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Articles

A Preliminary Microbial Survey of Ready-To-Eat Salads at Popular Food Establishments in Trinidad.

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Abstract

Ready-to-eat salads are becoming increasingly popular as they combine the healthy characteristics of fruits and vegetables with that of a short preparation time. This study seeks to determine the microbial quality of ready-to-eat salads that are sold in food establishments in Trinidad.

A total of 56 samples were collected from two supermarkets and two shopping malls and were analyzed using culture procedures. The total number of aerobic mesophilic bacteria and Escherichia coli colonies were determined for each salad sample. Samples were also tested for Salmonella spp.

The average number of aerobic bacteria was 6.3±1.1 logCFUg-1 with a range of 4.3 to 7.5 logCFUg-1. The level of aerobic bacteria in the salad samples was dependent on the food establishment from which it was purchased and varied significantly across food establishments (P<0.001). The shopping malls had higher levels of aerobic bacteria than supermarkets (P<0.001). E. coli was found in 100% of the salad samples analyzed and the level present varied significantly among food establishments (P<0.05). The average E. coli colony count was 3.7±0.7 logCFUg-1 ranging from 2.7 to 5.0 logCFUg-1. Salmonella spp. was detected in 67.86% of the samples analyzed. The number of salad samples contaminated with Salmonella varied significantly during the two periods of testing, week 1 and week 2 (P<0.05).

In this study, the researcher only sampled ready-to-eat salads from four of the many food establishments in Trinidad. Due to the high number of: aerobic bacteria and E.coli colonies, and samples contaminated with Salmonella found in the samples analyzed, it was recommended to increase the sample size in order to provide a general overview of the quality of ready-to-eat salads sold in Trinidad.

***Keywords**: Ready-To-Eat (RTE) Salads, Microbial Quality, Aerobic Mesophilic Bacteria, Escherichia Coli, Salmonella spp.*

Introduction

Food security has often been perceived as having enough food; and important elements, such as food safety and nutrition security were frequently overlooked (Eggersdorfer et al. 2016, 313). Today, food safety is receiving increasing recognition as an important link between food and health and is addressed simultaneously with other essential components to achieve and improve

food security (Unnevehr 2003). In addition, the globalization of food trade has amplified the chances that food produced in a particular location can affect the health of people living in another country (Tritscher et al. 2013). Food safety has become a joint concern for countries worldwide; Trinidad and Tobago included.

To increase food production and food security, the Government of Trinidad and Tobago is focused on the major challenges to the agricultural sector. One of these challenges include: the limited capacity among enterprises, both small and medium, in meeting internationally accepted standards relating to sanitary and phytosanitary measures and food safety (IICA 2011). This issue was proposed to be mediated by promoting and enabling the development of a more strengthened and integrated agricultural health and food safety system and by strengthening programs for improving quality, grades and standards in the areas of agricultural health and food safety (IICA 2011).

Global and national initiatives and promotions aiming to improve food nutrition security have encouraged people to increase their consumption of fruits and vegetables (Sapers, Gorny and Yousef 2006, 04). Consumption of low amounts of fruits and vegetables is one of the top ten selected factor for global mortality and therefore emphasis is placed on increasing the awareness and understanding on the importance of fruits and vegetables intake in an effort to prevent non-communicable diseases (WHO 2003). Due to the scientifically confirmed benefits of fruits and vegetables, the public has become more health conscious over the years (Sapers, Gorny and Yousef 2006, 04). However, outbreaks of foodborne diseases associated with the consumption of raw fruits and vegetables frequently occur in developing countries but have become numerous in developed countries (Beuchat 1998).

The occurrence of foodborne diseases may be influenced by: irrigation and other agronomic practices undertaken during production; the hygienic practices involved in handling fruits and vegetables; international travel and globalization of the supply and distribution of raw fruits and vegetables; the introduction of pathogens in new geographic areas; changes in the virulence and environmental resistance of the pathogens; decrease in the immunity of the population and; changes in the eating habits of the population (Beuchat 1998).

