Effects of heating on the antibacterial efficacy and physicochemical properties of plasma-activated water

Bohua Wang[1,2†], Wenjie Wang[1,2†], Qisen Xiang[1,2], Yanhong Bai[1,2*]

[1]College of Food and Bioengineering, Zhengzhou University of Light Industry, Zhengzhou, China; [2]Henan Key Laboratory of Cold Chain Food Quality and Safety Control, Zhengzhou, China

[†]These authors contributed equally to this work.

***Corresponding author:** Yanhong Bai, College of Food and Bioengineering, Zhengzhou University of Light Industry, Zhengzhou, China. Email: baiyanhong212@163.com

Received: 25 April 2022; Accepted: 3 October 2022; Published: 9 March 2023

RESEARCH ARTICLE

Abstract

Plasma-activated water (PAW), which is the water treated by cold plasma, represents a promising strategy for food decontamination. However, studies of the influences of heating on the antibacterial efficacy and physicochemical characteristics of PAW are limited. Therefore, the present work is aimed at determining the effect of heating on the bactericidal effects and physicochemical properties of PAW. PAW (1.0 mL) was heated in a water bath at 30–80°C for 10 min. After being cooled to room temperature, the antibacterial efficacy and physicochemical properties of PAW were measured. Heating at 40–80°C for 10 min caused a significant decrease in the antibacterial activity of PAW against *Listeria monocytogenes* and *Salmonella typhimurium*. After heating at 40–80°C for 10 min, the pH value and oxidation reduction potential (ORP) of PAW remained stable, and the level of nitrate and electrical conductivity of PAW remarkably increased, while hydrogen peroxide and nitrite contents significantly decreased. The combination treatment of PAW and mild heating (40–60°C for 4 min) showed greater antibacterial effect on *L. monocytogenes* and *S. typhimurium*. After the combined treatment of PAW with mild heating at 60°C for 4 min, the populations of *L. monocytogenes* and *S. typhimurium* decreased by 7.83 $\log_{10}$ CFU/mL and 9.35 $\log_{10}$ CFU/mL, respectively, which were significantly higher than that caused by PAW at 25°C or mild heating at 60°C alone. In summary, the antibacterial activity of PAW is significantly affected by the treatment temperature. This work provides a basis for the practical application of PAW in the food industry.

Keywords: plasma-activated water; mild heating; antibacterial activity; synergistic effect

Introduction

Microbiological contamination may occur at various points along the food chain, including production, processing, storage, distribution, and preparation. Spoilage microorganisms may result in the loss of nutritional and sensory properties of food products, such as discoloration, unpleasant odor, and off-flavor (Odeyemi *et al*., 2020). It is estimated that a significant portion of the total fruits and vegetables is wasted each year worldwide due to the microbial and biochemical spoilage of food (Alegbeleye *et al*., 2022), which causes immense economic losses for both producers and retailers. In addition, foodborne pathogens have become a serious public health threat in the last few years. The most common pathogenic microorganisms include *Escherichia coli*, *L. monocytogenes*, *Bacillus cereus*, *Clostridium botulinum*, *Staphylococcus aureus*, *Campylobacter jejuni*, and *Salmonella* spp. (Bintsis 2017). As noted by the World Bank, foodborne disease causes total economic loss of up to $95.2 billion within low- and middle-income countries, and the annual cost of curing foodborne illnesses is estimated at

ISSN 1757-837X online, DOI 10.15586/qas.v15i2.1115

$15 billion (James and Segovia, 2020). Therefore, microbial contamination is a challenging and significant issue for the food industry.

In recent years, various processing techniques have been applied to ensure the microbiological quality and safety of food products. Thermal sterilization has been one of the most widely utilized methods to achieve long-term shelf stability of food products by inactivating microorganisms and endogenous enzymes (Dong *et al*., 2021; Gavahian *et al*., 2020; Wu *et al*., 2020). However, conventional thermal processing operations also lead to the loss of heat-sensitive nutrients (such as vitamins and phenolic antioxidants) and the generation of potential toxic compounds such as acrylamide, furan, and acrolein, resulting in lowered nutritional value and sensory quality of food products (van Boekel *et al*., 2010).

In recent years, with an increase of consumers' demands for fresh and high-quality foods, nonthermal processing techniques, mainly pulsed electric fields, ultrasound, high hydrostatic pressure, cold plasma, and high-pressure carbon dioxide, have drawn considerable attention within the food industry (Anbarasan *et al*., 2022; Niveditha *et al*., 2021; Sruthi *et al*., 2022; Wu *et al*., 2020). Plasma-activated water (PAW), also known as plasma-treated water (PTW), is obtained by nonthermal plasma discharge over or in distilled water (Xiang *et al*., 2020). PAW exhibits excellent antimicrobial activity against various bacteria, yeast, mold, and viruses (Thirumdas *et al*., 2018). Because of its antibacterial efficacy and eco-friendly nature, PAW has been successfully applied to improve the microbial safety and quality of various food products, such as fruits, vegetables, meat and fish products, edible mushrooms, fish, eggs, and cereal products (Liao *et al*., 2019; Rahman *et al*., 2022; Xiang *et al*., 2019b). It has been reported that the antibacterial efficacy of PAW is influenced by the plasma discharge type, working gas, plasma activation time, and treatment time (Shaw *et al*., 2018; Xiang *et al*., 2018; Xu *et al*., 2016). In addition, certain environmental factors also affect the antimicrobial properties of PAW, such as organic matter (Xiang *et al*., 2019a) and temperature (Shen *et al*., 2016). Shen *et al*. (2016) discovered that the antibacterial activity of PAW against *S. aureus* decreased remarkably with increasing storage temperature (−80, −20, 4, and 25 °C). Similar findings were also observed by Tsoukou *et al*. (2020), who determined that storing PAW and plasma-activated saline at −80 or −150°C provided a better means to retain their bactericidal activity than higher temperatures (−16°C, 4°C, and room temperature) over long-term storage.

