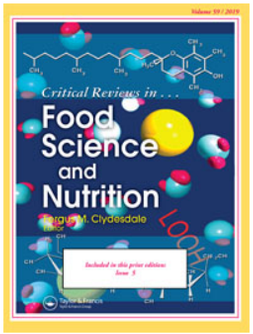

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REVIEW

Principles and recent applications of novel non-thermal processing technologies for the fish industry—a review

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ABSTRACT

Thermal treatment is a traditional method for food processing, which can kill microorganisms but also lead to physicochemical and sensory quality damage, especially to temperature-sensitive foods. Nowadays consumers' increasing interest in microbial safety products with premium appearance, flavor, great nutritional value and extended shelf-life has promoted the development of emerging non-thermal food processing technologies as alternative or substitution to traditional thermal methods. Fish is an important and world-favored food but has a short shelf-life due to its extremely perishable characteristic, and the microbial spoilage and oxidative process happen rapidly just from the moment of capture, making it dependent heavily on post-harvest preservation. The applications of novel non-thermal food processing technologies, including high pressure processing (HPP), ultrasound (US), pulsed electric fields (PEF), pulsed light (PL), cold plasma (CP) and ozone can extend the shelf-life by microbial inactivation and also keep good sensory quality attributes of fish, which is of high interest for the fish industry. This review presents the principles, developments of emerging non-thermal food processing technologies, and also their applications in fish industry, with the main focus on microbial inactivation and sensory quality. The promising results showed great potential to keep microbial safety while maintaining organoleptic attributes of fish products. What's more, the strengths and weaknesses of these technologies are also discussed. The combination of different food processing technologies or with advanced packaging methods can improve antimicrobial efficacy while not significantly affect other quality properties under optimized treatment.

KEYWORDS

non-thermal technology; fish; microbial inactivation; quality; HPP; US; PEF; PL; CP; ozone

Introduction

Among foods, fish is one of the most important commodities, which is popular all over the world due to its high nutritional value, being rich in proteins, vitamins and essential amino acids. Besides, fatty fish species are acknowledged as a natural food resource of omega-3 polyunsaturated fatty acids (PUFAS), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which cannot be synthetized by the human body and must be included in the diet (Murray and Burt, 2001). However, fish is highly perishable with a short shelf-life and depends heavily on post-harvest preservation methods, which is a challenge for the fish industry to avoid microbial spoilage while remaining high sensory quality parameters.

Nowadays, consumers are conscious of healthy food products with enhanced safety, great nutritional value, premium sensory quality, fresh-like and less or free of chemical additives. To meet consumers' Cooling (McDonald and Sun 2001; Hu and Sun 2000), Freezing (Kiani et al. 2012; Ma et al. 2015; Xie et al. 2015; Cheng, Sun and Pu 2016; Xie et al. 2016; Qu et al. 2017; Cheng et al. 2018) and drying (Yang, Sun and Cheng 2017; Pu and Sun 2016; Qu et al. 2017; Ma et al. 2017; Pu and Sun 2017) non-thermal food processing technologies, such as high pressure processing (HPP), ultrasound (US), pulsed electric fields (PEF), pulsed light (PL), cold plasma (CP) and ozone have attracted extensive attention from the food industry as well as researchers. These technologies have been shown to be effective on the inactivation of spoilage and pathogenic microorganisms while maintaining nutritional and sensory properties for a wide range of food products, such as fruit and vegetables (Ramazzina et al. 2015; Gomez-Lopez et al. 2005), fish (Erkan et al. 2011; Rode and Hovda 2016; Okpala 2014; Mikš-Krajnik et al. 2017), juice and milk (Yaldagard, Mortazavi, and Tabatabaie 2008; Jayathunge et al. 2015), or fresh and ready-to-eat meat products (Ganan et al. 2013; Bauer et al. 2017; McDonnell et al. 2014). Among them, HPP is the most successfully commercialized one, which is commonly applied on fruit juice or jams and also oyster (Huang et al. 2017; Voisin 2003; Murchie et al. 2005). US and PEF are also intensively used for extraction, freezing and drying (Musielak, Mierzwa, and Kroehnke 2016; Parniakov et al. 2016), while PEF is more suitable for liquid

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Table 1. Strengths and weaknesses of HPP, US, PEF, PL, CP and ozone.

