Growth potential of Salmonella spp. and Listeria monocytogenes in nine types of ready-to-eat vegetables stored at variable temperature conditions during shelf-life
Growth potential of *Salmonella* spp. and *Listeria monocytogenes* in nine types of ready-to-eat vegetables stored at variable temperature conditions during shelf-life

Anderson S. Sant’Ana *, Matheus S. Barbosa, Maria Teresa Destro, Mariza Landgraf, Bernadette D.G.M. Franco *

Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP - Brazil

**Abstract**

Growth potential (δ) is defined as the difference between the population of a microorganism at the end of shelf-life of specific food and its initial population. The determination of δ of *Salmonella* and *Listeria monocytogenes* in RTE vegetables can be very useful to determine likely threats to food safety. However, little is known on the behavior of these microorganisms in several RTE vegetables. Therefore, the aim of this study was to determine the δ of both pathogens in nine different types of RTE vegetables (escarole, collard green, spinach, watercress, arugula, grated carrot, green salad, and mix for yakisoba) stored at refrigeration (7 °C) and abuse temperature (15 °C). The population of aerobic microorganisms and lactic acid bacteria, including those showing antimicrobial activity has been also determined. Results indicated that L. monocytogenes was able to grow (δ ≥ 0.5 log10) in more storage conditions and vegetables than *Salmonella*. Both microorganisms were inhibited in carrots, although a more pronounced effect has been observed against *L. monocytogenes*. The highest δ values were obtained when the RTE vegetables were stored 15 °C/6 days in collard greens (δ = 3.3) and arugula (δ = 3.2) (*L. monocytogenes*) and arugula (δ = 4.1) and escarole (δ = 2.8) (*Salmonella*). In most vegetables and storage conditions studied, the counts of total aerobic microorganisms raised significantly independent of the temperature of storage (p < 0.05). Counts of lactic acid bacteria were higher in vegetables partially or fully stored at abuse temperature with recovery of isolates showing antimicrobial activity. In conclusion, the results of this study show that *Salmonella* and *L. monocytogenes* may grow and reach high populations in RTE vegetables depending on storage conditions and the definition of effective intervention strategies are needed to control their growth in these products.

© 2012 Elsevier B.V. All rights reserved.

**Introduction**

Surveillance data have shown that vegetables have been implicated in foodborne disease outbreaks caused by a variety of pathogenic microorganisms (*Sivapalasingam* et al., 2004; *Anon*, 2008a, 2010). As a result, numerous studies have been performed to determine the occurrence of microorganisms such as *Salmonella* (*Giusti* et al., 2010; *Corski* et al., 2011; *Sant’Ana* et al., 2011), pathogenic *Escherichia coli* (*Bohaychuk* et al., 2009; *Rúgeles* et al., 2011), and *Listeria monocytogenes* (*Little* et al., 2007; *Oliveira* et al., 2010; *Sant’Ana* et al., 2012) in different types of vegetables.

The contamination of vegetables by *Salmonella* spp. and *L. monocytogenes* might occur either in the field or during handling or processing (*Ailes* et al., 2008; *Caponigro* et al., 2010) as *Salmonella* is present in the intestinal tract of humans and animals (*Adley* et al., 2011; *Rostagno* and *Callaway*, 2011), and *L. monocytogenes* is a markedly ubiquitous microorganism being isolated from diverse sources (*Gandhi* and *Chikindas*, 2007). Despite presence in the vegetables, the capability of these microorganisms to survive, grow and cause disease will depend upon plant–microbe and microbe–microbe interactions (*Brandl*, 2006) and their responses to unfavorable conditions during minimal processing and storage (*Capozzi* et al., 2009).