Foodborne diseases – also known as foodborne illness or food poisoning – are the consequence of foodstuff contaminated with microorganisms or chemicals which can occur at any stage in the food chain: from food production to consumption. According to the Center for Foodborne Illness, Research and Prevention (CFI), there are more than 250 foodborne diseases that can be caused by bacteria, virus, parasites, harmful toxins or chemicals (CFI 2013). Foodborne diseases comprise of a wide range of illnesses and are responsible for high levels of morbidity and mortality worldwide especially the population at risk such as infants, young children, the elderly and the immuno-compromised (WHO 2014). The CFI estimated that globally, two billion people suffered from diarrheal disease each year, with many of these illnesses caused by contaminated food and/or water (CFI 2013). Foodborne diseases not only affects poor or developing countries; in the United States, it is estimated that 48 million persons or 1 in every 6 Americans gets sick, 128, 000 are hospitalized and 3000 die of foodborne diseases (CDC 2011).

Saperset al in 2006 stated that while the probability of being sickened by a foodborne illness via consumption of fresh fruits or vegetables is low; however, the probability still exists as these produce are most often eaten uncooked and contaminated produce cannot be completely disinfected by washing or rinsing the product in an aqueous solution (Saperset al 2006, 5). The Alliance for Food and Farming after analyzing the CDC United States databases during the period of 1990 to 2007, found that approximately 12.3% of all foodborne outbreaks were associated with produce and from that 10% were related with improper handling after leaving the farm and 2.2%

were associated with growing, packing, shipping or processing of the produce (Alliance for Food and Farming 2010). Most of the outbreaks (65% of outbreaks) associated with produce contaminated after leaving the farm was attributed to mishandling at the food service level and this was followed by mishandling at community events (14% of outbreaks) and mishandling in the home (13 % of outbreaks) (Alliance for Food and Farming 2010). Bacteria such as Clostridium botulinum, Bacillus cereus and Listeria monocytogenes are normal dwellers of many soil while Salmonella, Escherichia coli, Shigella and Campylobacter inhabits the intestinal tract of animals and are considered more likely to contaminate raw produce through contact with faeces, sewage, untreated/contaminated irrigation or surface water (Beuchat 1998).

This project aims to determine the microbial quality of ready-to-eat salads that are sold in food establishments in Trinidad. Ready-to-eat (RTE) foods are food and food products that have underwent some kind of processing and can be consumed without undergoing any further bacterial treatment such as heating. Salads are considered as a RTE food and are mixtures of minimally processed vegetables and/or fruits (raw vegetables / fruits which have been washed, peeled, sliced, chopped or shredded) with or without dressing. Due to their convenience value, RTE foods have become increasingly popular among consumers today (Jaroniet al. 2010). However, since these foods are minimally processed or are consumed raw, the microbial risks have also increased (Jaroni et al 2010). The four major bacterial pathogens associated with RTE foods are Salmonella spp, Escherichia coli, Listeria monocytogenes and Clostridium perfringens (Jaroni et al 2010). This study targeted two foodborne pathogens: Salmonella and E. coli, which are becoming increasingly popular, especially in fresh produce.

Materials and Methods

Sample Collection

Using the purposive sapling method, four (4) large-scale food establishments were selected for this study. Fourteen (14) ready-to-eat fruit and vegetable salads were collected from the four (4) food establishments in Trinidad during the period of August to September, 2014. Samples were collected from two supermarkets located in: Port of Spain and St. Augustine; and from restaurants in two shopping malls located in: Port of Spain and the Churchill Roosevelt Highway. Seven (7) samples were collected per week from each food outlet and the remaining seven samples were collected after one (1) consecutive week. Samples were collected during 11:00 hrs to 12:00 hrs. During collection, salads samples were placed in sterile, labeled sample bags and then transported to the laboratory for microbial analysis in a sterile, insulated cooler with ice. The salad samples were then tested for the presence of aerobic bacteria, Escherichia coli and Salmonella sp.

Aerobic Colony Count

The aerobic colony count was used as an indicator of the quality of the food rather than safety. It indicates the number of bacteria in the RTE salads that can grow in the presence of oxygen at mesophilic temperatures. Since there was no available guideline to determine the microbial quality of RTE salads in terms of the number of bacteria present, one was established by the researcher. The researcher classified the number of bacteria present according to high, medium and low (Table 1).