Technologies	Strengths	Weaknesses
HPP	• Wide range of microorganisms inactivation, including, spoilage or pathogenic bacteria, molds and yeasts, fungi and viruses; • Preservation of taste, nutrition and color, minor negative effect on sensory quality; • Shucking meat from shellfish; • Easy to commercialize and energy-efficient.	• Limited effect on spores; • Expensive equipment; • Batch process; • Limited packaging options.
US	• Simple; • Low pollution, green; • Non-invasive.	• Less disinfection ability
PEF	• Short time; • Low-energy.	• Limited effect on spores and viruses; • Higher cost; • Waste water treatment; • More suitable for liquid foods.
PL	• Cost-effective; • No residual on food; • Rapid inactivation on food surface, equipment, and packing materials.	• Low penetration capacity; • Sensory quality damage with closer distance.
CP	• Wide range of microorganisms inactivation, including, spoilage or pathogenic bacteria, molds yeasts, biofilms and spores; • Low temperature; • Rapid and effective.	• Low penetration depth; • Some alteration on sensory quality; • Possible residues on food.
Ozone	• Wide range of microorganisms inactivation, including, spoilage or pathogenic bacteria, molds, yeasts, fungi and spores; • Cost-effective; • Rapid decomposition without any residues left on food; • Capability for direct contact with food.	• Inability for storage, and thus generation and uses on -site; • Less effective when bacteria exist within organic materials; • Possible oxidize food components.

foods. PL, CP or ozone are also applied for decontamination of packing materials (Heinrich et al. 2015; Lee, Puligundla, and Mok 2015). The advantages and disadvantages of these technologies are compared in Table 1. To the best of our knowledge, this is the first review covering the applications of all the six emerging non-thermal food processing technologies in the fish industry. This review presents the principles, developments and applications of HPP, US, PEF, PL, CP and ozone in the fish industry, with a focus on their effects on microbial inactivation and other quality parameters of fish. Limitations and challenges are also discussed.

High-pressure processing (HPP)

Fundamentals

HPP is a batch process for solid food or a semi-continuous process for liquid food, in which typical applied pressure operates from 200 to 800 MPa at mild temperature of 5 to 35 °C, thus it has been evaluated as a novel non-thermal food processing technology compared with traditional heat-treated method (Hogan, Kelly and Sun 2005). When using HPP, many kinds of microorganisms are killed, and the advantage is that HPP does not break covalent bonds and has minor effect on low-molecular-weight food chemicals, such as pigments, vitamins, and flavor compounds. Therefore, HPP is capable of preserving food safely as well as maintaining high quality on appearance, nutritional value or taste. The first industrial application of HPP for commercial products was launched in Japan in 1991 (Yaldagard, Mortazavi, and Tabatabaie 2008), in which fruit purees and juice became the most widely used foods since these products normally have a low pH and water activity. HPP has been increasingly used on a wide range of food products in a large scale in the past 20 years, including meat products, fruit and vegetables, fish and seafood (Mùjica-Paz et al., 2011; Norton and Sun, 2008), which is mainly due to the innovative development in the availability of high pressure vessels that can withstand up to thousand pressure cycles (Patterson 2014). Besides, HPP has been approved by USDA (United States Department of Agriculture) as a legal process for eliminating *Listeria monocytogenes* in processed meat products (Simonin, Duranton, and DE Lamballerie 2012), and recognized by the State of California as a valid method to inactivate *Vibrio* bacteria in fish products (Campus 2010).

