Research Article

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Effect of plasma-activated water on microbial quality and physicochemical properties of fresh beef

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Abstract: This work studies the influence of plasma-activated water (PAW) on the decontamination of beef and its influence on the color, pH, the thiobarbituric acid reactive substance values (TBARS), and total volatile basic nitrogen (TVBN) values of meat. PAW was generated using non-thermal atmospheric pressure plasma jet (NTAPPJ). He + 0.2% N_2 and He + 0.2% O_2 were used as worker gas to generate PAW. The PAW produced by the He + O_2 plasma system exhibited a higher potential for decontamination of beef than that produced by the He + N_2 plasma system. The lightness value (L^*) of treated beef does not exhibit a noticeable difference with the control one. TBARS values of all treated beef were lower than the rancidity threshold but significantly greater than that of control samples. The TVBN value of control beef samples reached the decay threshold after 18 days of stockpiling, but treated beef remained good. This work reveals that PAW can potentially inhibit the growth of microorganisms in beef.

Keywords: non-thermal plasma, PAW, reactive species, TBARS

1 Introduction

The meat represents an essential source of protein for humans due to its unique nutrient composition, high water activity, and moderate pH [1]. Meat is the best growth medium for the different microorganisms [2]. The off-odor and the off-flavor of foods depend on some molecules generated by spoilage microorganisms. The aqueous meat phase contains some biocompounds, such as nucleotides, peptides, sugars, and amino acids, that cause the growth of microorganisms. The biochemical processes of peptides, sugars, and amino acids generate many chemical compounds which lead to meat decomposition [3]. Even though traditional methods can inactivate food-borne pathogens, these methods have a passive influence on the nutrient values. The deterioration of food quality, affecting such characteristics as aroma, taste, nutrition, color, and texture by thermal treatment, makes severe compromise in the original freshness, nutrition, and function of the food product [4].

One of the problems facing food systems is lipid oxidation, because it causes nutritional loss and the development of toxic compounds [5]. Thiobarbituric acid reactive substances (TBARSs) are considered simple and highly sensitive method as a marker for lipid peroxidation in foods [6]. The acceptance limit of TBARS for beef rancidity equals 1.0 mg/kg [7]. The total volatile basic nitrogen (TVBN) is a necessary measure for estimating the freshness of meat [8]. The TVBN quantity is utilized in many scientific applications to determine the range of degradation of meat, such that, as the TVBN amount increases, the activity of the harmful degradation of bacteria increases [9].

Hence, a significant consideration is now centered on creating new non-thermal strategies for decreasing food contamination by microorganisms [10]. The high-performance effect has been attained when the non-thermal plasma technique is used to inhibit bacterial lawns inoculated with resistant and non-resistant bacterial strains [11], to sterilize heat and chemical-sensitive medical devices [12], and to fight resistant bacterial strains [13]. Non-thermal plasma produces an electric field and generates reactive oxygen and nitrogen species [14,15]. It is then assumed that non-thermal plasma

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treatment could be an unusual way to deliver synchronously intense transient electric field and reactive species, which play an essential role in disinfection applications [16].

Recently, plasma-activated water (PAW) is used in many fields of research and applications [17]. PAW can be generated by direct treatment of water using cold plasma. The treated water converts into an acidified solution containing nitrogen oxide [18]. Moreover, this solution has many unique properties [18]. PAW might destroy microorganisms that direct plasma failed to kill [19]. Furthermore, PAW does not contain hazardous chemicals like traditional sanitizers [20]. However, many chemical components such as nitrate, nitrite, and hydrogen peroxide play essential roles in the decontamination process using PAW [21]. Moreover, the oxidative stress created by PAW represented the essential agent for annihilating microorganisms in food products [22]. Low pH is considered as the essential factor for increasing the potential of PAW to inhibit the growth of microbes [23]. Recently PAW played a worthy role in food preservation and its products. PAW is used in foodstuffs' decontamination instead of the conventional plasma procedures due to its many advantages [24]. Moreover, PAW has a higher potential for preserving meat and manufactured meat because it contains NO_2^-, NO_3^-, and H_2O_2 particles [25].

The objective of this experiment is to study the impact of PAW generated by non-thermal atmospheric pressure plasma jet (NTAPP) on beef properties. The produced particles in the plasma plume have been evaluated. The electrical conductivity, oxidation-reduction potential (ORP), and pH values and also the reactive species quantities in PAW were measured. The observed changes in color parameters of the samples were recorded. The microbe's inhibition using PAW on beef was achieved. pH, TBARS, and TVBN values of beef were measured.

2 Methods

2.1 Plasma source

In the current experiment, the NTAPPJ system was constructed and used to sterilize beef. Figure 1 depicts the NTAPPJ system, which has been illustrated in the previous studies [26–28]. The neon light transformer (Hb-C10) was used as a power supply to generate plasma plume. A voltage controller (TDGC2-3KVA Variac 220 V) was used to regulate the input voltage of the power supply from 0 to 220 V. The power supply delivers 10 kV, 30 mA output power and was operated in the kHz regime, *i.e.*, at 20 kHz in this work. The two mixtures (He + 0.2% N_2 [*HeI*], and He + 0.2% O_2 [*HeII*]) were used as worker gas to operate NTAPPJ in the current experiment. According to our previous work, the percentage of gases mentioned above has given the optimum conditions [28]. The purity

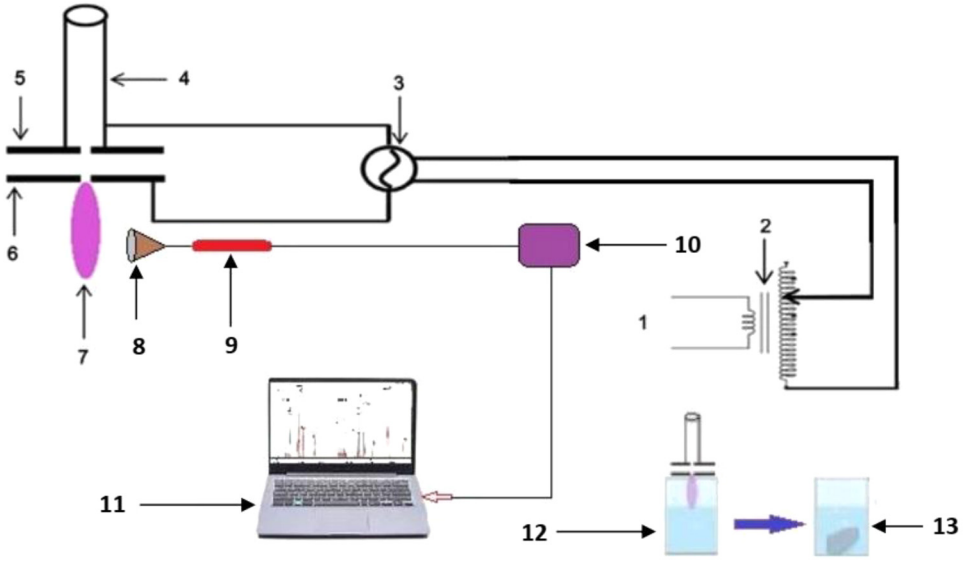

Figure 1: The electric circuit of NTAPPJ system: (1) input electric source 220 V, (2) voltage controllers, (3) neon power supply, (4) gas feeding through copper tube, (5) anode, (6) cathode, (7) plasma plume, (8) collimator, (9) optic fiber, (10) OES, (11) laptop, (12) production of PAW, and (13) beef treatment using PAW.

of N_2, He, and O_2 was 99.995, 99.997, and 99.996%, respectively.

