

Review

Non-Thermal Methods for Ensuring the Microbiological Quality and Safety of Seafood

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Abstract: A literature search and systematic review were conducted to present and discuss the most recent research studies for the past twenty years on the application of non-thermal methods for ensuring the microbiological safety and quality of fish and seafood. This review presents the principles and reveals the potential benefits of high hydrostatic pressure processing (HHP), ultrasounds (US), non-thermal atmospheric plasma (NTAP), pulsed electric fields (PEF), and electrolyzed water (EW) as alternative methods to conventional heat treatments. Some of these methods have already been adopted by the seafood industry, while others show promising results in inactivating microbial contaminants or spoilage bacteria from solid or liquid seafood products without affecting the biochemical or sensory quality. The main applications and mechanisms of action for each emerging technology are being discussed. Each of these technologies has a specific mode of microbial inactivation and a specific range of use. Thus, their knowledge is important to design a practical application plan focusing on producing safer, qualitative seafood products with added value following today's consumers' needs.

Keywords: non-thermal methods; microbiological safety; sensory quality; fish; seafood; high hydrostatic pressure; ultrasounds; non-thermal atmospheric plasma; pulsed electric fields; electrolyzed water



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1. Introduction

Raw fish and seafood products belong to the most traded foodstuff worldwide (FAO, 2020) and are much appreciated because of their high nutritional value. In general, it is becoming clear that there is a parallel trend in world fish production and the human demand for seafood products. Over 150 million tons of fish were utilized in 2018 for human consumption, and in 2030, this is expected to increase [1], showing the fish industry production dynamic. However, raw fish may lose their quality rapidly due to various physicochemical changes, enzyme activity, and microbial growth [2,3]. Together, particular care must be given to avoid seafood contamination during post-harvesting from chemicals and various bacterial pathogens [3].

Thermal methods are generally performed in the seafood industry to prolong the product's shelf life and inactivate the spoilage and pathogenic microorganisms. However, inhibition of spoilage microorganisms is not always the main objective, but it is an unavoidable positive effect [4]. Traditional thermal processing techniques, such as pasteurization and sterilization applied by the seafood industry, may be very efficient in inactivating or inhibiting bacterial pathogens but usually result in undesirable nutritional, chemical/biochemical, and sensorial changes in foods. These changes reduce the consumer's acceptance of now seeking minimally processed products with improved safety, added value, and increased shelf life [5,6].

As an outcome, to ensure safety and maintain the quality of seafood, several non-thermal techniques have been adopted and are continuously developing over the last

years. High hydrostatic pressure processing (HHP), ultrasounds (US), non-thermal atmospheric plasma (NTAP), pulsed electric fields (PEF), and electrolyzed water (EW) are some methods showing the potential to be applied by the seafood industry. Research on these non-thermal methods has shown significant bactericidal activity with minimal organoleptic changes [7–12]. Among them, HHP is the best-established method in the food industry, with many HHP processed products being commercially available in Europe, Japan, and the United States [13]. Among them, EW is another processing method with good results and notable research progress, providing safe and minimally affected raw fish and seafood products [14], while it is approved for use in the Japanese seafood industry [15]. The purpose of this review is to highlight and summarize past and recent studies on non-thermal technologies (HHP, US, NTAP, PEF, and EW), focusing on their efficacy to inactivate bacteria and to produce healthier seafood products with retained physicochemical properties.

2. High Hydrostatic Pressure—HHP

2.1. General Description of HHP Technology

High pressure processing (HPP), which is also known as ultra-high pressure (UHP) or high hydrostatic pressure (HHP), is a non-thermal technique that has emerged in food production within recent decades, and since the 1990s, the use of this technique has been extended to various types of foodstuff, including fish and seafood [16]. The critical advantage of HHP is the homogenous and instantaneous transmission of pressure in the treated solid or liquid product from all sides, leading to pathogens inactivation and shelf-life extension, while the overall sensory quality of the processed product is hardly affected [17]. During HHP treatment, the product is exposed to a pressure ranging from 100 to 1000 MPa for a short period (up to some minutes) and processing temperatures at -20 to 121 °C [13,18]. During HP treatment, the already sealed food product in its final package with flexible plastic material is placed in a cylindrical pressure vessel capable of sustaining the required pressure. The industrial food processing equipment is around 500 L capacity, capable of operating at a pressure higher than 1000 MPa [19]. Subsequently, the product is fully submerged in the pressure-transmitting liquid medium. Different pressure-transmitting media can be used, either pure substances or mixtures containing castor oil, silicone oil, sodium benzoate, ethanol, or glycerol. Industrial HP treatment is currently a batch or semi-continuous process [13]. The selection of equipment depends on the kind of food product to be processed.

The primary mechanism described for the bacterial inactivation under pressurizing time is the alteration of the cell membranes due to their structural changes in protein and membrane phospholipids leading to increased permeability, making the microorganism more vulnerable under the presence of antimicrobial agents [20,21]. It is well established that HHP processing depends on Le Chatelier's principle, where pressure applied and pressure within the food should be equal [16]. Additionally, during pressurization, the food undergoes isostatic compression, and when pressure is released, food returns to its initial shape [22,23]. These consecutive changes affect the cells' morphology and disorganize the large molecules without affecting the small molecules such as vitamins and flavor components [24].

In recent years, HP's treatment efficacy on bacterial inactivation has been extensively studied [25–28]. The degree of inactivation depends on the type of microorganism and their growth phase, the pressure level, the process temperature, and time (PTM), including the food or lab medium's pH and composition. As a general practice, increasing treatment pressure, holding time, or temperature will usually increase the number of microorganisms inactivated [29]. Even though, Gram-negative bacteria are more sensitive to high pressure than Gram-positive bacteria, many differences in barotolerance can be found among various strains of the same species [30–32]. Bacterial spores are highly resistant, yet there are limitations in killing them with HHP, but Barbosa-Canovas and Juliano (2008) [33] showed that when pressurization is carried out at temperatures close to 100 °C, spore-forming pathogens can be inactivated. Pressure inactivation of common spoilage and vegetative microorganisms is markedly enhanced at temperatures of 50 to 70 °C and subzero temperatures [34,35]. It has been generally observed that HHP and subzero temperatures (where the water exist in liquid phase, while pressure is increased, exert synergistic effects [36,37]. At ultra-low temperatures, and more specifically at temperatures below −22 °C, when the pressure increases above 200 MPa, an ice phase transition from type I to type III and further to type V, occurs [38]. This rapid phase shift of ice crystal structure enhances bacteria inactivation by causing membrane damage [37,39,40].

There is a large variation in the pressure resistance of spoilage bacteria and foodborne pathogens, such as *L. monocytogenes* and *Vibrio* spp., which are likely the two most investigated species in terms of the application of HHP on fish and seafood. The growth phase of the cells also affects its resistance to HP treatment. Microbial cells show the highest resistance during the stationary phase due to the well-formed membrane structure, with some exceptions mostly associated with RNA polymerase proteins in the cell wall [41]. The food matrix and the medium's pH also affect the cellular resistance during HHP treatment. In literature, references suggest that common and spore-forming bacteria [42] show a higher resistance at neutral pH [41,43,44]. The selection of the pressure temperature and time operational conditions (PTM) depends on the target microorganism and the product's nature to be processed, resulting in a final product with added value.