Table 1: Guidelines for the Microbial Quality of ready-to-eat salads – Aerobic Bacteria

Food Category	Criterion	Microbiological Quality (CFU per ml)		
		Low	Medium	High
Ready-to-eat Salad (fruits and Vegetables)	Aerobic Colony Count	$< 10^4$	10^4 to $<10^6$	$\geq 10^6$

Testing for E. coli

Ten grams (10g) of each sample was homogenized in a stomacher with Buffer Peptone Water (BPW).Decimal dilutions were prepared (10^{-1} to 10^{-5}). Each dilution was used to inoculate the MacConkey (MC) agar using the spread plate method. Plates were incubated at 37^0C for 24 hours. After incubation, the plates were examined for suspicious colonies of *E. coli*. Colonies appearing pink to red in colour with bile salt precipitate surrounding them on the MacConkey plates were confirmed using biochemical tests: indole test, Voges-Proskauer (VP) Test and Methyl Red test, Simmon Citrate test and the triple sugar iron agar test.

Testing for Salmonella spp.

Ten grams (10g) of sample was homogenized in a stomacher with Buffer Peptone Water (BPW). The mixture was then incubated at 35^0C±2^0C for 24±2.0 hours. 1 ml of the mixture was transferred to 10 ml tetrathionate Broth (TT) which was then incubated for 24±2 hours at 35^0C. After incubation, a 3 mm loopful of the inoculated TT broth was streaked on the Brilliant Green agar (BG) agar and the Xylose Lysine Desoxycholate (XLD) agar. The plates were incubated for 48±2 hours at 35^0C. After incubation, the plates were examined for the presence of suspicious *Salmonella* colonies. On the XLD agar: colonies appearing pink with or without black centers or colonies with large, glossy, black centers or almost completely black colonies were tested for *Salmonella*. On the BG agar: colonies appearing from red to pink-white surrounded by a brilliant/intense red zone were also tested for *Salmonella*. Suspicious colonies found on the BG agar and the XLD agar were confirmed using biochemical tests: triple sugar iron agar test, urease test, Lysine decarboxylase (LDC) test and the Indole Test.

Microbial Examination

Microbial results for the level of *Escherichia coli* and *Salmonella* were compared to the PHLS (Public Health Laboratory Service) guidelines for the microbial quality of ready-to-eat foods sampled at the point of sale (Table 2). Satisfactory results indicate good microbial quality; acceptable results are indices reflecting a marginal limit of microbial quality; and unsatisfactory results suggests that more sampling should be done and it warrants the inspection of the food production practices by health officers (PHLS 2000). Unacceptable results suggest that urgent attention is needed to identify the source of the problem and a risk assessment is advised (PHLS 2000).

Table 2: Guidelines for the Microbial Quality of ready-to-eat salads (PHLS 2002)

Food Category	Criterion	Microbiological Quality (CFU per gram)			
		Satisfactory	Acceptable	Unsatisfactory	Unacceptable / Potentially Hazardous
Ready-to-eat Salad (fruits and Vegetables)	*Escherichia coli*	<20	20 to <100	≥100	N/A
	Salmonella spp.	not detected in 25g			detected in 25g

N/A – not applicable

Statistical Analysis

All statistical analyses, including the means, standard deviation, medians, and graphs were conducted using the SPSS Statistics Base 16.0 program. One-way analysis of variance tests were performed to detect the statistical differences ($P < 0.05$) of the microbial levels among food establishments. The Tukey HSD test was used to perform multiple comparisons ($P<0.05$). The independent t-test was used to determine the significant differences in the microbial level of RTE salads collected from supermarkets and shopping malls. This test (independent t-test) was also used to compare the average microorganisms found in salads samples collected in week 1 and week 2. The Chi-square test was used to determine if the microbial quality of the salads were dependent on the food establishment from which they were purchased. Significance was measured at the 0.05 probability.

