Combination of peracetic acid and ultrasound reduces *Salmonella* Typhimurium on fresh lettuce (*Lactuca sativa* L. var. *crispa*)

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Abstract *Salmonella* outbreaks related to fruits and vegetables have been reported being lettuce one of the most contaminated. Peracetic acid (PA) at 50 mg/L, sodium dichloroisocyanurate (SD) at 100 mg/L, and the combination of SD at 100 mg/L and babaçu coconut (*Attalea speciosa*) oil detergent at 100 mg/L were applied to fresh lettuce. Natural contaminant microbiota, physicochemical characteristics, and sensory attributes were evaluated. PA and SD reduced mesophilic aerobic counts by 2.1 and 1.5 log cfu/g, respectively. The most efficient treatment in reducing natural microbiota (i.e., PA) was applied alone and in combination with ultrasound (US). It reduced *Salmonella enterica* Typhimurium counts to undetectable levels (< 1 log cfu/g). US further reduced *S. Typhimurium* counts by 0.6 log cfu/g in relation to PA, treatment which lessened the pH but increased the titratable acidity of lettuce, but did not cause total color difference. Therefore, the combination of PA and US holds a potential industrial application for sanitization purposes.

Keywords Peracetic acid · Sodium dichloroisocyanurate · Washing · Lettuce · Ultrasound

Introduction

Recent foodborne outbreaks have increased the concerns regarding risks to public health. *Salmonella* has been the most critical bacterium (CDC 2015). Although the most common vehicles for this pathogen are eggs, milk, and meat, several of outbreaks involving fruits and vegetables have been reported lately (CDC 2016; Harris et al. 2003). Lettuce and other ready-to-eat leafy greens have been recurrently associated to *Salmonella* outbreaks (Gajraj et al. 2012; Vestrheim et al. 2016). Sanitization stands out as an essential step to lessen outbreak risks and increase food safety (Araújo et al. 2015; Salgado et al. 2014). Chlorine-based compounds have been widely used for this purpose. However, European countries (e.g., Netherlands, Sweden, Germany, and Belgium) have banned some of these compounds that may lead to the production of carcinogens and pose public health threats (São José et al. 2014a, 2015). Additionally, the efficiency of these compounds in food decontamination is questionable (Meireles et al. 2016; São José et al. 2014a). In this context, studying and implementing alternative sanitization methods are of utmost importance.

Peracetic acid (PA) is a sanitizer regulated by the US Food and Drug Administration (FDA) under 21CFR173.315. Its use in ready-to-eat fruits and vegetables is allowed at concentrations not higher than 80 mg/L. PA rapidly decomposes into hydrogen peroxide and acetic acid, compounds which provide its high efficiency against bacteria, molds, viruses, and spores (Rosário et al. 2017; Srey et al. 2013). Furthermore, the rapid decomposition of hydrogen peroxide into water as well as the presence of acetic acid, which is generally recognized as safe (GRAS), prevents environmental damage (Kitis 2004; Srey et al. 2013). Sodium dichloroisocyanurate (SD) is an organic

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chlorine-based compound that efficiently inactivates microorganisms (Clasen and Edmondson 2006). There is no evidence that it can form carcinogenic compounds and pose health threats (Clasen and Edmondson 2006; Maffei et al. 2016). Also, detergents can act synergistically and boost the antimicrobial activity of chlorinated compounds (Srey et al. 2014) because of its ability to reduce the interfacial tension of the sanitizing solution in a way that the contact between the active ingredient and the target surface is increased.

Food decontamination by chemical compounds may be significantly improved when combined with an ultrasound treatment (Afari et al. 2016; Duarte et al. 2018; Francisco et al. 2017; Rosário et al. 2017; São José et al. 2014b). Ultrasound is a technology in which sound waves create a mechanical phenomenon called cavitation. This process forms microjets, which in turn may produce pores in microbial membrane as well as generate an intense pressure that facilitates sanitizer transport towards the intracellular medium. Moreover, cavitation can generate local heat (hot spots) and chemical (free radicals) effects that contribute to microbial inactivation (Awad et al. 2012; Gogate and Kabadi 2009; São José et al. 2014a).

In this paper, we report the investigation of the efficiency of PA, SD, and a combination of SD and a Babacu detergent in reducing the natural microbiota of lettuce, as well as at assessing their potential in preserving the physicochemical and sensory attributes of such food matrix. The effect of PA alone and in combination with ultrasound on the reduction of intentionally adhered *Salmonella* Typhimurium was also evaluated.

Materials and methods

Fresh lettuce (*Lactuca sativa* L.) var. *crispa* was purchased from the local market, between March and September 2016. After being transported in isothermal boxes, lettuce leaves were selected, washed, and cut in longitudinal 2-cm-wide strips. A total of 50 g was individually submitted to the following sanitizing procedures.