Although the operating and equipment costs of HHP remain high, over the last decade, there is a growing tendency for investment in industrial HP units worldwide, as reported by Huang et al. (2017) [45]. Considering the above, it is apparent that continuing research to improve our understanding of HHP effects on fresh and ready-to-eat (RTE) food and seafood products are essential to follow the market's needs and offer safer pathogen-free foods with added value.

2.2. Microbiological Quality and Safety

There are numerous strategies for the control of pathogens in fish and fishery products. HHP is used in the seafood industry, while it appears to be effective against the most common pathogenic and spoilage bacteria found on seafood products. Microbiological safety has been reported to be assured for many fish and seafood products with higher added value, such as shrimp, salmon, trout, cod, and oysters, among others. Table 1 presents some publications about HHP's application on fish and seafood products, showing targeted microorganisms and pressurization parameters (time, pressure level, and temperature). However, HHP treatment alone is often insufficient for substantial reduction or even inactivation of some microorganisms and needs to combine with other hurdles [46].

Table 1. Studies on the application of high hydrostatic pressure (HHP) treatment on fish and seafood products.

Products	Treatment Conditions	Target Microorganisms	References
Sea bass (<i>Dicentrarchus labrax</i>) fillets	100, 250, and 400 MPa/0, 5, 15, and 30 min/6 °C	Mesophilic aerobic bacteria	[47]
Sea bass (<i>D. labrax</i>) fillets	600 MPa/5 min/25 °C	Total aerobic viable count, <i>Pseudomonas</i> spp., <i>Brochothrix thermosphacta</i> , yeasts and molds, <i>Enterobacteriaceae</i> spp., H ₂ S-producing bacteria, and <i>Lactobacilli</i>	[48]
European catfish (<i>Silurus glanis</i>) fillets	200, 400, and 600/1 and 5 min/room temperature	<i>L. monocytogenes</i> , <i>Escherichia coli</i> and Mesophilic aerobic counts	[49]
Mild smoked rainbow trout (<i>Oncorhynchus mykiss</i>) fillets	200, 400, and 600/1 and 5 min/room temperature	<i>L. monocytogenes</i> , <i>E. coli</i> , and Mesophilic aerobic counts	[49]
Smoked salmon (<i>Salmo salar</i>) minced	Pressurization at low temperature *: 207 MPa/23 and 60 min/−5 to 6 °C * Pressurization at sub-zero temperature *: 207 MPa/60 min/−29 °C Freezing followed by pressurization **: 207 MPa/23 min/−29 °C	<i>L. innocua</i>	[36]
Cold-smoked sardine (<i>Sardina pilchardus</i>)	300 MPa/15 min/20 °C	Total aerobic viable count, H ₂ S-producing bacteria, and Luminescent bacteria, <i>Enterobacteriaceae</i> spp.	[50]
Cold-smoked salmon (<i>S. salar</i>)	400, 500, 600, 700, 800, and 900 MPa/10, 20, 30, and 60 s/room temperature	Total viable psychrotrophic count and Lactic acid bacteria <i>L. innocua</i>	[51]
Cold-Smoked Dolphinfish (<i>Coryphaena hippurus</i>)	300 MPa/15 min/20 °C	Total aerobic viable count, H ₂ S-producing bacteria, Luminescent bacteria, <i>Enterobacteriaceae</i> spp. <i>L. monocytogenes</i>	[52]
Coho Salmon (<i>O. kisutch</i>)	135, 170, and 200 MPa/30 s	Total aerobic viable count, mesophilic and psychrophilic aerobic counts, <i>Pseudomonas</i> spp., H ₂ S-producing bacteria (mainly <i>Shewanella putrefaciens</i>)	[53]
Black tiger shrimp (<i>P. monodon</i>)	300–600 MPa/30–50 °C/0–15 min	<i>Staphylococcus aureus</i>	[54]
Black tiger shrimp (<i>Penaeus monodon</i>)	300, 400, 500, and 600 MPa, 3, 6, 9, 12, and 15 min/room temperature (27 °C)	Mesophilic aerobic bacteria and total viable psychrotrophic count <i>E. coli</i> and <i>S. aureus</i>	[55]
Frozen cooked pink shrimps (<i>Pandalus jordani</i>)	250 MPa/0.5, 1.5, 3, and 10 min/−30 °C	<i>L. monocytogenes</i>	[56]
Fresh, shucked raw lobster (<i>Homarus americanus</i>) tails	150 and 350 MPa/10 min/4 °C	Total bacterial count and Lactic acid bacteria	[57]
Oysters homogenates	200, 250, and 300 MPa/5 and 10 min/1.5, 5, and 20 °C	<i>Vibrio parahaemolyticus</i>	[58]

Table 1. Cont.

Products	Treatment Conditions	Target Microorganisms	References
Oysters (<i>Crassostrea virginica</i>)	250 MPa/5 min, 300 MPa/2 min, and 350 MPa/1 min/−2, 1, 5, 10, 20, 30, 40, and 45 °C 5-log reduction parameters: 250, 300, 350, 400, and 450 MPa/2 min/1, 20, 30, and 40 °C	<i>V. parahaemolyticus</i>	[31]
Whole Gold Band Oysters ***	250 to 400 MPa/1 to 3 min/Room temperature	Total aerobic bacterial counts, presumptive <i>Vibrio</i> spp. count (PV), and presumptive <i>V. vulnificus</i> count (PVv)	[59]
Cooked oysters (<i>C. gigas</i>)	250 MPa/2, 5, 8, and 10 min and 300 MPa/0, 2, 5, 8, and 10 min/4 °C	Total aerobic counts, total anaerobic counts, and Coliforms	[60]
Four types of shellfish:			
- Mussels (<i>Mytilus edulis</i>)	300, 400, 500, and 600 MPa, 2 min/20 °C	Total aerobic viable count, Psychrotrophic count, <i>Pseudomonas</i> spp., and Coliforms	[61]
- Dublin bay prawns (<i>Nephrops norvegicus</i>)			
- Scallops (<i>Pecten maximus</i>)			
- Oysters (<i>C. gigas</i>)			
Abalone (<i>Haliotis rufescens</i>)	500 MPa/8 min and 550 MPa/3 and 5 min/room temperature	Total aerobic viable count, mesophilic and psychrophilic aerobic counts, <i>Pseudomonas</i> spp., H ₂ S-producing bacteria (mainly <i>S. putrefaciens</i>)	[53]
Six species of fresh edible seaweeds:			
- Two Chlorophyta (<i>Codium fragile</i> and <i>Ulva lactuca</i>)	400 and 600 MPa/5 min/room temperature	Total viable counts and heterotrophic marine bacteria	[62]
- Three Ochrophyta (<i>Himantalia elongata</i> , <i>Laminaria Ochroleuca</i> , and <i>Undaria pinnatifida</i>)			
- One Rhodophyta (<i>Chondrus crispus</i>)			
Pacific oysters (<i>C. gigas</i>)	300 MPa/2 min/20 °C	Total viable counts and heterotrophic marine bacteria	[63]

"0 min" pressure holding time refers to pressurization and immediate depressurization. * Smoked salmon minced used as a sample after thawing at 20 °C for 15 min. The temperature of the sample was about 7.0 °C. ** Smoked salmon minced used frozen at −29 °C. *** A commercially available HP-treated product.

