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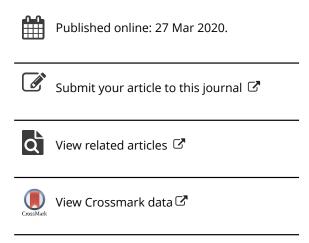
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#### **REVIEW**



# Functionalization of water as a nonthermal approach for ensuring safety and quality of meat and seafood products

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#### **ABSTRACT**

Meat and seafood products present a viable medium for microbial propagation, which contributes to foodborne illnesses and quality losses. The development of novel and effective techniques for microbial decontamination is therefore vital to the food industry. Water presents a unique advantage for large-scale applications, which can be functionalized to inactivate microbial growth, ensuring the safety and quality of meat and seafood products. By taking into account the increased popularity of functionalized water utilization through electrolysis, ozonation and cold plasma technology, relevant literature regarding their applications in meat and seafood safety and quality are reviewed. In addition, the principles of generating functionalized water are presented, and the safety issues associated with their uses are also discussed.

Functionalization of water is a promising approach for the microbiological safety and quality of meat and seafood products and possesses synergistic effects when combined with other decontamination approaches. However, functionalized water is often misused since the active antimicrobial component is applied at a much higher concentration, despite the availability of applicable regulations. Functionalized water also shows reduced antimicrobial efficiency and may produce disinfection by-products (DBPs) in the presence of organic matter, especially at a higher concentration of active microbial component. Utilization should be encouraged within regulated guidelines, especially as hurdle technology, while plasma functionalized water which emerges with great potentials should be exploited for future applications. It is hoped that this review should encourage the industry to adopt the functionalized water as an effective alternative technique for the food industry.

#### **KEYWORDS**

Antimicrobial; cold plasma; contamination; electrolytic; minimally processed; ozonized

#### Introduction

The demand for meat and seafood products is increasing in recent times due to their important nutritional values (Arya et al. 2018; Ekezie, Cheng, and Sun 2019; Ekezie, Sun, and Cheng 2019). Global meat production has rapidly increased by 25% in the past ten years to 323 Mt in 2017, and it is expected to grow by more than 48 Mt in 2027, while export is projected to be 20% higher. Meat per capita consumption is also expected to rise to 35.4 kg by 2027 (OECD-FAO 2018; FAOSTAT, 2019). Similarly, the global production of seafood continues to grow and reached 170.9 Mt in 2016, from 140.4 MT in 2007 (FAO 2018). Fish production is projected to increase slightly by 1% per annum to 195 Mt in 2027, while per capita consumption is forecast to rise slightly to 21.3 kg from 20.3 kg, providing about 3.2 billion people with nearly 20% of their average animal protein per capita intake. However, meat and seafood products are highly perishable, which presents a viable medium for microbial propagation, contributing to possible foodborne illnesses and quality deterioration. Therefore, common preservation methods such as cooling (McDonald and Sun, 2001; Zhu et al., 2019a, 2019b) and freezing (Xie et al., 2015, Tian et al., 2020; Mahato et al., 2019; Li, Zhu, and Sun 2018; Luo et al., 2018; Zhang, Zhu, and Sun 2018; Zhan et al., 2018, 2019a, 2019b) are used to maintain their quality and safety. On the other hand, growing health consciousness has necessitated the supply of minimally or nonthermally processed food products with fresh characteristics (Esua et al. 2019).

As such, the safety and quality of meat and seafood products have become a challenging preoccupation, which encourages the development of novel alternative technologies, especially nonthermal processing techniques (Liao et al. 2018). Although Brychcy et al. (2015) and Chen et al. (2016b) suggested that inhibitory processes of packaging and

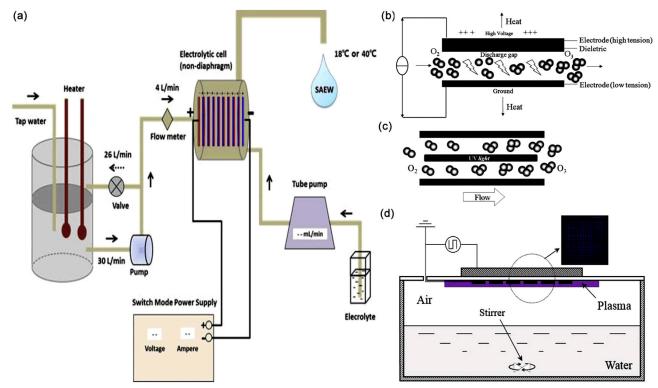


Figure 1. (a) Schematics of SAEW production (Forghani, Park, and Oh 2015) (b) Schematics of corona discharge method of OFW generation (c) Ultraviolet lamp method of OFW generation (Goncalves 2016) (d) Schematic representation of PFW generation (Jung et al. 2015a).

ionization radiation, control by moisture and temperature-based strategies typically characterize traditional approaches for meats and seafood preservation, water presents a unique advantage for large-scale applications and literature suggest that washing is a critical and most commonly used step during processing for microbial load reduction and dirt removal (Ding et al. 2010; Jo, Tango, and Oh 2018; Wang et al. 2018).

Recently, water has been functionalized for ensuring safety and quality of meat and seafood products with encouraging results through the use of chlorine-containing chemicals (Park, Chung, and Ha 2018), organic acids (Duan et al. 2017), electrolysis (Jo, Tango, and Oh 2018), ozone (Goncalves and Santos 2019), and cold plasma technology (Liao et al. 2018). Functionalized water through electrolysis, ozonation, and cold plasma technology have become a popular research topic with nonthermal processing effects (Ratana-Arporn, and Jommark 2014; Wang et al. 2018; Jo, Tango, and Oh 2018). However, some safety issues and interactions of functionalized water with substances in meat and seafood matrixes have been the subject of intense debate.

For example, chlorine-containing functionalized solutions from sodium hypochlorite, chlorine dioxide, and electrolysis may produce halogenated disinfection by-products (HDBPs) with health-related concerns due to chlorine reactivity with organic matter in meats and seafood (Gackowska et al. 2016). In addition, lipid oxidation, discolouration of surfaces, undesirable odour and toxicity of ozone have also been associated with functionalized water through ozonation, while mutagenic effects due to long term exposure, controllable dose, and regulatory concerns are some of the safety

issues surrounding functionalization of water by cold plasma technology (Cullen et al. 2018).

A considerable amount of literature exists on functionalized water applications through the use of electrolysis, ozone, and cold plasma technology, and significant progress has been made on using the water for ensuring the safety and quality of meat and seafood products. This review purposes to provide information regarding the progress in developing electrolyzed, ozonized and plasma functionalized water for meat and seafood products to ensure their quality and safety. The generation, principle and sanitizing efficacy of the functionalized water are presented, in addition to safety issues and challenges towards full-scale implementation. It is hoped that the current review should encourage further research and applications in the area for early adoption of the technology for the food industry as an effective alternative processing technique.

#### **Functionalization of water**

### Functionalization of water by electrolysis

Water can be functionalized by electrolysis. The electrolyzed functionalized water (EFW) is typically generated by passing a dilute salt solution (usually NaCl) through an electrolytic cell within a two-compartment electrolysis generator. This batch scale generator is separated into anode and cathode electrodes by an ion exchange diaphragm (Al-Holy and Rasco 2015; Al-Qadiri et al. 2016; Duan et al. 2017; Arya et al. 2018; Cichoski et al. 2019). Upon electrolysis, NaCl from the solution dissociates into positively (Na<sup>+</sup>) and

negatively (Cl<sup>-</sup>) charged ions, in addition to hydrogen (H<sup>+</sup>) and hydroxide (OH<sup>-</sup>) ions. The anode attracts the negative ions of OH and Cl, which loses electrons to produce chlorine gas (Cl<sub>2</sub>), hypochlorous acid (HOCl) and hydrochloric acid (HCl), thus generating acidic electrolyzed water (AEW) with pH of <2.7, available chlorine (AVC) of  $50 \pm 10$  mg/L and oxidation-reduction potential (ORP) of > 1100 mV (Duan et al. 2017; Rahman, Khan, and Oh 2016). On the other hand, positive ions of Na+ and H+ are attracted to the cathode and gain electrons to form hydrogen gas (H<sub>2</sub>) and sodium hydroxide (NaOH), leading to the generation of alkaline electrolyzed water (AiEW) with pH of 10 - 13 and ORP of < -900 mV (Rahman, Khan, and Oh 2016; Athayde et al. 2017; Rigdon, Hung, and Stelzleni 2017). Other variants of EFW include slightly acidic electrolyzed water (SAEW) produced from the electrolysis of HCl using a single chamber compartment without a membrane (Figure 1a), with pH of 5 - 6.5, AVC of 10 - 30 mg/L and ORP of 750 -850 mV (Forghani, Park, and Oh 2015; Duan et al. 2017) and neutral electrolyzed water (NEW) with pH and ORP in the range of 6 - 8 and 700 - 900 mV, respectively (Han, Hung, and Wang 2018). NEW is generated by the neutralization of positive ions (H<sup>+</sup>) from the anode and negative ions (OH-) from the cathode, and also by mixing AEW and AiEW to the required pH.

The antimicrobial actions of EFW are attributed to the ability of chlorine and reactive oxygen species to penetrate microbial cell membranes to cause oxidative damage to DNA and inhibit the activities of enzymes necessary for microbial growth, influenced by the synergistic actions of pH and ORP (Rahman, Khan, and Oh 2016). The formation of chlorine species and their reactive chemistry in EFW are dependent on the pH level, and since microbial cells survive at pH levels of 4 - 9 (Pan et al. 2019; Pan et al. 2020), low pH is likely to destroy the external layer of microbial cells, increasing permeability and susceptibility of the cells to HOCl. Neutral pH may result in reduced abrasion of contact surfaces and increased stability of the chlorine species (Ding and Liao 2019). Also, the membrane potential of microbial cells is usually in the range of -400 to 900 mV at their normal physiological state, thus a higher ORP environment can disrupt cell envelopes and result in the loss of intracellular cell components (Liao, Chen, and Xiao 2007; Quan et al. 2010).

EFW is an eco-friendly green-cleansing technology, and its on-site production ability and easy decomposition feature can prevent storage and waste disposal challenges (Park, Hung, and Brackett 2002; Duan et al. 2017). EFW has great potential for industrial applications as it constitutes less health hazard and can return to its original form as the chlorine species are expended when in contact with organic matter (Park, Hung, and Brackett 2002; Athayde et al. 2017).

