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Microbiological quality of ready-to-eat minimally processed vegetables consumed in Brazil

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A B S T R A C T

Minimally processed leafy vegetables are ready-to-eat (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. In the present study, indicator microorganisms, *Listeria* spp. and *Salmonella* spp. were determined for 162 samples of minimally processed leafy vegetables commercialized in Brazil. Psychrotrophic aerobic bacterial populations >5 log CFU/g were found in 96.7% of the samples, while total and thermotolerant coliforms were detected respectively in 132 (81.5%) and 107 (66%) of vegetables analyzed. *Escherichia coli* was present in 86 (53.1%) samples analyzed and *Listeria* spp. and *Salmonella* spp. were detected respectively in 6 (3.7%) and 2 (1.2%) samples. These results indicate the need of implementing quality programs in the production chain of RTE vegetables to improve shelf life and microbiological safety.

1. Introduction

Demographic changes related to aging of population and new trends of life style with an increasing demand for minimally processed ready-to-eat (RTE) foods has changed the scenario of foodborne diseases worldwide, with important economic and social impacts (Kennedy & Wall, 2007; Meng & Doyle, 2002). RTE vegetables can be obtained from the fresh products through selection, washing, peeling, cutting, sanitization, rinsing, drying and packaging, in order to extend shelf life and preserve nutritive and sensorial properties (Francis, Thomas, & O'Beirne, 1999). These steps may not be efficient to eliminate contamination of RTE vegetables with parasites and viruses and besides this, the storage under refrigeration may favor the growth of psychrotrophic pathogenic and spoilage microorganisms (Aguado, Vitas, & García-Jalón, 2004; Gleeson & O'Beirne, 2005).

In the USA, minimally processed vegetables are marketed since the 1970's, but in Brazil, this kind of product became commercially available only in the past two decades. Nowadays, RTE vegetables represent an important food market in Brazil and, the large supermarket chains are responsible for 10%–13% of total sales (Moretti, 2007; SEBRAE/ESPM, 2008; Silva et al., 2004). However, it is not simple to preserve the natural attributes of vegetables, since processing frequently causes mechanical injuries of tissues, leading to loss of water and color changes (browning or discoloration). Also, the formation of exudates rich in minerals, sugars, vitamins and other nutrients may support the growth of autochthonous microbiota (Soares & Geraldine, 2007).

Packing under modified atmosphere helps to extend shelf life of RTE vegetables and may act also as protective barrier against insects, rodents and microorganisms (Soares & Geraldline, 2007). Other technologies with potential to increase quality and safety of these products have been studied, such as ionizing radiation and the use of biopreservative cultures and/or their metabolites (Galvez, Lopez, Abriouel, Valdinia, & Ben Omar, 2008; Martins et al., 2004).

During harvest, the superficial microbiota of vegetables comprises mainly Gram negative saprophytes, but pathogenic microorganisms can also be found. Vegetables may harbor pathogenic *Escherichia coli* and *Salmonella* spp. – enteric bacteria involved in large foodborne outbreaks worldwide, causing symptoms of gastroenteritis, and even chronic infections (D'Aoust, 2007; Francis et al., 1999). *Listeria monocytogenes* can also contaminate RTE vegetables and it is a psychrotolerant and ubiquitous microorganism that causes listeriosis, an atypical infection with low mortality but high fatality rates among the elderly, pregnant women and immunocompromised individuals (Abadias, Usall, Anguera, Solsona, & Viñas, 2008; Beuchat, 1996; FDA, 2003).

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The objective of the present study was to assess the microbiological quality of RTE salads consumed in Brazil aiming to guide future improvement of food safety measures.

2. Material and methods

2.1. RTE vegetable samples

One hundred and sixty two samples of minimally processed leafy vegetables (300 g-packages), were randomly acquired from six supermarket chains in the city of Ribeirão Preto, São Paulo, located in the southeast region of Brazil and included: 30 kale, 28 cabbage, 26 lettuce, 22 mixtures of spring onion and parsley, 13 Chinese cabbage, 13 wild chicory, 11 chicory, 9 spinach, 6 arugula and 4 watercress. All the samples were obtained in the original package, within the shelf life of up to 8 days, as declared on labels. Samples were transported to the laboratory in insulated boxes and analyzed on the day of purchase.

2.2. Microbiological analyses

Aerobic psychrotrophic bacteria as well as total and thermotolerant coliforms and *Escherichia coli* were quantified using classical methodologies, with the results being expressed as colony forming units (CFU/g) and most probable number (MPN), respectively (Blodgett, 2006; Kornacki & Johnson, 2001; Swanson, Petran, & Hanlin, 2001).

