

Application of electrolysed oxidising water as a sanitiser to extend the shelf-life of seafood products: a review

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Abstract Electrolysed oxidising water (E.O. water) is produced by electrolysis of sodium chloride to yield primarily chlorine based oxidising products. At neutral pH this results in hypochlorous acid in the un-protonated form which has the greatest oxidising potential and ability to penetrate microbial cell walls to disrupt the cell membranes. E.O. water has been shown to be an effective method to reduce microbial contamination on food processing surfaces. The efficacy of E.O. water against pathogenic bacteria such as *Listeria monocytogenes*, *Escherichia coli* and *Vibrio parahaemolyticus* has also been extensively confirmed in growth studies of bacteria in culture where the sanitising agent can have direct contact with the bacteria. However it can only lower, but not eliminate, bacteria on processed seafoods. More research is required to understand and optimise the impacts of E.O. pre-treatment sanitation processes on subsequent microbial growth, shelf life, sensory and safety outcomes for packaged seafood products.

Keywords Electrolysed oxidising water · Shelf-life · Seafood products

Introduction

Food in general and seafood specifically contains high levels of nutrients such as protein, fats, and iron that support the growth of microorganisms resulting in spoilage and shortened shelf-life. The origins of the microbial contamination in fish are from the resident microbial flora associated with fish skin, intestinal content and gills, and from equipment surfaces and workers during processing (Cahill 1990; Gram and Huss 1996). There is a high possibility that contamination also comes from aerial sources in the food processing plants (Prendergast et al. 2004). The contamination initiates resulting microbiological spoilage of seafood products during storage causing off-odours, off-flavours, slime and discolouration (Gram and Huss 1996). Thus sanitisers are needed by the industry to control pre-packaging contamination by microorganisms to minimise the spoilage of products and maintain their safety and quality.

E.O. water, also known as electrolysed water, was first introduced into the market in Japan around 1980 as sanitation water stored in an automatic dispenser [Iseki et al. (2002) in Al-Haq et al. (2005)]. However, E.O. water was demonstrated earlier when Emswiler et al. (1976) found that chlorine water produced by the electrolysis of brine significantly reduced microbial numbers on beef carcasses. E.O. water has therefore been the subject of research because it has effective bactericidal activity with minimal residual impact on the products. Many researchers have studied this technology since 1980 (Al-Haq et al. 2005; Huang et al. 2008; Issa-Zacharia et al. 2010) with it gaining increasing acceptance as an alternative and effective sanitiser.

As a sanitising agent, hypochlorous acid (HOCl) in the E.O. water effectively inactivates both pathogenic and

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spoilage bacteria through oxidative damage to cell membranes (Wholey 2012). In vitro E.O. water studies on cell suspensions of bacteria and bacteria in biofilms have shown good results in their ability to kill food pathogens and spoilage organisms such as *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp., *Vibrio parahaemolyticus*, and *Pseudomonas* spp. (Kim et al. 2001; Ovissipour et al. 2015; Rahman et al. 2010). Research on the efficacy of E.O. water against those bacteria contaminating various food products has also shown excellent results in suppressing microbial contamination (Huang et al. 2006a; Kim and Hung 2012; Park et al. 2001; Pinto et al. 2015; Rahman et al. 2010; Shiroodi et al. 2016).

E.O. water in neutralised form has low corrosion potential. Corrosion on food-contact equipment surfaces can result in the formation of pits and cracks that become places for microorganisms to grow (Ayebah and Hung 2005). Ayebah and Hung (2005) showed that E.O. water did not cause a significant loss in weight for the stainless steel, aluminium and copper, but had significant impact on carbon steel. However, carbon steel is not commonly used in seafood processing plants due to its susceptibility to chloride corrosion.

Chlorine-based sanitisers have been the most commonly used system in the fish industry due to their low-cost and broad-spectrum bactericidal activity (ANZFA 2001; Eifert and Sanglay 2002) although peracetic acid formulations are also being applied. In practice chlorine-based and peracetic acid sanitisers come as highly concentrated chemical solutions which need to be diluted before use. This introduces health and safety issues in storage and application. E.O. water has gained uptake in food applications due to convenience for use in factories as well as its low corrosion potential. Additionally it improves safety for workers because it is manufactured in situ in a dilute form which lowers the risk of employee injuries from concentrated chemicals (Dickerson 2009). Commercial E.O. water generators that automatically produce hypochlorous acid sanitiser electrolytically from sodium chloride (NaCl) in aqueous solution have become widely available for use in the food industry.

The main issue for seafood producers is maintaining product quality and safety and having sufficient shelf-life for distribution and retail. E.O. water has been studied extensively for its bactericidal effects on seafood products (Huang et al. 2006b; Wang et al. 2014, 2015; Zhang et al. 2015). However, knowledge about the impact of E.O. water pre-treatment on the microbial ecology of stored seafood products is not well documented. This information is important as optimising the reduction in initial microbial contamination and influencing the type of microbial flora that grow on the seafood products influences the shelf-life as well as product safety and quality.

This review discusses the mechanisms and the applicability of E.O. water for extending the shelf-life of seafood products. The results of prior research are assessed and the impacts of properties such as oxidation–reduction potential (ORP), the changes in active chemical form of the chlorine as a function of pH and the efficacy of sanitation pre-treatments on the resultant quality of stored product are considered.