## Functionalization of water by ozone

The functionalization of water by ozone is achieved using air or oxygen (O2) as feed gas through ultraviolet technology, corona discharge and electrochemical process in water, or via a bubble generator to form micro/nano bubble ozonated water (Manousaridis et al. 2005; Rong et al. 2010; He et al. 2015; de Mendonca Silva, and Goncalves 2017; Goncalves, and Santos 2019). In the generation of ozonized functionalized water (OFW), a great amount of energy is usually required to break the O- O bond, thus the corona discharge and ultraviolet radiation methods (Figure 1b-c) are typically used for the excitation of O2 electrons. This induces splitting of the molecules to form ozone (O3) in combination with other O2 molecules in water as described (Manousaridis et al. 2005; Goncalves 2016; Brodowska, Nowak, and Smigielski 2018):

$$O_2 + e^- \to 2O \tag{1}$$

$$2O + 2O_2 \rightarrow 2O_3$$
 (2)

As O<sub>3</sub> is unstable in atmospheric state and solution state, depending on the water quality and purity, it can decompose into superperoxide  $(O_2^-)$ , hydroxyl (OH) and hydroperoxyl (HO<sub>2</sub>) radicals according to the following equations (Brodowska, Nowak, and Smigielski 2018):

The initiation reaction occurs first and includes the expedited decomposition of O<sub>3</sub> to O<sub>2</sub>-o and HO<sub>2</sub>o radical by an initiator such as OH molecule:

$$O_3 + OH^- \rightarrow O_2^{-o} + HO_2^o$$
 (3)

and further splitting of the radical when its acid/base equilibrium pKa of 4.8 is exceeded:

$$HO_2^o \to O_2^{-o} + H^+ \tag{4}$$

The radical chain reaction then follows, during which O<sub>3</sub> reacts with O2o anion to form O3o anion, and subsequent decomposition of O<sub>3</sub>-o to OHo radical via hydrogen trioxide (HO<sub>3</sub>°) as shown below:

$$O_3 + O_2^{-o} \to O_3^{-o} + O_2$$
 (5)

$$HO_3^o \leftrightarrow O_3^{-o} + H^+ \tag{6}$$

$$HO_3^o \rightarrow OH^o + O_2$$
 (7)

The OH° radical reacts further to generate HO<sub>2</sub>° radical, which initiates the reaction again from Eq. (4), setting up a chain reaction catalyzed by substances like organic molecules, which can convert OH radicals to superoxide radicals  $(O_2^{-o}).$ 

$$OH^o + O_3 \to HO_4^o \tag{8}$$

$$HO_4^o \rightarrow HO_2^o + O_2$$
 (9)

Radical scavengers such as organic and inorganic substances like HCO3 and CO32 react with the OH radical to generate secondary radicals incapable of producing superoxide radicals, thereby terminating the above chain reaction.

$$OH^o + HO_2^o \rightarrow O_2 + H_2O \tag{10}$$

As these radicals have strong oxidizing ability, they are responsible for the reactivity of OFW (Manousaridis et al. 2005; Chawla, Bell, and Janes 2007). The antimicrobial mechanism of OFW can be attributed to the reaction of reactive oxygen species and peroxides with unsaturated fatty the membrane of cells by ozonolysis. acids in

Polyunsaturated fatty acids and their residues in the cytoplasmic membrane of microorganisms undergo peroxidation by the actions of free radicals in OFW, causing the rearrangement of double bonds and the formation of conjugated bonds. This leads to complete oxidation of lipids, which alter their physical properties, triggering depolarization and inhibition of transport proteins and membrane enzymes. Excessive usage can produce oxidative spoilage, undesirable odours and surface discolouration (Manousaridis et al. 2005; Chawla, Bell, and Janes 2007; Brodowska, Nowak, and Smigielski 2018). Ozone is ecofriendly and safe to handle as it cannot be stored but generated on-site. Ozone can be applied to all types of food without a detrimental effect on quality at correct concentrations. The primary advantage of reactions involving OFW is that with O<sub>3</sub> incorporating into the oxidized product, a nontoxic compound from the unincorporated O2 is released as compared with hazardous carcinogenic chlorinated effluents associated with most other functionalized water from chemicals (Novak and Yuan 2003).

# Functionalization of water by cold plasma technology

The use of cold plasma technology is another method by which water or solutions of salts, buffers and organic acids can be functionalized (Ekezie, Sun, and Cheng 2017; Chen, Cheng, and Sun 2019). The plasma functionalized water (PFW) is produced by subjecting the water or these solutions to cold plasma discharge (Figure 1d) using two different modes either above or in water (Kim et al. 2016). During plasma discharge in water, high voltage application causes gases to undergo excitation, ionization and dissociation to form positive and negative species that react with water molecules to generate several radicals and unstable chemical species (Shen et al. 2019; Han, Cheng, and Sun 2019; Pan, Cheng, and Sun 2019). For air-operated plasma,  $O_2$  in the air to dissociate first into  $O_2$  atoms due to its lower electron energy requirement as compared with  $N_2$ .

The subsequent increase in treatment time results in energy accumulation necessary to dissociate  $N_2$ , and dissolution of nitrogen oxide from the gas phase dissociated reactions of  $O_2$  and  $N_2$ , generating nitrates ( $NO_3^-$ ) and nitrites ( $NO_2^-$ ), with the actual product concentration dependent on the pH of the solution. Dissociation and ionization of water molecules also occur as  $O_2$  atoms contact with water, and swift reaction with an additional water molecule in the gasliquid interface acidifies the solution, producing reactive species of  $OH^-$  and  $H_2O_2$ , according to the following reactions (Lukes et al. 2014; Liao et al. 2018; Shen et al. 2019):

$$O_2 \rightarrow O + O$$
 (11)

$$O_2 + O_2 + O \rightarrow O_3 + O_2$$
 (12)

$$N_2 \to N + N \tag{13}$$

$$N + O \rightarrow NO$$
 (14)

$$NO + O \rightarrow NO_2$$
 (15)

$$N_2 + O_2 \rightarrow 2NO \tag{16}$$

$$2NO + O_2 \rightarrow 2NO_2 \tag{17}$$

$$O + H_2O \rightarrow OH$$
 (18)

$$OH^{\cdot} + OH^{\cdot} \rightarrow H_2O_2 \tag{19}$$

$$NO_{2(aq)} + NO_{2(aq)} + H_2O_{(l)} \rightarrow NO_2^- + NO_3^- + 2H^+$$
 (20)

$$NO_{(aq)} + NO_{2(aq)} + H_2O_{(l)} \rightarrow 2NO_2^- + 2H^+$$
 (21)

Thus the  $NO_2^-$  content of PFW decreases with increasing post-discharge time, while  $NO_3^-$  increases as a result of  $NO_2^-$  disproportionation (pKa = 3.3) into  $NO_3^-$  with successive reaction under acidic conditions (Yong et al. 2018):

$$3NO_2^- + 3H^+ + H_2O \rightarrow 2NO + NO_3^- + H_3O^+$$
 (22)

The reactions of NO<sub>2</sub> with H<sub>2</sub>O<sub>2</sub>, NO<sub>2</sub> with OH radicals, and NO with superoxide anion radicals proceed to form peroxynitrites as detailed below. Peroxynitrites possess high reactivity and strong oxidation tendencies with biological molecules, either as peroxynitrite anion (ONOO<sup>-</sup>) or the protonated (ONOOH) peroxynitrous acid with a pKa of 6.8, contributing to the inactivation mechanism of PFW (Lukes et al. 2014; Shen et al. 2019).

$$NO_2^- + H_2O_2 \rightarrow ONOOH + H_2O$$
 (23)

$$ONOOH \leftrightarrow OH + NO_2$$
 (24)

$$OH + NO_2 \rightarrow ONOO^- + H^+ \tag{25}$$

$$O_2^- + NO \to ONOO^- \tag{26}$$

The generation of these abundant reactive species, ions, and free radicals from plasma discharge interaction with water is responsible for the antimicrobial properties of PFW and their concentration is dependent upon the working gas, plasma discharge type and the surrounding environment (Jung et al. 2015a; Kim et al. 2016; Liao et al. 2018; Ekezie, Cheng, and Sun 2018). Some of the species have relatively short half-lives (OH, and O2) with limited contribution to antimicrobial properties, while the long-term half-live species (O<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, NO<sub>2</sub>, and NO<sub>3</sub>), pH change and peroxynitrates are predominantly responsible for intracellular ROS generation, which causes oxidative stress in lipids, proteins, RNA and DNA (Yong et al. 2018; Liao et al. 2018). PFW is a green eco-friendly sanitizing technique, causing minimal or no damage to the quality and nutritional values of food. A variety of uses of PFW are continually being developed over direct plasma applications due to its ease of application, off-site generation, preservation and storage ability for largevolume processing.

# **Applications of functionalized water**

# Electrolyzed functionalized water

# Antimicrobial efficacy and application to meat safety and quality

The decontamination efficiency of various forms of EFW on meat products is illustrated in Table 1. The majority of the studies have utilized a soaking or immersion technique with a variable degree of success. For instance, Park, Hung, and

Table 1. Effects of electrolyzed functionalized water on meat and seafood safety and quality.