Regardless the low prevalence and counts of *L. monocytogenes* and *Salmonella* in RTE vegetables reported in several surveys (*Little* et al., 2007; *Oliveira* et al., 2010; *Sant’Ana* et al., 2011, 2012), populations as high as 10<sup>6</sup> CFU/g may be reached depending on storage conditions (*Koseki* and *Isobe*, 2005a,b). Although the infectivity of *Salmonella* is highly variable (*Mintz* et al., 1994; *Musher* and *Musher*, 2004), the infection by *L. monocytogenes* seems to be mostly associated with ingestion of high doses of this pathogen in healthy individuals (≥8 log CFU) or with low doses in susceptible individuals (2–3 log CFU) (*Takeuchi* et al., 2006; *Williams* et al., 2007; *Warriner* and *Namvar*, 2009). Given this, the knowledge of growth potential (δ) in RTE vegetables can be very useful to identify critical storage conditions to be respected to prevent the growth of pathogens in these products to unacceptable levels. In addition, this knowledge may help to determine the likely threats these microorganisms may pose to food safety.
Hence, the purpose of this study was to determine the growth potential (δ) of Salmonella and L. monocytogenes in nine types of RTE vegetables stored at different temperature conditions. In addition, the populations of aerobic microorganisms and lactic acid bacteria, including those showing antimicrobial activity were also investigated as they may influence the survival and growth of the pathogens.

2. Material and methods

2.1. Microorganisms and preparation of cell suspensions

Five strains of L. monocytogenes and five strains of Salmonella spp. were used in the study. The L. monocytogenes strains were isolated from RTE vegetables marketed in Sao Paulo, Brazil (Sant’Ana et al., 2012), and belonged serotypes 4b (strains 349, 564, 586 and 480) and 1/2b (strain 221) and ribotypes DUP 1038 (strains 349, 564 and 586), DUP 19191 (strain 480) and DUP 19175 (strain 221). Three Salmonella spp. strains were also isolated from RTE vegetables marketed in Sao Paulo, Brazil (Sant’Ana et al., 2011) and belonged to serovars S. Typhimurium (strain 227), S. Typhi (strain 386) and S. enterica subsp. enterica O:47:z4,z23:— (strain 994). Two Salmonella spp. strains (strains 2494 and 5711), from human and animal origin, kindly provided by Dr. Ernesto Hofer from Oswaldo Cruz Institute (Rio de Janeiro, Brazil), belonged to serovars S. Infantis IOC 2494 and S. Concord IOC 5711.

The strains of Salmonella spp. and L. monocytogenes were separately grown in 10 mL of tryptic soy broth (TSB) and TSB supplemented with 0.6% of yeast extract (TSB-YE), respectively, at 37 °C for 24 h under static conditions. One mL aliquots were transferred to fresh broths for incubation for extra 24 h, twice. The cultures were centrifuged at 8 °C for 10 min at 2810 x g (Mikro 22R, Hettich Zentrifugen, Germany), supernatants were discharged and pellets were washed three times with phosphate buffered saline pH 6.0. Pools of each pathogen were prepared mixing equal volumes of each washed suspension, to achieve an optical density at 630 nm equal to 0.5, corresponding to 10^6 CFU/mL, as checked by plate counting on TSA and TSA-YE.

2.2. Vegetables

Packages of RTE vegetables with no more than one day of storage after processing were acquired from supermarkets in the city of Sao Paulo, Brazil. Nine types of vegetables were selected either based on their consumption in Brazil (IBGE, 2011), prevalence of Salmonella spp. (Sant’Ana et al., 2011) and L. monocytogenes (Sant’Ana et al., 2012). All the RTE vegetables used (escarole, collard green, spinach, watercress, arugula, grated carrot, green salad (crisp, romaine and butter lettuces), and mix for yakisoba (broccoli, cabbage, cauliflower, leek, carrots and chard) were packaged under modified atmosphere, i.e., contained a mixture of gases such as O2, CO2 and N2. The packages were transported to the laboratory in isothermal boxes and kept under refrigeration until the experiments were performed. The experiments were performed in the same day of the purchase of the RTE vegetables.

2.3. Inoculation of vegetables, packaging and storage conditions

Portions of 25 g of each RTE vegetable were placed in plastic bags (62 µm thickness, O2 permeability of 1.375 m m⁻² day⁻¹ at 23 °C and water steam permeability of 3.5 g water m⁻² day⁻¹ at 38 °C and 90% relative humidity) and spot inoculated with 0.5 mL of the pools of Salmonella and L. monocytogenes properly diluted in 0.1% peptone water to achieve a final concentration of 10^3 CFU/mL in the products. The inoculation method and final concentration were according to the guidelines for challenge tests described in Anon (2003) and Anon (2008c). The bags containing the inoculated samples were sealed in a vacuum sealing machine AP 500 (Tecmaq, Sao Paulo, Brazil) under modified atmosphere containing 5% O2, 15% CO2 and 80% N2 (White Martins, Osasco, Brazil), which corresponds the gaseous composition commonly used by RTE vegetables processors in Sao Paulo.