Microbial growth and activity are important indexes to determine the shelf life and safety of various fresh and lightly preserved RTE seafood [64]. Therefore, HHP treatment was adopted to extend these products' shelf life by reducing the total aerobic bacterial counts attributed to the products' spoilage [65,66]. Yagiz et al. (2007) [67] reported that HHP at 450 and 600 MPa reduced the total aerobic counts by 4 to 6-log CFU/g on rainbow trout and mahi-mahi fillets during cold storage at 4 °C. In another recent study, when salmon, cod, and mackerel fish were treated with 200 or 500 MPa for 2 min, in compliance with other studies [50,68,69], it was found that the shelf life of the fish extended during storage at 0.5 °C [70]. Teixeira et al. (2014) [47] illustrated microbial reduction in 2-log CFU/g caused by HP treatment at 400 MPa for 30 min on sea bass (*D. labrax*) fillets and, as reported, might result in extended shelf life. Additionally, Tsironi et al. (2019) [48] found that treating sea bass fillets at a higher pressure for less time (600 MPa; 5 min; 25 °C) led to 5-log CFU/g reduction of TVC during storage at 2 °C, and the shelf life of HP-treated sea bass was extended up to 2 months, compared to 11 days for the untreated control fillets.

As we mentioned earlier, the successful combination of HHP with other hurdles can lead to the successful inactivation of spoilage and pathogenic bacteria. The shelf life of high added value lobster tails increased after treatment with HHP (150 or 300 MPa, 10 min) and subsequent sous vide cooking at 65 °C, stored under refrigeration up to 28 days [57]. Perez-Won et al. (2020) [71] found another successful combination to increase the microbial shelf life of Coho Salmon (*O. kisutch*). They focused on applying HHP (150 MPa, 5 min) combined with CO₂ (50, 70, 100%) atmosphere and found that the salmon's shelf life increased in parallel with the elevated CO₂ levels. HP treatment and CO₂ had no synergistic effect. The increased shelf life attributed to the ability of CO₂ to inhibit aerobic bacterial growth [72], while Briones et al. (2010) [53] also found that HP treatment < 200 MPa cannot extend the shelf life of chilled stored Coho salmon. When cold-smoked salmon fillets were treated with HHP at 600 MPa for 120 s in combination with nisin (10 µg/g) it resulted in 3.99 log CFU/g reduction in *L. innocua*, without any apparent synergistic effect [73]. Ekonomou et al. (2020) [28] investigated the synergistic effect of HHP at 200 MPa (15 min) with liquid smoke extracts and freezing and resulted in a more than 5-log reduction in CFU/g of the inoculated *L. monocytogenes* strain on the surface of hot smoked trout fillet.

In seafood products, the effects of HP processing on the inactivation of foodborne microorganisms are better documented for pressure treatments above 0 °C. However, the application of HHP at subzero temperatures to inactivate bacterial pathogens in seafood has been carried out, showing encouraging results. Satisfactory bacterial reduction with pressures as low as 200 to 250 MPa have been obtained [36,56]. The effectiveness was attributed to the transition of ice structure I to III occurring at temperatures below −25 °C. Regarding subzero temperatures, combined treatments with HHP led to a high reduction in inoculated *L. monocytogenes* on the surface of smoked salmon up to 4.89 log CFU/g (200 MPa, −18 °C, pH 4.5) [74]. The authors concluded that the bacterial reduction observed was due to the synergistic effect of pressure, temperature, and pH.

2.3. Effects of HHP on the Quality of Fish and Seafood

The most common treatments use pressure levels between 100 and 600 MPa for a period ranging from a few seconds to 10–15 min to avoid the product's sensory quality degradation. In general, it seems that the physicochemical parameters are not significantly affected by the applied conditions of HHP immediately. Mengden et al. (2015) [49] studied the effects of HHP (200, 400, and 600 MPa) on the overall sensory appearance of mild smoked rainbow trout fillets (*O. mykiss*) and fresh European catfish fillets (*S. glanis*). They reported that the sensory appearance was dependent on the intensity of HHP in the catfish fillets, while the smoked trout was almost unaffected. On the aim of improving the shelf life of six edible seaweed species, using HHP at 400 or 600 MPa for 5 min, del Olmo et al. (2020) [75] documented that HHP affected the physicochemical properties, color, and texture characteristics of the seaweeds at an acceptable level, while López-Pérez et al. (2020) [76] reported high odor characteristics and acceptance scores of HHP-treated kombu

seaweed (*L. ochroleuca*) stored at 5 °C for 180 days. Several authors investigated the effect of HHP on sensory quality parameters of fresh fish [28,47,48,57,68,77–79], mollusks [80–83], crustaceans [57,84–86], and RTE seafood products [28,87–91].

HP processing can retain or even improve the texture of seafood products and can be used to develop new products inducing desirable changes [92]. Furthermore, when HP treatment at 200 MPa applied to frozen raw fish to assist thawing, the water-holding capacity of HP-thawed fish in some cases increased [93]. The same phenomenon has been reported by several authors and may be due to an increase in hydration capacity of proteins during pressurization treatment [94–96]. However, according to Schubring et al. 2003 [93] the texture of HP-thawed raw salmon, cod, haddock, and trout fillets changed, showing a favorable increase in hardness in agreement with the results observed by Yagiz et al. (2009) [97] on Atlantic salmon. In addition, to increase the consumer acceptance of HP-treated seafood, it is well known that the retention of color is imperative. Therefore, many authors investigated the effects of HP treatment on fish color and found that many factors affect the final color of the product such as muscle hydration capacity and denaturation of proteins [98,99]. Among them, changes in the major pigments of astaxanthin and canthaxanthin (found on salmon, shrimps, etc.) [51,100,101], as well as myoglobin and porphyrin found in red meat fish [99] affect the color and are highly depended on the PTM conditions of HHP. Investigation of HP processing for a short time of some seconds showed no significant effect on cold-smoked salmon's redness [51]. Additionally, HHP at subzero temperatures retained the color of frozen cooked pink shrimps (*P. jordani*), exhibiting the positive effect of HHP on quality retention [56].

Consequently, HHP represents a highly important innovative technology for the seafood industry. HP treatment is an environmentally friendly application that can achieve high log reduction comparable with heat pasteurization, while maintaining the food's desirable organoleptic characteristics. Even though HP treatment is considered effective and commercially used, there are still numerous opportunities and challenges for food scientists to overcome, because HP application depends on the food matrix and the PTM parameters used.

3. Ultrasound—US

3.1. General Description of US

Ultrasound (US) is another thriving non-thermal method with increased interest in the food industry. US is a minimal process technology that can be implemented in several types of foods to inactivate foodborne pathogens and spoilage microorganisms without affecting the desired quality attributes of the product [102]. Ultrasonic waves used in the food industry are divided into low-energy ultrasounds, with high frequencies in the range of MHz (>1000 kHz), and high-energy ultrasounds, with low frequencies from 20 to 100 kHz [103]. Another parameter that influences US performance is the intensity of the treatment, where the application of low- and high-energy ultrasounds are generally performed at intensities lower or higher than 1 W/cm², respectively. The US technique is safe for human hearing, while it cannot detect frequencies lower than 20 Hz or higher than 20 kHz [104]. Whatever type of commercial system is used to apply high ultrasound to foods, it will consist of three necessary parts: generator, transducer, and the reactor to which it is coupled [103]. Epigrammatically, typical ultrasonic systems are the ultrasonic bath, the ultrasonic probes, the parallel vibrating plates, and the radial vibrating systems [103] and the airborne acoustic ultrasound systems first developed by Gallego-Juárez et al. (1978) [105].