Food Type	EFW Parameters	Log Reduction	Quality Attributes	References
	Li W raidilleteis	Log Neduction	Quality Attributes	Neierences
<b>Meats and Meat Products</b> Beef	AEW (pH: 3.03, Cl <sub>2</sub> : 34 mg/L, ORP: 760 mV, 720 s), AiEW	E. coli 0157:H7: 1.16-1.61		Arya et al. (2018)
	(pH: 10.73, ORP: -372, 720 s) SAEW (pH: 5.85, Cl <sub>2</sub> : 30 mg/L, ORP: 887 mV), 300 s	TAB: 2.35		Jo, Tango, and Oh (2018)
steaks)	AiEW (pH: 9.0, Cl <sub>2</sub> : 100 mg/L, 1% residual KCl), 90 s, vacuum packing, 4°C storage.	Yeast, coliforms, TBC, LAB: insignificant		Botta et al. (2018)
liver)	AEW (pH: 2.5, Cl <sub>2</sub> : 30 mg/L, ORP: 975 mV, 4 °C, 180 s) + AiEW (pH: 11.5, 25 °C, 180 s)	S. Enteritidis: 1.03–1.23; E. coli: 0.56–1.04; S. aureus: 0.62–1.28	Delayed lipid oxidation, improved elasticity	Shimamura et al. (2016)
	AEW (pH: 2.3, Cl <sub>2</sub> : 38 mg/L, 660 s, 22 °C)	E. coli: 1.4; TMC: 2.0; S. Typhimurium: 1.4; L. monocytogenes: 1.3		Al-Holy and Rasco (2015)
boneless)	AEW (pH: 2.31, Cl <sub>2</sub> : 25 mg/L, ORP: 1163 mV), SAEW (pH: 6.29, Cl <sub>2</sub> : 25 mg/L, ORP: 934 mV) + 0.5% FA, 40 °C, 180 s	TVC: 3.7; E. coli O157:H7, S. aureus, L. monocytogenes, S. Typhimurium: 0.63–2.62 9 days extended shelf life	Acceptable sensory properties	Tango et al. (2014)
hides)	SAEW (pH: 6.5, Cl <sub>2</sub> : 150 mg/L, 23 °C), AiEW (pH: 11.6, 43 °C)	APC: 1.01–1.60; E. coli O157:H7: 0.65–1.70; EBC: 1.50–2.2;		Jadeja and Hung (2014)
rib)	AEW (pH: 2.3, Cl <sub>2</sub> : 50 mg/L, ORP: 1110 mV), SAEW (pH: 6.2, ORP: 520 mV), 23 °C, 180 s	S. Typhimurium: 2.98 E. coli O157:H7: 1.64–1.72		Ding et al. (2010)
heads)	AEW (pH: 2.8, Cl <sub>2</sub> : 60 mg/L, ORP: 1190 mV), spray, 26 s.	E. coli O157:H7: 0.32.		Kalchayanand et al. (2008)
hides)	AiEW (pH: 11.20, 52 °C, 10 s) + AEW (pH: 2.40, Cl <sub>2</sub> : 70 mg/L, 60 °C, 10 s)	EBC: 4.30; APC: 3.50; E. coli O157:H7: 57% prevalence reduction		Bosilevac et al. (2005)
Chicken (breast)	SAEW (pH: 6.0, Cl <sub>2</sub> : 5 mg/L, ORP: 850 mV) + US (28 W/L), 300 s	Psychrotrophs: 0.76; LAB: 0.81; mesophiles: 0.98	Slight lipid oxidation, inhibit protein oxidation	Cichoski et al. (2019)
	SAEW (pH: 5.85, Cl <sub>2</sub> : 30 mg/L, ORP: 887 mV), 300 s	TAB: 2.43		Jo, Tango, and Oh (2018)
carcass)	AEW (pH: 2.46, Cl <sub>2</sub> : 58 mg/L, ORP: 1126 mV, 15 s), SAEW (pH: 5.98, Cl <sub>2</sub> : 30 mg/L, ORP: 865 mV, 15 s)	TVC: 0.47–0.83; Total coliforms: 0.49–0.96 2 days extended shelf life	Delayed lipid oxidation, quality maintained	Wang et al. (2018); Duan et al. (2017)
breast)	AEW (pH: 2.5, Cl <sub>2</sub> : 30 mg/L, ORP: 975 mV, 4 °C, 180 s) + AiEW (pH: 11.5, 25 °C, 180 s)	S. Enteritidis: 0.85–1.17; E. coli: 0.81–1.48; S. aureus: 0.43–0.56	Delayed lipid oxidation, improved elasticity	Shimamura et al. (2016)
frankfurters)	SAEW (pH: 5.37, Cl <sub>2</sub> : 29 mg/L, ORP: 786 mV, 60 s, 40 °C)	TBC: 1.35; <i>S.</i> Typhimurium: 1.45; <i>L. monocytogenes</i> : 1.00		Forghani, Park, and Oh (2015
legs)	AEW (pH: 2.3, Cl <sub>2</sub> : 38 mg/L, 660 s, 22 °C)	E. coli: 1.4; TMC: 1.9; S. Typhimurium: 1.5; L. monocytogenes: 1.1	Delayed lipid oxidation,	Al-Holy and Rasco (2015)
	AEW (pH: 2.54, Cl <sub>2</sub> : 50 mg/L, ORP: 1120 mV), SAEW (pH: 6.5, Cl <sub>2</sub> : 10 mg/L, ORP: 770 mV), 600 s, 22 °C, 4 °C storage	TVC: 1.49–2.64; <i>L. monocytogenes</i> : 1.2–2.54; S. Typhimurium: 1.93–2.25; 4 days extended shelf life	maintained sensory quality	Rahman et al. (2012)
wings)	AEW (pH: 2.57, Cl <sub>2</sub> : 50 mg/L, ORP: 1082 mV, 23 °C, 600 s)	C. jejuni: 2.96		Park, Hung, and Brackett (2002)
Chevon	AEW (pH: 3.03, Cl <sub>2</sub> : 34 mg/L, ORP: 760 mV, 720 s), AiEW (pH: 10.73, ORP: -372, 720 s)	E. coli O157:H7: 0.96–1.22		Arya et al. (2018)
Pork	SAEW (pH: 5.85, Cl <sub>2</sub> : 30 mg/L, ORP: 887 mV), 300 s	TAB: 2.20		Jo, Tango, and Oh (2018)
	AEW (pH: 3.03, Cl <sub>2</sub> : 34 mg/L, ORP: 760 mV, 720 s), AiEW (pH: 10.73, ORP: -372, 720 s)	E. coli 0157:H7: 1.30–1.52		Arya et al. (2018)
skin)	NEW (pH: 7.64, Cl <sub>2</sub> : 74 mg/L, ORP: 818 mV), 600 s	E. coli: 2.59; S. Enteritidis: 2.37; Y. enterocolitica: 1.81	Maintained colour properties	Han, Hung, and Wang (2018)
(loin)	AEW (pH: 2.60, Cl <sub>2</sub> : 74 mg/L, ORP: 1200 mV, 20 s) + AiEW (pH: 11.40, ORP: -830 mV, 20 s), 18 °C	Mesophilic count: 0.67–1.21; Psychrotrophs: 0.33–0.67	Delayed lipid oxidation, protein breakdown. Colour preserved	Athayde et al. (2017)

Table 1. Continued.

		Re		
Food Type	EFW Parameters	Log Reduction	Quality Attributes	References
(loin)	AiEW (pH: 10.92, ORP: -187 mV) + 2.5%		Improved water holding capacity and tenderness	Rigdon, Hung, and Stelzleni (2017)
(muscle)	Potassium lactate AEW (pH: 2.15, Cl <sub>2</sub> : 16.60 mg/ L, ORP: 1159 mV) + carrageenan hydrosols/gelatin hydrosols, 120 s	Psychrotrophs: 1.55–3.10; Improved antioxidant ac TNM: 2.08–3.25 colour deterioration Yeast & Mold: 1.75–2.68		Brychcy et al. (2015); (2016)
	AEW (pH: 2.31, Cl <sub>2</sub> : 30 mg/L, ORP: 1159 mV) + 0.05% FA, + 40 °C, 180 s, 4 °C storage	E. coli: 2.59; S. aureus: 2.38; L. monocytogenes: 2.69; S. Typhimurium: 2.99; 6 days extended shelf life	Improved sensory quality	Mansur et al. (2015)
(lean)	AEW (pH: 2.54, Cl <sub>2</sub> : 50 mg/L, ORP: 1130 mV), SAEW (pH: 6.8, Cl <sub>2</sub> : 10 mg/L, ORP: 700 mV), + 3% Calcium lactate, 23 °C, 600 s, 4 °C storage.	TVC: 1.4–2.2; Yeast & Mold: 1.07–1.57; L. monocytogenes: 1.72–3.17; E. coli O157:H7: 1.9–3.0; 6 days extended shelf life	Slight discolouration and off odour. Delayed lipid oxidation	Rahman, Wang, and Oh (2013)
Seafood	. c storage.			
Crustaceans (shrimp)	AEWice (pH: 2.38, Cl <sub>2</sub> : 44 mg/ L, ORP: 1153), 20 °C storage	Psychrobacter, Shewanella: 47.8–71.6% inhibition	Retard myofibrillar protein degradation Muscle tissue quality	Zhao et al. (2018)  Du et al. (2016)
	AEW (pH: 2.39, Cl <sub>2</sub> : 73 mg/L, ORP: 1168 mV) + HHP (400 mPa), 600 s	V. parahaemolyticus: 6.08; L. monocytogenes: 5.71; TAB: 5.66	maintained	Du et al. (2010)
(Pacific white shrimp)	SAEWice-glazing (pH: 6.4, Cl <sub>2</sub> : 6.4 mg/L, ORP: 420 mV, 10 s,	S. aureus: 1.22–2.1; TAB: 1.60–1.95	Retard muscle structure degradation and	Zhang et al. (2015)
(shrimp)	0°C) + MAP, -18°C storage AEWice (pH: 2.46, Cl <sub>2</sub> : 26 mg/ L, ORP: 1124), 18°C dark storage	lipid oxidation TVC: 1.50 Inhibition of melanotic reaction		Wang et al. (2014)
(Pacific white shrimp)	NEW (pH: 7.11, Cl <sub>2</sub> : 50 mg/L, ORP: 841 mV), 25 °C, 900 s.	V. parahaemolyticus: 4.16; Maintained sensory attributes V. vulnificus: 1.3. Maintained sensory attributes of colour, odour and texture.		Ratana-Arporn, and Jommark (2014)
	AEW (pH: 2.34, Cl <sub>2</sub> : 51 mg/L, ORP: 1163 mV) + 50 °C, 300 s	V. parahaemolyticus: 3.11; L. monocytogenes: 1.96; TAB: 1.44	Sensory properties maintained	Xie et al. (2012)
Fish (Atlantic salmon)	AEW (pH: 2.7, Cl <sub>2</sub> : 60 mg/L, ORP: 1150 mV), NEW (pH: 6.8, Cl <sub>2</sub> : 60 mg/L, ORP: 786 mV), 65 °C, 600 s	L. monocytogenes: 0.8–5.6		
(salmon)	AEW (pH: 2.6, Cl <sub>2</sub> : 65 mg/L, ORP: 1140 mV, 60 s) + UV-C + US	L. monocytogenes: 0.75; TVC: 0.64; Coliforms: 0.49	Firmness maintained. Slight deterioration in colour and odour	Miks-Krajnik et al. (2017)
(Atlantic salmon; dolphinfish)	AEW (pH: 2.8, Cl <sub>2</sub> : 50 mg/L, ORP: 1080 mV), 600 s	L. monocytogenes: 2.0; M. morganii: 2.0		McCarthy and Burkhardt (2012)
(Atlantic salmon; yellowfin tuna)	AEWice (pH: 2.5, Cl <sub>2</sub> : 100 mg/ L, ORP: 1173), 7200 s	E. aerogenes: 0.91–2.43; M. morganii: 1.24–3.50		Phuvasate and Su (2010)
(King George Whiting; Tasmanian Atlantic salmon)	NEW (pH: 7.0, ORP: 885 mV), 14 °C, 600 s, 4 °C storage	TPC, <i>Pseudomonas</i> spp., coliform: 0.5–2.0; 4 days extended shelf life	Acceptable overall appearance	Khazandi et al. (2017)
(Hairtail)	SAEW (pH: 4.5, $Cl_2$ : 30 mg/L, ORP: 867 mV, 900 s) + 1.5% chitosan, 900 s, $-3$ °C storage	TVC: 1.0; 6 – 7 days of extended shelf life	Retard protein deterioration, delay lipid oxidation. Quality and freshness maintained	Luan et al. (2017)
(Bombay duck)	SAEW (pH: 5.5, Cl <sub>2</sub> : 27 mg/L, ORP: 836 mV), + EBLCE, 300 s, 25 °C, 4 °C storage	TVC: 1.5–2.07. 12 days extended shelf life	Retarded lipid oxidation and formation of tertiary amines	Chen et al. (2016b)
(trout)	AEW (pH: 2.3, Cl <sub>2</sub> : 38 mg/L, 660 s, 22 °C)	E. col: 1.5; TMC: 1.6; Maintained sensory properties S. Typhimurium: 1.5;		Al-Holy and Rasco (2015)
(shad)	SAEW (pH: 5.8, Cl <sub>2</sub> : 30 mg/L, ORP: 810 mV, 300 s) + US	L. monocytogenes: 1.2 V. parahaemolyticus: 1.42; E. coli: 1.86	Inhibit lipid oxidation, improved textural properties	Park and Ha (2015)
(American shad)	(37 kHz, 0.071 W/cm <sup>3</sup> , 6000 s AEW (pH: 2.40, Cl <sub>2</sub> : 80 mg/L, ORP: 1185 mV, 900 s) + 2%	TVC: 0.25–2.40. 10 days of extended shelf life	Inhibit myofibril degradation and lipid oxidation.	Xu et al. (2014)
(obscure puffer)	CS, 600 s, 4 °C storage SAEW (pH: 6.1, Cl <sub>2</sub> : 21 mg/L, ORP: 947 mV), + CH, 330 s,	TVC: 1.10–1.56. 4 days of extended shelf life	Increased firmness Suppressed lipid oxidation, sensory properties maintained	Zhou et al. (2011)
(tilapia)	4 °C storage AEW (pH: 2.47, Cl <sub>2</sub> : 120 mg/L, ORP: 1159 mV), 23 °C, 600 s	V. parahaemolyticus: 3.84; E. coli: 1.68		Huang et al. (2006)
Molluscs (oyster)	SAEW (pH: 6.14, Cl <sub>2</sub> : 60 mg/L,	Salmonella spp., E. coli, V. parahaemolyticus:	Low pH, suppressed decline in colour, cutting strength,	Tantratian and Kaephen (2020)

