Microbiological Quality of Minimally Processed, Ready-to-Eat, Vegetables in Loja, Ecuador

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Abstract: Minimally processed, ready-to-eat (RTE) vegetables, are consumed raw so have to comply with microbiological requirements to ensure safety and avoid possible foodborne illnesses. The objective of this study was to evaluate the microbiological quality of RTE vegetables: lettuce, spinach, carrot, cabbage with carrot, parsley, parsley curd and coriander that are sold in supermarkets in the city of Loja, Ecuador. A total of 128 samples from 3 production batch were analyzed for total aerobic count, total coliform and *E. coli*. The aerobic count obtained averaged 6,6 $\log_{10}$ CFU/g and ranged from 4,57 to 7,82 $\log_{10}$ CFU/g. The coliform counts a ranged from 0,48 to > 5,04 $\log_{10}$ MPN/g and in 11 samples >5,04 $\log_{10}$ MPN/g was observed. Generic *E. coli* was detected in 32 samples at levels less than 6,2 MPN/g. In our study, 50% and 98% of the samples exceeded the reference limits for aerobic and coliform counts, respectively. The results of this study indicate that RTE vegetables have, in some cases, the same microbial load as packaged vegetables that are produced without a disinfection process, and maybe potential vehicles for pathogen transmissions, which means the necessity of regulate the quality assurance of RTE vegetables.

Keywords: microbiological quality, ready-to-eat vegetables, coliforms, *Escherichia coli*

Calidad Microbiológica de Vegetales Mínimamente Procesados Listos para el Consumo, en Loja, Ecuador

Resumen: Los vegetales listos para el consumo o mínimamente procesados (VMP), son consumidos crudos de modo que deben cumplir con requerimientos microbiológicos para asegurar la seguridad y evitar en lo posible enfermedades transmitidas por alimentos. El objetivo de este estudio fue evaluar la calidad microbiológica de VMP: lechuga, espinaca, zanahoria, col con zanahoria, perejil liso, perejil crespo y culantro que se comercializan en supermercados de la ciudad de Loja, Ecuador. 128 muestras provenientes de tres lotes de producción se analizaron en cuanto a aerobios totales, coliformes totales y *E. coli*. Se obtuvo un conteo de aerobios promedio de 6,6 $\log_{10}$ UFC/g en un rango entre 4,57 y 7,82 $\log_{10}$ UFC/g. El conteo de coliformes estuvo en el rango desde 0,48 hasta > 5,04 $\log_{10}$ NMP/g y en 11 muestras se observó valores > 5,04 $\log_{10}$ NMP/g, además se detectó la presencia de *E. coli* genérica en 32 muestras en niveles menores que 6,2 NMP/g. En nuestro estudio, el 50% y 90% de las muestras excedió los límites de referencia para aerobios y coliformes totales, respectivamente. Los resultados de este estudio indican que VMP tienen, en algunos casos, la misma carga microbiana que los vegetales empaquetados que se producen sin ningún proceso de desinfección, pudiendo ser potenciales vehículos para transmisión de patógenos siendo necesario regular el aseguramiento de la calidad de los vegetales listos para su consumo.

Palabras clave: calidad microbiológica, vegetales mínimamente procesados, coliformes, *Escherichia coli*,

1. INTRODUCTION

According Betts (2014) in the last few years, the market for RTE vegetables has increased explosively. The increasing demand for convenient, ready-to-eat (RTE) foods have greatly expanded the market demand for minimally processed foods, like bagged salads resulting in large scale production practices and broad distribution of fresh products. The main driving force for this market growth is the increasing consumer demand for fresh, healthy, convenient and additive-free prepared products (FAO, 2011). Minimally processed leafy vegetables RTE products very attractive to consumers looking for healthy and convenient meals. However, the microbiological safety of these foods is of special concern due to the absence of lethal treatments during processing (Oliveira 2011).

