STATISTICAL DISTRIBUTIONS DESCRIBING MICROBIAL QUALITY OF FRESH PRODUCE IN FOOD SERVICE FACILITIES

By

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ABSTRACT OF THE THESIS

Statistical Distributions Describing Microbial Quality of Fresh produce in Food Service Facilities

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Data on the microbial food quality of fresh Ready-to-eat produces were collected in Rutgers dining services from 2001 to 2013. Total aerobic plate count, total coliforms, confirmed *Bacillus cereus* count were determined using standard methods. Statistical analysis was performed on foods tested more than 35 times. Statistical distributions and histograms were generated using SigmaPlot and Microsoft Excel. All data could be described using normal distributions, once data above or below the upper or lower limit detection were considered separately. The mean value of total aerobic plate count of fresh produce were ranged from 3.40 to 6.45 log CFU/g. Among all the samples, spinach, pepper and carrot had higher mean count, while apple, onion and cauliflower had the lowest mean counts. Coliforms were most commonly found in apple, broccoli, romaine lettuce and spinach, and least commonly found in pepper. The average count of total coliform count (when present) ranged from 0.60 to 3.46 log CFU/g, with carrot having highest average, while apple having the lowest average count. *Bacillus cereus* were most commonly detected in onion and least detected in tomato. *B. cereus* counts (when present) were highest on average in broccoli and

lowest in pepper. Average *B. Cereus* counts for most produces were typically between ranged from 2 and 3 log CFU/g.

Acknowledgements

I would like to thank my advisor, Dr. Schaffner, for accepting me as his student when I started my Master's study. He always provided valuable advice and honest criticism. With his instruction and help during the past two years, not only have I learned to use statistical methods to analyze real world data in food microbiology research, but also I saw my potential and interest in academia, thus I have found my future career goal.

I would like to thank my committee members, Dr. Takhistov and Dr. Zylstra, for their precious suggestions, critique my thesis and their time.

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Chapter I - Literature Review

I.1 Foodborne disease associated with fresh produce

Fresh produce has been playing a more and more important part in American diet, due to their richness of vitamins and minerals, which is great promotion to people's health. It is reported that the consumption of fresh produce increased by over 30% during the past few years (1). However, with the increasing consumption of fresh produce, the proportion of produce-related outbreaks has also been increasing during the past decades (2). That may result from the higher perishability of fresh produce compared to processed food, and also the fact that produce are usually consumed raw, which means less chance for reducing possible microbial contamination of the produce, comparing with cooked food (3).

There are several source of foodborne pathogen contamination of Fresh produce. Preharvest contamination source including the usage of untreated irrigation water, contaminated organic fertilizers such as manure, runoff water from livestock operations, and invasion of wildlife or domestic animal which can occur anywhere in the farm (4, 5, 6). Postharvest contamination source including inappropriate operations during handling, washing, processing and packaging (7).

As a good source of important vitamins, minerals, and phyto-nutrients, the consumption of leafy green vegetables has been continuously increasing(8). However, based on a report from USDA Economic Research Service, leafy greens have been the category of produce that most likely to be associated with foodborne illness. In the

United States since 1996, it has been reported that 34% of outbreaks traced back to vegetables and fruits, 10% of cases of illness, and 33% of deaths were associated with leafy greens (9).

Several types of leafy green vegetables has been identified as vehicles of certain foodborne pathogens. Iceberg lettuce has been reported to be in associated with *Shigella sonnei*,*Yerisinia pseudotuberculosis*, and *Salmonella spp.* (7, 10, 11). Romaine lettuce has been association with *E. coli O157:H7* (12), and *E. coli O145* (13). Spinach has been linked to E. coli O157:H7 (9).

Besides leafy green vegetables, other types of fresh produce has been identified as vehicles of foodborne pathogens in the U.S., including apples, carrots, cucumber, melon, mushrooms, onions, peppers, potatoes, sprouts and tomatoes. The most commonly pathogens identified in produce-related outbreaks were Norovirus, *Salmonella* spp., and pathogenic *E. Coli* (14).

It has been reported that apple juice and cider were associated with *E.coli O157:H7* (15). Carrots has been associated with *Clostridium botulinum*, *Shigella sonnei*,*Yerisinia pseudotuberculosis* (16, 17, 18). Buchholz reported that cucumber was one of the vehicles associated with *E.coli O104:H4* in the large outbreak in German in 2011 (19). Based on a report from Center of Disease Control and Prevention in 2008, honeydew was associated with *Salmonella* spp. in the outbreak in New Jersey, 2007. Cantaloupe was reported to linked with *Listeria monocytogenes* and *Salmonella* spp. (20, 21).

I.2 Foodborne pathogens and indicator organisms

Produce-related foodborne disease outbreaks are mainly associated with bacterial pathogens. Pathogenic *Escherichia coli, Salmonella* spp., *Listeria monocytogenes, Clostridium perfingens, Staphylococcus aureus,* and *Bacillus cereus.*

I.2.a *Salmonella* spp.

Salmonella is a genus of rod-shaped, gram-negative, motile bacteria. They are found in worldwide cold-blood and warm-blood animals and in environment. Their optimal growth temperature is 38℃, and can survive in raw food, soil, water, factory and kitchen surfaces, and feces. They cause illnesses such as typhoid fever, paratyphoid fever, and gastrointestinal foodborne illness. *Salmonella* has been associated with a variety of fresh vegetables and fruits like tomatoes, alfalfa sprouts, melons and peppers in the past outbreaks (22, 23, 24, 25, 26).

I.2.b Pathogenic *Escherichia coli*

Pathogenic *E. coli* are a group of *E.coli* that cause foodborne illnesses of human, they are gram-negative, rod-shaped, facultative anaerobic bacteria. The most famous serotype of pathogenic *E. coli* are *E.coli O157:H7, E.coli O104:H4*. The specific produce that have been linked to pathogenic *E. coli* including lettuce, spinach, sprouts and apple juice. It was reported that from 1996, 83% of leafy green-related outbreaks were linked to *E. coli O157:H7* (9).

I.2.c *Listeria monocytogenes*

Listeria monocytogenes is gram-positive, facultative anaerobic bacterium that can cause Listeriosis, a serious infective disease with high fatality of over 20%, which makes it one of the most virulent foodborne pathogens (27). The disease predominantly strikes pregnant women, newborns, elderly and people with compromised immune systems.Outbreaks were mainly linked to ready-to-eat food including cheese, fresh celery and cantaloupe (28).

I.2.d *Clostridium perfingens*

Clostridium perfingens is estimated to cause one million cases of foodborne illnesses each year, and considered to be the second common bacteria that cause foodborne illnesses in the United States (29). Typically, *C.perfingens* spores germinate in raw or poorly cooked food under anaerobic conditions, or food that is properly cooked but let to stand to long. After ingestion, the bacteria start to form spores and produce *Clostridium perfingens* enterotoxin (CPE) which cause disease (30). It is reported that from 1998 to 2010, there were 289 confirmed *C. perfingens* outbreaks, 15,208 cases of illnesses, 83 hospitalizations, and 8 death. In most of these outbreaks *C. perfingens* is associated with meat and poultry (31).

I.2.e *Staphylococcus aureus*

One of the leading microorganisms that cause food poisoning is *Staphylococcus* *aureus*. They cause gastroenteritis resulting from consumption of contaminated food which contain the Staphylococcal enterotoxin. *Staphylococcus aureus* can survive and grow in a wide range of temperature an pH, which enables them to grow in a wide variety of foods. The food sources associated with *Staphylococcus aureus* are different among countries, as different countries have different consumption habits (32). In the United States, meat, salad, poultry, pastries, milk and seafood have been reported to responsible for Staphylococcal food poisoning (33).

I.2.f *Bacillus cereus*

Bacillus cereus is responsible for minority of foodborne illness in the United States, causing abdominal cramp, vomiting and diarrhea. Bacillus illnesses result from the survival of Bacillus endospores during improperly cooking which primes the spores to germinate, and subsequently improperly storage which allows vegetative growth of the bacteria. Bacterial growth then leads to production of enterotoxin that leads to Bacillus illness. In a report from 1998 to 2008, *B. cereus* is mainly linked to rice dishes, meat and poultry (34).

I.3 Indicator microorganisms

Since it is not feasible to detect all the foodborne pathogens in every food microbial quality test, the presence and amount of indicator microorganisms have been wildly used to suggest the sanitary level of food, as well as the risk of the presence of foodborne pathogens.

