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The antimicrobial effects of mist spraying and immersion on beef samples with plasma-activated water

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ABSTRACT

The use of plasma-activated water (PAW) as an antimicrobial agent to inactivate Salmonella Typhimurium on chilled beef during meat washing was evaluated. Two meat washing methods, spraying and immersion, were evaluated at contact times of 15, 30 and 60 s and meat storage times of 0, 1 and 7 days. The temperature of PAW was elevated to 55 °C for washing as it increased the microbial inactivation compared to ambient temperature. At the contact time of 60 s and meat storage time of 7 days, PAW spraying and immersion achieved 0.737-$\log_{10}$ and 0.710-$\log_{10}$ reductions against *Salmonella* Typhimurium, respectively; there were no significant differences between both washing methods, with spraying being preferred for commercial implementation. Compared to untreated and water-treated samples, meat washing with PAW alone improved the *S.* Typhimurium inactivation and did not cause negative impacts on the lightness and hue angle values, TBARS value, water holding capacity and pH. However, PAW reduced the redness, yellowness and chroma values with the decreased oxymyoglobin values of 44.1% at the storage time of 1 day. PAW spraying at 55 °C followed by additional water washing at 25 °C for 60 s achieved 0.696-$\log_{10}$ reduction and mitigated a reduction in (i) the redness value, from 11.3 to 18.2, (ii) the yellowness value, from 9.19 to 11.1, and (iii) the chroma value, from 14.5 to 21.3, without displaying colour differences (ΔE), as detected by human eyes, compared to water-treated samples. Moreover, the content of myoglobin forms was maintained by additional water washing.

1. Introduction

Meat contamination may occur during slaughter, post-slaughter stages (including carcass washing and chilling), transportation and storage. Raw meats and their products are the main sources of food-borne pathogens, such as *Salmonella* Typhimurium, which pose a potential threat to human health (Belov et al., 2022; Van Ba et al., 2018). In addition, meat discolouration and odour can be initiated by microbial spoilage, making the meat undesirable to consumers (Han et al., 2022). Safety measures from farm to table are required to ensure that meats are free from food-borne pathogens, preventing consumer exposure to microbial hazards (Lonergan, Topel, & Marple, 2019), and from spoilage microorganisms to prolong their shelf lives.

In the industry, the decontamination of meat carcasses relies on washing with high-pressure potable water or chemicals such as organic acids and chlorine (Das, Nanda, Das, & Biswas, 2019). Conventional water spraying is inefficient due to its excessive liquid consumption (Daniels, Modrow, Osburn, & Taylor, 2021) and its likelihood to spread bacteria (Orsoni et al., 2020). Alternatively, spraying with hot water or steam effectively reduces the number of microorganisms, but may cause adverse effects on the nutritional value and sensory quality of meat at long spraying times (Han et al., 2022).

On the other hand, the use of chemicals may produce undesirable and harmful by-products (Huang et al., 2019). Spraying with organic acids is applied at only one stage during the slaughter process, either after dehiding or during the final wash stage (Van Ba et al., 2018). Gonzalez, Geornaras, Nair, and Belk (2021) compared the decontamination efficacy of chemical treatments against *Campylobacter jejuni* on chicken wings between commercial spraying and immersion, indicating that immersion reduced the microbial inactivation efficacy compared to spraying. Moreover, immersion is more economical for small-scale meat producers, but spraying is more economical as the scale of production increases (Shen et al., 2019).

Recently, plasma-activated water (PAW) has been studied as an

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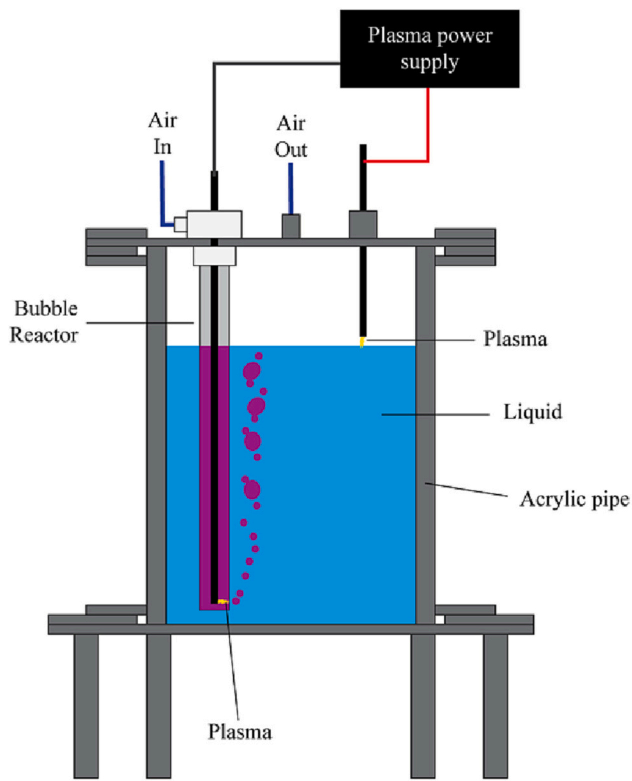

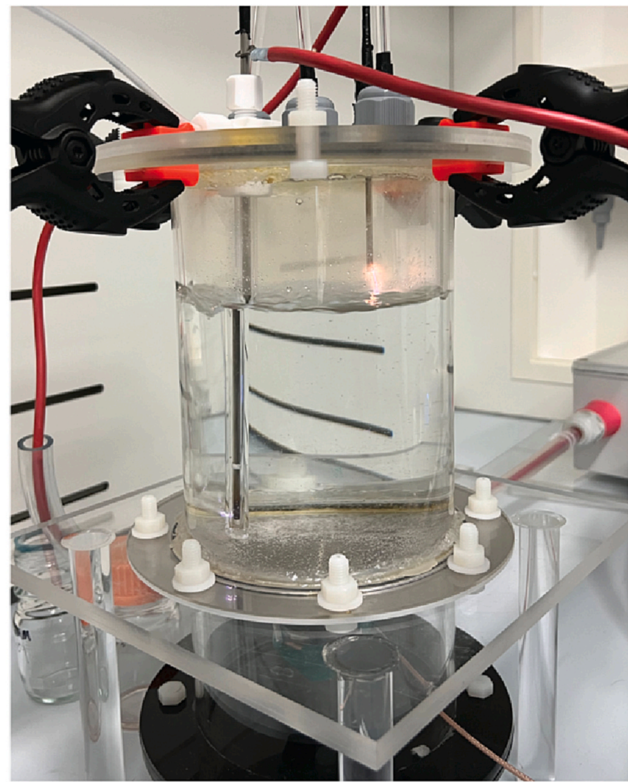

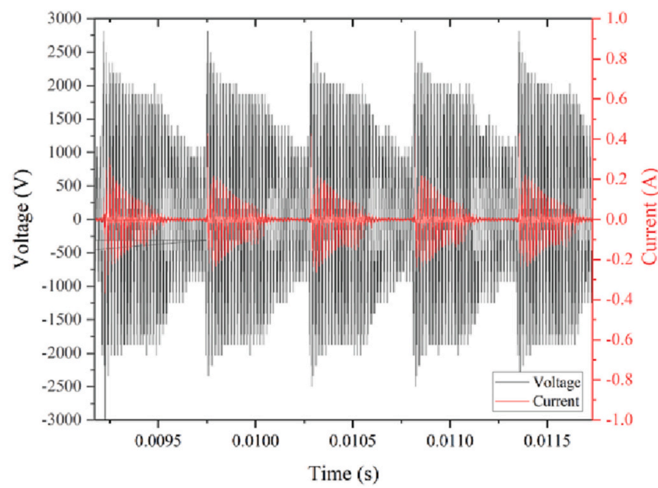

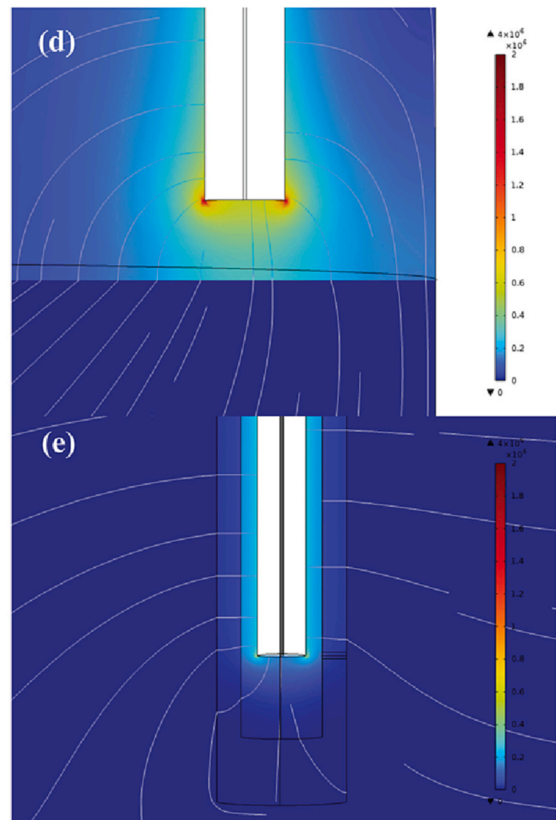

Fig. 1. (a) Experimental setup of a hybrid plasma discharge (HPD) reactor; (b) image of the HPD reactor; (c) Discharge voltage and current waveforms recorded by the HPD reactor; COMSOL results of the electric field strength distribution of (d) the high-voltage electrode and (e) the ground electrode of the HPD reactor at the output voltage of 2.81 kV and the discharge frequency of 2500 Hz.

antimicrobial agent for the disinfection of food products such as blueberry (Gan et al., 2022), tomato (Ali, Cheng, & Sun, 2021), cabbage (Choi et al., 2019), fresh beef (Barrales Astorga, Hadinoto, Cullen, Prescott, & Trujillo, 2022) and mushroom (Zhao et al., 2021; Zhao, Wang, & Ma, 2021). PAW is generated through the interaction between non-thermal plasma, which is generated by high electric field discharges in air, and water molecules, triggering chemical reactions at the gas-liquid interface and generating a cocktail of reactive oxygen and nitrogen species (RONS), including •OH, $•O_2^-$, $ONOO^-$, NO_2^-, NO_3^-, H_2O_2 and O_3 (Liao et al., 2020). The reactive species are the major contributors to the temporary bactericidal property of PAW, which can rupture the membrane of bacterial cells leading to cell death (Liu et al., 2021; Zhao, Oliveira, et al., 2021; Zhao, Wang, & Ma, 2021).