The above data suggested that temperature may affect the antimicrobial efficacy of PAW. However, to the best of our knowledge, the changes in the antibacterial and physicochemical properties of PAW after heating at different temperatures have not been well examined. Therefore, the current work aimed to investigate the influences of heating (30–80°C) on the antibacterial effects and physicochemical properties of PAW. In addition, the antimicrobial efficacy of PAW in combination with mild heating (40, 50, and 60°C) against *L. monocytogenes* and *S. typhimurium* was also investigated.

Materials and methods

Bacterial strains and chemicals

L. monocytogenes strain American Type Culture Collection (ATCC) 15313 used in this study was obtained from the ATCC. *S. typhimurium* strain China Center of Industrial Culture Collection (CICC) 21484 was purchased from the CICC (Beijing, China). Tryptic soy agar (TSA) and tryptic soy broth (TSB) were provided by Aobox Biotechnology Co., Ltd. (Beijing, China).

Cultivation of strains

L. monocytogenes (Gram-positive) and *S. typhimurium* (Gram-negative) were individually grown on TSA plates for 24 h at 37°C. One single colony of each strain was inoculated into 50 mL of TSB and incubated at 37°C overnight with constant shaking (120 rpm). Then, the cells were harvested by centrifugation at 5000 × *g* for 10 min at 4°C and washed thrice with sterile sodium chloride solution (0.85%, w/v). The cell pellets were then resuspended in 30 mL of sterile saline (0.85% NaCl) at a final density of approximately 9 $\log_{10}$ CFU/mL.

Preparation of PAW

In this work, PAW was prepared with an atmospheric pressure plasma jet (APPJ) system based on gliding arc discharge in air (Xiang *et al*., 2019b). The distance between the atmospheric-pressure plasma jet nozzle and the water surface was 5 mm. Compressed air (approximately 0.18 MPa) was selected as the working gas at a flow rate of 30 L/min at the jet outlet. Every 200 mL of sterile distilled water (SDW) was activated by plasma (discharge power 750 W) for 60 s to acquire PAW.

Influences of heating on the antibacterial efficacy of PAW

As shown in Figure 1, an aliquot of PAW (1.0 mL) was put into microcentrifuge tubes, which were incubated in a shaking water bath for 10 min at different temperatures (30, 40, 50, 60, 70, and 80°C). After incubation, the PAW samples

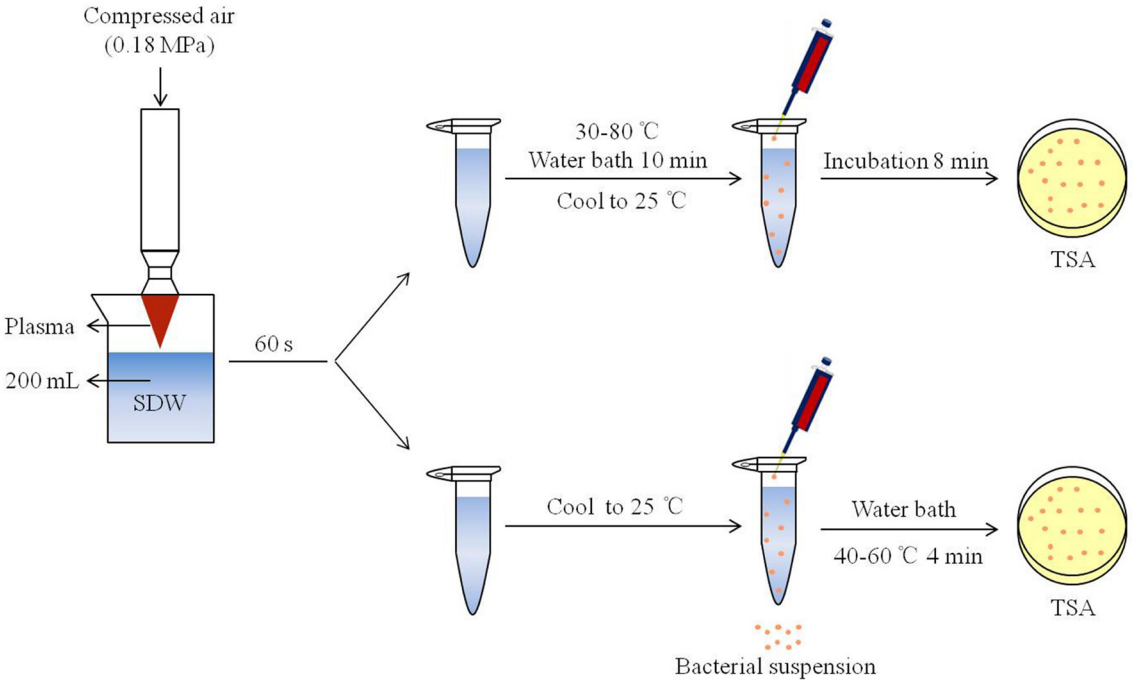

Figure 1. Schematic diagram of the plasma jet and experimental setup, including the generation of PAW and inactivation of PAW preheated or heated at different temperatures against bacteria. PAW, plasma-activated water; SDW, sterile distilled water.

were quickly cooled to room temperature (Xiang *et al.*, 2020). Thereafter, 100 µL of each bacterial suspension was violently mixed with 900 µL of the obtained PAW and then incubated for 8 min at ambient temperature (Xiang *et al.*, 2019a). Thereafter, the obtained mixtures were 10-fold serially diluted with sterile saline solution (0.85% NaCl). Then 100 µL of the appropriate dilution was spread plated onto TSA plates. Colonies on the plates were counted after incubation at 37°C for 24 h and the results were expressed as $\log_{10}$ CFU/mL. All of the tests were performed in triplicate.

Influences of heating on the physicochemical properties of PAW

An aliquot of PAW (1.0 mL) was put into the tubes and incubated in a shaking water bath for 10 min at different temperatures (30, 40, 50, 60, 70, and 80°C). Thereafter, the PAW samples were quickly cooled to room temperature and the main physicochemical properties of PAW were measured.

pH

The pH levels of PAW samples were measured using a SevenGo Duo pH Meter (Mettler-Toledo, Switzerland).