Chapter II - Statistical Distributions Describing Microbial Quality of Fresh produce in Food Service Facilities

II.1 Introduction

The number of produce-based foodborne illness outbreaks continues to be a source of concern. A report from Centers for Disease Control and Prevention (CDC) of the United States showed that reported outbreaks per year associated with fresh produce doubled between 1988 and 1992, compared to those between 1973 and 1987, and the percentage of illnesses and outbreaks associated with produce also increased, from 1% and 0.6%, to 12% and 6%, respectively (22). Another CDC study showed that from 1998 to 2008, nearly 46% foodborne illnesses, 38% hospitalizations and 23% deaths were attributed to produce (35). In a Canadian survey covering data collected between 2001 and 2009, 27 outbreaks and 1,549 cases of foodborne illness were linked to produce (36).

Produce-related foodborne outbreaks and illnesses are predominantly associated with bacterial pathogens. Pathogenic *Escherichia coli* is an important cause of foodborne human gastrointestinal disease, which is estimated to cause about 269,000 illnesses annually in the United States (37). Pathogenic *E. coli* has been linked to outbreaks with a wide variety of food products, including meat, juice, milk and fresh produce, which can be contaminated in the field through contaminated irrigation water, improperly composted manure or animal intrusion. Pathogenic *E. coli* has been specifically linked to lettuce, spinach, sprouts and apple juice.

Salmonella spp. causes more hospitalization and deaths from foodborne illness in the United States than other pathogens (38), with multiple outbreaks linked to produce since the 1990s. Before 1990, most *Salmonella* outbreaks were associated with poultry and poultry products (2), while in 2002-2003, it was reported that 31 *Salmonella* spp. outbreaks were produce-related, comparing to 29 outbreaks were attributed to poultry products (39). *Salmonella* has been associated with a variety of vegetables and fruits, with large outbreaks linked to tomatoes, alfalfa sprouts, cantaloupes, Serrano and jalapeno peppers and unpasteurized fruit juices (22, 23, 24, 25, 26}.

Listeria monocytogenes is the causative agent of Listeriosis, a serious infective disease with high mortality. The disease predominantly strikes pregnant women, newborns, elderly and people with compromised immune systems. A report from Centers for Disease Control and Prevention (CDC) shows 1651 cases of Listeriosis from 2009 to 2011, with a case fatality rate of 21%. Outbreaks are mainly associated with consumption of ready-to-eat food, and recent outbreaks were caused by cheese, fresh celery and cantaloupe (40).

Because foodborne pathogens may be present sporadically and at low concentrations, they can be difficult to detect. To address this issue, tests for “indicator” microorganisms, instead of pathogens, have been widely used to assess the microbial safety of food and water. Indicator organisms are useful because they thought to “indicate” the possible presence of pathogens, but are themselves present at higher concentrations and greater frequency that the pathogens they represent and are therefore more easily and reliably detected. The presence and amount of indicator microorganisms are thought to indicate the risk for the presence of foodborne pathogens, and are often use as a measure of quality control during food processing.

Different indicators can reveal different aspect of microbial quality of food. Aerobic plate count (APC) can indicate the time-temperature history and degree of microbiological control level during the production and transport of fresh produce. The total coliforms test include all aerobic and facultative anaerobic Gram-negative, nonspore-forming bacteria that can ferment lactose with production of acid and gas at 35 °C within 48h, and includes *E. coli, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae,* and *Klebsiella pneumoniae*. This classification is based on biochemical-reaction (or metabolism) rather than any genetic relatedness. Another widely used indicator test is for “fecal coliforms”, which include facultative anaerobic rod-shaped, Gram-negative nonspore-forming bacteria that can grow in the presence of bile salts, and ferment lactose to acid and gas at 44 °C within 48h. Historically called “fecal coliforms”, the term thermotolerant coliform is more correct, as microorganisms fitting this biochemical classification are not necessarily associated with feces. The most prominent thermotolerant or fecal coliform, generic *Escherichia coli* (also known as *E. coli* Biotype I) is generally considered the best indicator of fecal contamination because it is commonly found in the intestine of humans and warm-blooded animals. It is widely used in the assessment of the microbial quality of food and water samples. It is also considered more specific than fecal coliforms as its test does not detect non-fecal thermotolerant coliforms (41).

To assess the microbiological quality of food, especially ready-to-eat (RTE) food including fresh produce, many microbial surveys have been conducted over the years and around the world. In such surveys, researchers collect food sample from local markets, grocery stores, restaurants and/or cafeterias, and subject these samples to a variety of microbiological tests to determine the presence and/or concentration of foodborne pathogens and indicator microorganisms. The data and information gained in those surveys can be used subsequently to guide those facilities to improve their practices and reduce food safety risk. The knowledge gained can also support future food safety research and the development of food safety programs to control potential risk.

Numerous microbiological surveys have been conducted worldwide, including ones done in the United States, Canada, Mexico, China, and Spain (42, 43, 44, 45, 46). Rutgers University also has a long history of weekly, year-round microbiological quality inspection on the university dining halls for over 40 years, as part of food safety program in response to a foodborne disease outbreak which occurred in the 1960’s (47). Random inspections are currently conducted at six large dining halls and many smaller facilities at Rutgers. Data currently being collected and recorded include location, date and time of sampling, temperatures of ready-to-eat foods, total aerobic plate count, coliforms, *E. coli*, *C. perfringens*, *B. cereus*, *L. monocytogenes*, *S. aureus* counts, and presence of *Salmonella*. Our lab has previously published analyses on foods tested more than 50 times (primarily lunch meats and deli salads) and on surfaces tested more than 500 times (36 different surfaces types, including pastry brushes, cutting boards, and countertops) (48, 49).

With the recent interest in the microbial safety of fresh produce, the focus of Rutgers University dining hall food testing program shifted to include more fresh produce items. We have observed that produce items often do not meet the original testing criteria developed for ready-to-eat foods when the program was created (47). The object of this thesis is to characterize the microbial quality of ready-to-eat produce items in Rutgers University dining halls using the data collected over the past thirteen years, with an eye towards the development of new microbial quality standards for Rutgers University ready to eat produce.

II.2 Materials and Methods

Methods

Microbial analysis. All data were obtained from samples taken from dining facilities operated by Rutgers University from between 2001 to 2013. Facilities ranged from large dining halls serving thousands of meals every day to small cash operations. Temperatures of foods were obtained using a sterilized, calibrated thermometer (Thermapen, Thermoworks, Lindon, UT) before pickup. Foods were taken from serving lines or the kitchen and placed into sterile whirl-pack bags (Fisher Scientific, Pittsburg, PA) using sterilized utensils, then they were transported back to the laboratory in an insulated bag with ice, and stored in a lab refrigerator until testing.

Total aerobic count of foods was determined using Food and Drug Administration standard methods. Twenty-five grams of each food was weighed into a stomacher bag (Fisher brand, Pittsburgh, Pa.) with 225 ml of peptone water. The food was homogenized in a stomacher (Seward, London, UK) for 1 to 2 min depending on texture. Homogenate was serially diluted of 10^{-1}-10^{-5} of original homogenate and 0.1 ml of each diluted homogenate was spread on total aerobic count agar using sterile glass spreading rod.

Presumptive and confirmed total coliform and fecal coliform counts were determined using the most-probable-number (MPN) method. Aliquots of homogenate and dilutions up to 10^{-5} were added in triplicate to lauryl tryptose broth (Difco, BD) containing Durham tubes and incubated at 37 °C. At 24 and 48 h, tubes were checked for gas production, and transfers were made from positive tubes with a sterile loop to tubes containing brilliant green broth and *Escherichia coli* (EC) broth (Difco, BD). Brilliant green tubes were incubated at 37 °C and EC tubes were incubated at 45 °C. Tubes were checked for gas production at 24 and 48 h. An MPN calculator was used to determine number of presumptive and confirmed coliforms and presumptive *E. coli* count (50). All the samples containing gas were streaked onto Eosin-Methylene Blue (EMB) agar (Difco, BD) with a sterile loop. EMB plates were incubated at 35 °C for 24 h. Black, dark green colonies or colonies have a green sheen growing on EMB plates were Gram stained. Each colony consisting of gram-negative short rod shaped bacteria was used to inoculate in an Enterotube (Difco, BD), and incubated at 35 °C for 24 h. An Enterotube Interpretation Guide (codebook) was used to determine species of the inoculated bacterium.