PAW has been evaluated for the decontamination of pathogens on meat products such as chicken (Mai-Prochnow et al., 2020; Royintarat, Choi, Boonyawan, Seesuriyachan, & Wattanutchariya, 2020), fish (Gomez et al., 2019; Zhao, Oliveira, et al., 2021; Zhao, Wang, & Ma, 2021) and prawn (Herianto et al., 2022). Royintarat et al. (2020) reported that 0.46-$\log_{10}$ reduction of the inactivation against *Escherichia coli* was achieved when chicken was soaked in PAW at 40 °C for 60 min while maintaining the hardness, protein and lipid of the meat products. Similarly, Herianto et al. (2022) showed that PAW maintained the colour, firmness, pH, total volatile basic nitrogen (TVBN) and thiobarbituric acid reactive substances (TBARS) of shrimps while inhibiting the microbial growth after 9 days of storage. It is imperative to determine the most effective meat-washing approach to decontaminate raw meat that (i) improve the shelf-life by inactivating pathogens, (ii) does not change appearance, smell and taste; (iii) does not leave residues that may harm the environment, and (iv) is cheap and easily integrated in the meat processing line (Dincer & Baysal, 2004).

PAW has also been used as a thawing medium for reducing the bacterium for up to 1.17-$\log_{10}$ CFU·g^{-1} without degrading the proteins and the colours of chicken (Qian et al., 2022). Recently, PAW has been demonstrated as a safe media for the thawing of beef (Liao et al., 2020) and as an alternative to nitrites for the curing of beer jerky and its inactivation against *Listeria innocua* (Inguglia, Oliveira, Burgess, Kerry, & Tiwari, 2020). To our best knowledge, there are only three previous studies on the use of PAW for the microbial safety and quality preservation of beef (Barrales Astorga et al., 2022; Lotfy & Khalil, 2022; Zhao, Ojha, Burgess, Sun, & Tiwari, 2020); however, these studies produced a very low volume of PAW (from 40 to 200 ml), which can be performed only for small lab scale experimentation, and required long contact times for bacterial inactivation, from minutes to days, which is ineffective for industrial implementation. In addition, there are no studies on the effect of meat washing methods with PAW, such as spraying and immersion, on meat quality.

Therefore, the aim of this study is to assess the effect of PAW washing via spraying and immersion on the inactivation of *S.* Typhimurium on chilled beef at contact times of 15, 30 and 60 s. The liquid temperature of 55 °C was adopted during the meat washing in this study as it is commonly used by commercial organic acid spray washing (Efsa Panel on Food Contact Materials, E., Processing, A, et al., 2018) and because achieved greater microbial inactivation compared to ambient temperature in preliminary tests. These very short treatment times of 15, 30 and 60 s were selected to enhance the PAW adoption in the meat industry as an integrated part of the meat production line, which requires short production time and a minimal quality disruption to the food material. *S.* Typhimurium was also selected a model organism for meat safety in this study because it is an important cause of foodborne illness outbreaks in Australia and due to its prevalence in fresh raw meat and meat products (Ford et al., 2018).

The PAW inactivation against *S.* Typhimurium was evaluated at the meat storage time of 0. 1 and 7 days at 4 °C. Based on our previous work (Hadinoto et al., 2023), PAW was produced via a hybrid plasma discharge (HPD) reactor, which was energy efficient and achieved high inactivation against planktonic bacteria within 30 s of contact time. The physicochemical characteristics of the treated beef samples were evaluated based on changes in colour, content of myoglobin forms, lipid oxidation via TBARS, water holding capacity (WHC), pH and weight gain.

2. Materials and methods

2.1. Chemicals and materials

The following chemicals from Sigma-Aldrich were used: butylated hydoxytoluene, Griess' reagent for nitrite, hydrogen peroxide (30%), peptone water, potassium nitrate (99%), sodium azide, sodium chloride (99%), sodium nitrite (97%), sulfamic acid, thiobarbituric acid and titanium oxysulfate. Glacial acetic acid was purchased from ChemSupply Australia. MilliQ® water was made by MilliQ® IQ 700 (Merck Millipore, Australia).

2.2. Generation of plasma-activated water

Plasma-activated water was generated using a newly designed hybrid plasma discharge (HPD) reactor, shown in Fig. 1. The HPD reactor was powered using a high-voltage AC power source (Leap100, PlasmaLeap Technologies, Australia). The HPD reactor vessel consisted of a cylindrical acrylic pipe, a flat-end metal rod and a specially designed bubble reactor with a single orifice (400 μm diameter) and a gas flow controller (198–2981, RS Components, Australia). The reactor vessel in Fig. 1(a) was made from a cylindrical acrylic pipe with inner diameter, thickness and height of 123, 5 and 190 mm, respectively. The rod and bubble reactor were placed inside the acrylic pipe as shown as Fig. 1(a). The rod, made from 316 stainless steel with an outer diameter of 4 mm and a length of 179 mm, was insulated with a rubber tube and connected to the positive terminal of the power supply. The rod was referred as the high voltage (HV) electrode of the HPD reactor. The distance between the surface of the liquid and the end of the rod (high-voltage electrode) in all experiments was maintained at 6 mm as shown in Fig. 1(a). The bubble reactor contains a metallic rod enclosed on a quartz tube; the design of plasma-bubble reactors has been reported in the literature (Mai-Prochnow et al., 2020; Rao et al., 2023; Zhou et al., 2021; Zhou et al., 2021). Compressed air, retained at a flow rate of 0.8 L.min^{-1} via a flow controller, was supplied to the plasma-bubble reactor. The metal rod inside the bubble reactor was connected to the negative terminal of the power supply to complete the circuit. The following operational parameters were adopted for the production of PAW at 1.5 l with the addition of MilliQ® water and 8 mM NaCl: output voltage – 2.81 kV; resonance frequency – 60 kHz; input voltage – 200 V; discharge frequency – 2500 Hz; duty cycle – 50 μsec; discharge time – 30 min; average discharge power – 45.1 W, measured via Section 2.3 using the V—I graph in Fig. 1(c). 8 mM NaCl was added prior to plasma discharge to enhance the generation of RONS species in the liquid (Hadinoto et al., 2023).

2.3. Characterisation of plasma via optical emission spectra and discharge power analysis

The optical emission spectra (OES) of plasma discharges were performed to identify the RONS in plasma, using the method according to our previous work (Hadinoto et al., 2023). The applied voltage and current across the HPD reactor were determined by connecting the reactor with a voltage probe (P6015A, Tektronix, U.S.) and a current probe (4100, Pearson Electronics, U.S.) to a digital oscilloscope (DS-6104, RIGOL, China). The following expressions were used to determine the discharge power using the OriginPro® software (Mustafa et al., 2018; Sajib et al., 2019):

$$\text{Energy injection}\ \left(\text{J}\cdot\text{pulse}^{-1}\right), \text{E} = \int_{t_0}^{t_0+T} u(t)i(t)dt \tag{1}$$

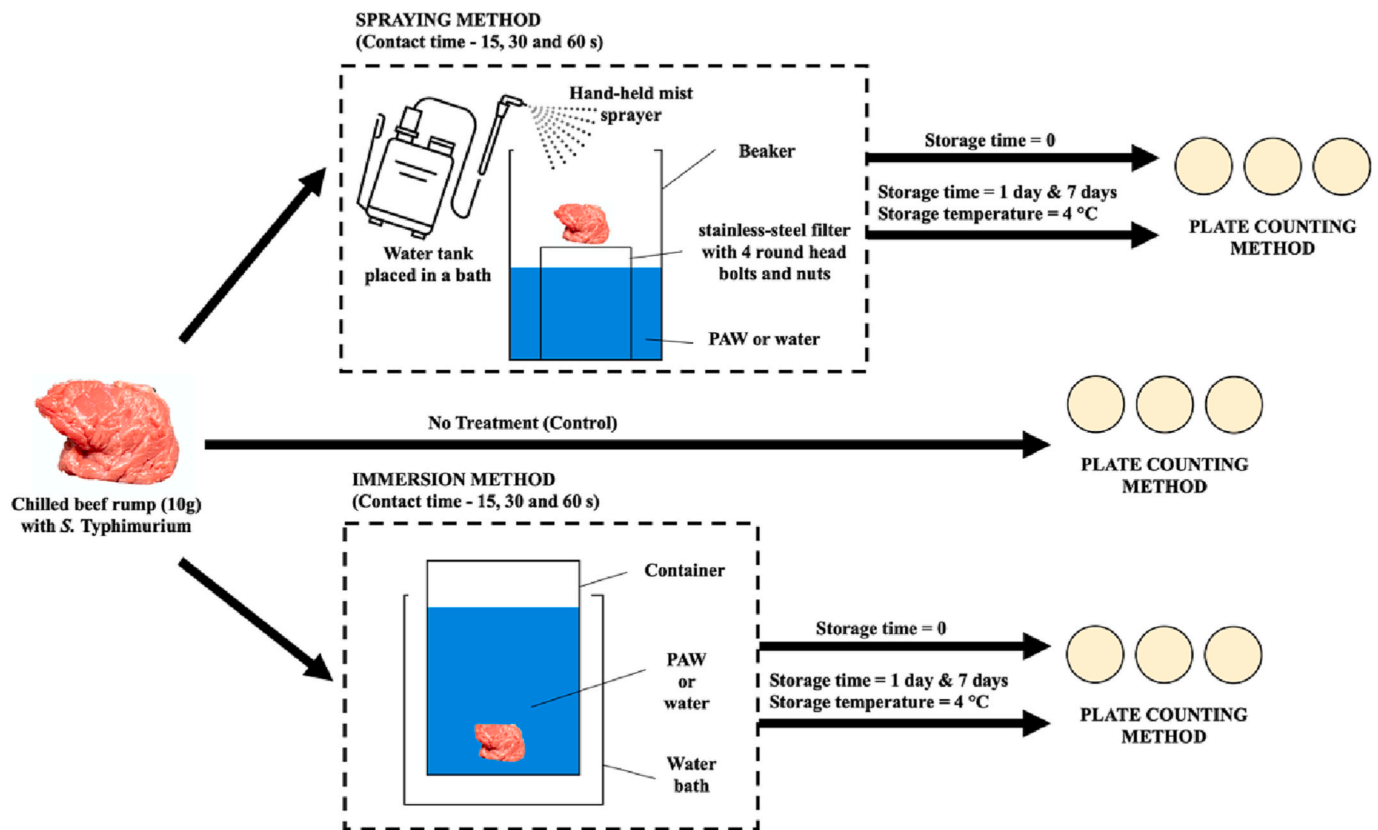

Fig. 2. Experimental setup of chilled beef samples treated with spraying and immersion methods.

$$\text{Discharge power (W)}, P = E\,f \tag{2}$$

where, u(t) is the voltage (V), i(t) is the current (A) and f is the pulse repetition frequency (Hz).

