Research

Combined peracetic acid: power ultrasound disinfection process enhances bioactive compounds and preserves quality attributes of fresh-cut lettuce (cv. Vera)

M. Irazoqui¹ · M. Colazzo² · E. Fender² · E. Budelli³ · S. Barrios³ · N. Pérez³ · P. Lema³

Received: 11 October 2023 / Accepted: 15 February 2024 Published online: 29 February 2024 © The Author(s) 2024 OPEN

Abstract

Lettuce (*Lactuca sativa*) is one of the most consumed vegetables, being an important source of carotenoids and polyphenols. Sanitization is a crucial step in fresh-cut production to ensure product safety, where non-thermal technologies like ultrasound (US) can enhance disinfectant effects. This study assessed the impact of combining peracetic acid (PAA) and power ultrasound (US) on the disinfection of fresh-cut lettuce and its quality parameters. Lettuce was treated with PAA (80 mg L⁻¹, 5 min) and PAA—US (5 min) at different acoustic power densities (APD) and frequencies: 25 W L⁻¹ at 25 kHz and 45 kHz, and 45 W L⁻¹ at 37 kHz and 80 kHz. Microbial load reduction ranged from 1.6 to 2.1 log CFU g⁻¹ for treated lettuce. While combined technologies generally improved microbial reduction, no clear trend was observed for APD or frequency variations. Wash water quality indicated the need for decontamination to prevent cross-contamination. Total Antioxidant Capacity (TAC), Total Phenolic Content (TPC), and lutein increased compared to untreated lettuce, possibly due to treatment-induced abiotic stress. Total chlorophyll decreased after treatment. Power US showed potential for disinfecting fresh-cut lettuce, inducing positive changes in relevant bioactive compounds with no significant impact on visual quality. Further optimization is needed to enhance microbial inactivation compared to PAA alone.

Keywords Disinfection · Ultrasound assisted process · Ready-to-eat · Minimally processed · Quality attributes

1 Introduction

Fresh vegetables are an essential part of human diet. In recent years, there has been an increasing demand for fresh-cut produce, being leafy vegetables the most consumed products. Particularly, lettuce (*Lactuca sativa*) is a leafy vegetable with important nutritional value, containing vitamins, minerals and phytonutrients [1]. Fresh-cut lettuce is the third most consumed vegetable in the US, and China is the largest producer in the world, representing 56% of world production [1]. Lettuce is one of the most difficult leafy vegetables to decontaminate since its curly and rough surface is very susceptible to microbial adhesion limiting shelf life [2].

Disinfection step is one of the most important operations in fresh-cut vegetables production, where sanitizers are applied to reduce or eliminate microbial hazards. Chlorine-based compounds, in the range of $50-200 \text{ mg L}^{-1}$ of active chlorine, are the most popular disinfection chemicals used in industry, with maximum exposure times of 5 min [3].

M. Irazoqui, mirazoqui@fing.edu.uy | ¹Departamento de Química del Litoral, CENUR Litoral Norte. Universidad de la República, 60000 Paysandú, CP, Uruguay. ²Licenciatura en Análisis Alimentario, Universidad Tecnológica, 60000 Paysandú, CP, Uruguay. ³Instituto de Ingeniería Química. Facultad de Ingeniería, Universidad de la República, 11300 Montevideo, CP, Uruguay.





Some countries have banned chlorine-based compounds becauseof health risk associations with by-products formation during disinfection processes [4, 5]. Alternative chemical-based disinfectants are peracetic acid (PAA), chlorine dioxide, electrolyzed oxidising water, hydrogen peroxide and ozone, among others. PAA is a weak acid commercially available as a mix of peracetic acid, acetic acid, hydrogen peroxide and water. It is a common substitute of chlorine because it is not expensive and has the advantage of decomposing rapidly into oxygen and acetic acid, which are generally recognized as safe (GRAS) [4, 6]. The Food and Drug Administration (FDA) allowed up to 80 mg L⁻¹ peracetic acid in water used in washing process of fruit and vegetables (21 CFR 173.315) [7]. Pahariya et al. [] support that PAA can achieve higher microbial reductions and lower browning effects on lettuce leaves when compared with disinfection treatments applying acidified sodium chlorine and 100 mg L⁻¹ sodium hypochlorite solutions for 1 to 5 min, showing that PAA is a good substitute to chlorine-based sanitizers [8].

At present, effective and eco-friendly preservation processes are needed for improving shelf-life and reducing nutritional losses of ready-to-eat, fresh-cut produce [9]. Non-thermal technologies such as power ultrasound (US) have gained popularity in the food industry due its capacity to inactivate microorganisms by cavitation effect and the ability of detach objects that adhere to the surface by cavitation bubbles [10]. Power ultrasound in combination with sanitizers has the ability to improve food decontamination by enhancing the antimicrobial effect of some chemical disinfectants [7]. Combining disinfection technologies is a convenient strategy for fresh-cut production, since a combination of chemical and physical factors can work simultaneously or sequentially seeking higher decontamination efficiency with milder effects on product quality (hurdle approach) [2].

In Rosário et al. [11] conducted a study where strawberries were exposed to a combined treatment of chemicals (acetic acid, peracetic and sodium dodecylbenzenesulfonate) and physical treatment (ultrasound—40 kHz, 500 W L⁻¹) at 7 °C for 5 min. They observed that the combination of US and PAA (40 mg L⁻¹) showed the best results in terms of reducing natural microbiota, while having no appreciable impact on physicochemical properties or sensory quality changes. Furthermore, Lu et al. [12] studied the effect of ultrasound (25 kHz, 26 W L⁻¹, treatment times of 1–4 min at ambient temperature) on secondary metabolites on tomato fruits. They found that application of US could have a beneficial health effect, enhancing the accumulation of some secondary metabolites and increasing antioxidant capacity. Combined application of US and PAA has been previously evaluated in lettuce by Oliveira Silveira et al. [4], who obtained reductions of 3 log CFU g⁻¹ for *Salmonella Typhimurium* when applying PAA (50 mg L⁻¹) and US (40 kHz, 500 W) to inoculated fresh-cut lettuce for 5 min, reporting no differences on product physicochemical characteristics such as pH, color and sensory attributes.

Since combined disinfection technologies can induce changes in phytochemical and sensory characteristics of the product, an evaluation of different process conditions focusing on microbial inactivation but also on nutritional and sensory changes of fresh-cut products is needed.

In this context, the aim of this study was to evaluate the effect of power ultrasound in combination with peracetic acid on the microbial load and quality of fresh-cut lettuce (cv. Vera), focusing on changes in phytoconstituents which represent lettuce nutritional quality. Different frequencies (f) and acoustic power densities (APD) were evaluated. For this objective we proposed three hypotheses: (i) combined treatments will have higher effects than PAA alone on product microbial quality; (ii) changing acoustic power density and frequency in combined washing treatments will have an impact on microbial reduction iii) US application will have an impact in phytochemical content showing differences among power densities and frequencies.

2 Materials and methods

2.1 Plant material

Ripe green lettuce (*Lactuca sativa* cv. Vera) was obtained from a farm located in Paysandú (Uruguay), cultivated for commercial purpose and following good agricultural practices (GAP). Outdoor grown lettuce was transported directly from farm to the laboratory of Tecnología de Alimentos (Departamento de Química del Litoral (DQL), CENUR Litoral Norte, UdelaR), and processed immediately.

