JTEP Jurnal Keteknikan Pertanian,
Vol. 14 No. 2, p 318-334, 2026
P-ISSN 2407-0475 E-ISSN 2338-8439

Available online:
http://journal.ipb.ac.id/index.php/jtep
DOI: 10.19028/jtep.014.2.318-334

Research Article

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Application of Plasma Activated Fine Bubble Water on Fresh-Cut Lettuce Quality and Safety

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Article Info

Submitted: 15 May 2026
Revised: 20 August 2026
Accepted: 4 September 2026
Available online: 14 September 2026
Published: June 2026

Keywords:
fresh-cut lettuce, plasma-activated water, postharvest quality, reactive oxygen and nitrogen species, washing technology

How to cite:
Hidayat, S. A., Purwanto, Y. A. ,& Sugiarto, A. T. (2026). Application of Plasma Activated Fine Bubble Water on Fresh-Cut Lettuce Quality and Safety. Jurnal Keteknikan Pertanian, 14(2): 318-334.
https://doi.org/*10.19028/jtep.014.2.318-334.*

Abstract

Lettuce (Lactuca sativa L.) is highly susceptible to postharvest deterioration, microbial contamination, and pesticide residues. This study evaluated the effectiveness of plasma-activated water (PAW) and plasma-activated fine bubble water (PAFBW) compared to distilled water (control) in maintaining the quality and safety of fresh-cut lettuce. A completely randomized design evaluated four factors: ozone concentration (1 and 2 ppm), immersion time (1 and 2 min), washing solution type (PAW and PAFBW), and storage temperature (15 °C and 25 °C). Distilled water controls were analyzed separately. Weight loss and total color difference (ΔE) were evaluated after two days of storage. Total plate count (TPC), total phenolics, and qualitative pesticide screening were measured immediately after washing and analyzed descriptively. One-Way ANOVA showed highly significant overall treatment effects ($p<0.001$) on weight loss ($F=10.90$) and color change ($F=61.94$). Factorial GLM identified storage temperature as the dominant factor. The 2 ppm PAW treatment with 2-min immersion at 15 °C produced the lowest weight loss (0.87 ± 0.11%) and smallest total color difference (0.40 ± 0.04). The lowest TPC was obtained for PAW 2 ppm 2 min (17,000 colony-forming units per gram [CFU/g]) and PAFBW 2 ppm 2 min (19,950 CFU/g). The highest total phenolic content was found in PAW 1 ppm 2 min (6.98 ± 0.05 mg gallic acid equivalents per gram [mg GAE/g]). PAW generally outperformed PAFBW in quantitative parameters, while PAFBW yielded the most favorable qualitative pesticide screening response, highlighting plasma technology's postharvest potential.

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1. Introduction

Lettuce (*Lactuca sativa* L.) is a high-value horticultural commodity widely consumed worldwide due to its nutritional content, refreshing flavor, and crunchy texture (Kim et al., 2016; Nikzad and Parastar, 2021). It is cultivated across diverse climates and serves as a primary ingredient in salads and side dishes, with continually increasing market demand (Shatilov et al., 2019). However, lettuce is highly susceptible to postharvest deterioration, particularly when minimally processed. Visual quality decline through enzymatic browning, weight loss due to transpiration, and microbial contamination significantly reduce its shelf life and commercial value (Teng et al., 2019).

Two major postharvest concerns for fresh-cut lettuce are pesticide residues and microbial contamination. Organophosphate pesticide residues can persist on lettuce due to off-label spraying and short pre-harvest intervals (Alen and Suharti, 2015), with up to 32% of fruits and vegetables exceeding maximum residue limits (Li et al., 2023). Foodborne outbreaks linked to leafy greens, including lettuce, have been documented extensively in the United States and the European Union (Callejón et al., 2015; Herman et al., 2015). Conventional chlorine-based sanitation poses concerns regarding the formation of harmful disinfection byproducts (Castro-Ibáñez et al., 2017), highlighting the need for safer and more effective alternatives.

Non-thermal plasma (NTP) technology has recently emerged as a promising postharvest intervention. Plasma-activated water (PAW), generated through plasma-liquid interactions, contains reactive oxygen and nitrogen species (RONS) including nitrate, nitrite, hydrogen peroxide, ozone, and hydroxyl radicals (Bruggeman et al., 2016). These species exhibit broad antimicrobial activity (Rothwell et al., 2023), inhibit enzymatic browning by suppressing hydrogen peroxide accumulation (Yun et al., 2021), and degrade organophosphate pesticides through hydroxyl radical-mediated oxidation (Sojithamporn et al., 2023; Zhou et al., 2019).

To enhance the stability and reactivity of RONS in solution, PAW can be combined with fine bubble technology to produce plasma-activated fine bubble water (PAFBW). Ultrafine bubbles are gas bubbles with diameters below 1 μm (ISO 20480-1, 2017) that provide a large interfacial area and stabilize reactive species in water (Tiwari et al., 2025). Rathore et al. (2023) demonstrated that an air bubble diffuser improved physicochemical properties of PAW, with up to a 300% increase in dissolved ozone. Gao et al. (2022) reported that microbubble-enhanced plasma activation achieved more than five-fold higher activation efficiency at the same energy input. Nemati et al. (2024) showed that plasma-activated microbubble water reduced Escherichia coli O157:H7 by 4.6 log CFU/g on romaine lettuce.

Despite these advances, comparative studies that simultaneously evaluate the performance of PAW and PAFBW on multiple postharvest quality parameters of fresh-cut lettuce remain limited, particularly under tropical postharvest conditions. The novelty of this study lies in the integrated assessment of weight loss, color change, microbial load, total phenolic content, and pesticide residue

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across distilled water, PAW, and PAFBW under varying ozone concentration, immersion time, and storage temperature. The objective of this research was to evaluate the effectiveness of PAW and PAFBW washing in reducing pesticide residues, suppressing microbial contamination, and maintaining visual quality to extend the shelf life of fresh-cut lettuce.

2. Material and Methods

This research was conducted from July to October 2025 at the Environmental and Biosystem Engineering Laboratory and the Food and Agricultural Product Processing Engineering Laboratory, Department of Mechanical and Biosystem Engineering, Faculty of Agricultural Engineering and Technology, IPB University, Bogor, Indonesia.

