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Antimicrobial effect of peracetic acid compared to sodium hypochlorite in the disinfection of ready-to-eat lettuce (*Lactuca sativa L.*)

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ABSTRACT

Ready-to-eat lettuces are highly demanded for salads but are exposed to factors that may pose health risks during production. There is an actual need to find alternatives to preserve fresh cut fruits and vegetables to improve the efficacy of the washing treatment. This study compared the antimicrobial effects of disinfection of raw ready-to-eat lettuce leaves with peracetic acid (CH_3CO_3H, PAA), sodium hypochlorite (NaClO) and washing with tap water. For disinfection, lettuce leaves were immersed in solutions of different concentration of PAA (40 and 80 ppm) and NaClO (100 and 150 ppm) for 2 min. The treated leaves were stored for 10 days under refrigeration (4 ºC). The aerobic plate count (APC), total coliforms, Salmonella spp. and total yeast and mold count (TYMC) were determined during this time. At the end of this period, samples treated with the lower PAA level had APC values like those obtained with both NaClO concentrations. The treatments with both disinfectants eliminated *Salmonella* spp., while the levels of APC, total coliforms and TYMC were below the limits of the Peruvian sanitary standard. It is concluded that PAA is a valid alternative to chlorine, and that higher concentrations could be used to achieve more significant population reductions

Keywords: Lettuce; Peracetic acid; Hypochlorite; Disinfection; Ready-to-eat.

1. Introduction

Lettuce (*Lactuca sativa* L.) is one of the most widely consumed vegetables worldwide, but its nutritional value has been underestimated despite being low in calories, fat and sodium. It is a good source of fiber, iron, vitamins, and other health-promoting bioactive compounds (Kim et al., 2016). Diet plays a critical role in strategies to reduce body weight and improve health. The prevalence of obesity has increased worldwide. In the United States, the prevalence of obesity in adults (men and women, for all age groups) was 42.4% between 2017-2018 (Hales et al., 2020); while in Peru obese people aged 15 years and older were 25.8% in 2021 (Instituto Nacional de Estadística e Informática, 2022). The incorporation of lettuce fiber in the diet helps weight loss because of its low caloric content, lowering the risk of cardiovascular diseases by reducing cholesterol and low-density lipoproteins (LDL), as well as the risk of diabetes by improving glucose metabolism besides decreasing the risk of colon cancer (Kim et al., 2016). In Peru, vegetable salads are consumed on average 3.3 times a week by people over 15 years of age (Instituto Nacional de Estadística e Informática, 2022).

As a result of changing consumer habits, the demand for minimally processed products has increased dramatically. The fresh produce production chain is complex and encompasses

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different crucial steps where microbial safety can be affected (Castro-Ibáñez et al., 2017). Washing fruits and vegetables is a common practice to remove soil, pesticide residues, and reduce microbial load. At the household level, this process involves rinsing under running water. A key difference in commercial operations is that fruits and vegetables are subject to handling and are typically immersed in an antimicrobial agent (Suslow, 1997). The EFSA Panel on Biological Hazards (BIOHAZ) (2013) identified combinations of foods and microbial pathogens generally associated with outbreaks in the European Union, finding that the first place was occupied by leafy green vegetables consumed raw and Salmonella spp. A similar result was obtained after the evaluation carried out by Da Silva Felício et al. (2015). Chlorine-based sanitizers are widely used for the decontamination of these fresh produce (Rico et al., 2007). The antimicrobial effect is due to the attack on thiol-containing enzymes and amino groups of proteins (Denyer & Stewart, 1998). However, incomplete oxidation of organic materials by chlorine can produce undesirable by-products, such as chloroform (CHCl3) or other trihalomethanes, which have known or suspected carcinogenic potential (Suslow, 1997). For this reason, several countries have restricted the use of chlorination for food washing. Concern about these chlorine reaction byproducts has led to increased efforts to evaluate and approve non-chlorine treatments for sanitization and postharvest handling, including their study in simulated lettuce washing water (Pablos et al., 2018). Survival of inoculated pathogens such as E. coli O157:H7 in lettuce has also been reported where common concentrations of chlorine (200 ppm) were used, obtaining results similar to those obtained by washing with deionized water (Beuchat, 1999). Alternative or modified methods have been proposed, such as addition of antioxidants, irradiation, ozone treatment, organic acids, modified atmosphere packaging, whey permeates, etc.; however, none has yet gained widespread industry acceptance (Gil et al., 2009). Among the disinfectants capable of replacing NaClO are peracetic acid (CH3CO3H, PAA), which is formed through the combination of hydrogen peroxide and acetic acid and has a broad oxidizing power and a wide spectrum of action and is effective in the presence of organic matter and hard water. It also has the advantage of not affecting the physicochemical, nutritional and sensory quality of the products (Ho et al., 2011; Nicolau-Lapeña et al., 2019). As a disinfectant, it has a proven a level of efficacy on fresh produce either alone or in combination with other organic acids (Lopez-Galvez et al., 2013). It was even able to eliminate two SARS- CoV-2 strains in lettuce, chicken and salmon samples inoculated with the virus (Jung et al., 2023). Its most common commercial presentation is in solution with hydrogen peroxide, another strong oxidant (Suslow, 1997).