Leaves with absence of defects (external and damaged leaves were eliminated) were selected to form a homogenous batch. Core was removed with a stainless-steel knife and lettuce leaves were cut in transversal long strips of 2–3 cm width. Cut lettuce strips were randomly assigned to the different disinfection treatments (c.f Sect. 2.2). A total of 24 heads (4 kg approximately) were used in the experiments. Non-disinfected cut lettuce was used as reference of fresh (untreated) lettuce.



2.2 Wash disinfection step

A combination of power ultrasound and peracetic acid solution (PAA—US) was used in wash disinfection of cut lettuce. Peracetic acid solution (80 mg L⁻¹) was prepared using a commercial formula of 3.5-5.5% acetic acid, 9-12% hydrogen peroxide and 23-30% distilled water. Treatment time was fixed in 5 min [13, 14].

Disinfection treatment was carried out in two stainless steel ultrasound units, assisted with a recirculating pump to facilitate convection (17 L min⁻¹) (Elma, Germany): Transsonic TI-H, working at frequencies of 25 kHz and 45 kHz, and Elmasonic P, working at frequencies of 37 kHz and 80 kHz. Transsonic TI-H unit worked at a nominal operating power of 100 W, while Elmasonic P worked at a nominal operating power of 120 W. In total, five treatments were applied on freshcut lettuce: PAA alone (considered as control) and four combined PAA—US treatments, as shown in Table 1.

A ratio of 1/15 w/w cut lettuce to solution was used in ultrasonic baths. Initial solution temperature was 16.0 \pm 0.7 °C. After application of PAA—US treatments, excess water was eliminated from samples using a domestic salad spinner. Sole chemical disinfection (80 mg L⁻¹ PAA) was assayed as control treatment, in order to discriminate the combined effect of both disinfection technologies. In control experiments, cut lettuce/solution ratio, temperature and treatment time was kept the same as described for combined PAA—US treatments.

2.3 Calorimetric determination of ultrasound parameters

The method commonly used for scaling the delivered power involves using power per unit of volume. For each experiment, ultrasound power dissipation (P) was determined calorimetrically recording temperature vs. time, and was calculated with Eq. 1 as previously described by Irazoqui et al. [15]. Specific energy was calculated as acoustic power density (APD) [16–18] using Eq. 2:

$$P = mC_P \left(\frac{\Delta T}{\Delta t}\right) \tag{1}$$

$$APD = \frac{P}{V}$$
(2)

where *m* and *V* are the mass and volume of ultrasonicated disinfection solution, C_{ρ} is the specific heat capacity of water (4.18 J g⁻¹ K⁻¹), ΔT is the increase of temperature during the process and Δt is process time. ΔT ranged between 1.8 and 1.9 °C for 100 W nominal power and between 2.9 and 3.1 for 120 W nominal power, Δt was fixed in 5 min for all treatments.

2.4 Wash water solution parameters

Table 1Treatments andexperimental conditions used

in the study

PAA concentration, pH and conductivity of the PAA solution in the ultrasonic bath was measured before and after wash disinfection step. For pH measurement, a digital pH meter PHS-3C (Orion, Belgium) was used. Conductivity was determined using a pocket conductivity, salinity and TDS meter EcoTestr[™] CTS (Oakton, USA). Peracetic acid concentration was determined by iodometric titration. Microbiological quality of disinfection solution after treatments was evaluated as described in Sect. 2.5.1. All measurements were performed at room temperature in triplicate.

Exp. Number	PAA (mg L^{-1})	Nominal power (W)	f (kHz)	Volume (L)	APD (W L^{-1}) ^a	Treatment name
1	80	_	_	3.0	-	PAA
2	80	100	25	3.0	25	P25_25
3	80	100	45	3.0	25	P25_45
4	80	120	37	3.0	42	P42_37
5	80	120	80	3.0	42	P42_80

^aSee Sect. 3.1 for APD experimental determination



2.5 Lettuce quality parameters

2.5.1 Microbiological analysis

10 g of cut-lettuce samples were homogenised for 1 min with 90 mL of sterile saline solution (0.85%) to generate the dilution 10⁻¹ in sterile stomacher bags. Official methods AOAC 990.12 and AOAC 997.02 for Petrifilm[™] (3 M[™], USA) were used for total mesophilic counts (TMC) and yeast (YC) and mold (MC) counts respectively [19, 20]. AFNOR 3 M 01/0609/97 for Petrifilm[™] (3 M[™], USA) was used for enterobacteriaceae counts (ENT) [21]. Incubation conditions were 35±1 °C for 48±3 h for aerobic mesophilic and for 24±2 h for enterobacteriaceae count plates, while for yeast and mold counts incubation conditions were 25 ± 1 °C for 5 d. Two dilutions were prepared for each replicate. Microbial reductions were calculated and expressed as log CFU g⁻¹. Microbial counts in untreated lettuce (c.f. Section 2.1) were determined as reference.

2.5.2 pH and color measurement

Lettuce samples were evaluated immediately after treatments. pH determination was performed using a digital pH meter in the filtered extract obtained after grinding 0.5 g mL⁻¹ of lettuce in distilled water for 2 min using a domestic mixer. Color measurements were analysed by direct reflectance readings using a surface colorimeter model CR-400 (Konica Minolta, USA). Calibration was performed using a white standard and D65 illuminant was used. L*, a* and b* parameters were obtained and total colour difference (ΔE) was calculated by Eq. 3, where "0" indicates values for untreated and "t" for treated lettuce samples:

$$\Delta E = \sqrt{\left(L_t - L_0\right)^2 + \left(a_t - a_0\right)^2 + \left(b_t - b_0\right)^2}$$
(3)

Browning index [] was calculated also by Eq. 4:

$$I = \frac{100 * (x - 0.31)}{0.17}, \text{ where } x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$$
(4)

Leaf and stem regions were analysed separately. For each treatment, 10 readings of each replicate were performed.

2.5.3 Electrolyte leakage

Electrolyte leakage rate (ER) was determined to quantify tissue deterioration as reported by Salgado et al. [22]. Briefly, 5 g of lettuce sample was stirred with 100 mL deionized water in an orbital shaker at 100 rpm (DragonLab, China). Electrical conductivity was measured at 1 min (C1) and 60 min (C60). Samples were then autoclaved (121 °C, 25 min) and total conductivity (C_{τ}) was measured after cooling. ER (%) was calculated by Eq. 5:

$$ER(\%) = \frac{(C_{60} - C_1)}{C_T} \times 100$$
(5)

2.5.4 Chlorophylls determination

Lettuce samples were freeze-dried in a freeze dryer (DW-10, China). Freeze-dried samples were processed in a coffee mill to obtain a homogeneous fine powder. For chlorophyll and carotenoid extraction (Sect. 2.5.5), 0.1 g of dried sample was extracted in 10 mL of 80% acetone. Mixture was vortexed for 1 min, sonicated for 10 min and finally centrifuged at 5500 × g for 5 min. Supernatant was separated and, if necessary, dilutions were made [12, 23].

Chlorophyll a (Chl A), chlorophyll b (Chl B), total chlorophyll (Chl T) was calculated by equations applied by Kotíková et al. [24] and proposed by Lichtenthaler and Wellburn [25], measuring absorbance at 470, 646 and 663 nm with a Genesys 10S UV–VIS spectrophotometer (Thermo Fisher Scientific, USA) [24, 25]. Results were expressed as mg g^{-1} (dry mass).