2.4. Simulation of electric field

The electric field in the HPD reactor was simulated using COMSOL Multiphysics AC/DC module version 6 (COMSOL Inc., MA, USA) as described in the literature (Song et al., 2016), with some modifications. The HPD reactor in Section 2.2 was modelled in a three-dimensional system and a physics-controlled mesh of extra finer elements was used to accomplish the simulation. The voltage of the high-voltage electrode was set as 2.81 kV and the voltage of the ground electrode was set as zero.

2.5. PAW characterisations

2.5.1. Physical properties of PAW

Electrical conductivity, pH and oxidation-reduction potential (ORP) were measured using a conductivity meter (Mettler-Toledo Ltd., Australia), and a benchtop pH/ORP meter kit (Hanna Instruments, Australia) (Zhao et al., 2020).

2.5.2. Reactive oxygen and nitrogen species

The reactive oxygen and nitrogen species (NO_2^-, NO_3^- and H_2O_2) in PAW were measured as described in our previous work (Hadinoto et al., 2021; Hadinoto et al., 2023). Briefly, a Griess' reagent and a titanium oxysulfate assay were used to measure NO_2^- at 548 nm and H_2O_2 at 403 nm, respectively, using a UV–visible microplate reader (SPECTROstar Nano, BMG Labtech, Australia). NO_3^- was determined by using a NO_3^--selective electrode (Cole-Parmer, Australia), Ionic Strength Adjuster (ISA) and sulfamic acid.

2.6. Bacterial evaluation

2.6.1. Preparation of planktonic bacterial suspension and bacterial analysis

Salmonella Typhimurium (NCTC74) were grown individually on nutrient agar plates at 37 °C for an incubation time of 24 h to obtain isolated colonies. To produce cell suspension, single colony was isolated and inoculated into 4.5 ml of peptone water. The cell suspension was incubated at 37 °C for 24 h.

For the bacterial analysis, 50 μl of the prepared *S.* Typhimurium suspension was added to 4.95 ml or sterile water (as a control) or prepared PAW (in Section 2.2) at 55 °C for the contact times ranging from 10 to 30 s. Three experiments with three technical replicates on different occasions were performed independently for each treatment. After treatment, samples were serially diluted in sterile peptone water. 100 μl of serially diluted solutions were spread onto nutrient agar in triplicates. These plates were incubated at 37 °C for 24 h. The surviving bacteria colonies were enumerated and expressed as $\log_{10}$ CFU·ml^{-1} for Section 2.6.4.

2.6.2. Sample preparation

Fresh grass-fed beef rump cuts were collected from a meat distributor (New South Wales, Australia) within a week of slaughter. Beefs were cut with a weight of approximately 10 g for the evaluation of bacterial inactivation. Similar to Section 2.6.1, *S.* Typhimurium were grown individually and then, a single colony of *S.* Typhimurium was incubated in peptone water. The prepared beef cut was then inoculated with 100 μl of *S.* Typhimurium suspension (Byun et al., 2022; Johnson Esua, Sun, Ajani, Cheng, & Keener, 2022). To enable the *S.* Typhimurium to attach to the meat surface, all samples were placed on a clean bench and air-dried for 30 min at room temperature.

2.6.3. Meat washing treatment and bacterial enumeration

To remove heat from the beef samples and not compromise meat

colour during treatment, all prepared beef samples with *S.* Typhimurium were chilled in a cold room at 4 °C for 24 h prior to treatment. Then, the chilled samples were exposed individually to either PAW or sterile water at 55 °C via two meat washing methods, spraying and immersion. A water bath (Grant Instruments, U.K.) was used to heat PAW and water to 55 °C. Both meat-washing methods were then compared with untreated chilled beef samples.

For the spraying method, a handheld mist sprayer (Illu-Mist Mist Battery Powered Sprayer, U.S.) with a liquid flow rate of 3.37 ml·s^{-1} was used. Before treatment, the chilled meat sample with *S.* Typhimurium was placed on top of a stainless-steel filter (737–4096, RS Components Pty Ltd., Australia) with 4 round head bolts and nuts, which was placed in a 500 ml borosilicate beaker, as shown in Fig. 2. The distance between the meat surface and the liquid outlet of the mist sprayer was kept at 70 mm.

For the immersion method, the chilled meat sample with *S.* Typhimurium was immersed in a flatbottom container (P246SU, Techno Plas Pty Ltd., Australia), containing 101 ml of water or PAW as shown in Fig. 2. The value of 101 ml was chosen based on the amount of liquid consumed by the spraying method at 30 s. Then, the container was placed in a water bath at 55 °C for 30 min (Fig. 2).

For all two meat washing methods with PAW and water, 3 contact times were used: 15, 30 and 60 s. The effect of storage time after treatment on the bacterial inactivation from the two washing methods was also investigated by storing the treated meat samples in a flat-bottom container (S5527SU, Techno Plas Pty Ltd., Australia) at 4 °C for 0, 1 and 7 days, shown in Fig. 2, and comparing them with the untreated meat samples at same storage conditions. For each treatment group (control, water, PAW), two washing methods (spraying and immersion), three contact times (15, 30 and 60 s) and three storage periods (0, 1 and 7 days) were performed. All experiments for the three treatment groups (control, water, PAW) were performed in three independent experiments on different occasions and in three technical replicates. The 8 mm NaCl solution was not chosen as a control in this study as because our previous works showed that the NaCl solution had no effect on the inactivation against planktonic bacteria (Hadinoto et al., 2021; Hadinoto et al., 2023). The total number of experiments and beef samples was 162: three treatments × two washing methods × three contact times × three storage times × three independent experiments. Based on the results in Section 3.2.1, the antibacterial effect of the first water/PAW washing followed by additional water washing at 25 °C for 60 s at the storage time of 1 day was investigated. For this set of experiments, 9 experiments, with corresponding beef samples, (three treatments x three independent experiments) were prepared.

Each of the plasma-treated, water-treated and untreated beef samples was homogenised with 90 ml D/E neutralising broth (Remel, U.S.) in a stomacher bag using a stomacher (Stomacher® 400 Circulator Lab Blender, Seward Ltd., U.K.) (Rothwell et al., 2022), for 2 min. Then, a serial dilution using sterile peptone water was performed and 100 μl of each dilution was spread onto XLD agar plates (PP2004, Thermo Fisher Scientific, Australia) in triplicates. Plates were incubated at 37 °C for 24 h. Bacterial colonies were then counted and expressed as $\log_{10}$ CFU·ml^{-1}.

2.6.4. Bacterial inactivation efficiency

The results obtained in Section 2.6.1 and Section 2.6.3 were reported as $\log_{10}$ CFU·ml^{-1}. $\log_{10}$ reduction were calculated using the following equation:

$$log_{10}\text{reduction} = log_{10}\text{N}_0 - log_{10}\text{N} \quad (3)$$

where, N is the microbial cell count for samples treated with PAW or water (CFU·ml^{-1}) and N_0 is the microbial cell count for untreated samples (CFU·ml^{-1}).

2.7. Quality analysis of beef

The best-performing contact time for the spraying method in Section 2.6.3 was chosen for the quality analysis of beef in Section 2.7.1–2.7.6. For quality analysis, PAW-treated beef samples were not only compared with samples treated with water (control) but also with untreated samples (or fresh raw beef) because this provides insights into the effect of meat washing methods, either with water or PAW, on meat quality.

2.7.1. Weight gain

The weight of plasma-treated, water-treated and untreated beef samples was measured to calculate the weight gain of treated samples, using the following equation (Barrales Astorga et al., 2022):

$$\text{Weight gain}\,(\%) = \frac{\text{meat sample after treatment}\,(\text{g}) - \text{intial meat sample}\,(\text{g})}{\text{initial meat sample}\,(\text{g})} \text{x}\,100 \quad (4)$$

2.7.2. Colour

The plasma-treated, water-treated and untreated beef samples (10 g) were allowed to bloom at a temperature of 20 °C for 30 min. After this time, the surface colours of the samples were measured using a ChromaMeter (CR-400, Komica Minolta Optics, Inc., Japan). The chromameter was set to illuminant D65, observer angle of 2° and aperture size of 8.0 mm and calibrated on a white standard plate before analysis. The colour of each sample was expressed as L^* (lightness), a^* (redness) and b^* (yellowness) values based on the reported methods (Barrales Astorga et al., 2022; Jo et al., 2020). For all of the storage times at 0, 1 and 7 days, the colour change (ΔE) of each sample was determined using eq. (5), which represents the degree of colour difference between treatments (Thangavelu, Tiwari, Kerry, & Alvarez, 2022).

$$\Delta E = \sqrt{\left(L_c^* - L_t^*\right)^2 + \left(a_c^* - a_t^*\right)^2 + \left(b_c^* - b_t^*\right)^2} \quad (5)$$

where, L_c*, a_c* and b_c* are the colour values of untreated sample at each storage time; and L_t*, a_t* and b_t* are the colour values of treated sample at each storage time (Xu, Tian, Ma, Liu, & Zhang, 2016).

Chroma (C, saturation index) was determined to indicate vivid or dull colour and hue angle (h*) was determined, using the following equations (Pogorzelska, Godziszewska, Brodowska, & Wierzbicka, 2018; Yong, Han, Kim, Suh, & Jo, 2018).

$$C = \sqrt{\left(a^*\right)^2 + \left(b^*\right)^2} \quad (6)$$

$$h^* = tan^{-1}\left(b^*/a^*\right) \quad (7)$$

2.7.3. Content of myoglobin forms

The compositions of myoglobin (deoxymyoglobin, oxymyoglobin and metmyoglobin) on treated and untreated beef samples were analysed according to the method from our previous work (Barrales Astorga et al., 2022; Jo et al., 2020), with some modifications. 5 g of each sample was homogenised with 10 ml phosphate buffer (pH 6.8) for 2 min and centrifuged at 3000 rpm for 10 min. The percentage of each myoglobin form was determined by measuring the absorbance of each supernatant at 503, 525, 557 and 582 nm using by a fiber-optic spectrometer (Ocean Optics, USB4000) and a semi-micro cuvette with a 10 mm path length (Sarstedt, Inc.).