Principles of production and chemistry of electrolysed water

E.O. water is produced by passing a saline solution (NaCl dissolved in H₂O) into a cell chamber containing an anode and a cathode which are normally separated by a membrane or diaphragm (Al-Haq et al. 2005; Huang et al. 2008; Rahman et al. 2016; Walker et al. 2005) as shown in Fig. 1. There are three types of electrolysed water based on pH which is governed by where in the system the water is collected from (Al-Haq et al. 2005; Ayebah and Hung 2005). Firstly, acidic electrolysed water (AEW), also known as electrolysed oxidising water (E.O. water), is collected from the anode side. Secondly, alkaline electrolysed water (ALEW), also known as electrolysed reducing water (E.R. water), is collected from the cathode side (Al-Haq et al. 2005; Botanical Food Company Pty Ltd 2014). Neutralised electrolysed water (NEW) is made by mixing the E.O. and E.R. water to the required pH. However, different terms have been used to define E.O. water such as acidic/slightly acidic electrolysed water (AEW/

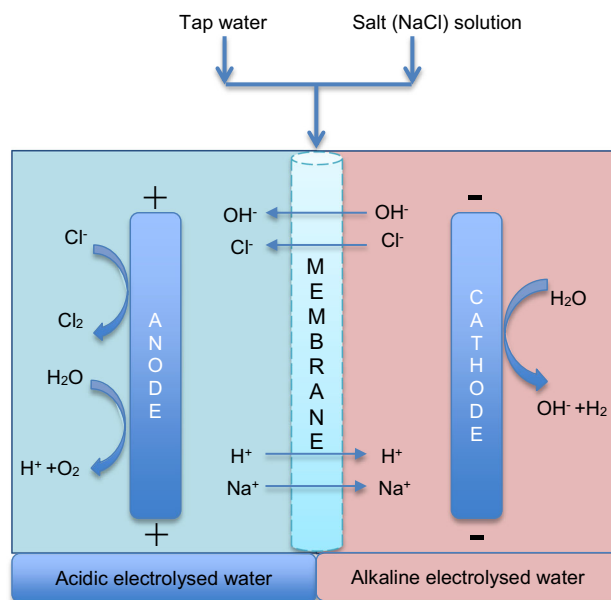


Fig. 1 Schematics of E.O. water generation and produced compounds

SAEW) (Xie et al. 2012; Zhang et al. 2015). It is also called low concentration electrolysed water (LcEW) (Rahman et al. 2010). In this paper E.O. water refers to neutralised or slightly acidic E.O. water unless otherwise stated.

Free chlorine is produced by the anode side, whereas the cathode side produces hydroxyl ions (OH^-). These migrate from the cathode to produce sodium hypochlorite (NaOCl) as shown in Fig. 2.

AEW usually has a low pH (2.3–2.7), high dissolved oxygen, high ORP (ORP, $>+1000$ mV) and an available chlorine content (ACC). E.O. water has strong bactericidal effects on pathogenic bacteria such as *L. monocytogenes*, *E. coli*, and *V. parahaemolyticus*, that occur in seafood products (Huang et al. 2006a; Ozer and Demirci 2006; Shiroodi et al. 2016; Xie et al. 2012; Yu et al. 2014). The bactericidal action is due to the high oxidation potential from chlorine and hypochlorous acid (HOCl) in the water that degrades the integrity of cell membranes. The bacterial cell membrane can then break due to osmotic forces (Fukuzaki 2006, Powitz 2010).

The cathode produces E.R water as about 10% v/v by-product of NEW or SAEW production. E.R water contains OH^- , hydrogen peroxide radical (H_2O_2^-) and hydroxyl radicals (HO^\cdot) (Al-Haq et al. 2005). As E.R water has an alkaline pH (10.0–11.5), high dissolved oxygen and low ORP (-800 to -900 mV), it can be used as an aid for cleaning equipment and surfaces. The alkaline pH removes dirt and grease due to fatty acid soap formation (Hsu 2005; Huang et al. 2006a). E.R water at high pH induces charges in organic molecules creating repulsion from surfaces of particles thus also causing dirt to be suspended in water (Powitz 2010).

Relation between ORP, pH, ACC and antimicrobial properties

The level of ORP in the solution is as an indicator of its ability to oxidise or reduce chemical compounds. Solutions with higher values of ORP have greater oxidising strength compared to weak ORP solutions (Jay et al. 2005). Jay et al. (2005) stated that the optimal ORP value for aerobic microorganisms growth is $+200$ to $+800$ mV, while

anaerobic microorganism prefer -30 to -550 mV to grow, and most facultative anaerobes optimally grow between $+200$ and -200 mV. For this reason E.O. water is an effective sanitiser against microorganisms because E.O. water has an ORP in range of $+900$ to >1000 mV depending on concentration and the species of chlorine present.

Len et al. (2002) proved that ORP, ACC and pH influence the efficacy of E.O. water. The ACC refers to the amount of active chlorine such as chlorine gas (Cl_2), HOCl and hypochlorite ions (^-OCl) in the E.O. water. Each of the parameters plays an important role in determining the ability of E.O. water to sanitise (Cao et al. 2009; Len et al. 2002; Phuvasate and Su 2010; Xie et al. 2012). pH determines the ratio of the chlorine compounds which vary in their reactive chemistry, and thus influences the efficacy of E.O. water (Cao et al. 2009; Rahman et al. 2016). A high pH will form predominately hypochlorite ions (^-OCl) while nearly neutral pH will produce HOCl and a low pH will form Cl_2 . (see Fig. 3) (Fukuzaki 2006; Rahman et al. 2016). A low pH can cause the loss of activity through off-gassing and degradation of free chlorine (Waters and Hung 2014).