Oxidation reduction potential (ORP)

The ORP value was determined using a 501 rechargeable ORP composite electrode (INESA Scientific Instrument Co., Ltd., Shanghai, China) connected to a SevenGo Duo pH Meter.

Electrical conductivity

The electrical conductivity was measured using a conductivity meter (INESA Scientific Instrument Co., Ltd., Shanghai, China).

H_2O_2

The H_2O_2 concentrations of PAW samples were measured using a colorimetric detection kit (D799773-0050) according to the manufacturer's protocol (Sangon Biotech Shanghai Co., Ltd., Shanghai, China).

NO_3^-

The NO_3^- content of PAW samples was determined by ultraviolet absorption spectrometry at a wavelength of 220 nm (Shen *et al.*, 2016).

NO_2^-

The NO_2^- contents of PAW samples were measured with a colorimetric assay kit (D799311-0050, Sangon Biotech Shanghai Co., Ltd., Shanghai, China) according to the manufacturer's instructions.

Antibacterial activity of PAW combined with mild heating

L. monocytogenes and *S. typhimurium* were selected to investigate the bactericidal efficacy of PAW in combination with mild heating. For PAW treatment alone, 900 µL of fresh PAW was mixed with 100 µL of bacterial suspension and incubated at 25°C for 4 min in a shaking

water bath. For mild heating treatment alone, 900 µL of sterile saline solution was mixed with 100 µL of bacterial suspension and then incubated in a shaking water bath at 40, 50, and 60°C for 4 min, respectively. For the combined treatments, 900 µL of PAW was blended with 100 µL of bacterial suspension and then incubated in a shaking water bath at 40, 50, and 60°C for 4 min, respectively. Then, the mixtures were immediately cooled to room temperature and the bacterial viability was evaluated with the previously described plating method. The SDW-treated cells served as the control.

Statistical analysis

All analyses were performed in at least triplicate. Data are presented as the mean ± standard deviation (SD) and were analyzed using SPSS version 24.0 for Windows (IBM SPSS Inc., Chicago, IL, USA). Statistically significant differences were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test with a level of $P < 0.05$.

Results and discussion

Influence of heating treatment on the inactivation efficacy of PAW

The influences of mild heating on the inactivation efficacy of PAW against *L. monocytogenes* and *S. typhimurium* are illustrated in Figure 2. The initial populations of *L. monocytogenes* and *S. typhimurium* vegetative cells were 9.21 $\log_{10}$ CFU/mL and 9.28 $\log_{10}$ CFU/mL, respectively. After the treatment with PAW preheated at 30°C, the population of *L. monocytogenes* decreased significantly by 1.54 $\log_{10}$ CFU/mL ($P < 0.05$). The heating treatment (40–80°C for 10 min) caused a significant decrease in the antimicrobial activity of PAW against *L. monocytogenes* (Figure 2A). After the treatments of PAW preheated at 40, 50, 60, 70, and 80°C, the populations of *L. monocytogenes* were markedly reduced by 1.40-, 1.17-, 1.20-, 1.02-, and 0.85-log values, respectively, which were lower than that of PAW preheated at 30°C ($P < 0.05$). A similar trend was also observed for *S. typhimurium* (Figure 2B). These data show that the heating treatment at 40–80°C for 10 min causes a significant decrease in the inactivation efficacy of PAW against *L. monocytogenes* and *S. typhimurium*. These data are consistent with previous findings of Shen *et al*. (2016) and Tsoukou *et al*. (2020) that PAW exhibits higher antibacterial activity when stored at low temperature.

Effect of heating treatment on the pH values of PAW

The influences of heating treatment on the antimicrobial activity of PAW may be attributed to the changes in the physicochemical properties of PAW. The acidification of plasma-treated liquid has been previously reported, and acidic pH is considered to play a crucial role in the bactericidal action of PAW (Thirumdas *et al*., 2018). As seen in Figure 3A, the pH value of PAW was 3.17 after plasma activation for 60 s, which was significantly ($P < 0.05$) lower than the 6.20 of SDW. The acidification of plasma-

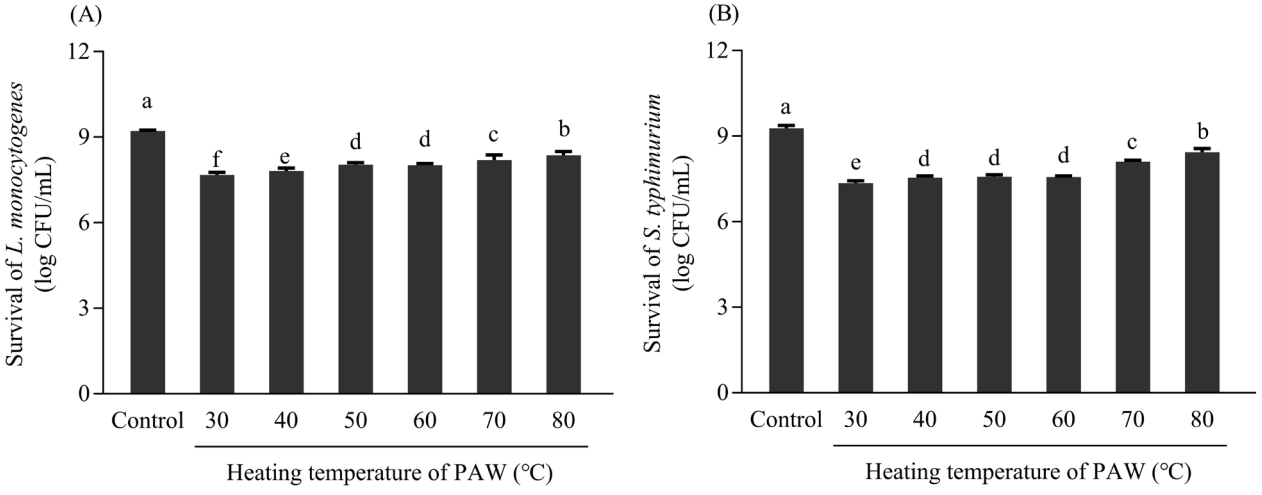

Figure 2. Influences of heating treatment on the inactivation efficacy of PAW against *L. monocytogenes* (A) and *S. typhimurium* (B). PAW samples were preheated at 30, 40, 50, 60, 70, and 80°C for 10 min. Then the antibacterial activity of these PAW samples was investigated as described in the Materials and Methods section. The SDW-treated cells served as the control. Values with different lowercase letters are significantly different ($P < 0.05$). PAW, plasma-activated water; SDW, sterile distilled water.