2.2 PAW preparation

NTAPPJ has been utilized to produce PAW. As seen in Figure 1, distilled water at 25°C was divided into two groups, the first group irradiated using *HeI* mixture to generate PAWX, and the second irradiated using *HeII* mixture to generate PAWY. The plasma dose of the *HeI* system was 2, 4, 6, 8, and 10 min to produce PAWX2, PAWX4, PAWX6, PAWX8, and PAWX10, respectively. Moreover, the plasma dose of the *HeII* system was 2, 4, 6, 8, and 10 min to produce PAWY2, PAWY4, PAWY6, PAWY8, and PAWY10, respectively. In all runs, the volume of distilled water was 160 mL. The irradiation of distilled water by NTAPPJ was operated concurrently with the stirring of distilled water using a magnetic stirrer. The temperature of PAWX and PAWY was measured using a thermocouple. The applied voltage on the two electrodes and gas flow rate were 3.5 kV and 14 L/m, respectively, for the two systems of carrier gas (*HeI* and *HeII*). The distilled water was filled in a Pyrex bowl, and it was tightly closed using a cork from the open side. The plasma jet was passed through the cork plug. The distance from the nozzle and distilled water was 3 mm. In addition, the length and the diameter of generated plasma were 7 and 3 mm, respectively. Moreover, there was no change in the weight of distilled water before and after the treatment.

2.3 Determination of the reactive species in the gas and liquid medium

The current experiment detected the emission spectra of generating plasma over a broad wavelength range of 200–900 nm using HR4000CG-UV-NIR (Ocean Optics) spectroscopy. Moreover, the spectrometer composes a lens connected to a detector *via* fiber optic cable (QP400-2-SR) with a diameter of 400 mm. The reactive species concentrations in the distilled water after treatment by NTAPPJ were also analyzed. Titanyl ions [29], Acorn Series ION 6 m (pH/mV/°C meter) nitrate electrode [30], and a Griess reagent were applied to quantify the H_2O_2, NO_3^- and NO_2^- concentration in PAW, respectively.

2.4 Characterization of PAW

Radiometer Analytical (CDM 210), the redox-sensitive electrode (waterproof-IP57-Taiwan), and Eutech instruments (pH 700 m) were applied to measure conductivity, ORP, and pH values for PAW, respectively.

2.5 Beef samples preparation

The beef was purchased from a domestic market. The surface layers of muscle beef (thickness 2 cm, length 5 cm, width 4 cm) from the chuck section were cut out using a meat-cutter electric machine (JIQI model). Then, the meat samples were frozen at –20°C for 24 h before treatment. After that the beef slices were divided into three groups. The first and second groups were soaked in PAWX and PAWY, respectively. Moreover, the third group (control beef) was soaked in untreated distilled water (160 mL). The soaking procedure continued until the beef samples' core temperature reached 0°C. The current work was carried out in the conditions of our laboratory at 50% relative humidity and temperature 24–25°C. Later, the treated and control beefs were stored at 5°C for 20 days. Moreover, microbial analysis, color measurement, and other physicochemical properties have been evaluated through the storage period.

2.6 Microbial analysis

The total aerobic bacteria were calculated for treated and untreated beef as the following, the homogenization of beef samples (5 g) with 45 mL of sterile saline solution was conducted using a stomacher BagMixer® 400. Then, the samples were decimally diluted using sterile saline solution. To compute the total number of aerobic microbes, the total plate count agar was prepared. After that the plates were incubated for 48 h at 37°C. All colonies were computed, and the numeral of microbes was identified as log CFU/g.

2.7 Color measurement

We measured the surface-color values of the samples by using a colorimeter (colorimeter CR-400 Chroma Meter).

Moreover, in International Commission on illumination (CIE), L^*, a^*, and b^* values representing lightness, redness, and yellowness, respectively. With an aperture size of 8.0 mm and illuminant D65, values are expressed by the following terminology: L^* (lightness), a^* (redness), and b^* (yellowness). The calibration of instrument was conducted using a standard white plate (L^* = 91.59, a^* = 0.3167, and b^* = 0.3331) [31].

Chroma (C^*) is the colorfulness of an area judged as a proportion of the brightness of a similarly illuminated area that appears white or highly transmitting. It is utilized to evaluate the degree of difference compared to grey color with the same lightness. Chroma (C^*) was determined by the following equation [30]:

$$c^* = \sqrt{a^{*2} + b^{*2}}. \quad (1)$$

A higher hue angle represents a lesser yellow character that was determined by the following equation.

$$h^* = \tan^{-1} = \left(\frac{b^*}{a^*}\right). \quad (2)$$

Total color difference can be measured as the modulus of the distance vector between the initial color values and the actual color coordinates.

$$\Delta E = \sqrt{L^{*2} + a^{*2} + b^{*2}}. \quad (3)$$

2.8 pH measurement of beef

Three grams of beef samples were mixed with 27 mL of distilled water for 60 s to determine the pH of current samples. Subsequently, pH values of the mixing were measured by a pH meter (Model 750, iSTEC, Seoul, Korea) [32].

2.9 TBARS measurement

5 g of samples were mixed with 25 mL of 20% trichloroacetic acid solution in a homogenizer for 30 s. The mixing was filtered to take off beef morsels from the filtrate. Then, 2 mL of 0.02 M aqueous TBA solution (3 g/L) was added to 2 mL of filtrate in a test tube. After that test tubes were incubated at 100°C for 30 min and cooled with running tap water. The absorbance of supernatant solutions was evaluated at 532 nm using a UV-VIS spectrophotometer (DU530; Beckman Instruments Inc., Brea, CA, USA). The TBA values (mg malondialdehyde/kg sample) were calculated using a standard curve.