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Callejón et al., (2015) mention that at the same time, outbreaks of foodborne illnesses associated with the consumption of fresh produce have increased. The complexities in production, processing, transportation and storage can be gaps for insanitation and contamination resulting in health risks. As a result, fresh produces have been implicated in many food borne outbreaks worldwide with *Listeria monocytogenes*, *Escherichia coli* serotype O157:H7 and *Salmonella (Feng and Reddy, 2013; Johnston et al., 2006)*. In recent years, the percentage of food poisonings induced by the consumption of contaminated vegetables and juices and other products thereof in the European Union ranged from 2,1% in 2009 to 7,1% in 2014 (EFSA, 2015).

For example, an outbreak of *E. coli* O157:H7 in 2006, in the United States caused by packed spinach, affected 23 states and some months later, another O157:H7 outbreak affected a chain of fast food restaurants and it was suspected to be due to the consumption of packed lettuce (Valentin-Bon et al., 2008). These incidences raised major public health concerns about the safety and quality of fresh produce. Accordingly, the PAHO/WHO Regional System of Information for Epidemiologic Watching of Diseases Transmitted by Foods (PAHO/WHO, On line) declared that in Latin America between 1998 to 2002, vegetables were the main vehicle involved in the outbreaks of diseases transmitted by food. This rising awareness has prompted many studies worldwide on the microbiological quality of fresh vegetable products, including in the United Kingdom (Sagoo et al., 2001, 2003), Spain (Soriano et al., 2006), the United States (Feng and Reddy, 2013; Valentin-Bon et al., 2008), Venezuela (Rincón et al., 2010), Chile (López et al., 2003) and Brazil (Fröder et al., 2007), but no studies have been done in Ecuador.
In this study, we examined various RTE vegetables that are sold in supermarkets to assess the microbiological quality of fresh produce sold in Loja, Ecuador.

2. MATERIALS AND METHODS

2.1 Produce samples

A total of 128 samples of RTE and packed vegetable samples were collected randomly from 3 supermarket chains from June to November 2014 in the city of Loja, Ecuador. The 60 RTE samples (some containing two to three ingredients, including lettuce, cabbage, carrot) consisted of packed, cut vegetables of lettuce (Creole, Roman and American), parsley (plain and curly) and coriander. These samples were obtained unopened in the original containers prior to their expiration date, and the production batches were verified and within the shelflife time frame of up to eight days, as indicated by the labels. Samples were placed in ice chests and transported immediately to the laboratory for analysis.

2.2 Microbiological analysis

The metadata on all the samples were recorded, including the name of the manufacturer, type of vegetable, lot number, date of expiration and type of packaging. Samples that were deteriorated or had visible damaged were discarded and not included in the analysis.

The surfaces of the containers were disinfected with 70% alcohol before sampling, to prevent cross-contamination.

The microbiological quality of the samples was determined with methods of the FDA Bacteriological Analytical Manual (BAM) (U.S., 2013) (http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949) briefly, 50 g of the product (from 3 production batches) were weighed and mixed with 450 mL of buffered peptone water, from which 1:10 serial dilutions were made until the final dilution of 10^7. The mesophilic aerobic count was performed by standard plate count using plate count agar (Difco BBL). The counts of coliform bacteria and *E. coli* were determined by the MPN method using Lauryl Sufate Tryptose broth, but modified with the use of ColiComplete disks (Biocontrol Bellevue, WA) (AOAC, 2012) as described in literature (Valentin-Bon et al., 2008). The disks contain X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) which is cleaved by β-galactosidase to produce a blue color that is indicative of coliform. The discs also contain 4-methylumbelliferone-ß-D-glucuronide, which is cleaved by *E. coli* ß-glucuronidase activity to produce blue fluorescence visible at 365-nm UV. After 48 h of incubation at 37°C, tubes with blue dye were counted as coliform positive and those with blue fluorescence were positive for *E. coli*. The level of coliform and *E. coli* present was estimated by MPN/g based on the combination of positive tubes.

The identification of the genus and the species of the gram-negative bacilli was carried out touching lightly the center of the colony with sterile inoculating needle and inoculate to Triple iron Agar (Difco BBL) slant, Lysine iron agar (Difco BBL) slant, Urea broth (BBL), Citrate medium (Difco BBL), and Sulfide indole motility agar (BBL) by streaking slant and stabbing butt. The culture was incubated 24 ± 2 h at 35°C and for the confirmation the Microgen GN-ID identification system was used.

