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# Combined effects of ultrasound, plasma-activated water, and peracetic acid on decontamination of mackerel fillets

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ABSTRACT

It is usually quite challenging to rely on one single intervention to achieve a satisfactory antimicrobial effect and maintain quality attributes of food. This study aimed to investigate the decontamination effectiveness of individual treatments, including ultrasound (US), plasma-activated water (PAW), and peracetic acid (PAA) and their combinations against native microbiota (total mesophilic bacteria (TMC) and total psychrotrophic bacteria (TPC)) and inoculated bacteria (*Escherichia coli*, *Listeria innocua,* and *Pseudomonas fluorescens*) on raw mackerel fillets. The impacts of the treatments on fish quality characteristics, such as colour, and lipid oxidation (primary and secondary products) were determined. Meanwhile, the physicochemical properties of PAW and plasma-activated PAA (PA-PAA), including pH, oxidation-reduction potential (ORP), conductivity, and reactive oxygen nitrogen species (RONS) after plasma treatment were examined. The results showed that combined treatments involving PAA tended to achieve higher inactivation rates, with the greatest inactivation of 0.72, 0.62, and 0.5 log CFU/g for *L. innocua, E. coli,* and *P. fluorescens* respectively. Significantly higher values of RONS and more acidic pH in PA-PAA were observed than that in PAW or PAA ($P \leq 0.05$), demonstrating the synergistic effect of the hurdle interventions, though the inactivation rates on the fish samples were not significantly higher than the individual treatments. Fish quality parameters were not notably affected compared to the control. The study showed promising results for fish decontamination, offering potential alternative options for future application.

## 1. Introduction

Fish contamination can occur in various ways, including contaminated water, processing (cutting boards, conveyor belts or skinner equipment, etc), transportation and storage. Many different kinds of pathogenic and spoilage bacteria can grow on fish, depending on where they are captured (marine or freshwater fish), how they are processed (vacuum-packaged or smoked fish), and storage conditions (chill or frozen etc), (Alfaro, Hernández, Le Marc, & Pin, 2013; Ampofo & Clerk, 2010; Gram & Huss, 1996; Lunestad et al., 2007). The contamination can lead to undesirable odours, soft texture, and off-flavours, rendering fish unacceptable to consumers, and the general treatment of water immersion only has limited decontamination efficacy for the fish (Gram & Dalgaard, 2002). Therefore, many strategies have been proposed to improve the decontamination rate, for example, washing fish in chemical antimicrobial agents, including chlorine-based sanitizers, hydrogen peroxide ($H_2O_2$), peracetic acid (PAA) or ozone, etc. Among them, chlorine-based agents and their by-products of haloacetic acids, trihalomethanes, and chloramines have raised huge concerns for human health and environmental safety (Singh, Singh, Bhunia & Stroshine, 2002). PAA is a mixture of acetic acid and hydrogen peroxide, both of which are responsible for the disinfection effect, and it is a green sanitiser without any residual. Moreover, PAA can maintain antimicrobial efficacy in the presence of organic materials, which is a big advantage over other chemical agents.

The application of novel non-thermal technologies for food

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decontamination has raised tremendous attention. Recently, Zhao, de Alba, Sun, and Tiwari (2019) reviewed the application of these technologies in the fish industry, including high-pressure processing (HPP), ultraviolet light (UV), ultrasound (US), pulsed electric field (PEF), and plasma, etc. Among them, plasma is the newest one, and it has been investigated for various food products ((Chen, Cheng, & Sun, 2020, Ekezie, Cheng, & Sun, 2017; Pan, Cheng, & Sun, 2021); Scholtz, Pazlarova, Souskova, Khun, & Julak, 2015). Plasma, the fourth state of matter, is composed of ions, electrons, reactive species, free radicals, and UV photons (Han, Cheng, & Sun, 2019; Jiang, Cheng, & Sun, 2020; Pan, Cheng, & Sun, 2019). Many different plasma instruments have been developed, such as plasma jet, corona discharge, dielectric barrier discharge (DBD), microwave plasma, etc ((Ali, Cheng, & Sun, 2021; Ekezie, Cheng, & Sun, 2019, 2018; Ekezie, Sun, & Cheng, 2019; Pan, Cheng, Lv, & Sun, 2019); Scholtz et al., 2015). Plasma-activated water (PAW) is generated by treating water with a plasma source; many different reactions between gaseous plasma and liquid water can be triggered during the treatment, leading to physiochemical property changes of water (Ali, Cheng, & Sun, 2021b; Esua, Cheng, & Sun, 2021). PAW has been proved to be capable of inactivating microorganisms in the planktonic form (Baek et al., 2020) (Ikawa, Kitano, & Hamaguchi, 2010; Zhang et al., 2013), biofilm form (Ercan et al., 2013) (Smet et al., 2019; Li et al., 2019), and also spores (Sun et al., 2012) (Los, Ziuzina, Boehm, Cullen & Bourke, 2020; Bai et al., 2020). These studies have demonstrated PAW as a potential alternative approach for food decontamination. Furthermore, the bactericidal property of PAW can last for some time depending on the storage temperature (Vlad & Anghel, 2017), which is beneficial for industrial implementation. US is a versatile technology that was developed decades ago, and the multiple applications of US, including decontamination in the food industry, have been reviewed by Huang et al. (2017).