HPP system includes two types, the vertical and horizontal ones. The horizontal system is commonly used in the food industry, which is made up of a pressure vessel, an external pressure generator, and a supporting unit for controlling pressure and temperature. As demonstrated in Figure 1, prepackaged food products are transferred into pressure vessel, and then the pressure-transmitting media, normally water, is pumped into the vessel from the bottom or both sides. After reaching to the desired pressure the pump is stopped, but the pressure level is held without further energy input, making HPP very energy efficient. Compared with heat-treated process, in which temperature gradients occur, food products regardless of geometry and size are subjected to pressure simultaneously and uniformly in all directions. HPP increases the food temperature, which is known as adiabatic heating. The increase of the temperature depends on the food composition and transmitting media, normally from 2 to 9 °C/100 MPa. For water-transmitted system, the increase is approximately 2 °C/100 MPa. Since HPP is used for prepackaged products, the possibility of secondary contamination is reduced while maintaining the freshness and nutritional value of products. However, it should be noticed that the packaging materials for HPP have to be flexible enough to withstand a reversible deformation without structure damage, since the food is

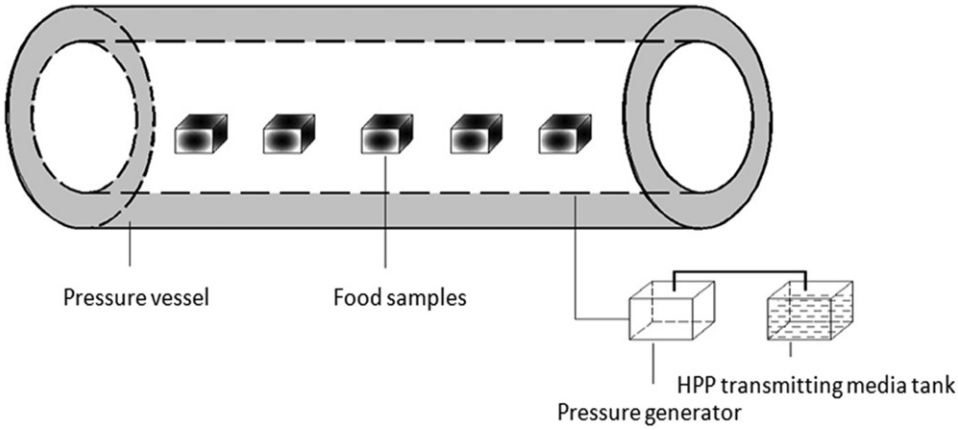

Figure 1. Schematic diagram of HPP equipment at industry scale.

compressed during pressure followed by the recovery to original size. Plastic packaging materials are suitable for this purpose due to the flexible integrity and reversible response to compression.

The mechanisms of microbial inactivation by HPP are agreed to being due to many processes happening simultaneously but are not completely known, however, it has been recognized that high pressure (HP) leads to the alteration of cell membrane, denaturation of proteins (including enzyme), and the changes of cell morphology, which are thought to be the primary reasons for microorganisms damage. High pressure alters the membrane permeability and causes leakage via internal and external membranes, which has been proved by increased sensitivity of the cells to sodium chloride and bile salts, and leakage of ATP after HP treatment (Smelt, Rijke, and Hayhurst 1994). HP can denature protein structure and corresponding enzyme activity. The disruption of key enzyme by HP results in the inactivation of microorganisms. In addition, HP causes changes of morphology and inner organization in cells. The altered distribution or degradation of DNA and the destruction of ribosome have been found in HP-treated cells (Niven, Miles, and Mackey 1999). Many vegetative bacteria including spoilage and pathogenic microorganisms, yeasts, molds and viruses, are sensitive to HP, however, it should be noticed that spores are very resistant to HP, for example, spores of *Clostridium botulinum* can still survive under extreme conditions of 827 MPa for 30 min at 75 °C (Farkas and Hoover 2000).

Many factors can affect the pasteurization by HP. The three main factors on treatment conditions are pressure, temperature and holding time. In general, higher pressure usually leads to greater possibility of microorganisms' death. However, it is noticeable that in many cases the inactivation curve does not follow first-order kinetics. The initial temperature applied in the industry is usually from 5 to 35 °C, and due to adiabatic heating, the temperature will increase during the process. As for the holding time, it is of great importance not to have a longer time than needed, in order to have the maximum economic profits.