3. RESULTS AND DISCUSSION

3.1 The mesophilic aerobic count

In the mesophilic aerobic bacteria count the mean microbial loads for RTE were lettuce 5,66±0,6, spinach 6,45±0,8, carrot 7,23±0,2, cabbage with carrot 7,31±0,3 $\log_{10}$ CFU/g, in the case of packaged vegetables, the mean microbial loads were: parsley smooth 5,58±0,6, parsley curli 6,09±0,4, coriander 6,03±0,5, lettuce creole 5,18±0,8, lettuce romaine 6,00±0,4 and spinach 6,00±0,4 $\log_{10}$ CFU/g, these values are shown in Table 1.

3.2 Total coliforms and E. coli

The number of total coliforms and *E. coli* in RTE and packaged vegetables are presented in Tables 2 and 3. Total coliforms were not detected in 6,7% of the vegetables (spinach).

All 5 samples (100%) of grated carrot and cabbage with carrot contained coliform levels between 5,04 and >5,04 $\log_{10}$ MPN/g.

E. coli was detected in 13 of 60 (21,7 %) RTE vegetable samples and in 19 of 68 (27,9 %) packaged vegetable samples (Table 3), but the levels were less than 6,2 MPN/g.

3.3 Discussion

The total counts of the lettuce samples we analyzed had a mean value of log 5,66 and ranged from 4,57 to 6,78 $\log_{10}$ CFU/g (Table 1). In Iran, RTE vegetables were examined and reported mean total counts of log 6.7 with ranges from 5,5 to 7,4 $\log_{10}$ CFU/g (Jeddi et al., 2014). In the Campinas area of Brazil, analysis of conventional and organic vegetables reported total count values from 4,00 to 6,89 $\log_{10}$ CFU/g (Maffei et al., 2013) and from 6,07 to 7,48 log10 CFU/g (Maistro et al., 2012), respectively. Our results are consistent with these data and with those reported from the United States in 55 samples of RTE salads (Hagenmaier and Baker, 1998) and in 100 samples of RTE vegetables (Valentin-Bon et al., 2008). In Spain, total counts between 3,1 to 7,81 $\log_{10}$ CFU/g were reported in 140 lettuce samples collected from 16 restaurants (Soriano et al., 2006). It has been noted that in all vegetable samples aerobial mesophylls were developed. In another study from Brazil, 21% of 133 samples of RTE vegetables showed > 6,0 $\log_{10}$ CFU/g for coliforms (Fröder et al., 2007). All these studies showed that RTE vegetables sampled worldwide contains high levels of total bacteria, often >7,0 $\log_{10}$ CFU/g.

Large variability in total counts not only between different samples but also within samples from the same producer and with the same production date have been previously described (Valentin-Bon et al., 2008). We found similar patterns in our study, all samples studied were positive for coliforms, where lettuce and spinach samples had values ranged from 4,57 to 6,78 and from 5,47 to 7,82 $\log_{10}$ CFU/g, respectively.

Table 1. Aerobic mesophilic counts in RTE and packed vegetables samples, with percentages of samples at the indicated levels.

Produce	No. of samples	Mean*	Range[a]	Percentages			
				$>10^7$	10^6 - 10^7	10^5-10^6	$<10^5$
Lettuce (RTE)	35	5,66±0,6	4,57-6,78	11	20	57	11
Spinach (RTE)	15	6,45±0,8	5,47-7,82	27	40	33	0
Carrot (RTE)	5	7,23±0,2	6,86-7,44	80	20	0	0
Cabbage with carrot (RTE)	5	7,31±0,3	6,80-7,56	80	20	0	0
Parsley smooth (packaged)	13	5,58±0,6	4,95-6,83	0	8	69	23
Parsley curly (packaged)	13	6,09±0,4	5,56-6,95	0	54	46	0
Coriander (packaged)	12	6,03±0,5	4,95-6,82	0	58	33	8
Lettuce creole (packaged)	10	5,18±0,8	4,95-6,82	0	20	40	40
Lettuce romaine (packaged)	10	6,00±0,4	5,05-6,40	0	80	20	0
American Spinach (packaged)	10	6,00±0,4	6,80-7,56	0	80	0	20

