AQ:1

Effects of the Plasma-Activated Water on the Quality and Preservation of Fresh-Cut Lettuce

Juan Camilo Chamorro, Gabriela Inés Denoya, Brenda Santamaría, Brenda Fina, Matías Ferreyra, Ezequiel Cejas, Anabel Rodríguez, Sergio Ramón Vaudagna, and Leandro Prevosto

***Abstract*— The effect of the application of plasma-activated water (PAW) on the quality and preservation of fresh-cut lettuce is reported in this article. PAW was produced by using a liquid-cathode air discharge. The average (bulk) water temperature was kept at ∼22 °C during the activation procedure and stored at 4 °C for up to five days. The pH value, electrical conductivity, and concentrations of H_2O_2 and NO_3^- in liquid at day 1 were 2.81, 1492μS/cm, and 77.8 and 223.4 mg/L, respectively, with slight variations over the whole storage time. No measurable amounts of NO_2^- were found. Twenty pieces of lettuce leaves were washed for 1 and 5 min in 1 L of PAW and stored for one and five days. PAW treatments were compared to tap water treatments. The lettuce samples were stored at 4 °C and analyzed on days 1, 3, and 7. The chromatic parameter results suggest that PAW treatments significantly reduce the degradation of lettuce chlorophyll from day 3 of refrigerated storage. The lettuce firmness was not significantly modified. The microbiological results of aerobic mesophilic, enterobacteriaceae, and psychrotrophs populations have shown that lettuce treated with PAW after three days of storage exhibited the strongest inactivation efficiency. Psychrotrophs counts were maintained for up to seven days. Similar inactivation efficiencies were found regardless of the PAW storage time. PAW treatments also favored both the antioxidant capacity FRAP, ABTS, and DPPH, and the total phenolic contents of lettuce at day 7 of storage.**

***Index Terms*— Food preservation, glow discharges, lettuce, plasma-activated water (PAW), washing agents.**

AQ:2 Manuscript received 5 December 2022; revised 31 May 2023; accepted 10 July 2023. This work was supported in part by the Universidad Tecnológica Nacional under Grant PID 5447 and Grant PID 5418, in part by the Agencia Nacional de Promoción Científica y Tecnológica under Grant PICT 2018-00702, in part by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) under Grant PIP 11220200100459CO, and in part by the Agencia Santafesina de Ciencia, Tecnología e Innovación under Grant DTT-2021-028 and Proyecto INTA PDI153. The review of this article was arranged by Senior Editor S. J. Gitomer. *(Corresponding author: Juan Camilo Chamorro.)*

AQ:3 Juan Camilo Chamorro, Brenda Santamaría, Brenda Fina, Matías Ferreyra, Ezequiel Cejas, and Leandro Prevosto are with the Facultad Regional Venado Tuerto, Departamento Ing. Electromecánica, Grupo de Descargas Eléctricas, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), AQ:4 Universidad Tecnológica Nacional, S3004 Venado Tuerto, Argentina (e-mail: jcchamorro@utp.edu.co).

AQ:5 Gabriela Inés Denoya, Anabel Rodríguez, and Sergio Ramón Vaudagna are with the Instituto Nacional de Tecnología Agropecuaria (INTA), Instituto AQ:6 de Tecnología de Alimentos, Hurlingham, Argentina, and also with ICyT-SAS, INTA, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), UEDD, Buenos Aires, Argentina.

Color versions of one or more figures in this article are available at https://doi.org/10.1109/TPS.2023.3297009.

Digital Object Identifier 10.1109/TPS.2023.3297009

I. Introduction

CHLORINE toxic residues present in fresh-cut fruit and vegetables, ready-to-eat, are a topical concern for the food industry [1], [2]. However, chlorine solutions are among the conventional treatments used by the industry to eliminate bacterial populations present in this type of food due to their simple implementation and low cost. Nowadays, there is a trend to minimize the use of chlorine in the industry due to environmental damage, the potential health implications of chlorine by-products, and consumer opposition. For example, a ban on chlorine for fresh-cut produce sanitation was introduced in the food legislation in Germany and Switzerland [3] and might be extrapolated in the future in the legislation of other countries, as well. Therefore, a novel preservation technology with high efficiency, uniformity, and low residues becomes necessary.

In order to replace these chlorine-based disinfection treatments, there is an increasing interest in nonthermal food processing technologies in the last decades, such as high hydrostatic pressures [4], pulsed electric fields [5], ultrasound power, and ultraviolet (UV) irradiation [6], [7]. In recent years, nonthermal plasmas have attracted a lot of attention in the food and agricultural industries, mainly for their applications in food sterilization and preservation [8]. Plasmas are partially ionized (quasi-neutral) gases, composed of molecules, atoms, UV photons, highly energetic electrons, charged particles, and reactive species, such as reactive nitrogen and oxygen reactive species (RONS) in air or similar gas mixtures. The nonthermal state is characterized by the presence of high-energy electrons (∼1–5 eV), while the gas in which the discharge occurs remains close to room temperature [9]. However, the highly irregular surface topography of food products offers numerous hidden places for microorganisms, thus increasing their resistance against direct plasma treatment [10]. To solve this problem, indirect plasma treatment through plasma-activated water (PAW) has been developed. During PAW generation, the RONS generated in the gas-phase plasma is transferred into the liquid by diffusion based on their solubility and induces the formation of secondary reactive species in the liquid, such as OH^-, H_2O_2, NO_2^-, and NO_3^- [11], [12].

The combined action of RONS and low pH contributes to the bactericidal activity of PAW [13]. Different types of solutions can also be activated with plasma to favor the generation of reactive species with bactericidal properties [14]. PAW has numerous advantages over traditional chemical sanitizers,

including being an environmental-friendly and cost-effective disinfectant that eliminates the need to store potentially hazardous chemicals [13]. Besides, PAW provides a series of advantages over direct treatment with plasma, e.g., dose control, ease of implementation, storage capacity, onsite/offsite generation, and sustainable production [15].

A relatively few number of papers have been devoted to investigate the pathogenic control on lettuce treated with PAW [16], [17], [18], [19], [20], [21]. However, to the best of our knowledge, the effect of the PAW treatments on the phenolic contents and the antioxidant capacity in fresh-cut lettuce has not been reported in the literature.

In this study, the effect of the application of PAW as a washing agent on the quality and preservation of fresh-cut lettuce was assessed. PAW was produced by using a liquid-cathode glow-type discharge in atmospheric pressure air. Both the physicochemical properties of PAW and its storage stability were measured. The antimicrobial activity of PAW against the natural microbiota of lettuce was evaluated. The physicochemical parameters were also evaluated based on the chromatic parameters and firmness of the lettuce (treated and controlled). Furthermore, the total phenolic content and antioxidant capacity were measured.

II. Materials and Methods

A. Plasma Device and PAW Generation

A millisecond pulsed-dc glow discharge in atmospheric pressure air, operating at a constant rms value of 100 mA, was used to generate PAW. The electrical circuit together with the power supply used to generate this discharge is similar to that used in [22] and [23]. The discharge was directed into the water vortex propelled by a magnetic stirrer bar ~720 rpm in order to optimize plasma–water interaction, thus enhancing the diffusion of RONS from the discharge toward the water [24]. The water acted as the cathode of the discharge. The liquid cathode was contained in a grounded stainless-steel reservoir. A cone-shaped thoriated tungsten (2 wt.%) electrode placed above the liquid reservoir was used as the anode; 1 L of distilled water (pH $\approx$ 5.2 and electrical conductivity <5 μS/cm) was exposed to the plasma discharge during an activation time of 60 min. The discharge length achieved on this condition was ~10 mm. The average (bulk) water temperature was kept constant at ~22 °C during the activation procedure by using a cooling system due to the thermally fragile chemistry of H_2O_2 [24]. However, boiling (as well as ion sputtering) phenomena induced by plasma are expected at the gas–liquid interface when the liquid electrode is the cathode because a large fraction of the discharge power is dissipated there [25]. Under the conditions considered, the (measured) water evaporation rate was low, ~1–2 mg/s. The photograph and schematic of glow discharge with a liquid cathode of distilled water are shown in Fig. 1(a) and (b), respectively.

Typical signals of current (I) and voltage (V) of the discharge are shown in Fig. 2. The signals corresponded to half of the activation time (30 min). The discharge current waveform oscillates with a frequency of 100 Hz, almost independent of the arc voltage evolution, because the discharge current is controlled by the high impedance (65 $\pm$ 2 kΩ) of the transformer. The voltage signal has also a frequency of 100 Hz, with large spikes at the beginning of each cycle (due to the quenching and reignition of the discharge). The discharge is probably ignited by a streamer-to-spark high-voltage transition, but, immediately after the breakdown, the voltage drops due to the transformer impedance, and a stable discharge was sustained. Besides, the voltage decreases when the current increases, thus leading to a negative slope in the voltage–current characteristic of the discharge. All these features suggest that this discharge regime may be considered to be a high-pressure glow-type discharge [22], [26].