The disinfectant effect of PAA, alone or in combination with other organic acids, against *E. coli* K12, *Enterobacter sakazakii*, *Salmonella* spp., *Listeria innocua*, and Lacobacillus plantarum inoculated on fruits and vegetables has been previously investigated (Kim et al., 2006; Yuk et al., 2006; Ho et al., 2011; Nicolau-Lapeña et al., 2019). In one of these studies, a synergistic effect was found with lactic acid (Ho et al., 2011). But others reported that the effect of PAA in combination with lactic acid is not different from that of washing with tap water in uninoculated iceberg lettuce (Lopez-Galvez et al., 2013). Nicolau-Lapeña et al. (2019) explained the absence of difference between yeast and mold populations of strawberries treated with NaClO (200 ppm), PAA (20 - 80 ppm) and the control, to the physical removal produced by the flow of water on the surface of the product. The same authors noted that the efficiency of PAA in removing natural microbiota (APC and TYMC) was lower than that of NaClO treatment, especially for microorganisms remaining in the washing water. Using lettuce inoculated with L. monocytogenes, Beuchat et al. (2004) reported that there was no significant difference for those treated with chlorine (100 µg/ml) or a PAA containing product (80 µg/ml), for each type of lettuce and inoculum level. Recently, Banach et al. (2021) performed a multi- criteria decision analysis to determine a control strategy for treating fresh-cut produce washing water, evaluating different agents such as ClO2, PAA, ozone, free chlorine and washing water without disinfectant. They found that PAA was the preferred control strategy, considering the criteria of effectiveness, technology, acceptability, economics and health issues.

Suslow (1997) stated that it is best to determine the disinfectant effects experimentally, for each type of product and local conditions. The present work aimed to determine the efficacy of two disinfectant compounds, sodium hypochlorite (NaClO) and peracetic acid (PAA), in solutions for washing fresh cut ready-to-eat lettuce stored in polyethylene bags under refrigerated conditions (4 ºC).

2. Methodology

2.1. Samples and conditioning

Whole iceberg lettuce samples were acquired from the Nueva Esperanza market in the city of Arequipa, Peru. Each lettuce was placed in polyethylene bags and transported in high-density polyethylene (HDPE) plastic boxes to the laboratories of the School of Food Industry Engineering at the Universidad Nacional de San Agustín de Arequipa. The whole samples were kept refrigerated (4 ºC) from the time they were received at the laboratory until they were processed on the same day of acquisition. Because the outer leaves of lettuce have APC values one logarithmic cycle higher than the rest (Adams et al., 1989), they were removed by cutting them with previously sterilized stainless-steel knives. Those that showed any type of mechanical damage or dark coloration were also removed from each lettuce. This handling and subsequent processing was performed using sterile nitrile gloves.