2.7.4. Lipid oxidation

The degree of lipid oxidation was assessed through the TBARS value of beef sample of about 10 g. The TBARS content was determined according to the reported method (Zeb & Ullah, 2016), with some modifications. Each sample was homogenised in 20 ml glacial acetic acid for 2 min with an addition of 1% butylated hydoxytoluene (BHT) to prevent further oxidation. The homogenised solution was centrifuged. After centrifugation, the supernatant was then filtered and reacted with

Table 1
P value of fixed main factor and interaction effects analysed for the inactivation efficiency of *S. typhimurium* on beef as the dependent variable. The contact time of 0 s was not included because there was no variation.

Inactivation Condition At	Washing Method (W)	Treatment (T)	Contact Time (C)	W × T	T × C
S = Day 0 & C = 15 s	0.0133	0.3339	–	0.8268	–
S = Day 0 & C = 30 s	0.0017	0.2187	–	0.2374	–
S = Day 0 & C = 60 s	0.0002	0.0173	–	0.5625	–
S = Day 1 & C = 15 s	0.0065	0.3878	–	0.9374	–
S = Day 1 & C = 30 s	0.002	0.3795	–	0.9675	–
S = Day 1 & C = 60 s	0.0006	0.204	–	0.7694	–
S = Day 7 & C = 15 s	<0.0001	0.0536	–	0.9031	–
S = Day 7 & C = 30 s	0.0008	0.3927	–	0.3805	–
S = Day 7 & C = 60 s	0.0002	0.7341	–	0.4517	–
S = Day 0	–	<0.0001	<0.0001	–	0.0051
S = Day 1	–	<0.0001	<0.0001	–	0.0223
S = Day 7	–	<0.0001	<0.0001	–	<0.0001

thiobarbituric acid (20 mM, 1:1 *v*/v). Samples with reagent were placed in a 95 °C water bath for 60 min before absorbance was read at 532 nm using a fiber-optic spectrometer (Ocean Optics, USB4000) and a semi-micro cuvette with a 10 mm path length (Sarstedt, Inc.). The TBARS value was calculated using a standard curve and expressed in mg MDA per kg of sample.

2.7.5. Water holding capacity

Water holding capacity (WHC) was conducted via a centrifugation method (Moutiq, Misra, Mendonca, & Keener, 2020), with some modifications. About 10 g of treated or untreated beef sample was mixed with 16 ml of sodium chloride solution (NaCl, 0.6 M) and homogenised using a stomacher (Stomacher® 80 Biomaster, Seward Ltd., U.K.) for 1 min. The meat slurry was then incubated at 4 °C for 30 min, followed by stirring for 1 min and centrifugation using a centrifuge (Centrifuge 5702, Eppendorf, Australia) at 4400 rpm for 20 min. After centrifugation, the supernatant layer was collected and measured by volume. The amount of added solution held by the beef is described as the water holding capacity in ml per 100 g of meat as shown in the equation:

$$WHC\left(\frac{ml}{100g}\right) = \frac{\text{Volumeof added solution (ml)} - \text{Volume of released solution (ml)}}{\text{Weight of sample (g)}} \text{x } 100 \quad (8)$$

2.7.6. pH

All the plasma-treated, water-treated and untreated beef samples of about 10 g were homogenised with 90 ml of MilliQ® water for 30 s using a stomacher (Stomacher® 400 Circulator Lab Blender, Seward Ltd., U. K.) according to the method from our previous work (Barrales Astorga et al., 2022). The pH of the sample was measured using a benchtop pH meter kit (Hanna Instruments, Australia) calibrated with three commercial buffer solutions of pH at 4.0, 7.0 and 10.0 before use (Barrales Astorga et al., 2022; Moutiq et al., 2020).

2.8. Statistical analysis

The research was carried out by applying 2 meat washing methods and 3 different treatments to the chilled beef meats, in 3 contact times and 3 storage times, and in 3 independent experiments on different occasions with 3 technical replicates. It was investigated whether there is a statistical difference between the washing method, treatment and storage time averages, and whether there are interactions between the factor levels. All experimental data (inactivation efficiency, weight gain,

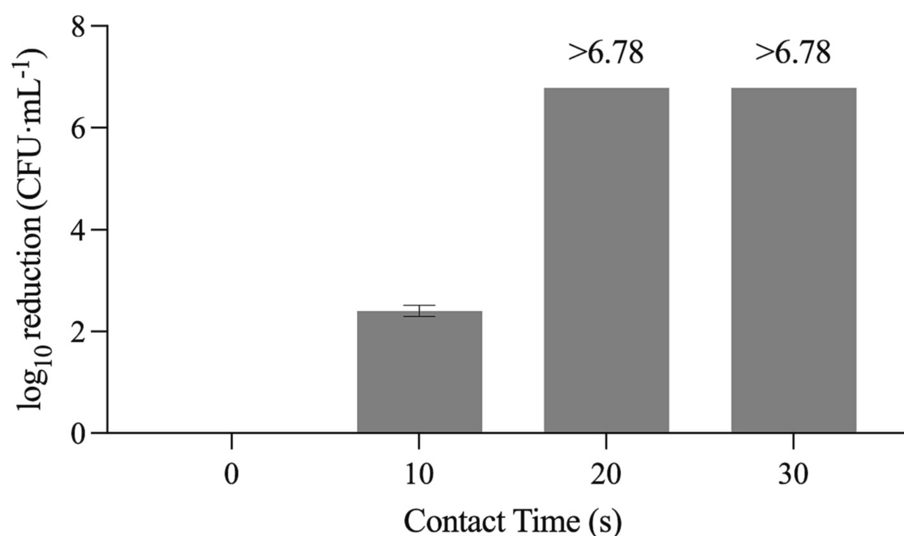

Fig. 3. Effect of PAW generated by the HPD reactor on the inactivation of planktonic *S.* Typhimurium cells for the contact times of 10, 20 and 30 s. At 20 and 30 s, the inactivation achieved >6.78-$\log_{10}$ reduction. Error bar represents standard error of the mean.

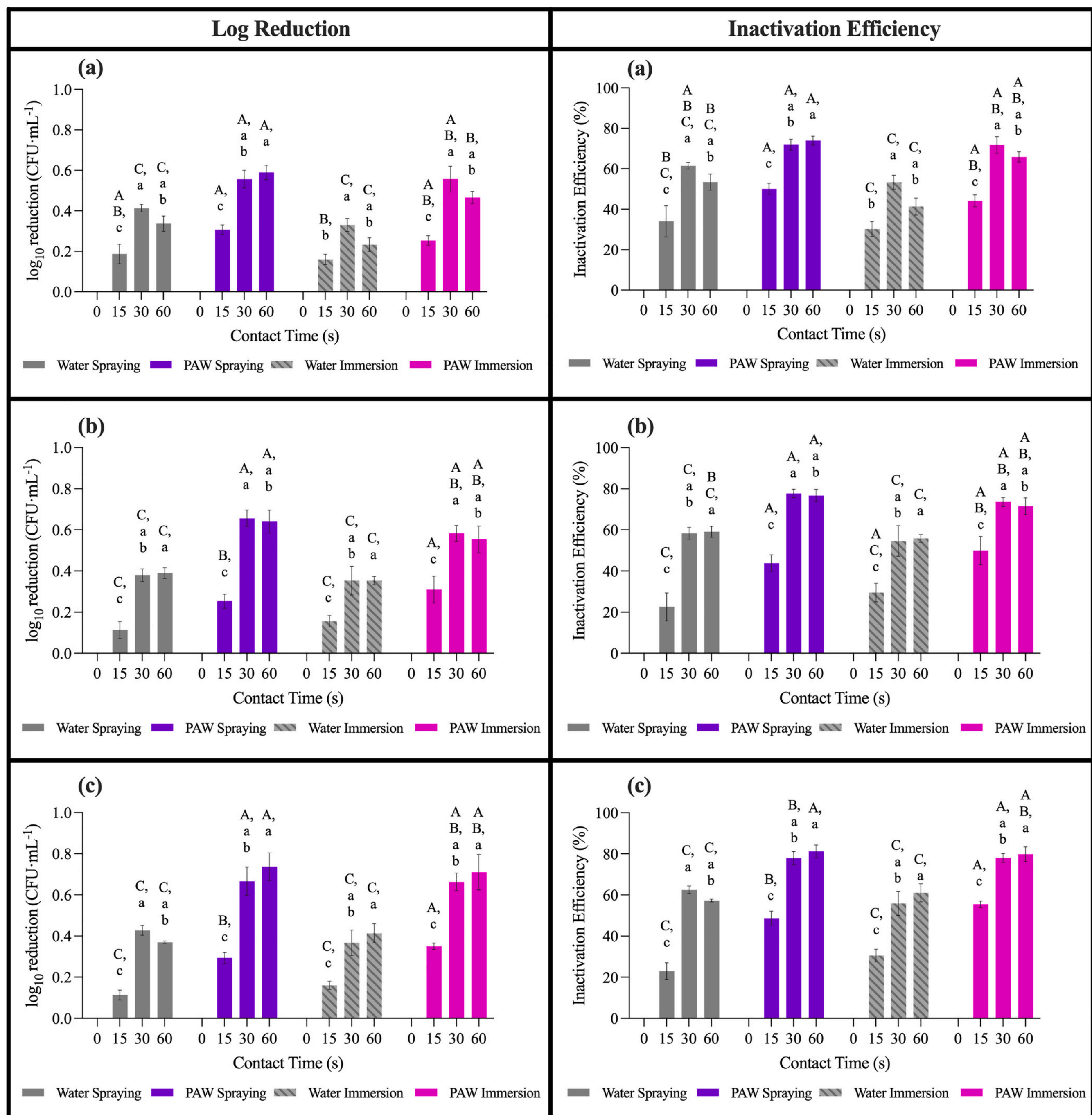

Fig. 4. Log_{10} reduction (left side) and inactivation efficiency (right side) of *S.* Typhimurium on beef by PAW and water using the two meat washing methods, mist spraying and immersion, with the contact times of 0, 15, 30 and 60 s and the beef storage times of (a) 0 day, (b) 1 day and (c) 7 days at 4 °C. Different uppercase letters (A, B, C, D) indicate statistically significant difference ($P < 0.05$) among the meat washing conditions with PAW and water within the same contact time while different lowercase letters (a, b, c) indicate statistically significant difference ($P < 0.05$) among the contact times within the same meat washing condition. Error bar represents standard error of the mean.

colour, content of myoglobin forms, TBARS, water holding capacity and pH) of the beef samples were expressed as the mean ($\overline{X}$) of experimental data in 3 independent experiments with the standard error (S.E.), $\overline{X} \pm$ S.E., and analysed by Two-way ANOVA. Statistical analysis of all data was performed using the Prism 8 software. The effects of washing method, treatment, contact time and storage time were considered as independent variables. Bacterial inactivation efficiency, weight gain, colour, content of myoglobin forms, TBARS, water holding capacity and pH were considered as dependent variables. The random effects were due to the beef samples and the replication of the experiments. Interaction terms for washing method × treatment, treatment × contact time and treatment × storage time were included. Significance differences between means were identified by the Tukey's honest significance difference (HSD) test with $P < 0.05$. P values for effects of fixed main factor (washing method, treatment, contact time and storage time) and their interactions are shown in Table 1 and Table 3.

