Short communication

Prevalence and counts of *Salmonella* spp. in minimally processed vegetables in São Paulo, Brazil

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**ABSTRACT**

Minimally processed vegetables (MPV) may be important vehicles of *Salmonella* spp. and cause disease. This study aimed at detecting and enumerating *Salmonella* spp. in MPV marketed in the city of São Paulo, Brazil. A total of 512 samples of MPV packages collected in retail stores were tested for *Salmonella* spp. and total coliforms and *Escherichia coli* as indication of the hygienic status. *Salmonella* spp. was detected in four samples, two using the detection method and two using the counting method, where the results were $8.8 \times 10^5$ CFU/g and $2.4 \times 10^5$ CFU/g. The serovars were *Salmonella* Typhimurium (three samples) and *Salmonella enterica* subsp. *enterica* O:47;Z4,Z23:-- (one sample). Fourteen samples (2.7%) presented counts of *E. coli* above the maximum limit established by the Brazilian regulation for MPV ($10^2$ CFU/g).

Therefore, tightened surveillance and effective intervention strategies are necessary in order to address consumers and governments concerns on safety of MPV.

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1. Introduction

Minimally processed vegetables (MPV) are those submitted to simple operations (slicing or shredding and disinfection), aiming to preserve their freshness, nutritional quality and sensorial properties (Cruz et al., 2006). It is expected that in addition to health aspects, minimal processing addresses consumers’ expectations for safe foods that are easy to use (Jacxsens et al., 2010).

The augmented demand and consumption of MPV and their increased association with foodborne disease outbreaks intensify the concerns with the safety of these products (Olsen et al., 2000; Little et al., 2003; Little and Gillespie, 2008). Careful management of process hygiene is crucial to avoid the presence of microbial hazards since minimal processing is not an end-point preservation treatment. In this sense, the use of indicator microorganisms such as *Escherichia coli* has been used to indicate the hygiene during processing (Little and Gillespie, 2008).

*Salmonella* is the leading cause of foodborne diseases worldwide (Greig and Ravel, 2009). Although the majority of outbreaks in which *Salmonella* spp. is the etiological agent are linked with ingestion of contaminated foods of animal origin (Greig and Ravel, 2009; Oliveira et al., 2010), the association with vegetables has been increasing in the last few years (WHO, 2008; Little and Gillespie, 2008; Lynch et al., 2009). The pathogen is a major challenge for the microbiological safety of MPV (Olsen et al., 2000; Krti = 007 et al., 2010), and has been found in several types of vegetables in Brazil and other countries (Fröder et al., 2007; Abadias et al., 2008; Meldrum et al., 2009; Giusti et al., 2010). Despite the fact that the prevalence of *Salmonella* spp. in fresh vegetables may be as high as 35% (FDA, 2001) and that outbreaks due to this pathogen–food combination are quite frequent (Harris et al., 2003), to our knowledge no study reported counts of *Salmonella* spp. in MPV. Thus, this study aimed at detecting and enumerating *Salmonella* spp. in MPV available in retail stores in São Paulo, Brazil.

2. Materials and methods

2.1. Collection of MPV samples

A total of 512 samples of MPV packages were purchased from retail stores of the five largest Brazilian supermarket chains in the city of São Paulo, Brazil. The samples belonged to the six most popular brands available in the market and only packages within expiration date stated in the label were collected. Each sample belonged to a different lot of production and contained vegetables grown in Brazil. MPV containing lettuce, carrot, collard green and cabbage were prioritized since these are the most consumed vegetables in Brazil (IBGE, 2007). All vegetables had been submitted to a sanitization step during processing and thus

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was carried out using API20E (Biomerieux, Marcy l’Étoile, France) and the presence of Salmonella indol, as recommended by ISO 6579 (Anonymous, 2002). Colonies were disinfected with 70% alcohol solution, opened under aseptic conditions and the vegetables analyzed for prevalence and enumeration of serovars. All culture media were from Merck (Darmstadt, Germany), except otherwise stated. For enumeration of Salmonella spp., the samples homogenate used for testing the presence of Salmonella spp. (10⁻¹) was plated in three different plates of XLD agar (portions of 0.3, 0.3 and 0.4 mL). Further decimal serial dilutions were prepared in 0.1% peptone water and aliquots of 0.3, 0.3 and 0.4 mL were spread onto XLD agar plates, and incubated at 37 ± 1°C for 24 ± 3 h. All culture media were from Merck (Darmstadt, Germany), except otherwise stated. For enumeration of Salmonella spp., the samples homogenate used for testing the presence of Salmonella spp. (10⁻¹) was plated in three different plates of XLD agar (portions of 0.3, 0.3 and 0.4 mL). Further decimal serial dilutions were prepared in 0.1% peptone water and aliquots of 0.1 mL were spread onto XLD agar plates, and incubated at 37 ± 1°C for 24 ± 3 h. Presumptive colonies in XLD and/or BS plates (five per plate in the case of enumeration) were then transferred to Nutrient Agar plates (Oxoid, Basingstoke, UK), incubated at 37 ± 1°C for 24 ± 3 h and then submitted to tests for fermentation of glucose, hydrolysis of urea, decarboxylation of lysine, production of β-galactosidase, production of acetoin (Voges–Proskauer test) and indol, as recommended by ISO 6579 (Anonymous, 2002). Colonies were also submitted to agglutination reaction using Salmonella polyvalent antisera (Provac, São Paulo, Brazil). Final confirmation was carried out using API20E (Biomerieux, Marcy l’Étoile, France) and PCR reaction, using primers ST 11 (AGC CAA CCA TTG CTA AAT TGG CCC A) and ST 15 (GGT AGA AAT TCC CAG CGG GTA CTG) selected from a Salmonella-specific fragment, JEO402-1 (Aabo et al., 1993) to amplify the 429 bp fragment specific for this microorganism. PCR tests were performed as described by Myint et al. (2006), and Salmonella Enteritidis ATCC 13076 and ultrapure water were used as positive and negative controls, respectively. Results were expressed as absence or presence of Salmonella spp. per 25 g (prevalence) or as CFU/g (enumeration). Only colonies confirmed as Salmonella spp. by biochemical and genetic tests simultaneously were considered to be positive for this pathogen. Confidence intervals (95% CI) for the prevalence of Salmonella spp. were estimated based on cumulative binomial distribution. Serotyping of Salmonella spp. isolates based on O- and H-group antigens was carried out in the National Center for Salmonella Serotyping in Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