(continued)

Table 1. Continued.

		Re	_	
Food Type	EFW Parameters	Log Reduction	Quality Attributes	References
	$4^{\circ}$ C, $1800\text{s}) + 5.0\mu\text{g/mL}$ epigallocatechin-3-gallate	undetectable levels. TVC: low counts. 7days extended shelf life	nitrogenous compound degradation	
(squid)	SAEWice (pH: 6.48, Cl <sub>2</sub> : 25 mg/L, ORP: 882 mV), 10°C storage	TVC: 1.46	Inhibit lipid oxidation. Delayed browning, softening	Xuan et al. (2017)
Clam, mussel)	AEW (pH: 3.1, Cl <sub>2</sub> : 20 mg/L, ORP: 1150 mV), AEW (pH: 3.55, Cl <sub>2</sub> : 10 mg/L, ORP: 950 mV)	E. coli O104:H4: 1.4–1.7; L. monocytogenes: 1.0–1.6; A. hydrophila: 1.3–1.6; V. parahaemolyticus: 1.0–1.5; C. jejuni: 1.5–2.2	•	Al-Qadiri et al. (2016)
(oysters)	AEW (pH: 2.82, Cl <sub>2</sub> : 30 mg/L, ORP: 1131 mV, 23 °C, 600 s)	V. parahaemolyticus: 0.8–1.1; V. vulnificus: 0.68–1.05		Ren and Su (2006)

Note: AEW(ice): Acidic electrolyzed water (frozen at -20 °C); ORP: Oxidation-reduction potential; AiEW: Alkaline electrolyzed water; TAB: Total aerobic bacteria; SAEW(ice)(ice-glazing): Slightly acidic electrolyzed water (frozen at -20°C) (Dip for 10 s at 0°C); TMC: Total mesophilic counts; FA: Fumaric acid; TVC: Total viable counts; EBC: Enterobacteriaceae; APC: Aerobic plate counts; US: Ultrasound technology; TBC: Total bacterial counts; NEW: Neutral electrolyzed water; TNM: Total number of microorganisms; HHP: High hydrostatic pressure; MAP: Modified atmosphere packaging; UV-C: Ultraviolet-C radiation; TPC: Total psychrotrophic count; EBLCE: Ebony-bamboo leaves complex extract; CH: Chitosan coating solution (1% chitosan, 1.5% acetic acid, 6% propylene glycol)

Brackett (2002) achieved a 2.96 log CFU/g reduction in Campylobacter jejuni on chicken using AEW. Arya et al. (2018) observed no significant bacterial reductions between different types of meat but reported the highest reductions of 1.16, 1.22 and 1.30 log CFU/ml in Escherichia coli (E. coli) on beef, chevon, and pork, respectively, for AEW as compared with AiEW. Despite having a neutral pH, NEW was a significantly better sanitizer on pork skin compared with pork chops, producing 1.81 - 2.59 log CFU/ml reduction in E. coli O157:H7, Salmonella Enteritidis (S. Enteritidis), and Yersinia enterocolitica, while maintaining the colour (Han, Hung, and Wang 2018). In the same vein, SAEW with pH close to the neutral value was effective for reducing the populations of total aerobic bacteria (TAB), the number of total bacterial count (TBC), total viable count (TVC) and total coliforms. The functionalized water also showed decontamination efficiency for yeast and mould, Listeria monocytogenes (L. monocytogenes), Staphylococcus aureus (S. aureus), Salmonella Typhimurium (S. Typhimurium), resulted in increased shelf life, enhanced acceptable sensory properties and delayed lipid oxidation in beef, chicken and pork (Ding et al. 2010; Rahman et al. 2012; Forghani, Park, and Oh 2015; Duan et al. 2017; Jo, Tango, and Oh 2018).

The limited effectiveness of AiEW and AEW on beef steaks and bovine heads have been reported (Botta et al. 2018; Kalchayanand et al. 2008). Spray technique of AiEW for 90 s on each side of beef steak in a slaughterhouse was unable to reduce the initial microbial counts to a significant level, while bovine heads sprayed with AEW for 26s could only result in 0.39 log CFU/cm<sup>2</sup> reduction of E. coli O157:H7. Low reductions in the number of TVC and coliforms (0.47-0.96 log CFU/cm<sup>2</sup>) found on chicken carcass were also observed with either AEW or SAEW spray for 15 s, in a cabinet designed for a large-scale chicken slaughter-line (Duan et al. 2017; Wang et al. 2018). In contrast, Jadeja and Hung (2014) reported as much as 1.34, 1.50, 1.09, and 0.65 log CFU/cm<sup>2</sup> reduction, respectively, in

number (APC), of aerobic plate count Enterobacteriaceae (EBC), S. Typhimurium and E. coli on beef hides sprayed with 60 ml of NEW. However, spraying with 32 ml of SAEW, followed by 28 ml of NEW was more effective, compared with individual applications of either 60 ml SAEW or NEW, yielding 1.01-2.98 log CFU/ cm<sup>2</sup> reduction. Taken together, results indicate that the acidity level may not be the only parameter influencing the antimicrobial efficacy of EFW, and the chlorine, ORP, application type and duration of application also contribute to the efficacy. In addition, organic matters especially protein depletes the free chlorine and reduces the sanitizing efficiency of EFW (Jo, Tango, and Oh 2018; Han, Hung, and Wang 2018).

Promising potential for synergy has also been demonstrated when EFW is combined with other intervention strategies. The population of Enterobacteriaceae (EBC) and the number of aerobic plate counts (APC) were effectively reduced by 4.3 and 3.5 log CFU/100 cm<sup>2</sup> respectively on cattle hides when AiEW was combined with AEW (Bosilevac et al. 2005). Shimamura et al. (2016) reported that lipid oxidation was delayed, meat elasticity was improved and more than 1 log CFU/g reduction was achieved in the initial population of bacteria on chicken breast and beef liver, irrespective of sample weights and available chlorine of the combined AiEW and AEW. Similar results were also reported by Athayde et al. (2017) for mesophilic counts and psychrotrophs on pork loin. Cichoski et al. (2019) recently observed improvement in the microbial quality of chicken breast treated with combined ultrasound (US) and SAEW, presenting the highest reductions of 0.98, 0.81, and 0.76 log for mesophilic bacteria, lactic acid bacteria (LAB) and psychrotrophic bacteria, respectively, and they also revealed that combined treatment could retard protein oxidation for chicken and caused no effect on anaerobic glycolysis process (Figure 2a). However, the combined treatment triggered slight lipid oxidation, which may not only limit the shelf life but also reduce the nutritive value of chicken. In another