Chlorine-based sanitisers that are widely used in the fish processing industry include calcium hypochlorite [$\text{Ca}(\text{ClO})_2$] (granular or powdered form) and sodium hypochlorite (NaClO) (liquid form) (Codex Alimentarius Commission 2000; FAO 2008). Calcium hypochlorite and sodium hypochlorite have hypochlorite ions (^-OCl) while the major active antimicrobial and sporicidal agent in neutralised E.O. water is HOCl . HOCl is the strongest oxidant due to its higher ORP and has 80 times more antimicrobial activity compared to ^-OCl (Cao et al. 2009). As a result HOCl is the main bactericidal agent in aqueous chlorine solutions (Codex Alimentarius Commission 2000; FAO 2008). Bacteria can be killed by high oxidising potentials that impact adenosine triphosphate (ATP) production through membrane rupture disturbing electron flow and creating disruption in cellular metabolic processes (Liao et al. 2007; McPherson 1993). HOCl is protonated allowing it to passively diffuse through the cell wall to penetrate the microbial lipid bilayer (Fig. 4) causing oxidative damage. In contrast ionized hypochlorite (^-OCl) can only react with the cell wall (Fukuzaki 2006; Huang et al. 2008).

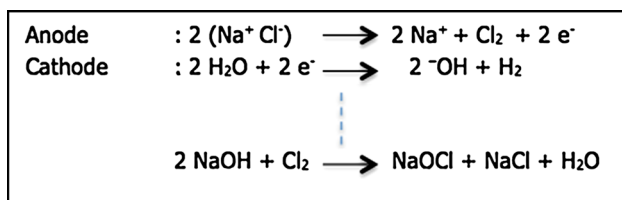


Fig. 2 The reaction producing sodium hypochlorite

Advantages and disadvantages of electrolysed water

E.O. water has been used widely in various sectors such as the food and medical industries due to being regarded as safe, environmentally friendly, low-cost and easily

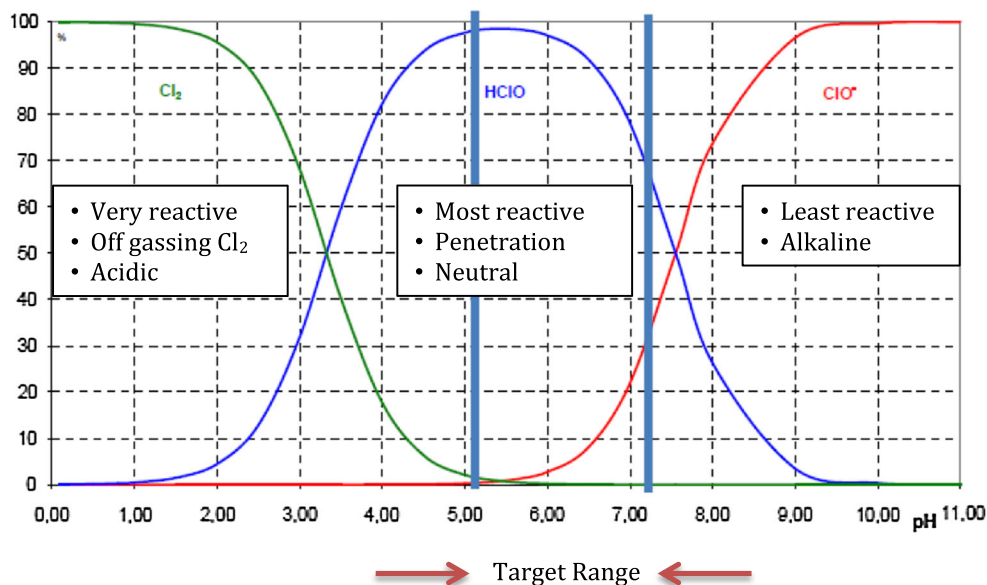
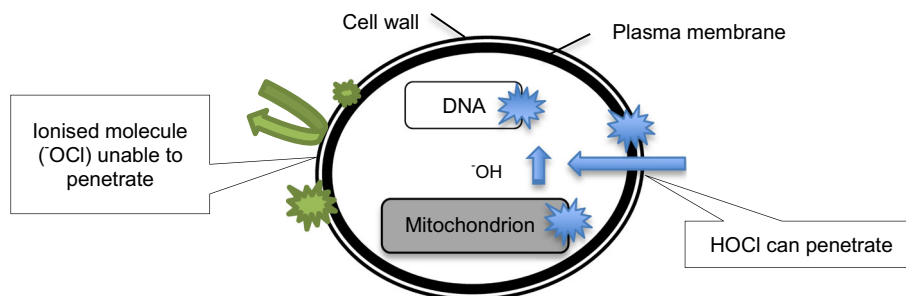


Fig. 3 The forms and activity of chlorine as a function of pH

Fig. 4 Hypochlorous action to break the microbial cell membrane



implementable (Al-Haq et al. 2005; Ayebah and Hung 2005; Huang et al. 2008; Powitz 2010; Walker et al. 2005). The ability for on-site production is the main advantage of E.O. water. This enhances the practicalities of seafood treatment under commercial operation. According to Rahman et al. (2016) in Japan the sushi industry has been able to reduce their expenses by millions of dollars by washing raw fish with E.O. water. This is one example of commercial application of E.O. water where any impact on seafood taste would be critical. Furthermore, as it can be produced on-site there is a reduced risk of chemical exposure to skin during transport, storage and handling of the chlorinated compounds (Rajeshwar and Ibanez 1997).