With increasing strict requirements on chemical sanitiser usage in food regulations, fish processing cannot only rely on a single intervention, while a hurdle approach combining several interventions can improve microbial safety without notably compromising quality characteristics and sensory attributes. Liao et al. (2020) reviewed plasma-based hurdle strategies, including mild temperature, organic acid, and US, etc. To the best of our knowledge, there has been no available literature that investigated the hurdle approach of US, PAA, and PAW on microbial decontamination of food products. This study aimed to evaluate the combined treatments of the three interventions against native microbiota, including total mesophilic counts (TMC), total psychrotrophic counts (TPC), and inoculated bacterial species, including *E. coli*, *L. innocua,* and *P. fluorescens* on raw mackerel fillets. The effects of the treatments on fish quality attributes, including colour and lipid oxidation were determined. Additionally, the physicochemical properties of PAW and plasma-activated PAA (PA-PAA), including pH, conductivity, oxidation-reduction potential (ORP), and the generation of reactive species ($H_2O_2$, $NO_2^-$, and $NO_3^-$) were measured.

## 2. Materials and methods

### 2.1. Bacterial inoculum preparation

Three bacterial species, including *E. coli* K12 DH5α, *L. innocua* 12210, and *P. fluorescens* DSM50090 were obtained from the culture collection at Teagasc Food Research Centre Ashtown (Dublin, Ireland), which were kept at −80 °C on protective beads. To grow the bacteria, a single bead of each strain was streaked on tryptic soya agar plates (TSA, Scharlau Chemie, Barcelona, Spain) and incubated at 37 °C for *E. coli* and *L. innocua*, and at 30 °C for *P. fluorescens* for 24 h. An isolated colony of each strain was inoculated into 20 mL sterile tryptic soy broth (TSB, Scharlau Chemie, Barcelona, Spain) and incubated at appropriate temperatures for 18 h until the early stationary phase was reached. Decimal dilutions of each strain were made in maximum recovery diluent (MRD, Scharlau Chemie, Barcelona, Spain), followed by centrifuging at 6000×*g* for 10 min. After centrifugation, the pellet of each strain was washed using sterile deionized water and mixed thoroughly on a vortex. The final concentration of each bacterial suspension was around 8.0 $\log_{10}$ CFU/mL, which was measured by colony counting on TSA plates. The three bacterial suspensions were mixed in a 1:1:1 (v/v/v) ratio to make an inoculum cocktail for subsequent fish inoculation (Kim et al., 2013).

### 2.2. Inoculation of mackerel fillets

Raw mackerel fillets were bought from a local supermarket on the day of the experiment, and delivered to the lab within 20 min. The fillets were cut aseptically into 4 × 4 × 1 cm (length x width x thickness) pieces (~10 g) using a sterilized knife on a sterile chopping board to avoid contamination. For inoculation, each fish piece was placed in a sterile Petri dish and spot inoculated with 100 μL of the prepared inoculum cocktail on one side of the fish piece (5 drops with 20 μL each). A sterile spreader was used to make sure the bacterial cocktail was spread evenly. The inoculated samples were left inside a laminar flow safety cabinet for 1 h with the Petri dishes lid open for bacterial attachment. Uninoculated fish pieces were used for native microbiota examination, that was, TMC and TPC.

### 2.3. Description of the devices and treatments

#### 2.3.1. US treatment

A low frequency (25 kHz) ultrasound bath at a power of 550 W was used, and the power was detected with the calorimetric method (Mantas, Pagán, & Raso, 2000). For US treatment, a piece of the fish cube was submerged in 30 mL of sterile water, and the container was placed in the ultrasonic bath for 10 min. The water in the ultrasonic bath was replaced with fresh cold water after each treatment, and the temperature of the water was below 25 °C after treatment, which minimised the impact of temperature on the results.

#### 2.3.2. Peracetic acid treatment

Peracetic acid (Sigma, 38%–40%) was diluted in sterile deionized water to a final concentration of 200 ppm, which was further confirmed using peracetic acid test strips (MQuant, 100–500 mg/L, Sigma Merck, Ireland). The working solution was prepared on the day the experiment was conducted and stored at 4 °C before usage. For PAA treatment, each fish cube was immersed in 30 mL of PAA solution for 10 min.

#### 2.3.3. PAW treatment

A plasma beam system (Diener Electronic GmbH & Co. KG, Ebhausen, Germany) operating at 20 kHz was used as the plasma source (Fig. 1). The device is composed of a high voltage generator (300 W), a power conductor in a flexible tube, and a plasma jet. A condenser connected with a cooling system was installed underneath the plasma jet to reduce the temperature of the plasma before it reached the liquid solution. Atmospheric ambient air was used as the working gas with a flow rate of 11 L/min. PAW was generated by treating 60 mL of sterile deionized water for 10 min, and the temperature of the water after treatment was under 30 °C, hence the temperature effect on fish decontamination can be neglected. For PAW treatment, 60 mL PAW was divided evenly into two parts immediately after generation, and one fish cube was submerged into the PAW for 10 min at room temperature.