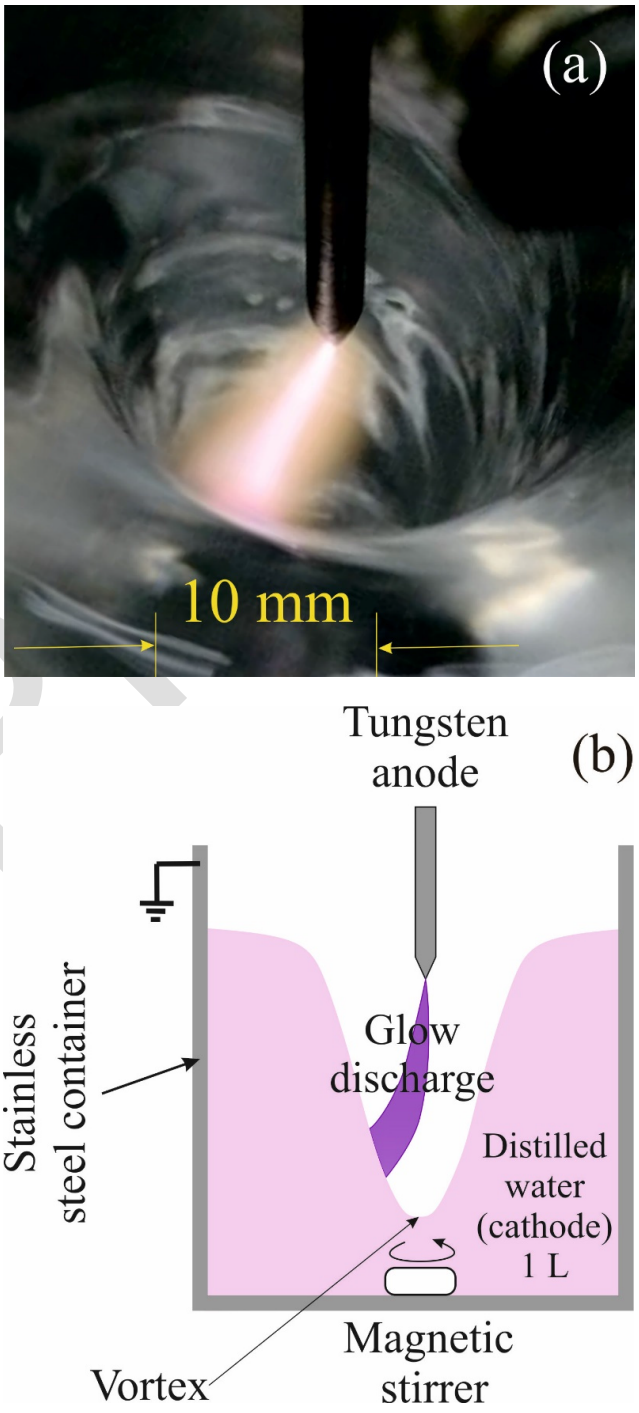

Fig. 1. (a) Photograph and (b) schematic of a millisecond pulsed-dc glow discharge in atmospheric pressure air with liquid cathode used for PAW generation.

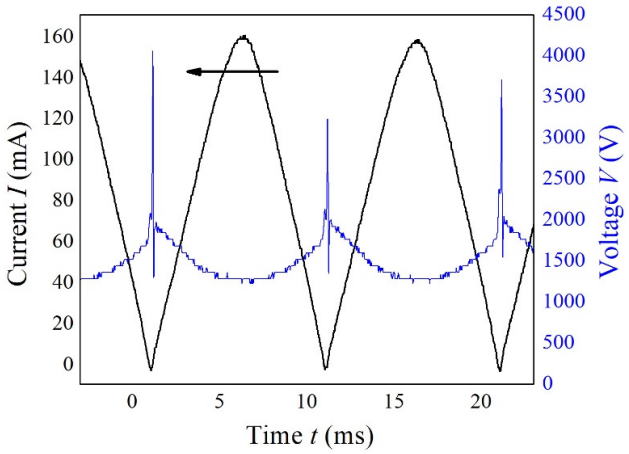

Fig. 2. Typical current and voltage waveforms of the discharge with the liquid cathode, captured for an activation time of 30 min.

The measured voltage in Fig. 2 includes not only the drop in the gas gap but also the drop in the equivalent resistance of the water electrode. At 0 min, the V–I characteristic curve has a positive slope, reaching a maximum voltage value of about 2 kV (data not shown), while, at 30 min (or longer times), the slope becomes negative, and the voltage drops to about 1.2 kV. This is expected because, as the exposure time increases, the conductivity of the water increases, and therefore, the resistive voltage drop in the water becomes small compared to that of the gas. Accordingly, the discharge operating power decreases as the water conductivity increases. Note that the found voltage drop value at 30 min (about 1.2 kV) is also consistent with the measured cathode voltage drop in distilled water (600–900 V) reported in the literature for a similar discharge [27]. The discharge operating power was calculated as

$$P = \frac{1}{\tau}\int I(t)V(t)dt \tag{1}$$

where τ is the period of the discharge current. The resulting power decreased from ∼160 W at $t = 0$ min to ∼100 W at 60 min. The energy per liter of water expended during the activation process was then calculated to be $\varepsilon \sim 416$ kJ/L.

The dc-excited discharges in a pin-to-water electrode geometry operating in air at rms current values of 100 mA and a power of 100 W typically exhibit gas temperatures exceeding 3000 K, electron temperatures of about 1 eV, and electron densities of the order of 10^{19} m^{-3} [25].

B. Storage Stability and Physicochemical Proprieties of PAW

1) Storage Stability of PAW: PAW stability was evaluated by measuring the temporal evolution of its physicochemical proprieties (concentration of reactive species, conductivity, and pH) over five days of refrigerated storage (at 4 °C ± 1 °C). In the experiments, PAW with one and five days of storage was used, defined as PAW1d and PAW5d, respectively.

2) pH and Electrical Conductivity: The levels of pH and electrical conductivity were determined using a HI 8314 pH meter (Hanna) with a range of 0–14 and a resolution of 0.01, and a CYBERSCAN COND 610 conductivity meter (Oakton Instruments, Vernon Hills, IL, USA) with a measurement range of 0–500 mS/cm with an accuracy of 1%. Both instruments were calibrated prior to the determinations using standard solutions (buffer pH: 7, buffer pH: 4, and KI 0.01-M solution with $\sigma = 1413$ μS/cm at 25 °C).

3) Hydrogen Peroxide Measurement: A method using peroxidase was used [28]. The method is based on the reaction of H_2O_2 with a mixture of 4-aminophenazone and phenol to give as a product a red quinoneimine (4-(p-benzoquinonamonoimino)-phenazone) that exhibits an absorption maximum at 505 nm.

4) Nitrate Measurement: The UV method was used [28]. Hydrochloric acid was added in the ratio of water: HCl = 50:1, and the absorbance at 220 nm (A_{220}) and 275 nm (A_{275}) was measured. These values were used to obtain the corrected absorbance ($A = A_{220} - 2\,A_{275}$).

C. Processing and PAW Treatments of Fresh-Cut Lettuce

Lettuce (*Lactuca sativa var. capitata*) was purchased at a local market (Buenos Aires, Argentina) and kept in a refrigerator at 4 °C until use. On the day of treatments, lettuces were rinsed gently with tap water by hand and air-dried. Afterward, the stems of the lettuce were removed, and the remaining leaves were cut into rectangles (7 × 8 cm) with a sharp stainless-steel knife. We assessed and compared three different lettuce treatments: 1) with one-day-stored PAW at 4 °C (PAW1d); 2) with five-day-stored PAW at 4 °C (PAW5d); and 3) tap water treatments, used as Control (C). The PAW was reutilized in the treatments. Physicochemical determinations on both PAW1d and PAW5d right after treatments showed variations <5% in species concentration, conductivity, and pH level.

Different lettuce samples were immersed in each PAW type (PAW1d and PAW5d) by using two soaking times (1 and 5 min). Each of these experiments was performed in triplicate. Fig. 3 shows the schematic of the experimental arrangement, including PAW generation and PAW treatment of fresh-cut lettuce. Twelve lettuce samples (∼40 g) were immersed in 1 L of PAW inside a beaker, mounted on a magnetic stirrer at ∼240 rpm. The mass-ratio lettuce/PAW was set at 1:25.

After treatments, samples were centrifuged in a home salad spinner for 5 min. Subsequently, treated samples were placed on sterile polypropylene trays, packed with a food-grade film of 9 μm thickness (70% polyvinyl chloride resin, permeability characteristics: $O_2 = 1536$ $cm^3 \cdot m^{-2}$ at 24 $h^{-1} \cdot atm^{-1}$; $CO_2 = 3690$ $cm^3 \cdot m^{-2}$ at 24 $h^{-1} \cdot atm^{-1}$; and water steam = 99 $g \cdot m^{-2}$ at 24 $h^{-1} \cdot atm^{-1}$) and stored at 4 °C ± 1 °C for up to seven days. The microbiological load, firmness, and chromatic parameters were evaluated at one, three, and seven days of refrigerated storage.

