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Prevalence and trends of bacterial contamination in fresh fruits and vegetables sold at retail in Canada

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ABSTRACT

In recent years, fresh fruits and vegetables have been linked to numerous foodborne illness outbreaks in different regions of the world, including in Canada. In light of rising concerns over the microbial safety of these commodities, the Canadian Food Inspection Agency conducted retail surveys to obtain information on the occurrence of bacterial pathogens in a wide range of produce available in the Canadian marketplace (local vs. imported, organic vs. conventional). Samples ($n = 31,329$) were collected across Canada over four years (2009–2013) and consisted of leafy vegetables ($n = 12,073$), leafy herbs ($n = 6032$), green onions ($n = 3381$), cantaloupes ($n = 3230$), tomatoes ($n = 4837$) and berries ($n = 1776$). These samples were analysed in ISO 17025-accredited laboratories for various bacterial pathogens (*Salmonella, Escherichia coli* O157, *Shigella*, *Campylobacter* and *Listeria monocytogenes*), as well as for generic *E. coli*, an indicator of fecal contamination. The Wilson confidence interval was used to determine the prevalence of the different micro-organisms in the commodities investigated. Control charts and seasonal indices, statistical tools adapted here to explore the large amount of data collected for each commodity, were used to identify potential adverse events or trends in bacterial contamination. The prevalence of bacterial contamination observed during this study in the six commodities examined was generally very low, with prevalence intervals ranging from [0, 0.08%] in tomatoes to [0.79, 1.30%] in leafy herbs. Most of the samples that were reported as "positive for bacterial contamination" had elevated (>100 CFU or MPN/g) levels of generic *E. coli*, but did not have detectable levels of the bacterial pathogens investigated. Of the samples that did have detectable levels of bacterial pathogens, the only bacteria that were both detected and isolated were *Salmonella* and *L. monocytogenes*. Despite the overall low prevalence of contamination seen in most produce, a notable seasonal trend was observed in the leafy vegetable group, where higher bacterial contamination rates were confirmed in the summer in organic as opposed to conventional products. These findings provide valuable baseline information that can support food safety decisions, and confirm that the vast majority of fresh fruits and vegetables available on the Canadian market are safe in terms of bacteriological hazards.

1. Introduction

1.1. International context

Fresh fruits and vegetables are well recognized as important parts of a nutritious and healthy diet and many countries, including Canada, have undertaken initiatives to encourage consumers to increase their consumption of these products. Consumers demand variety in and availability of these products all year round, which has impacted international trade, particularly for countries such as Canada where the growing season is short and many fresh fruits and vegetables are imported. Since the mid-1990s, there has been an increasing number of outbreaks of fresh produce-associated foodborne illness identified internationally and efforts are being made to resolve these food safety problems (Berger et al., 2010; Lynch, Tauxe, & Hedberg, 2009; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004).

A wide range of produce items has been implicated in human illness outbreaks worldwide and certain commodities are more frequently linked to these outbreaks; for example, leafy greens, such as lettuce and spinach, and fresh herbs, such as parsley and basil, are well-recognized potential sources of bacterial infections

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(FAO/WHO, 2008b). The USA has experienced several large high profile multi-state outbreaks attributable to leafy vegetables, including the 2006 outbreak of *Escherichia coli* (*E. coli*) O157:H7 infection, which was linked to the consumption of bagged spinach and resulted in almost 200 cases of food poisoning and three deaths (Grant et al., 2008; Wendel et al., 2009). In 2007, a microbiological study of fresh herbs sold at retail in the UK uncovered an international outbreak of *Salmonella* infection linked to contaminated basil from Israel that affected at least 51 individuals from England, Wales, Scotland, Denmark, the Netherlands and the USA (Elviss et al., 2009; Pezzoli et al., 2008). Fresh cut and whole melons have also been linked to several outbreaks in a number of countries (Hanning, Nutt, & Ricke, 2009; Lynch et al., 2009). In late 2011/early 2012, watermelon from Brazil was implicated in a multi-country outbreak of *Salmonella* infection in Europe, with 63 confirmed cases of food poisoning (Byrne et al., 2014). Fresh tomatoes have also been linked to several salmonellosis outbreaks in the USA over the past 10 years (Hanning et al., 2009).

1.2. Canadian context

Canada is a net importer of fresh fruits and vegetables (AAFC, 2014a, 2014b): approximately 88% of fruits and 41% of vegetables sold in Canada are imported (Statistics Canada, 2002). Free trade agreements, such as the North America Free Trade Agreement (NAFTA), have contributed to the increasing availability of fresh fruits and vegetables year round. According to 2011 import data, the main sources of produce imported into Canada are the USA for products such as leafy greens, soft fruits, citrus fruits, grapes, cauliflower, broccoli, onions, beans and carrots, Mexico for peppers, tomatoes, avocados, cucumbers and asparagus and Chile, Peru, Honduras, Guatemala, Costa Rica and China for a range of fresh produce (AAFC, 2014a, 2014b).

As with other industrialized countries, Canada has seen an increased number of foodborne illness outbreaks linked to fresh produce in the last decades. In a review of produce-associated outbreaks published in 2001, 11 outbreaks were described and attributed to a variety of commodities, including sprouts, cantaloupe, lettuce and fresh herbs, which were found to be contaminated with bacterial pathogens. Many of these outbreaks were inter-provincial and even international in scope, with countries such as the USA, Finland and Denmark reporting illnesses linked to the same products. The bacterial pathogens involved included *E. coli* O157:H7, *Salmonella, Listeria monocytogenes* (*L. monocytogenes*) and *Shigella* (Sewell & Farber, 2001). Since 2001, there have been a dozen other outbreaks of foodborne illness linked to produce contaminated by similar bacterial pathogens (Kozak, MacDonald, Landry, & Farber, 2013). Examples of more recent outbreaks that occurred in Canada include one caused by *E. coli* O157:H7 in lettuce imported from the USA, which affected 31 people in 2012 (PHAC, 2014), and one caused by *Salmonella* in domestic green onions, which resulted in 20 cases of foodborne illness in 2010 (PHAC, 2012).

1.3. A Canadian survey: rationale and objective

In 2007, the FAO and WHO convened an expert committee to establish priority commodities of concern in terms of microbiological hazards associated with fresh produce. Multiple factors were considered, including historical outbreaks, potential for contamination, exposure levels and potential for control, frequency and severity of disease and trade and economic impacts. Leafy vegetables and leafy herbs were given the highest level of priority, followed by berries, green onions, melons, sprouted seeds and tomatoes (FAO/WHO, 2008a). In 2008, the Canadian Food Inspection Agency's (CFIA) Food Safety Science Committee (FSSC) gathered experts from across Canada to assess and rank food–hazard combinations. In the area of microbiological hazards, contamination of fresh fruits and/or vegetables by various bacterial pathogens (*Salmonella* spp., *E. coli* O157:H7 and *Shigella* spp.) was seen as representing a significant food safety concern (CFIA, 2008).