2.10 Assay of the TVBN

100 g of beef were mixed in 200 mL of 7.5% aqueous trichloroacetic acid solution. Later, the mixing was centrifuged at 400×g for 5 min. Subsequently, a Whatman (number 3) filter paper was used to filter the supernatant liquid. Steam distillation was made utilizing a Kjeldahl-type distillator. 25 mL of the filtrate were loaded into the distillation tube, followed by 5 mL of 10% NaOH. A glass cylinder containing 10 mL of a 4% aqueous boric acid solution and 0.04 mL of methyl red and bromocresol green indicator for titration of ammonia were placed at the end of the condenser. The distillation process stopped when the volume of 50 mL was obtained. The distilled TVBN converted the boric acid solution green. This was titrated using a 0.01 mL graduated micro burette containing an aqueous 0.1 N H_2SO_4 solution. Complete neutralization was obtained when the color turned pink on adding a further drop of sulfuric acid: TVBN = n × 16.8 mg of nitrogen/100 g, where (n) is the volume of H_2SO_4 [33].

2.11 Statistical analysis

In this work, all experiments were performed by three replicates. One-way ANOVA analyzed the obtained data. The comparisons with $P < 0.05$ were considered significantly different.

3 Result and discussions

3.1 Reactive chemical particles in the gas medium

The optical emission spectrum of NTAPPJ for the *HeI* and *HeII* systems is shown in Figure 2. From this figure, UV emissions in the 200 nm and 300 nm range have not emerged. These results proved that the UV emission had not participated in this device's microbicidal process [34]. As shown in this figure, the reactive chemical particles of OH, OI, OII, N_2 1+, N_2 1−, N_2 2+, and He emerged in the UV-vis wavelength area. However, by comparing the spectroscopy of the present two systems, the *HeII* worker gas possesses an excess of reactive chemical particles. As shown in Figure 2, the OI band has arisen at 844 nm [35] for the *HeII* system but not observed for the

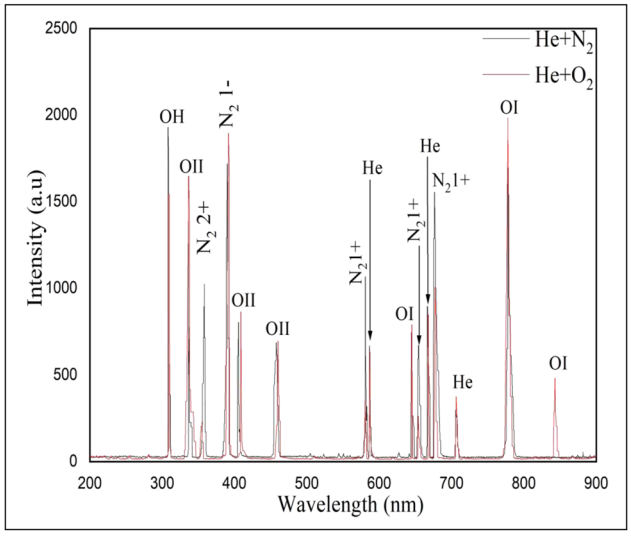

Figure 2: Emission spectra from 200 to 900 nm corresponding He + N_2 (*HeI*), and He + O_2 (*HeII*) systems at the same conditions.

HeI system. However, the emission intensity of OII at 337, 406, and 459 nm [34] for the *HeII* system has a higher value than that for the *HeI* system. O radical represents the primary factor in the increment of OII intensity. O radical has powerful oxidizability that provides it with the capacity to interact with other plasma components to create free radicals or other reactive particles [36].

3.2 The concentration of reactive species in PAW

In the present study, electrochemical analysis was utilized to evaluate the concentration of H_2O_2, NO_3^-, and NO_2^- in PAW. Figure 3(a–c) shows that the concentrations of H_2O_2, NO_3^-, and NO_2^- in PAWX and PAWY were increased by increasing the plasma irradiation time. The concentration of H_2O_2 in PAWX increased from 2,500 to 6,320 µM, while PAWY increased from 3,121 to 7,473 µM for 2 and 10 min, respectively. On the other hand, the concentration of NO_3^- in PAWX increased from 1,300–4,135 µM and from 2,100 to 5,212 µM in PAWY for 2 and 10 min, respectively. Moreover, the concentration of NO_2^- in PAWX increased from 1,100 to 3,862 µM and from 1,800

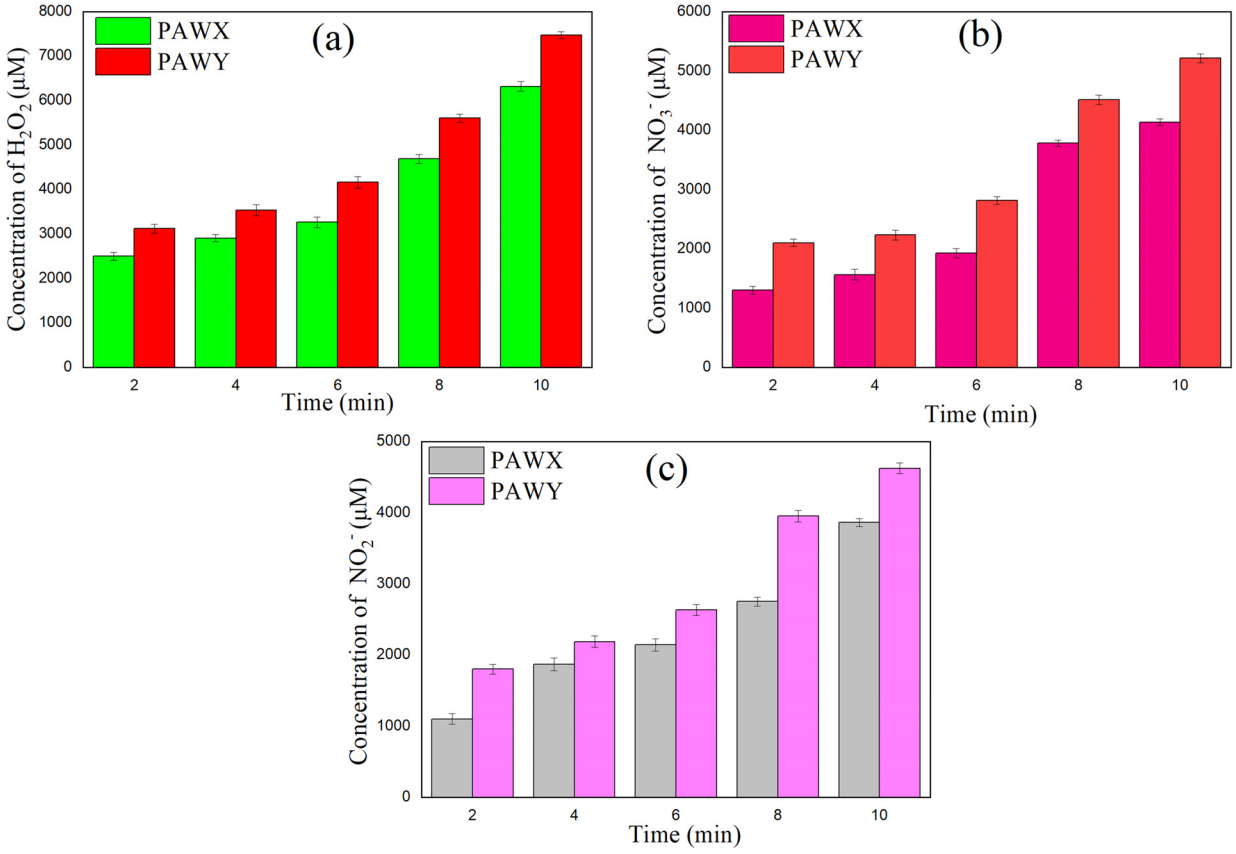

Figure 3: The concentration of reactive species in PAW; (a) H_2O_2, (b) NO_3^-, and (c) NO_2^-.