Sanitizing procedures

Peracetic acid (PA) (Proxitane® Paraná, Brazil) at 50 mg/L and sodium dichloroisocyanurate (SD) (JohnsonDiversey®, São Paulo, Brazil) at 100 mg/L isolated or combined with Babacu coconut (*Attalea speciosa*) oil detergent (DT) (Veros, São Paulo, Brazil) at 100 mg/L were applied to lettuce (*Lactuca sativa* L.) var. *crispa*. Additionally, PA was combined with an ultrasound (US) (Model Soniclean 15, Sanders Medical®, Minas Gerais, Brazil) at 40 kHz and 500 W. These treatments were applied by dipping lettuce

leaves for 5 min (Sagong et al. 2011) in 500 mL of sanitizing solution. Lettuce washed with drinking water (NS), sterilized water (SW), and US were used as controls (sanitizer-free treatments).

Microbiological evaluation

Naturally present contaminant microbiota

Twenty five grams of lettuce were dissolved in 225 mL of 0.1% peptone water (10^{-1}). The suspension was homogenized in a stomacher (Marconi®, Piracicaba, São Paulo, Brazil) for 1 min. Appropriate dilutions were prepared in 0.1% peptone water and then transferred to culture media specific for each microbial population. Mesophilic aerobic bacteria were plated on plate count agar (PCA) (HiMedia®, Mumbai, India) and incubated at 35 °C for 48 h. Molds and yeasts were plated on potato dextrose agar (PDA) (HiMedia®, Mumbai, India) and incubated at 25 °C for 7 days. After these periods, microbial counts were taken and the results were expressed as colony-forming units (cfu) per gram of lettuce (cfu/g) (Downes and Ito 2001).

Intentional contamination with Salmonella enterica Typhimurium

Inoculum preparation *Salmonella enterica* Typhimurium ATCC 13076 was kept in Eppendorf tubes containing 1 mL of Brain Heart Infusion (BHI) broth (HiMedia®, Mumbai, India) and glycerol (80:20) at -80 °C. This pathogen was activated in 10 mL of BHI broth by two consecutive subcultures that were incubated at 35 °C for 24 h. To obtain the inoculum, 10 mL of activated culture were centrifuged (Fanem, model 206 BL, São Paulo, Brazil) at 3000 g for 10 min. The supernatant was removed and the pellets were resuspended in 10 mL of 0.1% peptone water (Luo and Oh 2016). Subsequently, *S. Typhimurium* count in the inoculum was confirmed by plating on Hektoen Enteric Agar (HiMedia®, Mumbai, India).

Lettuce inoculation Lettuce leaves were selected and cut aseptically into 3×3 cm² pieces. A total of 0.1 mL of inoculum was added to each piece at different points. For each sanitization procedure, 25 g of contaminated lettuce were prepared. Contaminated samples were dried in a laminar flow hood for 30 min to allow *S. Typhimurium* adhesion on lettuce surface (Luo and Oh 2016) followed by sanitization as described in “Sanitizing procedures” section.

***S. Typhimurium* quantification** After each sanitizing procedure, 25 g of lettuce were diluted in 225 mL of a

0.1% peptone water (10^{-1}). Samples were homogenized in a stomacher (Marconi®, Piracicaba, São Paulo) for 1 min. Suitable dilutions were made in 0.1% peptone water and plated on Hektoen Enteric Agar (HiMedia®, Mumbai, India). *S. Typhimurium* colonies were counted after 18–24 h of incubation at 37 °C, the results being expressed as cfu/g (Downes and Ito 2001). The sanitizing solution was also plated and the cells were counted likewise. The results were expressed as log cfu/mL.

Titrateable acidity, pH, and sensory attributes

Total titrateable acidity (TTA) was determined through the titrimetric technique described in modified AOAC (AOAC 2005). The results were calculated as percent citric acid (%). pH was measured using a digital pH-meter (Model mPA-210, Tecnozon, São Paulo, Brazil) by inserting the electrode in 10 g of sample previously diluted in 100 mL of distilled water and homogenized. Sample color was measured right after sanitization by direct reflectance readings according to the rectangular coordinate system (L^* , a^* , b^* , Chroma, and Hue in the CIELAB color scale) on a Konica Minolta® (model CM-5, New Jersey, USA) colorimeter. L^* indicates brightness and ranges from black ($L^* = 0$) to white ($L^* = 100$), a^* indicates the intensity of red (+) and green (−) colors, and b^* refers to the intensity of yellow (+) and blue (−) colors. Chroma and Hue stand for saturation and tonality, respectively. The total color difference (TCD) was calculated by the following equation:

$$TCD = \left((L_T - L_0)^2 + (a_T - a_0)^2 + (b_T - b_0)^2 \right)^{1/2}$$

where in “0” indicates values obtained in the control (NS) and “T” indicates coordinate values for each treatment. Thus, TCD indicates the color difference between control (NS) and sanitized treatments.