The enumeration of total coliforms and E. coli was carried out using Petroflim® EC (3M, Saint Paul, USA), following the manufacturer’s instructions.

### 3. Results and discussion

Out of 512 packages of MPV tested in this study, four were Salmonella spp. positive. Two (0.4%, with 95% CI 0.05–1.4%) were positive in the prevalence study, and another two (0.4%) presented countable levels of this pathogen. In the prevalence study, Salmonella was detected in one pack of lettuce and arugula, and the serovars were Salmonella Typhimurium and Salmonella enterica subsp. enterica O:47:H2:22:3+, respectively. In the enumeration study, organic lettuce and mix of leafy vegetables (escarole and chicory) presented counts of the microorganism (8.8 × 10² CFU/g and 2.4 × 10² CFU/g, respectively) and both isolates belonged to serovar S. Typhimurium.

According to both Brazilian and international regulations (Brasil, 2001; Gilbert et al., 2000), the presence of Salmonella in ready-to-eat vegetables is unacceptable (absence/25 g) and may represent a risk for MPV consumers. Low prevalence of Salmonella in MPV as found in the present study has been reported by other authors in Brazil (Fröder et al., 2007), and in other countries as well (Abadias et al., 2008; Meldrum et al., 2009; Giusti et al., 2010). The prevalence of serovar S. Typhimurium is in agreement with data that show this serovar is one of the most common in humans or foods in Brazil (Geimba et al., 2004; Fernandes et al., 2006; Mürmann et al., 2008; Duarte et al., 2009).

In the period of this study, to the best of our knowledge neither cases of infection nor outbreaks linking consumption of MPV and Salmonella spp. have been reported in the State of São Paulo, Brazil. It is known that a dose in the range of 10⁵–10⁶ cells of Salmonella spp. may cause disease in humans (Blaser and Newman, 1982; Musher and Musher, 2004), with higher attack rates and severity resulting from higher doses in most of cases (Mintz et al., 1994; Hennessy et al., 1996). Although the counts of Salmonella found in some samples of MPV collected in this study could be enough to result in infection by this microorganism, several factors must be considered that affect the probability of infection by foodborne pathogens. One must consider that characteristics of the host, pathogen, food matrix and environment also play an important role in the probability of infection (Coleman and Marks, 1998). In addition, the heterogenic distribution of microorganisms in foods and their fate during commercialization and consumption steps will also influence the probability of occurrence of infection.

The populations of total coliforms in the tested MPV samples were above 10⁴ CFU/g in 56% of the samples (Table 2). E. coli was also detected, but only 14 (2.7%) samples surpassed the limit of 10² CFU/g established by the Brazilian Surveillance Agency (ANVISA) for ready-to-eat vegetables (Brasil, 2001). These results indicate that the samples tested in this study presented better hygienic conditions that those reported in other studies in Brazil (Fröder et al., 2007) or in other countries (Abadias et al., 2008; Meldrum et al., 2009; Giusti et al., 2010). Interestingly, the counts of E. coli in the four MPV samples harboring Salmonella spp. were below 10² CFU/g, indicating that the use of this microorganism as surrogate for Salmonella in MPV is of little use.
The findings reported in this study show that tightened surveillance and effective intervention strategies are necessary in order to address consumers and governments concerns on safety of MPV.

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References


Table 2

<table>
<thead>
<tr>
<th>Population (CFU/g)</th>
<th>Number of samples (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10³</td>
<td>102 (20)</td>
<td>119 (23.2)</td>
</tr>
<tr>
<td>10³–10⁴</td>
<td>80 (15.6)</td>
<td>9 (1.8)</td>
</tr>
<tr>
<td>10⁴–10⁵</td>
<td>103 (20.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>&gt;10⁵</td>
<td>3 (6.0)</td>
<td>512 (100)</td>
</tr>
<tr>
<td>E. coli</td>
<td>498 (97.2)</td>
<td>512 (100)</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>99 (19.3)</td>
<td>512 (100)</td>
</tr>
<tr>
<td></td>
<td>3 (0.6)</td>
<td>512 (100)</td>
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