to 4,625 µM in PAWY for 2 and 10 min, respectively. This character may be ascribed to the piling up of H_2O_2, NO_3^-, and NO_2^- in PAW generated from the reactions of reactive chemical particles in the gas medium with distilled water. Moreover, the results illustrated that the quantities of H_2O_2, NO_3^-, and NO_2^- in PAWX is lower than PAWY. The increment in H_2O_2, NO_3^-, and NO_2^- concentrations in PAWY is ascribed to the increment in chemical particles amount in the gas medium for this system. Moreover, an acceptable mechanism to produce hydrogen peroxide in the plasma-treated liquid is the recombination of hydroxyl ions created by plasma through electron collisions with water molecules [37]. On the other hand, many researchers have proved the existence of H_2O_2, NO_3^-, and NO_2^- in plasma-treated water [38].

3.3 Conductivity, pH, ORP, and temperature measurements of liquid medium

Conductivity, pH, ORP, and temperature of treated solutions are displayed in Figure 4. Rohit *et al.*, in 2018, have proved that the generation of reactive chemical molecules in water after plasma treatment led to an increment in conductivity of PAW [39]. Moreover, in the current experiment, as shown in Figure 4(a), in both *HeI* and *HeII* treatment methods, the conductivity of PAW increased after irradiation by NTAPPJ. The conductivity of PAWX was 253 µS/cm for untreated water but increased to 286, 378, 489, 650, and 790 µS/cm for PAWX2, PAWX4, PAWX6, PAWX8, and PAWX10, respectively. Also, the conductivity of PAWY increased to 496, 506, 690, 820, and 995 µS/cm

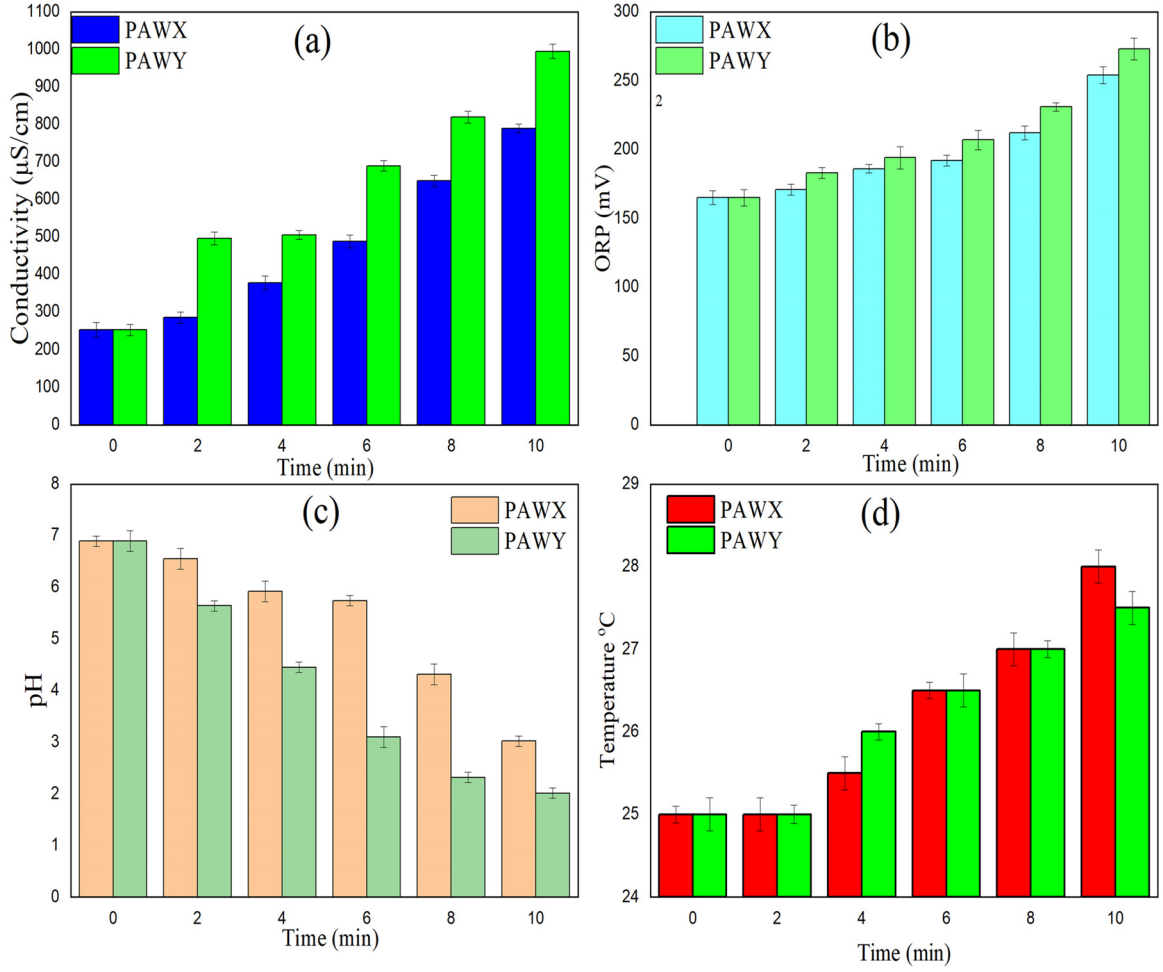

Figure 4: The properties of PAW: (a) conductivity, (b) ORP, (c) pH, and (d) temperature of PAW.

for PAWY2, PAWY4, PAWY6, PAWY8, and PAWY10, respectively. Further, the findings suggest that the increase in conductivity of PAWY is more significant than PAWX. The current results revealed that the conductivity of PAW is strongly dependent on the sort of working gas. As shown from the figure, for PAWY, the final values of the conductivity (995 μS/cm) are higher than that for PAWX (790 μS/cm).