The same year, the CFIA initiated the Food Safety Action Plan (FSAP) to modernize and strengthen Canada's food safety system (CFIA, 2013). Targeted surveys were launched as part of the FSAP initiative to obtain Canadian baseline data and information on the presence of priority and/or emerging microbiological hazards in food at the retail level. Based on the above recommendations from the FAO/WHO and the FSSC, in combination with a review of scientific literature and documented outbreaks of foodborne illness, fresh leafy vegetables, herbs, green onions, cantaloupes, tomatoes and berries were selected as priority commodities for targeted surveillance under the FSAP, with a focus on the bacterial pathogens *Salmonella* spp., *E. coli* O157 (except in cantaloupes), *L. monocytogenes* (in fresh-cut leafy vegetables and fresh-cut cantaloupes only), *Campylobacter* spp. (in leafy vegetables and herbs only) and *Shigella* spp., as well as generic *E. coli* as an indicator of fecal contamination. The aim of this paper is to present an analysis of the data generated by these surveys.

2. Materials and methods

2.1. Sampling design

Samples were collected between 2009 and 2013 for each of the six fresh fruits and vegetables commodity groups (leafy vegetables, leafy herbs, green onions, cantaloupes, berries and tomatoes) from a wide range of retail stores located in eleven major cities across Canada (Vancouver BC, Kelowna BC, Calgary AB, Saskatoon SK, Winnipeg MB, Toronto ON, Ottawa ON, Montreal QC, Quebec City QC, St. John NB and Halifax NS). The cities were selected based on geographic and demographic considerations and the corresponding number of samples was collected in proportion to the relative population of the respective areas. These cities encompassed four geographical areas: Atlantic (Halifax and St. John), Quebec (Quebec City, Montreal), Ontario (Ottawa, Toronto) and the western area (Vancouver, Kelowna, Calgary, Saskatoon and Winnipeg).

The goal of this sampling approach was to obtain a large set of samples that would be representative of the targeted food commodities available to Canadians at retail during the time of the survey. The number of samples collected in each season was impacted by the availability of the targeted commodity in the Canadian market at the time of sampling. Generally, domestic samples were collected during the summer months and imported samples were collected primarily in the fall, winter and spring months.

The target sample population consisted of all units of the targeted commodity (e.g., leafy vegetables) available at retail to Canadian consumers as per the sampling design. Since all units of the population could not be assumed to have an equal probability of selection, the commodity units at a store were drawn by the sampler as randomly as possible to be reasonably representative of the population. Consequently, the sampling method used for the targeted surveys is a non-probability sampling method, which does not allow standard statistical inferential methods to be invoked. The large number of samples taken throughout the surveys mitigates the lack of randomness inherent in our sampling approach. Accordingly, the statistical methods applied in the analyses of the ensuing data have been chosen due to their robustness with respect to randomization.

2.2. Bacterial analysis and reporting

Samples were analysed for the presence of up to six bacterial species (generic *E. coli* as an indicator of fecal contamination and five bacterial pathogens: *Salmonella* spp., *E. coli* O157:H7 and NM (non-motile), *Shigella* spp., *Campylobacter* spp. and *L. monocytogenes*) in ISO 17025-accredited laboratories using the methods published in Health Canada's Compendium of Analytical Methods for the Microbiological Analysis of Foods (HC, 2011a). These methods have been fully validated for the analysis of fresh fruits and vegetables and are used for the regulatory testing of foods and in food safety investigations. The analyses of bacterial pathogens were performed using enrichment methods, confirmed by isolation, purification and identification procedures. These analyses were done on 25 g of sample, except for whole cantaloupes, where the entire fruit was analysed. Briefly, the entire cantaloupe was placed in a sterile sample bag and a sufficient volume of enrichment broth was added in order to totally submerge the fruit. The sample was then mixed by manually rubbing the rind of the cantaloupe prior to incubation as described in the Health Canada Compendium methods used. The analysis of generic *E. coli* was performed using enumeration methods (most probable number, MPN, or direct plating procedure) with a lower reporting limit of 100 CFU or MPN/g, which corresponds to the maximum acceptable concentration of generic *E. coli* in fresh fruits and vegetables, as set by Health Canada (HC, 2008). Samples where bacterial pathogens were detected and confirmed in 25 g of products (or on the entire fruit in the case of whole cantaloupes), or where generic *E. coli* levels were found to be >100 CFU or MPN/g, were reported as positive.

3. Calculations

A statistical methodology was developed to investigate the presence of the six bacterial species in the chosen fresh fruits and vegetables, and to provide an overview of the results and general trends observed over the several years of sampling. This methodology combines the use of three statistical tools: 1) control charts, 2) seasonal indices and 3) confidence intervals for the prevalence. The analyses are based on the percentage of positive samples for a given bacterial species and for all bacterial species combined (i.e., global sample result).

3.1. Control charts

The first statistical tool was the control charts which are typically used as a process-monitoring technique for detection of the occurrence of assignable causes of process shifts, so that investigation of the process and corrective action may be undertaken before many nonconforming units are manufactured (Montgomery, 2009). In this study, a novel application of control charts was investigated as a potential tool to retrospectively look at a large amount of data collected over multiple years and to detect any potential events or trends. A few adjustments to what constitutes a typical control chart were made to suit the data:

1) to analyse the different commodity groups, a control chart was developed for each bacterium tested and for all the bacteria combined,
2) the control chart time intervals are in units of months,
3) the statistical parameter used to approximate the mean is the proportion (i.e., the overall percentage of positive results) since the analysis is a categorical data analysis,
4) the purpose of the control chart is to identify periods where the percentage of positive results falls above the upper control limit (UCL); therefore, the center line and the lower control limit are not shown, and
5) an upper control limit, plotted at two standard deviations from the center line, has been added; as a result, two upper control limits are calculated, providing greater flexibility in decision-making.

3.1.1. Calculating Upper control limits

In order to establish the limits of the control chart based on the percentage of positive results for each month (also called a p-chart), the count of positive results follows a binomial distribution with parameters n and p (where n is the number of samples and p the percentage of positive results). As stated in the last section, two UCLs have been established, which leads to three different areas:

1) an area of no concern (white area): the results obtained during this period warrant no further investigation,
2) an area of potential concern (yellow area): these results and their potential causes need some form of review, and
3) an area of concern (red area): these results must be investigated in detail.

It is important to note that the control chart is used primarily for two cases where i) $np(1-p)>5$ and $0.1<p<0.9$ or ii) $np(1-p)>25$ (Xie, Goh, & Kuralmani, 2002). Since the data do not always meet these requirements, some periods in the control chart, where sample size is low, have to be interpreted carefully since the approximation of the UCLs may be inaccurate.

The UCL's areas are calculated as follows:

$$\text{UCL of potential concern} = \overline{p} + 2\sqrt{\frac{\overline{p}(1-\overline{p})}{n}}$$

$$\text{UCL of concern} = \overline{p} + 3\sqrt{\frac{\overline{p}(1-\overline{p})}{n}}$$

3.1.2. Control limits with different sample sizes

Four types of control charts, accounting differently for the sample size variations, were compared and considered (see Appendix 1 in Supplemental Materials). The "Weighted Mean of Variable-Width Control Limits" was deemed the most appropriate:

$$\text{UCL} = \frac{\sum_{i=1}^{m} n_i \text{UCL}_i}{\sum_{i=1}^{m} n_i} \text{ such that } n_i \geq 1 \text{ for all } i$$

The "Weighted Mean of Variable-Width control Limits" had the lowest UCLs of the four methods considered when analysing variable sample sizes. Low UCL is preferable since it limits the risk of missing a trend or an event of potential significance.