Packages of RTE vegetables were exposed to three different storage conditions: I (100% of shelf-life at 7 °C ± 1 °C), II (30% at 7 °C ± 1 °C and 70% at 15 °C ± 1 °C) and III (100% at 15 °C ± 1 °C). These storage scenarios were chosen with the purpose to understand the behavior of Salmonella and L. monocytogenes under optimal storage conditions (≤ 7 °C) and under partial or full abuse temperature during shelf-life (15 °C). The shelf-life established by processors of RTE vegetables sold in Sao Paulo (6 days) was set as the maximum storage time. A total of 288 bags of RTE vegetables were prepared per replicate that consisted of: i) bags to be contaminated with both pathogens (n = 216), ii) control samples of the nine RTE vegetables (n = 36) and iii) samples of the nine RTE vegetables for total plate count and lactic acid bacteria enumeration (n = 36). The amount of packages prepared per condition, considered the storage conditions tested (n = 3), types of vegetables (n = 9), number of packages analyzed (n = 2 at the beginning and n = 2 at the end of the shelf-life) and pathogens tested (n = 2). For calculation of number of bags needed ii and iii, the following bases were considered: types of vegetables (n = 9), number of packages analyzed at the end of the shelf-life (n = 1), and storage conditions tested (n = 3 at the end of shelf-life). As at the beginning of the experiments all the vegetables were from the same lot, fewer bags were needed. Thus, for microbiological examinations at the beginning and at the end of shelf-life, 9 and 27 bags were needed (n = 36), respectively, for each of ii and iii. These experiments were carried out twice. Control samples comprehended vegetables spot inoculated with 0.5 mL of sterile distilled water. The pH values of the vegetables were determined using a pH meter (Láctea, LCP-210, Brazil) according to Scott et al. (2001).

2.4. Enumeration and detection of Salmonella spp. and L. monocytogenes

Salmonella spp. and L. monocytogenes were enumerated in two packages of RTE vegetables at time “0” (after inoculation) and at the end of shelf-life (day “6”). In addition, two packages were submitted to tests for presence–absence of both pathogens before inoculation and the end of shelf-life. Enumerations of Salmonella spp. and L. monocytogenes were performed by homogenizing 25 g of samples with 225 mL of 0.1% peptone water, following decimal dilutions and inoculation in duplicate plates of Mannitol Lysine Crystal Violet Brilliant Green (MLCB) agar (Salmonella spp.) and Oxford (OXA) agar added of selective supplement (SR0206) (L. monocytogenes). MLCB and OXA plates were incubated at 37 ± 1 °C for 24 h and 48 h, respectively. Mauve colonies with black centers in MLCB agar were counted as Salmonella, while brown colored colonies with aesculin hydrolysis in OXA were enumerated as L. monocytogenes. Up to 3 colonies per sample were selected for further confirmation of Salmonella spp. and L. monocytogenes by polyvalent serotyping and biochemical tests, respectively (Anon, 1996, 2002). The final results were expressed as log10 CFU/g. Samples of vegetables used for enumeration were kept frozen at −20 °C until the results were obtained. When no colonies were recovered by the quantification method (below the detection limit of 10^1 CFU/g), the samples were submitted to tests for presence–absence of Salmonella spp. and L. monocytogenes using ISO 6579 (Anon, 2002) and ISO 11290–1 (Anon, 1996) methods, respectively.