To isolate *Salmonella* spp. 25 g of each vegetable sample were transferred to sterile plastic bags and homogenized with 225 ml of 1% (w/v) buffered peptone water (BPW) (Merck, Germany) and kept at room temperature for 60 min. The pH was adjusted to 6.8 ± 0.2 and the isolation was carried out according to Andrews, Flowers, Silliker, and Bailey (2001). Results were obtained as Presence or Absence of *Salmonella* spp. in 25 g of food and the isolates were serotyped according to Popoff, Bockemühl, Brenner, and Gheesling (2001).

The immunoassay *Listeria* Rapid Test (Oxoid Ltd., Basingstoke, Hampshire, England) was used to detect *Listeria* spp., according to instructions of manufacturer. To check the performance of immunoassay and to score positive samples for Presence or Absence of *L. monocytogenes*, aliquots of Half Fraser enrichment broth were streaked on plates containing Oxford (OX) and Palcam (PAL) agars (Oxoid Ltd.) and incubated for 48 h at 35 °C, for presumptive detection of *Listeria* spp. Three to five typical black colonies from each agar were picked, purified on Tripticase Soy agar plus 0.6% yeast extract plates (Oxoid Ltd.) and those with blue appearance under oblique transmitted light were selected for biochemical identification (Hitchins, 2003).

3. Results and discussion

The quality and safety of minimally processed vegetables depend on the use of appropriate irrigation water and good practices during manipulation, but inherent risks of contamination due to cultivation in close contact with soil and organic fertilizers make difficult the control of pathogenic and spoilage microorganisms. Table 1 shows high populations of psychrotrophic aerobic bacteria were present in minimally processed vegetable samples analyzed in the present work, suggesting a short shelf life for the product and poor hygienic quality, likely due to the use of highly contaminated raw material, lack of good hygienic practices during processing and/or inadequate temperature of storage (Bruno, Queiróz, Andrade, Vasconcelos, & Borges, 2005). However, upon subjective visual and olfactory inspection, all the samples analyzed in the present work appeared to be suitable for consumption.

Table 1
Average populations of psychrotrophic aerobic bacteria present in the minimally processed leafy vegetable samples analyzed.

Samples	log CFU/g[a]
Arugula	9.4
Spring onion/parsley mixture	9.3
Spinach	9.0
Wild chicory	8.5
Chicory	8.2
Cabbage	8.2
Chinese cabbage	7.9
Kale	7.8
Lettuce	7.1
Watercress	7.1

[a] Colony Forming Units per gram of food.

In this study, coliforms were detected in the majority of samples of minimally processed vegetables (Table 2) with populations above 3 log MPN/g for 81.5% (data not shown), which may also contribute for decreasing the shelf life of products (Berbari, Paschoalino, & Silveira, 2001). Besides this, Table 2 reveals that thermotolerant coliforms were found in 66% of minimally processed vegetable samples, with populations higher than 2 log MPN/g for 69.2% of them (data not shown). Paula, Rodrigues, Tórtora, Uchoa, and Farage (2003) also reported high counts of thermotolerant coliforms (>2 log MPN/g) for the majority of samples of vegetables served in a restaurant in Rio de Janeiro, Brazil. The values obtained for thermotolerant coliforms is higher than the maximum value allowed by the Brazilian legislation for this type of food, which is 10^2 MPN/g for indicative samples (Brasil, 2001).

In our study, *E. coli* was detected in 53.1% of the samples (with populations ranging from 1 to 6 log MNP/g – data not shown) and the most contaminated vegetables were the mixtures of spring onion/parsley and kale, while lettuce samples were the less contaminated (Table 2). According to literature, *E. coli* is more suitable than thermotolerant coliforms as indicator of fecal contamination because it better correlates with potential contamination by enteric pathogens (Doyle & Erickson, 2006). The rate of contamination by *E. coli* found in the present study was higher than those reported by Abadias et al. (2008) for samples of fresh chopped vegetables in Spain (11.4%) and by Sagoo, Little, Ward, Gillespie, and Mitchell (2003) for vegetable salads from United Kingdom (1.3%). However, Prado et al. (2008) reported high of contamination by *E. coli* in Brazilian samples of minimally processed vegetables (30%).

Table 2
Occurrence of coliforms and *Escherichia coli* in samples of leafy vegetables minimally processed.