2.2. Minimal processing and disinfection

Each conditioned sample was cut lengthwise into strips of approximately 10 cm long by 1 cm wide. Except for the samples left unwashed and untreated, the rest were washed with tap water (free chlorine residual level: 0.40 mg/L, reported by Paco Choque and Pizarro Medina (2022)) by immersion in pre-washed HDPE plastic containers. For the treatments with both disinfectants, the washed lettuce samples were then immersed in additional HDPE plastic containers with the NaClO and PAA solutions. The ratio of cut leaves: solution volume was 1:10 in all cases, as reported by Vandekinderen et al. (2009) and Lopez-Galvez et al. (2013). The concentration levels of the NaClO solutions were 100 and 150 ppm (concentrations reported by Suslow (1997) as used for spraying of lettuce of different varieties) while those of PAA were 40 and 80 ppm. All solutions were prepared with distilled water boiled overnight and allowed to cool to room temperature. Preliminary tests were performed to compare the effect of contact time (1 and 2 min) on the disinfectant effect (APC) of NaClO and PAA, finding that there were no significant differences. Similar results were reported by Nicolau-Lapeña et al. (2019) with similar concentrations of PAA (20 - 80 ppm) and slightly higher NaClO concentration (200 ppm). In the current study, a contact time of 2 min was used for both disinfectants and the control. The treated leaves were then manually centrifuged for two minutes to remove excess solutions. Packaging was in heat-sealed polyethylene bags, each containing 150 g of fresh cut lettuce. The bags were stored (4 ºC) for up to 10 days in a dedicated refrigerator.

2.3. Microbiological analysis

The aerobic plate count (APC), total coliforms, Salmonella spp., and total fungi and yeasts count (TYMC) were determined for all samples including untreated and water-washed leaves. APC was performed according to the conventional method described in the FDA's Bacteriological Analytical Manual (BAM) (Maturin and Peeler, 2021). This value was used as a general indicator of the level of microorganisms. The total coliform (including E. coli) count was carried out using the technique described in the BAM (Feng et al., 2020), based on the use of 3M™ Petrifilm™. The interpretive guide from the manufacturer, which describes the coliform and *E. coli* count (except *E. coli* O157), was followed (3M, 2019). Isolation and identification of Salmonella spp. was also conducted according to the BAM (Andrews et al., 2023). This included an absence/presence test, where samples were preenriched with 0.1% (w/v) peptonized water, followed by enrichment in tetratrionate broth and selenite cystine broth. They were then seeded on brilliant green agar, Salmonella-Shigella agar and bismuth sulfite agar. Typical positive colonies that grew on at least one of the indicated agar media were considered suspicious and were seeded on triple iron sugar agar tubes. For the total yeast and mold count (TYMC), the plate dilution technique was used (Tournas et al., 2001). The results were expressed in colony forming units per gram of sample (CFU/g), while for Salmonella spp. the absence/presence was reported. All values were compared with the Peruvian sanitary standard (Peru, 2008) for semi-processed, refrigerated and/or frozen fresh fruits and vegetables. Three samples were taken for each treatment, time and concentration. The mean log reduction was calculated with respect to the untreated samples (Prado-Silva et al., 2015).

2.4. Statistical analysis

Analysis of variance and statistical tests were performed using the R programming language version 4.3.1 (R Core Team, 2023). Significant differences between means were tested using Tukey's (de Mendiburu, 2023) and/or Dunnet's (Signorell, 2023) tests at a significance level of $p < 0.05$, unless otherwise specified.

3. Results and discussion

3.1. Native microbiota

The APC and total coliform levels detected in the initial untreated samples (Tables 1 and 2) were below the Peruvian standard for semi-processed fresh fruits and vegetables (Peru, 2008). These values remained below the limit during storage at 5 and 10 days. Lopez-Galvez et al. (2013) reported higher APC levels, in the range of 2.6 - 3.5 log CFU/g, for cut leaves of iceberg lettuces. Even higher levels (4.17 log CFU/g) were reported by Vandekinderen et al. (2009), placing iceberg lettuce in the range observed by the same authors for samples of grated carrot, cut leeks and white cabbage (3.83 - 6.34 log CFU/g). The initial microbial load of Webb's lettuce was also reported to be higher (average of 6.65 log CFU/g, except for the outer layer of leaves) by Adams et al. (1989).