2.5.5 Carotenoid analysis by HPLC–DAD

Qualitative and quantitative analysis of carotenoids were performed using an Ultimate 3000 HPLC (Thermo Scientific) equipped with an automatic injector and diode array detector (DAD 3000). A reverse-phase C18 column (Agilent Eclipse Plus, 1.8 μ m and 4.6 × 100 mm) was used. Mobile phase consisted of water (solvent A) and acetonitrile (solvent B) and the flow rate was 1.2 mL min⁻¹. Elution program was a multistep gradient as follows: 5% A / 95% B for 0–3 min, 100% B from 3 to 45 min and 5% A/95% B from 46 to 50 min. Chromatograms were recorded at 448, 454 and 474 nm. Injection volume was 3 μ L. Identification of carotenoids from the extracts obtained in Sect. 2.5.4 was carried out by comparing their retention time and UV spectrum with beta-carotene (99% purity, HPC Standards GMbH, Germany), zeaxanthin (97% purity, ChromaDex., USA) and lutein (92% purity, PhytoLab GmbH & Co., Germany) standards. Quantification was performed when matches were found with an external standard calibration curve built for each identified compound in the range 0 to 50 mg L⁻¹. Results were expressed as mg g⁻¹ of dry sample.

2.5.6 Total phenolic content and total antioxidant capacity

Extraction procedure consisted in dissolving 0.25 g of freeze-dried sample in 8 mL of extraction solution acetone: water: acetic acid (70:29:1 v/v/v). Mixture was vortexed for 1 min, sonicated for 30 min, mixed for 2.5 min with a Micro Ball Mill (Grinder, China) at 650 rpm and sonicated again for 10 min. Finally, mixture was centrifuged at $5500 \times g$ for 15 min at 10 °C. Supernatant was transferred to a 10 mL volumetric flask and brought to volume with the extraction solution.

Folin–Ciocalteau assay was used to quantify total phenolic content (TPC) as described by Singleton et al. (44). 100 μ L of extract was incubated with 3000 μ L of sodium carbonate (2%) and 50 μ L of Folin-Ciocalteu reagent. Mixture was mixed and kept in the dark for 60 min at room temperature. Absorbance changes were measured using a Genesys 10S UV–VIS spectrophotometer (Thermo Fisher Scientific, USA) at 750 nm using the extraction solution as blank. A calibration curve was prepared with gallic acid standard (0–800 mg L⁻¹). TPC was expressed as mg gallic acid equivalents (GAE) per g of dry sample.

Total Antioxidant Capacity (TAC) was estimated by ABTS method according to Re et al. [26]. 7 mmol L⁻¹ of ABTS solution was activated with 140 mmol L⁻¹ of potassium persulfate solution. Solution was stored at room temperature in the dark for 14–16 h before use. Afterwards, ABTS⁺⁺ solution was diluted with buffer phosphate (5 mmol L⁻¹, pH 7.4) to obtain an absorbance of 0.70 ± 0.02 at 734 nm. A calibration curve was prepared with a Trolox standard (0–200 µmol L⁻¹). 300 µL of sample (or standard solution) was added to 2700 µL of ABTS⁺⁺ solution and absorbance was measured at 734 nm after 10 min using a Genesys 10S UV–VIS spectrophotometer (Thermo Fisher Scientific, USA). Results were expressed as µmol of Trolox equivalents (TE) per g of dry sample.

2.6 Experimental design and data analysis

First, an exploratory analysis was performed evaluating principal component analysis and cluster to determine the direction of the variance among the samples. Also, a MANOVA test was performed with a Pillai test to evaluate which variables had an impact when treatments were applied. Finally, ANOVA test was performed for each variable when significant differences were found. Results from all measured parameters were analysed considering treatments as variation factor. Significant differences between mean values were determined by applying Tukey test. Differences were considered significant when p < 0.05. All statistical analyses were performed using R Studio.

3 Results and discussion

3.1 Calorimetric determination of ultrasound parameters

Delivered power (P) and acoustic power density (APD) were calculated for the different frequencies and nominal power applied for the combined treatments. Initial temperature was 15.9 ± 1.4 °C and after treatments final temperature averaged 18.4 ± 0.8 °C. Table 2 shows nominal power, frequency, dissipated power and acoustic power density obtained for the four combined PAA—US treatments applied.

As observed in Table 2, for each of the two nominal power levels used (100 and 120 W), similar average power density (APD) was obtained despite the different frequencies applied. Based on these results, our study considered an average



Discover Food (2024) 4:14

Table 2Nominal power,frequency, dissipated powerand average acoustic powerdensity (APD) calculatedfor the different PAA—UStreatments applied

Nominal power (W)	f (kHz)	Dissipated power (W)	APD (W L ⁻¹)	Treatment name
100	25	82±3 ^a	23 ± 4^{a}	P25_25
100	45	83 ± 8^{a}	26 ± 3^{a}	P25_45
120	37	137 ± 3^{b}	43 ± 6^{b}	P42_37
120	80	128 ± 25^{b}	41 ± 9^{b}	P42_80

Results are expressed as average \pm standard deviation for treatments applied in triplicate (n = 3). Same letters in same column represent values which are not significantly different (Tukey test, p > 0.05)

APD of 25 W L^{-1} for a nominal power of 100 W and an average APD of 42 W L^{-1} for a nominal power of 120 W. This average APD will be the one considered in subsequent analysis of the data obtained for each experiment.

3.2 Wash disinfection solution quality parameters

Measurement of wash disinfection solution parameters can give information about the disinfection process and can also help determine if and when further treatment of residual wash water solution is necessary to avoid cross-contamination in the case water is to be re-used during the process.

Initial PAA concentration was $79.56 \pm 0.71 \text{ mg L}^{-1}$, which corresponded to an initial pH of 2.97 ± 0.06 . As expected, PAA concentration in disinfection solution decreased significantly after disinfection step for all treatments applied (p-value < 0.0001). PAA consumption ranged between 22.1 and 24.3%, and no significant differences were observed between control (PAA alone) and PAA—US treatments (p=0.868). Also, negligible effect of APD or frequency was observed in PAA consumption (p > 0.05).

Meanwhile, conductivity after treatments was higher for PAA—US treatments than in control experiments (PAA alone). PAA—US treatments showed between 50 and 61% higher conductivity in wash disinfection solution after treatments. An increase in conductivity following disinfection treatments could be associated with removal of soil and plant exudates. This could be explained by the cavitation effect produced by ultrasonic waves, where tiny bubbles implode on lettuce surface, enhancing removal of dirt, soil particles and plant exudates with respect to sole PAA disinfection. No significant effect of APD nor frequency was observed on disinfection solution conductivity (p > 0.05).

As for microbial quality of wash disinfection solution, Table 3 shows total mesophilic (TMC), enterobacteria (ENT), mold (MC) and yeast (YC) counts of the wash disinfection solution after treatment application.