Low-energy ultrasounds are used as a powerful tool at the laboratory and industry level for non-invasive analysis and monitoring of several food materials to improve the qualitative characteristics of high-quality foods and ensure safety [106]. Low-energy ultrasound has also been used to evaluate in a non-destructive way many measuring factors such as the concentration, viscosity, and composition of raw and fermented meat products [107,108], fish and seafood [109–111], and poultry [112]. On the other hand, high-energy ultrasound can disrupt the physical systems, cause different chemical or biochemical reactions, and lead to mechanical effects on food properties [103]. Ultrasonication is also aimed at microbial inactivation as a pasteurization method for liquid foods and a decontamination method of solid products [102]. The central mechanism of US is to inactivate microorganisms previously described by many authors [113–115] and is mainly due to cavitation phenomena leading to the breakdown of cell walls, disruption, and thinning of cell membranes caused by the generated shockwaves after the bubble collapsing, DNA damage through free radicals' production, and local heating.

Over the past few years, US has been applied for large-scale applications in the food industry, leading to a strategic advantage in the various stages of processing [116]. The effects of high ultrasound as an alternative to thermal pasteurization of liquid foods and as a method to inactivate microorganisms from solid products such as meat, poultry, fish, and seafood have been extensively studied in the last years. High ultrasound as a single-use technology cannot achieve the 5-log reduction in compliance with the FDA (2004) [117] requirements and is mainly used with mild thermal treatments. Therefore, high ultrasound is combined with other technologies to achieve a more effective bacterial log reduction and its mechanical, chemical, or biochemical effects as shown analytically in Table 2. Currently, US is considered an emerging and promising applicable technology in the food processing industry [118].

Table 2. Studies on the application of HP treatment on fish and seafood products.

Products	Treatment Conditions	Combination of Methods	Mechanical Effects	Chemical/Biochemical Effects	References
Red seabream (<i>Pagrus major</i>) fillets	40 kHz, 200 W, 10 °C	Ultrasound and thawing at 0 °C under vacuum (UVT)	No free water changes and improved physicochemical properties of proteins, actin had better thermal stability	-	[119]
Cod (<i>Gadus morhua</i>) fillets	(1) 25 kHz, 29.4 W/kg, 113.7 W, 20 min, 14 °C (2) 25 kHz, 14.7 W/kg, 64.3 W, 20 min, 14 °C (3) 25 kHz, 2.9 W/kg, 15.3 W, 20 min, 14 °C	Ultrasound and hydration medium's pH (from 8.5 to 10.5)	2.9 W/kg: produced the highest increments in WG (18.6%), US+pH 8.5: 1-day shorter hydration time	US+pH 8.5: improved microbial quality	[120]
Salted cod (<i>G. morhua</i>)	21.9 kHz, 20.5 kW/m ³ , 90 W, 1.2 m/s, −10 °C, 0, 10, and 20 °C	Ultrasound and low-temperature air drying (US+AIR)	US+AIR: softer texture, higher rehydration capacity, and color dependent on the drying temperature Faster (15%) cooking time, while F value remained the same Increased mass transfer-dirt removal (cleaner crabs) Enhanced salt extraction (reduced salt content in meat) Allergenicity decreased with increasing treatment time	-	[121]
Brown crab (<i>Cancer pagurus</i>) whole cooked	900 W ultrasonic bath, 45 min, 75 °C	Ultrasound and heat treatment at 75 °C, in water containing or not 5.0% NaCl (<i>w/v</i>)	(tropomyosin reduced 76% after 20 min of US treatment) Total antioxidant capacity strengthened US: reduced the time needed for MFD by 2 h	F ₇₀ ^{7.5} = 2 min and greater microbial reduction	[122]
Shrimps (<i>Litopenaeus vannamei</i> , whiteleg)	20 kHz, 400 W, 0, 5, 10, 15, 20 min, room temperature	Ultrasound and freeze drying at −20 °C	US: improved the chewiness property and the rehydration capability without significant deformation	-	[123]
Sea cucumber (<i>Stichopus japonicus</i>)	25 kHz, 160, 240, and 320 W, 0, 15, 30, and 45 min, 24 °C	Ultrasound (US) * and microwave freeze drying (MFD)		-	[124]

* Ultrasounds were used as a pretreatment method prior to microwave freeze drying (MFD).

3.2. Microbiological Quality and Safety

At present, ultrasonication, in combination with other methods, is rarely used to inactivate the microorganisms or extend the shelf-life of fish and seafood products during storage. To the best of the authors' knowledge, only a few studies have been published describing the US's antimicrobial effects on fish and seafood products, which are of exclusive interest in this review.

In a recent investigation, Antunes-Rohling et al. (2021) [120] studied the effect of high-power ultrasound as a new method to improve thawed cod fillets' quality. During this investigation, they evaluated the hydration medium's pH controlled with NaOH (from pH 8.5 to 10.5) combined with US at 25 kHz and its impact on the fillets' microbiological quality at the end of the hydration process. The combination of US and hydration media of pH 8.5 and 9.5 allowed to limit or even to inactivate the total aerobic bacteria of cod fillets when pH was higher (10.5) after five (5) days. It is mentioned that the use of NaOH to control the pH of the hydration media can have a high bactericidal effect [125]. It can be implemented with the simultaneous use of US, due to increased contact among microorganisms and the chemical agent [120]. It is also common to use US in combination with traditional heating to increase the microbial inactivation on different meat products, such as fish and seafood. In the case of ready-to-eat whole brown crab (*C. pagurus*), Condón-Abanto et al. (2018) [122] assessed for the first time the application of US (up to 900 W) in a water bath containing or not 5% (*w/v*) NaCl together with heat at 75 °C simulating the industrial heat processing of RTE crustaceans' products. In compliance with other authors, reporting the synergistic effect of US treatment with heat [126], they found that ultrasound-assisted cooking showed significantly higher microbial load reduction in white and brown crab meat. Particular mention has to be made in the study of Pedrós-Garrido et al. (2017) [127]. They explored the exclusive effect of high-intensity US at 30 kHz in an ultrasonic bath for 5 to 45 min in several fish species. After the application of US, the total psychrophilic and mesophilic counts of oily fish species, such as salmon (*S. salar*) and mackerel (*Scomber scombrus*), reduced up to 1.5 and 1.1 log CFU/g, respectively. The ultrasonication effect in white fish species, namely cod (*G. morhua*) and hake (*Merluccius merluccius*), was minimal and not higher than 0.5 log CFU/g.

3.3. Effects of US on the Quality of Fish and Seafood

As mentioned before, US treatment can be used in combination with other methods or individually to improve the physical properties [121,123,124] and the sensory quality [122,128] of fish and seafood products. Alternatively, it can be used to evaluate the fat content's measurement in live carps as a non-invasive method as reported by Maas et al. (2019) [129]. They used ultrasound at 5 MHz to measure the backfat thickness of carp (*Cyprinus carpio*) for the first time to ensure product quality and to achieve high customer acceptance. Shrimps are commonly blamed for causing allergic reactions [130,131], and high-intensity ultrasounds have been proved that can be used as a treatment to influence the allergenicity showing positive effects with a 76% reduction in tropomyosin [123]. Regarding the effectivity of US to improve the physical properties of cod fillets, Antunes-Rohling et al. (2021) [120] proposed the use of US to improve hydration and reduce the processing times. Sun et al. (2019) [128] combined US at 60 or 80 W with chitosan nanocomposite water-retaining agent (WRA) to study their cryoprotective effect on crayfish during frozen storage at −18 °C. They resulted in significant differences in the soaking weight gain, thawing loss, water content, and water activity between the control and WRA treated groups of frozen crayfish. Finally, they reported that US at 60 W and WRA improved the overall quality of the frozen crayfish at −18 °C.