Apart from the three factors aforementioned, the species of different microorganisms even the same species in different growth phases have different responses to high pressure. It has been demonstrated that gram-positive bacteria or cells in stationary growth phase tend to be more resistant to high pressure. The nature of food is also a significant factor, including the composition, pH, water activity (a_w) and so on. Lower pH is normally more sensitive to HP, which is the reason why fruit juice and jams became the first commercialized products. Therefore, when designing an optimum HPP procedure for safe and good sensory quality food, all the inter-acting factors should be taken into consideration.

Applications in the fish industry

The applications of HPP in the fish industry have received a great deal of attention in the past decade. HPP allows the inactivation of microorganisms as well as great sensory quality attributes. Many fish species have been studied, including fresh or smoked salmon, rainbow trout, red mullet, mackerel, etc. and also some bivalve shellfish, like oyster and lobster. Besides, HPP has the advantage of shucking meat from shellfish.

The microbial inactivation by HPP in the fish industry in the past decade is discussed in details in Table 2. In addition, HPP was also used to extend the shelf-life of fish products. Salmon is a well-welcomed aquaculture product all over the world, and smoking is one of the most popular methods to process it. Cold smoked salmon was treated with pressures of 220, 250 and 330 MPa under different temperatures for 5 and 10 min, both total psychrophilic counts (TPC) and total viable counts (TVC) were significantly inhibited and the shelf-life was increased to 8 weeks, which was two weeks longer compared with untreated controlled samples (Erkan et al. 2011). In addition, Karim et al. (2011) found that the shelf-life of herring and haddock treated at 200 MPa for 3 min and stored on ice at 2 °C was increased to 13 days compared to only 6 days for the control.

The effects of HPP on sensory quality of fish products can be seen in Table 3. After HPP treatment, a tendency of increase in lightness (L^*) and yellowness (b^*), and decrease in redness (a^*) were seen in many species of fish, resulting in paler and opaque appearance, like cooked products. The

Table 2. Microbial inactivation by HPP in fish products.

Products	Target microorganisms	Treatment conditions	Microbial reduction (log_{10}CFU/g)	Reference
Cold smoked salmon	*L. innocua*	450 and 600 MPa for 120 s at 4 °C	2.63 and 3.99 log_{10} CFU/g, respectively	Lebow et al. (2017)
Salmon, cod and mackerel	Aerobic bacteria count, H_2S-producing bacteria	200 and 500 MPa for 120 s at 8–9 °C	Undetectable level of aerobic counts in cod and mackerel and H_2S-producing bacteria in all fish when treated at 500 MPa	Rode and Hovda (2016)
Shucked abalone meat	Aerobic plate count	100 and 300 MPa for 5 or 10 min	Extending the shelf-life of 300 MPa treated samples to 35 d as compared with 14 d for controls and for 100 MPa treated samples	Hughes et al. (2016)
Hilsa fillets	Total plate count (TPC)	250, 350 MPa for 10 min at 27 °C	2.21 and 2.4 log cycles, respectively; 350 MPa increased the shelf-life to more than 25 d	Chouhan, Kaur, and Rao (2015)
Smoked rainbow trout and fresh catfish fillets	*L. monocytogenes, E. coli*	200, 400 or 600 MPa for 1 or 5 min at room temperature	>6 log_{10} CFU/g reductions in both fish products	Mengden et al. (2015)
Oyster homogenate	*Vibrio parahaemolyticus*	200 or 250 MPa for 5 min at 15, 5 and 1.5 °C	Decreasing the population for treatment at 250 MPa for 5 min and 5 °C to 6.2-7.4 log_{10} CFU/g; same treatment at 1.5 °C showing non-detectable (<10 CFU/g) levels	Phuvasate and Su (2015)
Chilled mackerel	Aerobic mesophilic and psychrophilic count, H_2S-producing bacteria	450 and 550MPa for 3 and 4 min at 20 °C	Significant extension of microbiological the shelf-life to 29 d and 40 d at 450 MPa/3 min and 550 MPa/4 min, respectively, as compared with 6 days for the control	Reyes et al. (2015)
Fresh herring and haddock	TVC, total psychrotrophic count	200, 250 and 300 MPa at 10 °C for 1 and 3 min. Storage on ice for up to 14 d	200 MPa for 3 min was the minimum treatment to increase the shelf-life in herring and haddock to 13 d on ice	Karim et al. (2011)
Cold smoked salmon	TVC, total psychrophilic counts (TPC)	220, 250 and 330 MPa, at 3, 7, 15 and 25 °C for 5 and 10 min	All treatments inhibited the growth of TVC and TPC and no unacceptable level were achieved during the storage	Erkan et al. (2011)
Red mullet	TVC, psychrotrophic bacteria	220 MPa for 5 min at 25 °C, 330 MPa for 5 min at 3 °C	Shelf-life extension up to 14 and 15 d, respectively	Erkan, Üretener, and Alpas (2010)
Squid	TVC	300 MPa at 20 °C for 20 min	1.26 log_{10} CFU/g	Gou, Lee, and Ahn (2010)
Cold smoked salmon	*L. innocua*	700, 800 and 900 MPa for 10 s	Inactivation of *L. innocua* until non-detectable level	Gudbjornsdottir et al. (2010)
Oyster	TVC, APC and H_2S-producing bacteria	260, 400 or 600 MPa for 5 min at 20 °C	Bacterial load initially reduced to levels below the detection limit. The growth rate was significantly reduced during storage on ice at 2 °C at 400 or 600 MPa	Cruz-Romero, Kerry, and Kelly (2008)
Cold-smoked dolphinfish	Aerobic bacteria, H_2S-producing bacteria, LAB	200, 300 and 400 MPa for 15 min at 20 °C	Aerobic bacteria and LAB were decreased below detection threshold for three weeks. H_2S-producing bacteria were not detected during chilled storage	Gómez-Estaca, Gómez-Guillén, and Montero (2007)