D. Lettuce Quality Analysis

1) Chromatic Parameters: Chromatic parameters of fresh-cut lettuce were measured with a Minolta CR-400 chromameter (Konica Minolta Sensing, Inc., Osaka, Japan) using the CIE scale $L^*C^*h^\circ$, where L^* represents the lightness, C^* represents the saturation or color intensity, and h° represents the hue or angle of color (90° = yellow and 180° = green) values. The equipment was set up for illuminant D_{65} and 2° observer angle, and calibrated using a standard white tile. The surface of six lettuce leaves was evaluated at four different positions

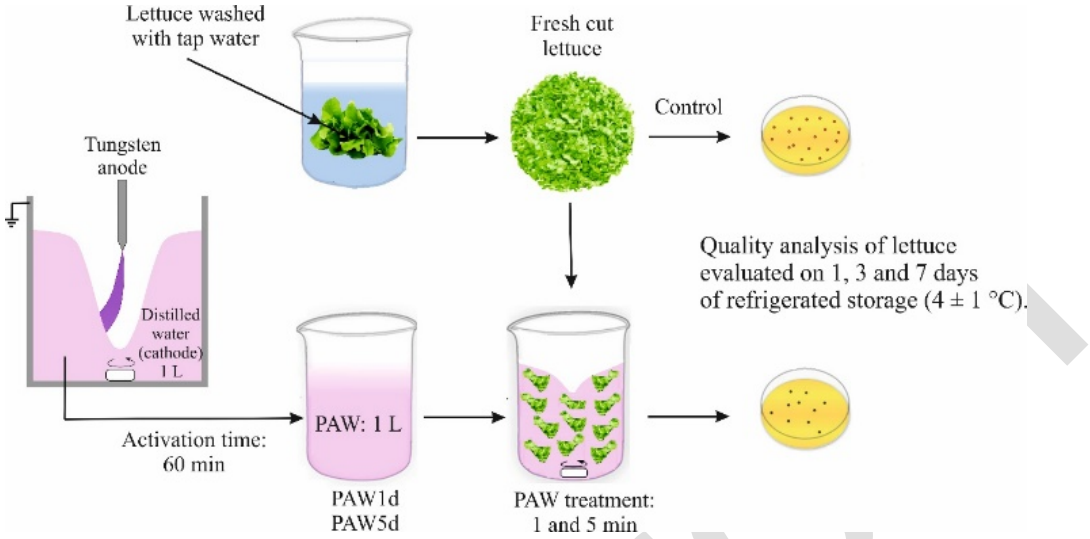

Fig. 3. Schematic of the experimental arrangement, including PAW generation and PAW treatment of fresh-cut lettuce.

from each. Chromatic parameters were evaluated in triplicate for each treatment (Control and PAW treatments: PAW1d and PAW5d) and on different days of refrigerated storage (one, three, and seven days).

2) Firmness: Lettuce leaf firmness was measured using a Texture Analyzer (TA.XT.plus Texture Analyser, Stable Micro Systems, London, U.K.) equipped with a Kramer Shear Press of ten blades with a 50-kg load cell. The velocity of the probe during the test was 0.5 mm s^{-1}. One leaf was placed into the sample holder (dimensions: 82 × 63 × 89 mm^3), and ten blades (1.5 mm thickness) were forced to cut the leaf. The maximum force (N) required to cut the leaf was recorded by the Texture Expert Software Program. Six leaves were evaluated (at room temperature) for each treatment (Control and PAW treatments: PAW1d and PAW5d) and on different days of refrigerated storage (one, three, and seven days).

3) Microbiological Analysis: All samples were serially diluted with a sterile 0.1% w/v peptone solution, and 1.0 mL of each dilution was plated into duplicate plates of appropriate agar. A plate count agar (PCA) medium (Merck, Germany) was used to determine the total aerobic mesophilic (RAM) and psychotropic counts after incubation at 37 °C for 48 h and 5 °C for 11 days, respectively. A red bile dextrose agar (VRBD) medium (Merck) was used for counting enterobacteriaceae after incubation at 37 °C for 24 h. The inactivation ability of PAW was determined from the log reduction (CFU/g), calculated from the following formula:

$$\log_{10}\text{reduction} = \log\left(\frac{N}{N_0}\right) \tag{2}$$

where N_0 is the number of microorganisms present in lettuce without any treatment, CFU/g, and N is the number of microorganisms in the treatment groups, CFU/g.

4) Total Phenolic Content and Antioxidant Capacity of Lettuce: The extraction for total phenol content and antioxidant capacity determinations were done according to [29] with slight modifications. Briefly, 2 g of the homogenized samples were mixed with 10 mL of aqueous methanol (90%, v/v). Afterward, the samples were vortexed for 2 min and centrifuged at 10 000 × g for 10 min at 4 °C. The supernatant obtained from each sample was used to carry out the following determinations. The antioxidant capacity was determined on the extracts based on three methods: ABTS and DPPH (electron and radical scavenging assay, respectively) and ferric reducing/antioxidant power (FRAP), focused on the reducing/oxidizing ability of the extracts. The content of total phenols was expressed as milligrams of Gallic acid equivalents (GAEs) per gram of vegetal tissue. The antioxidant capacity by the FRAP method was measured spectrophotometrically at 593 nm using a solution 10:10:1 300-mmol L^{-1} acetate buffer of pH 3.6, 20-mmol L^{-1} $FeCl_3$, and 10-mmol L^{-1} 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma-Aldrich, Steinheim, Germany) in 40-mmol L^{-1} HCl. The ferric [Fe (III)] TPTZ compound formed was reduced at its form Fe (II) by the antioxidants. The antioxidant capacity by the ABTS method was carried out spectrophotometrically at 734 nm with the 2, 2′ -azino-bis [3-ethylbenzothiazoline-6 sulfonic acid] diammonium salt (ABTS) reagent (Sigma-Aldrich). The ability of the extracts to neutralize the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was measured by the method used in [29]. The antioxidant capacity was expressed in Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents (TEAC): μM eq. Trolox/g tissue on a fresh weight basis. Trolox was purchased from Sigma-Aldrich.

E. Experimental Design and Statistical Analysis

The experimental factors were triplicate PAW treatments (PAW1d, PAW5d), per soaking times (1 and 5 min), and per evaluation day of lettuce (one, three, and seven days), corresponding to 36 trays. Besides, nine trays were prepared for controls, corresponding to three trays per storage day evaluated. Lettuce samples from different trays of the three replicates for each treatment and per day of evaluation were analyzed. The following quality determinations were carried out on the samples: chromatic parameters, microbiological counts, textural parameters, antioxidant capacity, and total phenolic contents.

Statistical analyses were performed with the R 4.1.1 software, R Core Team, and R Foundation for Statistical Computing (Vienna, Austria) [30]. Data are shown as mean values ± standard error of the mean (SEM). The significance

TABLE I
PHYSICOCHEMICAL PROPERTIES OF PAW FOR ONE AND FIVE DAYS OF REFRIGERATED STORAGE TIME AT 4 °C ± 1 °C. DATA ARE SHOWN AS MEAN VALUE ± STANDARD DEVIATION

	Distilled Water	Storage Time of PAW (at 4 °C)	
		1 day (PAW1d)	5 days (PAW5d)
pH	5.2 ± 0.30	2.81 ± 0.18	2.85 ± 0.14
Conductivity [μS/cm]	5.0 ± 0.25	1492 ± 76	1573 ± 95
H_2O_2 [mg/L]	Undetected	77.8 ± 2.0	67.9 ± 1.0
NO_3^- [mg/L]	Undetected	223.4 ± 3.0	227.9 ± 3.0

level cutoff was set at 95% ($p \leq 0.05$). One-way ANOVA and LSD (least significant difference) post-hoc tests were performed to examine the significant effects of treatments (PAW and Control) over the storage time. In the results, bars bearing different uppercase letters represent these significant differences ($p \leq 0.05$). On each storage day, two-way ANOVA and LSD post-hoc tests were used to examine the significant effects of both the treatments and soaking times. Bars bearing different lowercase letters represent these significant differences ($p \leq 0.05$).

III. RESULTS AND DISCUSSION

A. PAW Physicochemical Stability During Storage

Table I shows the physicochemical properties of PAW, corresponding to 60-min activation time and stored for one and five days at 4 °C ± 1 °C.

The pH showed a gentle increase over storage time from 2.81 to 2.85 for days 1 and 5, respectively. Electrical conductivity is an important indicator to determine the level of active ions that existed in PAW. These ions have been related to the presence of RONS and other reactive chemical species derived from chemical reactions between water molecules and plasma electrons [31]. A large part of the increase in PAW conductivity can be attributed to the change in pH since the contribution of H^+ ions (with a molar concentration given by $[H^+] = 10^{-pH}$) to the total conductivity is dominant due to their high specific conductance compared to that of the other ions [32]. Moreover, the antimicrobial activity of PAW was considered to be the combined action of high concentrations of reactive species and low pH that favors the reactive species to penetrate cell walls [33], [34], [35]. On the other hand, the presence of reactive species reduces the resistance of bacteria to acidic environments [36].

The aqueous concentration of H_2O_2 showed a gentle decrease over the storage time from 77.8 to 67.9 mg/L for days 1 and 5, respectively. On the other hand, the concentration of NO_3^- and the conductivity remained almost constant (within the statistical error) over the whole storage time. It is important to note that nitrite (NO_2^-) was not detected in the PAW, under the experimental conditions evaluated. This behavior could be attributed to the instability of nitrites in aqueous solutions containing hydrogen peroxide under acidic conditions [37], [38]. However, nitrite generation may be favored by adjusting the initial pH of the water (used for generating the PAW) to an alkaline level by using phosphate buffer [39].

By making the assumption that the ratio of (aqueous) RONS formation energy to expended energy is independent of the discharge power P, the energy yield of the discharge can be defined as C_i/ε, where C_i is the concentration of aqueous RONS of species i that is reached in a water volume V_w after an activation time Δt, and ε is the expended energy per liter of water. Therefore, the number of process parameters is reduced, and different experiments could be more easily compared to each other. The average energy yield of the discharge for the synthesis of NO_3^- and H_2O_2 was calculated from the corresponding concentrations (see Table I) to be 0.5 and 0.2 mg/kJ, respectively. These values are higher than the average energy yield values (0.06 and 0.05 mg/kJ for NO_3^- and H_2O_2, respectively) reported for the 150-W VitalFluid synthesizer [24] and also than those reported in [40].