treated solution is mostly attributed to the formation of NO_3^-, NO_2^-, and peroxynitrites from reactive nitrogen species (RNS) during plasma discharge (Liao *et al*., 2018; Thirumdas *et al*., 2018). After preheating at 30°C for 10 min, the pH value of PAW increased to 3.23. As shown in Figure 3A, there were no significant changes in the pH values of PAW samples after the heating treatment at 30–80°C for 10 min ($P > 0.05$). A similar finding has been reported in our previous study (Xiang *et al*., 2020), in which the pH values of PAW remained essentially stable after mild heating at 25–55°C for 30 min.

Influence of heating treatment on the ORP values of PAW

ORP is a measure of the ability of a solution to oxidize or reduce another substance and is related to the concentration of oxidizers as well as their activity and strength (Thirumdas *et al*., 2018). As presented in Figure 3B, after plasma activation for 60 s, the ORP value of SDW was 298.00 mV, and the ORP value of PAW was 571.83 mV ($P < 0.05$). Similar findings were reported by Joshi *et al*. (2018) that the ORP of PAW was 534.52 mV, which was significantly higher than the ORP of distilled water of 376.54 mV. According to previous reports, the higher ORP value of PAW is associated with the production of reactive species and plays a crucial role in the antibacterial action of PAW (Thirumdas *et al*., 2018). Thus, ORP serves to be a good indicator for the efficacy of PAW and its antimicrobial capacity (Joshi *et al*., 2018). As shown in Figure 3B, no significant changes in the ORP values of PAW were observed after heating at 30–80°C for 10 min ($P > 0.05$). Similar findings have been reported in our previous study (Xiang *et al*., 2020) and elsewhere (Shen *et al*., 2016). For example, there was no obvious change in the ORP of PAW samples stored at −80, −20, 4, or 25°C for up to 30 days (Shen *et al*., 2016).

Influence of heating treatment on the electrical conductivity of PAW

Electrical conductivity is commonly used to assess the ability of a liquid to conduct an electrical current and is directly related to the concentration of ions in the solution. As shown in Figure 3C, the electrical conductivity of PAW was 409.00 μS/cm after plasma activation for 60 s, which was significantly higher than the 4.22 μS/cm of SDW ($P < 0.05$). The increase in the conductivity of PAW may be attributed to the formation of active ions in water during plasma activation (Joshi *et al*., 2018; Suwal *et al*., 2019). Similarly, Sergeichev *et al*. (2021) also found a significant increase in the electrical conductivity of water after microwave discharge plasma exposure. Preheating at 30 or 40°C for 10 min caused no

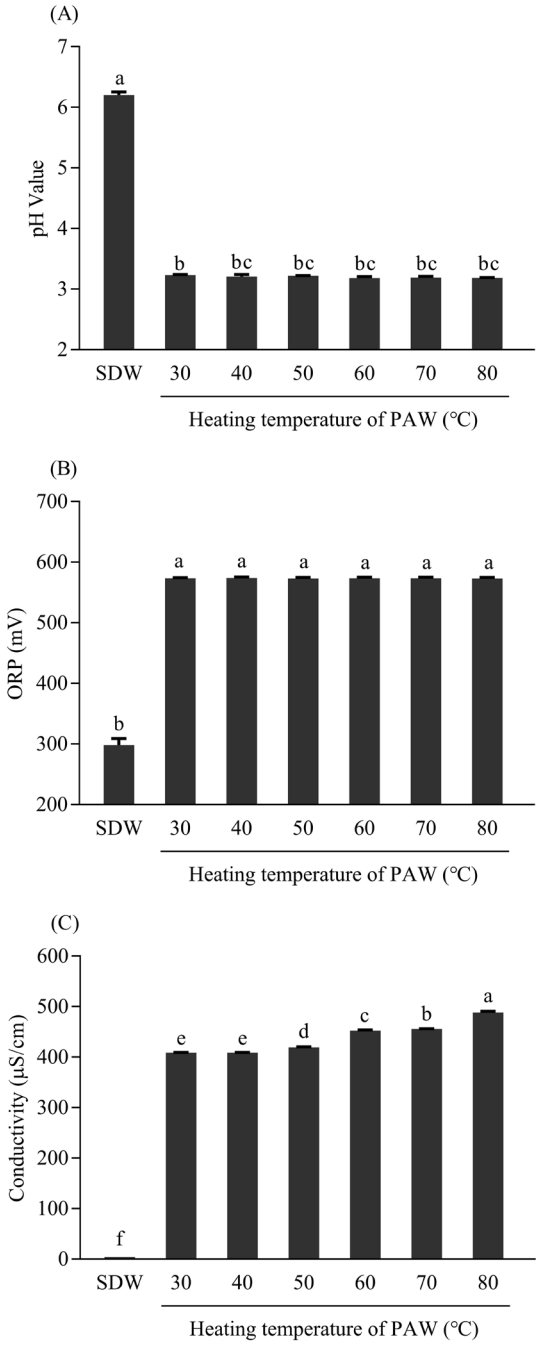

Figure 3. Influence of heating treatment on the pH (A), ORP (B), and electrical conductivity (C) of PAW. PAW samples were preheated at 30, 40, 50, 60, 70, and 80°C for 10 min. Values with different lowercase letters are significantly different ($P < 0.05$). ORP, oxidation reduction potential; PAW, plasma-activated water; SDW, sterile distilled water.

significant changes in the electrical conductivity of PAW ($P > 0.05$). However, an increase in the electrical conductivity of PAW was noticed after the heating treatment at 40–80°C for 10 min (Figure 3C). The electrical conductivity of PAW was increased after heating treatment at

40–80°C for 10 min ($P < 0.05$). In addition to the amount and composition of ionic species, the temperature also affects the electrical conductivity of a solution (Zhang *et al*., 2020b). At elevated temperatures, the ionization and the mobility speeds of the ions in solution may be accelerated, leading to an increase in their conductivity. Further investigations are still needed to understand the role of electrical conductivity in the antibacterial efficacy of PAW.