Sample	*N*[a]	Total coliforms	Thermotolerant coliforms	*Escherichia coli*
		n (%)[b]	*n* (%)[b]	*n* (%)[b]
Arugula	6	6 (100)	4 (66.7)	3 (50.0)
Spring onion/parsley mixture	22	22 (100)	22 (100)	21 (95.6)
Spinach	9	9 (100)	6 (66.7)	6 (66.7)
Wild chicory	13	13 (100)	11 (84.6)	6 (46.2)
Chicory	11	11 (100)	8 (72.7)	7 (63.3)
Cabbage	28	28 (100)	14 (50.0)	14 (50.0)
Chinese cabbage	13	13 (100)	7 (53.8)	2 (15.4)
Kale	30	30 (100)	25 (83.3)	21 (70.0)
Letucce	26	22 (78.6)	8 (30.8)	5 (19.2)
Watercress	4	4 (100)	2 (50.0)	1 (25.0)
Total	162	158 (97.5)	107 (66.0)	86 (53.1)

[a] Number of samples analyzed.
[b] Number and percentage of positive samples.

Populations of *E. coli* are often used for monitoring the sanitary conditions of foods, but selected serotypes of this species may also be involved in cases of foodborne diseases. In this paper, no further subtyping of the *E. coli* isolates was done, but EHEC (enterohemorrhagic *E. coli*) and EPEC (classical enteropathogenic *E. coli*) strains have emerged as important pathogens and have been responsible for foodborne outbreaks in many countries. As reported by the Centers for Disease Control and Prevention – USA, in 2006 and 2010 there were two major multiple states outbreaks involving *E. coli* O157:H7 and *E. coli* O145, associated with spinach and romaine lettuce, respectively (CDC, 2010).

With regard to *Salmonella* spp., 2 samples of wild chicories out of the samples of minimally processed vegetables analyzed in this study were contaminated (1.2%) and the isolates were serotyped as S. Madelia and S. London. The *Salmonella* positive samples also presented high populations of thermotolerant coliforms and aerobic psychrotrophic bacterial populations (data not shown). This rate of isolation is low if compared to results of Bruno et al. (2005) for samples of minimally processed vegetables commercialized in northeast Brazil (46.7%), but it is similar to findings of Fröder et al. (2007) for minimally processed leafy vegetables commercialized in city of São Paulo, Brazil (3%). These data are of special concern since *Salmonella* was the etiological agent identified in 34.7% of the foodborne outbreaks elucidated with laboratorial confirmation in State of São Paulo from 1999 to 2004 (SVS, 2005).

To date, there are no reported cases of listeriosis linked to consumption of foods in Brazil, but *L. monocytogenes* is recognized as an important foodborne pathogen worldwide. In the present work, *L. innocua* and *L. monocytogenes* were the most prevalent species, as reported by Farber and Peterkin (1991) for distribution of *Listeria* spp. in foods. *Listeria* spp. was present in 6 out of 162 samples of vegetables evaluated, with presence of *L. monocytogenes* in 2 (1.2%) samples (chopped kale and mixture of spring onion/parsley) and of *L. innocua* in 4 (2.4%) samples (kale, spinach, mixture of spring onion/parsley and Chinese cabbage). The positive samples for *L. monocytogenes* presented populations of aerobic psychrotrophic bacteria and thermotolerant coliforms above 6 log CFU or MPN/g and were also positive for *E. coli*.

Overall occurrence of *L. monocytogenes* in the vegetables analyzed in this study was low, in accordance with previous results presented by Porto and Eiroa (2001) and Fröder et al. (2007) who found respectively 3.2% and 0.6% of samples positive for *L. monocytogenes* (lettuce, parsley, watercress and spinach) among diverse vegetable samples from Brazilian markets surveyed. Fröder et al. (2007) also reported the isolation of *L. innocua* (0.9%) and *L. welshimeri* (0.6%) from lettuce samples. Data from USA reported by Lin, Fernando, and Wei (1996) reinforces that the prevalence of *L. monocytogenes* in salad samples is low (1.6%). However, conflicting results were reported by Ponniah et al. (2010) in a study in Malaysia, where *L. monocytogenes* was detected in 22.5% of minimally processed vegetables analyzed.

The low prevalence of *L. monocytogenes* in RTE foods must be carefully evaluated, since false negative results may be obtained due to low population of *Listeria* spp. in comparison with the background microbiota, the possibility of occurrence of sub-lethally injured listerial cells and to better growth of non-*monocytogenes* species in selective enrichment broths (Bruhn, Vogel, & Gram, 2005).

In conclusion, the presented results revealed that majority of the ready-to-eat leafy vegetable samples analyzed presented poor microbiological quality and it indicates the need of adoption of hygienic practices by food processors and consumers to minimize the risks of transmission of foodborne pathogens through this kind of foods.

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