#### 2.3.4. Combined treatment

Fish samples were subjected to the individual interventions, as well as their combinations, as shown in Table 1. Fish without any treatment was regarded as control, fish immersed in sterile deionized water was regarded as water treatment. After each treatment, the fish surface was wiped and dried with a paper towel to remove the attached liquid before microbial analysis. The experiment was repeated three times

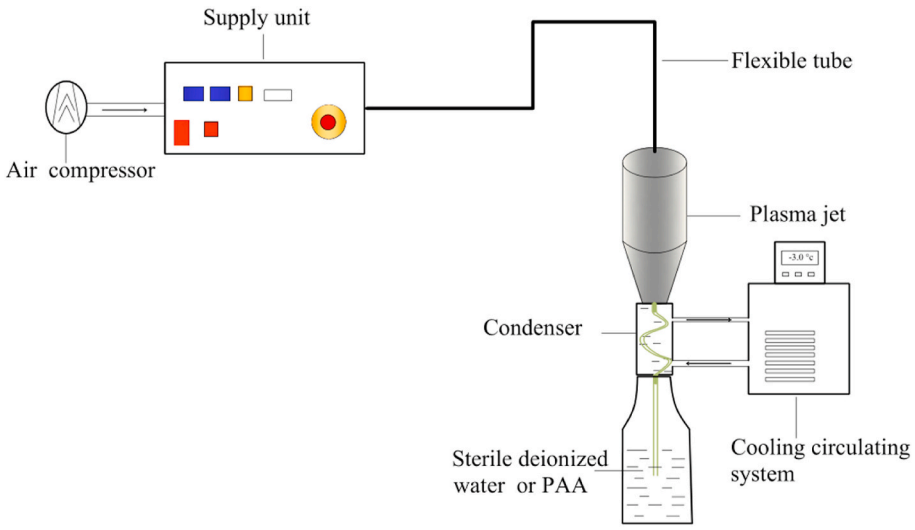


**Fig. 1.** Schematic diagram of the plasma device and plasma treatment to the solutions.

**Table 1**
Description of the treatments subjected to fish samples.

| Treatment | Description |
|---|---|
| Control | Without any treatment |
| Water | Immersed in deionized water for 10 min |
| US | Ultrasonic bath for 10 min |
| PAW | Immersed in PAW for 10 min |
| PAA | Immersed in PAA for 10 min |
| PAW + US | Immersed in PAW + ultrasonic bath for 10 min |
| PAA + US | Immersed in PAA + ultrasonic bath for 10 min |
| PA-PAA | Immersed in plasma-activated PAA for 10 min |
| PA-PAA + US | Immersed in plasma-activated PAA + ultrasonic bath for 10 min |

US: Ultrasound; PAW: Plasma activated water; PAA: Peracetic acid; PA-PAA: Plasma-activated Peracetic acid.

independently with two replicates for each treatment.

### 2.4. Microbial analysis

For microbial analysis of fish, each sample was transferred to a sterile blender bag after treatment, diluted (1:10) using MRD, and homogenized in a stomacher (VMR, Star-Blender, LB 400) for 30 s. Serial decimal dilutions were made in MRD for bacterial enumeration. Of note, for the treatments involving PAA, peracetic acid test strips (MQuant, 5–50 mg/L, Sigma Merck, Ireland) was used to detect the concentration of residual PAA in MRD to avoid extra antimicrobial effect on fish samples (Thi et al., 2015). Results showed that no detectable level of PAA was present, indicating no further neutralization was needed. Regarding native microbiota, 3 M Petrifilm ™ for Aerobic Count Plate (3 M, St. Paul, Minnesota, USA) was used for colony enumerating. For TMC and TPC, the plates were incubated at 30 °C for 48 h and 6.5 °C for 10 days, respectively. For the three inoculated bacteria, *E. coli* was enumerated by plating on MacConkey (Thermo Scientific Oxoid, UK) agar plates and incubated at 37 °C for 24 h; *L. innocua* was enumerated on Listeria selective agar base (Thermo Scientific Oxoid, UK) plates with Listeria selective supplement (Thermo Scientific Oxoid, UK) and incubated at 37 °C for 24 h; *P. fluorescens* was enumerated on Pseudomonas agar base (Thermo Scientific Oxoid, UK) plates with CFC supplement (Thermo Scientific Oxoid, UK) and incubated at 30 °C for 24 h. The colonies were counted, and the results were reported as $\log_{10}$ CFU/g. The inactivation effect of the treatments was calculated as follows:

$$\text{Log}_{10}\text{ reduction} = \text{Log}_{10}(\text{CFU}_{\text{Control}}) - \text{Log}_{10}(\text{CFU}_{\text{Treated}})$$

where the $\text{CFU}_{\text{Control}}$ is the population size without any treatment, and $\text{CFU}_{\text{Treated}}$ is the population after the treatment was applied.

### 2.5. Fish quality parameters

#### 2.5.1. Colour

The colour of mackerel fillets was investigated using a Minolta chromameter CR-400 (Konica-Minolta, Osaka, Japan). Before analysis, the instrument was calibrated with a standard white plate, and the parameters assessed were CIE $L^*$ (lightness-darkness), $a^*$ (redness-green), and $b^*$ (yellowness-blueness) (de l'Eclariage, 1975). The measurements were performed on three different locations on the surface of each mackerel piece.

#### 2.5.2. Peroxide value

The peroxide value (PV) is a measure of the primary products of lipid oxidation. To determine the PV of fish samples, lipids were extracted first. The fish samples were cut into small pieces and minced with a kitchen blender (Bosch 600 W, Gerlingen, Germany). A sample of 10 g of minced fish was taken for lipid extraction using the chloroform-methanol-water method (Bligh & Dyer, 1959). Chloroform extracts of lipids were collected and used for the determination of primary and secondary lipid oxidation products as described in Cropotova, Mozuraityte, Standal, and Rustad (2019). PV was measured using the iodometric titration method described in AOCS official methods (Cd 8b-90) (AOCS, 2003). The endpoint of titration was assessed potentiometrically with an automatic titrator (TitroLine 7800, Xylem Analytics, Mainz, Germany) coupled with a platinum electrode (Pt 62). The analysis was performed in three replicates and the results were expressed as the mean value of meq active $O_2$/kg lipids ± SD (SD, standard deviation).