E.O. water, as applied to food, has shown no adverse effects to humans or the environment (Al-Haq et al. 2005). It is not corrosive to the skin and mucous membranes, as it is produced at a low active concentration (Colangelo et al. 2015; Huang et al. 2008), and at a pH close to neutral where there is a low concentration of free chlorine [Venturini (2013) in Colangelo et al. (2015)]. E.O. water also becomes deactivated when it reacts with organic matter and can be diluted with tap water, or removed by reverse

osmosis (RO) water (Huang et al. 2008). Chlorine compounds can react with nitrogen groups in organic matter, which affects its bactericidal activity (Al-Holy and Rasco 2015). Chloroform is the main trihalomethane (THM) product which can result from a reaction between chlorine and organic compounds (Waters and Hung 2014). Trihalomethane is an environmental pollutant which is considered carcinogenic and has become a serious concern for researchers, industry and regulatory agencies as the consequence of production from the use of chlorine (Gómez-López et al. 2013). Chloroform and a halogenated carboxylic acid are formed by hydroxide ions which react with trihaloketone (Rook 1979). Although it can react with organic amines Gómez-López et al. (2013) provided evidence that under controlled conditions E.O. water (4.4 mg/l free chlorine, pH 6.5, ORP 814 mV) did not produce trihalomethane (THMs) during processing of baby spinach. In addition, E.O. water has low chemical impacts on the environment because it only uses low levels of salt solution with no other added chemical compounds (Kim et al. 2000).

Electrolysed water is also cost-effective (Al-Haq et al. 2005; Ayebah and Hung 2005; Walker et al. 2005). After capital costs for initial installation, the operating expenses for automated plants are only for maintenance, water, salt and electricity. It is possible to make a financial return on investment in 1 year compared to the cost of alternatives (Powitz 2010; Walker et al. 2005).

HOCl, produced by E.O. water generation has been approved by regulatory authorities for use as a sanitiser in the food industry. It also has been considered as an organic treatment. In 2015 the Botanical Food Company Pty Ltd submitted a petition to include HOCl produced by the electrochemical activation of sodium chloride and water to the USDA National Organic Program (Botanical Food Company Pty Ltd 2014). Technical information from the petition argued that HOCl does not exist without the presence of hypochlorite ions (OCl^-), therefore HOCl produced by the electrochemical activation of water and salt solution should be put alongside sodium and calcium hypochlorite as an approved organic status chlorine material on the USDA Organic list.

In seafood processing, chlorine-based compounds are mainly used as disinfectants/sanitiser before packaging and distribution. Its use on the edible portions of fish and shellfish is limited (FAO 2008). The recommendations allow up to 200 mg/l chlorine in water for washing of slaughtered fish pre-processing with an exposure time of up to 8 h if transport is needed (FAO 2008). However, there are diverse opinions and regulations across different countries on the application of E.O. water as a sanitising agent. European Union (EU) regulations do not allow chlorine-based compound in water on meat and fish products due to the potential for toxic residues from chlorine by products (Codex Alimentarius Commission 2000). Regardless, many companies internationally, including Enviro-lite[®], EAU Technologies Inc. and Viking Pure[™], have recognised the market for E.O. water and are producing E.O. water generators.

The use of E.O. water has some drawbacks. One of the concerns around using E.O. water in fish processing is the rapidity of the reaction of HOCl with organic and inorganic compounds. The reaction with organic compounds such as protein will reduce its effectiveness (Al-Holy and Rasco 2015). Furthermore, an electrophilic reaction of HOCl with inorganic substances activates aromatics, reduces sulphur and causes a reaction with amines (Deborde and von Gunten 2008). This could become a problem for the industry due to the production of a chlorine-like off odour. Another drawback is that HOCl is only stable for days within a certain range of pH values (Fig. 3). HOCl outside of this pH range results in the loss of free chlorine (OCl^- , HOCl, Cl_2 and chloramine compounds) (Waters and Hung 2014).

The inactivation of spoilage microbes in seafood products

Spoilage is a heterogeneous process as it involves numerous bacterial communities (Chaillou et al. 2015). The spoilage process in fish starts with autolytic degradation followed by microbial activity producing off odours and flavours (Gram and Huss 1996). Seafood products deteriorate rapidly through microbial growth. Even though many microorganisms occur in seafood post-harvest only specific spoilage organisms (SSO) are responsible for the degradation and limitation of shelf life (Gram and Huss 1996). Bacteria such as *Pseudomonas* spp., *Acinetobacter*, *Moraxella*, *Flavobacterium*, *Shewanella*, *Alcaligenes*, *Vibrio* and coliform are the main spoilage bacteria reported in fish, since these bacteria dominate in temperate water fish (Gram and Huss 1996; Ray and Bhunia 2008). To maintain the quality and freshness of products, and to improve shelf life, growth of the SSO must be controlled.

One of the existing mechanisms for control of microbial growth in the seafood industry is modified atmosphere packaging (MAP). MAP is a preservation technique in seafood processing that is known to prolong the shelf-life of products by inhibiting microbial growth. Carbon dioxide (CO_2) and nitrogen (N_2) are the most common gases used in MAP for seafood products (Sivertsvik et al. 2002). CO_2 in MAP has bacteriostatic and fungistatic properties and inhibits bacterial growth (Sivertsvik et al. 2002). However, MAP only suppresses growth and therefore it is essential that products have a low level of microorganisms before packaging. This is usually achieved through the use of cleaning processing and sanitiser.