3.2. Seasonal index

The second statistical tool was the seasonal index, which was used to detect and measure seasonal effects in a time series (i.e., a sequence of measurements, such as bacterial contamination, over a period of time). A seasonal effect is defined as the repetitive and predictable patterns of behaviour of data over a given time interval (e.g., days, weeks, months or quarters) (Sharma, 2010). The computation of the seasonal indices is illustrated in Appendix 2 of the Supplemental Materials and can be summarized in four steps (Hanke and Reitsch 1994):

1) The moving average for *x* periods is calculated,
2) The average of two consecutive moving averages is calculated in order to centre the moving average for one given period. This step is necessary since the number of periods is even (e.g., 4 seasons, 12 months, etc.),
3) The ratio-to-moving-average is calculated by dividing the original percentage by the centred moving average,
4) The seasonal index is then calculated by averaging and normalizing the ratios for each period.

For the purpose of our analyses, seasonal index graphs were designed to highlight the monthly seasonality of any bacterial contamination in each commodity group. Supplementary seasonal index graphs were produced when the seasonal index graph for the whole commodity showed a seasonal effect with a variation greater than 0.01, indicating a statistically significant influence (i.e., significantly different at the 95% confidence level, based on a dummy variable model). These graphs aim to compare the seasonal variations between commodity sub-groups, such as domestic *vs.* imported products, conventional *vs.* organic production and the geographical regions where the products were sampled.

Three conclusions may be drawn from the seasonal indices:

1) a seasonal index greater than one: there is a positive seasonal effect, meaning that the positive rate (% of positive samples) is higher than the average annual rate,
2) a seasonal index equal to one: there is no seasonal effect, and
3) a seasonal index less than one: there is a negative seasonal effect, meaning that the positive rate is lower than the average annual rate.

3.3. Confidence intervals for the prevalence

The third statistical tool used was the confidence intervals for the prevalence. The estimated prevalence is defined as the overall percentage of positive samples – either for a given bacterium or for all bacteria studied (global sample result). The Wilson confidence interval (i.e., a two-sided 95% confidence interval for the proportion) was used to estimate the prevalence of bacteria.

4. Results

A large number of fresh fruits and vegetables samples for six different commodity groups (leafy vegetables, tomatoes, leafy herbs, green onions, cantaloupes and berries) were collected and tested for the presence of a variety of bacteria over a four year period. Control charts and seasonal effects were calculated for each commodity and are presented in Section 4.1. Positive rates and prevalence of bacterial contamination in all commodities are summarized in Table 1 in Section 4.2.

4.1. Control charts and seasonality for each commodity

4.1.1. Leafy vegetables

From June 2009 to March 2013, 12,073 samples of fresh leafy vegetables were sampled across Canada. The samples consisted of imported and domestic products (68.0% and 32.0%, respectively), conventionally- and organically-grown products (80.8% and 19.2%) and whole and fresh-cut leafy vegetables (55.7% and 44.3%). The leafy vegetables consisted mainly of lettuces (head, leaf and mixes, 44.5%), salad mixes (30.0%) and other non-brassica leafy vegetables (mainly spinach, chard and arugula, 21.8%) (Tables 4 and 5 in Supplemental Materials). Note that only the fresh-cut leafy vegetables were tested for *L. monocytogenes*.

The control chart calculated for the leafy vegetables samples with all six bacterial results combined (Fig. 1) shows five periods that stand out: June and July of 2009 in the area of potential concern (yellow) and August 2009, July 2010 and 2012 in the area of concern (red). This means that the % of positive samples was significantly higher than average in several summer months during the four year period.

For all bacteria combined, the seasonal index for leafy vegetables was calculated for each month. Fig. 2 shows that July had a positive seasonal effect with a seasonal index above 1.01, meaning that the positive rate in July was statistically higher than the annual average. Additional seasonal indices were then calculated to compare the seasonal variations between the different commodity sub-groups. The comparison of imported and domestic leafy vegetables showed similar positive seasonal effects for the month of July (Fig. 3A), indicating that this trend applies to all products regardless of their country of origin. The comparison of conventional and organic leafy vegetables (Fig. 3B) showed a significant positive seasonal effect in July but only for organic leafy vegetables; this effect is strong, with an index of 1.08, and indicates that bacterial contamination was significantly more frequent in the organic produce in July in comparison with the other months of the year. The seasonal index by product types (Fig. 3C) showed that the positive effect seen in July was particularly strong in one of the main sub-types of leafy vegetables: the "other non-brassica leafy vegetable" group (mainly spinach, chard and arugula), with a seasonal index of 1.03. The same seasonal effect was observed to a lower degree in the salads group (seasonal index slightly above 1.01) but was not observed in the leaf and head lettuce groups. A comparison between the regions where samples were collected (Fig. 3D) shows that the positive seasonal effect was observed in July in all geographic areas except Ontario.

Individual control charts were also prepared for each of the six bacteria investigated. The control chart for generic *E. coli* in leafy vegetables shows four periods of interest: August 2009, indicating potential concern, and June 2009, July 2010 and 2012, indicating concern. These four periods had been flagged previously in the control chart calculated for the combination of all six bacteria. The seasonal indices for generic *E. coli* in leafy vegetables, globally and in the various sub-groups of interest (imported *vs*. domestic, organic *vs*. conventional, product types and sampling region), also showed the same trends as observed previously. The control chart for *L. monocytogenes* in fresh-cut leafy vegetables showed two periods in the area of concern: July and August of 2009; however, the seasonal index for *L. monocytogenes* in leafy vegetables did not identify any month indicating a statistically significant positive seasonal effect. The control chart for *Salmonella* spp. in leafy vegetables showed one period, December 2009, in the area of concern. The seasonal index calculated for this bacterium does not, however, indicate any significant seasonal effect.

The other bacterial pathogens investigated (i.e., *Shigella*, *E. coli* O157 and *Campylobacter*) were not detected in any of the leafy vegetables samples so no trends could be observed through the use of control charts or seasonal indices for these bacteria.

Contamination of leafy vegetables by the bacterial pathogens investigated was found to be a rare and sporadic occurrence; however, some trends emerged when the results were integrated, and included generic *E. coli*. Leafy vegetables appeared to be more frequently contaminated in the summer in most geographical regions. This trend applied to both imported and domestic leafy vegetables and was particularly strong in organic produce, but was not observed in head lettuces (e.g., Boston and iceberg), leaf lettuces (e.g., lamb and romaine) or brassica leafy vegetables (e.g., kale and broccoli leaves).

Table 1
Summary of positive rates and prevalence of bacterial contamination in all commodities.