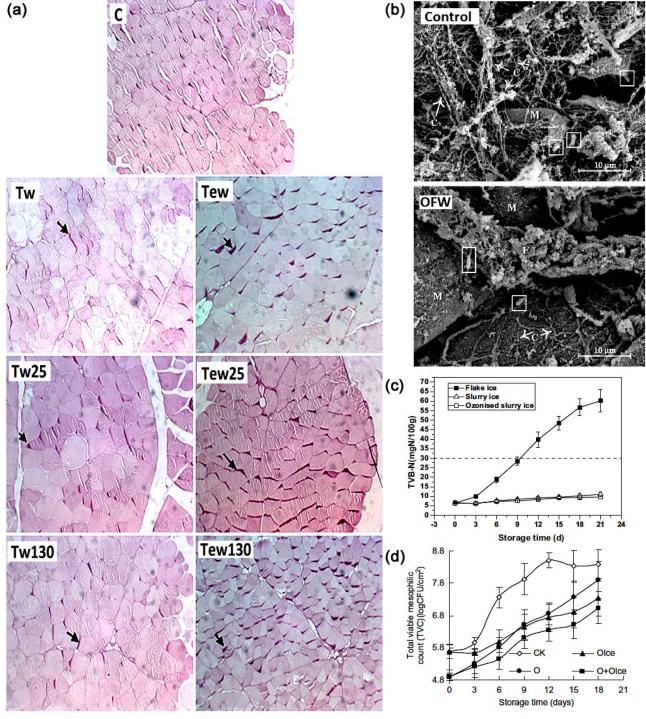


Figure 2. (a) Histological characterization of chicken breast, with the arrow indicating the presence of glycogen: C (control), Tw (sample immersed in water alone), Tw25 (US at 25 kHz), Tw130 (US at 130 kHz), TeW (SAEW application alone), Tew25 (SAEW and US at 25 kHz), TeW130 (SAEW and US at 130 kHz) (Cichoski et al. 2019) (b) Scanning electron micrographs of OFW treated beef depicting muscle tissue (M), fatty deposits (F) and collagen (C) (Novak and Yuan 2003). (b) Changes in TVB-N of bighead croaker stored in flaked ice, slurry ice and ozonized slurry ice (Chen et al. 2016a). (c) TVC changes in Japanese sea brass during storage (Lu et al. 2012).

study, Rahman, Wang, and Oh (2013) reported the effectiveness of the synergistic combination of AEW and calcium lactate on pork against yeast and mould, *L. monocytogenes*, and *E. coli*. Up to 3 log CFU/g reductions and 6 days extended shelf life were observed. The results are in agreement with those reported by Mansur et al. (2015), and Brychcy et al. (2015; 2016) for fresh pork, when AEW was combined with fumaric acid, and carrageenan/gelatin

hydrosols, which resulted in at least 2 log CFU/g reductions in *E. coli*, *S. aureus*, *L. monocytogenes*, *S. Typhimurium*, yeast and mold, and psychrotrophs. Six days of extended shelf life, improved antioxidant activity and sensory properties were also reported by these authors. These results are promising and indicate the capability of EFW in improving the safety and quality of meat as individual treatment and in combination with other approaches. However, these studies

were carried out in laboratory conditions and further investigations are needed for industrial applications.

# Antimicrobial efficacy and application to seafood safety and quality

Table 1 also illustrates the effectiveness of EFW utilization in seafood safety and quality. EFW has shown sanitizing efficiency in different microorganisms such as C. jejuni, Pseudomonas spp., E. coli, L. monocytogenes, Vibrio parahaemolyticus (V. parahaemolyticus), Aeromonas hydrophila (A. hydrophila), and Vibrio vulnificus (V. vulnificus) present on salmon, trout, tilapia, clam, shrimp, mussel, and oysters. When utilized in the acidic or neutral form, EFW caused reductions of 0.5 - 4.16 log CFU/g in these microorganisms with acceptable sensory properties and suppressed lipid oxidation (Huang et al. 2006; Ren and Su 2006; Xie et al. 2012; Ratana-Arporn and Jommark 2014; Al-Holy and Rasco 2015; Al-Qadiri et al. 2016; Khazandi et al. 2017). Results also revealed that EFW was more effective against pathogens on smoother surfaces. Even though the increase in AVC concentration and soaking time led to an increased microbial reduction, extended soaking time was detrimental to nutritional properties, while the use of agitation facilitated sanitization. The results also indicate that washing with functionalized water during evisceration before dipping was thought to have a significant effect. In contrast, Miks-Krajnik et al. (2017) reported that AEW as an individual treatment was not effective for salmon sanitization but was more effective in reducing populations of L monocytogenes and natural microbiota when combined with US and ultraviolet-C radiation (UV-C). Microbial reduction (0.49 - 0.79 log CFU/g) was rather low, which could be attributed to the fact that the combined treatment was applied sequentially. Miks-Krajnik et al. (2017) also revealed a slight deterioration in colour and odour of salmon, while salmon firmness was not affected by the combined treatment.

Du et al. (2016) investigated the use of AEW as a new pressure transmitting medium during high hydrostatic pressure (HPP) decontamination of shrimp and claimed that AEW enhanced the efficacy of HPP to 5.71 and 6.08 log CFU/g reductions of L. monocytogenes and V. parahaemolyticus, respectively, while maintaining muscle tissue quality. Likewise, SAEW as a primary disinfectant was shown to enhance the efficiency of US decontamination of freshly sliced shad, leading to 1.42 and 1.86 log CFU/g reductions of V. parahaemolyticus and E. coli, respectively (Park and Ha 2015), while the sensory properties were not altered under the harshest conditions of sequential combined treatment. Several other studies have also demonstrated the decontamination efficacy of EFW when combined with other approaches, especially mild heat on salmon (Ovissipour et al. 2018), botanic bio-preservative on Bombay duck (Chen et al. 2016b), and chitosan coating on obscure puffer (Zhou et al. 2011). Results indicate that NEW showed better antimicrobial properties when combined with mild heat (up to 5.6 log CFU/g reduction in L. monocytogenes) compared with the use of AEW alone, which could also suppress protein alteration (Ovissipour et al. 2018). When SAEW was combined with chitosan and botanic bio-preservative, lipid oxidation and myofibril protein degradation were retarded, sample firmness was increased, 2 log CFU/g reduction in TVC was achieved and shelf life was extended for at least 4 days (Zhou et al. 2011; Chen et al. 2016b; Luan et al. 2017). In addition, soaking oyster meat in epigallocatechin-3-gallate after washing with SAEW resulted in significantly low TVC counts, with undetectable levels of V. parahaemolyticus, Salmonella spp. and E. coli (Tantratian and Kaephen 2020). This combined with low pH suppressed reduction in cutting strength and nitrogenous compound degradation, leading to the extension of 7 days in shelf life.

For very delicate and highly perishable seafood products such as squids and shrimps, EFW is also used in the form of ice or ice-glazing. Zhang et al. (2015) revealed that forming an ice-glaze on shrimps from SAEW prolonged storage time of shrimp to 80 days, compared with only several hours achieved with individual SAEW application. It was believed that the phase change played a vital role in stabilizing active ions and dissolved hydrogen responsible for 1 - 2 log CFU/g reductions in S. aureus and TAB, the inhibition of muscle structure degradation and lipid oxidation. Similar results were also published by Xuan et al. (2017) and Zhao et al. (2018), reporting that SAEW and AEW ice presented the capability to delay browning and softening, inhibit lipid oxidation, retard myofibrillar protein degradation and produce 47-71% inhibition in Psychrobacter and Shewanella population. These results propose a new view of seafood refrigeration with a better guarantee of quality.

#### Challenges and safety issues

Free available chlorine (FAC) is present as hypochlorous acid (HOCl) at solution pH below 6.5 but dissociates to hypochlorite ions (OCl-) at high pH and to chlorine gas at low pH (Rahman, Wang, and Oh 2013; Veasey and Muriana 2016). Chlorine gas is corrosive, causes discomforts to operators and exhibits phytotoxic effects at pH levels lower than 5. However, HOCl and acidified chlorine, at concentrations not exceeding 50 ppm and pH of 5 - 10, fall under the latest update of safe and suitable listing of substances in the production of meat and poultry (USDA-FSIS 2019). The current Codex Alimentarius guideline also allows up to 10 mg/l and 100 mg/l chlorine for water in contact with fishery products and equipment cleaning purposes, respectively (FAO/WHO. 2000). The guideline also observes the absence of mutagens at chlorinated water levels of 20 mg/l for washing fish. It is necessary to apply EFW at AVC levels stipulated by regulating authorities, to avoid long term phytotoxic effects on operators and food handlers.

The presence of organic matter within inoculated cells is believed to shield microbial cells from HOCl in EFW, reducing its efficacy. Veasey and Muriana (2016) observed 65 and 96% reduction in FAC in EFW at protein levels of 0.025 and 0.05%, respectively, and complete elimination of FAC at 0.1% protein level. Similarly, Jo, Tango, and Oh (2018) noted that proteins caused a greater reduction in available chlorine compared with carbohydrates and lipids, but a notable reduction in the number of total aerobic counts (1.6 - 3 log CFU/g in beef, chicken and pork) was observed when SAEW was utilized.

Table 2. Effects of ozonized functionalized water on meat and seafood safety and quality.