No growth was observed for either enterobacteria or yeasts after the application of any of the tested treatments. These microbes were also significantly reduced in lettuce leaves (cf. Section 3.4), showing that all applied treatments led to the inactivation of enterobacteria and yeasts. Meanwhile, total mesophilic and mold counts in wash water were lower than 2 log CFU mL⁻¹, with no significant differences between PAA—US treatments and control. Considering the potential reuse of wash water during disinfection process, further treatments should be considered in washing solutions to avoid cross-contamination [26]. Consistent with our findings, Ssemanda et al. [27] reported difficulties for achieving inactivation of aerobic plate count (APC) when applying US and linked this to the formation of natural microflora biofilms and

(P42_37 and P42_80)					
Treatment	$\log TMC$ (log CFU mL ⁻¹)	$\log ENT (\log CFU mL^{-1})$	$\log MC (\log CFU mL^{-1})$	$\log YC (\log CFU mL^{-1})$	
PAA	1.65 ± 0.11^{a}	< 1.00 (3/3) ^a	0.89 ± 0.36^{a}	< 1.00 (3/3) ^a	
P25_25	1.61 ± 0.21^{a}	< 1.00 (3/3) ^a	1.03±0.36 (1/3) ^a	<1.00 (3/3) ^a	
P25_45	1.54 ± 0.22^{a}	< 1.00 (3/3) ^a	1.19 ± 0.36^{a}	< 1.00 (3/3) ^a	
P42_37	1.47 ± 0.22^{a}	< 1.00 (3/3) ^a	0.68 ± 0.14^{a}	<1.00 (3/3) ^a	
P42_80	1.76 ± 0.23^{a}	< 1.00 (3/3) ^a	0.98 ± 0.24^{a}	$0.77 \pm 0.40 (1/3)^{a}$	

Table 3 Total mesophilic Count (TMC), Enterobacteria (ENT), Mold Count (MC), and Yeast Count (YC) in wash disinfection solution after control (PAA) and combined PAA—US treatments: 25 W L⁻¹ at 25 kHz and 45 kHz (P25_25 and P25_45), and 42 W L⁻¹ at 37 kHz and 80 kHz (P42_37 and P42_80)

Numbers in parentheses represent replicates below detection limit out of the total number of replicates. For replicates below the detection limit, the detection limit was used in the calculation of means and standard deviations. Data are expressed as the mean of three replicates \pm standard deviation (n = 3). Same letters in same column represent values which are not significantly different (Tukey test, p > 0.05)



the ability of microbes to add-on to leaf matrix [27]. This may explain why US can effectively remove mesophiles, yeast and molds from lettuce surface but not destroy them, resulting in viable counts remaining in the water after processing.

Meanwhile, regarding the potential for cross-contamination during disinfection process, Huang et al. [28] studied the prevalence of Salmonella in wash water. They found that treatments involving chlorine wash combined with pulsed light and ultrasound could keep Salmonella in wash water below the detection limit when processing inoculated lettuce [28]. Therefore, ultrasound could be employed to enhance the microbial quality of wash water after disinfection treatments. Although our results for wash water quality are preliminary since only microbial quality was considered, it is important to recognize that eliminating microorganisms from fresh produce does not guarantee their complete inactivation and further wash water decontamination has to be considered.

In sum, combined PAA—US treatments assayed in this work showed no differences in PAA consumption or microbial counts in wash water compared to control treatment. These combined technologies were only more effective than control treatment in removing dirt and plant exudates. Differences with previously published results for fresh-cut lettuce may be attributed to the specific US conditions assayed in each study. Importantly, our results indicate the need for considering the washing solution quality when contemplating water reuse during the disinfection process.

3.3 Effect of APD and frequency on lettuce quality parameters: PCA and cluster study

A PCA test was performed with all quality parameters assayed in treated lettuce to determine whether they are affected by treatments and if there are correlations between them. Principal Component 1 (PC 1) represented 45.8% of the total variance, while second component (PC 2) represented 14.4% of the total variance. Adding a third component (PC 3) resulted in 11% additional representation of total variance. The three components presented eigenvalues greater than one (according to Kaiser criterion). The first two components allowed describing 60% of total variance, which represents a high explanation of the data, but adding a third component (71% of total variance) allowed to explain Total Phenolic Content and Browning Index with good modules ($\cos^2 > 0.6$). Nonetheless, pH and electrolyte leakage could not be well represented by these three dimensions. The two principal coordinates associated with parameters included in PCA analysis are shown in Fig. 1.



Fig. 1 PCA correlations with Dim 1 (PC1) and Dim 2 (PC 2) (right) and coordinates associated with each variable (left)



As can be appreciated, microbiological loads and carotenoids had a strong and positive correlation with PC1 and a positive but low correlation with PC2 (First quadrant). In contrast, chlorophylls had a strong and positive correlation with PC1 but a negative correlation with PC2 (fourth quadrant), where chlorophyll A was principally explained by PC2. Total Phenolic Content, Antioxidant Capacity, color difference, lutein and lutein-like compounds had a strong and negative correlation with PC1 (second and third quadrant). As was mentioned before, PC3 helped to explain the variance associated with Total Phenolic Content, showing a positive correlation, as well as Antioxidant Capacity and Browning Index (leaves and stem). In sum, the first two components can represent most of the variance associated to the studied variables. The third component is needed to have a better explanation of TPC and B.I., while pH and electrolyte leakage could not be well represented by any of these components. Also, it can be noted that positive PC1 can be associated with parameters which were negatively affected by disinfection treatments, whereas negative PC1 is associated with parameters which were positively affected by these treatments.

When a cluster analysis was performed, two groups were differentiated: the first one linked non-treated lettuce with treated lettuces at low frequencies (25 and 37 kHz). The other group involved lettuce treated at high frequencies (45 and 80 kHz).

A MANOVA test was performed to evaluate if measured variables were affected by assayed treatments. All lettuce parameters assayed presented p-values lower than 0.01 (**, Pillai test), with the exception of pH (p=0.017) and %EC (p=0.696). An ANOVA for each variable and study of the treatment effects are presented below.

3.4 Effect of ultrasound on microbiological quality of lettuce

Figure 2 shows microbial loads in fresh-cut lettuce leaves before and after the different disinfection treatments applied. Untreated lettuce (raw lettuce) counts were 6.67 ± 0.17 , 5.66 ± 0.11 , 5.16 ± 0.23 and 5.80 ± 0.21 log CFU g⁻¹ for total mesophilic, enterobacteria, mold and yeast counts respectively. Control (PAA alone) treatment and combined PAA—US treatments significantly reduced microbial loads with respect to untreated lettuce, with reductions ranging between 1.6 to 2.1 log CFU g⁻¹ (Tukey test, p < 0.001). Microbial reductions obtained in lettuce samples after disinfection treatments are in accordance with several published works, where it has been reported that sanitation step allows achieving 1 to 2 log cycles reduction on microbial load [29]. Fan et al. (2019) obtained similar reductions of 2.21, 1.35 and 1.88 log CFU g⁻¹ for total number of colonies, mold and yeast and total coliforms respectively, when applying a combined ϵ -polylysine (0.4 g L⁻¹) and ultrasound treatment (23 W L⁻¹, 10 min) to fresh-cut lettuce [10]. Oliveira Silveira et al. (2018) reported a significant reduction of 2.1 log CFU g⁻¹ for mesophilic aerobes when treating lettuce (var. Crispa) with peracetic acid (50 mg L⁻¹) for 5 min [4]. As for the effect of ultrasound, previous studies have shown that US application (130 W, 42 kHz) improved microbial reduction with respect to sole chlorine disinfection for process times longer than 10 min [15]. In other related work, Huang et al. (2018) simulated cross-contamination with inoculated lettuces and obtained a significant