4. Non-Thermal Atmospheric Plasma—NTAP

4.1. General Description of NTAP Technology

In 1972, Frank-Kamenetskiĭ [132] reported that the generated plasma is the fourth and unique state of matter. Treatment of foods with generated plasma is a novel processing technology gaining more industrial interest in recent years [133]. Plasma treatment can be categorized in thermal and non-thermal atmospheric plasma (NTAP) based on the conditions in which it is generated. When the plasma is generated at ambient pressure and temperature, it is termed cold plasma (CP), atmospheric cold plasma (ACP), or non-thermal atmospheric plasma (NTAP), while higher power and pressures need to obtain thermal plasma [134]. To generate NTAP, any kind of energy (electrical, thermal, optical, radioactive, and electromagnetic radiation) can be used to ionize the gases, but mainly electric and electromagnetic fields are used [135,136]. Plasma has a neutral ionized gaseous form constituted by ions, free electrons, gas atoms, and molecules, as well as UV photons depending on the process parameters and the gas employed [133,136,137]. Plasma occurs after the gas subjection through an electric field created between two electrodes (cathode and anode) separated by a small distance of 1 cm [7,138]. The gases mainly used for plasma generation and that can also affect its properties are air, oxygen (O₂), nitrogen (N₂), carbon dioxide (CO₂), and noble gases, individually or in combinations for optimal results.

NTAP has proved to be an additional tool for the successfully decontamination of abiotic food surfaces such as packaging materials and various food and seafood products [136]. NTAP can even be used toward the decontamination of food in their final package, with a dielectric barrier discharge (DBD) equipment [139,140]. NTAP can be used to inactivate many types of common foodborne pathogens [141–144], spoilage microorganisms [145,146] including yeasts and fungi [147,148], spores [147,149], and viruses [150]. A parameter that impacts the efficiency of NTAP is its low penetration efficiency making the method able to inactivate microorganisms only on the surface of solid foods [151]. In association with the previous parameter, when a foodstuff has high microbial loads forming multiple layers of bacteria on the surface, it cannot be destroyed because the upper layers of cells protect the underling from the NTAP [133]. Vatansver et al. (2013) [151] proposed three mechanisms of action for the inactivation of microorganisms with the use of NTAP: (a) the direct disruption of the cell membrane or wall, pushing to leakage of cellular components, (b) the oxidative damage to membranes or intracellular components, such as proteins and carbohydrates, and (c) the cellular DNA damage. Instead of the microbial inactivation, NTAP can extend the shelf life of foods, but more research is necessary before its adoption by the industry [76].

4.2. Microbiological Quality and Safety

NTAP has been used widely over the past ten years for the decontamination of food. However, there is a limited number of references investigating this technology in fish and seafood. Plasma technology offers high microbial inactivation in short processing times. Indeed, Albertos et al. (2017) [152] reported that cold atmospheric plasma generated by a dielectric barrier discharge (DBD) at 70 and 80 kV voltage resulted in significant microbial reduction after only 1-, 3-, and 5-min treatment. Particularly, the spoilage bacteria of fresh mackerel (*S. scombrus*) such as total aerobic psychrotrophic bacteria, *Pseudomonas* spp., and lactic acid bacteria (LAB) were reduced during storage. LAB were found to be the dominant microflora. Another study for microbial decontamination with promising results was presented by Choi et al. (2017) [153]. They used a corona discharge plasma jet (CDPJ) to induce microbial contaminants on the dried squid shreds' surface. CDPJ was generated using 20 kV pulsed DC voltage and at a 58 kHz frequency, applied for 0–3 min. The results of this study indicated that squid microbiota reduction was time-dependent, where total aerobic bacteria reduced more than 2-log, followed by marine bacteria, which were decreased by 1.6 log CFU/g. The NTAP treatment was evaluated as a possible method to improve the shelf-life of Asian sea bass slices (ASBS) stored at 4 °C treated with in-bag dielectric barrier discharge cold plasma (IB-DBD-CP) under two different gas combinations.

They reported that the shelf life of ASBS treated with IB-DBD-CP, packaged under gas A and B prolonged to 12 and 15 days, respectively [154].

It is encouraging that there is an increasing list of studies over the past five years demonstrating the antimicrobial properties of NTAP on fish [152,155–160] and RTE seafood products [136,153,161,162], which can lead to the adoption of this method from the seafood industry. NTAP has a high potential to be commercially used within many fields of the seafood industry. Kulawik and Kumar Tiwari (2019) [163] recommend the NTAP method for processing dried fish and seafood that have been better documented than the fresh fish.

4.3. Effects of NTAP on the Quality of Fish and Seafood

Regarding the overall quality of fresh fish and seafood products, the available information in the literature is variable. Color is one of the most important parameters, because it has been associated with the freshness of seafood products and their overall quality. When some authors investigated the color values of dried squid shreds [135] and fresh mackerel fillets [152], they found no significant changes in redness (a^*) and yellowness (b^*) after DBD and CDPJ plasma treatment, respectively. On the other hand, Olatunde et al. (2020) [154] observed that the lightness (L^*) and yellowness (b^*) of Asian sea bass slices (ASBS) packaged under different gas combinations during storage at 4 °C increased after treatment with in-bag dielectric barrier discharge CP (IB-DBD-CP). Different L^* and b^* values also observed for the ASBS samples treated in the bag with gas A (argon and oxygen, ratio 10:90) and gas B (carbon dioxide, argon, and oxygen, ratio 60:30:10) composition. Regardless of the gas composition used, all the plasma-treated samples had lower redness (a^*) value. The increase in L^* and b^* values for the samples in the presence of gas A attributed mainly to the existence of RNOS and ozone (O_3), which can cause myoglobin denaturation [164], while lipid and pigment oxidation occurs under CO_2 enriched environment (gas B). Furthermore, Singh and Benjakul (2020) [146] described that high voltage cold atmospheric plasma (HV-CAP) in combination with a gas mixture of argon and oxygen (90:10) for 5 min and chitooligosaccharide (COS) from squid (*Loligo formosana*) pen increased the sensory acceptability of ASBS at 4 °C. It was found that the appearance, color, and texture of the fish slices were higher than the acceptable limit of likeness (>5.0 score), in agreement with Olatunde et al. 2019b. However, the lipid oxidation and total volatile base (TVB) content resulted in unpleasant odor and off-flavor.

Fish and seafood processing with NTAP treatment can be more effective in microbial inactivation yet can be used without negatively affecting their quality parameters. To achieve this, all the aspects of the treatment and the type of food have to be considered carefully.