		Results			
Food Type	OFW Parameters	Log reduction	Quality Attributes	References	
Meat and Meat Products Pork (loins)	5 mg/L, 23 °C, 300 s,	TVC: 0.78; Yeast & Mold: 0.57;		Rahman, Wang, and	
	4°C storage	L. monocytogenes: 1.08; E. coli O157:H7: 1.01		Oh (2013)	
Beef (steaks)	6 mg/L, 4 °C, spray for 90 s, vacuum packing	Low counts of yeast, mold, coliforms, LAB, TAB		Botta et al. (2018)	
(head)	2.30 mg/L, 25 °C, spray at 170 kPa for 26 s	E. coli O157:H7: 0.39		Kalchayanand et al. (2008)	
(hides)	2 mg/L, $15 ^{\circ}$ C, spray at 4,800 kPa for $10 ^{\circ}$ S	APC: 2.1; EBC: 3.4; <i>E. coli</i> : 65% prevalence reduction		Bosilevac et al. (2005)	
(broil beef)	3 mg/L, $4$ °C, with agitation for 300 s	E. coli: 0.85; C. perfringens: 1.28. L. monocytogenes: 1.09		Novak and Yuan (2003)	
(carcass)	95 mg/L, 28 °C, spray at 552 kPa for 30 s	E. coli 0157:H7: 2.0-3.6; S. Typhimurium: 1.9-3.6		Castillo et al. (2003)	
(ground)	1%, 7.2 °C, 900 s, $+$ 0.5 % cetylpyridinium chloride	Coliforms: 1.88; <i>E. coli</i> : 1.68; S. Typhimurium: 1.77; APC: 1.50	Lighter colour, less oxymyoglobin, decrease in off-flavour	Pohlman et al. (2002)	
Chevon (boneless)	0.68 mg/L, pH: 6.8, ORP: 562.75 mV + AEW (pH: 2.73, ORP: 831. 55 mV), 60 - 720 s	E. coli O157:H7: 0.19 – 0.86		Degala et al. (2016)	
Seafood					
Crustaceans (shrimp)	1 mg/L, 600 s, 15 $^{\circ}$ C, $+$ MAP, 4 $^{\circ}$ C storage	TMC: 1.58–1.73; TPC: 0.61–0.65. 15 days extended shelf life	Lipid peroxidation delayed, freshness maintained	Goncalves and Santos (2019)	
	0.42 mg/L, 60–300 s, 25 °C, 4 °C storage	APC: 0.3–1.0; 2 days extended shelf life	Titratable acidity, colour, texture maintained. Retarded lipid oxidation	Okpala (2014); (2015)	
	1.6–1.9 mg/L, spray for 65 s, $19^{\circ}$ C, $+$ CC $+$ CF, $-20^{\circ}$ C storage	Psychrotrophs: 0.89–3.58; Mesophiles: 1.01–5.00; <i>L. innocua</i> : 1.01–5.00	.,	Guo et al. (2013)	
	1–3 mg/L, 60s, 10 °C	APC: 1.0–3.0; <i>P. fluorescens</i> : 2.0–2.9	Inhibited lipid oxidation	Chawla, Bell, and Janes (2007)	
Fish (tilapia)	4.5 mg/L, 1800 s, −18 °C storage	Low TVC levels. Shelf-life extension	Lipid peroxidation, preserved freshness and texture	Zhao et al. (2017)	
	1.5 mg/L, immersion for 900 s	TAMC: 4.13	Maintained colour, slight lipid oxidation	de Mendonca Silva and Goncalves (2017)	
(European anchovy, sardine)	Slurry ice, 0.30 mg/L, 900 s, −1 °C storage	Low population of  Lactobacillus spp. and EBC; TVC: 0.20-1.80;  Pseudomonas spp.: 0.30-1.10		Bono et al. (2017)	
(sardine)	Slurry ice, 660 mV, 0.17 mg/L, -1.5 °C, 2 °C storage	Low counts of coliforms, anaerobes, proteolytic and psychrotropic bacteria. 11 days extended shelf life		Campos et al. (2005)	
(bighead croaker)	Slurry ice, $0.10-0.20$ mg/L, $-1.5$ °C, $2$ °C storage	TVC: 1.38-2.00. 9 days Retarded lipid oxidation and extended shelf life protein degradation, texture maintained		Chen et al. (2016a)	
(bighead carp)	5–7.60 mg/L, 10 °C, immersion for 1200 s	Improved myofibrillar protein content, gel strength		Zhang et al. (2016)	
(Japanese sea bass)	5 mg/L, 5 °C, 1800 s $+$ flaked-ice, 2 °C storage	TVC: 1.40. 12 days extended Low lipid oxidation, shelf life myofibrillar protein maintained		Lu et al. (2012)	
(striped red mullet)	0.30 mg/L, 5 $^{\circ}$ C, agitation for 600 s, $+$ MAP, 1 $^{\circ}$ C storage	TVC: 1.2-2.6. 18 days extended shelf life	Low lipid oxidation. Quality and freshness maintained	Bono and Badalucco (2012)	
(sea bream)	1 mg/L, 600 s, 4 °C, + tea polyphenol, 4 °C storage	TVC: 0.90-2.80. 6 days Delayed lipid oxidation, extended shelf life protein breakdown.		Feng et al. (2012)	
(sea bream)	Slurry ice, 0.20 mg/L, 700 mV, 7200 s, 0 °C storage	TAMC, psychrotrophic and lipolytic bacteria: 0.30–0.56	Texture maintained Low biochemical activity, retarded lipid hydrolysis and oxidation	Alvarez et al. (2009)	
(salmon)	1.0–1.5 mg/L, spray for 60 s,	APC: 0.28-1.05;	Slight lipid oxidation,	Crowe et al. (2012)	
(turbot)	4°C storage. Slurry ice, 0.20 mg/L, –1.5°C, 2°C storage	L innocua: 0.45-1.17 Aerobes: 0.97; psychrotrophs: 1.51; Proteolytic bacteria: 0.96. 7 days extended	colour maintained Retarded lipid hydrolysis, oxidation	Aubourg et al. (2009)	
(rainbow trout)	1 mg/L, immersion for 5400 s, + vacuum packaging, 4 °C storage	shelf life H <sub>2</sub> S-producing bacteria, LAB, EBC, <i>B. thermosphacta</i> , <i>Pseudomonas</i> spp.: 1.0–1.2. 6	Delayed lipid oxidation	Nerantzaki et al. (2005)	
Molluscs (oyster)	9 mg/L, 600 s, 4 °C storage	days extended shelf life APC: 2.0. 15 days extended shelf life	Retarded lipid oxidation and protein denaturation	Chen et al. (2014)	

Table 2. Continued.

		Res			
Food Type	OFW Parameters	Log reduction	Quality Attributes	References	
(shucked mussel)	$0.005\text{mg/L},\ 120\text{s},\ +\ 5\text{g/L}$ chitosan, $600\text{s},\ 5^\circ\text{C}$ storage 1 mg/L, immersion for 5400 s, $4^\circ\text{C}$ storage	APC: > 1.0. 12 days extended shelf life APC: 0.7-2.1; EBC: 0.5-1.5 Pseudomonas spp.: 0.5-1.1 H <sub>2</sub> S bacteria: 1.1-2.5 B. thermosphacta: 0.3-1.4 3 days extended shelf life	Reduced lipid oxidation, freshness maintained Acceptable sensory qualities, retarded lipid oxidation	Rong et al. (2010) Manousaridis et al. (2005)	

Note: TAMC: Total aerobic mesophilic count; EBC: Enterobacteriaceae; LAB: Lactic acid bacteria; CC: Chitosan coating (lactic, acetic, levulinic acids); CF: Cryogenic freezing (Liquid nitrogen at -75 °C).

Dissolved organic matters as found in meat and seafood products can easily be converted to halogenated disinfection by-products, generally in the classes of trihalomethanes (THMs), haloacetic acids, haloacetonitriles (HANs), and haloketones (HKs) (Gackowska et al. 2016). Very few studies exist on the uptake of chlorine by meat and seafood products sanitized by chlorine-containing disinfectants, especially EFW. Still, Loan et al. (2015) investigated the chlorination of tyrosine as a result of hypochlorite washing in fish fillets and observed that 3-chlorotyrosine could be a potential marker for chlorine disinfectant detection in fish fillets. Results showed that 0.85 - 2.81% tyrosine in European plaice fillets were converted to 3-chlorotyrosine, depending on soaking time and concentration of hypochlorite acid. As much as 5.58 and 4.68 ppm of 3-chlorotyrosine, respectively, was formed on European plaice and gilthead seabream after treatment for 30 min at 2000 mg/l. Formation of 3-chlorotyrosine was also monitored at concentration levels recommended by CODEX and the results were below the limit of detection. However, 1.02 and 1.10 ppm of 3-chlorotyrosine were detected, respectively, at 31 and 62 mg/l OCl concentrations. The results advocates for the use of EFW and other chlorine-containing disinfectants within the recommended levels.

#### Ozonized functionalized water

# Antimicrobial efficacy and application to meat safety and quality

The effectiveness of OFW against the populations of spoilage and pathogenic microorganisms associated with meat and its related quality attributes have been demonstrated in a few studies (Table 2). Microbial reduction in the range of 0.85 - 3.6 log CFU/g and 0.57-2.1 log CFU/g, respectively, for pathogenic and spoilage microorganisms have been achieved without adverse effect on the quality. Rahman, Wang, and Oh (2013) reported the efficiency of OFW against the population of yeast and mould, L. monocytogenes, and E. coli on pork loins at an ozone concentration of 5 mg/L. Although OFW exhibited a low bactericidal effect on yeast and mould and the number of TVC (0.57 - 0.78 log CFU/g reduction), treatment resulted in a significant 1.08 log CFU/g reduction of L. monocytogenes and E. coli on pork. Similar results were obtained by Novak and Yuan (2003) for achieving 1.28 log CFU/g reduction of Clostridium perfringens (C. perfringens) and reduced viability  $(D_{60} = 7.64 \,\mathrm{min})$ , when compared with untreated samples of London, broil beef ( $D_{60} = 13.84 \,\mathrm{min}$ ) at  $3 \,\mathrm{mg/L}$  concentration and longer washing time. Ultrastructural analysis showed that treated samples had fewer collagen, exposing bacteria cells hidden in crevices (Figure 2b).

In contrast, Botta et al. (2018) reported the limited effectiveness of homogeneous spraying of all sides of beef steaks with 6 mg/L OFW for 90 s. Treatment had no effect on the reduction of the microbial population but slightly affected the intra-species distribution of Pseudomonas fragi and its oligotypes. Likewise, Kalchayanand et al. (2008) observed that a 6s high-pressure water wash at 1000 kPa, followed by OFW spray at 172 kPa for 20 s could only reduce E. coli O157:H7 on bovine heads by 0.39 log CFU/cm<sup>2</sup>. This limited effectiveness could be attributed to the application method and short exposure time, as sanitizer applications have been reported to be more effective during immersion applications with prolonged exposure time (Ratana-Arporn and Jommark 2014).

Furthermore, the antimicrobial effect of OFW at a higher ozone concentration (95 mg/L) has also been reported (Castillo et al. 2003). Pressurized OFW at 552 kPa significantly reduced the populations of E. coli and S. Typhimurium (1.9 - 3.6 log CFU/cm<sup>2</sup>) irrespective of the surface region of the beef carcass when compared with the initial microbial load. However, a lesser reduction was obtained from the inside round region of the beef carcass, while no significant difference was observed when compared with water spray at 2758 kPa. In another study, Bosilevac et al. (2005) reported 3.4 and 2.1 log CFU/100 cm<sup>2</sup> reduction in the population of EBC and the number of APC, respectively, on beef hides, in addition to a 65% prevalence reduction of E. coli at 2 mg/L ozone concentration, and they also observed that the efficiency of OFW could be increased by dislodging bacteria and removal of the competing organic matters in the meat matrix, which was achieved by applying OFW at a pressure of 4,800 kPa as the high pressure used in part attributed to the increased microbial reduction.