2.5. Determination of growth potential (δ) of Salmonella spp. and L. monocytogenes

The growth potential (δ) of Salmonella spp. and Listeria monocytogenes in each type of RTE vegetable was determined by the
2.6. Enumeration of aerobic microorganisms and lactic acid bacteria

Aerobic mesophilic microorganisms and lactic acid bacteria were quantified in two different packages of each type of vegetable and condition studied at the beginning (time “0”) and at the end of shelf-life (time “6”). Twenty five grams of each vegetable was homogenized with 225 mL of 0.1% peptone water, following decimal dilutions and pour plating in Plate Count (PCA) (Maturin and Peeler, 2001) and Man, Rogosa and Sharpe (MRS) agars (Hall et al., 2001), for counts of aerobic and lactic acid bacteria, respectively. MRS agar plates were overlaid with 15% agar–agar to create an environment with low oxygen tension. PCA and MRS plates were incubated at 37 ± 1 °C for 24 h and further examined for the presence of halos of inhibition. Any colonies showing ability to inhibit L. monocytogenes Scott A were selected, inoculated in MRS broth and purified by streaking onto MRS agar. Bacteriocin screening was performed by method described by Ivanova et al. (1998). After incubation at 30 ± 1 °C for 24 h in MRS broth, cell free supernatants were prepared, and pH was adjusted to 6.0 with 1 M NaOH to eliminate growth of the pathogen when δ values were negative or lower than 0.5 log_{10} (AFSSA, 2004; Anon, 2008a,b,c).

2.7. Determination of antimicrobial activity of lactic acid bacteria

The isolates of lactic acid bacteria were screened for antimicrobial activity using the method described by Todorov and Dicks (2005) with modifications. After incubation, MRS agar plates were overlaid with a thin layer of Brain Heart Infusion (BHI) containing 1% agar (w/v) inoculated with 10^{5}–10^{6} CFU/mL of L. monocytogenes Scott A. MRS plates were incubated at 37 ± 1 °C for 24 h and further examined for the presence of halos of inhibition. Any colonies showing ability to inhibit L. monocytogenes Scott A were selected, inoculated in MRS broth and purified by streaking onto MRS agar. Bacteriocin screening was performed by method described by Ivanova et al. (1998). After incubation at 30 ± 1 °C for 24 h in MRS broth, cell free supernatants were prepared, and pH was adjusted to 6.0 with 1 M NaOH to eliminate the antimicrobial activity due to acid production by the lactic acid bacteria. Antimicrobial activity due to production of proteinaceous bacteriocins was checked testing the sensitivity of the supernatant to 1 mg/mL of Proteinase K (Sigma-Aldrich, Saint Louis, USA), α-Chymotrypsin from bovine pancreas type II (Sigma) and Protease type XIV from Streptomyces griseus (Sigma) for 2 h. Antimicrobial activity was monitored according to the method described by Van Reenen et al. (2002). All the culture media used in this study were from Oxoid (Basingstoke, UK) unless otherwise stated, while all the enzymes were from Sigma-Aldrich (Saint Louis, USA).

2.8. Statistical analysis

The populations of total aerobic microorganisms and lactic acid bacteria at the beginning and at the end of shelf-life of RTE vegetables were assessed for significant statistical (p ≤ 0.05) differences using Shapiro–Wilk test followed by t test. The populations of total aerobic microorganisms and lactic acid bacteria obtained in different storage scenarios were checked for significant statistical (p ≤ 0.05) differences using one-factor analysis of variance (ANOVA) followed by Tukey’s test. Statistical analyses were carried out in Assistat version 7.5 free software (Campina Grande, Brazil) (Silva and Azevedo, 2002).

3. Results and discussion

Inappropriate storage temperature has been reported as one of the three most important faults contributing for the occurrence of outbreaks due to consumption of salads (Little and Gillespie, 2008). The occurrence of foodborne diseases outbreaks linked to vegetables have led to increased concerns over the safety of RTE vegetables (Harris et al., 2003; Sivapalasingam et al., 2004; Lynch et al., 2009). Two main outputs of the growth potential tests (δ) are the determination of the “growth or no growth” (positive or negative values of δ) of a microorganism under packaging and storage conditions studied and their classification into categories of potential risks when assessed along with data on the prevalence of pathogens (Uyttendaele et al., 2009; Skalina and Nikolajeva, 2010; Beaufort, 2011). Thus, the determination of the growth potential (δ) of Salmonella spp. and L. monocytogenes in different types of RTE vegetables stored at recommended (7 °C) and abuse temperature (15 °C) conditions may contribute for the assessment of consumer’s exposure to these microorganisms. Control samples ensured that no vegetables used in the experiments were previously contaminated with Salmonella spp. and L. monocytogenes. In addition, none of the control samples taken at the end of shelf-life indicated the presence of Salmonella spp. and L. monocytogenes.