2.1 Materials and Equipment

The lettuce used was curly lettuce (*Lactuca sativa* L.) obtained from a local traditional market in Bogor at commercial harvest maturity. Distilled water was used as the control washing medium and as the base solution for plasma activation. Reagents used in laboratory analyses included Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3) 2%, methanol, plate count agar (PCA) for total plate count assay, and an organophosphate pesticide test kit. The main equipment included a custom-built PAFBW reactor (45 VA, 220–240 V, with positive and negative electrodes; air flow rate 4 L/min and a fine bubble generator), a digital pH meter, a digital nitrate meter, an ozone analyzer, a dissolved oxygen meter, a chromameter (Konica Minolta CR-400), an analytical balance (0.0001 g resolution), 500-mL thin-wall polypropylene containers, a stopwatch, and a personal computer for data acquisition.

2.2 Experimental Design

The experiment was arranged using a completely randomized design (CRD) with four factors: (i) dissolved ozone concentration (P): P1 = 1 ppm, P2 = 2 ppm; (ii) immersion time (Q): Q1 = 1 min, Q2 = 2 min; (iii) washing solution type (R): R1 = distilled water (control), R2 = PAW, R3 = PAFBW; and (iv) storage temperature (S): S1 = 15 °C, S2 = 25 °C. The control treatment using distilled water (R1) did not include the ozone factor (P); therefore, PAW and PAFBW data were analyzed using a 2×2×2×2 factorial General Linear Model, while control comparisons were evaluated separately using One-Way ANOVA with Dunnett's test. Each treatment was performed in three replicates, except for total plate count and total phenolic content, which were both conducted via duplicate technical plating and analyzed descriptively as 10 separate treatments rather than evaluating storage effects.

2.3 Sample Preparation and Washing Procedure

Whole lettuce was transported and stored at 15 °C prior to treatment. The PAW was generated by passing air at 4 L/min through a custom-built dielectric barrier discharge reactor immersed in 500 mL of distilled water. Based on reactor calibration, the activation durations required to reach the nominal

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dissolved ozone endpoints were PAW 1 ppm = 25 min; PAW 2 ppm = 40 min. For PAFBW production, the same reactor was coupled with a fine-bubble generator that recirculated the activated water through a fine-bubble diffuser, producing the characteristic milky appearance. The corresponding activation durations were PAFBW 1 ppm = 30 min; PAFBW 2 ppm = 40 min. The achieved dissolved ozone concentrations were 1.12 and 2.34 mg/L for PAW and 1.08 and 2.53 mg/L for PAFBW at the nominal 1 and 2 ppm settings, respectively (Table 1). Multiple fresh-cut curly lettuce leaves were immersed in 1.5 L of either PAW or PAFBW solutions according to the designated ozone concentrations. Following the washing treatment, the samples were surface-dried, packed into 500 mL plastic containers (three samples per container), and stored at the assigned temperatures (15 or 25 °C) for 2 days.

2.4 Quality Parameter Measurements

2.4.1 Weight loss

Weight loss (%) was calculated as:

$$weight\ loss\ (\%) = \frac{W_0 - W_2}{W_0} \times 100\% \quad (1)$$

Where W_0 is the initial weight (g) and W_2 is the weight at day-2 of storage (Fan et al., 2019).

2.4.2 Color change

Color was measured using a chromameter that recorded the L*, a*, and b* values at three random points on each lettuce piece. The total color difference (ΔE) between day-0 and day-2 was calculated using the equation

$$\Delta E = \sqrt{(L_2 - L_0)^2 + (a_2 - a_0)^2 + (b_2 - b_0)^2} \quad (2)$$

Where subscripts 0 and 2 denote initial and final measurements (Nemati et al., 2024).

2.4.3 Total plate count (TPC)

Total plate count was determined using the pour-plate method on PCA. Lettuce samples (10 g) were homogenized in 90 mL sterile peptone water and serially diluted. Aliquots were plated in duplicate and incubated at 37 °C for 48 h. Colonies were enumerated and expressed as CFU/g; results were also reported as $\log_{10}$ CFU/g for descriptive analysis.

2.4.4 Total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu method (Malangngi et al., 2012). Methanolic extract (0.1 mL) was reacted with 0.1 mL of 50% Folin-Ciocalteu reagent and 2 mL of 2% Na_2CO_3, incubated for 30 min in the dark, and absorbance was read at 750 nm. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh sample (mg GAE/g).

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2.4.5 Pesticide residue

Pesticide residue was assessed qualitatively using a commercial organophosphate pesticide test kit as a rapid screening method rather than a compound-specific quantitative assay. Rapid enzyme-inhibition screening methods for organophosphorus residues in vegetable matrices have been described previously (Wang et al., 2012). Approximately 5 g of lettuce sample from each treatment was extracted following the kit instructions, and the resulting solution color was visually compared with a reference scale ranging from "-" (clear; no detectable response) to "+++" (high yellow-color intensity). The ordinal response was analyzed descriptively and was not interpreted as a residue concentration. Definitive identification and quantification require a validated multiresidue extraction, such as the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method, followed by gas or liquid chromatography (Anastassiades et al., 2003).

2.4.6 Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) and General Linear Model (GLM) procedures as specified in the experimental design. When significant treatment effects were detected, mean separation was performed using Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$), while Dunnett's test was used for comparisons with the control treatments. All statistical analyses were conducted using Minitab 17.1.0 version software.

3. Results and Discussion

3.1 Physicochemical Characteristics of Washing Media

Plasma activation significantly altered the physicochemical properties of distilled water (Table 1). PAFBW at 2 ppm exhibited the lowest pH (3.5) and the highest nitrate concentration (16 ppm), reflecting the most intensive RONS generation among the tested media. The decrease in pH is attributed to the formation of acidic species such as HNO_2 and HNO_3 as products of RONS reactions in water, which are recognized as key factors for antimicrobial activity (Oehmigen et al., 2010). The increase in dissolved ozone and nitrate from PAW to PAFBW at the same nominal ozone concentration confirms that the fine bubble diffuser enhanced the gas–liquid mass transfer and stabilized reactive species, in agreement with Rathore et al. (2023) and Gao et al. (2022).

Table 1. Physicochemical characteristics of distilled water, PAW, and PAFBW used as washing media.