Fig. 2 Microbial counts for untreated (Raw Lettuce) and the five treatments assayed: disinfected lettuce with peracetic acid alone (PAA), combined PAA—US at 25 W L⁻¹ and 25 kHz (P25 25), combined PAA—US at 25 W L⁻¹ and 45 kHz (P25_45); combined PAA—US at 42 W L⁻¹ and 37 kHz (P42 37) and combined PAA—US at 42 W L⁻¹ and 80 kHz (P42_80). Values are expressed as average ± standard deviation (n = 3). Same letters in same graph represent values which are not significantly different (Tukey test, p > 0.05)



(2024) 4:14

reduction of inoculated bacteria after treatment with ultrasound (42 kHz, 100 W) for 10 min [28]. Recently, Banach et al. (2020) reported limitations associated with PAA disinfection during fresh-cut processing of lettuce inoculated with *E. coli*. Contact time and dose of PAA were reported to be relevant factors and they showed that once *E coli*. is introduced during washing, it remains attached to lettuce [30]. In previous unpublished work conducted in our laboratories, it was observed that the application of ultrasound alone did not lead to a significant reduction in microbial load, even when the process time was extended up to 10 min. Oliveira Silveira et al. [4] found reductions of 0.6 and 0.3 log CFU g⁻¹ for mesophilic aerobic bacteria and molds and yeast respectively when lettuce was treated with US alone (40 kHz, 500 W, 5 min), showing that disinfectant effect of US in lettuce is limited [4].

In the present work, combined treatments resulted in higher microbial reductions than sole chemical disinfection only in some experimental conditions: an additional reduction of 0.8 log CFU g^{-1} was obtained in lettuce treated with 25 W L^{-1} , showing a synergic effect of chemical disinfection and US in these conditions. In combined treatments, a significant effect of APD was found for TMC, MC and YC, where treatments applying 25 W L^{-1} presented, in general, lower counts than treatments applying 42 W L^{-1} . However, no clear trend in the effect of frequency on microbial loads could be drawn out from data obtained. Different results were obtained for ENT, where no significant differences were found between applying sole chemical disinfection or combined US-PAA treatments. In this case, ENT counts showed a reduction average of 2.31 log CFU g^{-1} in all treatments applied. Results obtained for microbial loads in lettuce show that in the present work, conditions for a clear US effect on microbial load could not be determined.

Microbial reduction obtained in lettuce leaves for TMC and MC was very similar to the loads recovered in the disinfection solutions (cf. Section 3.2), suggesting that during disinfection step there is a physical removal from lettuce surface rather than an inactivation of these groups of microorganisms. Microbial load obtained in residual wash disinfection solution could be attributed to transfer of microorganisms from lettuce surface and corresponds to microorganisms which could not be inactivated either by PAA or US. Contrarily, ENT and YC are not recovered in residual wash water, meaning that disinfection treatments applied allowed removal and inactivation of these microorganisms (cf. Section 3.2).

Results obtained in the present work could be rendered positive from a classical disinfection point of view. The truth is that the global rise in foodborne illnesses related to fresh-cut vegetables, especially lettuce, underscores the need for urgent action [31, 32]. However, in the last years, concern has risen on the effect of traditional and green disinfection alternatives on the ecological balance of produce. Evidence suggests that the natural microbiota can inhibit gram-negative microorganisms, and lactic acid bacteria have been used for fresh-cut produce decontamination associated principally to their antimicrobial-producing capability [13, 33].

Public health concerns will continue to prompt exploration of multidisciplinary disinfection strategies, with a focus on greener alternatives with greater impact on produce preservation but with milder effects on quality and the environment. Further studies are necessary to comprehend how natural microflora control foodborne pathogens and how this knowledge can be used in disinfection processes. Continued investigation in these areas is crucial for advancing alternative disinfection approaches in fresh-cut lettuce. Additionally, exploring water disinfection in the produce washing process warrants deeper exploration.

3.5 pH, electrolyte leakage and color

Lettuce pH did not change significantly after disinfection treatments, obtaining an average of 5.97 ± 0.05 . Results obtained are in contrast with those reported by Oliveira Silveira et al. [4], who found a reduction in pH values after treatment with peracetic acid (50 mg L⁻¹) and the combination of peracetic acid and ultrasound (40 kHz, 500 W, 5 min) [4]. However, the study carried out by Lafarga et al. [34] did not show differences in pH when treating various vegetables (including lettuce) with ultrasound at 40 kHz and 250 W for 20 min [34].

Meanwhile, electrolyte leakage has been reported as a measurement of tissue deterioration or damage. In this work, PAA and PAA—US treatments yielded similar values of ER in lettuce leaves, averaging $2.11 \pm 0.92\%$. Comparable values were reported by Palma-Salgado et al. [35] when Romaine and Iceberg lettuce were treated with various disinfection solutions and US for 1 min [35]. Huang et al. [28] did not observed visible damage on lettuce surface when US treatments were applied for less than 10 min. However, when ultrasound treatment was extended to 20 min, injured surfaces became evident, raising ER values [28]. Based on these observations, US treatment time applied in the present work did not produce significant tissue damage.

Visual quality is one of the most important attributes in ready-to-eat vegetables, where color is a key physical parameter that impacts consumer acceptability. Our study assessed CIE Lab color parameters (L*, a*, b*), total color differences (ΔE) and browning index (B.I.) in lettuce stem and leaf. Results indicated a significant color difference between treated



and untreated lettuces. However, there were no significant differences observed between PAA treatment and the combined treatment of US and PAA, whether measured as ΔE or as B.I., on stem and leaf zones. Color variation in treated lettuces manifested higher L^{*} values (representing lightness) and b^{*} values (indicating blueness) compared to raw lettuce. Meanwhile a^{*} value was lower, indicating higher greenness compared to untreated lettuce.

Azam et al. [1] reported similar results, where no significant differences were obtained when applying different ultrasonic conditions to lettuce and attributed minor color differences to changes in lettuce surface [1]. Oliveira Silveira et al. [4] found some differences in a* values, where US-treated lettuce was greener than untreated lettuce and attributed these color differences to damages caused by hot spots and free radicals formed during cavitation [4]. Previous reports with confocal images evidenced that ultrasound treatment (130 W, 42 kHz) could affect lettuce's waxy layer (cv. Vera), but no differences on sensory attributes were found on processing day [15].

3.6 Chlorophyll content

Figure 3 illustrates the impact of PAA—US and PAA disinfection treatments on chlorophyll content in lettuce samples. The disinfection treatments notably affected chlorophyll content (p = 0.0245).

Regarding chlorophyll A (Chl A) content, treated lettuce exhibited similar levels to untreated lettuce, except for lettuce treated with 42 W L⁻¹ and 80 kHz (P42_80), which showed the lowest content of this compound.

Chlorophyll B (Chl B) was also significantly influenced by disinfection treatments, with a more pronounced degradation in the case of ultrasound application. An average reduction of 41 and 43% in Chl B was found when applying an APD of 25 W L⁻¹ and 42 W L⁻¹ respectively. However, PAA disinfection alone did not show significant differences with respect to untreated lettuce samples (p-value < 0.001). In treatments employing 42 W L⁻¹, increasing US frequency from 37 to 80 kHz negatively impacted Chl B content. A similar trend was observed in the case of 25 W L⁻¹ treatments: an increase in ultrasound frequency corresponded to a slight reduction in Chl B content, although in this case it wasn't statistically significant.