Effect of heating treatment on reactive species levels of PAW

Various reactive oxygen and nitrogen species (RONS) are generated in solutions during plasma activation, including H_2O_2, nitrite, nitrate, ozone, hydroxyl radicals, and superoxide anions (Thirumdas *et al*., 2018). As depicted in Table 1, the concentration of H_2O_2 in PAW was 38.13 μmol/L after 60 s of plasma activation, which was in agreement with previous studies (Sergeichev *et al*., 2021; Shen *et al*., 2016). Shen *et al*. (2016) observed that the H_2O_2 concentrations of PAW stored at −80 °C did not vary significantly over 30 days. In contrast, the H_2O_2 levels in PAW samples decreased to 6.0 μmol/L from approximately 24.4 μmol/L after 30-day storage at −20, 4, and 25°C (Shen *et al*., 2016). H_2O_2, a long-lived powerful oxidizing agent, is thought to be involved in the antimicrobial action of PAW. As one of the main reactive oxygen species in PAW, H_2O_2 is thought to be mainly generated by the following chemical reactions at the gas/liquid interface (Thirumdas *et al*., 2018).

$$H_2O + e^- \rightarrow H\bullet + \bullet OH + e^- \quad (1)$$

$$H_2O + e^- \rightarrow H^+ + \bullet OH + 2e^- \quad (2)$$

$$\bullet OH + \bullet OH \rightarrow H_2O_2 \quad (3)$$

As displayed in Table 1, the H_2O_2 levels of PAW decreased following heating treatment at 30–80°C for 10 min, which was in accordance with the decreasing trend of bactericidal activity (Figure 2). The concentration of H_2O_2 decreased from 38.13 to 29.00 μmol/L as the heating temperature increased from 30 to 80°C. As a reactive molecule, H_2O_2 is unstable and decomposes exothermally into water and oxygen gas by the following reaction:

$$2\,H_2O_2(aq) \rightarrow 2\,H_2O(l) + O_2(g) \quad (4)$$

Heating, light, and catalysts can accelerate this decomposition process (Anikin *et al*., 2022). Therefore, it can be speculated that the decrease in the bactericidal efficacy of PAW may be related to the demonstration of H_2O_2 accelerated by the heating treatment.

RNS, including NO_3^-, NO_2^-, and peroxynitrites, are also thought to play significant roles in the antimicrobial properties of PAW (Schnabel *et al*., 2014; Thirumdas *et al*., 2018). As shown in Table 1, the NO_3^- and NO_2^- levels of PAW increased to 921.18 and 812.29 μmol/L, respectively, after plasma treatment for 60 s. NO_3^- and NO_2^- in PAW are generated by the dissolution of nitrogen oxides formed by gas-phase reactions of N_2 and O_2 or H_2O in the air plasma (Pavlovich *et al*., 2014).

$$N_2(g) + O_2(g) \rightarrow 2NO(g) \quad (5)$$

$$2NO(g) + O_2(g) \rightarrow 2NO_2(g) \quad (6)$$

$$NO(g) + O_3(g) \rightarrow NO_2(g) + O_2(g) \quad (7)$$

$$2NO_2(g) + H_2O(l) \rightarrow NO_2^- + NO_3^- + 2H^+ \quad (8)$$

$$NO_2^- + H_2O_2 + H^+ \rightarrow NO_3^- + H_2O + H^+ \quad (9)$$

The heating treatment also caused remarkable changes in the levels of NO_3^- and NO_2^- in PAW. After incubation at indicated temperature (30–80°C) for 10 min, the NO_3^- amounts in PAW increased remarkably in a

Table 1. Influences of heating treatment on the H_2O_2, NO_3^-, and NO_2^- levels of PAW.

Group	Heating temperature (°C)	H_2O_2 (μmol/L)	NO_3^- (μmol/L)	NO_2^- (μmol/L)
SDW	30	ND	ND	ND
PAW	–	38.13 ± 0.48^a	921.18 ± 6.37^e	812.29±2.95^a
PAW	30	38.13 ± 0.85^a	932.00 ± 10.03^e	809.22 ± 4.37^a
PAW	40	36.38 ± 0.48^b	1002.53 ± 5.29^d	768.60 ± 9.39^b
PAW	50	33.50 ± 0.41^c	1012.47 ± 11.60^d	738.57 ± 9.52^c
PAW	60	33.38 ± 0.75^c	1072.63 ± 7.90^c	738.23 ± 4.37^c
PAW	70	31.88 ± 0.48^d	1116.47 ± 8.01^b	659.04 ± 6.54^d
PAW	80	29.00 ± 0.41^e	1135.29 ± 17.56^a	642.32 ± 5.05^e

–, indicates PAW was not heated. Means followed by different letters in the same column are statistically different ($P < 0.05$) by the Duncan's multiple range test. ND, not detected. PAW, plasma-activated water; SDW, sterile distilled water.

temperature-dependent manner (Table 1). As the heating temperature increased from 30 to 80°C, the content of NO_3^- increased from 932.00 to 1135.29 μmol/L. Similar findings were reported in previous studies, which showed that the NO_3^- concentration of PAW increased in a temperature-dependent manner following mild heating at 25–55°C for 30 min (Xiang *et al*., 2020). In addition, Shen *et al*. (2016) reported that the NO_3^- in PAW was more suitable during storage at higher temperatures, with 25°C > 4°C > −20°C > −80°C obtained in descending order for the NO_3^- contents in PAW. In contrast with the changing tendency of NO_3^-, the levels of NO_2^- in PAW decreased remarkably with increasing heating temperatures from 30 to 80°C (Table 1). The significant changes in the NO_2^- and NO_3^- levels may contribute to the decrease in the antibacterial activity of PAW (Figure 2).