As shown in Table 1, the pH values of the nine RTE vegetables studied (6.2–7.2) were in the range for optimum growth of Salmonella spp. and L. monocytogenes (Nguyen-the and Carlin, 1994). However, no relationship between pH values and growth potential (δ) has been found. In fact, it is known that the growth of foodborne pathogens at the surface of vegetables is dependent upon several factors and their complex interactions, such as epiphytic fitness, the physicochemical environment of plant surfaces, biofilm formation, and bacteria–bacteria and vegetable–bacteria relations (Brandl, 2006).

The fate of foodborne pathogens such as Salmonella spp. and/or L. monocytogenes has been studied in several vegetables such as broad leafy endives (Carlin et al., 1995), lettuces (Koseki and Isobe, 2005a,b),

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>pH</th>
<th>Storage conditionsa</th>
<th>δ (log_{10})b (sd)d</th>
<th>L. monocytogenes</th>
<th>Salmonella spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot</td>
<td>6.7</td>
<td>I</td>
<td>0.49 ± 0.4</td>
<td>1.03 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1.82 ± 0.03</td>
<td>1.91 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>1.92 ± 0.9</td>
<td>2.80 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabbage</td>
<td>7.2</td>
<td>I</td>
<td>−0.21 ± 0.9</td>
<td>−0.92 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.64 ± 1.1</td>
<td>−0.53 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>0.85 ± 0.9</td>
<td>0.31 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>6.2</td>
<td>I</td>
<td>0.88 ± 0.1</td>
<td>0.13 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1.19 ± 0.2</td>
<td>1.49 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>1.83 ± 0.2</td>
<td>1.68 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watercress</td>
<td>6.3</td>
<td>I</td>
<td>0.21 ± 0.6</td>
<td>−0.03 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1.13 ± 0.9</td>
<td>1.75 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>2.69 ± 0.3</td>
<td>2.39 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arugula</td>
<td>6.6</td>
<td>I</td>
<td>1.85 ± 0.0</td>
<td>2.11 ± 0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3.18 ± 0.2</td>
<td>2.81 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>3.22 ± 0.2</td>
<td>4.05 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrot</td>
<td>6.7</td>
<td>I</td>
<td>−3.61 ± 0.2</td>
<td>−0.44 ± 0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>−2.32 ± 0.1</td>
<td>−0.51 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>−1.37 ± 0.05</td>
<td>0.93 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green salad</td>
<td>6.4</td>
<td>I</td>
<td>0.51 ± 0.6</td>
<td>0.49 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2.39 ± 0.03</td>
<td>1.19 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>2.62 ± 0.3</td>
<td>2.61 ± 1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yakisoba</td>
<td>6.7</td>
<td>I</td>
<td>0.38 ± 0.8</td>
<td>0.05 ± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.97 ± 0.4</td>
<td>0.67 ± 0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>1.59 ± 0.4</td>
<td>1.33 ± 0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Storage conditions: I [100% of shelf-life (6 days) at 7 °C ± 1 °C], II [30% of shelf-life (6 days) at 7 °C ± 1 °C and 70% at 15 °C ± 1 °C] and III [100% of shelf-life (6 days) at 15 °C ± 1 °C].

b Growth was assigned when δ value was higher than 0.5 log_{10}.

c Composed by cauliflower, carrot, broccoli, cabbage and chard.

d Standard deviation.
carrots (Finn and Upton, 1997), cabbage (Finn and Upton, 1997), peppers and tomatoes (Ma et al., 2010), herbs (Hsu et al., 2006), spinach (Pirovani et al., 2000), and radish (Islam et al., 2004) but not in RTE collard greens, watercress, arugula and mix for yakisoba. Therefore, the current study reports for the first time the growth potential of Salmonella and L. monocytogenes in these RTE vegetables. Overall, data obtained indicate that growth potential (ϕ) of both pathogens was not related to the type of vegetable (Table 1). Among the nine vegetables studied, seven were leafy (escarole, collard greens, cabbage, spinach, watercress, arugula and green salad), one was root (carrot) and one (yakisoba) was a mix of in florescent (broccoli and cauliflower), leafy (cabbage), root (carrot) and stalk (chard) vegetables.