5. Pulsed Electric Fields—PEF

5.1. General Description of PEF Technology

Pulsed electric fields (PEF) is an emerging, cost-effective, and environmentally friendly method gaining importance, but further investigation is needed to allow the successful industrial application [115]. PEF is a non-thermal treatment that can be used as a decontamination technique and has minimal effects on the texture and other sensory properties of the food [165,166]. Additionally, PEF can improve the functional properties of seafood products, often containing high, unhealthy concentrations of additives such as NaCl through the alteration of the protein structure that leads to greater additive diffusion [167]. In PEF treatment, food is placed between two electrodes where a pulsed high-voltage electric field passes through for a short time period, ranging from 1–100 ns to several ms, and the electric field intensities ranging from 0.1 to 80 kV/cm [5]. The PEF process also produces ohmic heating due to the application of high-voltage pulses without causing any effects in the product [167,168]. The intensity and the treatment parameters of PEF can cause structural changes and reversible or irreversible permeabilization of the microbial cell membrane [169,170]. This phenomenon is called electroporation and results in altering membrane permeability through the formation of pores allowing the exchange of membrane components with the cell environment [171]. Regarding the sensory qualities of the

products, which are equally important with food safety, Toepfl et al. (2014) [172] suggested that PEF treatment possibly has less effect on meat and fish products than traditional thermal methods, and further investigation is necessary.

5.2. Microbiological Quality and Safety

In contrast with NTAP, which is mainly used on solid food [7], PEF is preferred to treat liquid food [173]. Still, it remains an effective method for the inactivation of various microorganisms such as foodborne pathogenic bacteria, commonly contaminating fish and seafood products [174]. The inadequate effect of PEF treatment on solid food matrices can be the main reason for the limited number of studies investigating the antimicrobial effect of PEF on fish and seafood products. To our knowledge, there are only two studies on fish and seafood products where the authors investigated the efficacy of PEF to decrease microbial growth.

In the first study, conducted in 2001 by Gudmundsson and Hafsteinsson [175], they found that the total bacteria counts of fresh lumpfish (*Cyclopterus lumpus*) roes reduced by 1 log₁₀ cycle when treated with PEF at 11 kV/cm and 12 pulses. However, the sequential exposure of the lumpfish roes at 13 kV/cm and 200 MPa doubled the bacterial reduction (2 log₁₀ cycles), exhibiting the potential impact of using HHP to increase the antimicrobial effect of PEF treatment in fresh seafood products in such low intensities. When PEF treatment is used alone, it needs to rise above 13 kV/cm to be effective against most microorganisms. In a recent study, Shiekh and Benjakul (2020) [176] tested the effect of PEF in the microbiological changes in Pacific white shrimp (*L. vannamei*), a very popular crustacean. The shrimps were stored at 4 °C for 10 days and were treated every two days with PEF at different densities (54–483 kJ/kg) and pulse numbers (200–600). Those included PEF-T1 (5 kV/cm, 200 pulses), PEF-T2 (10 kV/cm, 400 pulses), and PEF-T3 (15 kV/cm, 600 pulses) with the PEF specific energy of 54, 214, and 483 kJ/kg, respectively. The shrimps treated with PEF at the highest level (PEF-T3) (483 kJ/kg, 600 pulses) had the lowest bacterial load of 4.58 log CFU/g at the end of the storage period. Similarly, PEF-T3-treated shrimps had the lowest psychotropic bacterial counts after 10 days of storage at 4 °C. The authors concluded that PEF could be an effective method to inhibit the psychrophilic bacteria, which are the main cause of shrimp spoilage during refrigeration storage [177,178].

5.3. Effects of PEF on the Quality of Fish and Seafood

PEF, as a non-thermal method, can maintain the sensory properties and biochemical and nutritional value of treated foods [174,179]. Table 3 lists different studies investigating the application of PEF for the treatment of fish and seafood products. Kang et al. (2020) [12] investigated the physiochemical changes appearing in fresh, frozen-thawed, and supercooled yellowfin tuna fillets (*Thunnus albacares*) stored at 4.0 °C, −3.2 °C, and −18 °C (freezing) for 8 days, respectively. Supercooled tuna fillets assisted using PEF (3.5 V_{rms} at 20 kHz) and oscillating magnetic fields (OMF) (75 mT at 1 Hz). They concluded that PEF processing combined with supercooling could provide fresh tuna fillets with extended shelf life compared to conventional refrigeration. Similarly, Shiekh and Benjakul (2020) [178] documented higher sensory scores of PEF-treated shrimps at a higher electric field intensity (15 kV/cm, 600 pulses) than others after 10 days of storage at 4 °C.

In fish processing, PEF treatment is also applied to improve the extraction of various organic compounds from fish and seafood [180–183]. Franco et al. (2020) [184] recommended the use of PEF treatment for improved antioxidant extraction from fish residues (gills, bones, and heads) of two commercial species (sea bream and sea bass). Fish bones, together with gills, and heads are the main fish residues reaching about 20–75% of a fish's total weight [185]. These products are commonly rejected without any further process, resulting in environmental pollution and waste of potentially valuable resources. In this sense, He et al. (2014) [180] used PEF to extract chondroitin sulfate (CS) from fish bones and concluded that PEF extraction speed and CS content were much higher at 15–25 kV/cm.

Table 3. Studies on the application of pulsed electric fields (PEF) treatment on fish and seafood products.

Products	Treatment Conditions	Combination of Methods	Effects on Physicochemical Parameters	Effects on Sensory Characteristics	References
Asian sea bass skin	24 kV/cm, 72 ms, pulse width 0.1 ms, and pulse repetition times 20 ms	PEF and vacuum impregnation (VI) for 10, 15, 20, and 30 min by hydrolysis with porcine pancreas lipase (PPL)	PEF-VI-PPL: had lower monounsaturated and polyunsaturated fatty acid contents	PEF-VI-PPL: low fishy odor/flavor intensities of the resulting hydrolyzed collagen were observed, and overall reduced abundance of volatile compounds	[181]
<i>Arthrospira platensis</i>	38 kV/cm, pulse duration 232 μ s, frequency 158 Hz	PEF and heat at 35 °C	Extraction of phycobiliproteins was higher (0.15 mg/mL) and purer at lower treatment time (0.03 s compared to 5400 s)	NT	[186]
Fish wastes (fish bones)	5, 10, 15, 20, and 25 kV/cm, pulses 2, 4, 6, 8, 10, and 12	PEF and NaOH (1, 2, 3, 4, 5, and 6%), ratio of material to liquid (1:5, 1:10, 1:15, 1:20, and 1:25 g/mL)	PEF accelerated the extraction speed and improved the yield of chondroitin sulfate (CS) from fish bones CS purity was high, and the extract did not contain any other glycosaminoglycans PEF increased the antioxidant activity reaching values of 389.62 μ g Trolox/g	NT	[180]
Fish residues (gills, bones, and heads) from sea bass and sea bream	1.40 kV/cm, pulse duration 20 μ s, 100 pulses, frequency 10 Hz	Water extraction assisted by PEF	Water extraction assisted by PEF led to significant increases from heads, bones, and gills reaching 35.8, 68.6, and 33.8% for sea bream and 60.7, 71.8, and 22.1% for sea bass PEF: reduced lipid oxidation PEF+UAE: the highest lipid yield (30.34 g per 100g of solids) and higher content of PUFAs and carotenoids, such as astaxanthin, astaxanthin monoester, astaxanthin diester, canthaxanthin, and b-carotene	NT	[184]
Pacific white shrimp (<i>L. vannamei</i>)	4, 8, 12, and 16 kV/cm, 120, 160, 200, and 240 pulses	PEF and US-assisted extraction (UAE)		NT	[182]

Table 3. Cont.