Bacillus cereus was determined using Mannitol-Egg Yolk-Polymyxin (MYP) agar (Difco, BD) medium. Spread plates of MYP medium were prepared in duplicate, depositing 0.1ml of 10^{-1} dilution on each plate using sterile glass spreading rod. The plates were incubated at 30 °C for 24h. Pink-red colonies with a precipitation zone surrounding the colony were counted and further tested by Gram stain and hemolysis reaction. Samples with strong hemolytic activity and with production of a 2-4 mm zone of complete hemolysis surrounding the colony were counted.

Data analysis. Data were exported from the relational database in Access Data (Microsoft, Redmond, WA) to Excel (Microsoft) for preliminary analysis, and then from Excel to SigmaPlot (Systat Software, San Jose, CA), where histograms and box plot were created, and correlations were determined. Similar data sets were combined where appropriate. Distributions were created using SigmaPlot. The Anderson-Darling test was used to determine goodness of fit. After initial analysis, the normal distribution was selected to represent the log bacterial count on produce on the basis of its generally high statistical ranking, visually acceptable fits, and accepted use of describing microbial distributions in ready-to-eat produce. The normal distribution is a two-parameter distribution with parameters μ and σ, where μ is the mean and σ^2 is the variance.

II.3 Results

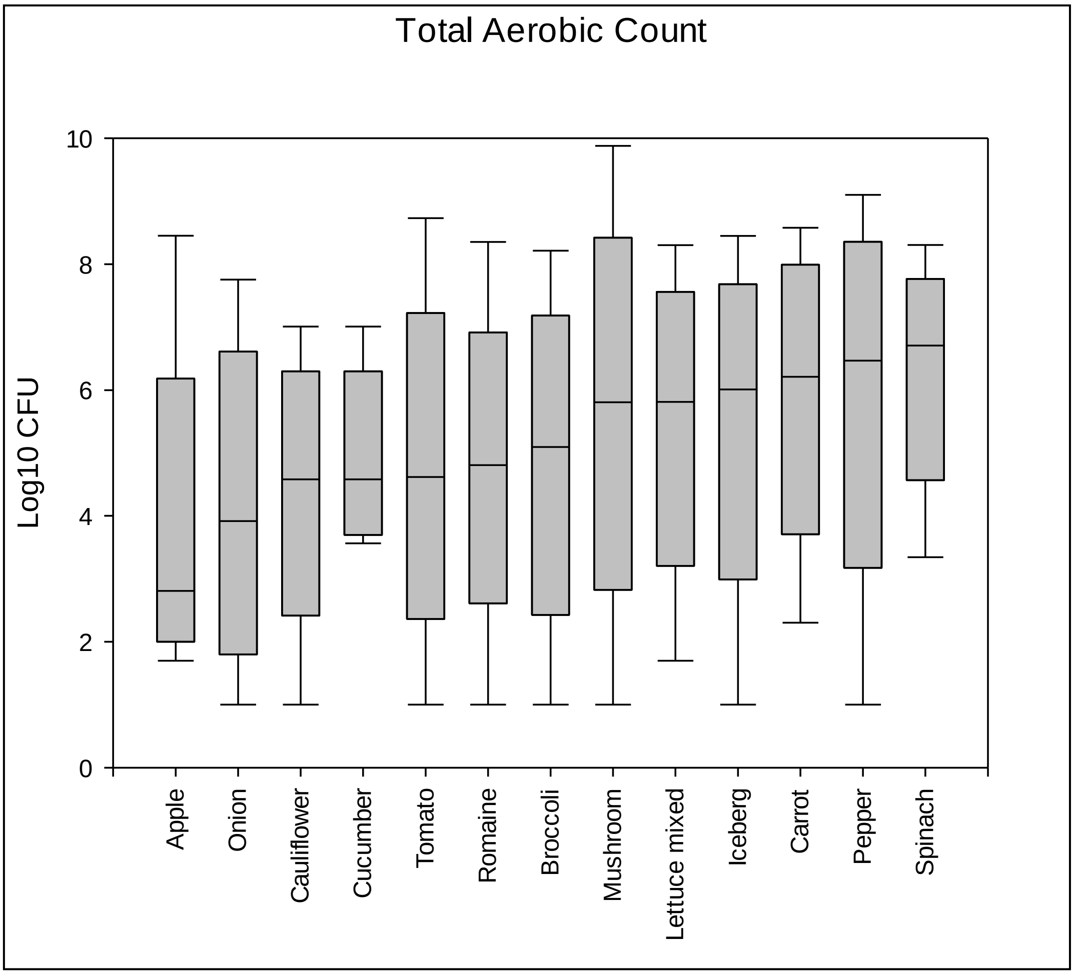

FIGURE 1. Box plot of total aerobic counts per gram (log scale) in different RTE produces.

The levels information of total aerobic count is shown in Figure 1 (sorted by median, from low to high). The counts range from 1 to 9.88 log CFU/g. Apple, onion and cauliflower had the lowest median of total aerobic counts of 2.81, 3.92, and 4.58 log CFU/g, respectively. Spinach, pepper and carrot had the highest medians of 6.71, 6.50, and 6.21 log CFU/g, respectively.

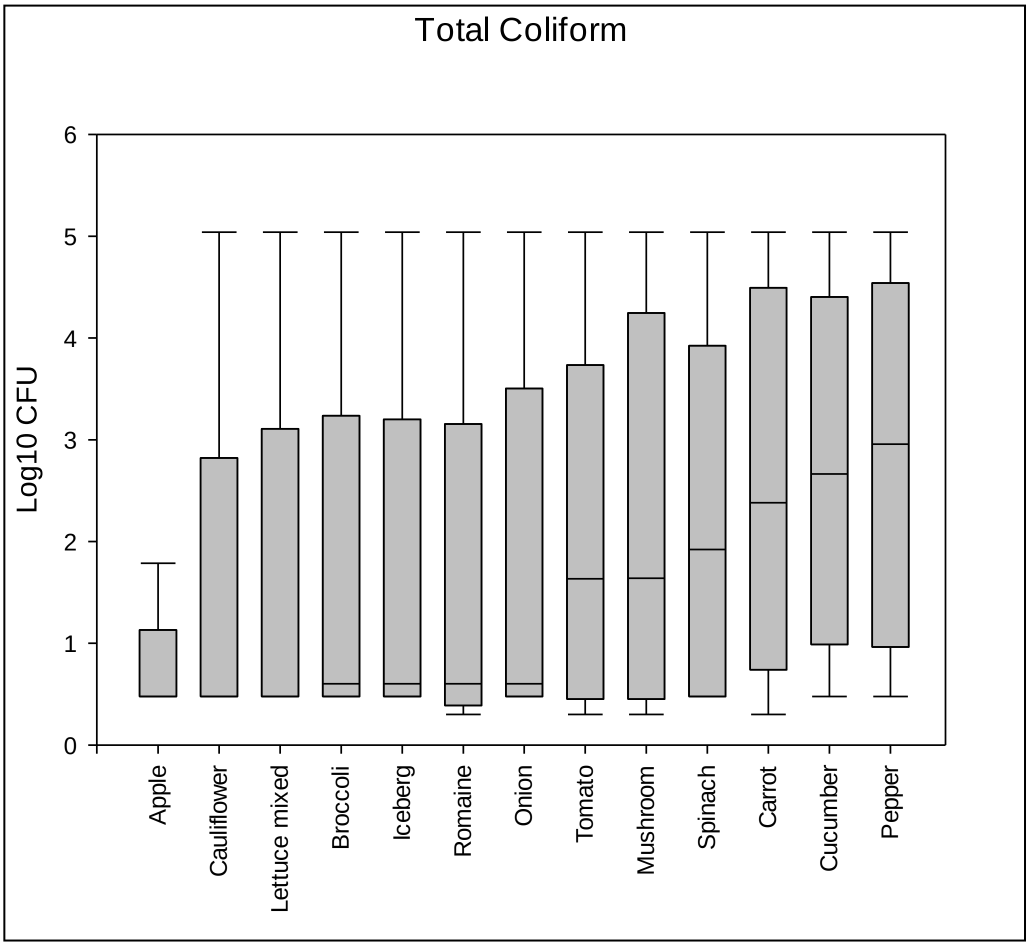

FIGURE 2. Box plot of total coliform counts per gram determined by a three-tube most-probable-number (MPN) method (log scale) in different RTE produces with positive samples.