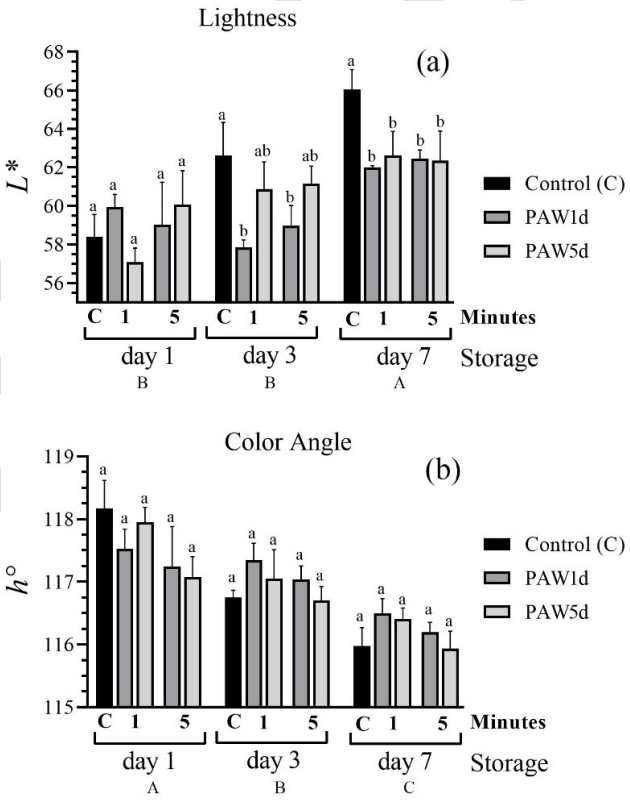

Fig. 4. Effect of PAW on (a) lightness L^* and (b) color angle $h°$ of fresh-cut lettuce during storage at 4 °C. PAW1d: one-day-stored PAW treatment; PAW5d: five-day-stored PAW; and Control (C): tap water treatment. Soaking times: 1 and 5 min. Different uppercase letters indicate significant effects ($p \leq 0.05$) of storage time on all treatments. For the same storage time (one, three, or seven days), bars bearing different lowercase letters are significantly different ($p \leq 0.05$).

B. Effects of PAW on the Lettuce Quality

1) Chromatic Parameters: Fig. 4 shows the evolution of the chromatic parameters of the lettuce treated with PAW and the

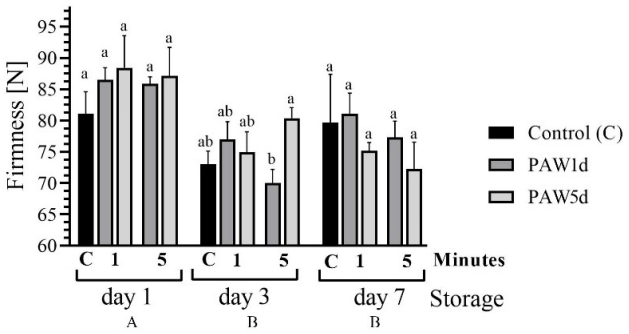

Fig. 5. Effect of PAW on firmness of fresh-cut lettuce during storage at 4 °C. PAW1d: one-day-stored PAW treatment; PAW5d: five-day-stored PAW; and Control (C): tap water treatment. Soaking times: 1 and 5 min. Different uppercase letters indicate significant effects ($p \leq 0.05$) of storage time on all treatments. For the same storage time (one, three, or seven days), bars bearing different lowercase letters are significantly different ($p \leq 0.05$).

control samples during seven days of storage at 4 °C. Lightness L^* [see Fig. 4(a)] remained without significant differences between days 1 and 3 of storage and then increased significantly for day 7. On the first day, no significant differences in L^* were observed between the control and PAW-treated samples. On day 3, the L^* values of the PAW-treated samples were lower than those corresponding to the control samples. However, significant differences were obtained only for the PAW1d-treated samples with both soaking times: 1 and 5 min. On the other hand, on day 7, the L^* values on PAW-treated samples were significantly lower than those of the control samples. As shown in Fig. 4(b), $h°$ decreased significantly over storage time. Although the statistical analysis did not show significant differences, the average h^* in PAW-treated samples was higher than the one of control samples on days 3 and 7 of storage. The color intensity C^* remained without significant differences over the whole storage time for all the treatments. Besides, no significant differences in C^* were observed between the control and PAW-treated samples on each of the storage days (these results are not shown). León et al. [41] reported a negative and positive linear correlation over the chlorophyll content of butterhead lettuce with L^* and $h°$, respectively. Considering these correlations, the behavior of L^* and $h°$ (see Fig. 4) suggests that the PAW treatment reduces the chlorophyll degradation in lettuce from day 3 of storage at 4 °C.

2) Firmness: Fig. 5 shows the firmness of PAW-treated and control fresh-cut lettuce samples during storage for seven days at 4 °C. Although the firmness decreased significantly with storage time, no significant differences between the firmness of control and PAW-treated samples were found for the evaluated storage days. This behavior suggests that the tissue structure of the lettuce remained intact after PAW treatment.

C. Effects of PAW on Microbiological Quality

Microorganisms distributed on lettuce surfaces are the main cause of postharvest deterioration, and some pathogenic bacteria are usually regarded as a serious hazard to human health [42]. Thus, the antimicrobial efficacy of PAW treatments against aerobic mesophilic (RAM), enterobacteriaceae, and psychrotrophs present in fresh-cut lettuce storage for seven days at 4 °C was evaluated (see Fig. 6). At harvest, the RAM, enterobacteriaceae, and psychrotrophs populations on

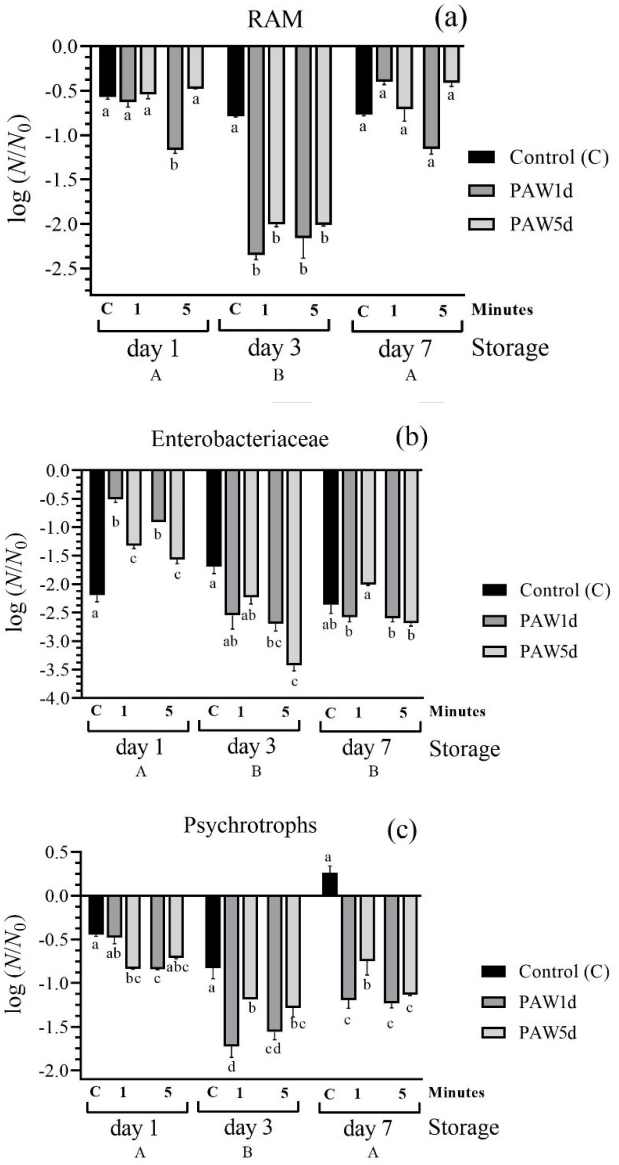

Fig. 6. Antimicrobial efficacy of PAW on fresh-cut lettuce during storage at 4 °C: (a) aerobic mesophilic, RAM, (b) enterobacteriaceae, and (c) psychrotrophs. PAW1d: one-day-stored PAW treatment; PAW5d: five-day-stored PAW; and Control (C): tap water treatment. Soaking times: 1 and 5 min. Different uppercase letters indicate significant effects ($p \leq 0.05$) of storage time on all treatments. For the same storage time (one, three, or seven days), bars bearing different lowercase letters are significantly different ($p \leq 0.05$).