Moreover, the ORP technique is utilized to measure the solution potential to oxidize or reduce another substance. The quantity of oxidizers and their strengths represent the fundamental factor of the powerful solution. The benefit of estimating the ORP of water is that it quickly evaluates the decontamination potential [8]. The waterpower to destroy the cell membrane of microbes increases as ORP increases. Moreover, McPherson *et al.*, in 1993, concluded that the ORP is considered a critical agent influencing microbial inhibition [40]. From Figure 4(b), the ORP of distilled water increased for both systems by increasing plasma dose. It can be observed that for PAWX, an increment in ORP value from 165 to 171 mV for 2 min of plasma treatment and a further increase in exposure times to 4, 6, 8, and 10 min lead to ORP values of 186, 192, 212, and 254 mV, respectively. However, ORP for PAWY increased to 183, 194, 207, 231, and 273 mV for 2, 4, 6, 8, and 10 min, respectively. The result suggests a significant increase in ORP values for PAWY compared to that for PAWX. However, Ma *et al.*, in 2015, examined the effectiveness of non-thermal plasma duration time on the ORP values for PAW [41]. They observed that as plasma dose increases, the values of ORP increase. Moreover, Burlicaa *et al.*, in 2006, concluded that PAW with higher values of ORP presents a powerful oxidizing capacity that possesses extraordinary antimicrobial potential, and water conductivity depends on water acidity [42].

From Figure 4(c), it can be noticed that as the plasma dose increase, the pH values of PAW has reduced, which means that PAW is getting acidic. Moreover, for PAWX, pH decreased from 6.98 to 3.02 after 10 min of NTAPPJ treatment. Further, PAWY exhibits the same response, but the decreasing pH for PAWY was higher than PAWX. These results declared that the H_2O_2, NO_3^-, and NO_2^- species play a worthy role in increasing conductivity and ORP values of PAW. Moreover, as the concentration of H_2O_2, NO_3^-, and NO_2^- increase, the acidity of the solutions increases because the pH values has decreased.

The temperature of PAW is illustrated in Figure 4(d). From this figure, the treatment of distilled water using NTAPPJ raised the temperature from 25 to 28°C as the treatment time increased from 2 to 10 min for both systems. The change in PAW temperature has not depended on the type of carrier gas. These results demonstrated that the small rise in PAW temperature could not be considered a factor in sterilizing beef and killing bacteria in the current experiment.

3.4 Total aerobic bacteria count

The effect of PAW on the lowering of microorganisms in treated and untreated beef is noticed in Figure 5. The results have revealed that reducing microorganisms of beef using PAW has increased with the increase in the treatment time in treated water. Moreover, these results can be concluded that PAW has a high potential to inhibit the growth of microbes on beef. The total aerobic bacteria count of beef was reduced from 3.1 to 2.4 and 2.3 log CFU/g after soaking in PAWX2 and PAWY2, respectively. As the irradiation dose for distilled water increased, the deficiency of microbe's number in soaked beef increased. The deficiency of microbes in beef has recorded 0.5 and 0.2 log CFU/g after soaking in PAWX10 and PAWY10, respectively.

Moreover, the preventing of bacteria growth in beef occurred due to the reactive chemical molecules generated in the PAW by NTAPPJ. These results indicated that PAWY exhibits high microbicidal than PAWX. The reduction in microorganisms has been recorded as 93.5% by PAWY10, while it has been recorded as 83.8% by PAWX10. The current results illustrated that the high ORP, high conductivity, and low pH of PAW have a strong influence on microbial growth. Thus, the produced active

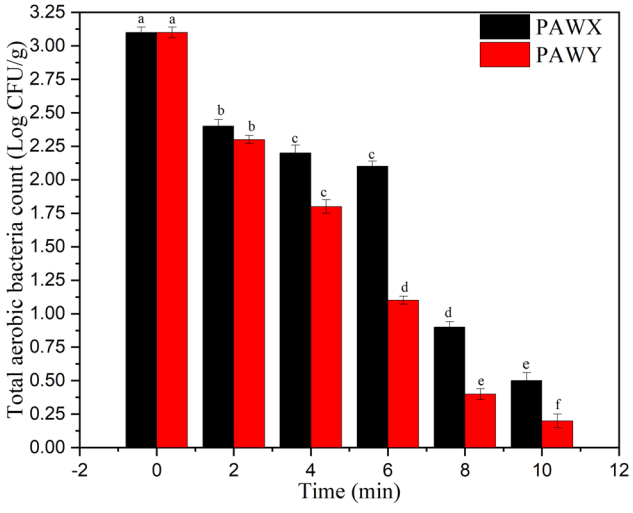

Figure 5: Total aerobic bacteria count for beef samples after treatment using PAW. Lowercase letters (a–f) in the same columns represent significance analysis; the different letters mean significant difference at $P < 0.05$.

species using plasma jet play a worthy role in inactivating microorganisms on the meat surface. These results also demonstrated that preventing the growth of microorganisms on meat surfaces using plasma treatment depends on the working gas and plasma dose. Moreover, Thirumdas *et al.*, in 2015, have concluded that reactive chemical molecules play a worthy role in membrane disruption, as they can drive protein denaturation [43]. The interaction of reactive chemical molecules with membrane lipids can stimulate the oxidation of amino acids, nucleic acids, and unsaturated fatty acid peroxides, which caused an alteration of the membrane function [44].

3.5 Surface color

Color is defined in three-dimensional space; the vertical axis defines lightness where the saturation and hue are given in a plane perpendicular to the lightness axis. The most used system for describing food color is the CIELAB system, where L^* describes lightness, a^* redness (–ve a^* = greenness), and b^* = yellowness (–ve b^* = blueness). The effect of PAW on the color parameter of beef samples is displayed in Figure 6(i– iii). The results revealed that PAWX or PAWY do not have a noticeable influence on the L^*-values of beef. There is no significant difference between control beef and treated beef. Also, the type of working gas has no remarkable impact on the L^*-values of treated beef, as shown in Figure 6(i). Moreover, previous studies revealed that the L^*-values of plasma-treated pork butt, beef loin [37], and cooked meat batter [45] do not change with untreated samples. Nevertheless, the L^*-value of plasma-treated bacon and stored pork loins decreased [45]. The effectiveness of PAW on the a^* values of beef is illustrated in Figure 6(ii). In the case of beef soaked in PAWX, it can be observed that there is no significant difference between untreated and