In this work, only the contents of long half-life reactive species (H_2O_2, NO_3^-, and NO_2^-) in PAW were measured after heating treatment. Many highly reactive species with short lifetimes may also be involved in the antimicrobial action of PAW. Therefore, in future studies, more attention should be paid to the stability of the short half-life of reactive species in PAW during heating treatment or storage.

Synergistic antibacterial efficacy of PAW with mild heating against bacteria

The antibacterial activity of PAW combined with mild heating (40, 50, and 60°C) against *L. monocytogenes* and *S. typhimurium* was also investigated. As exhibited in Figure 4, *L. monocytogenes* and *S. typhimurium* decreased by 0.83- and 1.29-log values, respectively, after PAW treatment alone at 25°C for 4 min. The population of *L. monocytogenes* decreased by 0.01, 0.30, and 2.06 $\log_{10}$ CFU/mL following the mild heat treatments at 40, 50, and 60°C for 4 min, respectively. Although mild heating decreased the antibacterial capacity of PAW (Figure 2), the combined treatment of PAW and mild heating showed a greater antibacterial effect against *L. monocytogenes* than any other single treatment. As indicated in Figure 4A, the populations of *L. monocytogenes* were reduced by 3.55, 6.61, and 7.83 $\log_{10}$ CFU/mL, followed by the PAW treatment combined with mild heating at 40, 50, and 60°C for 4 min, respectively. The enhanced antibacterial efficacy of PAW combined with mild heating was also observed for *S. typhimurium* (Figure 4B). Similarly, the populations of *S. typhimurium* cells were reduced by 4.70 and 7.35 $\log_{10}$ CFU/mL, respectively, after PAW treatment combined with mild heating at 40 and 50°C for 4 min. When treated with PAW at 60°C, the population of *S. typhimurium* was reduced from approximately 9.35 $\log_{10}$ CFU/mL to an undetectable level (Figure 4B). Similar findings were also reported in previous studies (Bai *et al*., 2020; Choi *et al*., 2019; Liao *et al*., 2020; Zhang *et al*., 2020a). For instance, PAW exhibited greater antimicrobial activity against *B. cereus* spores at higher temperatures (Bai *et al*., 2020). Zhang *et al*. (2020a) also found that *Saccharomyces cerevisiae* decreased by 4.40-log after the synergistic combination of PAW and mild heat at 50°C for 6 min, which was significantly higher than the individual treatments of PAW at 25°C (0.27-log) or mild heat at 50°C for 6 min (1.92-log).

Although preheating at 40–80°C for 10 min caused a significant decrease in the inactivation efficacy of

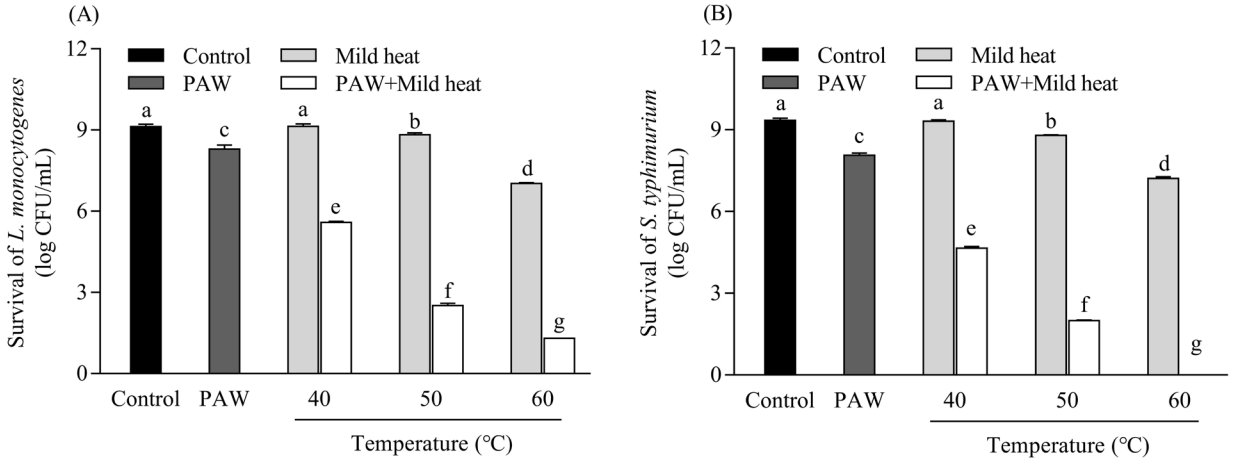

Figure 4. Inactivation of *L. monocytogenes* (A) and *S. typhimurium* (B) induced by PAW and mild heating, either alone or in combination for 4 min, respectively. Values with different superscript letters are significantly different ($P < 0.05$). PAW, plasma-activated water.

PAW, PAW combined with mild heating (40, 50, and 60°C) exhibited enhanced antibacterial activity against *L. monocytogenes* and *S. typhimurium* (Figure 4). Zhang *et al*. (2020a) found that the synergistic antifungal activity of PAW combined with mild heat against *S. cerevisiae* might be attributed to membrane damage, oxidative stress, and disruption of mitochondrial membrane potential. Liao *et al*. (2020) also observed the disruption of external structure and leakage of intracellular components in *B. cereus* spores after the combination treatment of PAW and mild heat. In the future, the mechanisms underlying the synergistic antimicrobial action of PAW and mild heating should be investigated with transcriptomic, proteomic, and metabolomic approaches. The practical application of PAW and mild heating has been reported in previous studies. For instance, the sequential application of PAW and mild heat was more effective in the inactivation of the naturally contaminating microorganisms as well as *L. monocytogenes* and *S. aureus* inoculated into salted kimchi cabbage (Choi *et al*., 2019). The combined treatment did not deteriorate the quality of salted Chinese cabbage, including the salinity, acidity, moisture content, and reducing sugar content. Similar findings were also reported by Liao *et al*. (2020) who found that PAW combined with mild heat (40 and 55°C) exhibited stronger inactivation efficacy against *B. cereus* spores in rice without adverse effects on the texture and sensory qualities of the rice after cooking. More attention should be paid to the application of this method in other perishable foods, such as fruits and vegetables. In addition, the influences of PAW combined with mild heat on the nutritional and sensory qualities of foods also need to be assessed in more detail.