Commodity (product types)	Results	Global sample result	*Campylobacter* spp.	*E. coli* O157:H7 & NM	*Listeria monocytogenes*	*Salmonella* spp.	*Shigella* spp.	Generic *E. coli*
Leafy Vegetables (e.g., leaf lettuce, head lettuce, mixed greens, spinach, etc.)	Positive rate[a]	48/12073[c]	0/5170	0/11392	14/4435	2/11400	0/5773	33/11869
	Prevalence[b]	0.40% [0.30, 0.53%]	0% [0, 0.07%]	0% [0, 0.03%]	0.32% [0.19, 0.53%]	0.02% [0, 0.06%]	0% [0, 0.07%]	0.28% [0.20, 0.39%]
Leafy Herbs (e.g., parsley, cilantro, basil, dill, mint, etc.)	Positive rate[a]	61/6032	0/3696	0/6022	Not Tested	5/6027	0/6020	56/6031
	Prevalence[b]	1.01% [0.79, 1.30%]	0% [0, 0.10%]	0% [0, 0.06%]	Not Tested	0.08% [0.04, 0.19%]	0% [0, 0.06%]	0.93% [0.72, 1.20%]
Tomatoes (fresh whole tomatoes)	Positive rate[a]	0/4837	Not Tested	0/2047	Not Tested	0/4416	0/4427	0/4837
	Prevalence[b]	0% [0, 0.08%]	Not Tested	0% [0, 0.19%]	Not Tested	0% [0, 0.09%]	0% [0, 0.09%]	0% [0, 0.08%]
Green Onions (fresh green onions)	Positive rate[a]	3/3381	Not Tested	0/2971	Not Tested	1/2963	0/2973	2/3381
	Prevalence[b]	0.09% [0.03, 0.26%]	Not Tested	0% [0, 0.13%]	Not Tested	0.03% [0.01, 0.19%]	0% [0, 0.13%]	0.06% [0.02, 0.22%]
Cantaloupes (whole and fresh-cut cantaloupes)	Positive rate[a]	5/3230	Not Tested	Not Tested	2/140	2/3215	0/2720	1/1029
	Prevalence[b]	0.15% [0.07, 0.36%]	Not Tested	Not Tested	1.43% [0.39, 5.06%]	0.06% [0.02, 0.23%]	0% [0, 0.14%]	0.10% [0.02, 0.55%]
Berries (e.g., blueberries, strawberries, blackberries, raspberries, other.)	Positive rate[a]	0/1776	Not Tested	0/1373	Not Tested	0/1370	0/1373	0/1776
	Prevalence[b]	0% [0, 0.22%]	Not Tested	0% [0, 0.28%]	Not Tested	0% [0, 0.28%]	0% [0, 0.28%]	0% [0, 0.22%]

[a] Number of positive samples over total number of samples.
[b] At a 95% confidence interval.
[c] One sample (lettuce-leaf) was positive for both generic *E. coli* and *L. monocytogenes*, which explains why the total number of positive samples (48) does not equate the total number of positive analytical results (49).

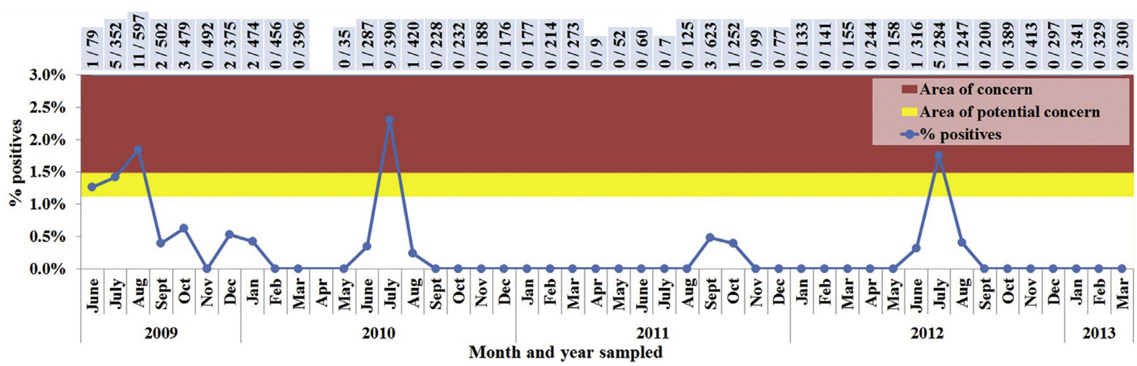

Fig. 1. Control Chart for Leafy Vegetables. The control chart calculated for the leafy vegetables samples with all six bacterial results combined showing five periods of interest.

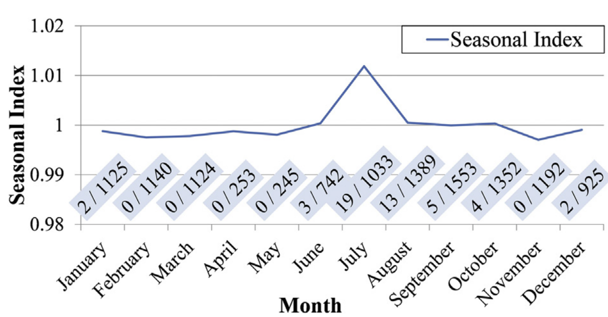

Fig. 2. Seasonal Index for Leafy Vegetables. The month by month seasonal index calculated for the leafy vegetables samples with all six bacterial results combined showing a positive seasonal effect in July indicating a statistically significant influence.

4.1.2. Leafy herbs

Between June 2009 and March 2013, 6032 samples of fresh leafy herbs were collected across Canada. The samples included imported and domestic products (66.0% and 34.0%) and products from conventional and organic production (71.7% and 28.3%). The samples collected consisted of herbs such as parsley, cilantro, basil, mint, dill, rosemary, oregano and chives (Tables 4 and 6 in Supplemental Materials). The global control chart calculated for the leafy herb samples for all bacteria combined (Fig. 4) shows that the sample positive rate was quite variable and went through many fluctuations during the four years of the survey. Three periods stand out: July 2009 and July 2010 in the area of potential concern and January 2010 in the area of concern.

The seasonal index for all bacteria combined for the leafy herb samples also shows fluctuations throughout the year, but no month showed a seasonal effect with a variation greater than 0.01 (Fig. 5).

Further analysis with additional indices for the leafy herb samples showed significant differences in some sub-groups: domestic leafy herbs had a seasonal index of 1.02 in July (2% above annual average), leafy herb samples from Ontario had a seasonal index of 1.01 in July (1% above annual average), and leafy herb samples from western Canada had a seasonal index of 1.03 in