Many types of bacteria may contaminate the seafood products during processing. The spoilage bacteria found in fish packed both in aerobic and in modified atmospheres are well documented. In fish packed in modified atmosphere, *Photobacterium* and lactic acid bacteria are reported to be the primary bacteria that are responsible for spoilage. In aerobically packed fish the main spoilage bacteria come from the genera *Pseudomonas* and *Shewanella* (Dalgaard et al. 1993; Emborg et al. 2002; Gram and Huss 1996). Furthermore, water-borne bacteria that belong to the genera *Flavobacterium*, *Chryseobacterium* and *Photobacterium* are the core community of seafood bacteria (Chaillou et al. 2015). In their study of food microbiomes, Chaillou et al. (2015) also stated that there is a noticeable difference in the seafood-specific cluster between the salmon-fillet specific bacteria which has less bacteria assigned to the genera *Shewanella*, *Phychrobacter* and *Arthrobacter* than the cod-fillet-specific cluster. The core microbial communities that exist in chilled seafood products are from the very specific group of psychrotrophic

bacteria that come from water reservoirs. In addition, Mørretrø et al. (2016) identified that specific spoilage organisms during pre-rigor processing of salmon are from the genera *Pseudomonas*, *Shewanella* and *Photobacterium*. Knowledge of the bacterial types that occur in packaged seafood products allows directed testing of sanitation and control methods to inactivate the inoculum of targeted microorganisms.

Studies by Powell and Tamplin (2012) and Milne and Powell (2014) observed no *Photobacterium* spp. including the specific spoilage organisms *Photobacterium phosphoreum*, in Atlantic salmon (*Salmo salar*) fillets from Tasmania packed in a modified atmosphere. Powell and Tamplin (2012) used culture-based and DNA-based techniques to study microbial communities in Atlantic salmon fillet and reported that, after 15 days, *Shewanella* spp. dominated the community. Milne and Powell (2014) used psychrotropic and mesophilic plate counts and molecular techniques to study the limited microbial growth in fresh chilled Atlantic salmon under a modified atmosphere packaging protocol (96%CO₂, 5:1 product gas ratio and stored for 38 days in less than 1 °C).

The main factors that influence shelf-life of MAP products are initial microbial load, temperature, gas mixture and the ratio of MAP gas to product (G/P). In contrast E.O. water is much more effective in reducing spoilage bacteria on seafood although all prior reports (Table 1) use acidified E.O. water (AEW) of low (<2.7) pH where dissolved chlorine will predominate in concentration over hypochlorous acid. Research on the efficacy of E.O. water against spoilage bacteria such as histamine-producing bacteria (HPB) (*Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Morganella morganii* and *Proteus hauseri*) on Atlantic salmon and yellow fin tuna skin, showed that soaking the fish skin in E.O. water (100 ppm chlorine for 120 min) reduced *E. aerogenes* and *M. morganii* populations by 1.3 and 2.2 log CFU/cm², respectively (Phuvasate and Su 2010). *E. cloacae*, *K. pneumoniae*, *P. hauseri* were not able to survive 120 min of soaking in E.O. water containing 50 ppm chlorine.

McCarthy and Burkhardt-III (2012) carried out research on the efficacy of E.O. water (50 ppm chlorine) against *M. morganii* biofilms on mahi-mahi surfaces and *L. monocytogenes* biofilms on salmon surfaces. Their results showed that E.O. water reduced the number of *M. morganii* and *L. monocytogenes* from 4.02 to 2.48 log₁₀ CFU/g and from 4.47 to 2.33 log₁₀ CFU/g, respectively in the same day that samples were inoculated with bacteria. However, the efficacy of the E.O. water was lost by the following day as the *M. morganii* and *L. monocytogenes* populations increased again. Organic compounds in the fish flesh could be the factor that reduced the efficacy of the E.O. water (Liu et al. 2006).

The inactivation of pathogenic bacteria in seafood products

Food safety is a particular area of concern in the production of seafood products after its quality. Novotny et al. (2004) listed 10 pathogenic bacteria that are associated with fish and fish products, namely, *V. parahaemolyticus*, *Vibrio cholerae*, *E. coli*, *Aeromonas* spp., *Salmonella* spp., *Staphylococcus aureus*, *L. monocytogenes*, *Clostridium botulinum*, *Clostridium perfringens* and *Campylobacter jejuni*. To avoid the presence of pathogenic bacteria in food, safe practises must be used during the production process. E.O. waters with different pH, ORP and free chlorine concentrations have been tested extensively over decades for ability to reduce microbial numbers, especially of pathogenic bacteria in seafood products (Huang et al. 2006a, b; Ozer and Demirci 2006; Phuvasate and Su 2010; Shiroodi et al. 2016; Xie et al. 2012) (Table 1).

E.O. water (pH 2.7, ORP 1.150 mV, free chlorine 60 ppm) was also used to inactivate 6 log₁₀ CFU/g *L. monocytogenes* in cold-smoke salmon fillets (Shiroodi et al. 2016). The result showed that salmon fillet immersed in E.O. water for 10 min at 40 °C in a pre-treatment stage, reduced the *L. monocytogenes* population by 2.9 log₁₀ - CFU/g. This research indicates that 10 min is the minimum time to inactivate *L. monocytogenes* populations in fish fillets using E.O. water. Even though it might be expected that a long treatment time may have significant impacts on sensory properties, the research indicated no significant changes in sensory and textural properties compare to control fillets.