The levels information of coliform count is shown in Figure 2 (sorted by median from low to high). The counts range from 0.30 to 5.04 log MPN/g. Apple, cauliflower and mixed lettuce had the lowest median of total aerobic counts (0.48, 0.48, and 0.48 log MPN/g, respectively), while pepper, cucumber and carrot had the highest medians (2.96, 2.66, and 2.38 log MPN/g, respectively).

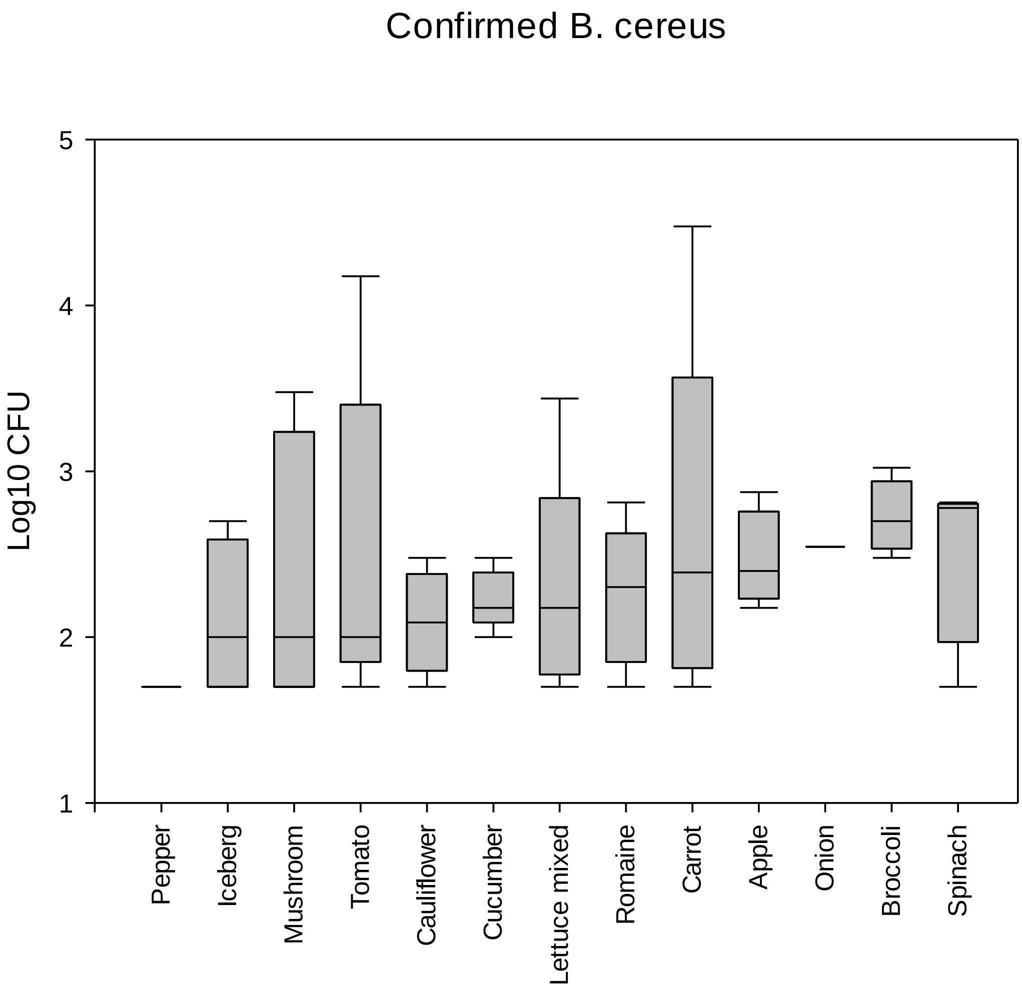

FIGURE 3. Box plot of *Bacillus cereus* counts per gram determined by MYP agar selection and hemolysis test (log scale) in different RTE products with positive samples.

The levels information of *Bacillus cereus* count is shown in Figure 2 (sorted by median from low to high). The counts range from 1.70 to 4.48 log CFU/g. Pepper, iceberg lettuce, mushroom and tomato had the lowest median of total aerobic counts (1.70, 2.00, 2.00, and 2.00 log CFU/g, respectively), while spinach, broccoli and onion had the highest medians (2.78, 2.70, and 2.54 log CFU/g, respectively).

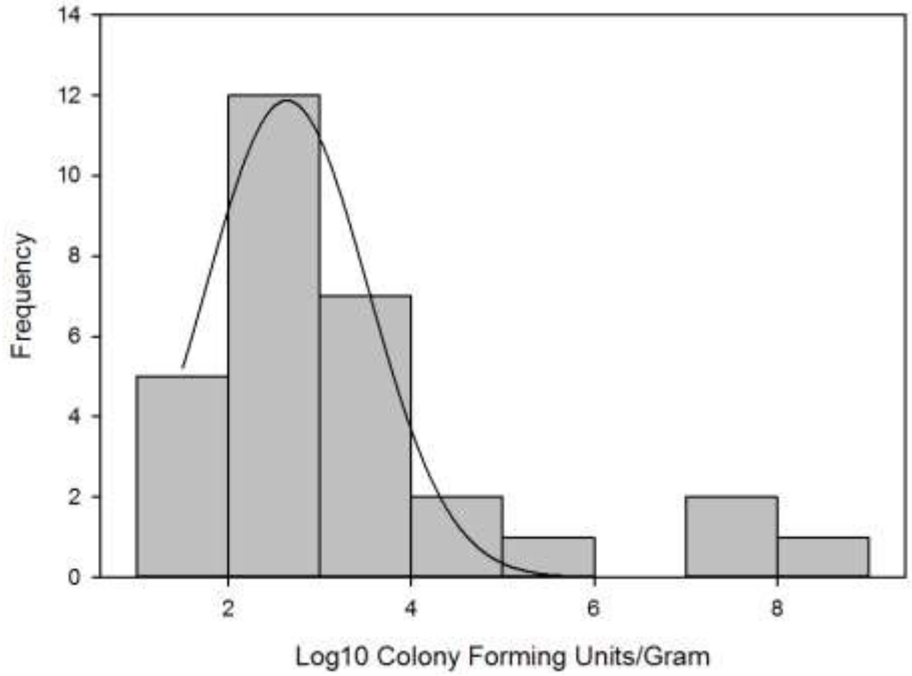

FIGURE 4. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 30 samples of apple.

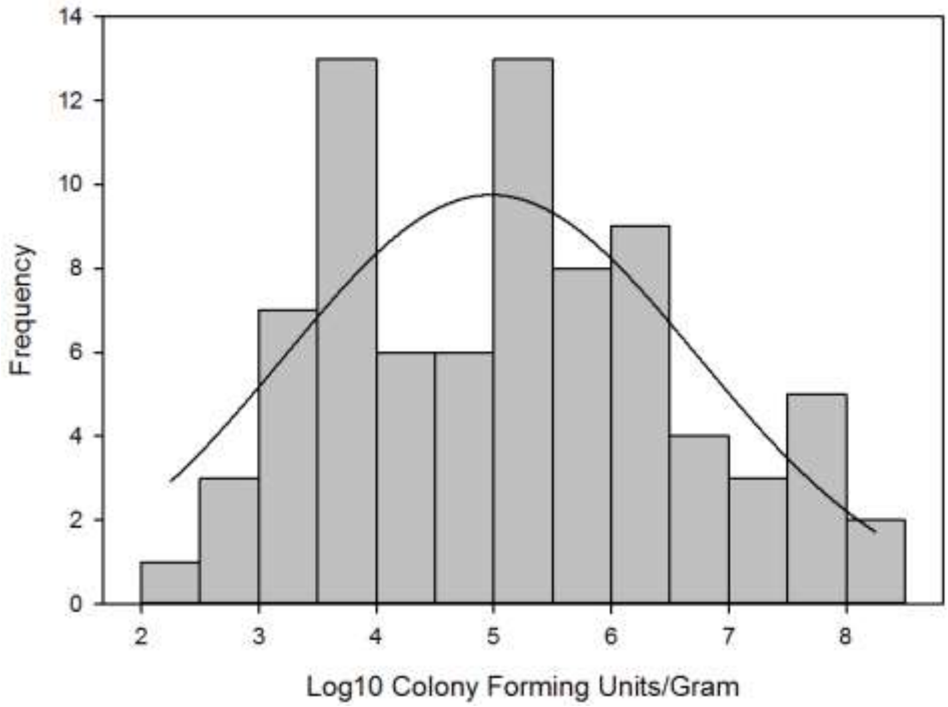

FIGURE 5. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 80 samples of broccoli.