Favourable results have also been reported when OFW is combined with other sanitizers. When combined with AEW, 0.68 mg/L concentration of OFW could significantly improve the inactivation rate of E. coli K12 on boneless chevon, yielding 0.86 log CFU/ml reduction, compared with 0.52 log CFU/ml obtained with individual application of OFW (Degala et al. 2016). Also, Pohlman et al. (2002) noted that



the combined application of OFW and cetylpyridinium chloride on beef trimmings prior to grinding could effectively reduce bacterial counts regardless of the nature of the microorganism. The combined treatment was more sensitive for coliforms with 1.88 log CFU/g reduction and less sensitive for E. coli with 1.68 log CFU/g reduction. Combined treatment also resulted in a brighter colour of beef, less oxymyoglobin and a decrease in off-flavour. It can be argued that the destruction of bacteria cells is attributed to lysis and cellular permeability changes as a result of oxidation.

# Antimicrobial efficacy and application to seafood safety and quality

The treatment of seafood products with OFW has been extensively studied with promising results as observed in Table 2. Okpala (2014, 2015) obtained 1.0 log CFU/g reduction in the number of APC on shrimp with shelf life extension of up to 2 days and delayed lipid oxidation using OFW at a low concentration of 0.42 mg/L. Chawla, Bell, and Janes (2007) examined the effect of OFW at concentrations of 1 -3 mg/L on shrimp and observed that increasing the concentration of OFW and soaking of shrimps in OFW, rather than spraying applications produced greater log reductions of spoilage bacteria. The number of APC and population of Pseudomonas fluorescens on shrimps was reduced by 1 - 3 and 2.0 - 2.9 log CFU/g, respectively, in addition to inhibition of lipid oxidation. de Mendonca Silva and Goncalves (2017) obtained a 4.13 log CFU/g reduction in the number of total aerobic mesophilic count (TAMC) and observed slight lipid oxidation in tilapia fish at OFW concentration of 1.5 mg/L. Comparatively, a similar reduction in the number of APC in addition to 15 days extended shelf life and retarded protein denaturation was obtained at a higher concentration level of 9 mg/L (Chen et al. 2014). Together, results indicate that the antimicrobial efficacy of ozone is not dependent on concentration alone, but also the dynamics of the microbial population.

In a most recent study, Goncalves and Santos (2019) reported that for shrimp treated with a low concentration (1 mg/L) of OFW under MAP, an extension of shelf life for 15 days, reduction of 0.61 – 1.73 log CFU/g in mesophilic and psychrotrophic counts with low melanosis index and improvement in freshness were achieved. Similar results were also reported for H<sub>2</sub>S-producing bacteria, Brochothrix thermosphacta, Pseudomonas spp. and the numbers of TVC and APC on striped red mullet, sea bream, rainbow trout, and oyster when OFW at low concentrations, ranging from 0.005 – 1 mg/L was combined with MAP, tea polyphenol, vacuum packaging and chitosan (Nerantzaki et al. 2005; Rong et al. 2010; Bono and Badalucco 2012; Feng et al. 2012). These studies confirm that OFW combined with other antimicrobial approaches is capable of better maintenance of storage quality and post-mortem shelf-life extension compared with the individual application. To reduce the physical damage suffered by highly perishable seafood items and improve protection against the action of oxygen, OFW is also used in the form of slurry and flaked ice so as to achieve faster chilling rate and rapid heat removal (Campos et al. 2005; Alvarez et al. 2009; Aubourg et al. 2009; Chen et al.

2016a; Lu et al. 2012; Bono et al. 2017). Besides decrease in microbial proliferation, longer period of shelf life extension and enhanced fish sensory quality (Figure 2c-d), these studies, on one hand, confirm the antimicrobial efficacy of OFW at low concentrations (0.10 - 0.30 mg/L) and on the other hand makes a case for the use of functionalized water in a binary mixture of water and ice maintained at super-chilling temperatures. Super-chilling temperature is believed to expedite the mechanism of ozone decomposition to the inhibition phase, which is responsible for activating the scavenging activity of bicarbonate ions towards oxidative destruction of the cells of microorganisms (Bono et al. 2017).

# Challenges and safety issues

The use of O<sub>3</sub> as an antimicrobial agent in food processing in the aqueous or gaseous phase is approved by regulating authorities (USFDA 2001). The USA Occupational Safety and Health Administration (OSHA) guidelines for workplace suggests 0.3 and 0.1 ppm for short term (15 min) and long term (8 h) exposures, respectively, while levels of 5 ppm and above are regarded as dangerous to health by the USA National Institute for Occupational Safety and Health (NIOSH) (CDC 2014; Pandiselvam et al. 2017). O<sub>3</sub> is highly corrosive and should be properly handled, as the sensitivity to certain allergens, bronchitis and asthma may be increased, although partial tolerance may be developed with continual exposures. The use of OFW at  $O_3$  concentration levels regarded as detrimental to health should be discouraged, especially at the industrial scale. Low concentration OFW can be applied as a hurdle technology with other techniques to improve its effectiveness.

Furthermore, the antimicrobial effect of OFW varies with the form of OFW applications, microbial contaminants, additives in food, contact time, temperature, and type of food surface. The effect is further reduced due to self-depletion, resulting from reactions with various organic matters in foods (Novak and Yuan 2003; Nerantzaki et al. 2005; Goncalves and Santos 2019). Limited studies exist on the reaction of  $O_3$  with organic matter during meat and seafood processing with OFW. Korany et al. (2018) reported that organic matter countered a biocidal efficacy of L. monocytogenes strains and biofilm. The oxidation of bromide ion to hypobromous acid/ hypobromite ion is also possible, depending on the water characteristics (Jarvis, Parsons, and Smith 2007).

However, Manousaridis et al. (2005) argued that it was difficult to predict the reaction of O<sub>3</sub> with the organic matter due to its high reactivity. De Mendonca Silva (2017) also observed that increasing the contact time between OFW and samples led to a decline in efficacy, attributed to subsequent dissociation of O<sub>3</sub> to O<sub>2</sub>. They also reported the highest bacterial reduction between the first five minutes, suggesting that the effect of OFW was greater within the first few minutes of exposure.

In addition, excessive usage or long time exposure of OFW may encourage discolouration, surface oxidation, and undesirable odour development (Manousaridis et al. 2005). Novak and Yuan (2003) noted that the use of OFW at concentrations of > 3 mg/L could be challenging, as discolouration, and lipid



and protein oxidation could occur, and they also observed that at OFW concentration of 3 mg/L, polysaccharides, fibres and collagen were removed from broil beef surface.

#### Plasma functionalized water

# Antimicrobial efficacy and application to meat safety and quality

Table 3 highlights the published data on PFW applications in meat and seafood quality and safety. Recently, Zhao et al. (2020) observed that fresh beef sprayed with PFW maintained significantly low pH and lipid oxidation and improved textural properties, in addition to 3.1 log CFU/g reduction of microbial colonies and 6 days of extended shelf life. A significant 1.05 log CFU/g reduction of Pseudomonas deceptionensis (P. deceptionensis) CM2 on chicken breast, after immersion in PFW for 12 min was similarly reported by Kang et al. (2019). Chicken breast also exhibited significantly low pH, enhanced brightness, and caused insignificant changes in textural properties. Synergistic effect of reactive species in PFW-mediated treatment is perceived to disrupt the outer and cytoplasmic membrane of P. deceptionensis CM2, leading to the severe leakage of intracellular components. The potential of PFW as a thawing media for beef has been explored (Xinyu et al. 2020). Compared with microwave and SAEW thawing, PFW caused 0.83-1.76 log inactivation in fungi, yeast and bacteria population, and retarded protein and lipid oxidation. Thawing is regarded as an uneven and slow process that can enable cross-contamination of products, thus the use of PFW is a novel and encouraging approach.

In another study, Qian et al. (2019) enhanced the antimicrobial efficacy of PFW by exposing low levels of lactic acid in water to plasma jet, suggesting that plasma functionalized lactic acid (PFL) was more stable, with added antimicrobial species for beef decontamination, achieving a range of 1.24 to 3.52 log CFU/g reduction of S. Enteritidis spot-inoculated on beef slices. The PFL had no apparent negative effects on the quality parameters of beef.

Hurdle technology including PFW and US has also been investigated. Royintarat et al. (2020) soaked chicken meat in PFW and sonicated for 60 min, and observed that ultrasonic cavitation caused membrane disruption of bacteria, which aided PFW reactivity. They achieved 0.83 and 1.33 log CFU/ ml inactivation of S. aureus and E. coli K12, respectively as compared with 0.33 and 0.46 log CFU/ml obtained from the individual application of PFW.

Yong et al. (2018) explored the prospect of PFW as an alternative to synthetic sodium nitrite during meat products manufacture and observed that an alkaline solution exposed to 120 min plasma treatment led to an increase in the redness in loin ham with low bacteria and residual nitrite content when compared with brine solutions produced from

Table 3. Effects of plasma functionalized water on meat and seafood safety and quality.