Table 3. Effect of HPP on sensory quality parameters of fish products.

Products	Quality parameters	Treatment	Salient findings	Reference
Salmon, cod and mackerel	Texture, color, liquid loss, water holding capacity (WHC), protein denaturation	200 and 500 MPa for 120 s at 8–9 °C	Hardness and *L** increased at 500 MPa and *a** decreased; Highest liquid loss and lowest WHC in mackerel; severe protein denaturation	Christensen et al. (2017)
Red abalone; shucked abalone meat	TVBN, K-value, and biogenic amines; color and texture	300 MPa for 10 min; 100 and 300 MPa for 5 or 10 min	Much lower TVBN value after 300 MPa for 10 min treatment; increase in K-value over time but no significant differences among all the treatments; no detection of biogenic amines during 35 days under 300 MPa treatment; no significant effects on color and texture after treatments	Hughes et al. (2016)
Salmon, cod and mackerel	pH, lipid oxidation, acid phosphatase (ACP)	200 and 500 MPa for 120 s at 8–9 °C	Small differences in pH compared to untreated samples; increased lipid oxidation in cod samples at 200 MPa during storage; 500 MPa increased lipid oxidation in salmon; high level of lipid oxidation in unprocessed and HPP-treated mackerel	Rode and Hovda (2016)
Mild smoked rainbow trout and fresh European catfish fillets	Sensory analysis	200, 400, or 600 MPa for 1 or 5 min at room temperature	Minor effect on trout fillets; catfish tended to became paler and like cooked product	Mengden et al. (2015)
Hilsa fillets	TVBN and TMA-N, lipid oxidation, free fatty acid (FFA), color, texture,	250, 350 MPa for 10 min at 27 °C	350 MPa reduced the production of TVB-N; HPP delayed TMA-N production; higher increase in lipid oxidation and lower values of FFA during storage at 350 MPa; increase in *L**and *b** and decrease in *a**; increase in hardness	Chouhan, Kaur, and Rao (2015)
Sea bass	Lipid oxidation, color, TVBN and TMA-N, pH	250 and 400 MPa for 5 min at 6 °C	Increased in lipid oxidation and whiteness; and decreased in translucency; no effect was observed in volatile bases or in pH values	Teixeira et al.(2014)
Herring and haddock	TVBN, TMA	200, 250, 300 MPa for 1 and 3 min at 10 °C	Delay in TVBN and TMA production	Karim et al. (2011)
Atlantic salmon	Lipid oxidation, fatty acid profile, color, texture	150, 300 MPa for 15 min at room temperature. Storage 6 d at 4 °C	Increase of *L** and *b**, decrease of *a** when pressure increased; increase in hardness, gumminess and chewiness, decrease in adhesiveness when pressure level increased; TBARS increased during storage; no significant difference in total saturated or unsaturated fatty acid composition between control and HPP-treated samples	Yagiz et al. (2009)
Oyster	Color, pH, texture, lipid oxidation	260, 400, 600 MPa at 20 °C for 5 min. Storage during 31 d at 2 °C	Increase of *L** by HPP and *b** increased during storage; increase of pH; increase of cutting strength with increasing pressure during storage; increase in lipid oxidation dependent on pressure	Cruz-Romero, Kerry, and Kelly (2008)
Cold-smoked dolphinfish	WHC, texture, color, lipid oxidation, sensory analysis	Smoking combined with 200, 300 or 400 MPa for 15 min at 20 °C	WHC decreased at 400 MPa; shear strength and *L**, *a** and *b** increased with all treatments; lipid oxidation was prevented by smoking; best sensory attributes scores for smoked samples were treated at 300 MPa	Gómez-Estaca, Gómez-Guillén, and Montero (2007)