Experimental design and statistical analysis

To investigate the antimicrobial effectiveness of the sanitizers on the natural contaminant microbiota, PA, SD, SD + DT, and US were applied. PA was applied separately and in combination with US to verify the efficiency of US in improving the effect of PA on the inactivation of *S. Typhimurium* in both lettuce surface and sanitizing solution. Physicochemical and sensory attributes of all treatments were determined to assess possible changes. The results were submitted to one-way analysis of variance (ANOVA) and Duncan’s test using the Assistat freeware (Silva and Azevedo 2016). All analyses were carried out with three repetitions and in duplicate, immediately after sanitization. The level of significance was 0.05.

Results and discussion

Effects on the natural contaminant microbiota

The natural microbiota of lettuce is comprised of mesophilic aerobic bacteria, molds, and yeasts. Because it may cause food spoilage, the current investigation was set out to study strategies of reducing the natural microbiota of lettuce. The initial counts of mesophilic aerobic bacteria as well as molds and yeast were equal to 6.8 and 4.3 log cfu/g, respectively. Concerning mesophilic aerobic bacteria, the greatest reduction ($p < 0.05$) (i.e., 2.1 log cfu/g) was achieved by PA (Fig. 1). Bachelli et al. (2013) obtained a 3.0 log cfu/g reduction in mesophilic aerobics in lettuce by applying PA at 100 mg/L for 15 min. SD and SD + DT reduced the counts of such microorganisms by 1.5 and 1.1 log cfu/g, respectively, these values not being significantly different ($p > 0.05$). Therefore, DT did not contribute to the antimicrobial effect of SD. As for molds and yeasts, PA, SD, and SD + DT decreased their counts by 1.6, 1.5, and 1.4 log cfu/g, respectively, values which did not differ ($p > 0.05$) from each other (Fig. 1). São José and Vanetti (2015) found similar reductions in watercress when testing SD at 200 mg/L and PA at 40 mg/L for 10 min (1.9 and 2.5 log of cfu/g, respectively). Consequently, both sanitizers may also be employed to efficiently reduce the development of this group of microorganisms. US decreased the counts of mesophilic aerobic bacteria and molds and yeasts by 0.6 and 0.3 log cfu/g, respectively,

Fig. 1 Decimal reductions of mesophilic aerobic bacteria and, molds and yeasts relative to the control treatment (no sanitizer) in lettuce sanitized through different methods. SW sterilized water, US ultrasound (40 kHz), SD sodium dichloroisocyanurate (100 mg/L), DT detergent (100 mg/L), PA peracetic acid (50 mg/L). Mean values followed by same lower case letter for aerobic mesophilic and uppercase letter for molds and yeast do not differ significantly ($p > 0.05$)

values which did not differ significantly ($p > 0.05$) from their SW counterpart. US is known to inactivate microorganisms, but the reductions lower than 1.0 log cfu/g demonstrated that US alone presented limited efficiency in lettuce.

Effect on *Salmonella enterica* Typhimurium

Several pathogenic bacteria are associated with outbreaks resulting from the consumption of vegetables. Sanitization stands out as an important approach of lessening this risk. Based on the natural microbiota found here, the best sanitizer (i.e., PA) was selected to be applied in combination with US in the reduction of *S. Typhimurium* on lettuce surface. The initial *S. Typhimurium* count on lettuce was 7.5 log cfu/g. Sterilized water (control) reduced 0.8 log cfu/g in *S. Typhimurium* count on lettuce surface and the count in the sanitizing solution was 5.0 log cfu/mL. PA and PA + US significantly reduced ($p < 0.05$) cells adhered on lettuce by 2.4 and 3.0 log cfu/g, respectively. Besides that the pathogen remained undetectable on sanitizing solution after sanitizing with PA and PA + US. Sagong et al. (2011) obtained a reduction of 2.0 log cfu/g in *S. Typhimurium* counts in lettuce by using 0.5% lactic acid combined with US at 40 kHz for 5 min. US further reduced by 0.6 log cfu/g to the bactericidal action of PA in *S. Typhimurium* inactivation, but this reduction was not significant ($p > 0.05$). Although cavitation has been proven to remove cells adhered onto food surfaces, pathogens may penetrate fruits and vegetables and become inaccessible to sanitizers and sound waves (Bartz et al. 2015; Rosário et al. 2017). Another factor that may impair sanitizers' action is the formation of biofilms on food surface, once this structure is resistant to chemical agents (São José and Vanetti 2012; Rosário et al. 2017). In this study, 5 min was probably not enough for US mechanical action to remove *S. Typhimurium* cells adhered on lettuce.