Treatment	pH	Temperature (°C)	Nitrate (ppm)	O_3 (mg/L)
Distilled water	6.5	29.5	6	0.00
PAW 1 ppm	4.1	31.2	8	1.12
PAW 2 ppm	3.9	31.4	11	2.34
PAFBW 1 ppm	3.7	31.6	13	1.08
PAFBW 2 ppm	3.5	31.8	16	2.53

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3.2 Weight Loss

Weight loss is a key indicator of freshness loss governed by transpiration and respiration (Fan et al., 2019). Analysis of variance demonstrated that the treatment had a highly significant effect on weight loss at day-2 of storage ($F_{19,40} = 10.90$, $p < 0.001$). Furthermore, the factorial GLM applied to the plasma treatments revealed that ozone concentration ($F = 6.07$, $p = 0.019$) and storage temperature ($F = 91.63$, $p < 0.001$), significantly affected weight loss, while immersion type and washing solution showed no significant effects. Weight loss results across treatments are presented in Table 2.

Table 2. Weight loss (%) of fresh-cut lettuce at day-2 of storage. Treatment codes: P (ozone concentration: 1 or 2 ppm), Q (immersion time: 1 or 2 min), R (washing solution: R1 = distilled water, R2 = PAW, R3 = PAFBW), S (storage temperature: 1 = 15 °C, 2 = 25 °C). Values are means ± SD (n=3).

Treatment	Mean (%)	Treatment	Mean (%)
P2Q2R2S1	0.87 ± 0.11	P1Q2R2S2	2.98 ± 0.25
P2Q2R3S1	1.02 ± 0.07	P2Q2R3S2	3.00 ± 0.14
P2Q1R2S1	1.37 ± 0.07	P2Q2R2S2	3.00 ± 0.13
P2Q1R3S1	1.41 ± 0.09	P2Q1R2S2	3.11 ± 0.15
P1Q1R2S1	1.48 ± 0.04	Q1R1S1	3.20 ± 0.00
P1Q2R3S1	1.56 ± 0.06	P1Q1R2S2	3.28 ± 0.12
P1Q2R2S1	1.78 ± 0.22	P1Q1R3S2	3.31 ± 0.23
Q2R1S1	2.01 ± 1.73	P2Q1R3S2	3.34 ± 0.23
P1Q1R3S1	2.59 ± 2.39	P1Q2R3S2	3.62 ± 0.31
		Q2R1S2	5.08 ± 0.46
		Q1R1S2	5.92 ± 0.05

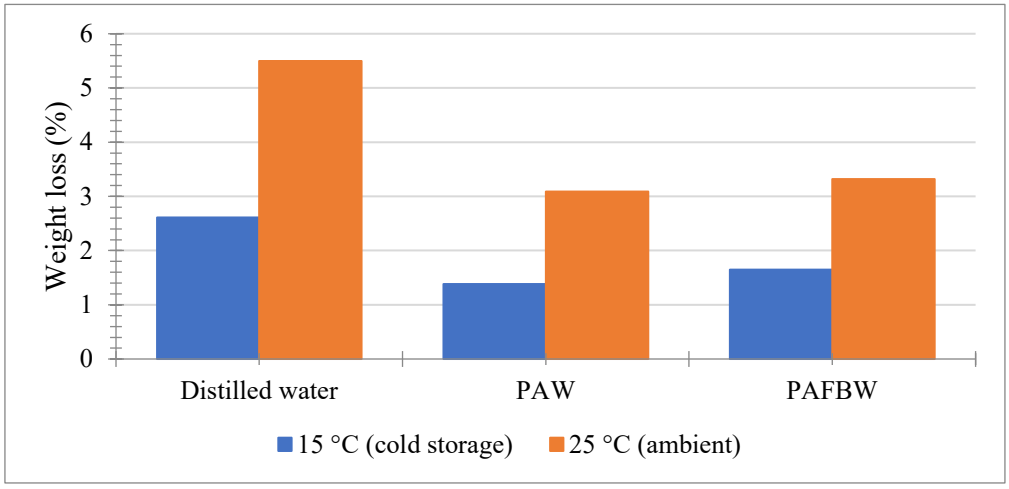

Figure 1. Mean weight loss (%) of fresh-cut lettuce at day-2 of storage by washing solution and storage temperature. Values are means averaged across ozone concentration and immersion time. Cold storage (15 °C) markedly suppressed weight loss across all washing treatments.

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As shown in Table 2 and Figure 1, storage temperature was the dominant factor governing weight loss. Cold storage (15 °C; S1) consistently produced lower weight loss than ambient storage (25 °C; S2), with an average S2/S1 ratio of approximately 2.12. The lowest weight loss was obtained for treatment P2Q2R2S1 (PAW, 2 ppm, 2 min, 15 °C) at 0.87%, while the highest was obtained for the distilled water control at ambient temperature (Q1R1S2) at 5.92%. At 15 °C, PAW treatments (mean 1.38%) produced lower weight loss than PAFBW (mean 1.65%) and distilled water (mean 2.61%); a similar pattern was observed at 25 °C.

The effectiveness of PAW in suppressing weight loss is presumed to be related to the deposition of RONS on the lettuce surface, which reduces the metabolic rate of plant tissue and consequently lowers water loss (Fagundes et al., 2013). Plasma-based treatments have also been reported to inhibit cell wall pectin metabolism, thereby maintaining firmness and quality of postharvest produce (Cheng et al., 2023). Singh and Thakur (2024) similarly reported that cold plasma treatment suppresses respiration and transpiration in fresh horticultural products. The findings of the current study are consistent with Nemati et al. (2024), who demonstrated that plasma-activated microbubble water maintained the texture of romaine lettuce during 9-day storage at 15 °C.

3.3 Color Change

One-Way ANOVA across all 20 treatments showed a highly significant overall treatment effect on ΔE at day-2 of storage ($F_{19,40} = 61.94$, $p < 0.001$). Additionally, the factorial GLM confirmed that storage temperature was the most dominant factor affecting color change ($F = 354.73$, $p < 0.001$), with significant interaction effects among the factors. Color change results across all treatments are summarized in Table 3.

Table 3. Total color difference (ΔE) of fresh-cut lettuce at day-2 of storage. Values are means ± SD (n=3).