Table 2
L^* (lightness), a^* (redness) and b^* (yellowness), chroma (C), hue angle (h^*) and colour change (ΔE) of beef samples with various treatment conditions at the contact time of 30 s and the beef storage times of 1 day and 7 days at 4 °C. Different lowercase letters (a, b, c) indicate statistically ssignificant difference ($P < 0.05$) among the washing conditions within the same colour coordinate value and storage time. Data were presented as the mean ± standard error.

Treatment No.	Storage Time	1 day						7 days					
	Meat Washing Method	L^*	a^*	b^*	C	h^*	ΔE	L^*	a^*	b^*	C	h^*	ΔE
T1	Untreated beef	49.8^a ± 1.7	21.1^{ab} ± 0.5	11.6^{ab} ± 0.3	24.1^{ab} ± 0.5	1.64^a ± 0.02	–	47.0^a ± 1.1	19.2^a ± 1.7	10.5^a ± 0.8	21.9^a ± 1.9	1.64^a ± 0.06	–
T2	Water spraying at 55 °C for 30 s	48.6^a ± 0.1	21.7^a ± 0.1	12.0^a ± 0.2	24.8^a ± 0.1	1.63^a ± 0.03	2.37^a ± 1.04	50.4^a ± 0.7	15.4^{ab} ± 2.8	9.43^a ± 0.77	18.1^{ab} ± 2.8	1.38^a ± 0.21	5.67^a ± 2.14
T3	PAW spraying at 55 °C for 30 s	48.4^a ± 0.6	11.3^d ± 0.3	9.19^{bc} ± 0.50	14.5^d ± 0.5	0.948^a ± 0.053	10.7^b ± 0.8	46.9^a ± 0.4	13.3^b ± 0.2	9.21^a ± 0.23	16.2^b ± 0.1	1.21^a ± 0.06	6.15^a ± 1.98
T4	PAW spraying at 55 °C for 30 s + water spraying at 25 °C for 60 s	50.5^a ± 0.7	18.2^c ± 0.4	11.1^{ac} ± 0.4	21.3c ± 0.5	1.44^a ± 0.04	3.45^a ± 0.34	47.0^a ± 2.4	12.2^b ± 1.6	9.07^a ± 2.13	15.3^b ± 1.3	1.10^a ± 0.25	10.5^a ± 2.13

3. Results and discussion

3.1. Evaluation of spraying and immersion methods with water and PAW for reduction of Salmonella Typhimurium on chilled beef

The two techniques of meat washing, spraying and immersion, were studied with PAW and water as the control. Meat samples were inoculated with *S.* Typhimurium, and chilled to 4 °C. Then, the meat washing was performed for 0, 15, 30 and 60 s. The meat samples were subsequently stored for 0, 1 and 7 days in a fridge at 4 °C. Fig. 3 shows that the inactivation of *S.* Typhimurium in a planktonic state by PAW achieved a complete reduction of >6-$\log_{10}$ reduction after 20 s of contact time, which performed better than the inactivation of adhered cells, shown in Fig. 4, with <1-$\log_{10}$ reduction after 60 s of contact time. This is expected because the attachments of *S.* Typhimurium cells on meat surfaces form biofilms, preventing cell injury and increasing the resistance of bacterial pathogens to antimicrobial treatments (Bridier et al., 2015; Giaouris et al., 2014). Hence, PAW treatment was less efficient on adhered cells compared to planktonic cells.

Irrespective of the meat-washing technique and the meat storage time, washing the beef samples with PAW at contact times of 30 and 60 s resulted in a reduction in the *S.* Typhimurium load significantly higher than those with water (control) as shown in Fig. 4. For instance, at the meat storage time of 0 day, spraying the beef samples with PAW and water for 30 s achieved 0.556-$\log_{10}$ reduction (71.9% inactivation efficiency) and 0.415-$\log_{10}$ reduction (61.5% inactivation efficiency), respectively (Fig. 4). Similarly, the immersion of the beef samples in PAW and water for 30 s reached 0.558-$\log_{10}$ reduction (71.7% inactivation efficiency) and 0.334-$\log_{10}$ reduction (53.4% inactivation efficiency), respectively, at the storage time of 0 day (Fig. 4). The main effects of treatment and contact time were significant with the significant interaction effect of treatment × contact time as shown in Table 1. Overall, PAW improved the inactivation.

The PAW of this experiment was obtained with the hybrid plasma discharge (HPD), from Hadinoto et al. (2023), which resulted on the acidification of liquid with low pH and generated long-lived reactive species in PAW including NO_2^-, NO_3^- and H_2O_2 with NO_2^- as the dominant species at the liquid salinity of 8 mM NaCl. The physiochemical properties of PAW are shown in Table A1. The RONS production in PAW was attributed to the simultaneous plasma discharge in both the high-voltage (HV) electrode and the ground electrode by the HPD reactor, which was revealed by the computational simulation of electric field distribution in the HPD reactor in Fig. 1(d) and Fig. 1(e). The calculated electric field in the middle point between the liquid surface and the tip of the HV electrode was 3.96×10^5 V·m^{-1} [Fig. 1(d)] while the electric field around the side of the ground electrode was 1.89×10^5 V·m^{-1} [Fig. 1(e)]. The synergetic effect of RONS and acidic pH during the generation of PAW at high salinity contributed to the enhanced bacterial inactivation, resulting in the induction of oxidative stress in the cell membrane and the leakage of intracellular components, which led to cell death (Barrales Astorga et al., 2022; Kang et al., 2019).

Table 3
P value of fixed main factor and interaction effects.

Dependent Variable	Treatment (T)	Storage Time (S)	T × S
Colour external L^*	0.932	0.4804	0.1471
Colour external a^*	0.0053	0.0002	0.0202
Colour external b^*	0.002	0.0166	0.127
Deoxymyoglobin	<0.0001	0.0518	0.341
Oxymyoglobin	0.0046	0.0136	0.2566
Metmyoglobin	<0.0001	0.0467	0.1358
TBARS values	0.0427	0.4229	0.7139
Weight gain	0.5122	0.1255	0.6175
Water holding capacity	0.1193	0.1622	0.3252
pH	0.4098	0.0461	0.4225

In addition, increasing the contact time from 15 s to 30 s improved the antibacterial ability of PAW against *S.* Typhimurium cells on chilled beef rumps for both the spraying and immersion methods at the storage times of 0, 1 and 7 days (Fig. 4); this is due to the increased interactions of reactive species with the bacterial cells and higher induced oxidative damage of cells at higher contact times (Perinban, Orsat, & Raghavan, 2022; Zhao, Ojha, et al., 2020; Zhao, Zhao, et al., 2020). Nonetheless, our results demonstrated that the bacterial reductions at 30 s and 60 s in Fig. 4 were not significantly different. For instance, the bacterial reductions of spraying the beef samples with PAW for 30 and 60 s were 0.656-$\log_{10}$ reduction (77.6% inactivation efficiency) and 0.666-$\log_{10}$ reduction (77.9% inactivation efficiency), respectively, at the meat storage time of 1 day (Fig. 4). This may be associated with the lag period of *S.* Typhimurium cells on beef at very short treatment time (Juneja & Eblen, 2000; Juneja, Hwang, & Friedman, 2010), and necessitates further studies developing a kinetic model to evaluate the kinetic behaviours of adhered *S.* Typhimurium on beef during the meat washing with PAW. Based on this, the contact time of 30 s was chosen as the optimal condition for the quality analysis of beef samples in Section 2.7.

Washing the beef samples via the spraying method was insignificantly different from those via the immersion method irrespective of the meat storage time and the liquid type (Fig. 4). This can be supported by the washing method × treatment interaction effect that was insignificant for any contact times and storage, shown in Table 1. For instance, for the meat washing with PAW for 60 s at the storage time of 7 days, the spraying method resulted on a 0.737-$\log_{10}$ reduction (81.2% inactivation efficiency) while the immersion method resulted on 0.710-$\log_{10}$ reduction (79.77% inactivation efficiency) (Fig. 4). Shen et al. (2019) evaluated the effects of commercial immersion and spraying methods on the inactivation against *Campylobacter jejuni* on chicken at the contact times of 20–30 s, indicating that the spraying method is more suitable

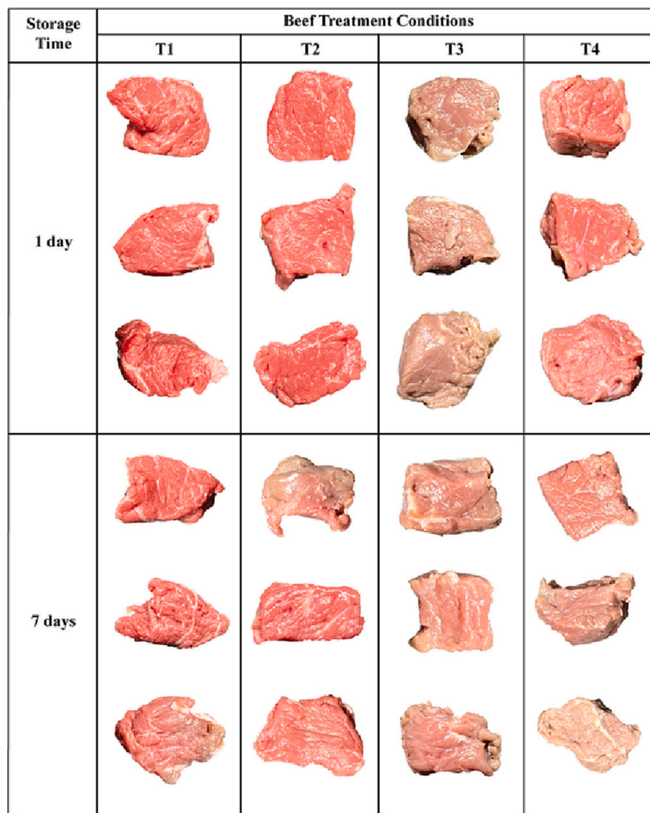

Fig. 5. Photographs of untreated beef (T1, control) and beef surface samples treated with the water spraying method at 55 °C for 30 s (T2), the PAW spraying method at 55 °C for 30 s (T3) and the PAW spraying method at 55 °C for 30 s followed by the additional water spraying method at 25 °C for 60 s (T4) at the meat storage times of 1 day and 7 days at 4 °C.

for large-scale producers. Moreover, the conventional immersion method consumes more water and produces wastewater than the spraying method (Bailone, Borra, Fukushima, & Aguiar, 2022). Therefore, the spraying method at 30 s was selected for the analysis of meat quality in Section 2.7.