	Plasma Type /		Results		
Food Type	Exposure Time (min)	PFW Parameters	Log reduction	Quality Attributes	References
Meat and Meat Products					
Fresh cut beef	Microplasma jet, 8 kHz, 10 kV/30 min	pH: 2.5, NO <sub>2</sub> <sup>-</sup> : 2.6 μmol/ L, NO <sub>3</sub> <sup>-</sup> : 90.4 mg/L, O <sub>3</sub> : 0.11 mmol/L, H <sub>2</sub> O <sub>2</sub> : 116.2 mg/L	Total bacteria: 3.01. 4–6 days extended shelf life	Improved texture, low pH, TVB-N and lipid oxidation.	Zhao et al. (2020)
Frozen beef	Atmospheric pressure plasma jet, 600 W/1 min	J	TVC, yeast and fungi: 0.83–1.76 log	Moisture gain, lipid and protein oxidation retarded, pale colour, reduced hardness	Xinyu et al. (2020)
Beef	Plasma jet, 19.2 kV, 0.46 W, 0.02 mA/0 - 1.6 min	pH: 2.69, H <sub>2</sub> O <sub>2</sub> : 0.62 μM, NO <sub>3</sub> : 500 μM, NO <sub>2</sub> : 1990 μM/20 min	S. Enteritidis: 1.24–3.52	Mild lipid oxidation, maintained colour and protein content	Qian et al. (2019)
Chicken skin, breast	Argon underwater plasma jet, 5 mm, 1.5 kHz, 6.8 kV/6.5 min	pH: 5.5, ORP: 380 mV, EC: 8.7 $\mu$ S/cm, H <sub>2</sub> O <sub>2</sub> : 2.8 ppm; + US (40 Hz, 220 W), 40 °C/60 min	S. aureus: 0.30-0.86 E. coli K12: 0.46-1.33	Maintained hardness, protein and lipids, distinct colour change	Royintarat et al. (2020)
Chicken breast	Gliding arc discharge plasma jet, 5 mm, 5 kV, 40 kHz, 750 W/1 min	3–12 min	P. deceptionensis: 1.05	Low pH, increased brightness, maintained textural characteristics	Kang et al. (2019)
Pork (loin ham)	DBD, 5 cm, 15 kHz/120 min	pH: 9.01, NO <sub>2</sub> : 782 ppm, NO <sub>3</sub> : 358 ppm	TAB:0.33. No genotoxicity	Increased redness, lower residual nitrite content	Yong et al. (2018)
(meat batter)	S-DBD, 3.14 - 200 W, 15 kHz/30 min	$NO_2$ : 46 ppm, $HNO_2$ : 45 ppm + 80 °C, 1800 s		Increased redness	Jung et al. (2015a)
(emulsion-type sausage)	S-DBD, 3.14 – 200 W, 15 kHz/120–240 min	pH: 9.01, NO <sub>2</sub> : 782 ppm, NO <sub>3</sub> : 358 ppm	TAB: low counts	No immune toxicity, lower residual nitrite, no change in colour and peroxide value	Jung et al. (2015b); Kim et al. (2016)
Seafood				F	
Crustaceans (shrimp)	DBD, 30 W, 5 mm distance/10	lce, pH: 3.04, ORP: 485 mV, EC: 427 μs/cm, H <sub>2</sub> O <sub>2</sub> : 2.15 mg/L, O <sub>3</sub> : 8.60 mg/L, NO <sub>3</sub> <sup>-</sup> : 78.2 mg/L	TVC: 2.1. 8 days extended shelf life	Delayed texture and protein degradation, slight lipid peroxidation	Liao et al. (2018)

Note: (S-)DBD: (Surface-) Dielectric barrier discharge; EC: Electrical conductivity.

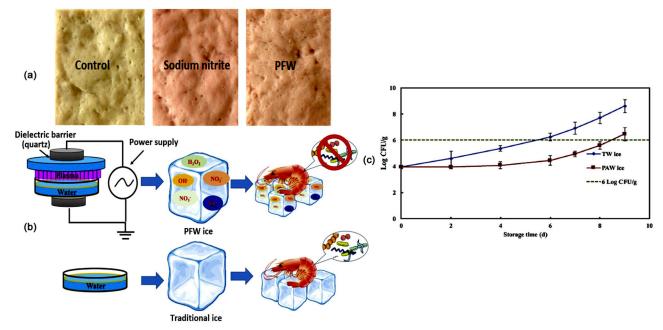


Figure 3. (a) Color of cooked meat batters treated with sodium nitrite and PFW (Jung et al. 2015a) (b) Experimental presentation for PFW ice preservation of shrimps (d) Changes in TVC of shrimp during storage with PFW ice (Liao et al. 2018).

sodium nitrite. Moreover, only 0.33 log CFU/g reduction in the population of TAB was achieved, and the weak antimicrobial effect of PFW was because the PFW was made from an alkaline solution. The use of the alkaline solution was to prevent the decrease of pH in PFW since the number of nitrite ions in PFW decreased with an increase in acidity. In the same vein, Jung et al. (2015a), Jung et al. (2015b) and Kim et al. (2016) confirmed that the concentration of nitrite generated during the interaction of plasma with liquid increased with increasing treatment time, which was responsible for the low counts of TAB in meat batter and emulsion-type sausage without mutagenicity and noticeable change in peroxide values while maintaining low residual nitrite. Nitrites are usually responsible for the distinctive colour of cured meat (Figure 3a), in addition to inhibiting the oxidation of lipids and the growth of pathogenic and spoilage microorganisms (Jung et al. 2015a). Microbial safety concerns, formulations, limited availability, added cost and increased incubation time associated with natural and conventional curing methods have encouraged alternative curing processes or sources of nitrite (Kim et al. 2016). Collectively, these studies demonstrate that PFW can be considered as a potential cost-effective substitute to vegetable formulations containing nitrite and synthetic nitrite for the natural process of meat curing without concerns of limited availability and microbial safety.

# Antimicrobial efficacy and application to seafood safety and auality

The concern for seafood quality, especially for longer storage periods and its high perishability, has driven research for the novel, safe and low-cost alternatives like PFW. However, only a few studies are available regarding the use of PFW on seafood (Table 3). Nevertheless, Liao et al. (2018) were

able to demonstrate that water functionalized by cold plasma for 10 min and frozen at -20 °C for 24 h (Figure 3b) significantly delayed microbial growth in shrimps, probably attributed to ample stable reactive species of nitrite, nitrate, O<sub>3</sub>, and H<sub>2</sub>O<sub>2</sub> in PFW. The quality of seafood items treated with PFW might effectively depend on treatment time, mode of utilization and washing time. In its ice form, PFW successfully caused a 2 log CFU/g reduction in the number TVC (Figure 3c) and mitigated melanosis progression in shrimp. Melanosis progression is often expressed in the form of faded colour, delay in texture and protein degradation, and reduction in the production of volatile basic nitrogen (Liao et al. 2018). However, the lack of research warrants further studies, especially the chemical properties before and after sample washing to establish the viability of PFW for seafood quality and safety.

## Challenges and safety issues

The nitrite from PFW is seen as a potential substitute for the use of nitrites from chemical compounds and some natural substances for meat product processing (Jung et al. 2015a). Nitrates are comparatively non-toxic, while nitrites decrease rapidly in meats over time and are converted to nitric oxide and N-nitroso compounds (NOCs) such as nitrosoamines and nitrosoamides with potential health effects (Govari and Pexara 2015). Although regulatory guidelines for PFW utilization is lacking, the USFDA approves 200 and 500 ppm for nitrite and nitrate, respectively, on the edible portion of finished products (USFDA, 2002). Residual nitrites in meats and seafood products from PFW processing are a challenging issue, as an appropriate amount needs to be maintained for antibotulism protection, while higher levels would favour the potential formation of carcinogenic NOCs (Govari and Pexara 2015; Yong et al. 2018).

Nevertheless, low residual nitrites have been associated with PFW applications in meat products while studies are yet to be conducted on seafood products. For example, PFW treatment for 2h with 782 and 358 ppm of nitrite and nitrates, respectively, led to significantly lower residual nitrite of 10.36 - 32.38 ppm in emulsion-type sausage and loin ham during storage for 28 days (Jung et al. 2015b; Yong et al. 2018). Meat products processed with PFW had lower residual nitrites concentration as compared with products treated with sodium nitrite and celery powder. Similarly, Jung et al. (2015a) observed a residual nitrite of 9.2 ppm from meat batter cured with PFW having 46 and 45 ppm of nitrite and nitrous acid, respectively, compared with 70 ppm obtained from curing with sodium nitrite. Thus, even though PFW contains high amounts of nitrites and nitrates, which could leave residues in meat and seafood products, the residual nitrites from the finished products fall within the recommended levels. Nevertheless, processing with PFW should still be encouraged at low amounts of nitrites and nitrates to ensure low residual nitrites in finished products.

Additionally, the presence of organic matter could decrease the efficiency of PFW, as affirmed in a recent study by Xiang et al. (2019). The addition of peptone or beef extract to PFW before mixing with bacterial suspension increased the pH value of PFW, which could significantly reduce NO<sub>2</sub> concentration and ORP but had no significant effect on H<sub>2</sub>O<sub>2</sub> concentration. S. aureus and E. coli O157:H7 were significantly reduced by 2.32 and 3.70 log CFU/ml, respectively in PFW without organic matter. Inactivation was reduced to a range of 1.19-1.41 log CFU/ml and 0.84-2.8 log CFU/ml, respectively for S. aureus and E. coli O157:H7 in the presence of beef extract, and 0.78-1.5 log CFU/ml and 1.46-2.33 log CFU/ml in the presence of peptone.

# **Conclusions and future perspective**

With increasing safety and sanitary challenges, functionalized water holds a potential significance for the meat and seafood industry since washing is a critical stage during processing and water can be exploited for large-scale applications. All the functionalized water exhibits strong antimicrobial effects against spoilage and pathogenic microbes, while PFW emerges with great potentials for meat and seafood safety and quality.

However, despite recommendations from regulating authorities, functionalized water is often misused since the active antimicrobial component is applied at a much higher concentration. Guidelines allow up to 10 mg/l Cl<sub>2</sub> for water in contact with products, but EFW has been used at the levels of up to 120 mg/l Cl<sub>2</sub>. The use of O<sub>3</sub> at levels of 5 ppm and above is regarded as dangerous to health, yet OFW is applied at levels as high as 95 ppm. The few studies available for PFW applications also show the use of large amounts of nitrites and nitrates, although the residual nitrites on finished products are within the recommended limits. A higher concentration of the active component can normally

translate to increased microbial safety of meat and seafood products, but the potential health hazard on operators and consumers requires proper attention.

Functionalized water application should be encouraged at low concentration of active components and especially within the limits of regulating guidelines. For instance, variants of EFW such as AiEW, SAEW and NEW with very low available chlorine and less acidic or near-neutral pH may be potential substitutes to AEW. In addition, the use of combination approaches has revealed synergy and proven more effective over individual application. To this end, hurdle technology involving functionalized water at very low concentrations of the active component is promising for better safety, quality and shelf-life extension of meat and seafood products. Furthermore, due to the possible disinfection by-products (DBPs) formation, a post rinsing treatment with deionized water could also be introduced after functionalized water applications to reduce amounts of active components capable of forming DBPs on meats and seafood products. There is also a need for coordinated research efforts to substantiate these facts and actively pursue the identification and quantification of DBPs in meat and seafood products subjected to functionalized water applications.

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