*Results are expressed as mean±SD of the two replicates; Counts are given in terms of $\log_{10}$ CFU/g of products; [a] range in $\log_{10}$ CFU/g of product

In some samples, condensations in the bags, probably from humidity, were observed at the bottom of the bags, and this may have provided moisture which can stimulate microbial growth. These results suggest that the microbiological qualities of the packaged vegetables reported from the various countries appear to be similar. The mesophyll aerobic count is used by some countries as an indicator of safety, but has no direct relation with the presence of pathogens or their toxins. In Ecuador, there are no regulations for mesophyll aerobic count for this type of products, but others have suggested 5,0 $\log_{10}$ CFU/g as acceptable for aerobic counts (Solberg et al., 1990). The National Standard of Iran uses aerobic counts of less than 6,0 $\log_{10}$ CFU/g as being considered safe for consumption. In this context, our study showed that 50% of the produce samples in Loja, Ecuador would have exceeded this norm. Moisture was observed in the bottom of many of the bags of lettuce and spinach we tested, which may have favored microbial growth and contributed to the high counts. The RTE plant products may be contaminated at each stage of their production process (both pre- and post-harvest and processing). Berthold-Pluta et al., (2017) indicated that potential sources of contamination include soil, water, feces (human and animal origin), animals (for example insects and birds), handling of the products, harvesting and processing equipment and transport.

Table 2. Total coliform counts in fresh RTE and minimally – processed packaged vegetables, with percentages of samples at the indicated levels

Produce	No. of samples	Mean*	Range[a] MPN/g	Percentages					
				$>10^4$	$10^3 – 10^4$	$10^2 – 10^3$	10^1- 10^2	$<3x10^1$	<3
Lettuce (RTE)	35	4,02±1,1	2,18 - 5,66	48	23	29	0	0	0
Spinach (RTE)	15	3,99±0,9	0,48 - 5,04	53	33	0	7	0	7
Carrot (RTE)	5	TNTC	>5,04	100	0	0	0	0	0
Cabbage with carrot (RTE)	5	TNTC	5,04 - >5,04	100	0	0	0	0	0
Parsley smooth (packaged)	13	3,81±0,7	2,87 - >5,04	38	31	31	0	0	0
Parsley curly (packaged)	13	3,14±0,9	1,56 - 4,66	15	31	39	15	0	0
Coriander (packaged)	12	4,52±0,5	3,38 - 5,04	92	8	0	0	0	0
Lettuce creole (packaged)	10	3,01±1,5	0,48 - 4,66	30	30	20	0	20	0
Lettuce romaine (packaged)	10	2,98±1,0	1,48 - >5,04	20	30	40	10	0	0
American Spinach (packaged)	10	3,75±1,2	2,36 - >5,04	80	10	10	0	0	0

*Results are expressed as mean±SD corresponded to each vegetable, counts are given in terms of $\log_{10}$ MPN/g of products; [a] range in $\log_{10}$ MPN/g of product; TNTC, too numerous to count

Table 3. Percentages of fresh, RTE and minimally – processed bagged vegetables that contained *E. coli*.

Food item (No of samples)	No of positive samples	*Concentration (MPN/g)*
Lettuce (RTE) (35)	9	6,2
Spinach (RTE) (15)	2	6,2
Carrot (RTE) (5)	1	6,2
Cabbage with carrot (RTE) (5)	1	6,2
Parsley smooth (packaged) (13)	0	--
Parsley curly (packaged) (13)	3	6,2
Coriander (packaged) (12)	3	6,2
Lettuce creole (packaged) (10)	2	6,2
Lettuce romaine (packaged) (10)	5	6,2
American Spinach (packaged) (10)	6	6,2

The greatest applications of coliform, *Enterobacteriaceae*, and *E.coli* testing is in assessment of the overall quality of a food and the hygienic conditions present during food processing. The *Enterobacteriaceae*, coliforms, and Escherichia coli are used with as Quality and Safety indicators.