Results and Discussion

A total of 56 ready-to-eat (RTE) salad samples were collected during the year 2014. Salad samples were obtained from two supermarkets and two shopping malls in Trinidad. Each food store/outlet was sampled twice. The RTE salad samples were mixed, consisting of both fruits and vegetables such as: carrots, cucumbers, lettuce, tomatoes, and cabbages. The salad samples were analyzed for their microbial quality using culture techniques and confirmed using biochemical tests at the University of the West Indies Microbiology Laboratory.

Microbial Quality of Samples from the four Food Establishments

Table 3: The average aerobic and *E. coli* colonies in salad samples and the average number of samples contaminated with *Salmonella* for each food establishment.

Food Sore	Mean Aerobic Colony Count (log $CFUg^{-1}$)	Mean No. of *E. coli* colonies (log $CFUg^{-1}$)	Mean No. of Samples Contaminated with *Salmonella spp.*
Supermarket A	5.3 ± 1.1^{a}	3.7 ± 0.6^{a}	0.5 ± 0.5^{a}
Supermarket B	5.9 ± 0.8^{a}	3.9 ± 0.9^{a}	0.8 ± 0.4^{a}
Shopping Mall C	6.9 ± 0.5^{b}	3.2 ± 0.6^{b}	0.6 ± 0.5^{a}
Shopping Mall D	7.2 ± 0.4^{b}	4.0 ± 0.4^{ac}	0.8 ± 0.4^{a}
Total	6.3 ± 1.1	3.7 ± 0.7	0.7 ± 0.5
P-value	0.001	0.008	0.322

Escherichia coli isolates were observed in 100% (56) of the salad samples surveyed, and the number of colonies found in samples for the various food establishments differ significantly from each other, P = 0.008 (Table 3). All samples (56) recorded microorganism levels above 4.0 log CFUg-1 with samples from the two shopping malls recording significantly higher number of colonies than samples from the supermarkets, P = 0.001 (Table 3). On the other hand, there were no significant differences in the average number of samples contaminated with Salmonella spp., P = 0.322 (Table 3).

Aerobic Bacteria

The aerobic colony count (AAC) is also known as the total viable count or the standard plate count and it is an indicator of the quality, not the safety of the salads; thus does not contribute towards a safety assessment (Health Protection Agency 2009). However, the AAC can be used to provide information on the remaining shelf-life of the food product and can emphasize potential handling and storage problems (Health Protection Agency 2009).

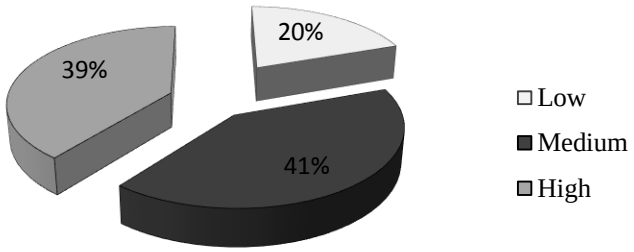

Figure 1: Microbial Quality of RTE Salads – Aerobic Bacteria

In this experiment, the aerobic colony count is used as an indicator of the sanitation processes involved in the preparation of the food product (RTE salads). A high aerobic colony count may be an indication that the product may have been prepared un-hygienically or stored inappropriately (New South Wales Food Authority 2009). A low aerobic colony count indicated that there may be no sanitation problems; however, it is not a guarantee that the sample is free of pathogens (New South Wales Food Authority 2009). In this study, the number of aerobic bacteria found in the RTE salads was placed into 3 categories: high, medium and low. 19.6% (11) samples had low levels of aerobic bacteria, 41.1% (23) had medium level of bacteria and 39.3% (22) recorded high levels of aerobic bacteria (Figure 1).

Escherichia coli and Salmonella spp.

Escherichia coli is an indicator organism which confirms the presence of faecal contamination (Ashbolt et al 2001). Food tested positive for E. coli can be contaminated by many factors such as: natural contamination, actual growth in the food product, personnel contamination and poor sanitation practices (Hayes 1992, 196).