3.2. Quality attributes of beef

Based on Section 3.1, 3 treatment conditions were used to evaluate the quality attributes of meat:

- Untreated beef (referred as "T1")
- Water spraying at 55 °C for 30 s (referred as "T2")
- PAW spraying at 55 °C for 30 s (referred as "T3")
- PAW spraying at 55 °C for 30 s followed by an additional water spraying at 25 °C for 60 s (referred as "T4")

3.2.1. Colour and myoglobin forms

The surface colour of meat plays a major role in consumer purchasing decisions. Colour is the primary quality attribute of meat to indicate the freshness and wholesomeness of the meat products. The lightness or L^* values of all beef samples T1, T2 and T3 were in the range of 46.7–50.4, as shown in Table 2, with insignificant main effects of treatment and storage time as well as the insignificant interaction effect of treatment × storage time as shown in Table 3. This means that all samples displayed the same reflectivity of the beef surface (Pogorzelska et al., 2018; Qian et al., 2022). This result is in agreement with Herianto et al. (2022), who found that PAW maintained the L^* values of shrimp meats.

Compared to the untreated (T1) and water-treated beef samples (T2), the PAW-treated samples (T3) had a significant reduction (i) in the redness, or a^* values, from 21.1– 21.7 to 11.3, and (ii) in the yellowness, or b^* values, from 11.6– 12.0 to 9.19, at the meat storage time of 1 day, as seen in Table 2. The main effects of treatment and storage time were significant for a^* and b^* values as shown in Table 3. A discolouration of the meat was also observed on the surface of PAW-treated samples (T3) at the storage time of 1 day in Fig. 5. PAW acidification was reported to cause the coagulation of surface protein, which affected the discolouration of meat products with PAW (Chaijan et al., 2022). Qian et al. (2022) stated that the RONS in PAW accelerated the metmyoglobin (MetMb) formation in beef samples, resulting in a decreased a^* value. However, this cannot support the observed phenomenon of reduced a* values in this study because the difference of MetMb values between T1, T2 and T3 samples were insignificant as shown in Fig. 6(c). In addition, the presence of oxymyoglobin (OxyMb) on the surface of beef samples influences the bright red colour appearance of meat (Huang, Chang, & Hsu, 2021; Wang et al., 2021). The main effects of treatment and storage time and the interaction effect of treatment × storage time for a^* values were significant as shown in Table 3.

The decreased redness of the PAW-treated samples was attributed to a significant reduction in OxyMb, shown in Fig. 6(b). For instance, the OxyMb decreased from 63.6% (T1) to 44.1% (T3) at the storage time of 1 day [Fig. 6(b)]. In addition, the reduced OxyMb caused a reduction in the b^* value (Fernández-López, Sayas-Barberá, Pérez-Alvarez, & Aranda-Catalá, 2004), which was confirmed by the results in Table 2 and Fig. 6(b). The main effects of treatment and storage time for both OxyMb and MetMb were significant but not their interaction (Table 3). Fortunately, the reduced redness and yellowness values were mitigated by introducing an additional meat washing with water right after spraying with PAW (T4 in Table 2 and Fig. 5), which increased (i) the a^* value to 18.2 (from 11.3 in T3) and (ii) the b^* value to 11.1 (from 9.13 in T3) at the storage time of 1 day.

At 7 days of storage, there was a discolouration process for all T1, T2, T3 and T4 samples, shown in Fig. 5, which showed the browning effect. This phenomenon is due to heme oxidation in meat and the effect of oxygen content during storage as all beef samples were not vacuum sealed, resulting in chemical changes in myoglobin and brown off-colours (Pogorzelska et al., 2018). The changes in myoglobin were also supported by the decreased deoxymyoglobin (DeoMb) and the increased MetMb with the meat storage time of 7 days compared to those stored for 1 day (Fig. 6). During storage, H_2O_2 accelerates the oxidation of myoglobin and damage the secondary structure of myoglobin (Huang et al., 2019). The concomitant oxygenation of DeoMb and the acceleration in the MetMb formation gives a brown colour to the surface of beef samples (Chaijan et al., 2021; Pogorzelska-Nowicka et al., 2022). This is consistent with Chaijan et al. (2022), who found that the MetMb formation and browning colouring of Asian sea bass steak increased during 30-day of storage. In this study, all beef samples were stored for up to 7 days in a sealed container with the presence of oxygen, which inevitably lead to the browning of meat during storage in all cases including the control samples. In our previous work, we evaluated the effect of PAW on beef samples under vacuum packaging for up to 4 weeks, indicating good retention in colour over the first 3 weeks compared to the water-treated samples (Barrales Astorga et al., 2022). Moreover, there was no significant change observed in the yellowness or b^* values for all beef samples T1, T2, T3, T4 at the storage time of 7 days (Table 2) with insignificant interaction effect of treatment × storage time (Table 3).

Compared to the untreated samples (T1 in Table 2) and water-treated samples (T2), PAW (T3 and T4) had significant impacts on the chroma (C) values of the sample at the storage time of 1 day but did not significantly change the hue angle (h^*) values. After storing the meat samples for 7 days, there was a significant reduction in the C values by the spraying method with PAW (16.2, Table 2) compared to the untreated samples (C = 21.9). The reduced C values indicate that the PAW-treated samples had a less vivid colour. Moreover, the h^* values of all

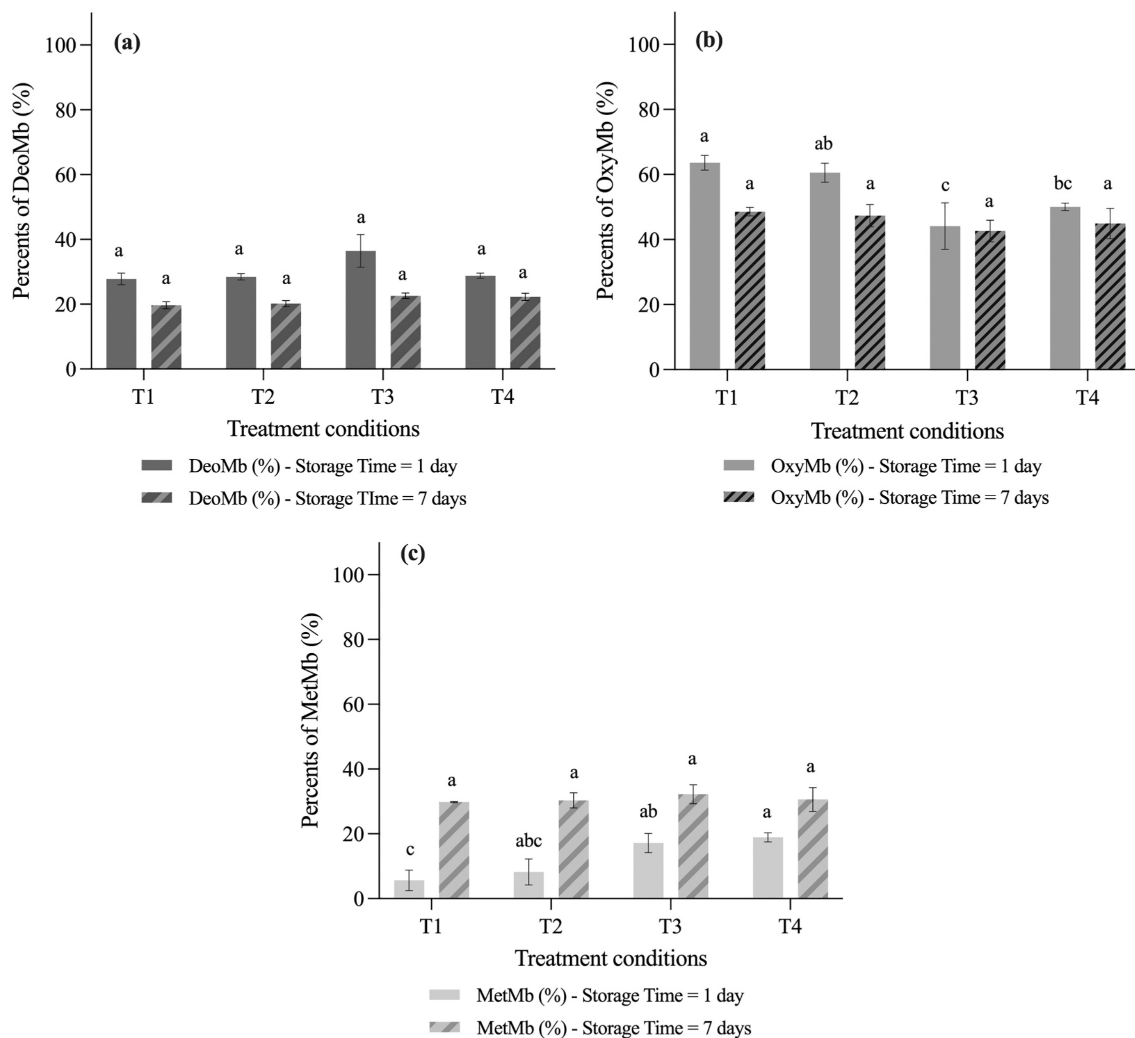

Fig. 6. (a) Deoxymyoglobin (%DeoMb), (b) oxymyoglobin (%OxyMb), and (c) metmyoglobin (%MetMb) of untreated beef (T1, control) and beef samples treated with the water spraying method at 55 °C for 30 s (T2), the PAW spraying method at 55 °C for 30 s (T3) and the PAW spraying method at 55 °C for 30 s followed by the additional water spraying method at 25 °C for 60 s (T4) at the beef storage times of 1 day and 7 days at 4 °C. Different lowercase letters (a, b, c) indicate statistically significant difference (P < 0.05) among the meat washing conditions with PAW and water within the same beef storage time. Error bar represents standard error of the mean.

samples were in the range of 0.948–1.64 with insignificant differences at both storage times of 1 and 7 days (Table 2).