Treatment	ΔE	Treatment	ΔE
P2Q2R2S1	0.40 ± 0.04	P2Q2R3S1	1.91 ± 0.05
P2Q1R2S1	0.41 ± 0.02	P2Q2R2S2	1.95 ± 0.03
P1Q2R3S1	0.45 ± 0.03	P1Q2R2S2	1.98 ± 0.08
P2Q1R3S1	0.45 ± 0.04	P1Q2R3S2	2.00 ± 0.15
P1Q2R2S1	0.46 ± 0.02	P1Q1R3S2	2.01 ± 0.03
P1Q1R3S1	0.47 ± 0.03	P1Q1R2S2	2.05 ± 0.01
P1Q1R2S1	0.47 ± 0.02	Q2R1S2	2.19 ± 0.32
Q2R1S1	0.69 ± 0.09	Q1R1S2	2.30 ± 0.63
Q1R1S1	0.84 ± 0.16		
P2Q2R3S2	1.86 ± 0.03		
P2Q1R3S2	1.89 ± 0.05		
P2Q1R2S2	1.90 ± 0.03		

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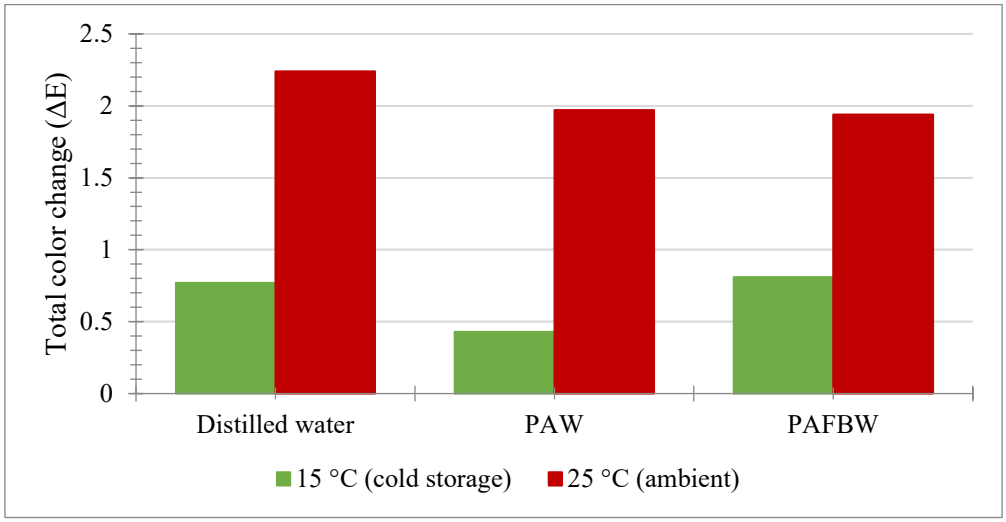

Figure 2. Mean total color difference (ΔE) of fresh-cut lettuce at day-2 of storage by washing solution and storage temperature. Cold storage clearly separated lettuce into a low-ΔE group (<0.85), while ambient storage produced ΔE values >1.86 across all treatments. The PAFBW group at 15 °C is elevated by an outlier (P2Q2R3S1, ΔE = 1.91).

A clear separation was observed between the cold-storage group (ΔE < 0.85) and the ambient-storage group (ΔE > 1.86), confirming that storage temperature was the dominant factor for color stability. At 15 °C, all washing treatments produced relatively small color changes, with P2Q2R2S1 yielding the lowest ΔE (0.40) and Q1R1S1 yielding the highest (0.84). One notable anomaly is treatment P2Q2R3S1 (PAFBW 2 ppm, 2 min, 15 °C), which produced a ΔE of 1.91 — a value comparable to ambient-storage samples. This isolated high value may reflect natural biological variation among lettuce leaves and should be interpreted cautiously; because tissue damage was not measured directly, its cause cannot be determined from the present dataset and requires further replicated investigation.

At 25 °C, PAW and PAFBW treatments showed slight advantages in inhibiting browning compared to the distilled water control, although the differences were modest. This is consistent with Nemati et al. (2024), who reported that plasma-activated microbubble water significantly reduced PPO and POD activities in lettuce, thereby suppressing enzymatic browning. Lower chlorophyll degradation in plasma-treated samples may also have contributed to color stability (Eckardt, 2009). Iakimova and Woltering (2015) showed that reactive nitrogen species, particularly nitric oxide, inhibit wound-induced browning in fresh-cut lettuce by suppressing hydrogen peroxide accumulation. The interaction between cold plasma and food enzymes has been comprehensively reviewed by Misra et al. (2016).

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3.4 Total Plate Count

Total plate count results are presented in Table 4. Because TPC was measured using the Plate Count Agar (PCA) method in technical duplicates without true biological replicates, the data were analyzed descriptively rather than statistically.

Table 4. Total plate count (TPC) of fresh-cut lettuce immediately after washing treatment.

Treatment	CFU/g	$\log_{10}$ (CFU/g)
P2Q2R2 (PAW 2 ppm, 2 min)	17,000	4.23
P2Q2R3 (PAFBW 2 ppm, 2 min)	19,950	4.30
P2Q1R3 (PAFBW 2 ppm, 1 min)	22,270	4.35
P1Q1R2 (PAW 1 ppm, 1 min)	23,640	4.37
Q1R1 (Distilled, 1 min)	29,270	4.47
P1Q2R2 (PAW 1 ppm, 2 min)	30,360	4.48
P1Q1R3 (PAFBW 1 ppm, 1 min)	33,450	4.52
P2Q1R2 (PAW 2 ppm, 1 min)	36,410	4.56
Q2R1 (Distilled, 2 min)	37,730	4.58
P1Q2R3 (PAFBW 1 ppm, 2 min)	45,550	4.66