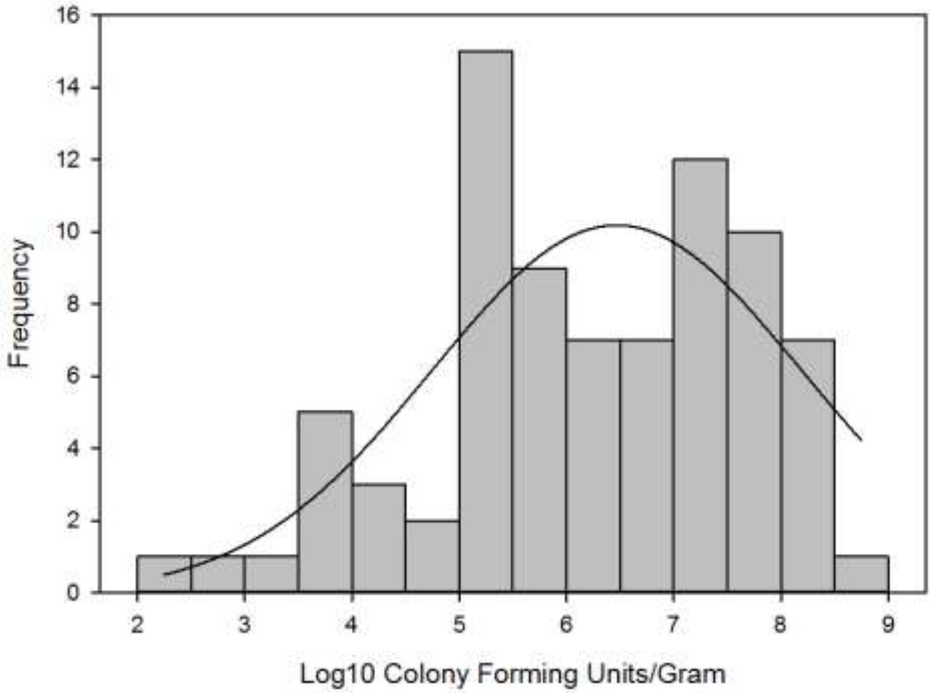

FIGURE 6. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 81 samples of carrot.

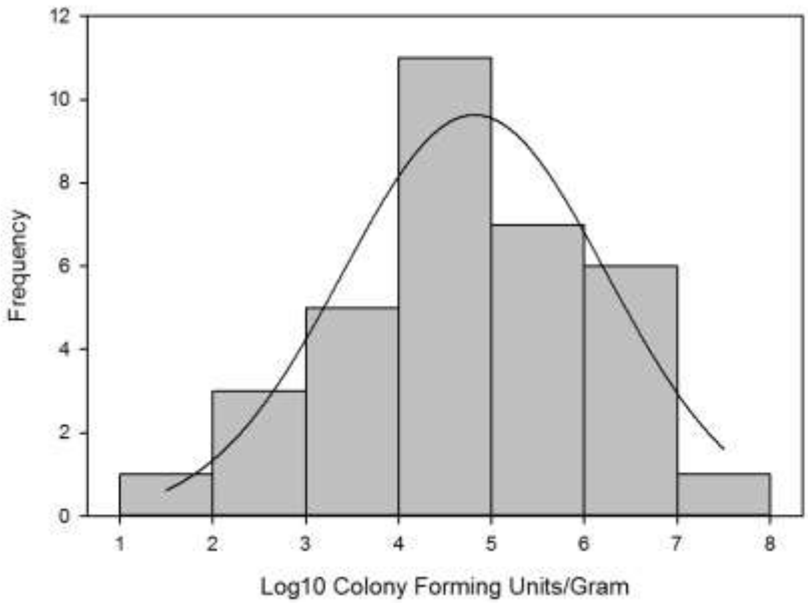

FIGURE 7. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 34 samples of cauliflower.

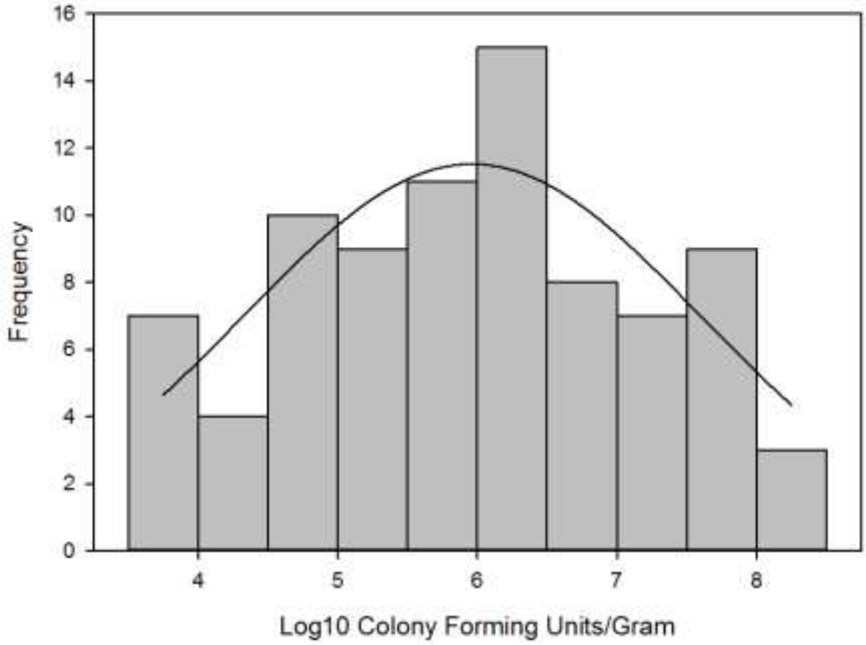

FIGURE 8. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 83 samples of cucumber.

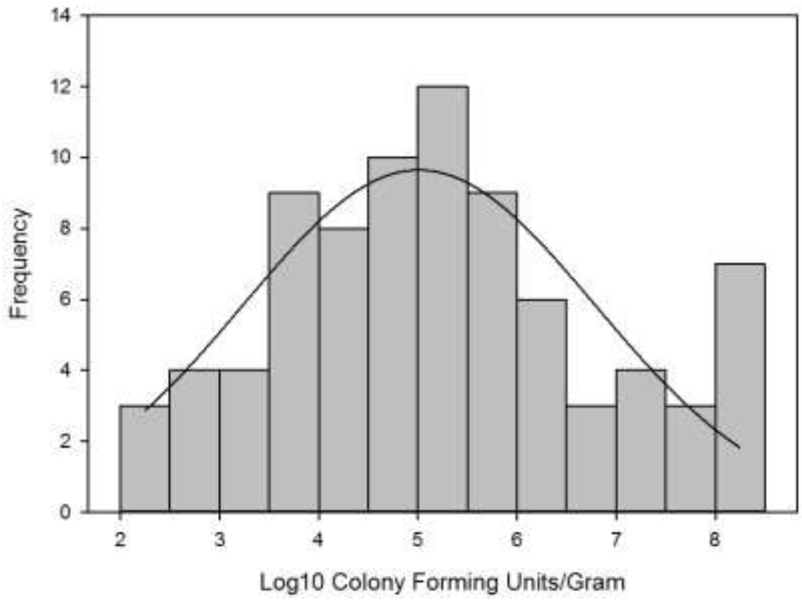

FIGURE 9. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 82 samples of iceberg lettuce.

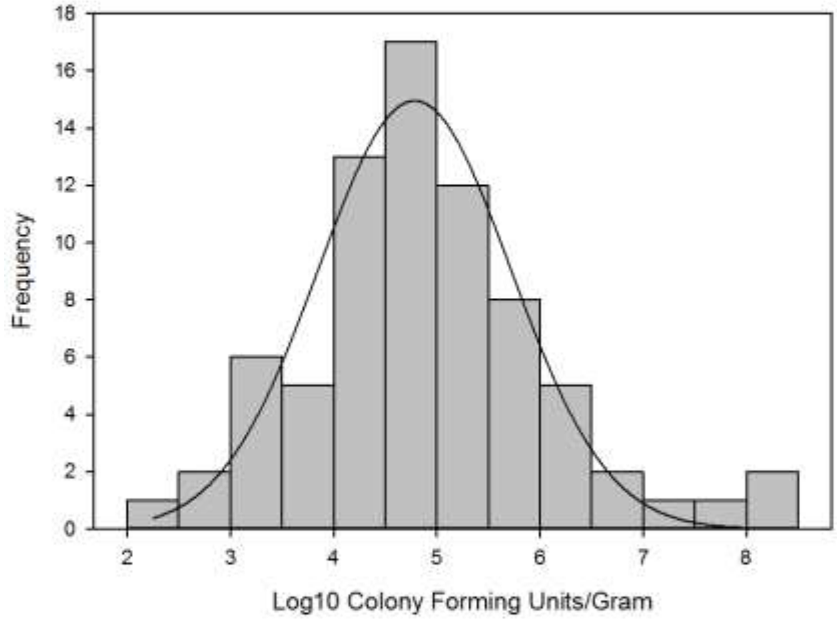

FIGURE 10. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 75 samples of romaine lettuce.