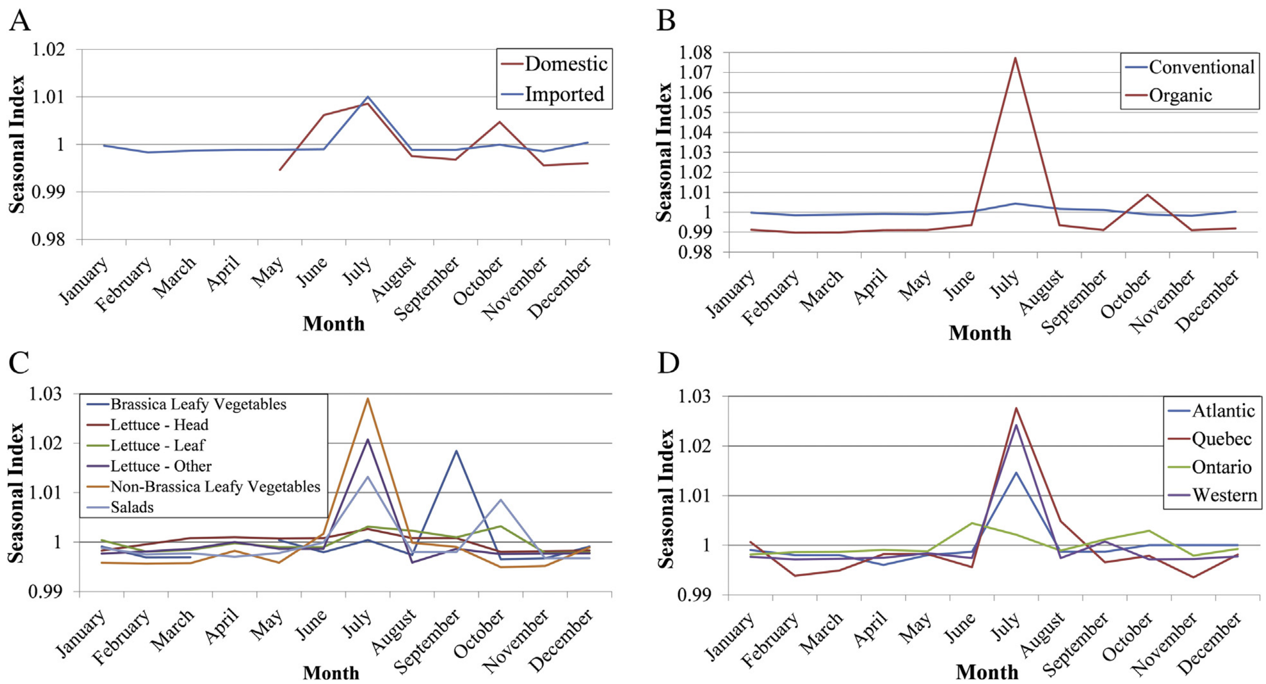

Fig. 3. A: Seasonal Index - Comparison between Product Origin. The month by month seasonal index comparing domestic and imported leafy vegetables samples (all six bacterial results combined) showing similar positive seasonal effects in July. B: **Seasonal Index - Comparison between Organic and Conventional Leafy Vegetables**. The month by month seasonal index comparing organic and conventional leafy vegetables samples (all six bacterial results combined) showing a positive seasonal effect in July but only for organic leafy vegetables. C: **Seasonal Index - Comparison between Product Types**. The month by month seasonal index comparing the product types of the leafy vegetables samples (all six bacterial results combined) showing a positive seasonal effect in July in "non-brassica leafy vegetable" group and in "salads" group. D: Seasonal Index - Comparison between Geographic Areas. The month by month seasonal index comparing the geographic areas of the leafy vegetables samples (all six bacterial results combined) showing a positive seasonal effect in July in all geographic areas except Ontario.

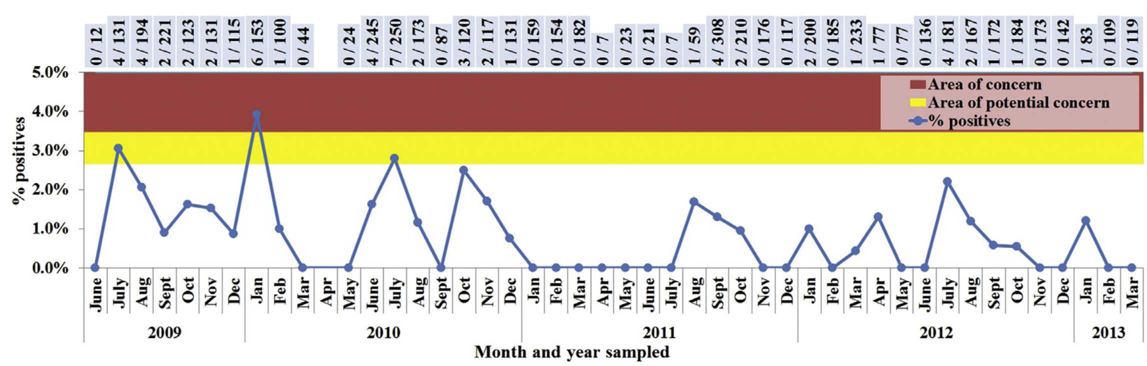

Fig. 4. Control Chart for Leafy Herbs. The control chart calculated for the leafy herbs samples with five bacterial results combined showing three periods of interest.

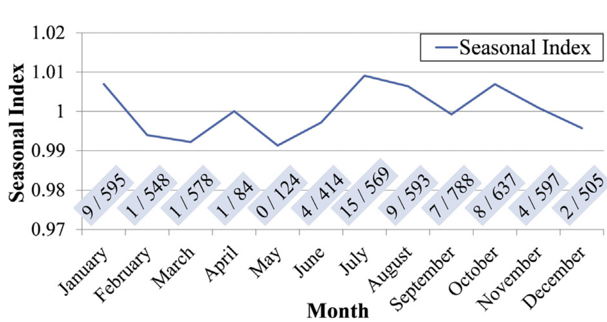

Fig. 5. Seasonal Index for Leafy Herbs. The month by month seasonal index calculated for the leafy herbs samples with five bacterial results combined showing no month with a positive seasonal effect indicating a statistically significant influence.

January (3% above annual average) (data not shown).

The control chart and the seasonal index for generic *E. coli* in leafy herb samples were virtually identical to those calculated for all bacteria combined (data and figures not shown).

The control chart for *Salmonella* (figure not shown) showed two months with a positive rate; however, no more than one sample was found to be positive for this pathogen in each instance and therefore no period was identified as being of particular interest. The seasonal index calculated for *Salmonella* showed no seasonal effect for this pathogen. The other bacterial pathogens investigated in leafy herbs (i.e., *Shigella*, *E. coli* O157 and *Campylobacter*) were not detected in any of the samples and, consequently, no trends could be observed through the use of control charts and seasonal indices for these bacteria.

In summary, bacterial contamination of leafy herbs was found to occur on a regular basis but at a low rate. A few trends were observed: domestic leafy herb samples obtained from Ontario had higher contamination rates in July and contamination of samples obtained from western Canada appeared to be more frequent in January.

4.1.3. Cantaloupes

Between June 2009 and March 2013, 3230 samples of cantaloupes were collected across Canada. The samples included imported and domestic cantaloupes (75.3% and 24.7%) and whole and fresh-cut cantaloupes (74.8% and 25.2%). Most cantaloupes sampled were from conventional production (98.5%) rather than from organic production (1.5%) (Table 4 in Supplemental Materials). The global control chart for the cantaloupe samples (Fig. 6) showed that the positive rate was consistently very low to nil, although two periods, December 2010 and June 2012, were in the area of concern, and one, September 2011, in the area of potential concern. In each time period, only one sample was found to be contaminated. Because bacterial contamination was observed infrequently in the cantaloupe samples, data from global and individual control charts and seasonal indices were inconclusive.

Bacterial contamination of the cantaloupes sampled over the four years of surveys was very low; consequently, no trends in bacterial contamination could be observed.

4.1.4. Green onions

Between May 2010 and March 2013, 3381 samples of green onions were collected across Canada. The samples included imported and domestic green onions (62.8% and 37.2%) and from conventional and organic production (77.8% and 22.2%) (Table 4 in Supplemental Materials). The global control chart calculated for the green onions shows that the positive rate was greater than zero for three months; however, only one sample was found to be positive in each of these instances (Fig. 7).