From Table 1 it can be depicted that L. monocytogenes grew better (ϕ > 0.5 log10) than Salmonella spp. in the studied vegetables, which can be explained by the psychrophilic behavior of L. monocytogenes (Gandhi and Chikindas, 2007). When the vegetables were stored at 7 °C during the whole shelf-life (condition I) the growth of L. monocytogenes was not supported in escarole, collard green, watercress and yakisoba. At storage condition I, a complete inhibition of L. monocytogenes was observed only in carrots. In these samples, the populations of L. monocytogenes were lower than the limit of enumeration of the method used (10^3 CFU/g). However, after 6 days of shelf-life the survival of this microorganism was confirmed by the positive results obtained in the detection method (data not shown). The anti-listerial activity of raw carrots has been linked to antimicrobial compounds found in the carrot tissue, which have been found to show lethal effects in low temperatures (4 and 10 °C) (Noriega et al., 2010). As the levels of L. monocytogenes in vegetables are barely above 10^3 CFU/g (Crépet et al., 2009), if carrots are stored at 7 °C inhibition of this pathogen during shelf-life is expected. Still at 7 °C, L. monocytogenes was able to grow (ϕ > 0.5 log10) in green salad, spinach and arugula. Despite this, the increase in the population of this microorganism was not higher than 1 and 2 log10a, highlighting the suppressant effect of temperature on the growth of this bacterium. The results shown in Table 1 indicate that shredding (lettuce) or not (arugula and spinach) seems to not correlate with growth potential of this pathogen in the vegetables. Some authors have reported that the attachment and further survival and growth of pathogen are more prominent when vegetables are cut or shredded during minimal processing (Ells and Hansen, 2006; Brandl, 2008). In addition to providing sites for the attachment of microorganisms (Patel and Sharma, 2010; Goldberg et al., 2011), cutting and shredding causes release of exudates from vegetables nourishing the attached bacteria that may grow and reach high populations (Teplitski et al., 2011; Koseki and Isobe, 2005a,b).

At 7 °C, Salmonella was able to grow (ϕ > 0.5 log10) in escarole and arugula during shelf-life (condition I). The increase in the population of this microorganism in escarole and arugula were ≥ 1 and ≥ 2 log10a, respectively (Table 1). Green salad, spinach, yakisoba, collard greens, cabbage, watercress and carrots stored at 7 °C did not support the growth of Salmonella during shelf-life. Particularly in the case of cabbage and carrots, the populations of Salmonella were reduced when vegetables were stored at 7 °C (Table 1). Even though the presence of antimicrobial compounds in carrots and cabbage has been reported (Gogo et al., 2010; Noriega et al., 2010), it is known that inhibitory compounds found in carrots are particularly effective against L. monocytogenes (Noriega et al., 2010), while cabbage antimicrobial activity is more pronounced against Salmonella and Gram-negative bacteria (Gogo et al., 2010). Besides, the fact that cabbage did not support the growth of Salmonella at all storage conditions may be related to the low attachment strength of this bacterium to surface of cabbages. According to Patel and Sharma (2010), Salmonella presented the overall lower attachment strength on cabbage (0.12) when compared to iceberg (0.23) and Romaine lettuce (0.34) after 24 h at 10 °C. When the growth potential (ϕ) of both pathogens was investigated at partial or full abuse temperature during shelf-life (Table 1), results indicated that L. monocytogenes was able to grow in all RTE vegetables studied, excepting carrots when partially or fully exposed to abuse temperature (15 °C) during shelf-life, i.e., conditions II and III. In these scenarios, the increase in the populations of L. monocytogenes at the end of shelf-life was ≥ 2 log10 in collard green, arugula and green salad (conditions II and III) and watercress (condition III). On the other hand, an increase of L. monocytogenes population ≤ 2 log10 was observed in escarole, cabbage, spinach and yakisoba (conditions II and III) and watercress (condition II). The highest growth potential (ϕ) values for L. monocytogenes were observed at storage condition III in collard greens (ϕ = 3.3) and arugula (ϕ = 3.2), while the lowest values were observed in cabbage. At abuse storage conditions (II and III), negative values of growth potential (ϕ) of L. monocytogenes were only observed in carrots. Although cabbage extract has been shown highly inhibitory for Gram-negative microorganisms, it presents some suppressant effects against the growth of Gram-positive microorganisms such as L. monocytogenes (Gogo et al., 2010). On the other hand, the growth of this microorganism in all the other types of RTE vegetables with rises in its population of up to 3 log10 CFU/g concerns these vegetables are consumed raw. Although the highest increase in the population of L. monocytogenes has been observed in collard greens at condition III, the exposure of consumers to these microorganisms will depend, among other factors, on the mode of preparation.