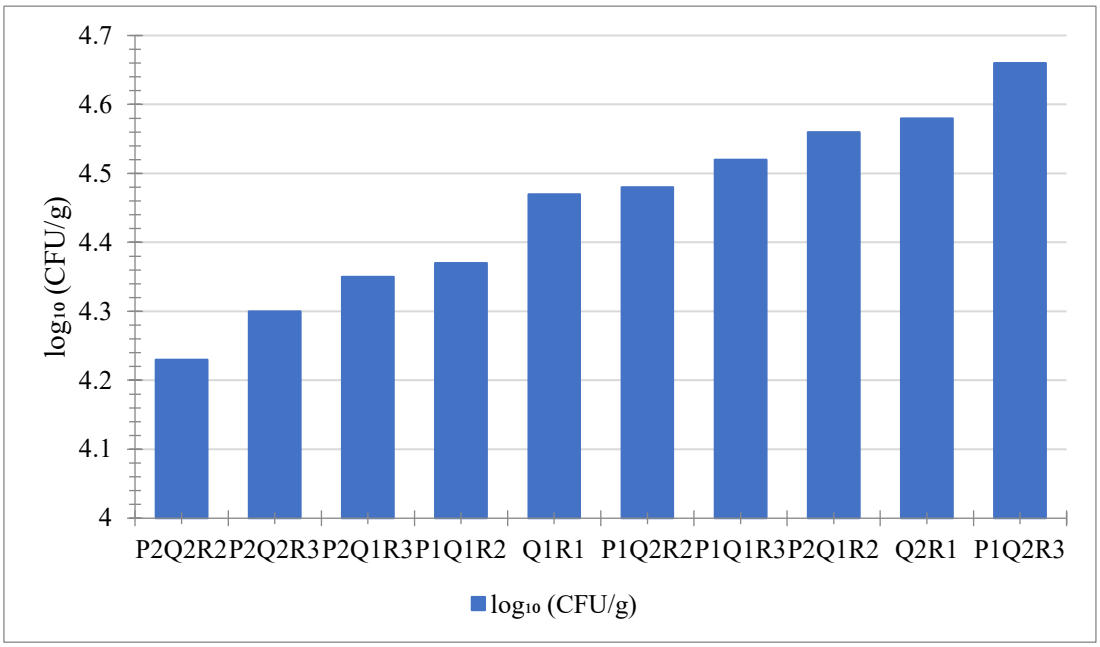

Figure 3. Total plate count ($\log_{10}$ CFU/g) of fresh-cut lettuce after various washing treatments, sorted from lowest to highest. PAW 2 ppm with 2-min immersion (P2Q2R2) produced the lowest microbial load.

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Treatment P2Q2R2 (PAW 2 ppm, 2 min) yielded the lowest TPC at 17,000 CFU/g (4.23 log), followed by P2Q2R3 (PAFBW 2 ppm, 2 min) at 19,950 CFU/g (4.30 log). The distilled water controls (Q1R1 and Q2R1) recorded TPC values of 29,270 and 37,730 CFU/g, respectively. Notably, the average TPC for PAW (26,852 CFU/g) was lower than that for PAFBW (30,305 CFU/g). However, treatment P1Q2R3 (PAFBW 1 ppm, 2 min) produced the highest TPC (45,550 CFU/g), exceeding the distilled water controls. This finding indicates that antimicrobial efficacy is not solely determined by the type of plasma-activated medium (PAW vs. PAFBW) but also depends strongly on the combination of ozone concentration and immersion time.

The antimicrobial mechanism of PAW and PAFBW is associated with the combined action of low pH and RONS, particularly ozone and hydroxyl radicals, which disrupt cell membrane integrity (Małajowicz et al., 2022). Nemati et al. (2024) demonstrated by FE-SEM that plasma-activated microbubble water induced complete cell lysis in E. coli O157:H7. Although Wang et al. (2023) reported that PAW achieved disinfection efficacy comparable to free chlorine on fresh produce, the present results emphasize that operational parameters must be carefully optimized to avoid suboptimal performance, particularly at lower ozone concentrations.

3.5 Total Phenolic Content

Phenolic content was measured immediately after washing without storage, and therefore reflects the direct effect of the washing treatment. Results are summarized in Table 5.

Table 5. Total phenolic content (mg GAE/g) of fresh-cut lettuce immediately after washing treatment.

Treatment	Total phenolic (mg GAE/g)
P1Q2R2 (PAW 1 ppm, 2 min)	6.98 ± 0.05
P1Q1R3 (PAFBW 1 ppm, 1 min)	6.06 ± 0.07
P2Q1R3 (PAFBW 2 ppm, 1 min)	5.74 ± 0.06
P1Q2R3 (PAFBW 1 ppm, 2 min)	5.17 ± 0.06
P2Q2R2 (PAW 2 ppm, 2 min)	5.06 ± 0.06
P1Q1R2 (PAW 1 ppm, 1 min)	4.23 ± 0.04
Q2R1 (Distilled, 2 min)	4.15 ± 0.04
P2Q1R2 (PAW 2 ppm, 1 min)	4.09 ± 0.04
P2Q2R3 (PAFBW 2 ppm, 2 min)	4.03 ± 0.07
Q1R1 (Distilled, 1 min)	3.55 ± 0.04

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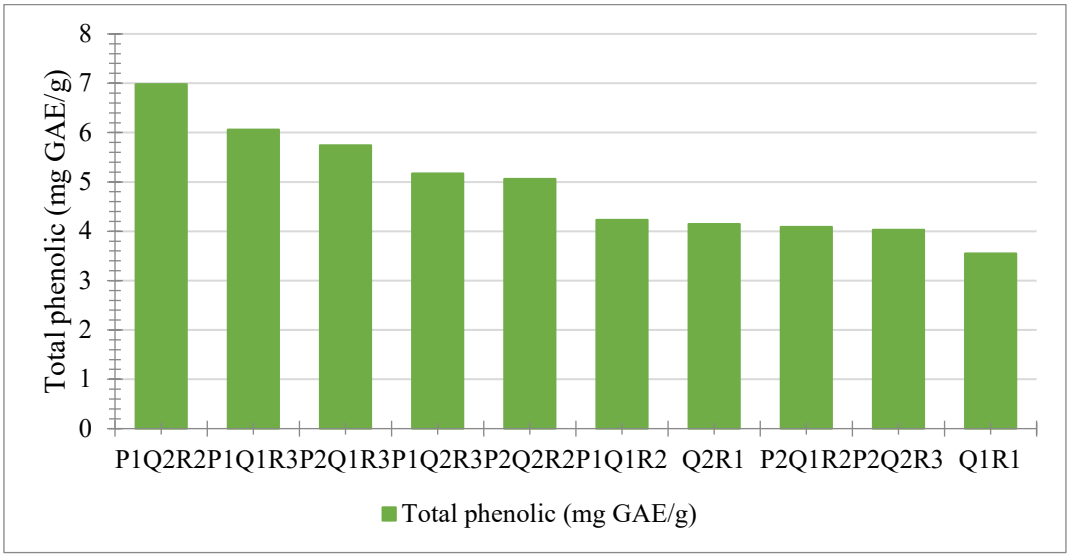

Figure 4. Total phenolic content (mg GAE/g) of fresh-cut lettuce immediately after various washing treatments, sorted from highest to lowest. PAW at 1 ppm with 2-min immersion (P1Q2R2) produced the highest phenolic content.