Table 2 also revealed the colour difference (ΔE) values of all samples at the meat storage times of 1 day and 7 days, which describe small differences between colours as detected by human eyes: trace ($0<\Delta E<0.5$), slight ($0.5<\Delta E<1.5$), noticeable ($1.5<\Delta E<3.0$), appreciable ($3.0<\Delta E<6.0$), much ($6.0<\Delta E<12$), and very much ($\Delta E>12$) (Pogorzelska et al., 2018). At the storage time of 1 day, PAW (T3) exhibited a "much" difference (10.7 in Table 2) compared to the control (untreated samples). This was mitigated by the additional water spraying after PAW spraying (T4), which exhibited an "appreciable" change with the ΔE value of 3.45 (Table 2). It must be noted that the spraying method with water (T2) induced a "noticeable" change (2.37, Table 2), which is also not significantly different to T4. The above results demonstrated that PAW retained most of the colour values in the beef samples, except redness, but this was alleviated, although not eliminated completely, by introducing the additional water spraying after PAW treatment. Compared to untreated and water-treated samples, water washing slightly reduced the redness and chroma values and maintained the yellowness value after storing the samples for 1 day, but this did not significantly alter the overall colour difference detected by human eyes as represented by ΔE.

3.2.2. *Lipid oxidation*

As PAW is rich in reactive species, the interaction between its reactive species and the fatty acids of meat may induce lipid oxidation forming malondialdehyde (MDA) as a product of polyunsaturated fatty acid degradation, which results in off-odours and rancid off-flavours. The MDA in meat was determined via the TBARS analysis, which is the key biomarker to detect lipid oxidation and freshness of meat (Bauer et al., 2017; Thangavelu et al., 2022). Fig. 7 shows that the difference in TBARS values, expressed in mg MDA·kg^{-1} sample, was insignificant between untreated, water-treated and PAW-treated beef samples at the meat storage times of 0, 1 and 7 days. For instance, at the storage time of 1 day, the TBARS values of T1, T2, T3 and T4 samples were 0.277, 0.261, 0.257 and 0.454 mg MDA·kg^{-1} sample, respectively. Moreover, the treatment × storage time interaction effect for TBARS values were insignificant (Table 3). PAW was reported to accelerate the lipid oxidation of chicken meat because of the generated peroxides in PAW (Qian et al., 2022).

Another study indicated that the breakdown of lipid primary oxidation products into aldehydes and ketones caused increased lipid oxidation in fish meat (Maqsood & Benjakul, 2011). It has been reported that the oxidation rate of lipids in food products is influenced by the nature of the food matrix and the conditions of plasma treatment

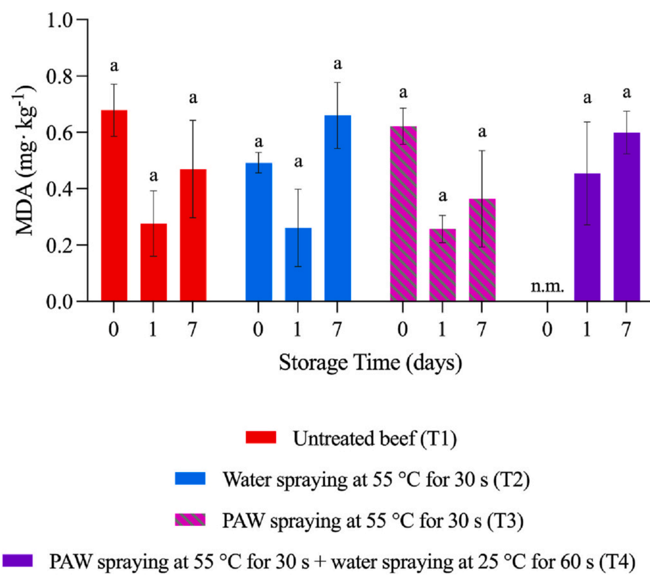

Fig. 7. TBARS values (in mg MDA·kg^{-1}) of untreated beef (T1, control) and beef samples treated with the water spraying method at 55 °C for 30 s (T2), the PAW spraying method at 55 °C for 30 s (T3) and the PAW spraying method at 55 °C for 30 s followed by the additional water spraying method at 25 °C for 60 s (T4) at the beef storage times of 1 day and 7 days at 4 °C. Different lowercase letters (a, b, c) indicate statistically significant difference ($P < 0.05$) among the meat washing conditions with PAW and water within the same beef storage time. n.m. represents "not measured". Error bar represents standard error of the mean.

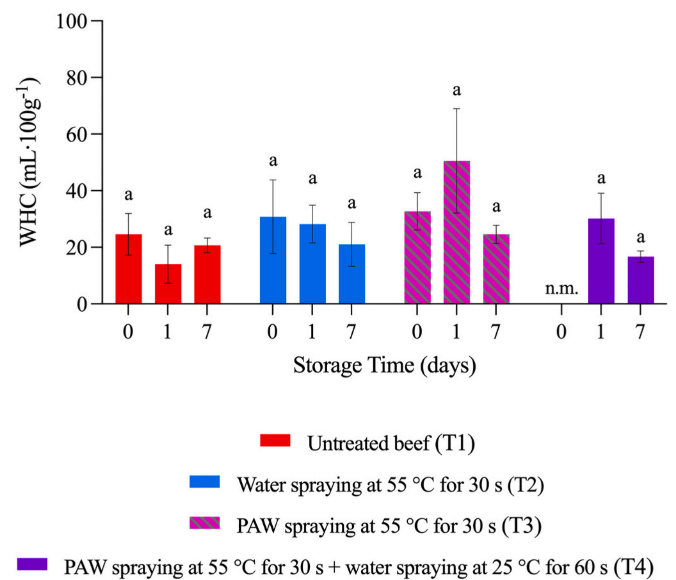

Fig. 9. Water holding capacities of untreated beef (T1, control) and beef samples treated with the water spraying method at 55 °C for 30 s (T2), the PAW spraying method at 55 °C for 30 s (T3) and the PAW spraying method at 55 °C for 30 s followed by the additional water spraying method at 25 °C for 60 s (T4) at the beef storage times of 1 day and 7 days at 4 °C. Different lowercase letters (a, b, c) indicate statistically significant difference ($P < 0.05$) among the meat washing conditions with PAW and water within the same beef storage time. n.m. represents "not measured". Error bar represents standard error of the mean.

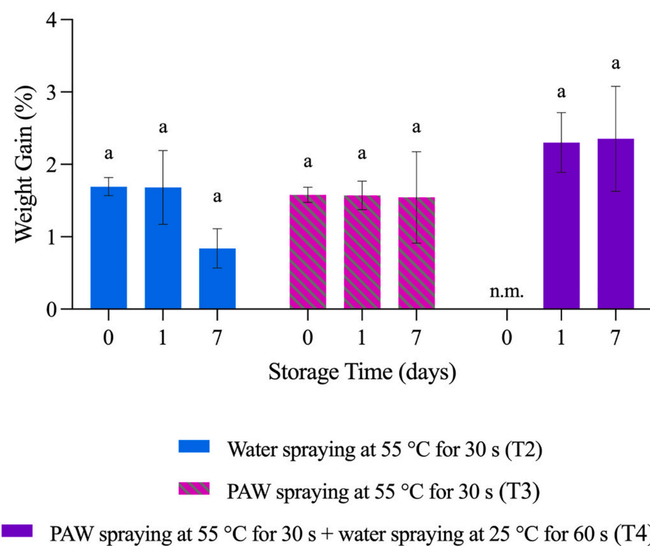

Fig. 8. Weight gains of beef samples treated with the water spraying method at 55 °C for 30 s (T2), the PAW spraying method at 55 °C for 30 s (T3) and the PAW spraying method at 55 °C for 30 s followed by the additional water spraying method at 25 °C for 60 s (T4) at the beef storage times of 1 day and 7 days at 4 °C. Different lowercase letters (a, b, c) indicate statistically significant difference ($P < 0.05$) among the meat washing conditions with PAW and water within the same beef storage time. n.m. represents "not measured". Error bar represents standard error of the mean.

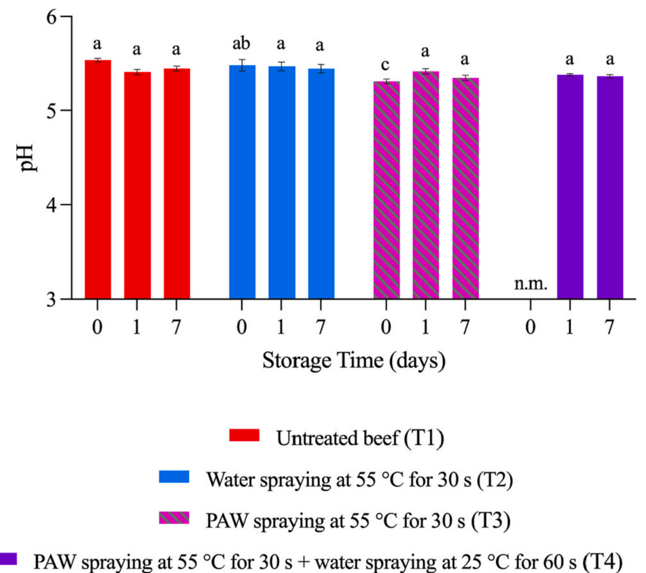

Fig. 10. pH of untreated beef (T1, control) and beef samples treated with the water spraying method at 55 °C for 30 s (T2), the PAW spraying method at 55 °C for 30 s (T3) and the PAW spraying method at 55 °C for 30 s followed by the additional water spraying method at 25 °C for 60 s (T4) at the beef storage times of 1 day and 7 days at 4 °C. Different lowercase letters (a, b, c) indicate statistically significant difference ($P < 0.05$) among the meat washing conditions with PAW and water within the same beef storage time. n.m. represents "not measured". Error bar represents standard error of the mean.

(Gavahian, Chu, Mousavi Khaneghah, Barba, & Misra, 2018), eventually affecting the cell structure and integrity (Yadav & Roopesh, 2022). Our results showed that PAW preserved the lipid oxidation of beef, which is in line with reported PAW studies (Chaijan et al., 2021; Herianto et al., 2022; Liao et al., 2020; Marcinkowska-Lesiak et al., 2022; Muhammad et al., 2019).