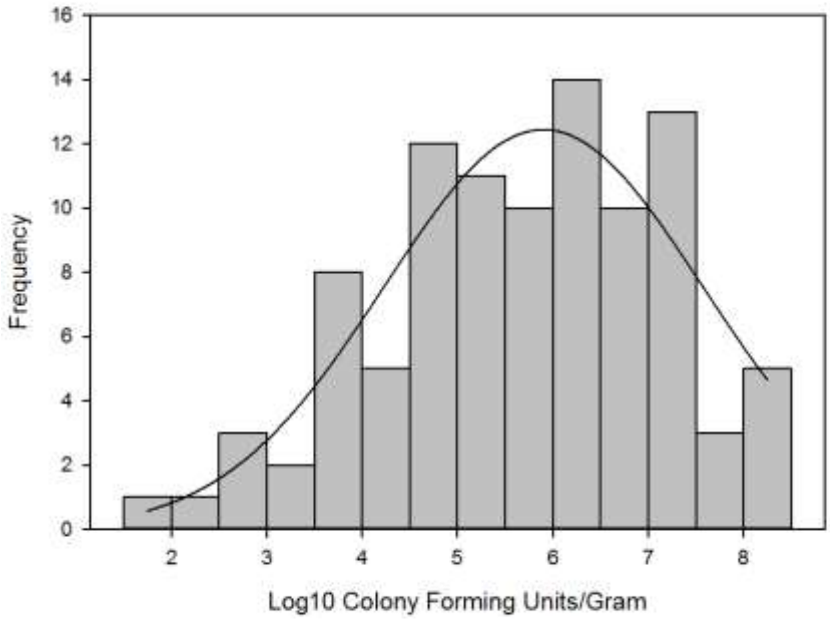

FIGURE 11. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 98 samples of mixed lettuce.

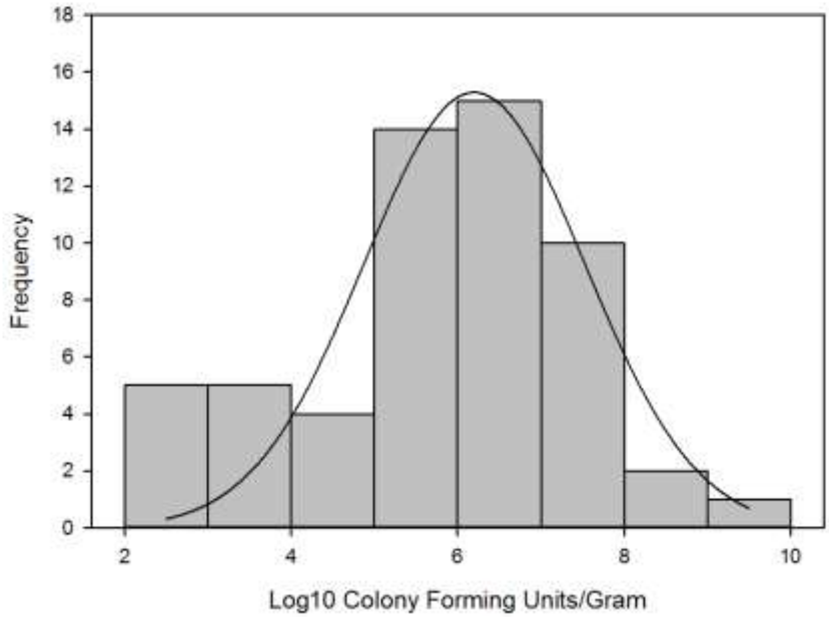

FIGURE 12. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 56 samples of mushroom.

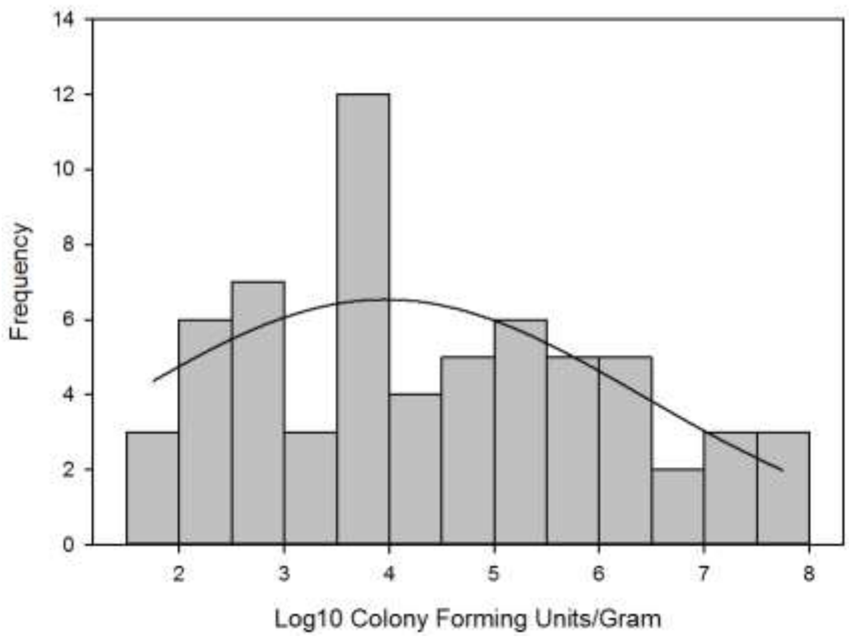

FIGURE 13. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 64 samples of onion.

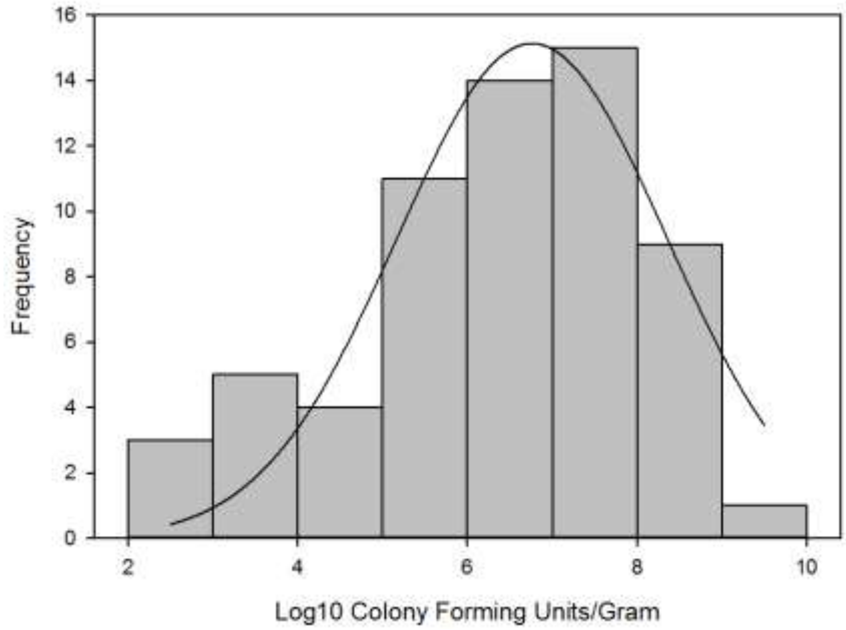

FIGURE 14. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 62 samples of pepper.