The highest total phenolic content was obtained for P1Q2R2 (PAW 1 ppm, 2 min) at 6.98 mg GAE/g, followed by P1Q1R3 (PAFBW 1 ppm, 1 min) at 6.06 mg GAE/g. The distilled water control Q1R1 produced the lowest content (3.55 mg GAE/g). The increase in extractable phenolics in plasma-treated samples is presumed to be associated with the release of bound phenolic compounds through interactions with RONS, consistent with Dantas et al. (2021), who reported enhanced bioaccessibility of phenolic compounds in açai pulp following cold plasma treatment. Ahmadian et al. (2023) showed that plasma pretreatment can modify cell wall polysaccharide distribution to enhance phenolic extraction. However, treatment P2Q2R3 (PAFBW 2 ppm, 2 min) yielded a phenolic content (4.03 mg GAE/g) lower than the distilled water control Q2R1 (4.15 mg GAE/g), suggesting that excessively high RONS concentrations in PAFBW may oxidatively degrade phenolic compounds themselves. This dual effect of RONS promoting extraction at low concentrations and degrading phenolics at high concentrations, highlights the importance of optimizing plasma activation parameters.

3.6 Pesticide Residue

The qualitative pesticide test kit produced its clearest response for PAFBW at 2 ppm with 2-min immersion (Table 6). According to the kit's ordinal reference scale, this was the most favorable screening response among the tested treatments. Because the assay was qualitative and non-compound-specific, the result should be interpreted as evidence of lower screening intensity rather than definitive proof or quantification of pesticide degradation.

JTEP Jurnal Keteknikan Pertanian,
Vol. 14 No. 2, p 318-334, 2026
P-ISSN 2407-0475 E-ISSN 2338-8439
Available online:
http://journal.ipb.ac.id/index.php/jtep
DOI: 10.19028/jtep.014.2.318-334

Table 6. Qualitative pesticide residue assessment using a commercial organophosphate test kit. Symbols: − (clear, no detectable residue); + (low intensity); ++ (high intensity); +++ (very high intensity).

Washing solution	Immersion time	Result
Distilled water	1 min	+++
Distilled water	2 min	+++
PAW 2 ppm	2 min	++
PAFBW 1 ppm	1 min	+
PAFBW 1 ppm	2 min	+
PAFBW 2 ppm	1 min	+
PAW 1 ppm	1 min	−
PAW 1 ppm	2 min	−
PAW 2 ppm	1 min	−
PAFBW 2 ppm	2 min	−

The favorable screening response observed for PAFBW at 2 ppm and 2-min immersion may be associated with its higher RONS concentration and the capacity of fine bubbles to increase interfacial contact and radical distribution on the lettuce surface. These conditions could promote pesticide oxidation, consistent with reports on cold plasma-assisted degradation processes (Lukes et al., 2014; Oehmigen et al., 2010; Sojithamporn et al., 2023). At 1 ppm, PAFBW with 2-min immersion was more effective than 1-min immersion, indicating that contact time plays an important role at lower RONS intensity. This is consistent with Zhou et al. (2019), who reported that increased plasma exposure time enhances pesticide degradation.

In contrast, PAW at both 1 and 2 ppm produced clearer screening responses after 1 min than after 2 min. Given the assay's qualitative nature, this non-monotonic pattern may reflect matrix interference or visual-reading variability and should not be interpreted as evidence that longer washing increased residues. The distilled-water responses should likewise be interpreted descriptively. Overall, PAFBW at 2 ppm for 2 min produced the most favorable qualitative screening response, but the finding requires chromatographic confirmation using, for example, gas chromatography-electron capture detection (GC-ECD) or ultra-high-performance liquid chromatography-photodiode array detection (UHPLC-PDA) (Avram et al., 2025).

3.7 General Discussion and Implications.

Three principal findings emerge from this study. First, storage temperature was the dominant factor affecting weight loss and color change, with cold storage at 15 °C consistently producing better outcomes than ambient storage. This is consistent with the general postharvest principle that low temperature suppresses metabolism and transpiration, and aligns with the recommended storage temperature for fresh-cut leafy greens reported by Gorski et al. (2022) and Agusta (2022).

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DOI: 10.19028/jtep.014.2.318-334

Second, contrary to the initial hypothesis that PAFBW would outperform PAW due to enhanced RONS stability, PAW consistently produced equal or better results across most quantitative parameters (weight loss, ΔE, TPC, total phenolic). PAFBW only produced the most favorable qualitative organophosphate screening response. The underlying cause of its weaker performance for the quantitative quality parameters cannot be resolved from the present dataset. Factors for future testing include RONS dose and distribution, bubble-size characteristics, and their interaction with sensitive cut-leaf tissue. These hypotheses warrant systematic investigation, including bubble size characterization, lipid peroxidation analysis, and electron microscopy of leaf surfaces. Nikzadfar et al. (2024) similarly emphasized the importance of optimizing plasma treatment parameters in postharvest applications.

Third, treatments at 2 ppm ozone with 2-min immersion generally produced the best results for TPC, indicating that antimicrobial efficacy increases with both RONS concentration and contact duration. This study has limitations, including a relatively short storage period (2 days) and the absence of TPC replication, which should be addressed in future research. Practical implications include the potential adoption of plasma-activated washing in fresh-cut lettuce processing lines, particularly when combined with appropriate cold-chain management. Implementation at industrial scale will require optimization of reactor energy consumption (Rathore et al., 2024), wash water recycling considerations (Patange et al., 2019), and food safety verification.

4. Conclusion

Plasma-activated water (PAW) and plasma-activated fine bubble water (PAFBW) treatments significantly affected ($p < 0.001$) weight loss and color change (ΔE) of fresh-cut lettuce. Storage temperature was the dominant factor for these parameters, with 15 °C consistently outperforming 25 °C. Descriptively, PAW yielded better or comparable results to PAFBW on TPC and total phenolic content. The best overall treatment was PAW at 2 ppm and 2-min immersion at 15 °C, producing weight loss of 0.87%, ΔE of 0.40 and the lowest microbial load (17,000 CFU/g). Meanwhile, the highest total phenolic content (6.98 mg GAE/g) was achieved using PAW at 1 ppm with 2-min immersion. PAFBW at 2 ppm with 2-min immersion produced the most favorable qualitative organophosphate screening response. This finding indicates potential for residue reduction but does not establish compound-specific degradation or compliance with maximum residue limits. Antimicrobial efficacy increased with both ozone concentration and immersion time. These findings imply that plasma-activated washing is a promising postharvest technology for fresh-cut lettuce; however, optimization of fine bubble parameters, longer storage trials, and quantitative pesticide analysis are required to confirm and extend these findings.