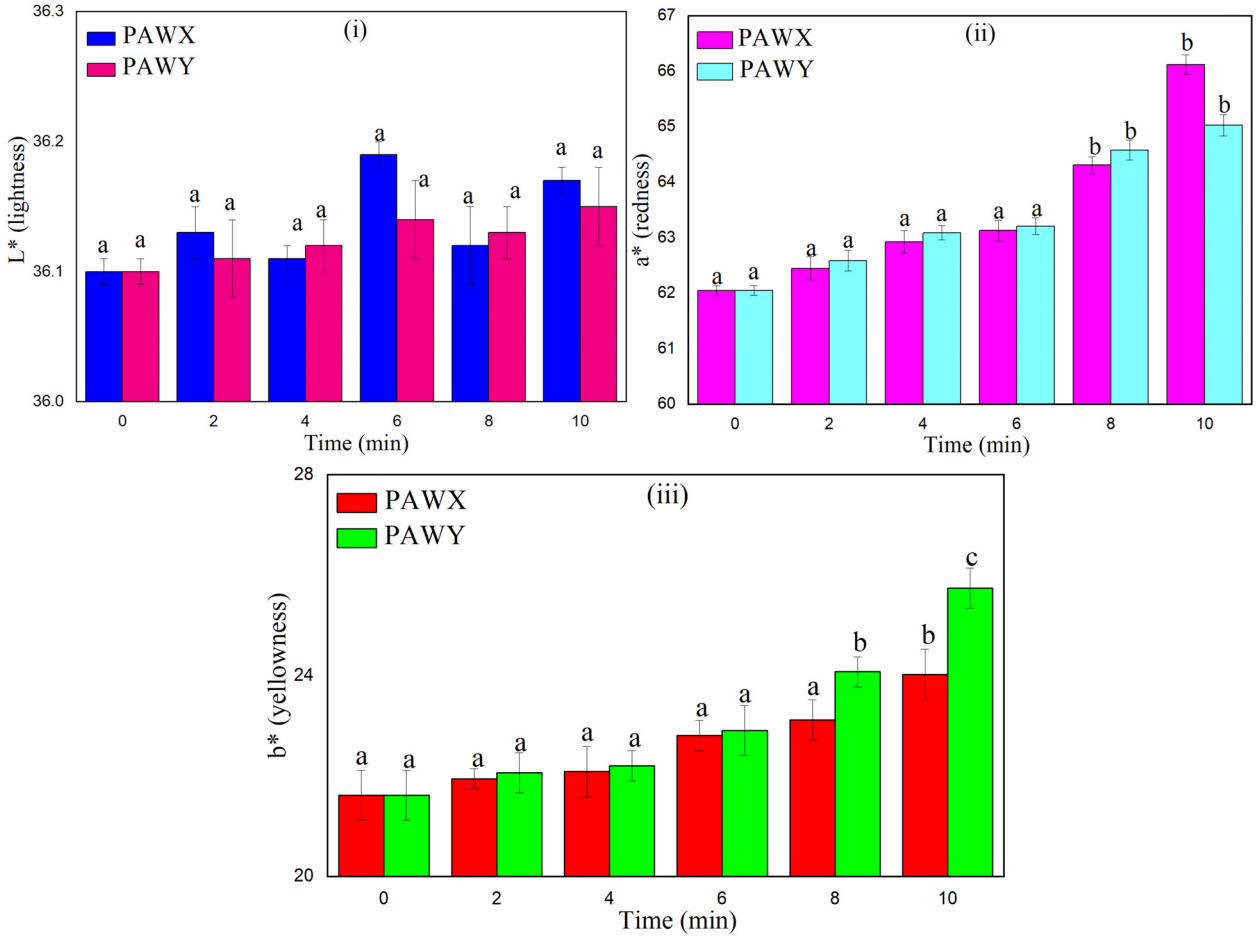

Figure 6: Color changes in the beef surface after treatment using PAW: (i) L^* (lightness), (ii) a^* (redness), and (iii) b^* (yellowness). Lower case letters (a–c) in the same column represents significance analysis; the different letters mean significant difference at $P < 0.05$.

the soaked beef in PAWX2, PAWX4, and PAWX6. However, there is a significant difference between PAWX8 and PAWX10 with the untreated beef and the soaked beef in PAWX2, PAWX4, and PAWX6. The same character can observe for the beef soaked in PAWY. Previous researchers have illustrated that the a^* values of meat increase as the plasma dose increases [45], also increase at a higher input power and exposure time [46]. In contrast, the a^* values significantly decreased with increasing stockpiling time [47]. Moreover, the effect of PAW on b^* values of beef can be seen in Figure 6(iii). There is a significant difference between the soaked beef in PAWX10 and the soaked beef in PAWX at a lower plasma dose. On the other hand, there is a significant difference between soaked beef in PAWY8 and PAWY10 with the soaked beef in PAWY at a lower plasma dose. On the other hand, Natalia *et al.*, in 2012, found that non-thermal plasma did not impact the b^* value of meat [48]. Moreover, Table 1 shows the values of chroma (C^*), hue angle (h^*), and total color difference (ΔE). However, there is a significant difference in chroma (C^*) between the beef soaked in PAWX8 and PAWX10 with the untreated beef and the beef soaked in PAWX2, PAWX4 and PAWX6. Moreover, as the chroma values increase, the color intensity of samples distinguished by humans increases [32]. The same trend has been observed for the beef soaked in PAWY. On the other hand, there is a significant hue angle difference between the PAWX10 with the untreated beef and the beef soaked in PAWX2, PAWX4, PAWX6, and PAWX8. Nevertheless, there is a significant hue angle difference between the PAWY8 and PAWY10 with the untreated beef and the beef soaked in PAWY2, PAWY4, and PAWY6. Moreover, the total color difference indicates the magnitude of color difference between the stored and control samples [49]. From these results in the case of the soaked beef in PAWX, the color differences are not obvious to the human between untreated beef and the soaked beef in PAWX2, PAWX4, PAWX6, and PAWX8 because of $\Delta E < 3$. On the other hand, the color differences are obvious to the human eye between untreated beef and PAWX10 because $\Delta E > 3$. Moreover, in the case of the soaked beef in PAWY, the color differences are not obvious to the human between untreated beef and the soaked beef in PAWY2, PAWY4, and PAWY6. However, the color differences are obvious to the human eye between untreated beef and PAWY8 ($\Delta E > 3$). However, color differences were visually noticeable between untreated beef and PAWY10 ($\Delta E > 4$). So, the change in beef color from purplish red to red for PAWX has been observed after soaking beef in PAWX10. However, for PAWY, the

Table 1: Color parameter of the beef surface after treatment using PAW

Treatment time (min)	PAWX				PAWY			
	C^*	h^*	ΔE	Color	C^*	h^*	ΔE	Color
0	65.70 ± 1.05a	19.20 ± 1.33a		Purplish red	65.70 ± 1.05a	19.20 ± 1.33a		Purplish red
2	66.19 ± 1.52a	19.36 ± 1.43a	0.51 ± 1.02a	Purplish red	66.36 ± 1.65a	19.42 ± 1.08a	0.70 ± 1.05a	Purplish red
4	66.69 ± 1.34a	19.34 ± 1.02a	0.99 ± 1.22a	Purplish red	66.88 ± 1.52a	19.39 ± 0.87a	1.19 ± 1.45a	Purplish red
6	67.12 ± 1.24a	19.86 ± 0.88a	1.60 ± 1.02a	Purplish red	67.2 ± 1.74a	19.92 ± 1.02a	1.73 ± 1.33a	Purplish red
8	68.33 ± 1.61b	19.77 ± 1.96a	2.71 ± 1.14a	Purplish red	68.92 ± 1.84b	20.44 ± 1.65b	3.52 ± 1.01b	Red
10	70.35 ± 1.66b	19.97 ± 1.12b	4.73 ± 1.44b	Red	69.94 ± 1.71b	21.60 ± 1.55b	5.09 ± 1.50c	Red

[(a–c)]Lower case letters (a–c) in the same column represents significance analysis; the different letters mean significant difference at $P < 0.05$

change inf color beef from purplish red to red has been observed after soaking beef in PAWY8 and after soaking beef in PAWY10. These results have indicated that the change in color of beef treated by PAW did not occur at a low plasma dose.

