

Effectiveness of chlorine alternatives to inactivate *Salmonella* Typhimurium in leafy vegetables

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The consumption of leafy vegetables is associated with a healthy diet. However, they are susceptible to several sources of contamination throughout the production chain. Washing-disinfection is essential to promote the microbiological safety of these vegetables, with chlorine-based products being widely used. However, due to their potential to generate toxic by-products, there has been an increased interest in chlorine alternatives, such as organic acids and hydrogen peroxide. To assess the effectiveness of chlorine alternatives to wash vegetables, focusing on the inactivation of *Salmonella* Typhimurium. The experiments were conducted with a strain of *S. Typhimurium* (ATCC 14028) previously electro-transformed to express the plasmid pGFPuv. Briefly, a portion of lettuce (~150 g) was contaminated with *Salmonella* (6 logs CFU/mL) through immersion in experimentally contaminated water (4 L at 25 °C) for 30 min. Next, it was shaken manually to remove excess liquid and divided into five portions (25 g each). The first portion was washed by immersion in static water, while the others were immersed in water containing the tested products: acetic acid, lactic acid and hydrogen peroxide at concentrations of 0.5% and 2.0%, as well as chlorine (sodium dichloroisocyanurate) at 250 ppm, for 5 and 15 min. Subsequently, the portions were rinsed in static water and dried using a manual salad spinner. The samples underwent enumeration of *S. Typhimurium* by plating onto Xylose Lysine Deoxycholate (XLD) agar supplemented with carbenicillin (50 µg/mL). They were also tested for the presence of this bacterium using the ISO 6579-1:2017 method. All tested products reduced *S. Typhimurium* counts compared to washing with only water, which achieved reductions <0.5 logs CFU/g. The alternative products showed reductions slightly lower but comparable to those obtained with chlorine, which effected a reduction ranging from ~2.0 to ~3.3 logs CFU/g. The greatest reductions were achieved at a concentration of 2.0% for 15 min: 3.3, 3.1, and 2.9 logs CFU/g for lactic acid, acetic acid, and hydrogen peroxide, respectively. On the other hand, when testing the lowest concentration of products for a shorter time (0.5% for 5 min), the reductions were 1.3, 0.7 and 0.9 logs CFU/g for lactic acid, acetic acid, and hydrogen peroxide, respectively. Overall, as expected, the effectiveness of the products decreased as the concentration and contact time were reduced. However, under all tested conditions, lactic acid exhibited better effectiveness compared to acetic acid and hydrogen peroxide. The three alternative products tested effectively reduced the population of *S. Typhimurium* in the lettuce samples. However, lactic acid demonstrated the highest efficacy, similar to that achieved with chlorine. These results indicate their potential for practical application, highlighting the importance of further studies in this area.

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