#### 2.5.3. Thiobarbituric acid reactive substances (TBARS)

The secondary products of lipid oxidation of fish samples were measured according to the method described by Ke and Woyewoda (1979) using a GENESYS 10 S UV-VIS spectrophotometer (Thermo Scientific, Pittsburgh, PA, USA). The results were expressed as the mean value of mg of malonaldehyde (MDA)/kg lipid ± SD.

### 2.6. Physicochemical analysis of plasma-activated solutions

The pH, ORP, and conductivity of PAW and PA-PAA were measured before and immediately after plasma treatment using a pH meter (Orion, 420APLUS, UK), an ORP electrode (HANNA, HI3230B, UK), and an electric conductivity meter (Jenway, 4070, UK) respectively. In addition, the concentration of reactive species, including hydrogen peroxide

($H_2O_2$), nitrites ($NO_2^-$), and nitrates ($NO_3^-$) generated in PAW and PA-PAA were also measured. The measurement was based on colourimetric methods using a microplate reader (BioTek, EPOCH2C, USA), as described in detail in a previous study (Zhao, Ojha, Burgess, Sun, & Tiwari, 2020b).

### 2.7. Statistical analysis

To compare the effects of the different treatments, data obtained from the microbiological, quality parameter, and physicochemical properties were subjected to one-way ANOVA using SPSS 24.0 (SPSS Inc., Chicago, IL). Fisher LSD (least significant difference) test was applied, and the statistical difference was determined at $P \leq 0.05$ level.

## 3. Results

### 3.1. Decontamination efficacy

The decontamination efficacy of the treatments against natural microbiota (TMC and TPC) on raw mackerel fillets are shown in Table 2. Initial populations of TMC and TPC on raw fillets were similar, with values around 6.0 log CFU/g (data not shown). Overall, the greatest reduction for TMC and TPC was 0.41 and 0.70 log CFU/g, respectively. Deionized water immersion for 10 min resulted in almost no reduction (<0.1 log) for the natural microbiota. For TMC decontamination, all the treatments showed no significant difference, although treatments involving PAA tended to obtain greater inactivation, with 0.41 and 0.38 log CFU/g reduction for PAA and PA-PAA treatments respectively. For TPC decontamination, PAA, PA-PAA, and PA-PAA + US resulted in 0.59, 0.68, and 0.70 log CFU/g reduction, respectively, which were significantly greater compared to other treatments ($P \leq 0.05$).

The reduction of *L. innocua*, *E. coli,* and *P. fluorescens* after individual and combined treatments is shown in Fig. 2. After inoculation, the attached population of *L. innocua*, *E. coli,* and *P. fluorescens* was around 5.45, 5.62, and 6.20 log respectively (data not shown). Similar to the natural microbiota, water immersion resulted in the least reduction, with around 0.10 log CFU/g reduction for the inoculated bacteria. For L. *innocua*, the treatments involving PAA achieved significantly greater reduction comparing to water treatment ($P \leq 0.05$), with the reduction ranging from 0.59 to 0.72 CFU/g, with no significant difference observed between the PAA-involved treatments. The individual or combined US or PAW treatments led to no significant reduction when compared to water immersion. For *E. coli*, the only significant reduction was observed after the combined treatment of PA-PAA + US ($P \leq 0.05$), with a 0.61 log reduction achieved. Similarly, treatments involving PAA also tended to obtain greater inactivation, with a reduction of 0.59 and 0.53 log CFU/g for PAA + US and PAA treatment respectively. For *P. fluorescens* inactivation, all the treatments resulted in no significant difference comparing to water immersion, and the reduction was ranged from 0.14 to 0.50 log CFU/g, slightly lower than the other two bacterial species.

**Table 2**
Reduction of total mesophilic counts (TMC) and total psychrotrophic counts (TPC) on mackerel fillets subjected to different treatments. Data are expressed as the mean value of three independent experiments ± SD.

| Treatment | Reduction ($log_{10}$ CFU/g) | |
|---|---|---|
| | TMC | TPC |
| Water | <0.10 [a] | <0.10 [a] |
| US | 0.30 ± 0.01 [a] | 0.32 ± 0.12 [ab] |
| PAW | 0.15 ± 0.07 [a] | 0.24 ± 0.05 [ab] |
| PAA | 0.41 ± 0.27 [a] | 0.59 ± 0.19 [b] |
| PAW + US | 0.27 ± 0.18 [a] | 0.38 ± 0.01 [ab] |
| PAA + US | 0.32 ± 0.02 [a] | 0.52 ± 0.37 [ab] |
| PA-PAA | 0.38 ± 0.25 [a] | 0.68 ± 0.30 [b] |
| PA-PAA + US | 0.17 ± 0.05 [a] | 0.70 ± 0.34 [b] |

US: Ultrasound; PAW: Plasma activated water; PAA: Peracetic acid; PA-PAA: Plasma-activated Peracetic acid.
Different letters in the same column indicate a significant difference ($P \leq 0.05$).