only exception happened in cold-smoked dolphinfish, where an increase in all parameters of L^*, a^* and b^* were observed ((Gómez-Estaca, Gómez-Guillén, and Montero 2007)). The increase in brightness was thought to be due to the loss of active pigment and protein coagulation. The protein coagulation can alter the surface attributes of food matrixes, which leads to the increase in light reflection thus forming a white appearance (Kruk et al. 2011). The changes of appearance in fish were in positive correlation with pressure applied (Mengden et al. 2015). Hardness is an important texture property in meat or seafood. HPP-treated seafood products were reported to have increased hardness, cutting or shear strength, and also higher springiness and chewiness as seen in Table 3. The effect of harder texture on fish by HPP may result from myofibrillar protein denaturation and aggregation. Similar to color, higher pressure intensity normally leads to harder texture in fish. HPP seems to increase the lipid oxidation as shown in Table 3, which can be estimated by the production of thiobarbituric acid reactive substances (TBARS).

Ultrasound (US)

Fundamentals

Ultrasound is sound waves with frequency exceed human audible limit (20 kHz), and it has been widely used in food processing, preservation and safety, including microbial inactivation, texture tenderization, emulsification, extraction, freezing and thawing, etc (Tao and sun 2015; Kiani, Sun and Zhang 2013; Delgado, Zheng and Sun 2009; Zheng and Sun 2006; Sun and Li 2003; Li and Sun 2002). Despite heat generated, US can be considered as an emerging non-thermal technology for food preservation. According to the frequency range, the applied ultrasound can be classified into two groups: low energy (frequency: 5–10 MHz, intensity <1W/cm^2) and high energy ultrasound (frequency: 20–100 kHz, intensity >1W/cm^2). Low energy ultrasound is not that widely used but as an analytical tool for quality evaluation and nondestructive inspection. High energy ultrasound is disruptive, thus can induce physical, chemical/biochemical or mechanical effects on food properties, and has been used for microbial inactivation and quality improvement (Char et al. 2010; Chandrapala et al. 2012; Awad et al. 2012; Musielak, Mierzwa, and Kroehnke 2016; Ozuna et al. 2015; Tiwari 2015; Cheng et al. 2015).

The US equipment at laboratory scale usually consists of ultrasonic generator, oscilloscope, and sample room. However, most of US treatments have not reached industrial level, though US applications in food research have shown promising effects. This is mainly because ultrasonic equipment has to be specifically designed for each single application, which leads to the lack of appreciation by the food industry (Mawson and Knoerzer 2007). Therefore, collaboration between laboratory research and industry scale is necessary in the future.