Salmonella spp. belongs to the family of Enterobacteriaceae and can be commonly found in the intestinal tract of animals (New South Wales Authority 2009). These enteric bacteria are most

often associated with raw foods of animal origin; however it can be found in a wide range of food and food products (New South Wales Authority 2009). The presence of Salmonella spp. in ready-to-eat salads can be due to: poor handling practices and cross contamination (New South Wales Authority 2009).

To determine the microbial quality, the number of E. coli colonies found in the RTE salads from the 4 food establishments was placed into 3 categories (according to the PHLS guidelines): satisfactory, acceptable and unsatisfactory. Microbial quality for Salmonella spp. was placed into 2 categories: satisfactory and unacceptable / potentially hazardous (according to the PHLS guidelines).

It was observed that all, 100%, RTE salad samples had an unsatisfactory level of E. coli present (Table 4). On the other hand, 32.1% (18) of the samples were free of Salmonella spp. and were classified as satisfactory while 67.9% (38) samples tested positive for Salmonella and were therefore placed in the unacceptable / potentially hazardous category (Table 4).

Table 4: Frequency of Microbial Quality – *E. coli* and *Salmonella spp*.

Microbial Quality	% of RTE salads contaminated with *E. coli* (n=56)	% of RTE salads contaminated with *Salmonella spp.* (n=56)
Satisfactory	0.0 (0)	32.1 (18)
Acceptable	0.0 (0)	N/A
Unsatisfactory	100.0 (56)	N/A
Unacceptable / Potentially Hazardous	N/A	67.9 (38)
Total	100.0 (56)	100.0 (56)

N/A means not applicable.

Dependence of Microbial Quality on Food Establishments

Escherichia coli

The level of E. coli contamination for the four food establishment was unsatisfactory, as 100% of the RTE salad samples from each food store/outlet had exceeded 2.0 logCFGg-1. Therefore, the dependence of E.coli contamination on food establishment was undetermined.

Salmonella spp.

Based on the results (Table 5), it can be observed that the microbial quality of the salad samples in terms of Salmonella presence, is not dependent on the food establishment from which it was obtained, $\chi 2$ (3, N=56) = 3.60, p=0.31.

Table 5: The Microbial Quality of Each Food Establishment – *Salmonella spp*.

Food Establishment	% of RTE Salads that were Satisfactory	% of RTE Salads that were Unacceptable/Potentially Hazardous
Supermarket A	50.0 (7)	50.0 (7)
Supermarket B	21.4 (3)	78.6 (11)
Shopping Mall C	35.7 (5)	64.3 (9)
Shopping Mall D	21.4 (3)	78.6 (11)
Total	32.1 (18)	67.7 (38)

$\chi^2 = 3.603$ df= 3 P=0.308

Aerobic Bacteria

Table 6 demonstrated that the microbial quality of the salad samples in terms of aerobic bacteria, is affected or dependent on the food establishment from which it was purchased, $\chi 2$ (6, N=56) = 30.18, p<0.001.

Table 6: The Microbial Quality of Each Food Establishment – Aerobic Bacteria

Food Establishment	% of RTE Salad Sample that were		
	Low	Medium	High
Supermarket A	57.1 (8)	21.4 (3)	21.4 (3)
Supermarket B	21.4 (3)	71.4 (10)	7.1 (1)
Shopping Mall C	0.0 (0)	42.9 (6)	57.1 (8)
Shopping Mall D	0.0 (0)	28.6 (4)	71.4 (10)
Total	19.6 (11)	41.1 (23)	39.3 (22)

$\chi^2 = 30.18$ df= 6 P<0.001

Microbial Quality of RTE Salads obtained from Supermarkets versus Shopping Malls

Table 7: The average aerobic and *E. coli* colonies in salad samples and the average number of samples contaminated with *Salmonella* for supermarkets and shopping malls.