Although the studies on E.O. water efficacy against pathogenic and aerobic bacteria are well documented, knowledge on the efficacy of E.O. water against specific spoilage organisms (SSO), particularly psychrotrophic and lactic acid-producing bacteria, and its impact on subsequent growth patterns in seafood products is insufficient to optimise its application.

Efficacy of E.O. ice

In seafood processing maintaining the cold-chain is vital. Seafood producers use ice to retain the quality and suppress microbial growth in the products during processing. E.O. water efficacy rapidly decreases when it reacts with organic matter (Al-Holy and Rasco 2015). To overcome this issue hypochlorous acid in ice (E.O. ice) can be used. Active E.O. water will be released slowly during melting allowing it to function as a sanitiser longer than simple E.O. water. E.O. ice could be thus be used to replace ordinary ice in

Table 1 Inactivation of microbial flora on seafood by electrolysed oxidising water

Material	Immersion condition	Indicator	Effectiveness	E.O. water property				Sources
				pH	ORP	Free chlorine (mg/l)	Temperature (°C)	
Salmon fillet	65 min	<i>Escherichia coli</i>	++	2.6	1150	90	35	Ozer and Demirci (2006)
Salmon fillet	67 min	<i>Listeria monocytogenes</i>	++	2.6	1150	90	35	
Whole Tilapia	5–10 min	<i>Escherichia coli</i>	++	2.5	1159	120	23	Huang et al. (2006a)
Whole Tilapia	1–10 min	<i>Vibro parahaemolyticus</i>	++	2.5	1159	120	23	
Tuna fillet	5 min	<i>Aerobic bacteria counts</i>	+	2.5	1105	50	3	Huang et al. (2006b)
Tuna fillet	5 min	<i>Aerobic bacteria counts</i>	+	2.2	1135	100	3	
Atlantic salmon skin	120 min	<i>Enterobacter aerogenes</i>	++	2.7	1160	50	Room temperature	Phuvasate and Su (2010)
Atlantic salmon skin	120 min	<i>Enterobacter cloacae</i>	++++	2.7	1160	50	Room temperature	
Atlantic salmon skin	120 min	<i>Klebsiella pneumoniae</i>	++++	2.7	1160	50	Room temperature	
Atlantic salmon skin	120 min	<i>Morganella morganii</i>	+	2.7	1160	50	Room temperature	
Atlantic salmon skin	120 min	<i>Proteus hauseri</i>	+++	2.7	1160	50	Room temperature	
Atlantic salmon skin	24 h on E.O. ice	<i>Enterobacter aerogenes</i>	++	2.5	1173	100	NA	
Atlantic salmon skin	24 h on E.O. ice	<i>Morganella morganii</i>	+++	2.5	1173	100	NA	
Yellowfin tuna skin	24 h on E.O. ice	<i>Enterobacter aerogenes</i>	+++	2.5	1173	100	NA	
Yellowfin tuna skin	24 h on E.O. ice	<i>Morganella morganii</i>	+++	2.5	1173	100	NA	
Trout skin on fillet	10 min	<i>Salmonella typhirium</i>	++	2.3	NA	38	22	Al-Holy and Rasco (2015)
Trout skin on fillet	10 min	<i>Listeria monocytogenes</i>	++	2.3	NA	38	22	
Whole Yellow croaker	25 min	<i>Aerobic bacteria counts</i>	++	2.6	1250	45.12	NA	Hu et al. (2015)
Salmon fillet	2 days	<i>Listeria monocytogenes</i>	+	2.8	1080	50	4	McCarthy and Burkhardt-III (2012)
Mahi-mahi fillet	2 days	<i>Morganella morganii</i>	+	2.8	1080	50	4	

++++, bacterial reduction being more than 4 log CFU/per unit; +++, between 2 and 4 CFU/per unit; ++, bacterial reduction being between 1 and 2 CFU/per unit; bacterial reduction being +, bacterial reduction being less than 1 log CFU/per unit. –, not measured. The indicators were inoculated on fish surface (skin)

seafood production to give ice a greater strength in inhibiting microbial growth during processing.

Feliciano et al. (2010) observed the efficacy of E.O. ice for inhibiting the microbial growth on whole tilapia, tilapia fillet and water during storage. This study used E.O. ice made from E.O. water containing 150 ppm chorine at pH 6.8 and frozen at -40°C . The result showed that E.O. ice has the ability to reduce the numbers of *E. coli* K12,

Listeria innocua and *Pseudomonas putida* on whole tilapia, tilapia fillets and the water during 72 h storage.

In addition, many studies have shown that E.O. ice helps seafood products to maintain quality. Research projects conducted on the effects of E.O. ice on seafood quality such as Pacific saury (Kim et al. 2006), shrimps (Lin et al. 2013; Wang et al. 2014, 2015; Zhang et al. 2015) investigated not only microbiological changes but also

physicochemical, enzymatic activities, sarcoplasmic proteins sensory properties and shelf-life.