Available online:
http://journal.ipb.ac.id/index.php/jtep
DOI: 10.19028/jtep.014.2.318-334

5. Acknowledgement

This research was funded by the Ministry of Higher Education, Science, and Technology (Kemendiktisaintek) through the Student Thesis Research grant (contract No. 006/C3/DT.05.00/PL/2025) and the Indonesia Endowment Fund for Education Agency (LPDP), Ministry of Finance of the Republic of Indonesia.

6. AI Writing Statement

The authors utilized ChatGPT (version 5.5) and Claude (Sonnet 5) during the initial stages to assist in generating a structural outline and exploring relevant literature. However, the entire final draft, data analysis, and data interpretation were exclusively authored by the researchers. The authors assume full responsibility for the accuracy, originality, and integrity of the article's content.

7. References

Agusta, W. (2022). Pengaruh teknik pencucian dan suhu ruang terhadap kualitas selada (Lactuca sativa L.) selama penyimpanan. Jurnal Agribisnis Perikanan, 14(2), 416–426. https://doi.org/10.52046/agrikan.v14i2.416-426

Ahmadian, S., Kenari, R. E., Amiri, Z. R., Sohbatzadeh, F., & Khodaparast, M. H. H. (2023). Effect of ultrasound-assisted cold plasma pretreatment on cell wall polysaccharides distribution and extraction of phenolic compounds from hyssop. International Journal of Biological Macromolecules, 233, 123557. https://doi.org/10.1016/j.ijbiomac.2023.123557

Alen, Y., & Suharti, N. (2015). Pemeriksaan residu pestisida profenofos pada selada (Lactuca sativa L.) dengan metode kromatografi gas. Jurnal Sains Farmasi & Klinis, 1(2), 140–149.

Anastassiades, M., Lehotay, S. J., Stajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International, 86(2), 412-431. https://doi.org/10.1093/jaoac/86.2.412

Avram, C. V., Molnar, C., & Covaciu, F. D. (2025). Integrated UHPLC-PDA and GC-ECD approaches for detecting pesticide residues in frozen fruits and vegetables. Journal of Food Composition and Analysis, 145, 107715. https://doi.org/10.1016/j.jfca.2025.107715

Bruggeman, P. J., Kushner, M. J., Locke, B. R., Gardeniers, J. G. E., Graham, W. G., Graves, D. B., et al. (2016). Plasma–liquid interactions: A review and roadmap. Plasma Sources Science and Technology, 25(5), 053002. https://doi.org/10.1088/0963-0252/25/5/053002

Callejón, R. M., Rodríguez-Naranjo, M. I., Ubeda, C., Hornedo-Ortega, R., García-Parrilla, M. C., & Troncoso, A. M. (2015). Reported foodborne outbreaks due to fresh produce in the United States and European Union. Foodborne Pathogens and Disease, 12(1), 32–38. https://doi.org/10.1089/fpd.2014.1821

Available online:
http://journal.ipb.ac.id/index.php/jtep
DOI: 10.19028/jtep.014.2.318-334

Castro-Ibáñez, I., Gil, M. I., & Allende, A. (2017). Ready-to-eat vegetables: Current problems and potential solutions to reduce microbial risk in the production chain. LWT, 85, 284–292. https://doi.org/10.1016/j.lwt.2016.11.073

Cheng, J. H., He, L., Sun, D. W., Pan, Y., & Ma, J. (2023). Inhibition of cell wall pectin metabolism by plasma activated water to maintain firmness and quality of postharvest blueberry. Plant Physiology and Biochemistry, 201, 107803. https://doi.org/10.1016/j.plaphy.2023.107803

Dantas, A. M., Batista, J. D. F., Lima, M. D. S., Fernandes, F. A. N., Rodrigues, S., Magnani, M., & Borges, G. (2021). Effect of cold plasma on açai pulp: Enzymatic activity, color and bioaccessibility of phenolic compounds. LWT, 149, 111883. https://doi.org/10.1016/j.lwt.2021.111883

Eckardt, N. A. (2009). A new chlorophyll degradation pathway. The Plant Cell, 21(3), 700. https://doi.org/10.1105/tpc.109.210313

Fagundes, C., Carciofi, B. A. M., & Monteiro, A. R. (2013). Estimate of respiration rate and physicochemical changes of fresh-cut apples stored under different temperatures. Food Science and Technology, 33(1), 60–67.

Fan, K., Zhang, M., Bhandari, B., & Jiang, F. (2019). A combination treatment of ultrasound and ε-polylysine to improve microorganisms and storage quality of fresh-cut lettuce. LWT, 113, 108315. https://doi.org/10.1016/j.lwt.2019.108315

Gao, Y., Li, M., Sun, C., & Zhang, X. (2022). Microbubble-enhanced water activation by cold plasma. Chemical Engineering Journal, 446, 137318. https://doi.org/10.1016/j.cej.2022.137318

Gorski, L., Cooley, M. B., Oryang, D., Carychao, D., Nguyen, K., Luo, Y., et al. (2022). Prevalence and clonal diversity of Listeria monocytogenes isolates collected from public access waters near produce production areas. Applied and Environmental Microbiology, 88(8), e00357-22. https://doi.org/10.1128/aem.00357-22

Herman, K. M., Hall, A. J., & Gould, L. H. (2015). Outbreaks attributed to fresh leafy vegetables, United States, 1973–2012. Epidemiology and Infection, 143(14), 3011–3021. https://doi.org/10.1017/S0950268815000047

Iakimova, E. T., & Woltering, E. J. (2015). Nitric oxide prevents wound-induced browning and delays senescence in fresh-cut lettuce. Innovative Food Science & Emerging Technologies, 30, 157–169. https://doi.org/10.1016/j.ifset.2015.06.001

ISO. (2017). Fine bubble technology — General principles for usage and measurement of fine bubbles — Part 1: Terminology (ISO 20480-1:2017). International Organization for Standardization.

