INACTIVATION OF SALMONELLA ENTERITIDIS ON LETTUCE BY PROCEDURES USED BY MINIMALLY PROCESSED VEGETABLES INDUSTRIES

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RESUMO – Métodos de lavagem e desinfecção utilizados pelas indústrias de vegetais minimamente processados foram reproduzidas em laboratório, a fim de verificar a sua eficácia na redução de Salmonella Enteritidis SE86 (SE86) em alface. O procedimento de lavagem reduziu, em média, 1 log CFU/g na população da SE86. Diferentes tempos de imersão (1, 2, 5 e 15 minutos) em solução desinfetante demonstraram reduções entre 2,06 ± 0,10 log UFC/g e 3,01 ± 0,21 log CFU/g. O enxágue final foi capaz de reduzir a contagem bacteriana entre 0,12 ± 0,63 log CFU/g e 1,90 ± 1,07 log CFU/g. Dos cinco métodos testados apenas um foi capaz de eliminar a população de SE86, sendo este lavagem seguida de desinfecção com hipoclorito de sódio a 200 ppm por 15 minutos e enxágue final.

ABSTRACT – Washing and disinfection methods used by minimally processed vegetable industries were reproduced in laboratory in order to verify its effectiveness to reduce Salmonella Enteritidis SE86 (SE86) on lettuce. The washing procedure alone decreased approximately 1 log CFU/g of SE86 population and immersion times of 1, 2, 5, and 15 minutes in disinfectant solution demonstrated reduction rates ranging from 2.06 ± 0.10 log UFC/g to 3.01 ± 0.21 log CFU/g. Rinsing alone was able to reduce counts from 0.12 ± 0.63 log CFU/g to 1.90 ± 1.07 log CFU/g. From the five methods reproduced only one was able to eliminate the population of SE86, being washing, disinfection with 200 ppm sodium hypochlorite for 15 minutes and final rinse.

PALAVRAS-CHAVE: Indústrias de vegetais minimamente processados; lavagem e desinfecção; hipoclorito de sódio; Salmonella Enteritidis SE86

KEYWORDS: Minimally Processed Vegetable industries; washing and disinfection; sodium hypochlorite; Salmonella Enteritidis SE86

1. INTRODUÇÃO

Fresh produce consumption has increased expressively in the last years worldwide, at the same time that increased the number of foodborne diseases associated to these kind of foods (FDA 2015). Among the most important pathogens associated with fresh products are Salmonella spp.
strains, which has being responsible for several foodborne outbreaks (Olaimat and Holley 2012; Tomas-Callejas et al., 2012; Sant’Ana et al., 2011; Bennett et al., 2015).

In Brazil, from 2000 to 2014, 38.2% of registered foodborne outbreaks were caused by *Salmonella* spp. (BRASIL 2015). And a specific strain of *Salmonella* Enteritidis (SE86) was identified as responsible for several Salmonellosis outbreaks since 1999 to 2013, in the State of Rio Grande do Sul (RS), Southern Brazil (Tondo and Ritter, 2012; Tondo et al., 2015). This is probably the most studied foodborne pathogen of Southern Brazil, during the last 15 years (Tondo et al., 2015).

During the last decade, the number of minimally processed vegetable industries increased expressively in Brazil. In these kind of industry, microbial contamination present on vegetables is typically reduced through washing and sanitization procedures (FAO/WHO, 2008), which correspond to the main critical points of processing (Gil et al., 2015). According to the Codex Alimentarius, minimally processed vegetable industries can use different methods and products for disinfection (CAC/RCP, 2003), and chlorine compounds seems to be the best option because they are cheap and present broad-spectrum microbial inactivation (Van Haute et al., 2015). Currently, in Brazil, there is no official regulation recommending sanitizers or how to wash and disinfect vegetables in minimally processed vegetable industries, and several methods have been used without a comparison regarding its effectiveness against important food pathogens.

Thus, the objective of this research was to evaluate the inactivation of *Salmonella* Enteritidis SE86 on lettuces submitted to different methods of washing and disinfection used by industries of minimally processed vegetables of Southern Brazil.

2. **MATERIAIS E MÉTODOS**

2.1 **Investigation of processing characteristics**

The processing characteristics of the investigated companies were followed. Each industrial plant was visited two times, between October 2013 and January 2014 where the washing and disinfection steps were carefully accompanied. Then, each process was reproduced in the laboratory in order to verify the effectiveness on the inactivation of SE86 on lettuces. The washing and rinsing steps were reproduced at laboratory using 250 mL of potable water. The sanitizer usually found in industries (Água Sanitária Qboa®) was chose to perform the tests, being the immersion conducted in 500 mL of 200 ppm of this sanitizer.

2.2 **Experimental design**

One specific strain of *Salmonella* Enteritidis, the SE86 was used as inoculum. This strain was originally isolated from a cabbage responsible for a foodborne outbreak occurred in 1999 in Southern Brazil and was responsible for more than 90% of Salmonellosis identified in the State of RS during the last years (Tondo and Ritter, 2012; Tondo et al., 2015).