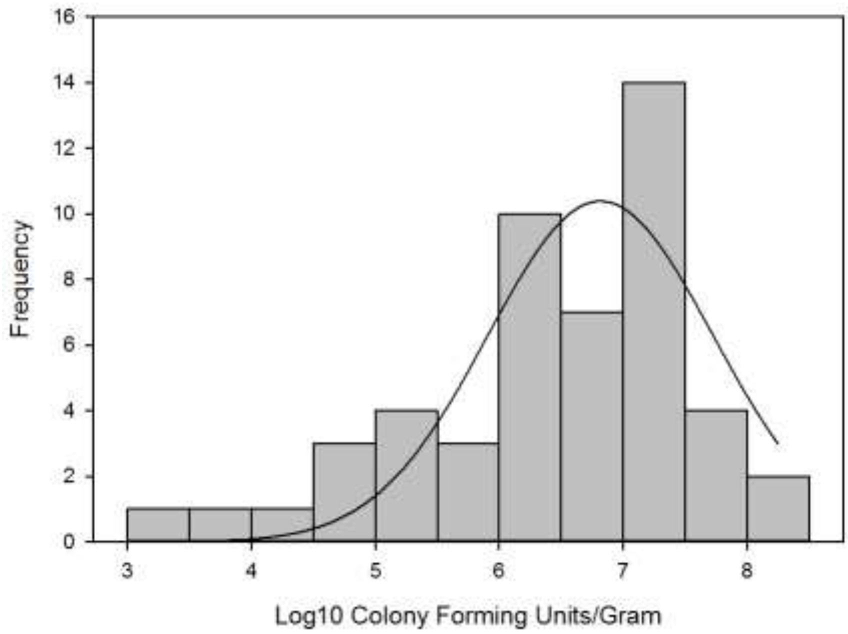

FIGURE 15. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 50 samples of spinach.

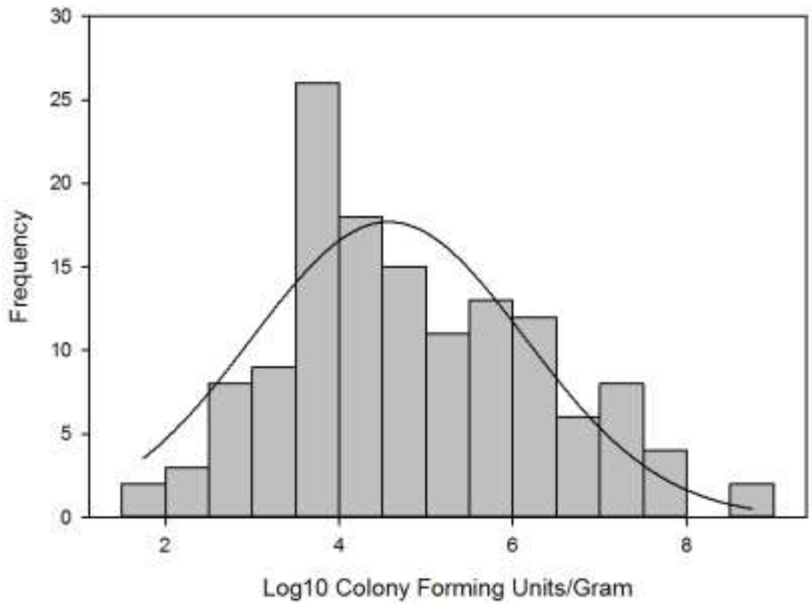

FIGURE 16. Normal distribution (line) fit to raw data (bars) for log total aerobic count per gram of 137 samples of tomato.

An example of total aerobic count data fit to normal distribution is given in Figure 4 for tomato (n=137), the most frequently tested RTE produce. The parameters of the normal distribution were μ=4.83 and σ=1.49.

TABLE 1. Statistical analysis of log total aerobic count per gram for RTE produces picked up from Rutgers University dining facilities (2001-2013); data represent normal distribution parameters (log CFU/g) or the percentage of samples below the limit of detection

Produce	Normal parameters (μ,σ^2)	Below limit of detection (%)	No. of samples
Apple	(3.40, 1.78)	46.4	56
Broccoli	(5.03, 1.59)	3.6	83
Carrot	(6.19, 1.48)	1.2	82
Cauliflower	(4.52, 1.57)	5.6	36
Cucumber	(5.96, 1.26)	1.2	84
Iceberg	(5.17, 1.67)	2.4	84
Romaine	(4.85, 1.27)	1.3	76
Lettuce mixed	(5.65, 1.46)	1.0	99
Mushroom	(5.65, 1.77)	1.8	57
Onion	(4.08, 1.90)	11.1	72
Pepper	(6.20, 1.81)	6.1	66
Spinach	(6.45, 1.11)	0.0	50
Tomato	(4.77, 1.55)	2.8	141

Normal distribution parameters for all RTE produces are given in Table 1. Little variation was seen between total aerobic count distributions for the produces (Figure. 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16). Apple had a lower mean in total aerobic count of 3.40 log CFU/g compared with the other produces, and this difference is also apparent from Figure. 4. Carrot, pepper and spinach had higher mean in total aerobic counts of 6.19, 6.20 and 6.45 log CFU/g, respectively (Figure. 6, 14 and 15).

TABLE 2. Summary of total coliform most probable number per gram for RTE produces picked up from Rutgers University dining facilities (2001-2013)

Produce	Average	SD	Below limit of detection (%)	No. of samples
Apple	0.60	0.32	0.0	56
Broccoli	1.74	1.52	0.0	83
Carrot	3.46	1.51	1.2	82
Cauliflower	1.07	1.08	2.8	36
Cucumber	3.12	1.36	1.2	84
Iceberg	1.62	1.52	2.4	84
Romaine	1.31	1.17	0.0	76
Lettuce mixed	1.48	1.32	1.0	99
Mushroom	2.58	1.49	1.8	56
Onion	1.66	1.43	2.8	72
Pepper	3.13	1.65	3.0	66
Spinach	2.48	1.61	0.0	50
Tomato	2.06	1.33	1.4	141

A summary of total coliform results for all RTE produces tested is given in Table 2. Apple, broccoli, romaine lettuce and spinach contained detectable coliforms in all samples. Coliform counts (when present) were highest on average in carrot (3.46 log MPN/g) and lowest in apple (0.60 log MPN/g). Average coliform counts for most products were typically between 1 and 4 log MPN/g.

TABLE 3. Summary of *Bacillus cereus* count per gram for RTE produces picked up from Rutgers University dining facilities (2001-2013)

Produce	Average	SD	Below limit of detection (%)	No. of samples
Apple	2.48	0.36	94.6	56
Broccoli	2.73	0.27	96.4	83
Carrot	2.51	0.90	90.2	82
Cauliflower	2.09	0.55	94.4	36
Cucumber	2.23	0.18	94.0	84
Iceberg	2.11	0.46	94.0	84
Romaine	2.24	0.37	90.8	76
Lettuce mixed	2.21	0.59	92.9	99
Mushroom	2.38	0.82	91.1	56
Onion	2.54	--(*)	98.6	72
Pepper	1.70	0.00	95.5	66
Spinach	2.43	0.63	94.0	50
Tomato	2.32	0.60	89.4	141

**B. cereus* presented in only one sample.

A summary of *Bacillus cereus* results for all RTE produces tested is given in Table 3. *B. cereus most* commonly present in onion. *B. cereus* counts (when present) were highest on average in broccoli (2.73 log CFU/g) and lowest in pepper (1.70 log CFU/g). Average *B. cereus* counts for most products were typically between 2 and 3 log CFU/g.

II.4 Discussion

Total Aerobic Plate Count

Table 1 shows result of total aerobic count in the samples analyzed, the results showed that there was a high variability among samples of each produce, and the result in this study was similar with that of several recent studies.

Previous research has shown varying results. In a study of fresh vegetables and fruits in Singapore, apple were found to have a median of 3.4 log CFU/g, with a range of

2.1 to 5.1 log CFU/g, which is consistent with the levels of contamination that we found for apple. The Singapore study also reported carrot to have a range from 2.6 to 6.9 log CFU/g with a median of 4.8 log CFU/g (51), which is lower than the bacterial level we found. Other studies showed similar or higher level, such as medians of 5.5 log CFU/g, 6 log CFU/, 7 log CFU/g and 7.8 log CFU/g. These results were higher than we found in this study.

Lettuce were found to have relatively high total aerobic count level in our study. Similarly, in a survey of Ready-to-eat lettuce in Swiss market, the lettuce were found to have a range of 5 to 6 log CFU/g in total aerobic count (52). Another survey found leafy lettuce to have total aerobic count ranging from 6.23 to 6.36 log CFU/g (53). In a survey from Singapore, the total aerobic count ranged from 1.6 to 9.1 log CFU/g, with a median of 5.8 log CFU/g for lettuce (51), which is consistent with our results. It was also reported that lettuce mix had total aerobic count ranging from 4.5 to 8.0 log CFU/g. Similarly, a study from Spain showed that the total bacterial counts for RTE lettuce at 16 university restaurants ranged from 3.01 to 7.81 log CFU/g (54).