lettuce without any treatments (N_0) were 8.51, 8.20, and 8.95 log CFU/g, respectively. The RAM [see Fig. 6(a)] showed significant reductions from day 1 to day 3 of storage, probably due to PAW treatments, and then increased significantly for day 7. On day 1 of storage, only the PAW1d-treated samples with a soaking time of 5 min showed a significant reduction in RAM of 0.59 log compared with the control. On day 3 of storage, all treatments with PAW achieved significant reductions in RAM compared with the control, reaching a maximal reduction of 1.57 log for PAW1d with a soaking time of 1 min. On day 7 of storage, the PAW treatments lost reduction efficiency and the RAM population increased. No significant differences were found between the control and the PAW treatments. The enterobacteriaceaes [see Fig. 6(b)] showed a significant reduction from day 1 to day 3 of

storage caused by the PAW treatments and remained without significant changes to day 7. Unexpectedly, the PAW-treated samples showed a lower reduction than the control on day 1 of storage. On day 3 of storage, all PAW-treated samples achieved higher RAM reductions than control samples. However, only significant differences were achieved with both PAW1d and PAW5d treatments and soaking times of 5 min reaching reductions of 1.0 log and 1.73 log, respectively. On day 7 of storage, the reduction level of the PAW-treated samples was maintained, but no significant differences with the control were found. The psychrotrophs [see Fig. 6(c)] showed significant reductions from day 1 to day 3 of storage, probably due to PAW treatments, and then increased significantly for day 7, but there was also an increase in the control samples on that day. On day 1 of storage, significant differences in psychrotrophs were observed between the control and PAW-treated samples but only for PAW1d and PAW5d with soaking times of 5 and 1 min, respectively. On day 3 of storage, all treatments with PAW achieved significant reductions in psychrotrophs compared with the control, reaching a maximal reduction of 0.9 log for PAW1d with a soaking time of 1 min. On day 7 of storage, all treatments with PAW achieved significant reductions in psychrotrophs compared with the control, reaching a maximal reduction of 1.45 log for PAW1d with a soaking time of 5 min. These results show that PAW treatments achieved reductions of RAM, enterobacteriaceae, and psychrotrophs populations from day 3 of storage of lettuce at 4 °C compared with the control samples. However, the PAW treatments maintained the efficiency for reducing psychrotroph populations on day 7 of storage.

Though the effectiveness of PAW as a food disinfection agent depends not only on the experimental conditions of the treatments (e.g., PAW composition, mass-ratio sample/PAW, and soaking time) but also on the type of microorganism and substrate treated, it could be valuable to compare the results obtained with those reported by other authors using PAW, as well as other conventional methods of food decontamination. For instance, Schnabel et al. [17], [43] reported that PAW treatments of fresh-cut lettuces achieved ∼2–3 log reduction in native microflora higher than those obtained with tap water and ClO_2 controls (with similar reductions to each other), when applied at the initial stage of a washing process using a soaking time of 3 min. Chen et al. [44] showed that PAW treatments on pears with a soaking time of 5 min significantly inhibited the growth of total aerobic bacteria, reaching reductions of ∼0.41 and ∼0.74 log higher than NaClO controls (solution: 200 μL L^{-1}) on four and eight days of refrigerated storage, respectively. Choi et al. [45] reported that 10-min PAW treatments of cabbage achieved reductions of mesophilic aerobic bacteria and *S. aureus* of ∼1.8 and 0.9 log higher than tap water, respectively, and up to ∼1.5 and 0.6 log higher than NaClO, respectively. On the other hand, Shirron et al. [46] provide an experimental overview of the efficacy of common sanitation methods against natural bacteria and human enteric pathogens on cucumber and parsley based on a solution of peracetic acid (PA) and hydrogen peroxide with a concentration of 0.024% PA and an exposition time of 5 min, sodium dichloroisocyanurate (NaDCC) with a concentration of 0.015% and an exposition time of 30 min, and the quaternary ammonium compound didecyldimethylammonium chloride (DDAC) with a concentration of 0.0125% and an exposition time of 3 min. Compared with washing parsley and cucumbers with water, treatments with all three sanitizers were not effective, resulting in a maximal reduction of only 0.7 log CFU of *Salmonella typhimurium*. These sanitizers were also not effective in the removal of natural bacteria from parsley (maximal reduction was 0.7 log CFU). Sanitation of cucumbers was more successful; PA showed the most effective result, with a reduction of 2.7 log in aerobic microorganisms compared with cucumbers washed with tap water. Nascimento et al. [47] reported mesophilic aerobic reduction values in lettuce up to 3.13, 1.07, 2.43, 1.85, and 2.11 log compared with tap water, using aqueous solutions of acetic acid (4%) PA (80 ppm), NaDCC (200 ppm), NaClO (200 ppm), and vinegar (50%), respectively, with a soaking time of 15 min. As a whole, the obtained reduction level's result (see Fig. 6) is comparable with those reported for PAW treatments for similar soaking times ((1)–5 min) [17], [43], [44], [45], [46] and also for conventional sanitizing treatments for longer soaking times (10–30 min) [46], [47], but without the use of chlorine compounds.

Effects of reactive species (e.g., nitrates, nitrites, and H_2O_2) in acidic environments are primarily responsible for the antimicrobial efficiency of PAW [48], [49], which is attributed to oxidative damage of the cell membrane, cell wall breakdown (intramolecular bonds of peptidoglycan), cell shrinkage and cytoplasmic leakage, and DNA breakdown, besides mutagenic and cytotoxic damage [15]. Our results showed that the PAW treatments on day 3 of storage exhibited stronger inactivation efficiency than on day 1, suggesting that bacteria under lethal doses of PAW were incapable of repairing themselves during three days of refrigerated storage. Besides, the effectiveness of PAW treatments for reducing psychrotrophs was maintained during the seven days of refrigerated storage. Ma et al. [48] reported that maximal reductions of *Staphylococcus aureus* inoculated on strawberries were achieved four days after the application of the PAW treatments. However, several papers report that bacterial maximal reductions are achieved immediately after PAW treatments [10].

Under the evaluated conditions, no clear trend between the reduction of microorganisms and the soaking time (treatment time) was found. Xu et al. [49] reported similar results on mushrooms treated with PAW. This lack of response to soaking time may be related to different aspects. PAW is a relatively nonpenetrating treatment, and the lettuce surface has uneven locations where the microorganisms could be shielded from the PAW treatment. In addition, the antioxidant constituents of lettuce may scavenge the free radicals present in the PAW, thereby reducing the bactericidal effect. This issue requires further investigation into the kinetics of degradation of the active species in PAW due to the complexity of PAW solutions in which multiple chemical components exert varied biological effects on differing time scales [50]. Besides, PAW treatments with refrigerated storage times of one and five days (PAW1d and PAW5d) showed similar microorganism reduction levels. This allows us to think that the PAW activation process could be done in a different place from the one in which the treatments should be done. The low variation level of PAW properties with the storage time (<5%; see Table I) did not significantly alter microbial reduction efficiency. PAW treatment

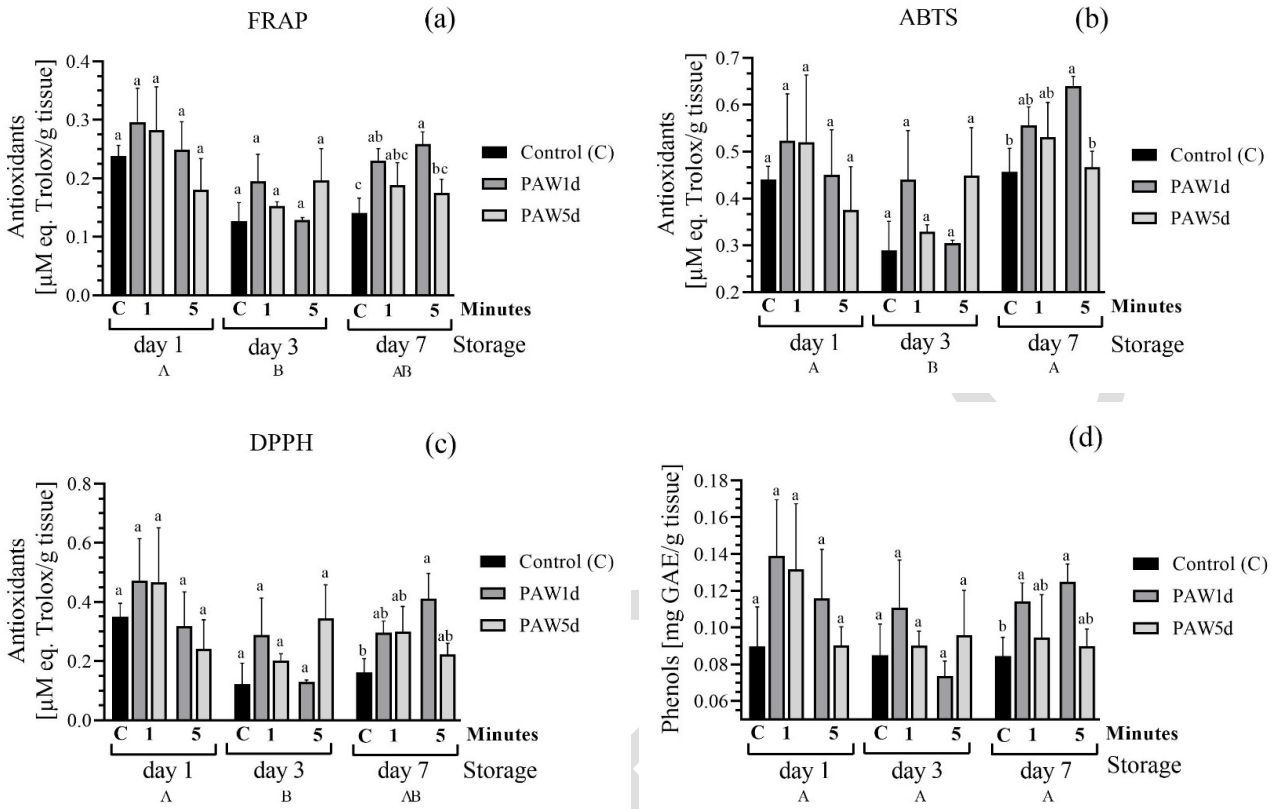

Fig. 7. Effect of PAW on antioxidant capacity and total phenolic contents of fresh-cut lettuce during storage at 4 °C: (a) FRAP, (b) ABTS, (c) DPPH methods, and (d) total phenols. PAW1d: one-day-stored PAW treatment; PAW5d: five-day-stored PAW; and Control (C): tap water treatment. Soaking times: 1 and 5 min. Different uppercase letters indicate significant effects ($p \leq 0.05$) of storage time on all treatments. For the same storage time (one, three, or seven days), bars bearing different lowercase letters are significantly different ($p \leq 0.05$).

was clearly more effective in reducing microorganisms on lettuce than tap water treatment, improving the general quality after seven days of storage at 4 °C.