Food Establishment	Average Aerobic Bacteria Colonies (log $CFUg^{-1}$)	Average *E. coli* Colonies (log $CFUg^{-1}$)	Average No. of Samples contaminated with *Salmonella*
Supermarket	5.6 ± 1.0^a	3.8 ± 0.8^a	1.6 ± 0.5^a
Shopping Mall	7.1 ± 0.5^b	3.6 ± 0.6^a	1.7 ± 0.5^a
P-value	0.0001	0.22	0.27

*Results are expressed as mean ± standard deviation
*Means with the same letter are not significantly different from each other

An independent t-test was done to compare the means of the microorganisms (E. coli, Salmonella spp., aerobic bacteria) found in salads samples from supermarkets against the means of the microorganisms found the samples from shopping malls (Table 7). It was observed that there was no significant difference (p=0.22) in the mean number of E. coli colonies found in the samples from the supermarkets (3.8 ± 0.8 log CFUg-1) and shopping malls (3.6 ± 0.6 log CFUg1). Similar results was obtained when samples were tested for Salmonella (p=0.27). However, there was significant difference (p<0.001) in the average number of aerobic bacteria colonies of salads purchased from supermarkets (5.6 ± 0.99 log CFUg-1) when compared to those purchased from shopping malls (7.1 ± 0.45 log CFUg-1). This may be due to the unhygienic handling of the food in the shopping malls which was observed during the sample collection.

Comparison of the Average Microorganisms found in the RTE Salads for Two Durations

Table 8: The average aerobic and *E. coli* colonies in salad samples and the average number of samples contaminated with *Salmonella* for week 1 and week 2.

Microorganism	Week	Mean	P-value
Average No. of Samples contaminated with *Salmonella*	Week 1	0.57±0.5[a]	0.02
	Week 2	0.79±0.4[b]	
Average *E. coli* Colonies (log $CFUg^{-1}$)	Week 1	3.9±0.7[a]	0.49
	Week 2	3.5±0.6[a]	
Average Aerobic Bacteria Colonies (log $CFUg^{-1}$)	Week 1	6.4±1.0[a]	0.95
	Week 2	6.3±1.0[a]	

*Results are expressed as mean ± standard deviation

*Means with the same letter are not significantly different from each other

Based on the independent t-test it was observed that there was no significant differences in the average aerobic bacteria colony count (p=0.95) and the average number of *E. coli* colonies ((p=0.49) for the salad samples tested during the periods of week 1 and week 2 (Table 8). However, the average number of samples contaminated with *Salmonella* differ significantly (p=0.02) between the two periods of testing (Table 8). In week 1, 16 of the 28 samples tested were contaminated with *Salmonella*; while in week 2, 22 of the 28 samples were contaminated with *Salmonella*.

Conclusions

This study takes an initial step towards understanding the relationship between the microbial quality of ready-to-eat salads and food-service establishments. Fifty-six (56) samples of ready-to-eat salads were collected twice from each food establishment and were tested for: aerobic mesophilic bacteria, Escherichia coli and Salmonella spp.

It was found that the number of colonies of aerobic mesophilic bacteria varied significantly among the four food establishments and was dependent on the store/outlet from which it was purchased. E. coli was present in all samples at an unsatisfactory level and also varied significantly among the four stores/outlets. Salmonella on the other hand, did not vary and the organism's presence in food samples was not dependent on the place from which it was bought.

Samples obtained from shopping malls and those from supermarkets have similar levels of E.coli and Salmonella contamination; however, RTE salads from the shopping malls had significantly higher levels of aerobic bacteria.

The week in which the samples were collected from each food establishment did not affect the number of aerobic bacteria and E. coli colonies found in the salads. However, the number of samples contaminated with Salmonella in week 2 was significantly higher than that of week 1.

In this study, the researcher only sampled ready-to-eat salads from four of the many food establishments in Trinidad. Due to the high number of: aerobic bacteria and E.coli colonies, and samples contaminated with Salmonella found in the samples analyzed, it was recommended to increase the sample size in order to provide a general overview of the quality of ready-to-eat salads sold in Trinidad.

It was also noted that fruits and vegetables used in the preparation of the RTE salads were subjected to various conditions across the food chain (growth, harvesting, transportation, and preparation) that could increase contamination; and treating these products by disinfection does not assure complete removal of pathogenic microorganisms. It was suggested that measures such as: good agricultural practices (GAP), good hygienic practices (GHP), good manufacturing practices (GMP) and Hazard Analysis and Critical Control Points (HACCP); be implemented to minimize the risk of microbial contamination from farm-to-fork.

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