The total coliform counts we obtained were from 0,47 to >5,04 $\log_{10}$ MPN/g (Table 2), which are higher than the 0,47 to 3,38 $\log_{10}$ MPN/g reported in Spain (Soriano et al., 2006) and the <0,47 to >4,0 $\log_{10}$ MPN/g reported in the United States (Valentin-Bon et al., 2008). In our study, it was not possible to obtain a mean coliform value because 13 of the 60 samples have exceed the counting limit of >5,05 $\log_{10}$ MPN/g. There are no permissible limits of indicator bacteria for RTE in Ecuador, but again, using the National Standard of Iran, 98% of our produce samples have exceeded that standards.

Generic *E. coli* bacteria were detected in 9 samples of lettuce, 2 samples of spinach, 1 sample of cut carrot and 1 sample of cabbage with chopped carrot (Table 3). In Brazil, the standard for minimally processed products for consumption has a limit of 100 CFU/g for fecal coliforms and in one analysis of 133 salad samples, 73% had exceeded that limit (Fröder et al., 2007). In the United Kingdom, the Public Health laboratory has set *E. coli* limits for RTE foods, including bagged produce, where *E. coli* of 20 CFU/g is satisfactory, from 20 to <100 CFU/g as acceptable and ≥ 100 CFU/g as unsatisfactory (Sagoo et al., 2003). In a few studies, 3200 organic salads and 3852 conventional RTE salads were sampled and analyzed in the United Kingdom and the results showed that only 0,5% had exceeded the permissible maximum *E. coli* limit of 100 CFU/g and were qualified as unsatisfactory (Sagoo et al., 2001, 2003). In our study, 25% of the samples had *E. coli* but did not exceeded the value of 6,2 MPN/g. This is similar to the results obtained in the United States (Jeddi et al., 2014; Valentin-Bon et al., 2008) where *E. coli* was detected in 21 samples (18,1%) but at low levels, suggesting that *E. coli* are not usually present at high levels in bagged produce.

The common foodborne genera of the Family *Enterobacteriaceae* include *Citrobacter*, *Enterobacter*, *Erwinia*, *Escherichia*, *Hafnia*, *Klebsiella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella*, and *Yersinia*, we also found the presence of other Gram negative bacteria in produce but in different percentages, *Pseudomonas spp* (13%), *Enterobacter aerogenes* (45%), *Enterobacter cloacae* (8,3%), *Klebsiella Oxytoca* (1,6%), *Citrobacter freundi* (3,3%), and *Enterobacter spp* (1,6%). These results are consistent with those reported in literature (Seow et al., 2012; Soriano et al., 2006). The presence of these microorganisms in foods, may be indicators of lack of food quality, indicator microorganisms are considered to be all groups or species of microorganisms involved in diseases of alimentary origin that are usually enteric pathogens, which means that they can survive in the gastrointestinal tract of humans, as well as animals and birds, a directly or indirectly contaminated with fecal material from any of these sources, is indicative of the possible presence of enteric pathogens (Ray and Bhunia, 2010).

The results from all these studies showed that the flora and bacterial content in RTE products are highly variable and complex. The microbiological quality of fresh RTE products from production to packaging has been studied (Johnston et al., 2006) and showed that indicator levels in mustard and spinach leaves remained relatively constant, whereas in coriander, parsley and, above all, melons had varied counts demonstrating that the microbial load not only vary according to processing, but also depends on the type of product. The processing procedures for each one of the produce products is different moreover, variations in the microbial load can also occur during storage.

Prevention of foodborne contamination in fresh-cut processing starts in the field by identifying and eliminating possible sources of contamination. Principles of Good Agricultural Practices (GAP) and Good Handling Practices (GHP) remain the pre-requisite cornerstones of food safety management strategies to address challenges posed at the pre-harvest level (Castro-Ibañez et al 2017).

In conclusion, there is a wide range and variations in the microbial counts in the fresh produce samples in Ecuador and even between samples with the same use-by-dates. These RTE produce products can contain very high levels of microbial content and these products are difficult to disinfect, hence can pose health risks if contaminated with pathogens. To improve the safety of fresh vegetables in the industry some effective measures should be applied such as chemical and physical treatments, refrigeration and packaging in modified atmosphere can inhibit or retard bacterial growth.

For the prevention of contamination in fresh processed vegetables, possible sources of contamination should be identified, Principles of Good Agricultural Practices (GAP) should be applied, Good Handling Practices (GHP), food safety management based on the previous analysis of the harvest.

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