### 3.2. Fish quality parameters after treatments

The impacts of the treatments on colour parameters of mackerel fillets are shown in Fig. 3. The results showed that $L^*$ and $b^*$ values were slightly reduced compared to the controls. A significant reduction of $L^*$ value was observed after US treatment ($P \leq 0.05$), and no significant differences were observed for the other treatments. Similarly, there was no significant difference for $a^*$ and $b^*$ values after all the treatments compared to the controls.

PV and TBARS analysis are widely employed for the determination of primary and secondary products of lipid oxidation. The results of the lipid oxidation after treatments are shown in Table 3. The PV results were in the range of 0.5–7.2 meq $O_2$/kg lipid, and no significant differences were observed between different treatments. Control samples showed a value of 4.4 meq $O_2$/kg lipid, while Water, US, and PAW + US treatments showed lower values of 2.2, 1.7, and 0.5 meq $O_2$/kg lipid, respectively. The highest PV value of 7.2 meq $O_2$/kg lipid was observed after PAA treatment. TBARS values ranged from 0.1 to 7.6 mg of malondialdehyde (MDA)/kg lipid, and no significant differences were observed between different treatments. Values of <1.0 mg MDA/kg lipid were observed for the control, PAA, PAW + US, PA-PAA and PA-PAA + US treatments. Two of the highest TBARS values, 4.9 and 7.6 mg MDA/kg lipid were observed after PAW and PAA + US treatment respectively.

### 3.3. Physicochemical parameters of PAW and PA-PAA

The physicochemical properties of water and PAA, including pH, ORP, and conductivity before and after plasma treatment are shown in Table 4. Overall, plasma treatment resulted in significant differences for all the parameters measured ($P \leq 0.05$). For example, the pH of the water and PAA significantly decreased from 6.22 to 3.11, and from 3.48 to 2.84, respectively. ORP and conductivity were significantly increased after plasma treatment. Moreover, significant differences in the physicochemical properties were observed between PAW and PA-PAA.

The concentration of long-living reactive species after plasma treatment is presented in Fig. 4. The values of the three reactive species were significantly higher for PA-PAA than PAW except for $NO_2^-$ ($P \leq 0.05$). For example, the concentration of $NO_3^-$ for PA-PAA and PAW was 1320 and 300 μM respectively. Similarly, the concentration of $H_2O_2$ for PA-PAA was 140.56 μM, which is more than 10 times higher than that in PAW with a value of 13.43 μM. While regarding $NO_2^-$, the value was 290 μM for PA-PAA, lower than 420 μM for PAW.

## 4. Discussion

The study aimed to investigate the decontamination efficacy of individual US, PAW, PAA treatments and their combinations on mackerel, as well as the impact on fish quality parameters. In this study, water immersion had a very limited antimicrobial effect (<0.1 log) whether for native microbiota or inoculated species, and US treatment improved the decontamination efficacy compared to water immersion. US for decontamination purposes has been applied to various food products. Recently, Pedrós-Garrido et al. (2017) investigated the surface decontamination effect of US treatment at 30 kHz applied on oily fish (salmon and mackerel) and white fish (cod and hake) for 5–45 min, and the greatest reduction of 1.00 and 1.50 log CFU/g for mesophilic and psychrophilic counts was observed respectively on oily fish. The inactivation effects in their study were higher than our work, the reason may be because they used continuous water flow, and the fish samples were treated for 45 min, while our treatment was in a static bath and treated for 10 min.

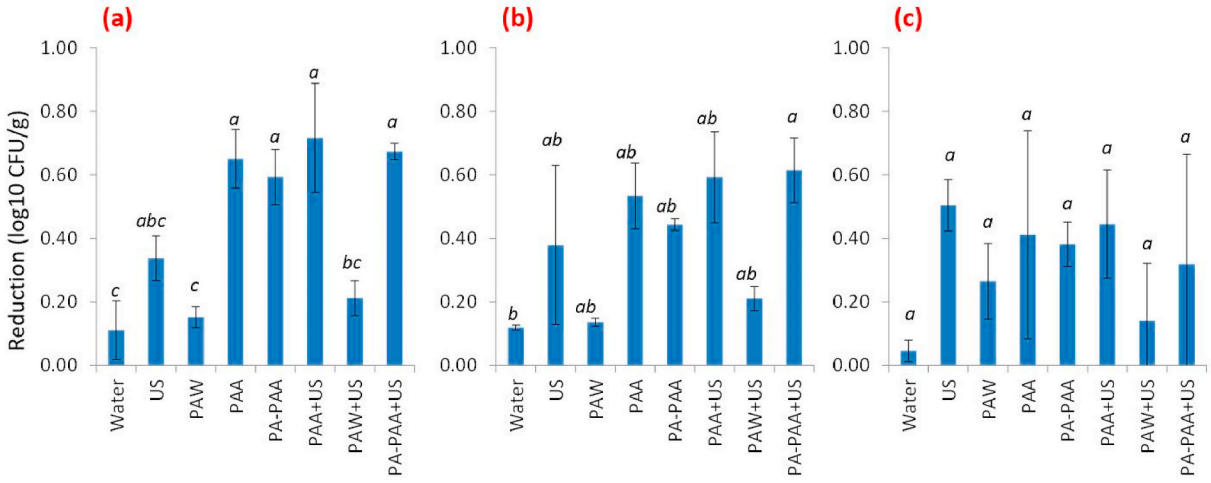


**Fig. 2.** Reduction of (a) *L. innocua*, (b) *E. coli* K12, and (c) *P. fluorescens* after different treatments on fish. Data are expressed as the mean value of three independent experiments ± SD. Different letters in the same graph indicate a significant difference ($P \leq 0.05$).