The antioxidant effect of nitrite from PAW helps to bind the iron centre of myoglobin to decrease the amount of free iron available, inhibiting lipid oxidation (Igene, Yamauchi, Pearson, Gray, & Aust, 1985; Inguglia et al., 2020; Marcinkowska-Lesiak et al., 2022). The

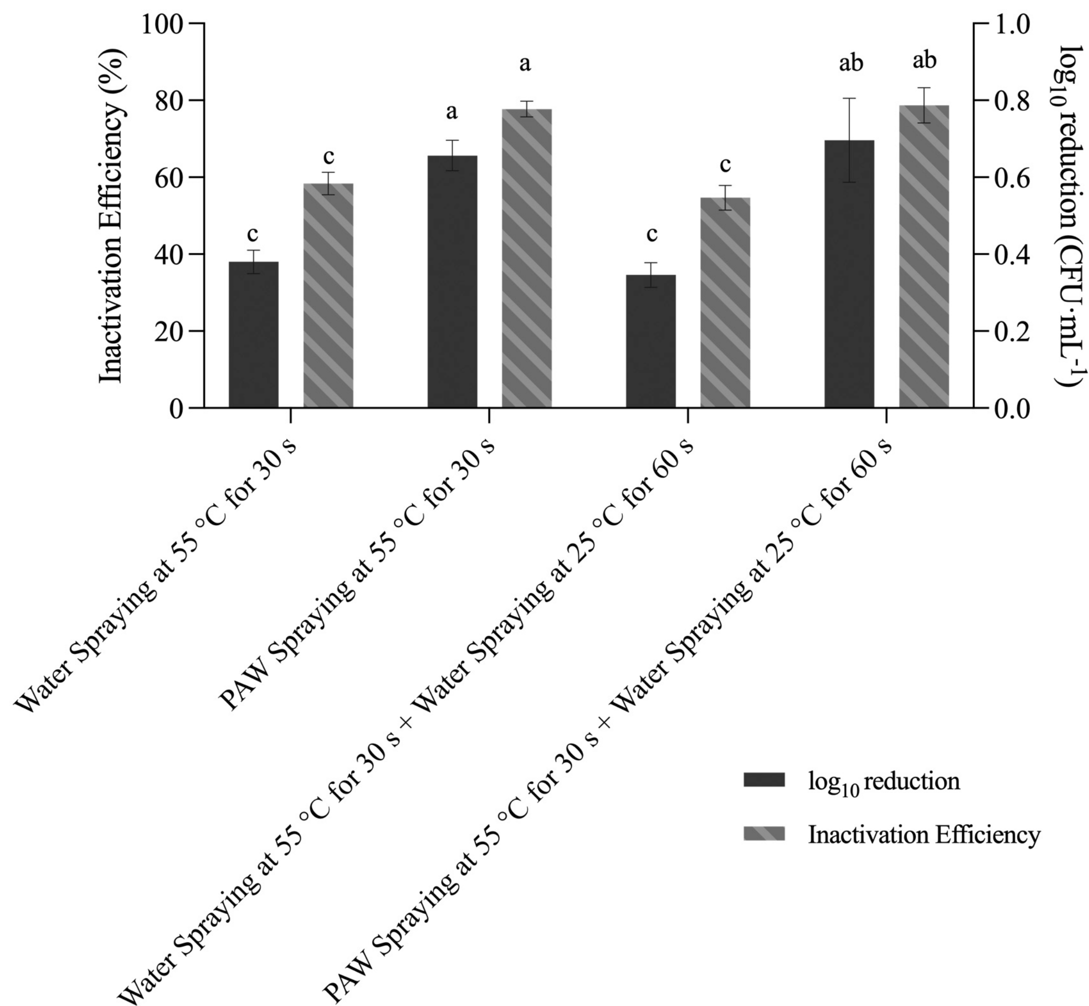

Fig. 11. Log_{10} reduction (right side) and Inactivation efficiency (left side) of beef surface samples treated with the water spraying method at 55 °C for 30 s, the PAW spraying method at 55 °C for 30 s, the water spraying method at 55 °C for 30 s followed by the water spraying method at 25 °C for 60 s and the PAW spraying method at 55 °C for 30 s followed by the additional water spraying method at 25 °C for 60 s at the beef storage times of 1 day at 4 °C. Different lowercase letters (a, b, c) indicate statistically significant difference ($P < 0.05$) among the meat washing conditions within the same beef storage time. Error bar represents standard error of the mean.

chemical properties of PAW generated by the HPD reactor were fully discussed in our previous work (Hadinoto et al., 2023). The measured concentration of NO_2^- in PAW was at 75.1 mg·l^{-1} (Table A1), whereas the concentrations of NO_3^- and H_2O_2 were at 17.9 mg·l^{-1} and 0 mg·l^{-1}, respectively. The antimicrobial capacity of PAW during meat washing, presented in Section 3.1, may also contribute to the insignificant change in the TBARS values (Herianto et al., 2022). Overall, all the reported TBARS values in Fig. 7 were in the range of 0.256 to 0.679 and below the threshold TBARS value of 2–2.5 mg MDA·kg^{-1} for rancidity flavour (Ripoll, Alcalde, Horcada, & Panea, 2011), indicating that all T1, T2, T3 and T4 beef samples were acceptable.

3.2.3. *Weight gain, water holding capacity and pH*

Compared to untreated samples, all meat washing conditions T2, T3 and T4 resulted in a weight gain of beef samples, in the range of 0.84 to 2.35%, with all significant difference between T2, T3 and T4 (Fig. 8). To determine the textural characteristic of meat products, water holding capacity (WHC) values of all beef samples in Fig. 9 were measured. PAW was reported to affect the texture of fish by decreasing the WHC of muscle proteins in the meat due to the reactive oxygen species (ROS) in PAW (Chaijan et al., 2021), For chicken meat, PAW reduced the WHC with loss of soluble proteins by increasing the gap between muscle fibres (Qian et al., 2022). Fig. 9 demonstrated that the difference between untreated samples (T1) and treated samples (T2, T3 and T4) was insignificant. Results show that at the storage time of 7 days, the WHC values of T1, T2, T3 and T4 samples were 20.7, 21.0, 24.6 and 16.7 ml·100 g^{-1} sample, respectively, which means that PAW maintained the space in the myofibril compartment (Moutiq et al., 2020), as well as the tender texture and drip loss of the meat products (Barrales Astorga et al., 2022).

During PAW treatment, the interactions between RONS and meat surfaces can contribute to changes in the pH of treated meat products. pH can be used as an indicator of beef freshness, which is typically in the range of 5.30–5.70 (MLA, 2011). Average pH values of the beef samples treated with water and PAW were in the range of 5.31–5.48 as shown in Fig. 10, with an insignificant difference with the untreated samples (5.40–5.54), suggesting that all T1, T2, T3 and T4 beef samples were acceptable. The insignificant difference in the pH values between untreated and treated samples (Fig. 10) supported the insignificant change in the L^* values in Section 3.2.1, in which acidification affects meat colour, influencing the variability of colour lightness measurements (Jankowiak, Cebulska, & Bocian, 2021). Meat products with low pH can have higher reflectance compared to those at high pH (Swatland, 2008). There is also a linkage between WHC and pH of the meat. When the pH reaches the isoelectric point (IEP) of major proteins in beef at about 5.5, the polarised group within the muscle proteins are drawn to each other

and a decrease in water amount is formed (Liao et al., 2020; Rao & Gault, 1990). No significant differences in pH were identified between the T1, T2, T3 and T4 samples during storage time (Fig. 10), supporting the insignificant change in the WHC of beef (Fig. 9). Additionally, the treatment × storage time interaction effect for weight gain, water holding capacity and pH were insignificant as shown in Table 3. Overall, PAW slightly increased the meat weight and preserved the water holding capacity and pH of meat.

Fig. 11 shows the inactivation of *S.* Typhimurium on chilled beef samples with water spraying for 30 s, PAW spraying for 30 s, water spraying for 30 s followed by water spraying for 60 s and PAW spraying for 30 s followed by water spraying for 60 s at the meat storage time of 1 day. It shows that the additional meat washing with water had no impact in the antibacterial efficiency, but the second washing improved the redness, yellowness and chroma values. For instance, the additional water spraying after the first meat washing with water (0.346-$\log_{10}$ reduction or 54.7% inactivation efficiency, Fig. 11) had no significant impact on the bacterial reduction compared to washing with water alone (0.383-$\log_{10}$ reduction or 58.4% inactivation efficiency). However, the colour difference (ΔE) value, the yellowness value and the content of myoglobin forms were maintained by the additional water washing. The redness value improved from 11.3 (T3) to 18.2 (T4), as explained in Section 3.2.1, but the value was still lower than control with a* value of 21.1 (T2).

3.3. Comparing with other studies

The PAW inactivation at 30 s observed in this study performed (i) 33.9% higher than the PAW inactivation against *S.* Typhimurium on chicken at a 15-min contact time (Sammanee et al., 2022), (ii) 720% higher than the PAW inactivation against *S.* Typhimurium on pork at a 15-min contact time (Sammanee et al., 2022), (iii) 112% higher than the inactivation via direct non-thermal plasma against *S.* Typhimurium on pork at a 2.5-min contact time (Jayasena et al., 2015) and (iv) 64% higher than the inactivation via direct non-thermal plasma against *S.* Typhimurium on beef at a 2.5-min contact time (Jayasena et al., 2015). It is important to note that the generation of PAW consumed a very low discharge power of 45.1 W [Fig. 1(c)] and for the first time, it has been successfully demonstrated to inactivate pathogens on beef in very short contact times while preserving most of the quality attributes of the meat.

4. Conclusions

This work evaluated the use of plasma-activated water (PAW) for washing chilled beef rumps to inactivate foodborne pathogens. Two meat washing methods, spraying and immersion, was tested at contact times of 15, 30 and 60 s. The efficiency of PAW against adhered *S.* Typhimurium cells was lower than those in a planktonic state with no significant difference between the two mashing methods or contact times. Spraying with PAW for 30 s did not negatively impact the lightness and hue angle values compared to untreated beef samples and water-treated samples, but it reduced the redness, yellowness and chroma values. However, the negative effects were mitigated by introducing additional water spraying at 25 °C for 60 s. PAW spraying with and without additional water spraying achieved 0.696- and 0.656-$\log_{10}$ reduction in *S.* Typhimurium, respectively, without negative impacts on the quality of the meat. Overall, PAW spraying is a promising method for inactivating foodborne pathogens in meat washing.

CRediT authorship contribution statement

Koentadi Hadinoto: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Hanxia Yang:** Validation. **Tianqi Zhang:** Resources. **Patrick J. Cullen:** Writing – review & editing, Supervision. **Stuart Prescott:** Writing – review & editing, Supervision. **Francisco J. Trujillo:** Conceptualization, Software, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

Author Patrick J. Cullen is the CEO of PlasmaLeap Technologies, the supplier of the Leap100 power supply and bubble reactors used in this study.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2023.109165.

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