Meanwhile, results of total chlorophyll content primarily reflected the variations found for Chl B, with reductions of up to 39% observed in lettuce treated with 42 W L⁻¹ at 80 kHz. Decrease in total chlorophyll content could be linked to oxidation processes, with a direct action of reactive oxygen species (ROS) [36]. These ROS are reported to be generated during the application of power ultrasound, as free radicals formed when cavitation bubbles collapse [37]. Chlorophylls are pigments responsible for the green coloration, therefore differences in lettuce chlorophyll content could be visually manifested, especially in terms of greenness. In our work, no significant differences were observed in color measurement (color differences and browning index) for lettuce stems and leaves in US-treated samples. Consequently, the reduction in chlorophyll observed in treated lettuce did not have a significant impact on stem and leaf color. Other studies found in the literature show no significant differences in chlorophyll content when comparing fresh-cut lettuce treated with

Fig. 3 Chlorophyll A (Chl A), Chlorophyll (Chl B) and total chlorophyll (ChIT) content for untreated (Raw lettuce) and for the five treatments assayed: disinfected lettuce with peracetic acid alone (PAA), combined PAA—US at 25 W L⁻¹ and 25 kHz (P25 25), combined PAA—US at 25 W L⁻¹ and 45 kHz (P25_45), combined PAA—US at 42 W L⁻¹ and 37 kHz (P42_37) and combined PAA—US at 42 W L⁻¹ and 80 kHz (P42_80). Values are expressed as average ± standard deviation (n=3). Same letters are not significantly different (test de Tukey, p > 0.05)





 ϵ -polylysine (PL), US and combined PL and US [10]. Meanwhile, Azam et al. [1] reported an initial chlorophyll content of 7 mg g⁻¹ in raw Romaine lettuce (considering 95% of water), with an approximately 5% reduction after applying various US treatments for 8 min [1]. In our study, untreated (raw) lettuce exhibited an average total chlorophyll content of 5.12 ± 0.10 mg g⁻¹, with Chl B being more affected by disinfection treatments than Chl A. Our results are consistent with reported research, emphasizing the influence of different treatments on chlorophyll levels. We suggest that the observed reduction might not be significant enough to appreciably affect the visual appearance of lettuce.

3.7 Carotenoid profile

Green leafy vegetables are widely recognized for being an important source of carotenoids, renowned for both their antioxidant capacity and provitamin activity [38, 39]. The carotenoid profile in lettuce samples was assessed by HPLC analysis (Fig. 4).

With the standard compounds available, only lutein (Peak 1) was identified, with no matches found for zeaxanthin or beta-carotene. As can be observed in Fig. 4, lutein emerges as the most predominant carotenoid in the analysed lettuce



Fig. 4 Chromatogram example of lettuce sample at 474 nm with lutein peak identified at 4.08 min for lettuce sample and standard



(cv. Vera). Our results are in accordance with those of Caldwell & Britz [38], who also reported no zeaxanthin in the carotenoid profiles of eight lettuce varieties, highlighting violaxanthin and lutein as the major components.

At 474 nm, several "carotenoid-like compounds" were observed. Through HPLC MS–MS, four peaks subsequent to lutein were identified and recognized as having a lutein-like formula. Table 4 presents the lutein content determined by quantifying Peak 1 and the content of lutein-like compounds quantified by establishing a relationship between their peak area and the lutein peak area.

Significant increases in both lutein content and lutein-like compounds were observed after PAA disinfection and some combined disinfection treatments compared to untreated lettuce (Raw lettuce). For PAA disinfection and combined disinfection treatments at 45 and 80 kHz frequencies, lutein content was notably enhanced, representing a more than 200% increase compared to untreated lettuce. On the other hand, lettuce treated at 25 kHz and 37 kHz exhibited similar lutein content to untreated lettuce. In both applied power densities (APD) assayed, an increase in frequency resulted in a rise in lutein content. However, increasing APD from 25 to 42 W L⁻¹ led to a reduction in average lutein content.

It is important to emphasize that lutein consumption plays a vital role in protecting vision. Lutein serves as a preventive agent against cataract formation and macular degradation, highlighting the importance of controlling its degradation during food processing [40]. The results obtained indicated that treated lettuce exhibited higher contents compared to untreated lettuce, demonstrating the beneficial effects of processing fresh-cut lettuce. Nevertheless, it is essential to study specific processing conditions to avoid potential detrimental effects.

3.8 Total phenolic content (TPC) and total antioxidant capacity (TAC)

Results for TPC and TAC following the disinfection treatments are shown in Fig. 5. In terms of TPC, untreated lettuce exhibited an average of 5.57 ± 0.35 mg gallic acid per gram dry sample. This value is in accordance with previous reports, where total phenols ranged between 3.64 and 51.8 mg gallic acid per g dry sample for different lettuce varieties (assuming 95% of water content) [41]. Concerning TPC in treated lettuce, a significant increase in phenolic content was observed, except for the 25 kHz treatment. PAA treatment showed the greatest TPC increase (100%), while US-treated lettuce demonstrated a 28% and 71% average increase with 25 W L⁻¹ and 42 W L⁻¹ APD respectively. Yu et al. [42] reported similar results when treating whole lettuce leaves with ultrasound (25 kHz, 26 W L⁻¹) for 1–3 min, achieving a significant enhancement of between 26 and 35% in phenolic content compared to untreated lettuce [42]. Consistent findings were reported by Lafarga et al. [34], who obtained higher TPC values for various vegetables, including lettuce. They reported a 55% higher TPC in lettuce treated with US (40 kHz, 250 W, 20 min) [34].

In the case of TAC, untreated lettuce showed an average value of $81.3 \pm 1.7 \mu$ mol Trolox equivalent per gram dry sample. Treated lettuce presented an increase of approximately 50% in TAC compared to untreated lettuce, except for the treatment with frequencies of 25 kHz, where no significant difference was observed. Lafarga et al. (2019) reported no significant differences in TAC between treated lettuce (40 kHz, 250 W, 20 min) and untreated lettuce employing FRAP technique. However, when the TAC assay was conducted using DPPH technique, treated lettuce revealed higher values [34].