Effect on the physicochemical and sensory attributes

The major objective of sanitization is the reduction of pathogenic and spoilage microorganisms. However, knowing the physicochemical and sensory changes that may occur in this step is of great importance. DT and SD provided lettuce with intense soapy and chlorine odors, respectively, whereas PA did not result in any undesirable odors. The physicochemical and sensory attributes of lettuce are presented in Table 1. PA and PA + US reduced lettuce pH ($p < 0.05$). This effect can be attributed to the low pH of PA (López-Gálvez et al. 2009). A similar outcome was observed for TTA, which was possibly a result of significant residual effects ($p < 0.05$).

Color is a food quality indicator that may be a decisive factor for the consumer acceptability of ready-to-eat lettuce. None of the treatments changed ($p > 0.05$) L^* (lightness), b^* (predominance of yellow or blue), and Chroma (predominant color saturation) values. Zhang and Yang (2017) also found no difference in L^* and b^* after the application of hydrogen peroxide and citric acid on lettuce grown in organic and conventional systems. Negative a^* values indicated the presence of green, with PA, SD, US, and PA + US showing the highest ($p < 0.05$) a^* values compared to the control. Therefore, one may state that these treatments reduced the green staining in lettuce. São José and Vanetti (2015) also found that PA at 40 mg/L, SD at 200 mg/L and US at 45 kHz, when applied for 10 min, altered the a^* value of watercress. Hue values close to 100° indicate a green Hue. The lower the Hue value, the higher the tendency towards a yellow Hue. US and PA + US resulted in the lowest Hue values. This outcome suggests that free radicals and hot spots formed during cavitation may have damaged lettuce. TCD indicates the overall color difference visually noticeable and is described by the total distance of the three-dimensional colors of CIELab scale. TCD values equal to or greater than five indicate easily detectable color differences (Obón et al. 2009). Although some color parameters have been modified at certain sani-

Table 1 Physicochemical and sensory attributes of sanitized lettuce

	pH	TTA	L^*	a^*	b^*	Chroma	Hue	TCD
NS	5.92 ± 0.08 a	0.69 ± 0.04 cd	25.87 ± 0.70 a	− 4.83 ± 0.40 bc	17.85 ± 0.74 a	18.50 ± 0.80 a	105.13 ± 0.86 a	–
SW	5.96 ± 0.05 a	0.70 ± 0.03 cd	26.05 ± 1.18 a	− 4.83 ± 0.52 bc	19.08 ± 1.19 a	19.68 ± 1.26 a	104.18 ± 0.83 ab	1.24
PA	5.37 ± 0.06 b	0.91 ± 0.02 b	25.65 ± 3.44 a	− 4.36 ± 0.42 ab	18.20 ± 3.48 a	18.73 ± 3.45 a	103.66 ± 1.77 ab	0.63
SD	5.93 ± 0.13 a	0.76 ± 0.03 c	24.05 ± 1.15 a	− 4.42 ± 0.43 ab	17.46 ± 0.96 a	17.86 ± 0.63 a	104.39 ± 1.60 ab	1.90
US	5.93 ± 0.05 a	0.72 ± 0.04 cd	23.44 ± 0.82 a	− 3.66 ± 0.64 a	17.21 ± 0.24 a	17.50 ± 0.30 a	101.87 ± 2.34 c	2.77
PA + US	5.35 ± 0.06 b	0.99 ± 0.02 a	23.81 ± 1.79 a	− 4.00 ± 0.31 ab	17.76 ± 1.89 a	18.19 ± 1.91 a	102.71 ± 0.84 bc	2.22
SD + DT	5.77 ± 0.07 a	0.66 ± 0.01 d	23.38 ± 0.09 a	− 4.71 ± 0.19 bc	16.55 ± 0.21 a	17.21 ± 0.17 a	105.88 ± 0.75 a	2.81

NS no sanitizer, SW sterilized water, PA peracetic acid, SD sodium dichloroisocyanurate, US ultrasound, DT detergent. Mean values followed by the same letters within a column do not differ significantly ($p > 0.05$). TTA total titratable acidity (percent of citric acid, %), TCD total color difference

tizing conditions, TCD values remained lower than five for all treatments (Table 1). Therefore, color changes in lettuce were overall undetected.

Conclusion

PA was the most effective treatment in reducing aerobic mesophilic bacteria in lettuce. This compound deserves special attention since it is biodegradable and also due to recent restrictions on chlorinated compounds. A reduction of 3 log cfu/g on *S. Typhimurium* cells in lettuce after application of PA + US as well as total inactivation of such pathogens in the sanitizing solution were observed. Despite its many advantages, PA caused physical and chemical changes in lettuce, but still sensory changes were not perceived. In summary, the combination of PA and US presented a high capacity for being used in industrial applications for sanitization purposes to improve the safety of lettuce consumption.

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