3.6 pH measurement of beef samples

pH value of beef before treatment has been measured, and it recorded 5.56. The changes in pH-values for control beef during stockpiling at 5°C can be observed from Figure 7(i and ii). The pH of the control beef was 5.55 after the second day of stockpiling. Moreover, pH significantly increased to a value of 6.39 after 20 days of stockpiling. According to Ockerman and Cahill, 1977, the increase in pH can be ascribed to the growth of microorganisms [50]. Moreover, when ammonia is released from bacteria during amino acid metabolism, this leads to increased pH of chilled meat [51]. On the other hand, Figure 7(i) reveals the impact of plasma treatment on pH values of soaked beef in PAWX. It can be observed that there is a significant difference between control and treated beef $P < 0.05$. On the other hand, there is no significant difference between soaked beef in PAWX2 and PAWX4 through the storage period. From the 16th day of storage, there is no significant difference between soaked beef in PAWX2, PAWX4, and PAWX6. Moreover, it can be observed that for beef soaked in PAWX2 after the second day of stockpiling, pH significantly decreased from 5.56 to 5.41. Further, as the preparation time of PAWX increased, the pH values of soaked beef after the second day of storage decreased. Moreover, the pH of soaked beef increased continuously from 5.41 and 4.72 after the second day of stockpiling to a recorded value of 5.85 and 4.99 after 20 days of stockpiling for beef soaked in PAWX2 and PAWX10, respectively. The change in pH values for soaked beef in PAWY is illustrated in Figure 7(ii). There is a significant difference in pH values between control and soaked beef in PAWY. However, there is no significant difference in pH values between soaked beef in PAWY8 and PAWY10. From Figure 7(i and ii), it can be observed that, after the same storage period, the reduction rate of pH values for beef soaked in PAWY was higher than that soaked in PAWX. From these results, it can concluded that, after the same storage period, the pH of beef diminished continuously by increasing the plasma dose of PAW. On the other hand, the pH of soaked beef in PAWY was significantly lower than PAWX at the same plasma dose. These results reasonably agree with the results of conductivity and ORP for PAW. Moreover, the longer the plasma processing of beef, the more acidogenic molecules will be produced, leading to a diminish in the pH values [32]. However, the impact of PAW efficacy on beef decontamination can be ascribed to the killing of microorganisms that occurs on the surface of the beef by PAW particles. The acidic medium produced by the PAW reduces the quantity of ammonia [52].

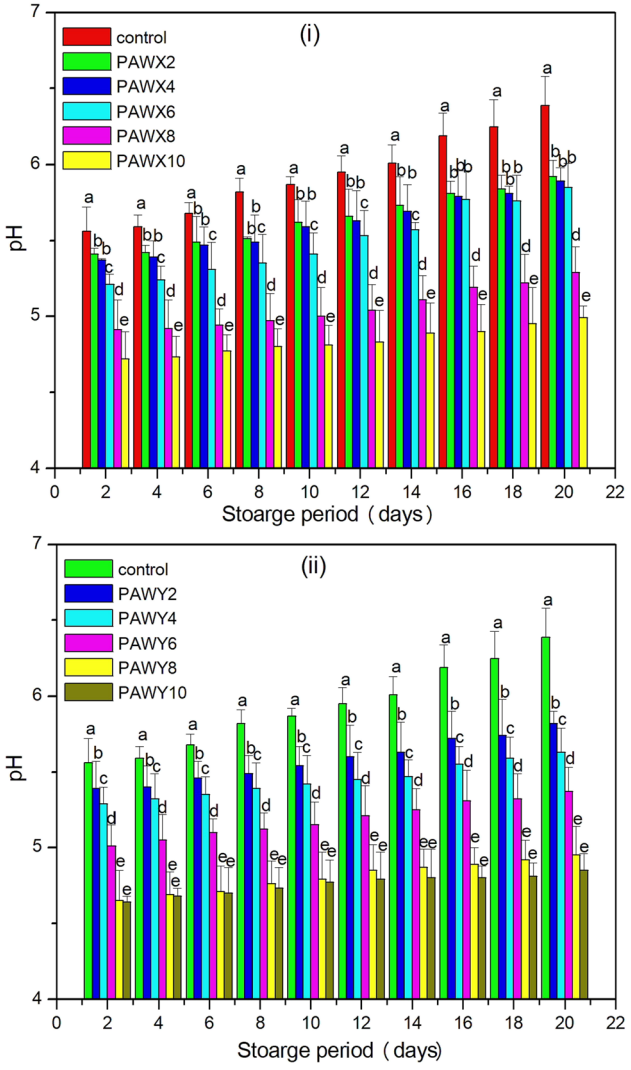

Figure 7: pH of beef after treatment using (i) PAWX and (ii) PAWY. Lower case letters (a–e) in the same column at the same time and the same working gas represent significance analysis; the different letters mean significant difference at $P < 0.05$.

3.7 TBARS measurement

Figure 8(i and ii) displays the effect of PAW treatment on the TBARS values of beef samples. TBARS values of