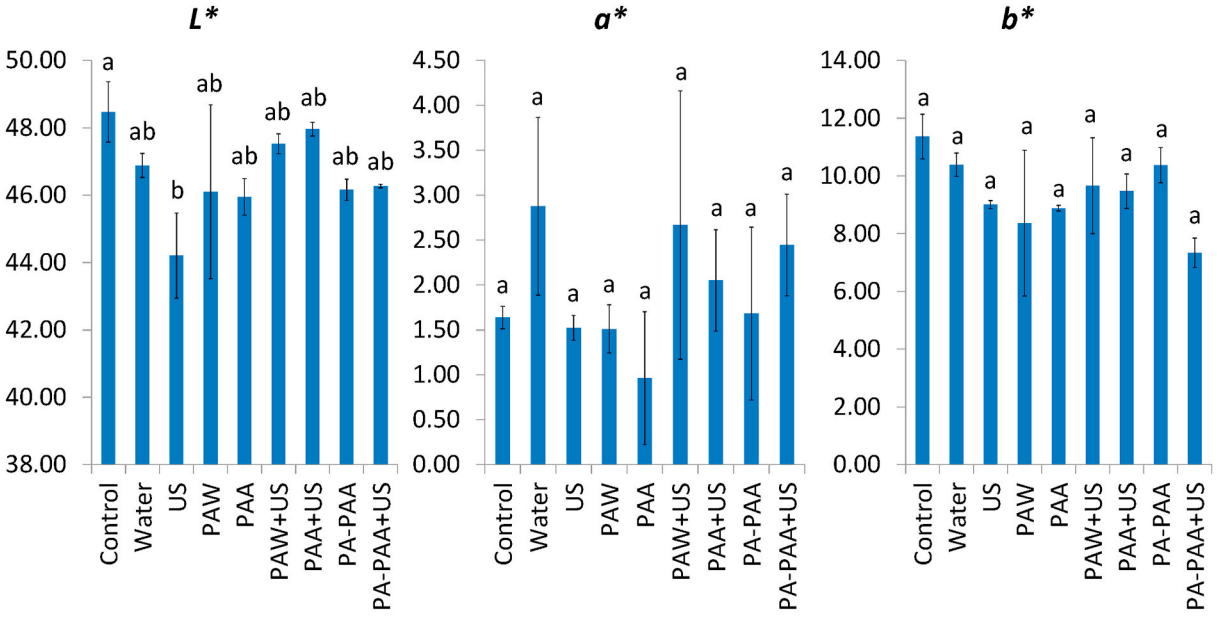


**Fig. 3.** Colourimetric parameters of $L^*$, $a^*$, and $b^*$ values of mackerel subjected to different treatments. Data are expressed as the mean value of three independent experiments ± SD. Different letters in the same graph indicate a significant difference ($P \leq 0.05$).

**Table 3**
Peroxide value (PV) and TBARS of mackerel fillets subjected to different treatments. Data are expressed as the mean value of three independent experiments ± SD.

| Analysis | Control | Water | US | PAW | PAA | PAW + US | PAA + US | PA-PAA | PA-PAA + US |
|---|---|---|---|---|---|---|---|---|---|
| PV (meq $O_2$/kg lipid) | 4.4 ± 1.8$^{ab}$ | 2.2 ± 1.1$^{ab}$ | 1.7 ± 0.8$^{b}$ | n.a. | 7.2 ± 0.3$^{a}$ | 0.5 ± 0.2 $^{b}$ | n.a. | n.a. | 5.6 ± 2.2$^{ab}$ |
| TBARS (mg MDA/kg lipid) | 0.1 ± 0.1$^{a}$ | 1.2 ± 1.4$^{a,b}$ | 1.3 ± 1.8$^{a,b}$ | 4.9 ± 3.9$^{c}$ | 0.4 ± 0.4$^{a}$ | 0.1 ± 0.1$^{a}$ | 7.6 ± 11.5$^{d}$ | 0.6 ± 0.7$^{a}$ | 0.3 ± 0.4$^{a}$ |

US: Ultrasound; PAW: Plasma activated water; PAA: Peracetic acid; PA-PAA: Plasma-activated Peracetic acid; PV: Peroxide value; TBARS: Thiobarbituric acid reactive substances.
n.a means not available.
Different letters in the same row indicate a significant difference ($P \leq 0.05$).