Similar to what was observed for cantaloupes, bacterial contamination in the green onions sampled during the four years of the survey period was so infrequent that it was not possible to detect any seasonality in the data (seasonal index not shown).

4.1.5. Tomatoes and berries

From June 2009 to March 2010 and from May 2011 to March 2013, 4837 samples of tomatoes were collected across Canada. Samples were imported and domestic (56.8% and 43.2%) and from conventional and organic production (57.3% and 42.7%) (Table 4 in Supplemental Materials).

From May 2010 to March 2013, 1776 samples of berries were collected across Canada. The samples included imported and domestic products (42.6% and 57.4%), mostly from conventional production (97.3% as opposed to 2.7% from organic production or harvested from the wild). Berries samples consisted mainly of blueberries, strawberries, blackberries and raspberries (Tables 4 and 7 in Supplemental Materials).

No pathogen or generic *E. coli* (at levels >100 CFU or MPN/g) were detected in the tomatoes or berries sampled during the survey period; consequently, no trends could be observed through the use of control charts and seasonal indices.

4.2. Prevalence data for all commodities

Prevalence data for all bacteria studied (individually and in combination) for the 12,073 samples of leafy vegetables, 4837 tomatoes, 6032 leafy herbs, 3381 green onions, 3230 cantaloupes and 1776 berries collected over the four year survey period are summarized in Table 1. Both the positive rate (the number of positive samples over the total number of samples) and the prevalence (% of positive samples; both average and range of prevalence values for different product types) are included in the table. Overall, the results show that the prevalence of bacterial contamination in the commodities studied is very low (between 0% and 1.01%), the highest being in leafy herbs (confidence interval: 0.79–1.30%), followed by leafy vegetables (0.30–0.53%), cantaloupes (0.07–0.36%), green onions (0.03–0.26%), berries (0–0.22%) and tomatoes (0–0.08%).

No bacterial pathogens or generic *E. coli* (at levels above 100 CFU or MPN/g) were detected in the samples of tomatoes and berries collected during the study. The bacterial pathogens *E. coli* O157 and *Shigella* were not detected in any the samples of fresh fruits and vegetables examined for these bacteria. *Campylobacter* was not detected in any of the samples of leafy herbs and leafy vegetables analysed for this pathogen. *Salmonella* was detected only at very low levels (prevalence <0.08%) in leafy herbs, cantaloupes, green onions and leafy vegetables. *L. monocytogenes*, which was only analysed in samples of fresh-cut cantaloupes and fresh-cut leafy vegetables since the current Canadian guideline for this pathogen only applies to produce processed and sold as ready-to-eat (HC, 2011b), was found at low levels (prevalence 1.43% in cantaloupes and 0.32% in leafy vegetables). Enumeration of this pathogen was done in almost all positive samples (12 out of 16 samples) and was found to be below 100 CFU/g, a level deemed to represent little risk for human health, in all but one sample (a sample of fresh-cut cantaloupes, where levels were 160 CFU/g). Generic *E. coli*, an indicator of fecal contamination, was found sporadically at levels >100 CFU or MPN/g and was more often found in leafy herbs (0.93%), followed by leafy vegetables (0.28%), cantaloupes (0.10%) and green onions (0.06%). Less than a third of these positive

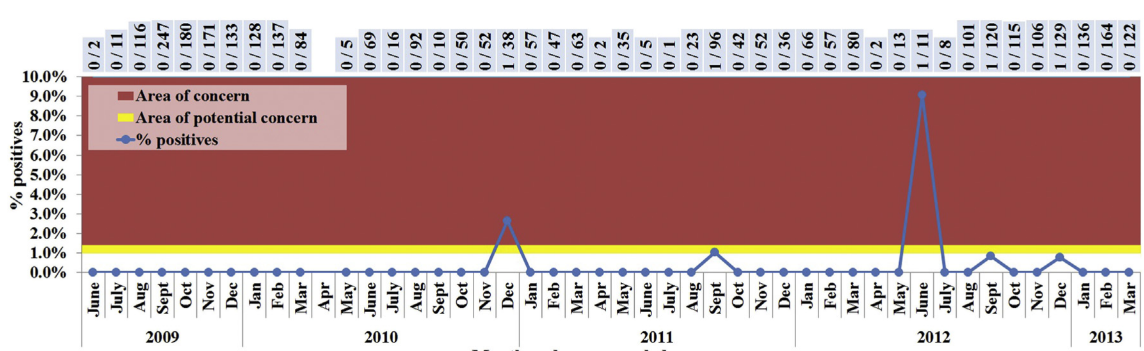

Fig. 6. Control Chart for Cantaloupes. The control chart calculated for the cantaloupes samples with four bacterial results combined showing three periods of interest in which only one sample was found to be contaminated in each of these instances.

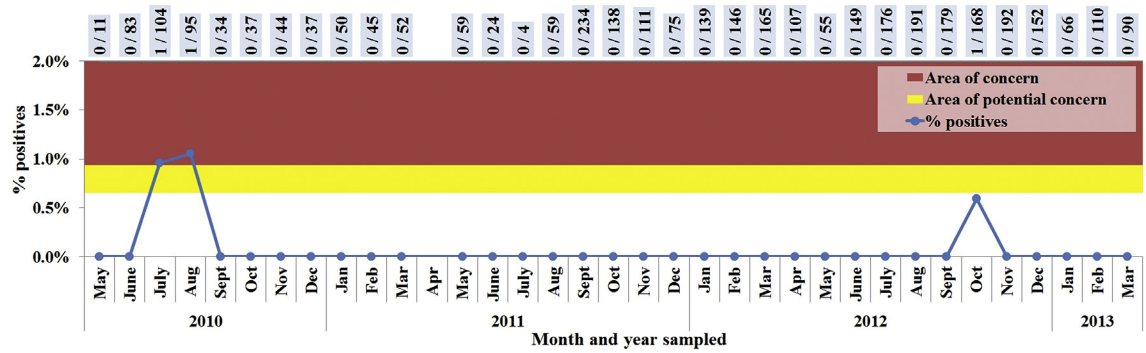

Fig. 7. Control Chart for Green Onions. The control chart calculated for the green onions samples with four bacterial results combined showing three periods of interest in which only one sample was found to be contaminated in each of these instances.

samples presented levels >1000 CFU or MPN/g, levels that could represent a health risk (HC, 2008). A list of the positive samples and their characteristics is provided in Table 8 in the Supplemental Materials.

5. Discussion

The aim of this paper was to present the analysis of the data obtained from four years of retail surveys investigating the bacterial contamination of a wide range of fresh produce commodities available at Canadian retail that have previously been implicated in foodborne illness outbreaks: leafy vegetables, leafy herbs, green onions, tomatoes, cantaloupes and berries. The objective of the analysis was to provide an overview of the results and trends observed and generate a benchmark to support food safety decisions.

5.1. Prevalence of bacterial pathogens and generic E. coli

The prevalence of bacterial contamination observed during this study in the six commodities examined was generally very low. Bacterial pathogens were rarely detected and isolated. Three pathogens were not detected in any of the samples examined: *Shigella* (23,286 samples analysed), *E. coli* O157 (23,805 samples analysed) and *Campylobacter* (8866 samples analysed). As a result, the prevalence intervals calculated for these pathogens in the various produce groups studied were found to be as low as [0, 0.03%], for *E. coli* O157 in leafy vegetables, the group with the largest sample size, and as high as [0, 0.28%] for *Shigella* in berries, the group with the smallest sample size.