From the results obtained in the present work, it is evident that both TPC and TAC experienced a significant increase after combined PAA—US treatments. As previously mentioned, lutein is the main carotenoid in lettuce, and carotenoids are known for their antioxidant properties. The results demonstrated an increase in lutein after treatments assayed, which could contribute to the observed increments in TAC for treated lettuce. This observation may

Table 4Lutein and lutein-likecompound content ($\mu g g^{-1}$)for the different disinfectiontreatments

Treatment	Lutein (µg g ⁻¹)	Lutein-like compounds (µg g ⁻¹)
p-value	< 0.001	< 0.001
Raw lettuce	$45.4 \pm 3.8^{\circ}$	55.8 ± 1.2^{c}
PAA	110.9 ± 10.2^{ab}	66.7 ± 0.3^{a}
P25_25	51.5±3.2 ^c	58.3 ± 0.5^{bc}
P25_45	129.2±17.1 ^a	70.0 ± 4.9^{a}
P42_37	39.5±5.2 ^c	64.3 ± 1.8^{ab}
P42_80	89.7 ± 7.2^{b}	65.6 ± 1.3^{a}

Values are expressed as average \pm standard deviation (n = 3). For each column, same letters are not significantly different (Tukey test, p > 0.05)



Fig. 5 Total phenolic content (left) and total antioxidant capacity (right) for untreated (Raw lettuce) and for the five treatments assayed: disinfected lettuce with peracetic acid alone (PAA), combined PAA—US at 25 W L⁻¹ and 25 kHz (P25_25), combined PAA—US at 25 W L⁻¹ and 45 kHz (P25_45), combined PAA—US at 42 W L⁻¹ and 37 kHz (P42_37) and combined PAA—US at 42 W L⁻¹ and 80 kHz (P42_80). Values are expressed as average ± standard deviation (n = 3). Same letters are not significantly different (test de Tukey, p > 0.05)

be attributed to stress induced by the exposure of lettuce to US and PAA treatments. Several reports have indicated that different chemical and/or physical treatments generate abiotic stress, activating secondary pathways such as the biosynthesis of phenolic compounds and other metabolites with antioxidant properties [12, 34, 42, 43].

Results from our work highlight the relationship between stress responses and the improvement of antioxidant compounds in lettuce, offering valuable insights into the potential benefits of the applied disinfection treatments.

4 Conclusion

The present study provides new insights into the impact of power US on the quality of fresh-cut lettuce during disinfection process. Combined PAA—US treatments demonstrated enhanced microbial inactivation, particularly for selected microorganism groups (TMC, MC and YC). However, no discernible trend was observed with increasing APD or frequency. Interestingly, there were no significant differences noted in PAA consumption or microbial loads in residual wash water between PAA sole disinfection and combined PAA—US treatments. Microbial loads obtained in the wash water underscore the importance of treating wash water after disinfection steps to ensure safety.

In terms of product quality, instrumental color measurement remained unaffected by combined PAA—US treatments. However, these treatments positively influenced the levels of bioactive compounds, leading to an increase in lutein content, TPC and TAC, potentially attributed to induced abiotic stress. Particularly, an increase in APD resulted in a decrease in total lutein and an increase in TPC. Meanwhile, the degradation of chlorophylls was evident in UStreated samples.

The obtained results suggest that power US can be effectively applied for the disinfection of fresh-cut lettuce, with the added benefit of inducing positive changes in relevant bioactive compounds while showing no appreciable effects on visual quality. Further experiments are needed to optimise the application of combined PAA—US treatments to achieve a more significant enhancement in microbial inactivation compared to PAA disinfection alone.

Author contributions All authors design the experimental work. MI, SB and EB wrote the main manuscript text and prepared figures associated with results. MI, MC and EF carried out the experimental work, collaborating in the acquisition, analysis, process and interpretation of data. NP and PL participated in discussion of results and revised critically the manuscript. All authors reviewed the manuscript.



Funding This work was supported by Espacio Interdisciplinario (El, Universidad de la República).

Data availability The authors declare that the data supporting the findings of this study are available within the paper and if there are any raw data files needed in another format, they are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate We confirm that the collection of plant materials for this study was conducted in accordance with local and national guidelines.

Competing interests The authors declare they have no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

- 1. Azam SMR, Ma H, Xu B, Devi S, Stanley SL, Siddique MAB, et al. Multi-frequency multi-mode ultrasound treatment for removing pesticides from lettuce (Lactuca sativa L.) and effects on product quality. Lwt. 2021;143:111147. https://doi.org/10.1016/j.lwt.2021.111147.
- 2. Unal Turhan E, Polat S, Erginkaya Z, Konuray G. Investigation of synergistic antibacterial effect of organic acids and ultrasound against pathogen biofilms on lettuce. Food Biosci. 2022;47:101643.
- 3. Meireles A, Giaouris E, Simões M. Alternative disinfection methods to chlorine for use in the fresh-cut industry. Food Res Int. 2016;82:71–85. https://doi.org/10.1016/j.foodres.2016.01.021.
- 4. Oliveira Silveira L, do Alves Rosário DK, Garcia Giori AC, Silva Oliveira SB, da Mutz Silva Y, Suprani Marques C, et al. Combination of peracetic acid and ultrasound reduces Salmonella Typhimurium on fresh lettuce (Lactuca sativa L. var. crispa). J Food Sci Technol. 2018;55:1535–40.
- 5. Wang J, Zhang Y, Yu Y, Wu Z, Wang H. Combination of ozone and ultrasonic-assisted aerosolization sanitizer as a sanitizing process to disinfect fresh-cut lettuce. Ultrason Sonochem. 2021;76:105622. https://doi.org/10.1016/j.ultsonch.2021.105622.
- 6. Marçal S, Campos DA, Pintado M. Washing with sodium hypochlorite or peracetic acid: its impact on microbiological quality, phytochemical composition and antioxidant activity of mango peels. Food Control. 2022;139:109080.
- 7. Rosario DKA, Bernardo YAA, Mutz YS, Tiwari B, Rajkovic A, Bernardes PC, et al. Modelling inactivation of Staphylococcus spp. on sliced Brazilian dry-cured loin with thermosonication and peracetic acid combined treatment. Int J Food Microbiol. 2019;309:108328.
- 8. Pahariya P, Fisher DJ, Choudhary R. Comparative analyses of sanitizing solutions on microbial reduction and quality of leafy greens. Lwt. 2022;154:112696. https://doi.org/10.1016/j.lwt.2021.112696.
- 9. Fan K, Wu J, Chen L. Ultrasound and its combined application in the improvement of microbial and physicochemical quality of fruits and vegetables: a review. Ultrason Sonochem. 2021;80:105838. https://doi.org/10.1016/j.ultsonch.2021.105838.
- 10. Fan K, Zhang M, Bhandari B, Jiang F. A combination treatment of ultrasound and ε-polylysine to improve microorganisms and storage quality of fresh-cut lettuce. Lwt. 2019;113:108315. https://doi.org/10.1016/j.lwt.2019.108315.
- 11. do Rosário DKA, da Silva Mutz Y, Peixoto JMC, Oliveira SBS, de Carvalho RV, Carneiro JCS, et al. Ultrasound improves chemical reduction of natural contaminant microbiota and Salmonella enterica subsp. enterica on strawberries. Int J Food Microbiol. 2017;241:23–9. https://doi.org/10.1016/j.ijfoodmicro.2016.10.009.
- 12. Lu C, Ding J, Park HK, Feng H. High intensity ultrasound as a physical elicitor affects secondary metabolites and antioxidant capacity of tomato fruits. Food Control. 2020;113:107176. https://doi.org/10.1016/j.foodcont.2020.107176.
- 13. Artés F, Gómez P, Aguayo E, Escalona V, Artés-Hernández F. Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. Postharvest Biol Technol. 2009;51:287–96.
- 14. Van de Velde F, Güemes DR, Pirovani ME. Optimisation of the peracetic acid washing disinfection of fresh-cut strawberries based on microbial load reduction and bioactive compounds retention. Int J Food Sci Technol. 2014;49:634–40.
- 15. Irazoqui M, Romero M, Paulsen E, Barrios S, Pérez N, Faccio R, et al. Effect of power ultrasound on quality of fresh-cut lettuce (cv. Vera) packaged in passive modified atmosphere. Food Bioprod Process. 2019;117:138–48. https://doi.org/10.1016/j.fbp.2019.07.004.
- 16. Anese M, Maifreni M, Bot F, Bartolomeoli I, Nicoli MC. Power ultrasound decontamination of wastewater from fresh-cut lettuce washing for potential water recycling. Innov Food Sci Emerg Technol. 2015;32:121–6.
- 17. Gao S, Lewis GD, Ashokkumar M, Hemar Y. Inactivation of microorganisms by low-frequency high-power ultrasound: 1. Effect of growth phase and capsule properties of the bacteria. Ultrason Sonochem. 2014;21:446–53. https://doi.org/10.1016/j.ultsonch.2013.06.006.
- 18. Zhou B, Feng H, Pearlstein AJ. Continuous-flow ultrasonic washing system for fresh produce surface decontamination. Innov Food Sci Emerg Technol. 2012;16:427–35. https://doi.org/10.1016/j.ifset.2012.09.007.
- 19. 3M Petrifilm FS. Aerobic Count Plate. Product Instructions. US. 2017. www.3M.com/foodsafety.
- 20. 3M Petrifilm FS. Rapid Yeast and Mold Count Plate. 3M. 2018. www.3M.com/foodsafety. Accessed on 16 Oct 2023
- 21. 3M Petrifilm FS. Enterobacteriaceae Count Plate. 3M. 2018. www.3M.com/foodsafety. Accessed on 16 Oct 2023
- 22. Salgado SP, Pearlstein AJ, Luo Y, Feng H. Quality of Iceberg (Lactuca sativa L.) and Romaine (L. sativa L. var. longifolial) lettuce treated by combinations of sanitizer, surfactant, and ultrasound. LWT Food Sci Technol. 2014;56:261–8. Accessed on 16 Oct 2023