Kim et al. (2006) investigated the effects of E.O. ice compared to tap water ice (TW-ice) on microbial growth, chemical and sensory properties of Pacific saury. They used E.O. ice with pH 5, ORP 866 mV and active chlorine at 47 ppm. The result revealed that the E.O. ice can inhibit microbial growth and delayed formation of volatile-based nitrogen (TVBN) and thiobarbituric acid reactive substances (TBARS). Sensory scores indicated that E.O. ice could prolong the shelf-life of Pacific saury 4–5 days longer compared to TW ice. Volatile amines such as TVBN and trimethylamine oxide (TMAO) are the characteristic molecules that produce fishy odour in seafood products. Inhibiting the formation of TVBN and TMAO with E.O. ice will improve the sensory quality of seafoods. Furthermore, E.O. ice with physicochemical properties pH 2.5, ORP 1124 mV, and ACC 26 ppm (Lin et al. 2013; Wang et al. 2014) significantly retarded the formation of TVBN and colour changes in shrimps during 6 days storage. The diversity of bacteria revealed by the polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) method reduced significantly in the shrimp that were stored with E.O. ice in both studies. Wang et al. (2015) used E.O. ice with pH 2.3, ORP 1153 mV, and ACC 44 ppm to improve the quality and safety of shrimp. They found that E.O. ice could inhibit enzymatic activities (cathepsin and polyphenol oxidase (PPO)) and did not cause any noticeable changes in sarcoplasmic protein profiles.

The effect of E.O. ice glazing on shelf-life of shrimp was investigated by Zhang et al. (2015) who combined E.O. ice glazing (pH of 6.5, ORP 530 mV, ACC 6.5 mg/l) with MAP (40%CO₂, 10%O₂, 50%N₂ and 30%CO₂, 20%O₂, 50%N₂) to extend the shelf-life of peeled frozen shrimp. The combination successfully inhibited relevant indicators of deterioration, such as microbial growth, values of TVBN, TMA, TBARS, texture and colour. However, this research did not provide any information about how long the shelf-life of the product could be extended with these methods. While the above findings have shown that E.O. ice is a promising technology to improve the quality of seafood products there are not many published studies of the effects of E.O. ice on seafood products especially under commercial conditions. Further studies in this area are needed to establish a stronger evidence-base.

Inactivation of microbes on food-contact surfaces

E.O. water is effective in sanitising equipment (Table 2). Processing equipment and utensils can cause cross contamination during processing. Sanitation and hygiene

practices must be maintained and monitored. There are many studies using E.O. water as a surface disinfectant. Venkitanarayanan et al. (1999) studied the efficacy of E.O. water as a disinfectant against *E. coli* O157:H7 and *L. monocytogenes* on plastic cutting boards. The results showed that soaking the boards in E.O. water at 23 °C for 20 min, 35 °C for 10 min, and 45 °C for 10 min significantly reduced *E. coli* O157:H7 and *L. monocytogenes* populations.

E.O. water can be an effective sanitiser on glass, steel, glazed and unglazed ceramic tiles and vitreous china (Park et al. 2002). The result from Park et al. (2002) showed that no viable cells of *E. aerogenes* and *S. aureus* were found on tested surfaces after immersion for 5 min with 50 rpm agitation. The agitation may provide E.O. water the opportunity to better contact and penetrate the bacteria cell surfaces.

E.O. water has also been studied for its affect against pathogenic bacteria such *L. monocytogenes* in biofilms and cell suspensions (Kim et al. 2001; Ovissipour et al. 2015; Rahman et al. 2010; Shiroodi et al. 2016) with promising results. The ability of E.O. water to inactivate a 5-strain mixture of *L. monocytogenes* biofilms on stainless steel surfaces (2 mm thickness with 7.5 by 11 cm) showed that E.O. water with pH 2.6, ORP 1160 mV and residual chlorine 56 mg/l inactivated the bacteria to a level of 1–5 CFU/coupon after 300 s of E.O. water treatment from initial inoculum of 10.1 log₁₀ CFU/ml (Kim et al. 2001). The 300 s treatment was able to inactivate the *L. monocytogenes* biofilm since E.O. water penetrated inside the bacterial clumps.

However, the information on the efficacy of E.O. water against survivor microbes on food contact surfaces in fish-processing plants is insufficient to optimise methods to minimise cross contamination during product processing. Recent research showed that among Gram-negative genera (*Listeria*, *Stenotrophonas*, *Brochothrix*, *Serratia*, *Acinenobacter*, *Rhodococcus* and *Chryseobacterium*) *Pseudomonas* spp. is the dominant survivor genera in the salmon-processing conveyor belts (Langsrud et al. 2015). The study also concluded that *L. monocytogenes* cannot be eliminated in microbial biofilms. Thus, sanitation procedures need to be improved, as current practical sanitation methods can leave high numbers of diverse survivor microbial flora.

Chlorine, in the form of hypochlorite, is currently the most common sanitiser in the seafood industry. However, compared to chlorine, soaking for 5 min in E.O. water was more effective in inactivating *L. monocytogenes* on stainless steel sheets, ceramic tiles and floor tile surfaces (Liu et al. 2006). These seafood processing surfaces were studied with and without crabmeat residue inoculated with *L. monocytogenes*. The results showed

Table 2 Inactivation of microbial on food contact surfaces by electrolysed oxidising water