Kim, M. J., Moon, Y., Tou, J. C., Mou, B., & Waterland, N. L. (2016). Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). Journal of Food Composition and Analysis, 49, 19–34. https://doi.org/10.1016/j.jfca.2016.03.004

Available online:
http://journal.ipb.ac.id/index.php/jtep
DOI: 10.19028/jtep.014.2.318-334

Li, W., Chen, J., Linli, F., Wang, X., Hu, S., & Zhao, L. (2023). Organophosphorus pesticide contaminants in fruits and vegetables: A review. Food Chemistry: X, 20, 101014. https://doi.org/10.1016/j.fochx.2023.101014

Lukes, P., Dolezalova, E., Sisrova, I., & Clupek, M. (2014). Aqueous-phase chemistry and bactericidal effects from an air discharge plasma in contact with water. Plasma Sources Science and Technology, 23(1), 015019. https://doi.org/10.1088/0963-0252/23/1/015019

Malangngi, L. P., Sangi, M. S., & Paendong, J. J. E. (2012). Penentuan kandungan tanin dan uji aktivitas antioksidan ekstrak biji buah alpukat. Jurnal MIPA Unsrat Online, 1(1), 5–10.

Małajowicz, J., Khachatryan, K., & Kozłowska, M. (2022). Properties of water activated with low-temperature plasma in the context of microbial activity. Beverages, 8(4), 63. https://doi.org/10.3390/beverages8040063

Misra, N. N., Pankaj, S. K., Segat, A., & Ishikawa, K. (2016). Cold plasma interactions with enzymes in foods and model systems. Trends in Food Science & Technology, 55, 39–47. https://doi.org/10.1016/j.tifs.2016.07.001

Nemati, V., Sadeghi, A., & Mozafarpour, R. (2024). Plasma-activated water and microbubble as postharvest technology: Effects on E. coli O157:H7 reduction, functional and storage quality of romaine lettuce. Journal of Agriculture and Food Research, 17, 101259. https://doi.org/10.1016/j.jafr.2024.101259

Nikzad, N., & Parastar, H. (2021). Evaluation of organic pollutants effect on antioxidant activity and total phenolic content of lettuce using UV-Vis spectrophotometry. Microchemical Journal, 170, 106632. https://doi.org/10.1016/j.microc.2021.106632

Nikzadfar, M., Kazemi, A., Abooei, R., Abbaszadeh, R., Firouz, M. S., Akbarnia, A., & Rashvand, M. (2024). Application of cold plasma technology on the postharvest preservation of in-packaged fresh fruit and vegetables. Food and Bioprocess Technology, 17(12), 4473–4505.

Oehmigen, K., Hähnel, M., Brandenburg, R., Wilke, C., Weltmann, K. D., & von Woedtke, T. (2010). The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. Plasma Processes and Polymers, 7(3–4), 250–257. https://doi.org/10.1002/ppap.200900077

Patange, A., Lu, P., Boehm, D., Cullen, P. J., & Bourke, P. (2019). Efficacy of cold plasma functionalised water for improving microbiological safety of fresh produce and wash water recycling. Food Microbiology, 84, 103226. https://doi.org/10.1016/j.fm.2019.05.010

Rathore, V., Jamnapara, N. I., & Nema, S. K. (2023). Enhancing the physicochemical properties and reactive species concentration of plasma activated water using an air bubble diffuser. Physics Letters A, 482, 129035. https://doi.org/10.1016/j.physleta.2023.129035

Rathore, V., Patil, C., & Nema, S. K. (2024). Production of large quantity of plasma activated water using multiple plasma device setup. Current Applied Physics, 61, 121–129. https://doi.org/10.1016/j.cap.2024.03.002

Available online:
http://journal.ipb.ac.id/index.php/jtep
DOI: 10.19028/jtep.014.2.318-334

Rothwell, J. G., Hong, J., Morrison, S. J., Nilesh, K., & Xia, B. (2023). An effective sanitizer for fresh produce production: In situ plasma-activated water treatment inactivates pathogenic bacteria. Microbiology Spectrum, 11(4), e00523-23.

Shatilov, M. V., Razin, A. F., & Ivanova, M. I. (2019). Analysis of the world lettuce market. IOP Conference Series: Earth and Environmental Science, 395(1), 012053. https://doi.org/10.1088/1755-1315/395/1/012053

Singh, S. P., & Thakur, R. (2024). Postharvest applications of cold plasma treatment for improving food safety and sustainability. Postharvest Biology and Technology, 209, 112694. https://doi.org/10.1016/j.postharvbio.2023.112694

Sojithamporn, P., Leksakul, K., Sawangrat, C., Charoenchai, N., & Boonyawan, D. (2023). Degradation of pesticide residues in water, soil, and food products via cold plasma technology. Foods, 12(24), 4386. https://doi.org/10.3390/foods12244386

Teng, Z., Luo, Y., Bornhorst, E. R., Zhou, B., Simko, I., & Trouth, F. (2019). Identification of romaine lettuce cultivars with reduced browning for fresh-cut processing. Postharvest Biology and Technology, 156, 110931. https://doi.org/10.1016/j.postharvbio.2019.110931

Tiwari, S., Patel, A., & Kumar, N. (2025). Ultrafine bubbles for enhanced stability of reactive species in plasma-activated water. Journal of Physics D: Applied Physics, 58(3), 035201.

Wang, J. L., Xia, Q., Zhang, A. P., Hu, X. Y., & Lin, C. M. (2012). Determination of organophosphorus pesticide residues in vegetables by an enzyme inhibition method using alpha-naphthyl acetate esterase extracted from wheat flour. Journal of Zhejiang University-SCIENCE B, 13, 267-273. https://doi.org/10.1631/jzus.B11a0180

Wang, J., Wang, L., Maimaitiyiming, R., & Aihaiti, A. (2023). Comparison of plasma-activated water and free chlorine in disinfecting E. coli O157:H7 and Salmonella Typhimurium inoculated on fresh produce. LWT, 187, 115384. https://doi.org/10.1016/j.lwt.2023.115384

Yun, Z., Gao, H., Chen, X., Duan, X., & Jiang, Y. (2021). Effects of hydrogen water treatment on antioxidant system of litchi fruit during the pericarp browning. Food Chemistry, 336, 127618. https://doi.org/10.1016/j.foodchem.2020.127618

Zhou, R., Zhou, R., Wang, P., Xian, Y., Mai-Prochnow, A., Lu, X., Cullen, P. J., Ostrikov, K., & Bazaka, K. (2019). Degradation of pesticide residues on fresh produce using cold plasma: Mechanisms and application. Trends in Food Science & Technology, 85, 112–120.