D. Effect of PAW on Antioxidant Capacity and Total Phenolic Contents

Fig. 7 shows antioxidant capacity and total phenolic contents for PAW-treated lettuce and control samples during seven days of storage at 4 °C. Antioxidant capacity by the FRAP method [see Fig. 7(a)] showed significant reductions from day 1 to day 3 of storage and then increased gently for day 7. Although no significant differences between the FRAP scavenging capacity of control and PAW-treated samples were found for the days 1 and 3 of refrigerated storage of lettuce, the samples treated with PAW1d achieved values significantly higher than the control on day 7: 64% and 84% for soaking times of 1 and 5 min, respectively.

Antioxidant capacity by ABTS [see Fig. 7(b)] and DPPH [see Fig. 7(c)] showed similar behaviors over the storage time, reaching significant reductions from day 1 to day 3 and then increasing for day 7 (only significantly for ABTS). Although no significant differences between control and PAW-treated samples were found for days 1 and 3 of refrigerated storage of lettuce, PAW1d-treated samples with a soaking time of 5 min achieved significantly higher antioxidant capacity than control on day 7: 40% and 155% for ABTS and DPPH methods, respectively. This antioxidant capacity increment may be associated with a natural defense mechanism of lettuce against oxidative stress induced by the PAW. This behavior pattern has been observed in treatments with PAW of pears [44], mushrooms [49], and Chinese bayberries [51]; however, the mechanism to explain the effect of PAW on antioxidant capacity is only speculative.

Phenols [see Fig. 7(d)] remained without significant differences over storage time. Although no significant differences between the phenols of control and PAW-treated samples were found for days 1 and 3 of refrigerated storage of lettuce, the samples treated with PAW1d achieved values significantly higher than the control on day 7: 36% and 48% for soaking times of 1 and 5 min, respectively.

Siddiq et al. [52] and Mongi et al. [53] reported a significant positive correlation between the total phenolic contents and the antioxidant capacity of fresh-cut products. Thus, the phenols increment of lettuce treated with PAW may be related to the antioxidant capacity by FRAP, ABTS, and DPPH methods on day 7 of storage. Besides, plants can activate polyphenol synthesis in response to stress, such as injury, pathogen attack, or low nutrients [54]. Therefore, the increment of RAM, enterobacteriaceae, and psychrotrophs of PAW-treated samples on day 7 of storage (see Fig. 6) could favor the phenols generation on this storage day, as well.

IV. Conclusion

This article suggests that PAW produced by using a liquid-cathode glow-type discharge in atmospheric pressure air has the potential to control microbial contamination and

maintain lettuce quality during seven days of refrigerated storage at 4 °C. For an activation time of 60 min, PAW H_2O_2 and NO_3^- concentrations achieved values of 77.8 and 223.4 mg/L, respectively, while pH and electrical conductivity were 2.81 and 1492 μS/cm, respectively. No measurable amounts of NO_2^- were found.

The stability of PAW upon five days of refrigerated storage at 4 °C was evaluated. The aqueous concentration of H_2O_2 showed a gentle decrease over the storage time from 77.8 to 67.9 mg/L for days 1 and 5, respectively. On the other hand, the concentration of NO_3^-, conductivity, and pH remained almost constant (within the statistical error) over the whole storage time.

The chromatic parameters results suggest that PAW treatments reduce the chlorophyll of lettuce degradation for seven days of storage at 4 °C. Besides, no significant differences between the firmness of control and PAW-treated samples were found during refrigerated storage.

The lettuce microbiological results of aerobic mesophilic (RAM), enterobacteriaceae, and psychrotrophs populations show that the PAW treatments on day 3 of storage exhibited stronger inactivation efficiency than on day 1, indicating that bacteria under the lethal PAW dose were unable to repair themselves during three days of refrigerated storage. However, psychrotrophs inactivation efficiency was maintained for up to seven days. PAW treatments with refrigerated storage times of one and five days show a similar reduction level, which is consistent with the low variation levels of PAW properties (<5%) with the storage times. This allows us to think that the PAW activation process could be done in a different place from the one in which the treatments should be done. No clear trend between the reduction of microorganisms and the soaking time (treatment time) was found for the evaluated conditions.

PAW treatments significantly favored both the antioxidant capacity (FRAP, ABTS, and DPPH methods) and the production of total phenolic contents of lettuce toward day 7 of refrigerated storage.

As a whole, the results suggest that PAW could be used as a promising substitute for traditional sanitizer to maintain the quality of fresh-cut lettuce during refrigerated storage.

V. Acknowledgment

Juan Camilo Chamorro and Ezequiel Cejas thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) for their post-doctoral fellowships. Gabriela Inés Denoya, Brenda Fina, Anabel Rodríguez, Sergio Ramón Vaudagna, and Leandro Prevosto are members of CONICET. Brenda Santamaría and Matías Ferreyra thank CONICET for their doctoral fellowships.

References

[1] A. Raffo and F. Paoletti, "Fresh-cut vegetables processing: Environmental sustainability and food safety issues in a comprehensive perspective," *Frontiers Sustain. Food Syst.*, vol. 5, pp. 1–16, Jan. 2022, doi: 10.3389/fsufs.2021.681459.

[2] X. Chen and Y. Hung, "Development of a chlorine dosing strategy for fresh produce washing process to maintain microbial food safety and minimize residual chlorine," *J. Food Sci.*, vol. 83, no. 6, pp. 1701–1706, May 2018, doi: 10.1111/1750-3841.14189.

[3] R. Stead, "Time to replace chlorine with nature's approach and reap the marketing and environmental benefits that are available," Campden Chorleywood Food Res. Assoc. Group, Newsletter, Chippin Campden, Gloucestershire, U.K., Tech. Rep. 3, 2004.

[4] G. Katsaros and P. Taoukis, "Microbial control by high pressure processing for shelf-life extension of packed meat products in the cold chain: Modeling and case studies," *Appl. Sci.*, vol. 11, no. 3, p. 1317, Feb. 2021, doi: 10.3390/app11031317.

[5] F. J. Barba et al., "Current applications and new opportunities for the use of pulsed electric fields in food science and industry," *Food Res. Int.*, vol. 77, pp. 773–798, Nov. 2015, doi: 10.1016/j.foodres.2015.09.015.

[6] M. R. Corbo, A. Bevilacqua, D. Campaniello, D. D'Amato, B. Speranza, and M. Sinigaglia, "Prolonging microbial shelf life of foods through the use of natural compounds and non-thermal approaches—A review," *Int. J. Food Sci. Technol.*, vol. 44, no. 2, pp. 223–241, Feb. 2009, doi: 10.1111/j.1365-2621.2008.01883.x.

[7] D. Knorr, A. Froehling, H. Jaeger, K. Reineke, O. Schlueter, and K. Schoessler, "Emerging technologies in food processing," *Annu. Rev. Food Sci. Technol.*, vol. 2, no. 1, pp. 203–235, Apr. 2011, doi: 10.1146/annurev.food.102308.124129.

[8] X. Liao et al., "Inactivation mechanisms of non-thermal plasma on microbes: A review," *Food Control*, vol. 75, pp. 83–91, May 2017, doi: 10.1016/j.foodcont.2016.12.021.

[9] M. Boulos, P. Fauchais, and E. Pfender, *Thermal Plasmas: Fundamentals and Applications*, vol. 1. New York, NY, USA: Plenum, 1994.

[10] Q. Xiang, L. Fan, Y. Li, S. Dong, K. Li, and Y. Bai, "A review on recent advances in plasma-activated water for food safety: Current applications and future trends," *Crit. Rev. Food Sci. Nutrition*, vol. 62, no. 8, pp. 2250–2268, Dec. 2020, doi: 10.1080/10408398.2020.1852173.

[11] J. Luo, L. Nie, D. Liu, and X. Lu, "Electrodeless discharge in water: Reactive species in liquid and gas phase and energy cost for nitrogen fixation," *Plasma Processes Polym.*, vol. 20, no. 3, Mar. 2023, Art. no. 2200181, doi: 10.1002/ppap.202200181.

[12] H. Cheng et al., "On the dose of plasma medicine: Plasma-activated medium (PAM) and its effect on cell viability," *Phys. Plasmas*, vol. 29, no. 6, Jun. 2022, Art. no. 063506, doi: 10.1063/5.0089357.

[13] J. Shen et al., "Bactericidal effects against *S.aureus* and physicochemical properties of plasma activated water stored at different temperatures," *Sci. Rep.*, vol. 6, no. 1, p. 28505, Jun. 2016, doi: 10.1038/srep28505.

[14] Y. Li, L. Nie, D. Liu, S. Kim, and X. Lu, "Plasma-activated chemical solutions and their bactericidal effects," *Plasma Processes Polym.*, vol. 19, no. 11, Nov. 2022, Art. no. 2100248, doi: 10.1002/ppap.202100248.