Similarly, *Salmonella* was detected and isolated in only ten of the 29,391 samples analysed. These positive samples were found in fresh leafy herbs, cantaloupes, green onions and leafy vegetables. The prevalence calculated for *Salmonella* confirmed that its occurrence in fresh produce was rare. The highest positive rates were for leafy herbs and cantaloupes, with prevalence intervals of [0.04, 0.29%] and [0.02, 0.23%], respectively.

L. monocytogenes was detected and isolated in 14 out of 4435 samples of fresh-cut leafy vegetables and two out of 140 samples of fresh-cut cantaloupes. Enumeration results (obtained for 12 out of 16 samples) were found to be below 100 CFU/g, a level posing very little risk (HC, 2011b), in all but one sample.

The prevalence of generic *E. coli* at levels above the maximum acceptable concentration of 100 CFU or MPN/g was also found to be below 1% in all commodities targeted, which is considered to be very low. Generic *E. coli* is typically found in the intestinal tracts and in the feces of warm-blooded animals, including humans, and, as such, is often used as an indicator of fecal contamination due to insufficient cleanliness during production, distribution and/or sale of produce. Higher levels of generic *E. coli* imply that there is a possibility that other enteric bacteria, such as the pathogens investigated in this study, could be present.

Regardless of the low pathogen prevalence identified in produce through this study, detection and isolation of any pathogen is unacceptable and in violation of Canadian food safety requirements. As such, all samples that were confirmed positive for bacterial pathogens or for levels of generic *E. coli* that could represent a health risk (>1000 MPN or CFU/g) were subject to follow-up actions by the CFIA, which included recalls of the affected products when still available on the marketplace.

The prevalence rates found in this study are consistent with values previously published for jurisdictions benefiting from an equivalent level of protection in terms of food safety (e.g., the USA and EU). The USDA's extensive Microbiological Data Program, which ran from 2001 to 2012 and collected information on the prevalence of bacterial pathogens in fresh produce sold in the USA, also rarely detected and isolated bacterial pathogens (USDA, 2014); for example, *Shigella*, which was tested for in cantaloupes (n = 2037), leafy vegetables (n = 4156) and tomatoes (n = 2702) in 2008, was not detected in any of the samples collected. Similarly, *E. coli* O157, which was tested for over a period of several years in cantaloupes, (n = 9169), leafy vegetables (n = 17,027), tomatoes (n = 9.842) and green onions (n = 7192), was also never detected. *Salmonella*, however, was detected and isolated in several commodities (in total, approximately 70,000 samples were analysed), but its occurrence was rare (<0.1%) in all commodities examined (leafy vegetables, cantaloupes, tomatoes and green onions), with the exception of leafy herbs, where the occurrence was slightly higher (0.36%). This last finding, pointing to leafy herbs as being the most contaminated produce group, is consistent with what we observed during our surveys.

5.2. Trend analysis

Trend analysis was conducted using control charts and seasonal indices, as opposed to traditional inferential statistical methods due to the limitations of our sampling approach in terms of randomness (as detailed in Section 2.1). As generally seen in most surveillance activities of food bacterial pathogens, the large number of samples with negative results represents a challenge in conducting trend analyses, as a very large sample size is required to observe any potentially significant events or trends. Nonetheless, the methods developed and presented in this paper proved to be easy to use, interpret and replicate, and provided a good overview of the data at hand. These methods helped identify some trends worth noting and exploring further, notably in leafy vegetables, the group with the largest sample size (n = 12,073). The control chart for leafy vegetables showed spikes of concern in levels of bacterial contamination over the summer (e.g., July) for multiple years of the study. The global seasonal index (combined data for all six bacteria) and the seasonal index for generic *E. coli* alone confirmed that there was a significantly higher rate of bacterial contamination in July, and that this trend is driven by the results for generic *E. coli*. Further analyses by commodity sub-groups showed that this trend was evident in the organically-grown leafy vegetable group and not in the conventionally-grown leafy vegetables. Due to the more common usage of animal manure as fertilizer in organic production, the microbial safety of organic produce (in comparison with conventionally-grown produce) has been questioned over the years. Several studies on this subject (Mukherjee, Speh, Dyck, & Diez-Gonzalez, 2004; Oliveira et al., 2010; Tango, Choi, Chung, & Oh, 2014) found that the hygienic quality of produce was impacted by organic production and that organic produce was more susceptible to fecal contamination. Our findings support this observation and indicate that summertime is the prime period to observe this difference in hygienic quality in organic leafy vegetables.

It was also interesting to observe that the seasonal indices for domestic and imported leafy vegetables were very similar, indicating that the trend in bacterial contamination did not differ based on the product origin.

Bacterial contamination in leafy herbs appeared to fluctuate over time, but no remarkable seasonal trend could be observed. Generally speaking, of the commodity groups investigated in these surveys, leafy herbs were the most contaminated and the data seem to indicate that this trend is fairly consistent throughout the seasons.

Detection of bacterial pathogens and generic *E. coli* (at levels above 100 CFU or MPN/g) was so seldom in the other commodity groups studied that, not surprisingly, no particularly notable events in bacterial contamination emerged. Considering that tomatoes have been involved in multiple major outbreaks of *Salmonella* infection in North America (Hanning et al., 2009), it is quite remarkable that none of the 4837 samples collected over three years of surveillance presented any contamination with the bacteria examined.

6. Conclusion

The results of this study indicate that the contamination of fresh fruits and vegetables with bacteria at levels representing a risk to public health is rare in the Canadian marketplace. This finding suggests that food safety practices carried out by the different players along the food supply chain, from agricultural practices by the farmers to handling practices by the food distributors and vendors are generally good. Contamination of produce with bacterial pathogens such as *Salmonella* and *L. monocytogenes* occurs only very sporadically, which is still unacceptable as it represents a food safety risk.

Food producers, distributors and vendors are responsible for ensuring that their products meet all applicable food safety requirements and many resources are available to the food industry to aid in the production, transportation, storage and sale of fresh fruits and vegetables of acceptable quality and safety (Canada GAP, 2015; CFIA, 2014; CODEX, Revised 2013; JUS, 2008, 2011; OMAFRA, 2015). The CFIA's role is to provide food safety oversight of the regulated parties and to promote safe production and handling of foods throughout the entire food production chain.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodcont.2016.02.047.

References

AAFC Agriculture and Agri-Food Canada. (2014a). *Statistical overview of the Canadian fruit industry 2013*. Retrieved from http://www5.agr.gc.ca/resources/prod/doc/horticulture/fruit_report_2013-en.pdf.

AAFC Agriculture and Agri-Food Canada. (2014b). *Statistical overview of the Canadian vegetable Industry 2013*. Retrieved from http://www5.agr.gc.ca/resources/prod/doc/pdf/st_ovrv_veg_2013_eng.pdf.

Berger, C. N., Sodha, S. V., Shaw, R. K., Griffin, P. M., Pink, D., Hand, P., et al. (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology, 12*(9), 2385–2397.