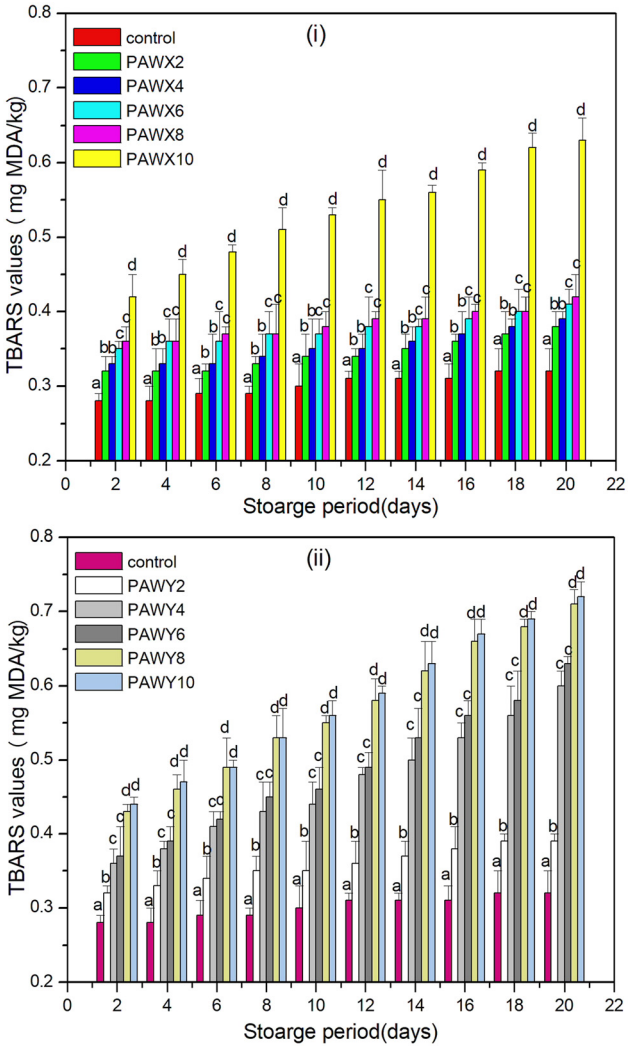

Figure 8: TBARS values (mg MDA/kg) after treatment using (i) PAWX and (ii) PAWY. Lower case letters (a–d) in the same column at the same time represent significance analysis; the different letters mean significant difference at $P < 0.05$.

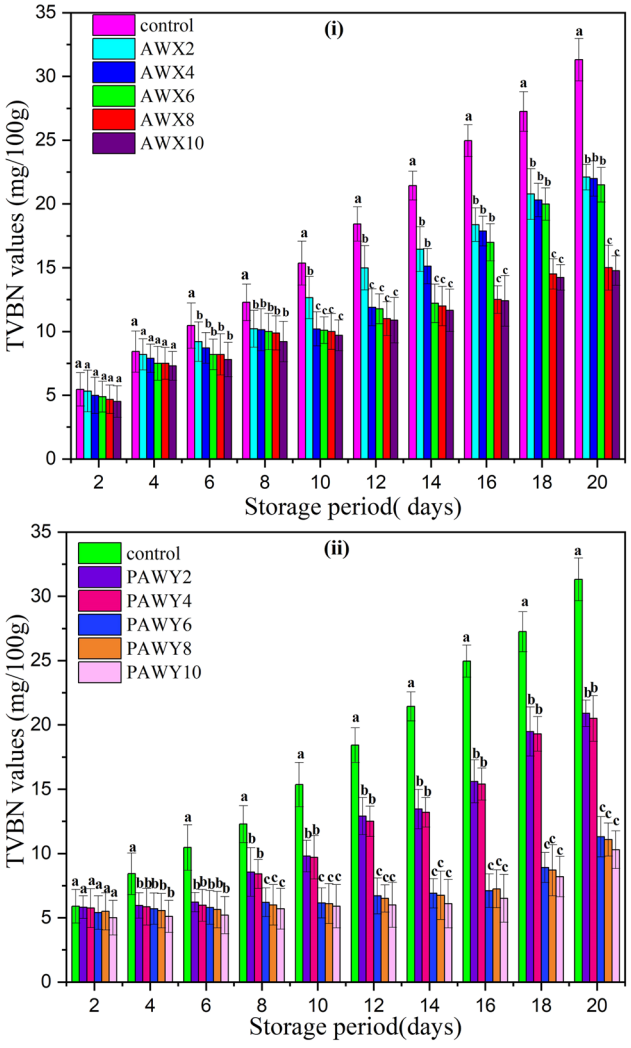

Figure 9: TVBN values (mg/100 g) after treatment using (i) PAWX and (ii) PAWY. Lower case letters (a–c) in the same column at the same time and the same working gas represent significance analysis; the different letters mean significant difference at $P < 0.05$.

control samples during the storage period have no significant change. From Figure 8(i) we can notice that the soaked beef in PAWX2 and PAWX4 has no significant change. Also, the soaked beef in PAWX6 and PAWX8 has no significant change. The soaked beef in PAWX10 has higher values of TBARS; it has increased from 0.42 mg MDA/kg after the second day of storage to 0.63 mg MDA/kg after the twentieth day of storage. Figure 8(ii) shows that the soaked beef in PAWX4 and PAWX6 has no significant change. Also, the soaked beef in PAWX8 and PAWX10 has no significant change.

On the other hand, the TBARS of the soaked beef in PAWY is higher than that of PAWX. TBARS of the soaked beef in PAWY10 has increased from 0.44 mg MDA/kg after the second day of storage to 0.72 mg MDA/kg after the twentieth day of storage. Moreover, TBARS values of stockpiling samples increased continuously as stockpiling time increased for both treatment systems. In this experiment, according to Rahman *et al.*, 2015, TBARS values ranged from 0.28 to 0.72 mg MDA/kg, so that the TBARS values of all treated and untreated beef were still at a low level and did not reach the threshold rancidity (1.0 mg/kg) [7]. The change in TBARS of treated beef can be attributed to oxidative stress produced by reactive PAW species. However, oxidative stress produced by reactive species plays a critical role in the lipid oxidation of treated beef; in this work, the change in TBARS did not reach the harmful effect.

3.8 Total VBN content

From Figure 9, it can be noticed that there are significant changes in TVBN values for control samples through stockpiling at 5°C. The TVBN of control beef was 5.46 mg/100 g after the second day of stockpiling. Moreover, after 20 days of stockpiling, the TVBN significantly increased to a value of 31.31 mg/100 g. From these results, according to Yao *et al.*, it can be noticed that, after 18 days of stockpiling, control samples reach the decay threshold (>25 mg/100 g) [52]. From these results, in the case of soaked beef in PAWX after the second and fourth day of stockpiling, there is no significant difference between treated and untreated beef, as shown in Figure 9. However, in the case of beef in PAWY after the second and fourth day of stockpiling, it can be seen that there is no significant difference between treated and untreated beef. TVBN values were increased for all treated samples as stockpiling time increased. TVBN values of treated samples do not reach the decay threshold, revealing that the fresh beef was of good quality. These results indicated that the soaked beef in PAW exhibits the best TVBN values with higher quality.

4 Conclusion

It is clear from these results that the microbial safety of beef can be enhanced using PAW treatment. Optimization of NTAPPJ parameters is considered an essential procedure to significantly reduce the number of microorganisms. One can conclude from our study that all two carrier gases (*i.e.*, *HeI*, and *HeII*) can enhance microbial safety. Out of the tested conditions in the current work, the use of PAW produced by *HeII* plasma has a higher microbes reduction than that produced by the *HeI* system. Reactive species generated in distilled water play a worthy role in inhibiting microorganism's growth in beef. The effect of PAW on the TBARS values of soaked beef was considered negligible, indicating that lipid oxidation was minimal. PAW treatment will be used to preserve the beef from decay or spoiling during stockpiling widely.

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