[15] R. Thirumdas et al., "Plasma activated water (PAW): Chemistry, physico-chemical properties, applications in food and agriculture," *Trends Food Sci. Technol.*, vol. 77, pp. 21–31, Jul. 2018, doi: 10.1016/j.tifs.2018.05.007. AQ:7

[16] M. Andrasch, J. Stachowiak, O. Schlüter, U. Schnabel, and J. Ehlbeck, "Scale-up to pilot plant dimensions of plasma processed water generation for fresh-cut lettuce treatment," *Food Packag. Shelf Life*, vol. 14, pp. 40–45, Dec. 2017, doi: 10.1016/j.fpsl.2017.08.007.

[17] U. Schnabel et al., "Plasma-functionalized water: From bench to prototype for fresh-cut lettuce," *Food Eng. Rev.*, vol. 13, no. 1, pp. 115–135, Mar. 2021, doi: 10.1007/s12393-020-09238-9.

[18] U. Schnabel et al., "Sanitation of fresh-cut endive lettuce by plasma processed tap water (PPtW)—Up-scaling to industrial level," *Innov. Food Sci. Emerg. Technol.*, vol. 53, pp. 45–55, May 2019, doi: 10.1016/j.ifset.2017.11.014.

[19] M. S. I. Khan and Y.-J. Kim, "Inactivation mechanism of *Salmonella* typhimurium on the surface of lettuce and physicochemical quality assessment of samples treated by micro-plasma discharged water," *Innov. Food Sci. Emerg. Technol.*, vol. 52, pp. 17–24, Mar. 2019, doi: 10.1016/j.ifset.2018.11.011.

[20] A. Fröhling, J. Ehlbeck, and O. Schlüter, "Impact of a pilot-scale plasma-assisted washing process on the culturable microbial community dynamics related to fresh-cut endive lettuce," *Appl. Sci.*, vol. 8, no. 11, p. 2225, Nov. 2018, doi: 10.3390/app8112225.

[21] A. Patange, P. Lu, D. Boehm, P. J. Cullen, and P. Bourke, "Efficacy of cold plasma functionalised water for improving microbiological safety of fresh produce and wash water recycling," *Food Microbiol.*, vol. 84, Dec. 2019, Art. no. 103226, doi: 10.1016/j.fm.2019.05.010.

[22] L. Prevosto, H. Kelly, B. Mancinelli, J. C. Chamorro, and E. Cejas, "On the physical processes ruling an atmospheric pressure air glow discharge operating in an intermediate current regime," *Phys. Plasmas*, vol. 22, no. 2, Feb. 2015, Art. no. 023504, doi: 10.1063/1.4907661.

[23] E. Cejas, J. C. Chamorro, and L. Prevosto, "Quantitative schlieren diagnostics applied to a millisecond pulsed-DC hybrid discharge in atmospheric pressure air," *Plasma Chem. Plasma Process.*, vol. 42, no. 3, pp. 657–670, Feb. 2022, doi: 10.1007/s11090-022-10233-7.

[24] W. F. L. M. Hoeben, P. P. van Ooij, D. C. Schram, T. Huiskamp, A. J. M. Pemen, and P. Lukeš, "On the possibilities of straightforward characterization of plasma activated water," *Plasma Chem. Plasma Process.*, vol. 39, no. 3, pp. 597–626, Apr. 2019, doi: 10.1007/s11090-019-09976-7.

[25] P. J. Bruggeman et al., "Plasma–liquid interactions: A review and roadmap," *Plasma Sources Sci. Technol.*, vol. 25, no. 5, Sep. 2016, Art. no. 053002, doi: 10.1088/0963-0252/25/5/053002.

[26] Y. Akishev, M. Grushin, V. Karalnik, A. Petryakov, and N. Trushkin, "Non-equilibrium constricted DC glow discharge in N_2 flow at atmospheric pressure: Stable and unstable regimes," *J. Phys. D: Appl. Phys.*, vol. 43, no. 7, Feb. 2010, Art. no. 075202, doi: 10.1088/0022-3727/43/7/075202.

[27] P. Bruggeman et al., "Characteristics of atmospheric pressure air discharges with a liquid cathode and a metal anode," *Plasma Sources Sci. Technol.*, vol. 17, no. 2, May 2008, Art. no. 025012, doi: 10.1088/0963-0252/17/2/025012.

[28] R. Baird, A. Eaton, and E. Rice, *Standard Methods for the Examination of Water and Wastewater*, 23rd ed. Washington, DC, USA: American Public Health Association, American Water Works Association, Water Environment Federation, 2017.

[29] G. I. Denoya, G. A. Polenta, N. M. Apóstolo, C. O. Budde, M. Cristianini, and S. R. Vaudagna, "High-pressure processing applied for enhancing the antioxidant content of minimally processed peaches," *Int. J. Food Sci. Technol.*, vol. 57, no. 1, pp. 684–694, Nov. 2021, doi: 10.1111/ijfs.15448.

[30] R Core Team. (2022). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. [Online]. Available: https://www.R-project.org/

[31] Y. Maeda, N. Igura, M. Shimoda, and I. Hayakawa, "Bactericidal effect of atmospheric gas plasma on *Escherichia coli* K12," *Int. J. Food Sci. Technol.*, vol. 38, no. 8, pp. 889–892, Dec. 2003, doi: 10.1046/j.1365-2621.2003.00746.x.

[32] P. Lukes, B. R. Locke, and J. L. Brisset, "Aqueous-phase chemistry of electrical discharge plasma in water and in gas-liquid environments," in *Plasma Chemistry and Catalysis in Gases and Liquids*. Hoboken, NJ, USA: Wiley, 2012, ch. 6, pp. 243–308, doi: 10.1002/9783527649525.ch7.

[33] S. Ikawa, K. Kitano, and S. Hamaguchi, "Effects of pH on bacterial inactivation in aqueous solutions due to low-temperature atmospheric pressure plasma application," *Plasma Processes Polym.*, vol. 7, no. 1, pp. 33–42, Jan. 2010, doi: 10.1002/ppap.200900090.

[34] Z. C. Liu et al., "Physicochemical processes in the indirect interaction between surface air plasma and deionized water," *J. Phys. D: Appl. Phys.*, vol. 48, no. 49, Dec. 2015, Art. no. 495201, doi: 10.1088/0022-3727/48/49/495201.

[35] R. Zhou et al., "Plasma-activated water: Generation, origin of reactive species and biological applications," *J. Phys. D: Appl. Phys.*, vol. 53, no. 30, Jul. 2020, Art. no. 303001, doi: 10.1088/1361-6463/ab81cf.

[36] K. Oehmigen, M. Hähnel, R. Brandenburg, C. Wilke, K.-D. Weltmann, and T. von Woedtke, "The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids," *Plasma Processes Polym.*, vol. 7, nos. 3–4, pp. 250–257, Mar. 2010, doi: 10.1002/ppap.200900077.

[37] D. Thomas and J. Vanderschuren, "Modeling of NO_x absorption into nitric acid solutions containing hydrogen peroxide," *Ind. Eng. Chem. Res.*, vol. 36, no. 8, pp. 3315–3322, Aug. 1997, doi: 10.1021/ie960436g.

[38] M. S. Rayson, J. C. Mackie, E. M. Kennedy, and B. Z. Dlugogorski, "Accurate rate constants for decomposition of aqueous nitrous acid," *Inorganic Chem.*, vol. 51, no. 4, pp. 2178–2185, Feb. 2012, doi: 10.1021/ic202081z.

[39] S. Jung et al., "The use of atmospheric pressure plasma-treated water as a source of nitrite for emulsion-type sausage," *Meat Sci.*, vol. 108, pp. 132–137, Oct. 2015, doi: 10.1016/j.meatsci.2015.06.009.

[40] Z. Machala, B. Tarabová, D. Sersenová, M. Janda, and K. Hensel, "Chemical and antibacterial effects of plasma activated water: Correlation with gaseous and aqueous reactive oxygen and nitrogen species, plasma sources and air flow conditions," *J. Phys. D: Appl. Phys.*, vol. 52, no. 3, Jan. 2019, Art. no. 034002, doi: 10.1088/1361-6463/aae807.

[41] A. P. León, S. Z. Viña, D. Frezza, A. Chaves, and A. Chiesa, "Estimation of chlorophyll contents by correlations between SPAD-502 meter and chroma meter in butterhead lettuce," *Commun. Soil Sci. Plant Anal.*, vol. 38, nos. 19–20, pp. 2877–2885, Nov. 2007, doi: 10.1080/00103620701663115.

[42] E. A. Szabo, K. J. Scurrah, and J. M. Burrows, "Survey for psychrotrophic bacterial pathogens in minimally processed lettuce," *Lett. Appl. Microbiol.*, vol. 30, no. 6, pp. 456–460, Jun. 2000, doi: 10.1046/j.1472-765x.2000.00747.x.

[43] U. Schnabel et al., "Optimizing the application of plasma functionalised water (PFW) for microbial safety in fresh-cut endive processing," *Innov. Food Sci. Emerg. Technol.*, vol. 72, Aug. 2021, Art. no. 102745, doi: 10.1016/j.ifset.2021.102745.

[44] C. Chen et al., "The effects of cold plasma-activated water treatment on the microbial growth and antioxidant properties of fresh-cut pears," *Food Bioprocess Technol.*, vol. 12, no. 11, pp. 1842–1851, Sep. 2019, doi: 10.1007/s11947-019-02331-w.