Curly lettuces were purchased from local markets and transported to the laboratory. The lettuce leaves were not washed or disinfected before inoculation, since it is not happen in real conditions. Samples of internal leaves weighting 10 g were added to sterile plastic bags and inoculated with 100 µL SE86 suspension, reaching a final concentration of $10^5$ – $10^6$ CFU/g. Inoculated lettuces were let for 30 minutes at room temperature and submitted to procedures described in Table 1. Each procedure was repeated two times with three replicates per trial.

| Table 1: Washing and disinfection processes used at different minimally processing |
vegetable industries of Southern Brazil which were reproduced in the Laboratory in order to inactive *Salmonella Enteritidis* SE86 on lettuces.

<table>
<thead>
<tr>
<th>Industry</th>
<th>Process</th>
</tr>
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<tbody>
<tr>
<td>Industry 1</td>
<td>Washing followed by immersion b in 200 ppm of sodium hypochlorite for 2 minutes b</td>
</tr>
<tr>
<td>Industry 2</td>
<td>Immersion b in 200 ppm of sodium hypochlorite for 15 minutes d</td>
</tr>
<tr>
<td>Industry 3</td>
<td>Washing followed by immersion b in 200 ppm of sodium hypochlorite sanitizer for 15 minutes h</td>
</tr>
<tr>
<td>Industry 4</td>
<td>Washing followed by immersion b in 200 ppm of sodium hypochlorite sanitizer for 1 minute j</td>
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<tr>
<td>Industry 5</td>
<td>Washing followed by immersion b in 200 ppm of sodium hypochlorite sanitizer for 5 minutes l</td>
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</tbody>
</table>

a, c: Performed with 250 mL of potable water at 22 °C; b: Immersion in 500 mL of 200 ppm of free chlorine solution at 22 °C.

After each step of each procedure, lettuce samples were added to a new sterile plastic bag containing 90 ml of 0.1% peptone water added with 0.9% NaCl. Each sample was homogenized using a stomacher (Seward®) for 30 s and was serially diluted in 0.1% peptone water and plated (20 μL), in triplicate, by the droplet-method on XLD (HiMedia®) agar plates (Malheiros et al., 2009). The plates were incubated at 37 °C for 24 hours.

3. RESULTADOS E DISCUSSÃO

The results of initial microbiological loads showed average counts of 5.83 ± 0.83 log CFU/g (Figure 1).

**Figure 1:** Kinetics of *Salmonella Enteritidis* SE86 reduction due to different washing and disinfections procedures observed in minimally processing vegetable industries.
In minimally processed vegetable industries the initial washing should be done and can be achieved very simply by spraying with potable water or immersion of product in chilled water (1-10 °C) (Artes and Allende, 2014). When performed in the laboratory, the washing step alone decreased significantly the SE86 population on lettuce, being the reduction approximately 1 log CFU/g (Figure 1). Other authors also reported that the washing step reduces around 1 log CFU/g of the microbial loads present on lettuces (Beuchat et al., 2001; Van Haute et al., 2013).

Only Industry 2 did not perform the initial washing with potable water, and, instead of that, this Industry started the process directly with immersion in 200 ppm sodium hypochlorite for 15 minutes, followed by using coconut soap for 1 minute. This procedure was not verified at any other industry, scientific literature or official regulation.

The disinfection performed using 200 ppm sodium hypochlorite was able to reduce 1 to 3 log CFU/g of SE86, depending the immersion time used (Figure 1). Corroborating these results, other studies have shown that chlorine solution significantly reduces the microbial load of minimally processed vegetables. The reduction observed in other studies ranged from 1 log CFU/g to 3.15 log CFU/g (Baur et al., 2005; Beltrán et al., 2005; Casteel et al. 2008; Luo et al., 2011; Tirpanalan et al., 2012; Thomas-Callejas et al., 2012).

In Brazil there is no specific legislation for the use of sanitizing to disinfect fresh-cut vegetables in minimally processed industries, neither regarding the contact time and concentrations. Typically, the minimally processed vegetable industries follow the recommendation from the regulation of Good Manufacturing Practices for food services, which considers the following steps: a) washing using potable water, b) disinfection by immersion in 200 ppm of free chlorine solution, for 15 minutes, and c) rinsing with potable water (RIO GRANDE DO SUL, 2009).
The last procedure adopted by all industries was rinse. The rinse must be being this step is recommended by the Codex Alimentarius (CAC/RCP, 2003). Beyond this purpose, reductions were demonstrated by rinse, 0.12 ± 0.63 log CFU/g to 1.90 ± 1.07 log CFU/g (Figure 1).

Regarding the whole procedure only the demonstrated by Industry 3 was able to eliminate the population of SE86. This Industry followed washing by disinfection with immersion 200 ppm sodium hypochlorite for 15 minutes and final rinsing.

4. CONCLUSÕES

Regarding the different washing and disinfection procedures observed in minimally processed vegetable industries investigated, only one was able to eliminate all the population of SE86, being washing, disinfection with 200 ppm sodium hypochlorite for 15 minutes and final rinse. However immersion times of 1, 2, and 5 minutes in 200 ppm sodium hypochlorite showed similar reduction rate of Salmonella Enteritidis SE86. This indicates that industries could adopt shorter times of disinfection in order to reduce costs of process and also significantly reducing the numbers of Salmonella on lettuce leaves.

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6. REFERÊNCIAS BIBLIOGRÁFICAS

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