Products	Treatment Conditions	Combination of Methods	Effects on Physicochemical Parameters	Effects on Sensory Characteristics	References
Freshwater mussels	10, 15, 20, 25, 30, and 35 kV/cm, 2, 4, 6, 8, 10, and 12 pulses	PEF and enzymolysis (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h)	PEF showed higher extraction speed and up to 77.08% higher protein extraction at 20 kV/cm and 8 pulses and 2h enzymolysis	PEF: Off-odor of treated mussel samples reduced PEF-OMF-Supercooling: - no undesirable microstructural changes - no significant difference between the refrigerated and supercooled samples on drip loss - the K-value * (26.4%) indicated that supercooling preserved the fish fillets	[187]
Yellowfin tuna (<i>T. albacares</i>) fillets	3.5 V _{rms} at 20 kHz	PEF and oscillating magnetic fields (OMF), 75 mT at 1 Hz and supercooling at −3.2 °C	NT	PEF: slightly affected the firmness of the roes PEF (4–12 kV/cm): damaged roes were less than 8.0% with greater damage with higher pulse numbers PEF (0.35 kV/cm, 20–40 pulses): the muscle cells decreased in size, and gaping occurred with collagen leakage in the gap PEF (0.35 kV/cm, 20–40 pulses) + HHP (300 MPa): produced gaping without visible collagen leakage in the gap	[12]
Lumpfish (<i>C. lumpus</i>) roes	4, 8, 10, 12, 13, 15 kV/cm, 2, 4, 6, 10, 12 pulses	PEF at 13 and 15 kV with 5 and 10 pulses and HHP at 200	PEF (11 kV/cm and 12 pulses): 1 log ₁₀ TVC reduction PEF (13 kV/cm, 12 pulses) + HHP (200 MPa): 2 log ₁₀ reduction	PEF: slightly affected the firmness of the roes PEF (4–12 kV/cm): damaged roes were less than 8.0% with greater damage with higher pulse numbers PEF (0.35 kV/cm, 20–40 pulses): the muscle cells decreased in size, and gaping occurred with collagen leakage in the gap PEF (0.35 kV/cm, 20–40 pulses) + HHP (300 MPa): produced gaping without visible collagen leakage in the gap	[175]
Salmon	0.35 kV/cm, 60 pulses, pulse duration 2 ms	PEF and HHP at 200 or 300 MPa and vice versa	NT	PEF (0.35 kV/cm, 20–40 pulses): the muscle cells decreased in size, and gaping occurred with collagen leakage in the gap PEF (0.35 kV/cm, 20–40 pulses) + HHP (300 MPa): produced gaping without visible collagen leakage in the gap	[175]

* K-value represents the cutoff (spoilage) grade for tuna fillets is set 50% [188]; NT—variable was not included.

PEF treatment could be used as a pretreatment for maintaining the quality parameters of perishable seafood that undergoes changes. Still, it shows high aptitudes that can be widely used in the seafood industry as an eco-friendly and cost-efficient extraction method.

6. Electrolyzed Water—EW

6.1. General Description of EW Technology

Cross-contamination of food and seafood products remains a major problem for the food industry that needs to be solved. Although numerous effective techniques have been developed or improved in recent years, effective sanitizers are widely used for the inactivation of foodborne pathogens that commonly contaminate the food during the different processing stages or preparation of final products [189,190]. However, it is commonly accepted that common sanitizers used are becoming less effective against resistant microorganisms, may have high cost, and can produce chemical residues. Therefore, the food processors and food safety experts must use innovative, safer, and more effective sanitizers to surpass the problems mentioned above. Electrolyzed water (EW) is considered a new non-thermal and environmentally friendly food sanitizer [6,191].

Electrolyzed Water (EW) is produced by a process where electricity is used to make a chemical change called electrolysis. The electrolysis occurs in a specially designed chamber containing water with dissolved sodium chloride (NaCl). However, this process is not spontaneous, and electrical power from a generator passes through the submerged electrodes (separated by a membrane) in the solution [192]. In the EW generator, the voltage and current values are normally set at 9–10 V and 8–10 A, respectively [193]. During the process of electrolysis, NaCl is breaking apart into sodium metal (Na) and chlorine gas (Cl₂), while water (H₂O) is breaking apart using electrolysis into hydrogen (H₂) and oxygen (O₂) gasses. Analytically, the negatively charged ions of chloride (Cl⁻) and hydroxide (OH⁻) are losing their electrons (e⁻) through the anode of the EW generator. During this oxidation process, hypochlorous acid (HOCl), hypochlorite ion (-OCl), hydrochloric acid (HCl), oxygen gas (O₂), and chlorine gas (Cl₂) are generated. On the opposite, positively charged ions (Na⁺ and H⁺) gain electrons (e⁻) pushed out the cathode, and reduction takes place, resulting in the generation of sodium hydroxide (NaOH) and hydrogen gas (H₂). Inside the chamber, two types of EW are produced: (1) at the anode, acidic electrolyzed water (AEW) or electrolyzed oxidizing water (EOW) with pH value 2–3, oxidation-reduction potential (ORP) >1100 mV, and available chlorine concentration (ACC) of 10 to 90 ppm, and (2) at the cathode, basic electrolyzed oxidizing water (BEW) with pH value ranging from 10 to 13 and ORP of -800 to -900 mV is generated [15]. Meanwhile, other solutions of EW have been reported and used, such as neutral electrolyzed water (NEW) with a pH value of 7–8, and ORP equal to 750 to 900 mV, produced in a single cell chamber and the anodic solution is mixed with OH⁻ [193]. Slightly acidified electrolyzed water (SAEW) with a pH value of 5–6.5 and ORP of nearly 850 mV [194] is also produced in a single cell chamber by the electrolysis of HCl alone or in combination with NaCl. The most common types of EW currently used for their desirable effects are summarized in Table 4. The physicochemical effects of the generated EW may vary depending on the type and concentration of the solution, voltage and current value, water flow, and time of electrolysis [192].

Table 4. Types and physicochemical properties of electrolyzed water EW used on fish and seafood products.

Types of EW	pH	ORP (mV)	ACC (mg/L)	Dilute Solution	References
AEW	2.22	1137	Approx. 41	NaCl 0.1%	[195]
	2.30	>1100	38	KCl 0.1%	[196]
AEWice ¹	2.64	1124	26	NaCl 0.1%	[197]
AIEW ²	11.40	−795	NT	NaCl 12.0%	[198]
NEW	6.80	786	60	NT	[199]
SAEW	6.37	980	30	NaCl 0.5% and HCl 0.05%	[200]
SAEWice ¹	6.48	882	25	NaCl 0.2% and HCl 0.04%	[201]
WAEW ¹	3.55	950	10	NaCl 40 mg/L	[202]
ECAS4 ³	7.00	850	300	NaCl 0.4–0.5%	[203]

NT—variable was not included; ¹ after generation, the EW is poured to freeze at −18 to −20 °C; ² AIEW means alkaline EW; ³ WAEW means weak or weakly AEW.

EW shows high antimicrobial activity against the most common bacteria, spores, fungi, and viruses found on food and the general environment of food processing plants and has a minimal effect on the physicochemical and sensory quality of the food [204–209]. The antimicrobial activity and the mode of action of EW against bacteria are still not fully described. However, it is known that chlorine and reactive oxygen can disrupt the microbial cell membrane and cause oxidative damage to DNA [210]. It is not clear whether the antimicrobial efficacy of EW is due to chlorine compounds [211], oxidation-reduction potential (ORP) [193,212], pH [195,211], or the synergistic effect of these factors.