In a Japanese survey of Iceberg lettuce, it was found to have a range of 5 to 6 log CFU/g for aerobic count (55), which was similar to our result. Romaine lettuce obtained from Brazil markets were found to have a range of 6.50 to 6.81 log CFU/g in total aerobic count, which was higher than our results (56). In another study, it was reported that romaine lettuce had total aerobic count ranging from 2.4 to 7.3 log CFU/g.

Among all the produces, spinach had a median total aerobic count of 6.45 log CFU/g, resulting in the highest mean count. Likewise, Kase and Borenstein found baby spinach to have total aerobic count levels ranging from 3.9 to 8.2 log CFU/g (57). Similarly, Valentin-Bon et al. reported that spinach had total bacterial counts of 7.2 to 7.7 log CFU/g and a broad range of <4 to 8.3 log CFU/g (42).

Our result shown that bell pepper had one of the highest total aerobic counts. Prior study done by Cárdenas et al. found serrano and jalapeño pepper has a range of 4.4 and 4.7 log CFU/g (58), which was lower than what we found. Liao et al. reported jalapeño pepper obtained from grocers in Pennsylvania had levels of total aerobic count ranging from 4.7 to 6.3 log CFU/g with a median of 5.6 log CFU/g. Another study in Mexico found 7.2 and 6.4 log CFU/g for serrano and jalapeño pepper (59).

The median of total aerobic count of tomato was 4.77 log CFU/g. This was also consistent with the results from a Singapore survey, in which tomato was reported to have total aerobic count ranging from 2.4 to 5.5 log CFU/g with a median of 4.2 log CFU/g (51). In contrast, it was reported bola and saladette tomatoes have total aerobic count of 3.2 and 3.6 log CFU/g, respectively (60).

Coliforms

In this study, the produces had mean coliform counts ranging from 0.60 to 3.46 log MPN/g (Table 2). The highest mean coliform counts were obtained in the carrot, pepper and cucumber with 3.46, 3.13 and 3.12 log MPN/g, respectively. Recent studies also have varying results in total coliforms. In Singapore study, it was reported to have coliform levels of 0.1 log CFU/g in apple, 2.7 log CFU/g in carrot (51), which were much lower than we found.

Romaine lettuce obtained from Brazil markets was found to have a range of 3.23 to 3.50 log MPN/g in coliforms (61). In another survey, spinach and lettuce mix had coliforms ranging from <0.47 to >4.0 log MPN/g, similar to the range of <0.47 to 3.38 log MPN/g reported for RTE lettuce in a Spain survey (62), which was a little higher than our results. Iceberg lettuce was found to have coliform ranging from 1 to 4 log MPN/g (55), which was much higher than our results.

Cárdenas et al. found 3.3 and 3.7 log CFU/g in pepper with a range from <1 to 3 log CFU/g (60), which is consistent with our results, and much lower than those reported for 3.0 to 8.1log CFU per sample from popular markets in Hidalgo, Mexico (63).

The concentrations of coliforms on spinach from the US ranged from undetectable to 4.0 log CFU/g (64). Likewise, Valentin-Bon et al. found that spinach have a range from <0.47 to ≥4.0 log MPN/g. Both of them had a lower coliform level than our result (42).

In the Singapore survey, tomato was reported to have a median of 2.1 log CFU/g (51), which is very similar to our results. Tomato is also reported to have coliform contamination of 2.6 log CFU/g with a range from <2 to 5 log CFU/g (60), which is higher than what we found in this study.

Other pathogenic indicators

These surveys showed that the microbial flora of RTE produce is highly variable and complex. Among all the 955 samples, No *Clostridium perfringens* was isolated. *Staphylococcus aureus* was isolated in 2.53% of all the samples, while *Escherichia coli* was isolated from 1.36% of all the samples, and *E. coli O157:H7* was isolated only once from spinach in the past ten years. *Salmonella* spp. was isolated from 0.73% of all the samples, *Listeria monocytogenes* was isolated from 0.31% of all the samples, and *Bacillus cereus* was isolated from 6.81% of all the samples.

The microbial level of those pathogenic indicators was lower than other studies. In an survey in Canada, *E. coli* was isolated from 8.2% of RTE produces including lettuce, spinach, carrots, and green onions (43). Another survey in Swiss showed *E.coli* was isolated from 3.52% of RTE lettuce and fruits samples, and non-O157 STEC was isolated once in an lettuce sample (52), which also reported a higher level (3.52%) of *L.monocytogenes* compared to our results. A study in Ontario collected from retail distribution centers and farmers' markets found 5.3% positive for *E.coli* of the fresh produces samples positive for *E. coli*, and 0.17% to be positive for *Salmonella* (65). In the USDA Microbiological Data Program a total of 7,646 produce samples from terminal markets and wholesale distribution centers were tested, and a positive rate of 20.8% for *E.coli* was reported with an enzyme-based detection method, which was higher than our result. *Salmonella* was isolated from 0.04% of the samples, which was lower than *Salmonella* isolated in our study. Other researches have reported positive rates for *E. coli* higher than that reported here (46, 66). Among those studies, none of the samples was positive for *E. coli O157:H7*. The bacteria counts varied largely among studies, which could due to different testing methodologies, produce type, and geographic positions. Overall, the levels of *E. coli* found in this study are lower than those reported by several other researchers. Besides, no *Salmonella* or *E. coli O157:H7* were detected in fresh produce from Spain (46), Norway (67), Ireland (68), or the United Kingdom (69). Several studies have been conducted in recent years in the United States to evaluate the microbiological quality of fresh produce, and no or very low levels of pathogenic bacteria have been reported (66,70).

Cho et al. found positive rates for *S. aureus* and *B. cereus* of 1.72% and 2.18% in RTE foods, respectively, both of which were lower higher than we found in this study (71). According to the study of Chung et al., the detection rates of *B. cereus*, *Salmonella* spp, *L. monocytogenes*, and *S. aureus* in RTE foods in Korea were less than 4%, which was similar to our results. A survey in Mexico found that 1.25% of RTE tomato and peppers were positive for *Salmonella* (60), which was higher than our results. In another study conducted in Portugal, the positive rate for *Salmonella*, *L. monocytogenes* and *B. cereus* were 1.99%, 0.66%, and 22.7%, respectively (72). In another study in Japan, Hara-kudo et al. reported a positive rate of *Salmonella* of 0.1-0.2% isolated from cucumber, lettuce, sprout and tomatoes (73), which was lower than our results. Although the microbial load of most of the indicator bacteria were lower in this study compared to other studies, demonstrates that there is room for improvement throughout the produce processing and storage.

There are a few possible explanations for the relatively high total aerobic counts in lettuces, spinach, pepper and carrot. First, lettuces and spinach are leafy greens with large surface areas and folds. This makes them more susceptible to bacterial contamination and adhesion; and when untreated manure is utilized as soil fertilizers in the farm, it is also easy for their open leaves to contact with soil and irrigation water, thus result in microorganisms transfer on to their leaves (74). Second, the reason for high aerobic count in pepper could result from the cross-contamination between pepper stems and pepper flesh when they are prepared for serving, as pepper stems accounted for a greater contamination level of aerobic bacteria; in additional, the stem is the contact area of pepper used to pluck it from the plant during harvest, which could also cause cross-contamination (60). Last, for carrot, since it is a subterranean crop and in direct contact with soil, those bacteria in irrigation water, manure and fertilizers could easily attach to it (46).

We were unable to compare our results of the microbial load on cauliflower and broccoli with those of other studies, for there are very few papers reporting microbial load on those produces, that may due to few outbreaks they caused as they are usually consumed cooked.

Furthermore, the high aerobic microbial load could also result from processing, storage and handling. Microbial surveys can demonstrate that products handled under good sanitary conditions generally evidence microbial counts significantly lower than those of products produced in plants operating under poor sanitary conditions (75).

The result in this study showed that mean total aerobic count for the broccoli, carrot, cucumber, iceberg lettuce, lettuce mixed, mushroom, pepper and spinach examined exceeded 5 log CFU/g (Table 1), indicating that those produces were in unsatisfactory condition to consumption, because the “acceptable” level of aerobic bacterial count for ready-to-eat food is not more than 5 log CFU/g at Rutgers Dinning halls (48). Therefore, these results suggest that effective control measures should be implemented in production facilities and subsequent processes to enhance the microbiological quality of fresh produce provided in Rutgers dining facilities.

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