The antimicrobial effect of US treatment can be mainly explained by cavitation phenomena, shear disruption, localized heating and free radicals formation. The propagation of ultrasound in the medium creates a series of compressions. When the energy reaches a certain point, the rarefaction will exceed the attractive forces among molecules, followed by the generation of cavitation bubbles (Cheng et al. 2015). The unstable bubbles collapse violently and produce outward propagating shockwaves, which can destroy the cell walls of microorganisms or break polymer chains easily. These effects can also lead to the breakdown of water molecules (Riesz and Kondo 1992), causing highly reactive free radicals ($H_2O \rightarrow H + {}^{\bullet}OH$). These free radicals can modify or damage intracellular components including DNA (Liao et al. 2018).The effects of US treatment depend on ultrasonic intensity, ultrasonic frequency, temperature, processing time and the waveforms, that is, pulsed or continuous wave. Besides, the microbial species and growing phase are also important factors.

Applications in fish industry

The individual applications of US or its combination with other decontamination technologies have been studied in different fish products in order to improve microbiological safety and enhance sensory quality.

Pedrós-Garrido et al. (2017) investigated the effects of US treatment at 30 kHz in an ultrasonic bath for 5 to 45 min on oily fish (salmon and mackerel) and white fish (cod and hake). A much higher microbial reduction was found in oily fish than white fish, which was assumed that oily fish has a higher fat content thus can help promote bacterial detachment when treated by US, since previous studies demonstrated that US could increase the lipid extraction in plants or seeds. After 45 min of US treatment, there was no significant change in lipid content in the four species, but a significant reduction of TBARS (thiobarbituric acid reactive substances), and a decrease of redness (a^*) and yellowness (b^*) was detected in salmon, however, an increase of redness (a^*) was observed in hake. Moisture level did not change significantly except for hake. These results demonstrated that US could be used as a surface decontamination technology. Mikš-Krajnik et al. (2017) found that US combined with ultraviolet light (UV) or the combination of US with UV and acidic electrolyzed water (AEW) were the most effective treatments to decrease *Listeria monocytogenes* and total viable counts (TVC) on raw salmon fillets. However, the individual application of US or AEW did not affect microbial load significantly. Similarly to aforementioned study, a decrease in redness (a^*) and yellowness (b^*) was observed in salmon fillets, but texture and firmness were not significantly affected.

Sashimi is a conventional and popular Japanese dish, which is composed of sliced raw seafood and a simple dipping sauce. Without any extra tastes or flavors, texture is the critical sensory quality to determine consumers' acceptance. Chang and Wong (2012) used ultrasonic bath to tenderize cobia sashimi, and optimal firmness was achieved under the treatment of 60 and 90 min. Compared with traditional aging process, in which softer texture was obtained on day 7 but lower freshness, US is much more efficient

and also allows the freshness and good sensory quality of fish. In addition, US was used to tenderize jumbo squid, where response surface methodology (RSM) was employed to determine the optimal processing conditions. A satisfied tenderization effect was obtained through histological assay and SDS-PAGE (Hu et al. 2014).

Pulsed electric field (PEF)

Fundamentals

PEF processing includes the application of short-time electric pulses (1–100 μs) in different ranges of electric field intensities, that is, reversible permeabilization in plant cells (0.1 – 1 kV cm^{-1}) , irreversible permeabilization in animal and plant tissue(0.5 – 3 kV cm^{-1}) , and irreversible permeabilization of microbial cells (15–70 kV cm^{-1}). Pulsed electric field (PEF) is a novel non-thermal and fast (milliseconds) technology (Wang, Sun and Zhu 2018), which offers safety and high quality, fresh-like liquid foods with great nutritional value, excellent flavors and pro-longed shelf-life. Therefore, it has been widely applied on liquid matrices, such as milk (McAuley et al. 2016; Halpin et al. 2013; Walkling-Ribeiro et al. 2011), fruit juice (Sampedro et al. 2013; Charles-Rodríguez et al. 2007), alcoholic beverage (Yang et al. 2016), and vegetable juice (Quitão-Teixeira et al., 2008) and soups (Sampedro et al. 2013). Recently, PEF has also been shown to be effective in solid food matrices, such as chicken (Haughton et al. 2012), beef (Arroyo et al. 2015; O'Dowd et al. 2013), pork (McDonnell et al. 2014). PEF has been shown capable of inactivating numerous microorganisms including *Escherichia coli*, *Staphyloccus aureus*, or *Bacillus subtilis* (Alkhafaji and Farid. 2008; Sobrino et al., 2006; Heinz and Knorr 2000). However, no effect has been observed on viruses and bacterial spores, and the effect of PEF on enzymes is also quite limited.