- 23. Hasperué JH, Guardianelli L, Rodoni LM, Chaves AR, Martínez GA. Continuous white-blue LED light exposition delays postharvest senescence of broccoli. LWT - Food Sci Technol. 2016;65:495–502.
- 24. Kotíková Z, Lachman J, Hejtmánková A, Hejtmánková K. Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. Lwt. 2011;44:1703–10.
- 25. Lichtenthaler HK, Wellburn AR. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc Trans. 1983;11:591–2.
- 26. Elizaquível P, Sánchez G, Selma MV, Aznar R. Application of propidium monoazide-qPCR to evaluate the ultrasonic inactivation of Escherichia coli O157:H7 in fresh-cut vegetable wash water. Food Microbiol. 2012;30:316–20. https://doi.org/10.1016/j.fm.2011.10.008.
- 27. Ssemanda JN, Joosten H, Bagabe MC, Zwietering MH, Reij MW. Reduction of microbial counts during kitchen scale washing and sanitization of salad vegetables. Food Control. 2018;85:495–503. https://doi.org/10.1016/j.foodcont.2017.10.004.
- 28. Huang K, Wrenn S, Tikekar R, Nitin N. Efficacy of decontamination and a reduced risk of cross-contamination during ultrasound-assisted washing of fresh produce. J Food Eng. 2018;224:95–104.
- 29. Gil MI, Selma MV, López-Gálvez F, Allende A. Fresh-cut product sanitation and wash water disinfection: Problems and solutions. Int J Food Microbiol. 2009;134:37–45. https://doi.org/10.1016/j.ijfoodmicro.2009.05.021.
- Banach JL, de Veen HVB, van Overbeek LS, van der Zouwen PS, Zwietering MH, van der Fels-Klerx HJ. Effectiveness of a peracetic acid solution on Escherichia coli reduction during fresh-cut lettuce processing at the laboratory and industrial scales. Int J Food Microbiol. 2020;321:108537. https://doi.org/10.1016/j.ijfoodmicro.2020.108537.
- 31. Pang H, Lambertini E, Buchanan RL, Schaffner DW, Pradhan AK. Quantitative microbial risk assessment for Escherichia coli O157:H7 in fresh-cut lettuce. J Food Prot. 2017;80:302–11.
- 32. Ma L, Zhang M, Bhandari B, Gao Z. Recent developments in novel shelf life extension technologies of fresh-cut fruits and vegetables. Trends Food Sci Technol. 2017;64:23–38. https://doi.org/10.1016/j.tifs.2017.03.005.
- 33. Critzer FJ, Doyle MP. Microbial ecology of foodborne pathogens associated with produce. Curr Opin Biotechnol. 2010;21:125–30. https://doi.org/10.1016/j.copbio.2010.01.006.
- Lafarga T, Rodríguez-Roque MJ, Bobo G, Villaró S, Aguiló-Aguayo I. Effect of ultrasound processing on the bioaccessibility of phenolic compounds and antioxidant capacity of selected vegetables. Food Sci Biotechnol. 2019;28:1713–21.
- 35. Palma-Salgado S, Pearlstein AJ, Luo Y, Park HK, Feng H. Whole-head washing, prior to cutting, provides sanitization advantages for freshcut Iceberg lettuce (Latuca sativa L.). Int J Food Microbiol. 2014;179:18–23.
- Guo Z, Liu H, Chen X, Huang L, Fan J, Zhou J, et al. Modified-atmosphere packaging maintains the quality of postharvest whole lettuce (Lactuca sativa L. Grand Rapid) by mediating the dynamic equilibrium of the electron transport chain and protecting mitochondrial structure and function. Postharvest Biol Technol. 2019;147:206–13. https://doi.org/10.1016/j.postharvbio.2018.09.001.
- 37. Jiang Q, Zhang M, Xu B. Application of ultrasonic technology in postharvested fruits and vegetables storage: a review. Ultrason Sonochem. 2020;69:105261. https://doi.org/10.1016/j.ultsonch.2020.105261.
- 38. Caldwell CR, Britz SJ. Effect of supplemental ultraviolet radiation on the carotenoid and chlorophyll composition of green house-grown leaf lettuce (Lactuca sativa L.) cultivars. J Food Compos Anal. 2006;19:637–44.
- 39. Cruz R, Baptista P, Cunha S, Pereira JA, Casal S. Carotenoids of lettuce (Lactuca sativa L.) grown on soil enriched with spent coffee grounds. Molecules. 2012;17:1535–47.
- Sledz M, Wiktor A, Rybak K, Nowacka M, Witrowa-Rajchert D. The impact of ultrasound and steam blanching pre-treatments on the drying kinetics, energy consumption and selected properties of parsley leaves. Appl Acoust. 2016;103:148–56. https://doi.org/10.1016/j.apaco ust.2015.05.006.
- 41. Llorach R, Martínez-Sánchez A, Tomás-Barberán FA, Gil MI, Ferreres F. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem. 2008;108:1028–38.
- 42. Yu J, Engeseth NJ, Feng H. High intensity ultrasound as an abiotic elicitor—effects on antioxidant capacity and overall quality of romaine lettuce. Food Bioprocess Technol. 2016;9:262–73.
- 43. Agüero MV, Jagus RJ, Martín-Belloso O, Soliva-Fortuny R. Surface decontamination of spinach by intense pulsed light treatments: impact on quality attributes. Postharvest Biol Technol. 2016;121:118–25. https://doi.org/10.1016/j.postharvbio.2016.07.018.
- 44. Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology, 1999;(14):152–178. https://doi.org/10.1016/s0076-6879(99)99017-1.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