Other variants of EW are also being developed and applied, such as water functionalization by ozone [213] or plasma [214], showing great antimicrobial and sensorial results on foods. Overall, EW has various food industry applications and minimum health hazards, while the dangerous chlorine compounds produced can return to its original form after contact with organic matter [160,215]. EW could have a significant impact on the seafood industry, translating into health and economic benefits by reducing food spoilage bacteria.

6.2. Microbiological Quality and Safety

EW application offers great antimicrobial effect and can be used in association or not with other methods [197,216] and antimicrobial agents such as bacteriocins [217] to achieve high microbial reduction numbers and extend the shelf life of fish and seafood products. Linked with those mentioned above, American shad (*Alosa sapidissima*) can be stored at 4 °C for 25 days, coated with chitosan (CH) solution (2% *w/v*; pH 3.7) after washing with EOW (pH 2.4; ORP = 1185 mV; free chlorine, 70–80 ppm). The results revealed that EOW+CH was more effective than either treatment alone [218] (Xu et al., 2014). During refrigeration, the total microbial population of untreated samples surpassed the 7-log CFU/g on day 10. At the same time, EOW, CH, or EOW+CH-treated samples did not reach this value until day 15 or 20, respectively. The lower numbers of TVC bacteria reported may be due to the formation of hypochlorous acid (HOCl) and the low pH of the EW leading to the cells' outer membrane damage [219] and the ability of CH to disrupt the lipopolysaccharide layer of the outer membrane of Gram-negative microorganisms [220]. These findings can also be used to serve as comparisons with other studies showing that after treatment of fish with EW, the total microbial counts reduced and extended the shelf life [221–224].

EW is also known for its efficacy against foodborne pathogens commonly found on seafood or seafood processing surfaces [207–210]. Regarding this, Al-Qadiri et al. (2016) [202] investigated the effect of strong (SAEW) and weak (WEAW) electrolyzed water on *E. coli* O104:H4, *L. monocytogenes*, *A. hydrophila*, *V. parahaemolyticus*, and *Campylobacter jejuni* inoculated on the surface of live clam (*Venerupis philippinarum*) and mussel (*M. edulis*). This study revealed the potential to use EW with strong ORP and chlorine concentration of 10–20 mg/L to inhibit the activity of pathogenic bacteria from important aquaculture shellfish, which are known to be contaminated or even accumulate these microorganisms by filtering the environmental water [225,226]. EW studies on *E. coli* O157:H7 and

L. monocytogenes inoculated on raw salmon fillets also showed comparable results as the shellfish findings from this study, with Ozer and Demicri (2006) [198] reporting reductions ranging from 0.47 to 1.07 log CFU/g and 0.40 to 1.12 log CFU/g, respectively. The maximum decrease was with acidic EW at 35 °C, pH 2.6, ORP 1150 mV, and 90 ppm free chlorine. Ovissipour et al. (2018) [199] investigated the effect of different electrolyzed water solutions (AEW and NEW) in combination with mild thermal treatment at 50, 55, 60, or 65 °C for 2, 6, or 10 min to reduce *L. monocytogenes* on Atlantic salmon (*S. salar*). They observed that the NEW application for 10 min at 65 °C reduced the counts of *L. monocytogenes* by 5.60 log CFU/g. These results comply with other authors also presented a high reduction in *L. monocytogenes* population on the surface of RTE cold-smoked salmon fillets after the combined treatment of AEW (pH 2.7; ORP 1150 mV; free chlorine 60 ppm) and mild temperature at 40 °C for 10 min, reaching 2.85 log CFU/g [216]. The higher reductions observed in both studies while increasing the temperature may be due to the changes in the bacterial cell's physicochemical properties, which can lead to the faster entrance of the EW into the cell [204]. Besides, when inoculated shrimps with *V. parahaemolyticus* treated simultaneously with basic and acidic EW at 4, 30, and 50 °C, it was found that 5 min pretreatment of BEW followed by 1 min treatment of AEW at 50 °C had the greatest antimicrobial activity against the pathogen [227].

6.3. Effects of EW on the Quality of Fish and Seafood

The treatment of fish and seafood products with various types of EW has been extensively studied with great antimicrobial results, as observed above. Still, food safety has to be accompanied by quality and sensory retention. When Wang et al. (2014) [197] investigated the effect of acidic EW ice, a relatively new concept, on the quality of shrimp (*L. vannamei*) in dark condition, they reported that AEW ice could be a proper post-harvest treatment for preserving the quality of seafood in dark condition. The raw shrimp results showed that AEW ice, besides its bactericidal effect on TVC, inhibited the pH changes, the formation of total volatile basic nitrogen (TVB-N), and the activity of Polyphenol oxidase (PPO) after 6 days of storage. These results in agreement with Lin et al. (2013) [228] indicate that AEW ice can serve as an innovative method to retain the quality of shrimps. In addition, lower TVB-N, trimethylamine (TMA), and thiobarbituric acid reactive substance (TBARS) values also reported for raw puffer fish (*Takifugu obscurus*) stored at 4 °C after treatment with weakly acidic EW (WAEW) and modified atmosphere packaging (MAP) [229]. Overall, Li et al. (2020) [229] concluded that WAEW and MAP (60% CO₂; 5% O₂; 35% N₂) significantly extended the shelf life and maintained the better quality of the farmed puffer fish. Khazandi et al. (2017) [203] similarly reported increased shelf life of Southern Australian King George Whiting (KGW) and Tasmanian Atlantic Salmon (TAS) refrigerated (4 °C) fillets by 2 and 4 days, respectively, after treatment with pH neutral EW solution of 15 and 50%. Finally, numerous studies indicating the potential of using different types of EW on fish and seafood products to retain their main physicochemical [14,199,216,223,229–231] and sensory characteristics [198,205,215,218] playing an essential role in consumer acceptance.

7. Conclusions

Consumer demand for safe, high-quality seafood with added value has made the non-thermal emerging technologies a vital tool for the seafood industry to ensure food safety and quality. Historically, ice has been used for the preservation of perishable seafood from ancient times. However, nowadays, it is not a practical solution, because seafood is shipped worldwide and needs to retain its microbiological safety without adverse effects on the sensorial and nutritional values. On the other hand, thermal technologies can successfully reduce the microbial pathogens of interest, but high-temperature treatments can affect the quality parameters at a non-acceptable level for the consumer or the industry demands.

Non-thermal technologies such as HHP, US, PEF, NTAP, and EW reviewed in the current paper have shown to be capable of inactivating the most common foodborne pathogens found on fish and seafood products maintaining the organoleptic and sensory characteristics. There is an emphasis on applying HHP and PEF to pre-packed and liquid seafood products within the food industry to retain their high quality on appearance and sensory attributes. At the same time, most US, NTAP, and EW studies have focused on the effects of fish processing, showing minor quality degradation compared with conventional thermal treatments. Several advantages accompany all these: reduced energy consumption, reduced product/waste products, reduced costs, improved product quality, and shorter production times, resulting in increased production. The synergistic effects among non-thermal and other technologies offer new potentials in the seafood industry, aiming to enhance the quality of seafood throughout the application of hurdle technology, while preserving the important foods' naturalness. Besides, their application has some limitations, as not all these methods are in the same development stage. The seafood industry must consider these issues following the consumers' willingness to buy natural products with excellent sensory experience. Therefore, further research is necessary to improve the promising non-thermal emerging technologies to the level that will be applied universally as safe by the seafood industry and produce seafood products accepted as natural by consumers.

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