Since the first study of PAW decontamination potential on strawberry was reported by Ma et al. (2015), many other food products, including fruits and vegetables, beef and shrimp have been investigated (Zhao, Patange, Sun, & Tiwari, 2020). The inactivation effect of PAW is due to the acidic pH and reactive oxygen-nitrogen species (RONS) generated in water. Plasma treatment of a liquid solution triggers a series of chemical reactions, leading to the primary and secondary reactive species, and also the other physiochemical property changes, as presented in section 3.3. The decontamination efficacy of PAW will be significantly compromised in the presence of organic materials. For example, Zhao, Ojha, Burgess, Sun, & Tiwari (2020c) observed a significant decrease in the efficacy of PAW in the presence of fish gelation and fish homogenate, and the inactivation rate was in negative correlation to the percentage of organic materials. When applying PAW on

**Table 4**
Physicochemical parameters of solutions before and after plasma treatment. Data are expressed as the mean value of three independent experiments ± SD.

| Solutions | Parameters | | |
|---|---|---|---|
| | pH | ORP (mV) | Conductivity (μs/cm) |
| PAW | 3.11 ± 0.02$^{a, A}$ | 561.00 ± 1.41$^{a, A}$ | 365.00 ± 21.21$^{a, A}$ |
| Water | 6.22 ± 0.03$^{b}$ | 378.55 ± 2.90$^{b}$ | 4.00 ± 0.00$^{b}$ |
| PA-PAA | 2.84 ± 0.01$^{a, B}$ | 660.95 ± 4.60$^{a, B}$ | 557.00 ± 29.70$^{a, B}$ |
| PAA | 3.48 ± 0.02$^{b}$ | 555.90 ± 22.20$^{b}$ | 171.00 ± 2.83$^{b}$ |

PAW: Plasma activated water; PAA: Peracetic acid; PA-PAA: Plasma-activated Peracetic acid.
Different lowercase letters for each parameter indicate a significant difference after plasma treatment ($P \leq 0.05$). Different uppercase letters for each parameter indicate a significant difference between PAW and PA-PAA ($P \leq 0.05$).

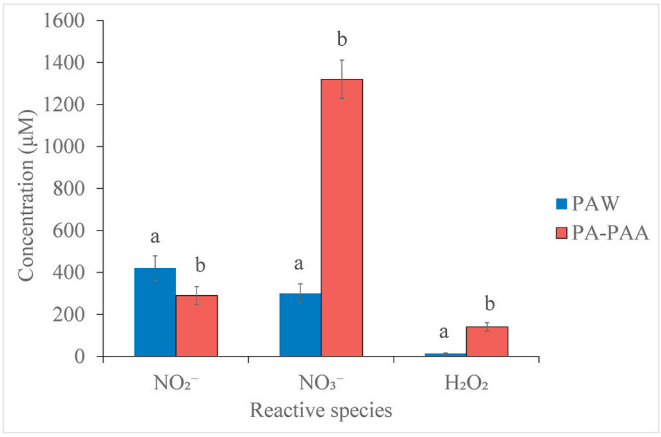


**Fig. 4.** The concentration of reactive species ($NO_2^-$, $NO_3^-$, and $H_2O_2$) generated in PAW and PA-PAA. Data are expressed as the mean value of three independent experiments ± SD. Different letters for the same reactive species indicate a significant difference ($P \leq 0.05$).

food products, many factors can influence the inactivation efficacy, including the processing parameters, the microbial characteristics, and also the food sample attributes; moreover, the differences in the plasma devices general used makes it difficult to compare the inactivation effects between different studies.

PAA has been widely used as a washing sanitiser on fruits, vegetables, fish, and poultry (Bauermeister, Bowers, Townsend, & McKee, 2008; Thi et al., 2015; Van de Velde, Piagentini, Güemes, & Pirovani, 2013). In our study, PAA treatment achieved 0.65, 0.53, and 0.41 log CFU/g reduction for *L. innocua*, *E. coli,* and *P. fluorescens* respectively, being the most effective individual treatment, which was mainly because PAA was less affected by the presence of organic materials (Kitis, 2004). Thi et al. (2015) evaluated the efficacy of PAA washing on *Pangasius* fish, and 0.40–1.40 log CFU/g reduction of *E. coli* was achieved, whereas almost no reduction of total psychotropic counts, lactic acid bacteria, and coliforms was observed.

Plasma-based hurdle technology has attracted increasing attention in recentyears, for example, the combination of plasma with an organic acid. Qian et al. (2019) treated lactic acid (0.05–0.20%) by plasma jet for 40–100 s to generate plasma-activated lactic acid (PALA). Results showed that beef immersion in PALA for 20 s led to 1.24–3.52 log reduction of *Salmonella* Enteritidis depending on LA concentration and plasma treatment time, and no negative effect on beef quality, including colour, pH, lipid oxidation, or protein structure was observed. Chaplot, Yadav, Jeon, & Roopesh (2019) evaluated the synergistic effect of atmospheric cold plasma (ACP) with PAA on *S.* Enteritidis-inoculated raw poultry meat, wherein two concentrations of PAA (100 and 200 ppm), along with different orders of ACP and PAA treatments were conducted. Significant reductions were observed from the hurdle interventions ($P \leq 0.05$), ranging from 2.3 to 5.3 log CFU/cm$^2$, higher than any of the individual treatments. The synergistic decontamination effect was attributed to the breaking down of the covalent bonds of the reactive species in PAA (such as, C–O, O–O) to form into new radical species, such as singlet oxygen and OH$^{\cdot}$, which can further form into ozone and $H_2O_2$, leading to enhanced inactivation efficacy (Winter et al., 2014). Our study in section 3.3 confirmed the significantly higher value of $H_2O_2$, $NO_2^-$, and more acidic pH in PA-PAA than PAW or PAA alone. They also claimed that the combination of PA-PAA can achieve greater decontamination effectiveness than gathering the single treatment one by one. Apart from organic acid, plasma can also be combined with ultrasound. Liao et al. (2018) observed significantly higher inactivation efficacy on *S. aureus* from the hurdle technology of ACP and US, and plasma treatment followed by US showed a higher inactivation rate than the opposite order. The authors explained that subsequent US treatment helped reactive species generate in the medium to inject into the *S. aureus* cells, hence the inactivation efficacy was enhanced. When US was implemented first, *S. aureus* cells were more likely to develop resistance to the following plasma treatment, indicating the order of the treatment is also an important concern when multiple interventions are combined. Chen, Lee, Chen, Chen, & Chang (2009) developed an instrument that can implement plasma and US simultaneously, and the authors proposed that electric discharge occurs inside the bubble with the plasma-assisted US, and the intensity is more than hundreds of times higher than that in water, which is the mechanism for the synergistic inactivation effect. No significant higher reduction was observed in our study compared to individual treatments, maybe because the microbial loads were very high, for example, the initial native microbiota was around 6.0 log, hence it was difficult to obtain obvious reduction. Nonetheless a trend of increased inactivation from the combined treatment can be observed.