Byrne, L., Fisher, I., Peters, T., Mather, A., Thomson, N., Rosner, B., et al. (2014). A multi-country outbreak of *Salmonella* Newport gastroenteritis in Europe associated with watermelon from Brazil, confirmed by whole genome sequencing: October 2011 to January 2012. *Euro surveillance, 19*(31), 6–13.

Canada GAP. (2015). *Food safety for fresh fruits and vegetables*. Retrieved from http://www.canadagap.ca/.

CFIA Canadian Food Inspection Agency. (2008). *Food safety science committee summary report 2008*. Retrieved from http://merlin.cfia-acia.inspection.gc.ca/english/fssa/invenq/guidoce.asp#refman5.

CFIA Canadian Food Inspection Agency. (2013). *ARCHIVED – Food safety action plan evaluation*. Retrieved from http://www.inspection.gc.ca/about-the-cfia/accountability/other-activities/audits-reviews-and-evaluations/fsap/report/eng/1385242045975/1385242106674.

CFIA Canadian Food Inspection Agency. (2014). *Fresh fruits and vegetables – Food safety*. Retrieved from http://www.inspection.gc.ca/food/fresh-fruits-and-vegetables/food-safety/eng/1299853525490/1299854225881.

CODEX Alimentarius Commission. (Revised 2013). The code of hygienic practice for fresh fruits and vegetables (CAC/RCP 53-2003). Retrieved from www.codexalimentarius.org/input/download/.../CXP_053e_2013.pdf.

Elviss, N. C., Little, C. L., Hucklesby, L., Sagoo, S., Surman-Lee, S., de Pinna, E., et al. (2009). Microbiological study of fresh herbs from retail premises uncovers an international outbreak of salmonellosis. *International Journal of Food Microbiology, 134*(1–2), 83–88.

FAO/WHO. (2008a). *Microbiological hazards in fresh fruits and vegetables*. Retrieved from http://www.fao.org/fileadmin/templates/agns/pdf/jemra/FFV_2007_Final.pdf.

FAO/WHO. (2008b). *Microbiological risk assessment series 14: Microbiological hazards in fresh leafy vegetables and herbs*. Retrieved from ftp://ftp.fao.org/docrep/fao/011/i0452e/i0452e00.pdf.

Grant, J., Wendelboe, A. M., Wendel, A., Jepson, B., Torres, P., Smelser, C., et al. (2008). Spinach-associated *Escherichia coli* O157:H7 outbreak, Utah and New Mexico, 2006. *Emerging Infectious Diseases, 14*(10), 1633–1636.

Hanke, J. E., & Reitsch, A. G. (1994). *Understanding business statistics* (2nd ed.). Boston, Massachusetts: Richard D. Irwin, Inc.

Hanning, I. B., Nutt, J. D., & Ricke, S. C. (2009). Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. *Foodborne Pathogens and Disease, 6*(6), 635–648.

HC Health Canada. (2008). *Health products and food Branch standards and guidelines for the microbiological safety of food – An interpretive summary*. Retrieved from http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume1-eng.php.

HC Health Canada. (2011a). *Compendium of analytical methods*. Retrieved from http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php.

HC Health Canada. (2011b). *Policy on Listeria monocytogenes in ready-to-eat foods*. Retrieved from http://www.hc-sc.gc.ca/fn-an/legislation/pol/policy_listeria_monocytogenes_2011-eng.php.

JUS Department of Justice Canada. (2008). *Food and drugs act*. Retrieved from http://laws-lois.justice.gc.ca/eng/acts/F-27/.

JUS Department of Justice Canada. (2011). *Fresh fruit and vegetable regulations*. Retrieved from http://laws-lois.justice.gc.ca/eng/regulations/C.R.C.,_c._285/index.html.

Kozak, G. K., MacDonald, D., Landry, L., & Farber, J. M. (2013). Foodborne outbreaks in Canada linked to produce: 2001 through 2009. *Journal of Food Protection, 76*(1), 173–183.

Lynch, M. F., Tauxe, R. V., & Hedberg, C. W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection, 137*(3), 307–315.

Montgomery, D. C. (2009). *Introduction to statistical quality control* (6th ed.). USA: John Wiley & Sons, Inc.

Mukherjee, A., Speh, D., Dyck, E., & Diez-Gonzalez, F. (2004). Preharvest evaluation of coliforms, *Escherichia coli, Salmonella*, and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *Journal of Food Protection, 67*(5), 894–900.

Oliveira, M., Usall, J., Vinas, I., Anguera, M., Gatius, F., & Abadias, M. (2010). Microbiological quality of fresh lettuce from organic and conventional production. *Food Microbiology, 27*(5), 679–684.

OMAFRA Ontario ministry of agriculture food and rural affairs. (2015). *Food Safety*. Retrieved from http://www.omafra.gov.on.ca/english/infores/foodsafe/safety.html.

Pezzoli, L., Elson, R., Little, C. L., Yip, H., Fisher, I., Yishai, R., et al. (2008). Packed with *Salmonella*-investigation of an international outbreak of *Salmonella* Senftenberg infection linked to contamination of prepacked basil in 2007. *Foodborne Pathogens and Disease, 5*(5), 661–668.

PHAC Public Health Agency of Canada. (2012). *Salmonella outbreak linked to green onions, personal communication*.

PHAC Public Health Agency of Canada. (2014). *Outbreak of E. coli O157:H7 associated with lettuce served at fast food chains in the Maritimes and Ontario, Canada, Dec 2012*. Canada Communicable Disease Report CCDR, 40 S-1 http://phac-aspc.gc.ca/publicat/ccdr-rmtc/14vol40/dr-rm40s-1/dr-rm40s-1-ecoli-eng.php.

Sewell, A. M., & Farber, J. M. (2001). Foodborne outbreaks in Canada linked to produce. *Journal of Food Protection, 64*(11), 1863–1877.

Sharma, J. K. (2010). *Fundamentals of business statistics* (1st ed.). India: Pearson Education India.

Sivapalasingam, S., Friedman, C. R., Cohen, L., & Tauxe, R. V. (2004). Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection, 67*(10), 2342–2353.

Statistics Canada. (2002). *Food consumption in Canada Part II 2001*. Retrieved from http://www5.statcan.gc.ca/olc-cel/olc.action?objId=32-230-X&objType=2&lang=en&limit=0.

Tango, C. N., Choi, N. J., Chung, M. S., & Oh, D. H. (2014). Bacteriological quality of vegetables from organic and conventional production in different areas of Korea. *Journal of Food Protection, 77*(8), 1411–1417.

USDA United States Department of Agriculture. (2014). *Microbiological data Program*. Retrieved from http://www.ams.usda.gov/AMSv1.0/mdp.

Wendel, A. M., Johnson, D. H., Sharapov, U., Grant, J., Archer, J. R., Monson, T., et al. (2009). Multistate outbreak of *Escherichia coli* O157:H7 infection associated with consumption of packaged spinach, August-September 2006: the Wisconsin investigation. *Clinical Infectious Diseases, 48*(8), 1079–1086.

Xie, M., Goh, T. N., & Kuralmani, V. (2002). *Statistical model and control chart for high quality processes* (1st ed.). USA: Kluer Academic Publishers.