The presence of *Salmonella* spp. was not detected in any of the samples. The adoption of Good Handling Practices (GHP) and Hazard Analysis and Critical Control Points (HACCP) could have minimized the initial contamination of the samples (Chaves et al., 2016). TYMC values (Table 3) were not detectable at the beginning or throughout storage of untreated lettuce leaf samples. Lopez-Galvez et al. (2013) also showed TYMC levels below the detection limit for some iceberg lettuce samples, up to a maximum of 3.2 log CFU/g.

The storage period had no significant effect on the level of APC and TYMC in the untreated lettuce samples. Lopez-Galvez et al. (2013) observed slight growth for both groups of microorganisms in samples stored at a higher temperature (7 ºC) from the fourth day onwards.

Table 1
Values of APC (log CFU/g) of the untreated and treated samples

Disinfectant	Concentration (ppm)	Time (days)		
		0	5	10
Untreated	-	2.4 ± 0.4	2.3 ± 0.5	2.4 ± 0.4
Wter	-	1.8 ± 0.5	1.6 ± 0.5	2.6 ± 0.2
PAA	40	1.5 ± 0.4	1.9 ± 0.6	2.3 ± 0.3
	80	2.2 ± 0.5	2.1 ± 0.4	2.4 ± 0.1
NaClO	100	1.7 ± 0.8	2.2 ± 0.2	2.2 ± 0.4
	150	1.8 ± 0.0	2.1 ± 0.1	2.2 ± 0.4

Table 2
Values of total coliforms (log CFU/g) of the untreated and treated samples

Disinfectant	Concentration (ppm)	Time (days)		
		0	5	10
Untreated	-	1.6	BDL	BDL
Water	-	BDL	1.7 ± 0.9	1.7
PAA	40	BDL	BDL	BDL
	80	BDL	BDL	BDL
NaClO	100	BDL	BDL	BDL
	150	BDL	BDL	BDL

BDL: Below detection limit.

Table 3
Values TYMC (log CFU/g) of the untreated and treated samples

Disinfectant	Concentration (ppm)	Time (days)		
		0	5	10
Untreated	-	BDL	BDL	BDL
Water	-	1.8	BDL	BDL
PAA	40	3.0	BDL	BDL
	80	BDL	2.0	BDL
NaClO	100	BDL	BDL	BDL
	150	BDL	BDL	BDL

BDL: Below detection limit.

3.2. Effect of treatments on the amount of APC, total coliforms, total coliforms, and Salmonella spp.

All observed values of APC and total coliforms in the treated samples were below the microbiological limits of the Peruvian standard for semi-processed fresh fruits and vegetables. (Peru, 2008). Only the time factor (days) had a significant effect (p = 0.058) for APC values. Initially, both NaClO treatments achieved a reduction similar to that of the water wash, below 1 log as indicated by Parish et al. (2003). Samples treated with PAA (40 ppm) showed the greatest initial reduction. For the tap water wash treatment, the mean APC level decreased slightly after 5 days relative to the level measured after the initial wash, then rose sharply after 10 days. This reduction (Figure 1), which was initially the third in magnitude after that of both NaClO treatments, became the largest of all treatments at 5 days and the smallest of all at 10 days. Lopez-Galvez et al. (2013) also reported that APC values in iceberg lettuce treated with disinfectants based on PAA, hydrogen peroxide, organic acids, propylene glycol and NaClO (contact time = 1 min) were not significantly different from those obtained with water washing after the fifth day. The APC results we obtained after 10 days were lower than those reported by these authors after 7 days at 7 ºC (4.9 - 6.9 log CFU/g). Also, for lettuce, Vandekinderen et al. (2009) showed that for a wider range of contact times (1 - 10 min) a higher PAA concentration (100 - 250 mg/L) corresponds with a linear increase in the reduction of APC values. The largest APC reductions of the PAA-treated samples were obtained with the lowest concentration (40 ppm), with the gap narrowing after 5 and 10 days of storage, after which they were similar to those obtained with both NaClO concentrations. Furthermore, Nicolau-Lapeña et al. (2019) reported that there was no significant difference in the APC values of strawberries treated with PAA (20 - 80 ppm) and NaClO (200 ppm) for 1 or 2 min. Coliforms (Table 2) were initially detected in the untreated samples at the beginning of the experiment. Samples washed with tap water showed growth in 5 days, and only one of these samples maintained a similar value on the tenth day. With respect to the disinfectants, colonies were only detected in one of the samples treated with PAA (80 ppm) after 10 days. Kim et al. (2006) reported the reduction of a pathogenic coliform (*E. sakazakii*) in lettuce inoculated and treated with either chlorinated water, chlorine dioxide or a PAA and hydrogen peroxide-based sanitizer.