Another major issue regarding the presence of L. monocytogenes in RTE vegetables is their further use to prepare salad-based meals that are not stored at adequate refrigeration temperatures nor consumed quickly, providing adequate nutritional and environmental conditions for the growth of this pathogen. This is of particular concern since the growth of L. monocytogenes in salad-based meals has been reported in the literature (Uyttendaele et al., 2009; Skalina and Nikolajeva, 2010). Whereas vegetables have been placed in a category of low risk based on probability of representing a listeriosis hazard (Warriner and Namvar, 2009), the implications of the growth potential of L. monocytogenes in these products to food safety should not be underestimated.

The exposure of the RTE vegetables to abuse temperature (15 °C) remarkably influenced the growth potential (ϕ) of Salmonella (Table 1). The microorganism was able to grow in escarole, collard green, spinach, watercress, arugula, green salad and yakisoba partially exposed at abuse temperature (15 °C) (condition II). In these vegetables and storage condition, the increase in Salmonella population was not higher than 2 log10 except in arugula in which the growth of the microorganism was 2.8 log10 (Table 1). At storage condition III (15 °C/6 days), Salmonella was able to grow in all vegetables (ϕ > 0.5 log10), except on cabbage where ϕ = 0.5 log10. The highest ϕ values for Salmonella spp. were obtained at storage condition III in arugula (ϕ = 4.1), followed by escarole (ϕ = 2.8), collard green (ϕ = 2.6) and green salad (ϕ = 2.6). Even though the detection of a single cell of Salmonella in foods is unacceptable from a regulatory point of view (zero tolerance) (Anon, 2001), the determination of its growth potential in vegetables is of major importance as Salmonella is the leading etiological agent of outbreaks of fresh produce illnesses (Harris et al., 2003; Little and Gillespie, 2008). In order to investigate the role of background microbiota of RTE vegetables on the behavior of Salmonella and L. monocytogenes, the populations of total aerobic microorganisms and lactic acid bacteria at the beginning and end of shelf-life were investigated (Table 2). Populations of total aerobic microorganisms varied from 5.5 to 8.1 log10 CFU/g and 7.1 to 10 log10 CFU/g at the beginning and at the end of shelf-life, respectively. In most vegetables and storage conditions studied, the counts of total aerobic microorganisms raised significantly despite the temperature of storage (p < 0.05). On the other
4. Conclusions

Currently, little information on the behavior of Salmonella and L. monocytogenes in these RTE vegetables has been available. Thus, the results obtained in this study highlight that Salmonella and L. monocytogenes are able to grow and reach high populations in several types of RTE vegetables. It has been found that arugula, collard greens and green salad constituted the best substrates for the growth of these pathogens, which has been greatly potentialized whenever exposition at abuse temperature (15 °C) has been tested. Although field operations are likely to represent the main source of contamination of fresh vegetables (Farrar and Guzewich, 2009; Matthews, 2009), conditions and practices during processing, commercialization and consumption will define the fate of pathogens and likely risks to consumers. Therefore, it can be concluded that the growth of Salmonella and L. monocytogenes in RTE vegetables could be controlled by ensuring that these products are stored in temperature below 7 °C during commercialization. This measure will be more effective if the contamination of vegetables during field operations is prevented.

Acknowledgements

The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Projects 07/54891–2 and 07/54890 6), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support. The authors also thank Dr. Ernesto Hofer, form Fundação Oswaldo Cruz, for providing the Salmonella strains.

References