Regarding quality parameters, the colour of food has a great influence on consumers' acceptance. A significant decrease of $L^*$ value was observed after US treatment. This was probably due to the mechanical denaturation of myofibrillar proteins, leading to their aggregation and changes of light reflection properties (García & Paulo, 2005). The decreasing trend of lightness for fish samples after US treatment (Pedrós-Garrido et al., 2017) and PAA treatment (Thi et al., 2015) has been previously reported. There was no significant difference for $a^*$ and $b^*$ values among all the treatments, indicating that the treatments in this study did not significantly influence the fish colour. Lipid oxidation is one of the main reasons for quality deterioration especially for fish products, which can affect the colour, flavour, safety, and nutritive value. PV analysis is applied as a quality assessment for oils and lipid-containing foods. The acceptable limit of peroxide value for marine lipids is 5 meq/kg (CODEX, 2017), so the value of 7.2 for PAA and 5.6 for PA-PAA + US meant the fish samples exceeded the acceptable limit of PV after treatments. PV values for all the other treatments were below the acceptable limit, and the values did not vary significantly between the different treatments. Pérez-Andrés et al. (2020) investigated the effects of cold plasma treatment on mackerel lipid oxidation during storage, and an acceptable limit of 1–2 mg MDA/kg lipid for TBARS was reported, beyond which fish muscle would produce an unpleasant volatile flavour. Our results showed that lipid oxidation of TBARS exceeded the acceptable limit after PAA + US and PAW treatment, and the values after all the other treatments were under the acceptable limit. According to the results observed, lipid was least oxidized for US and PAW + US regarding both PV and TBARS. PV was remarkably increased for the treatment involving PAA compared to the control and other treatments. This phenomenon was probably because PAA is a strong oxidizer, which can attach the lipids and lead to formation of lipid peroxides. Lipid oxidation is a complex process involving the formation and breakdown of lipid oxidation compounds at the same time. The lowest lipid oxidation values for both PV and TBARS obtained after US and PAW + US treatment could be due to an antioxidative mechanism involving the

inactivation of prooxidative enzymes (Pazos et al., 2015).

Oehmigen et al. (2011) investigated the possible bactericidal mechanisms in plasma-treated liquids and reported that the reactive species from the plasma phase react with the aqueous liquids leading to acidification and generation of $H_2O_2$, $NO_2^-$ and $NO_3^-$. The reduced pH of PAW and PA-AA, as well as the generation of RONS were also observed in our study. It was reported that ORP plays an important role in microbial inactivation by damaging the cell membrane (McFerson, 1993). Additionally, the bactericidal effect of plasma-activated solutions is generally associated with the combination of high ORP and low pH (Tian et al., 2015). The more acidic pH and higher values of ORP and conductivity were consistent with the greater inactivation efficacy of PA-PAA than PAW. The lower concentration of $NO_2^-$ generated in PA-PAA than in PAW is mainly due to the instability of $NO_2^-$, thus it will be transformed to the stable state of $NO_3^-$, and the more acidic environment of PA-PAA accelerated the transformation process, especially as PAA is a strong oxidizer.

## 5. Conclusions

This study evaluated the potential of US, PAW, and PAA-based hurdle interventions to ensure raw mackerel safety, and to the best of our knowledge, this is the first study that measured the inactivation effectiveness of the three technologies together. Our results demonstrated that individual treatments applied to fish fillets had limited antimicrobial efficacy, while a trend of enhanced inactivation was observed in the combined interventions. The treatments involving PAA tended to achieve greater inactivation as organic material has less effect on the PAA inactivation rate. The quality parameters of fish were not markedly changed after the treatments. The significantly higher values of reactive species, and more acidic pH in PA-PAA than PAW or PAA demonstrated the synergistic effect of hurdle technologies. Additional optimization would be necessary to improve the inactivation efficacy of the methods assessed and to control the lipid oxidation caused by the radicals formed.

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## CRediT authorship contribution statement

**Yi-Ming Zhao:** Writing – original draft, Formal analysis, Investigation. **Marcia Oliveira:** Formal analysis, Investigation, Writing – review & editing. **Catherine M. Burgess:** Formal analysis, Investigation, Writing – review & editing. **Janna Cropotova:** Formal analysis, Investigation. **Turid Rustad:** Formal analysis, Investigation. **Da-Wen Sun:** Supervision, Funding acquisition, Writing – review & editing. **Brijesh K. Tiwari:** Supervision, Funding acquisition, Resources, Writing – review & editing.

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