Material	Immersion condition	Indicator	Effectiveness	E.O. water property				Sources
				pH	ORP (mV)	Free chlorine (mg/l)	Temperature (°C)	
Stainless steel containing seafood residue	5 s	<i>Listeria monocytogenes</i>	+++	2.5	1150	50	23	Liu et al. (2006)
Ceramic tile containing seafood residue	5 s	<i>Listeria monocytogenes</i>	+++	2.5	1150	50	23	
Floor tile containing seafood residue	5 s	<i>Listeria monocytogenes</i>	++	2.5	1150	50	23	
Plastic cutting board	10 min	<i>E. Coli</i> O157:H7	++++	2.5	1165	87	35	Venkitanarayanan et al. (1999)
Plastic cutting board	10 min	<i>Listeria monocytogenes</i>	++++	2.4	1156	66	35	
Stainless steel	5 min	<i>S. aerogenes</i>	+++	2.5	1181	53	23	Park et al. (2002)
Stainless steel	5 min with agitation at 50 rpm	<i>S. aerogenes</i>	++++	2.5	1181	53	23	
Stainless steel	5 min	<i>S. aureus</i>	++	2.5	1181	53	23	
Stainless steel	5 min with agitation at 50 rpm	<i>S. aureus</i>	++++	2.5	1181	53	23	
Glazed ceramic tiles	5 min	<i>Enterobacter aerogenes</i>	++++	2.7	1160	50	Room temperature	Phuvasate and Su (2010)
Glazed ceramic tiles	5 min	<i>Enterobacter cloacae</i>	++++	2.7	1160	50	Room temperature	
Glazed ceramic tiles	5 min	<i>Klebsiella pneumoniae</i>	++++	2.7	1160	50	Room temperature	
Glazed ceramic tiles	5 min	<i>Morganella morganii</i>	++++	2.7	1160	50	Room temperature	
Glazed ceramic tiles	5 min	<i>Proteus hauseri</i>	++++	2.7	1160	50	Room temperature	
Stainless steel	5 min	<i>Enterobacter aerogenes</i>	++++	2.7	1160	50	Room temperature	
Stainless steel	5 min	<i>Enterobacter cloacae</i>	++++	2.7	1160	50	Room temperature	
Stainless steel	5 min	<i>Klebsiella pneumoniae</i>	++++	2.7	1160	50	Room temperature	
Stainless steel	5 min	<i>Morganella morganii</i>	++++	2.7	1160	50	Room temperature	
Stainless steel	5 min	<i>Proteus hauseri</i>	++++	2.7	1160	50	Room temperature	
Conveyor belt coupon	5 min	<i>Listeria monocytogenes</i>	+++	2.8	1080	50	Room temperature	McCarthy and Burkhardt-III (2012)
Conveyor belt coupon	5 min	<i>Morganella morganii</i>	+++	2.8	1080	50	Room temperature	
Dirty fish retailer in fish market	10 min	Aerobic bacteria counts	++++	2.2	1145	200	23	Huang et al. (2006a)
Dirty fish retailer in fish market	10 min	Aerobic bacteria counts	++++	2.5	1120	100	23	

++++, bacterial reduction being more than 4 log CFU/per unit; +++, between 2 and 4 CFU/per unit; ++, bacterial reduction being between 1 and 2 CFU/per unit; bacterial reduction being +, bacterial reduction being less than 1 log CFU/per unit. –, not measured. The indicators were inoculated on surface of the materials

that the chlorine content and ORP had a positive impact on the bactericidal activity of E.O. water. However, further research is required to understand the mechanism behind this effect.

Combination with other treatments and new techniques of E.O. water application

In practise, multiple inhibitory steps are needed to optimise shelf-life. This principle is known as hurdle technology: combining several preservation technologies to extend the shelf-life of products. In the seafood industry, this usually involves combining MAP with other technologies since MAP is the most widely used mechanism for preserving fresh seafood products. There are many stages involved in processing seafood starting from pre-treatment followed by processing, packaging and ending with distribution of the products. To maintain freshness and the quality of products control of microbial growth is required. This needs continual synergy with other treatments during producing, packaging, distribution and display in the store until the products are consumed.

In the last decade, many studies combining E.O. water with other treatments have been carried out to optimise the efficacy of E.O. water and to evaluate the effects of treatments on subsequent product quality (Gomez-Lopez et al. 2015; Mahmoud et al. 2007; Xie et al. 2012; Zhou et al. 2011). For example, there has been study on a combination of E.O. water with 1% of essential oil (0.5% carvacrol + 0.5% thymol) in carp fillets. This study indicated that dipping the fillet into E.O. water and essential oil for 15 min did not affect the nutritional components of the carp fillets. It also suggested that the combination could be a good alternative to preserve fish fillets. In addition, the combination of E.O. water containing of 50 mg/l free chlorine and CO gas treatment for 48 h during refrigeration and frozen storage of tuna slices, was an effective treatment for maintaining quality and freshness of the products and in prolonging the shelf-life (Huang et al. 2006b).

Combinations of E.O. water with natural preservation agents may give good results. The combination of E.O. water and chitosan to preserve puffer fish (*Takifugu obscurus*) during storage, showed that the combination gave a better result compared to the combination of carboxymethyl chitosan and E.O. water for inhibiting microbial populations growth, degradation of myofibrils and maintaining the freshness of the product (Zhou et al. 2011). This study also concluded that the treatment with chitosan and E.O. water could extend the product shelf-life by up to 50% of its original shelf-life.

Conclusion

Extending the shelf-life of seafood products needs an integrated system from the initial stage of processing until display of the product in the supermarket. Lowering the initial bacterial load in the pre-treatment stage of seafood processing is crucial, but the next stages of production also influence the quality of products. E.O. water is a promising technology with considerable potential to prolong the shelf-life of fresh, frozen and live seafood products. Many research programs have proven that E.O. water and E.O. ice are effective sanitisers due to their bactericidal properties with no noticeable changes in product sensory qualities. However, the influence of E.O. water and E.O. ice on the microbial ecology of subsequent packaged product is not yet fully understood. Knowing the changes to the microbial ecology in the products will give a better solution to find improved methods for extending the shelf-life of seafood products.

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