Conclusion

In summary, heating treatment at 40–80°C for 10 min caused a significant decrease in the antibacterial efficacy of PAW against *L. monocytogenes* and *S. typhimurium*. Heating treatment caused significant changes in the physicochemical properties of PAW, mainly including electrical conductivity, H_2O_2, NO_2^-, and NO_3^-, which may contribute to a reduction in its antibacterial effect. These data suggest that temperature is one of the important factors affecting the antibacterial activity of PAW. Interestingly, however, PAW combined with mild heating (40–60°C for 4 min) exhibited enhanced antibacterial activity against *L. monocytogenes* and *S. typhimurium*. The results in this work provide a basis for the practical application of PAW in the food industry. In future studies, more attention should be paid to the application of this method to various foods and its influences on the nutritional and sensory qualities of foods. In addition, more research is needed to reveal the mechanisms underlying the synergistic antimicrobial action of PAW and mild heat.

Acknowledgments

The work was financially supported by the Henan Special Research Fund for Non Profit Sector (201300110100) and the National Natural Science Foundation of China (32072356).

Conflict of interest

The authors declare that there are no conflicts of interest.

References

Alegbeleye, O., Odeyemi, O.A., Strateva, M. and Stratev, D., 2022. Microbial spoilage of vegetables, fruits and cereals. Applied Food Research 2(1): 100122. https/doi.org/10.1016/j.afres.2022.100122

Anbarasan, R., Jaspin, S., Bhavadharini, B., Pare, A., Pandiselvam, R. and Mahendran, R., 2022. Chlorpyrifos pesticide reduction in soybean using cold plasma and ozone treatments. LWT-Food Science and Technology 159: 113193. https/doi.org/10.1016/j.lwt.2022.113193

Anikin, O.V., Bolotov, A.V., Minkhanov, I.F., Varfolomeev, M.A., Tazeev, A.R., Chalin, V.V., et al., 2022. Factors influencing hydrogen peroxide decomposition dynamics for thermochemical treatment of bottomhole zone. Journal of Petroleum Exploration and Production Technology 12: 2587–2598. https/doi.org/10.1007/s13202-022-01507-z

Bai, Y., Muhammad, A.I., Hu, Y.Q., Koseki, S., Liao, X.Y., Chen, S.G., et al., 2020. Inactivation kinetics of *Bacillus cereus* spores by plasma activated water (PAW). Food Research International 131: 109041. https/doi.org/10.1016/j.foodres.2020.109041

Bintsis, T., 2017. Foodborne pathogens. AIMS Microbiology 3(3): 529–563. https/doi.org/10.3934/microbiol.2017.3.529

Choi, E.J., Park, H.W., Kim, S.B., Ryu, S., Lim, J., Hong, E.J. et al., 2018. 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 98: 501–509. https://doi.org/10.1016/j.foodcont.2018.12.007

Dong, S.S., Ma, Y.F., Li, Y.F. and Xiang, Q.S., 2021. Effect of dielectric barrier discharge (DBD) plasma on the activity and structural changes of horseradish peroxidase. Quality Assurance and Safety of Crops & Foods 13(3): 92–101. https://doi.org/10.15586/qas.v13i3.934

Gavahian, M., Meng-Jen, T. and Khaneghah, A.M., 2020. Emerging techniques in food science: the resistance of chlorpyrifos pesticide pollution against arc and dielectric barrier discharge plasma. Quality Assurance and Safety of Crops & Foods 12(SP1): 9–17. https://doi.org/10.15586/qas.v12iSP1.807

James, H.S. and Segovia, M.S., 2020. Behavioral ethics and the incidence of foodborne illness outbreaks. Journal of Agricultural and Environmental Ethics 33(3–6): 531–548. https://doi.org/10.1007/s10806-020-09837-w

Joshi, I., Salvi, D., Schaffner, D.W. and Karwe, M.V., 2018. Characterization of microbial inactivation using plasma-activated

water and plasma-activated acidified buffer. Journal of Food Protection 81(9): 1472–1480. https://doi.org/10.4315/0362-028X.JFP-17-487

Liao, X.Y., Bai, Y., Muhammad, A.I., Liu, D.H., Hu, Y.Q. and Ding, T., 2020. The application of plasma-activated water combined with mild heat for the decontamination of *Bacillus cereus* spores in rice (*Oryza sativa* L. ssp. *japonica*). Journal of Physics D-Applied Physics 53(6): 064003. https://doi.org/10.1088/1361-6463/ab573a

Liao, X.Y., Su, Y., Liu, D.H., Chen, S.G., Hu, Y.Q., Ye, X.Q., et al., 2018. Application of atmospheric cold plasma-activated water (PAW) ice for preservation of shrimps (*Metapenaeus ensis*). Food Control 94: 307–314. https://doi.org/10.1016/j.foodcont.2018.07.026

Liao, X.Y., Xiang, Q.S., Cullen, P. J., Su, Y., Chen, S.G., Ye, X.Q., et al., 2019. Plasma-activated water (PAW) and slightly acidic electrolyzed water (SAEW) as beef thawing media for enhancing microbiological safety. LWT-Food Science and Technology 117: 108649. https://doi.org/10.1016/j.lwt.2019.108649

Niveditha, A., Pandiselvam, R., Prasath, V.A., Singh, SK., Gul, K. and Kothakota, A., 2021. Application of cold plasma and ozone technology for decontamination of *Escherichia coli* in foods – a review. Food Control 130: 108338. https://doi.org/10.1016/j.foodcont.2021.108338

Odeyemi, O.A., Alegbeleye, O.O., Strateva, M. and Stratev, D., 2020. Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. Comprehensive Reviews in Food Science and Food Safety 19(2): 311–331. https://doi.org/10.1111/1541-4337.12526

