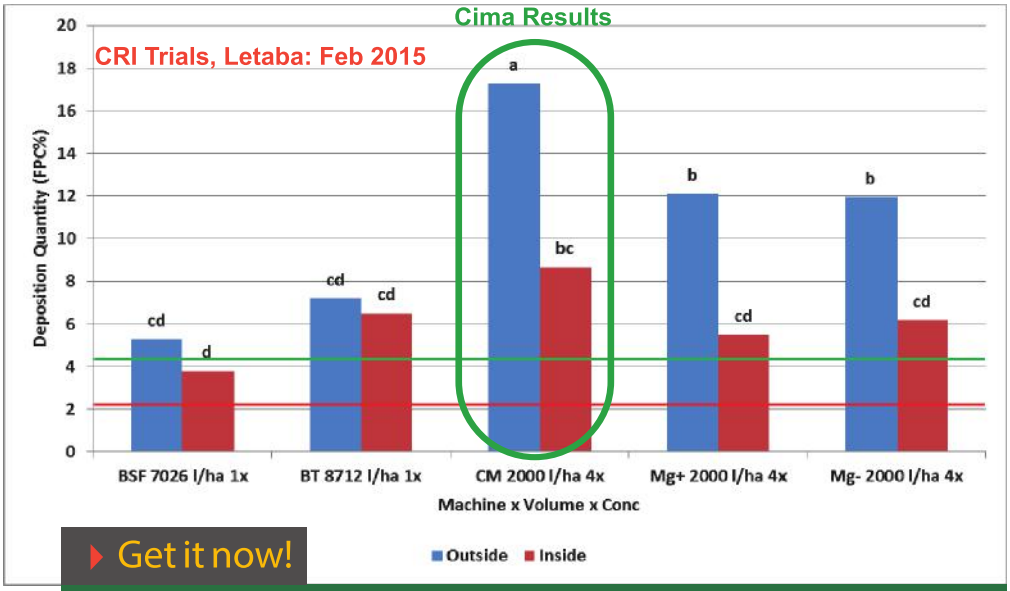

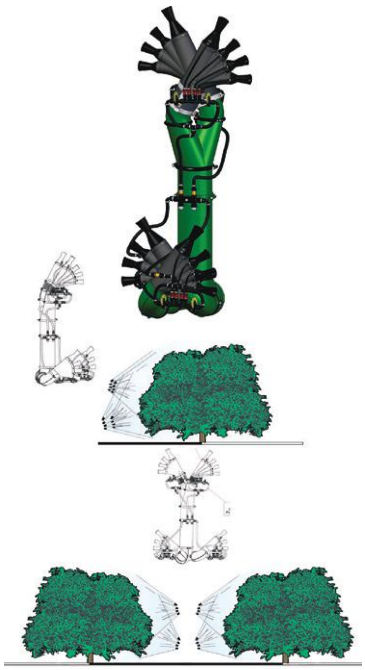

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