[45] E. J. Choi et al., "Sequential application of plasma-activated water and mild heating improves microbiological quality of ready-to-use shredded salted kimchi cabbage (*Brassica pekinensis* L.)," *Food Control*, vol. 98, pp. 501–509, Apr. 2019, doi: 10.1016/j.foodcont.2018.12.007.

[46] N. Shirron, G. Kisluk, Y. Zelikovich, I. Eivin, E. Shimoni, and S. Yaron, "A comparative study assaying commonly used sanitizers for antimicrobial activity against indicator bacteria and a *Salmonella* typhimurium strain on fresh produce," *J. Food Protection*, vol. 72, no. 11, pp. 2413–2417, Nov. 2009, doi: 10.4315/0362-028x-72.11.2413.

[47] M. S. Nascimento, N. Silva, M. P. L. M. Catanozi, and K. C. Silva, "Effects of different disinfection treatments on the natural microbiota of lettuce," *J. Food Protection*, vol. 66, no. 9, pp. 1697–1700, Sep. 2003, doi: 10.4315/0362-028x-66.9.1697.

[48] R. Ma, G. Wang, Y. Tian, K. Wang, J. Zhang, and J. Fang, "Non-thermal plasma-activated water inactivation of food-borne pathogen on fresh produce," *J. Hazardous Mater.*, vol. 300, pp. 643–651, Dec. 2015, doi: 10.1016/j.jhazmat.2015.07.061.

[49] Y. Xu, Y. Tian, R. Ma, Q. Liu, and J. Zhang, "Effect of plasma activated water on the postharvest quality of button mushrooms, Agaricus bisporus," *Food Chem.*, vol. 197, pp. 436–444, Apr. 2016, doi: 10.1016/j.foodchem.2015.10.144.

[50] M. J. Traylor et al., "Long-term antibacterial efficacy of air plasma-activated water," *J. Phys. D: Appl. Phys.*, vol. 44, no. 47, Nov. 2011, Art. no. 472001, doi: 10.1088/0022-3727/44/47/472001.

[51] R. Ma et al., "Effect of non-thermal plasma-activated water on fruit decay and quality in postharvest Chinese bayberries," *Food Bioprocess Technol.*, vol. 9, no. 11, pp. 1825–1834, Jul. 2016, doi: 10.1007/s11947-016-1761-7.

[52] M. Siddiq, S. Roidoung, D. S. Sogi, and K. D. Dolan, "Total phenolics, antioxidant properties and quality of fresh-cut onions (*Allium cepa* L.) treated with mild-heat," *Food Chem.*, vol. 136, no. 2, pp. 803–806, Jan. 2013, doi: 10.1016/j.foodchem.2012.09.023.

[53] J. M. Richard, K. N. Bernadette, W. Trude, M. C. Lucy, and E. C. Bernard, "Effect of solar drying methods on total phenolic contents and antioxidant activity of commonly consumed fruits and vegetable (mango, banana, pineapple and tomato) in Tanzania," *Afr. J. Food Sci.*, vol. 9, no. 5, pp. 291–300, May 2015, doi: 10.5897/AJFS2015.1232.

[54] R. A. Dixon and N. L. Paiva, "Stress-induced phenylpropanoid metabolism," *Plant Cell*, vol. 7, pp. 1085–1097, Jul. 1995, doi: 10.1105/tpc.7.7.1085.

Juan Camilo Chamorro was born in Riosucio, Colombia, in March 1989. He received the degree in physics engineering from the Technological University of Pereira, Pereira, Colombia, in 2013, and the Ph.D. degree in engineering from the National University of Rosario, Rosario, Argentina, in 2021.

He currently holds a post-doctoral fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Venado Tuerto, Argentina. Since 2014, he has been part of the Electrical Discharge Group, National Technological University, Venado Tuerto. In 2019, he joined National Technological University as a Professor. His research focuses on plasma optical diagnostics, computational modeling, and the applications of nonthermal electric discharges.

AQ:8

Gabriela Inés Denoya received the degree in food science and technology from the School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina, in 2008, and the Ph.D. degree in biochemical sciences from the University of Buenos Aires in 2015.

She joined the Food Technology Institute, Agroindustry Research Center, National Institute of Agricultural Technology (INTA), Buenos Aires, in 2009, with a scholarship to work on the topic "preservation of minimally processed fruit and vegetable products." She currently works as a Researcher and a Coordinator of the processing, physical and sensory analysis area of the Food Technology Institute, INTA, where she is also a Researcher for the National Council for Scientific and Technical Research (CONICET). She is also responsible for research lines at INTA and participates in other projects. Regarding teaching, she is a Professor of the bachelor's degree in food science and technology at the National University of Hurlingham, Buenos Aires. She works especially in food processing, both for preservation and transformation. She carries out research lines related to the application of nonthermal technologies, mainly in fruit and vegetable products.

Brenda Santamaría was born in Venado Tuerto, Argentina, in 1989. She received the Engineering degree in chemical engineering from the Rosario Regional Faculty, National Technological University, Rosario, Argentina, in 2020.

Since 2021, she has been a Doctoral Fellow with the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Venado Tuerto, Argentina. She is conducting their doctoral studies with the Electrical Discharge Group (GDE), National Technological University, Venado Tuerto. Her research interests include nonthermal discharge in contact with liquids and applications of nonthermal plasma, in particular, decontamination of organic compounds in water.

Brenda Fina was born in Colón, Argentina, in 1986. She received the Diploma degree in biotechnology and the Ph.D. degree in biological sciences from Rosario National University, Rosario, Argentina, in 2010 and 2015, respectively.

She has been a Professor with Rosario National University and National Technological University, Venado Tuerto, Argentina, where she is part of the Electrical Discharge Group. Since 2021, she has been a Researcher with the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Venado Tuerto. She has worked on toxicological studies of fluoride in plants and animals. Her research interests include the biological applications of nonthermal plasma, including microbial disinfection, decontamination of organic compounds in water, and the application of activated water in seeds and fruits.

Matías Ferreyra was born in Canals, Argentina, in 1992. He received the Engineering degree in electromechanical engineering from the Venado Tuerto Regional Faculty, National Technological University, Venado Tuerto, Argentina, in 2018.

Since 2019, he has been a Doctoral Fellow with the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Venado Tuerto. He is conducting his doctoral studies at the Electrical Discharge Group (GDE), National Technological University, and the Institute of Agricultural and Environmental Biosciences Research (INBA), Faculty of Agronomy, University of Buenos Aires, Buenos Aires, Argentina. Since 2022, he has been a Professor with National Technological University. His research interests include nonthermal discharge in contact with liquids, agriculture applications of plasma-activated water, optical diagnostics, and plasma technology.

Ezequiel Cejas was born in Los Quirquinchos, Argentina, in 1987. He received the Engineering degree in electromechanical engineering from the Venado Tuerto Regional Faculty, National Technological University, Venado Tuerto, Argentina, in 2017, and the Ph.D. degree in engineering from the National University of Rosario, Rosario, Argentina, in 2022.

Since 2022, he has been a Post-Doctoral Fellow with the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Venado Tuerto. He is conducting post-doctoral studies in the area of food preservation and alimentary security employed nonthermal plasmas at atmospheric pressure at the Electrical Discharge Group, National Technological University. His research interests include plasma diagnostics, numerical modeling, and plasma technology.

Anabel Rodríguez was born in Buenos Aires, Argentina, in 1983. She received the Ph.D. degree in food engineering from the National University of La Plata, La Plata, Argentina, in 2014.

She was a Researcher with the Center for Research and Development in Food Cryotechnology (CIDCA), Buenos Aires, Argentina, until 2014. Since 2014, she has been working at the National Institute of Agricultural Technology (INTA), Buenos Aires. Her research interests include quality control, food processing, biochemical and nutritional analysis of food, and new product development.

Sergio Ramón Vaudagna was born in Ceres, Argentina, in 1965. He received the bachelor's degree in chemical engineering and the Ph.D. degree in chemical engineering from the School of Chemical Engineering, National University of the Litoral, Santa Fe, Argentina, in 1992 and 1997, respectively.

He joined the Food Technology Institute of the Agroindustry Research Center, National Institute of Agricultural Technology (INTA), Buenos Aires, Argentina, in 1997. Since 2000, he has been a Researcher with the National Council for Scientific and Technical Research (CONICET), Buenos Aires. Since 2014, he has been the Head of the Food Technology Institute, INTA. He is also the Director of the master's degree in food technology at the Buenos Aires Regional School, National Technological University, Venado Tuerto, Argentina, and an Associate Professor with the National University of Hurlingham, Buenos Aires. His research interests include the application of thermal (pasteurization, sterilization, and vacuum cooking) and nonthermal (high hydrostatic pressure, gamma irradiation, cold plasma, chemical compounds, and bacteriocins) technologies in food processing.

Leandro Prevosto was born in Venado Tuerto, Argentina, in 1971. He received the Diploma degree in electromechanical engineering from National Technological University, Venado Tuerto, in 2005, and the Ph.D. degree (Hons.) in engineering from the University of Buenos Aires, Buenos Aires, Argentina, in 2009.

Since 2010, he has been a Professor with National Technological University, where he is currently the Director of the Electrical Discharge Group. Since 2012, he has been a Researcher with the Consejo Nacional de Ciencias y Tecnología (CONICET), Venado Tuerto. His research interests include thermal and nonthermal electrical discharges, plasma diagnostics, and plasma applications, including plasmas in agriculture.