Pavlovich, M.J., Ono, T., Galleher, C., Curtis, B., Clark, D.S., Machala, Z. and Graves, D.B., 2014. Air spark-like plasma source for antimicrobial NOx generation. Journal of Physics D: Applied Physics 47(50): 505202. https://doi.org/10.1088/0022-3727/47/50/505202

Rahman, M., Hasan, M.S., Islam, R., Rana, R., Sayem, A., Sad, M.A.A., et al., 2022. Plasma-activated water for food safety and quality: a review of recent developments. International Journal of Environmental Research and Public Health 19(11): 6630. https://doi.org/10.3390/ijerph19116630

Schnabel, U., Andrasch, M., Weltmann, K.D. and Ehlbeck, J., 2014. Inactivation of vegetative microorganisms and *Bacillus atrophaeus* endospores by reactive nitrogen species (RNS). Plasma Processes and Polymers 11(11): 110–116. https://doi.org/10.1002/ppap.201300072

Sergeichev, K.F., Lukina, N.A., Sarimov, R.M., Smirnov, I.G., Simakin, A.V., Dorokhov, A.S., et al., 2021. Physicochemical properties of pure water treated by pure argon plasma jet generated by microwave discharge in opened atmosphere. Frontiers in Physics 8: 614684. https://doi.org/10.3389/fphy.2020.614684

Shaw, P., Kumar, N., Kwak, H.S., Park, J.H., Uhm, H.S., Bogaerts, A., et al., 2018. Bacterial inactivation by plasma treated water enhanced by reactive nitrogen species. Scientific Reports 8: 11268. https://doi.org/10.1038/s41598-018-29549-6

Shen, J., Tian, Y., Li, Y.L., Ma, R.N., Zhang, Q., Zhang, J., et al., 2016. Bactericidal effects against *S. aureus* and physicochemical properties of plasma activated water stored at different temperatures. Scientific Reports 6: 28505. https://doi.org/10.1038/srep28505

Sruthi, N.U., Josna, K., Pandiselvam, R., Kothakota, A., Gavahian, M. and Khaneghah, A.M., 2022. Impacts of cold plasma treatment on physicochemical, functional, bioactive, textural, and sensory attributes of food: a comprehensive review. Food Chemistry 368: 130809. https://doi.org/10.1016/j.foodchem.2021.130809

Suwal, S., Coronel-Aguilera, C.P., Auer, J., Applegate, B., Garner, A.L. and Huang, J.Y., 2019. Mechanism characterization of bacterial inactivation of atmospheric air plasma gas and activated water using bioluminescence technology. Innovative Food Science & Emerging Technologies 53: 18–25. https://doi.org/10.1016/j.ifset.2018.01.007

Thirumdas, R., Kothakota, A., Annapure, U., Siliveru, K., Blundell, R., Gatt, R., et al., 2018. Plasma activated water (PAW): chemistry, physico-chemical properties, applications in food and agriculture. Trends in Food Science & Technology 77: 21–31. https://doi.org/10.1016/j.tifs.2018.05.007

Tsoukou, E., Bourke, P. and Boehm, D., 2020. Temperature stability and effectiveness of plasma-activated liquids over an 18 months period. Water 12(11): 3021. https://doi.org/10.3390/w12113021

van Boekel, M., Fogliano, V., Pellegrini, N., Stanton, C., Scholz, G., Lalljie, S., et al., 2010. A review on the beneficial aspects of food processing. Molecular Nutrition & Food Research 54(9): 1215–1247. https://doi.org/10.1002/mnfr.200900608

Wu, D., Forghani, F., Daliri, E.B.M., Li, J., Liao, X.Y., Liu, D.H., et al., 2020. Microbial response to some nonthermal physical technologies. Trends in Food Science & Technology 95: 107–117. https://doi.org/10.1016/j.tifs.2019.11.012

Xiang, Q.S., Kang, C.D., Niu, L.Y., Zhao, D.B., Li, K. and Bai, Y.H., 2018. Antibacterial activity and a membrane damage mechanism of plasma-activated water against *Pseudomonas deceptionensis* CM2. LWT-Food Science and Technology 96: 395–401. https://doi.org/10.1016/j.lwt.2018.05.059

Xiang, Q.S., Kang, C.D., Zhao, D.B., Niu, L.Y., Liu, X. and Bai, Y.H., 2019a. Influence of organic matters on the inactivation efficacy of plasma-activated water against *E. coli* O157: H7 and *S. aureus*. Food Control 99: 28–33. https://doi.org/10.1016/j.foodcont.2018.12.019

Xiang, Q.S., Liu, X.F., Liu, S.N., Ma, Y.F., Xu, C.Q. and Bai, Y.H., 2019b. Effect of plasma-activated water on microbial quality and physicochemical characteristics of mung bean sprouts. Innovative Food Science & Emerging Technologies 52: 49–56. https://doi.org/10.1016/J.IFSET.2018.11.012

Xiang, Q.S., Zhang R., Fan, L.M., Ma, Y.F., Wu, D., Li, K., et al., 2020. Microbial inactivation and quality of grapes treated by plasma-activated water combined with mild heat. LWT-Food Science and Technology 126: 109336. http://doi.org/10.1016/j.lwt.2020.109336

Xu, Y.Y., Tian, Y., Ma, R.N., Liu, Q.H., Zhang, J., 2016. Effect of plasma activated water on the postharvest quality of button mushrooms, *Agaricus bisporus*. Food Chemistry, 197: 436–444.

Zhang, R., Ma, Y.F., Wu, D., Fan, L.M., Bai, Y.H. and Xiang, Q.S., 2020a. Synergistic inactivation mechanism of combined plasma-activated water and mild heat against *Saccharomyces cerevisiae*. Journal of Food Protection 83(8): 1307–1314. https://doi.org/10.4315/JFP-20-065

Zhang, W.T., Chen, X., Wang, Y., Wu, L.Y. and Hu, Y.D., 2020b. Experimental and modeling of conductivity for electrolyte solution systems. ACS Omega 5(35): 22465–22474. https://doi.org/10.1021/acsomega.0c03013