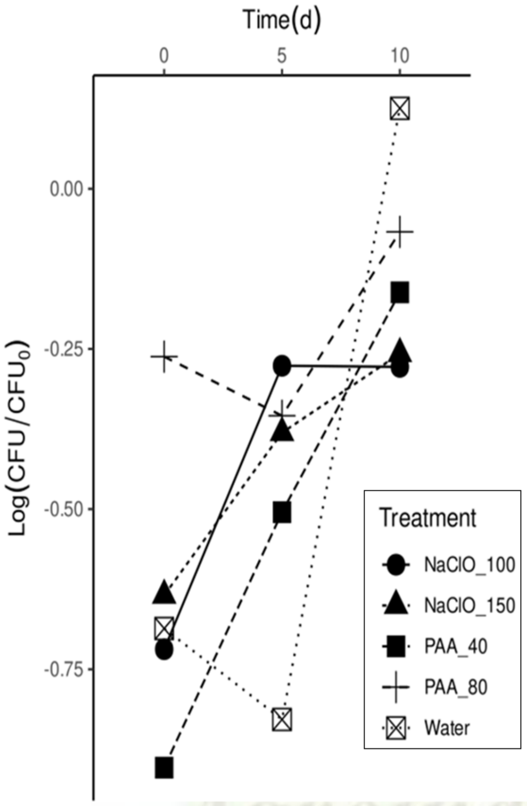

Figure 1. APC reductions for the treated samples during the experiment

These authors indicated that chlorine was less effective in reducing this coliform, but all treatments reduced the population of *E. sakazakii* below the detection limit. They also indicated that the washing process is less efficient in removing *E. sakazakii* from lettuce compared to apples or tomatoes. In turn, Truchado et al. (2021) pointed out for cut lettuce that the operational limits of chlorine in the process wash water (20 - 25 mg/L as free chlorine) are adequate for the inactivation of pathogens (*E. coli* and *L. monocytogenes*), but that higher contact times or concentrations would be required in the case of PAA and ClO2 treatments, in order to reduce the induction of viable but non-culturable pathogenic bacteria cells. In all cases, the values reported in the present study were lower or within the range of the Peruvian standard (Peru, 2008). None of the untreated or treated samples with water or disinfectants reported the presence of *Salmonella* spp. at the beginning or during storage.

3.3. Effect of disinfectant treatments on total yeast and mold counts

TYMC values were not detected in the untreated lettuce samples (Table 3), nor in those treated with NaClO, but were detected in only one of the samples washed with water initially and in individual samples treated with PAA (40 ppm) at the beginning and after 5 days (80 ppm). Lopez-Galvez et al. (2013) reported initial differences between yeast counts in lettuce washed with water or with disinfectants, but noted that these differences disappeared after the fifth day. For all treatments in the present study, no detectable TYMC values were observed in the samples after 10 days.

4. Conclusions

Ready-to-eat lettuces are raw products, exposed to many risk factors. Contamination prevention is very important as there is a continuous risk throughout the production chain. Especially in the city of Arequipa, Peru, biological and chemical contamination factors in irrigation water must be considered.

The reductions obtained by the treatments were similar to those previously reported for water washing, which was also compared. Samples treated with both NaClO concentrations produced similar results throughout the observation period. Those treated with PAA had better initial results in APC reduction when the lower concentration (40 ppm) was used, but the difference with those treated with the higher concentration decreased visibly with time.

It is recommended to study higher concentration levels of NaClO (e.g., 300 ppm chlorine) at pilot scale, similar to what is done in the industry to maintain a level of available chlorine throughout the process. Higher PAA concentrations should also be evaluated, especially since they do not affect the sensory perception of iceberg lettuce.

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