Although a large number of scientific papers have been published showing the benefits of PEF treatment, there is still rare report about the industrial use of PEF. Due to the higher cost compared to traditional thermal processing, waste water treatment and the difficulty to scale up to industrial level, PEF has not been widely implemented in the food industry. Several PEF treated juice companies are available in the USA. The PEF equipment in laboratory normally includes electric pulses generator, treatment chamber and two electrodes. PEF process involves the usage of high voltage electric pulses on food products, which are placed between or passed through two electrodes. Several designs are available including the parallel, the co-linear, and the co-axial ones.

In terms of disinfection by PEF, there is no confirmed evidence on mechanisms at cellular level, but the most accepted mechanism is the breakdown of cell membrane (Zimmermann, Pilwat, and Riemann 1974). Pores on cell membrane are formed by high intensity electric field pulses discharges and as a consequence, the permeability of membrane increases. With the increase in the number of pores, loss of cell content or intrusion of surrounding media occurs, which is also called cell electroporation. The antimicrobial effect of PEF depends on extrinsic factors such as electric field intensity, treatment time, temperature, pulse width, shape, electrical conductivity and pH, and also on intrinsic factors of microorganisms, such as type, size, microbial load and growth stage.

Applications in fish industry

To our knowledge, the effect of PEF on decontamination of fish is scarce. According to Guerrero-Beltran and Welti-Chanes (2016), meat and fish products do not resist to PEF strength beyond 10 kV/cm, since higher intensity tend to change the muscle texture. This is in accordance with (Gudmundsson and Hafsteinsson 2001), who investigated the effect of PEF and its combination with high pressure on microstructure of salmon and lumpfish roes. They concluded that low field strength (less than 2 kV/cm and 20-40 pulses) was effective to decrease microbial growth, but texture and microstructure of salmon was negatively affected. However, the texture and microstructure of lumpfish roes, which are often used in many kinds of fish roe and also caviar-like products, were found not to be affected even after PEF treatment of 12 kV/cm and 12 pulses, probably because of the three main membrane layers, which offers much strength and protection. Therefore, PEF treatment could be valuable as a pretreatment for roes and to extract substances from waste material of the fish industry.

Pulsed light (PL)

Fundamentals

Pulsed light (PL), also known as pulsed ultraviolet (UV) light, is effective to inactive microorganisms on surface non-thermally, such as food or food-contact materials (Oms-Oliu et al. 2010). PL employs short time, high-peak-power pulsed light of wide spectra, ranging from 100 to 1100 nm, which includes 54% UV light (100–400 nm), 26% visible light (400–700 nm), and 20% near-infrared light (700–1100 nm). In UV wavelength range, short wave UV-C (200-280 nm) has been shown to be the most lethal and germicidal to microorganisms as it has the maximum absorption capacity for pyrimidine bases of nucleic acids, which interrupts the replication of DNA, causing cell death (Bintsis et al., 2000). PL treatment only takes few seconds thus it is quite cost-effective. Besides, it does not leave any unwanted residual compounds on foods during the process. The applications of PL have been studied in liquid food, such as fruit juice and milk (Kasahara, Carrasco, and Aguilar 2015; Ferrario, Alzamora, and Guerrero 2015; Ochoa-Velasco, Cruz-González, and Guerrero-Beltrán 2014; Koutchma 2009), solid food, including fruit and vegetables (Gomez-Lopez et al. 2005, Marquenie et al. 2003), regular or ready-to-eat meat (Ganan et al. 2013, Wambura and Verghese 2011), and food packaging materials (Heinrich et al. 2015). The main limitation of PL to solid food surface decontamination is its low penetration capacity, which limits the antimicrobial effect. In 1999, PL method was approved by the United States

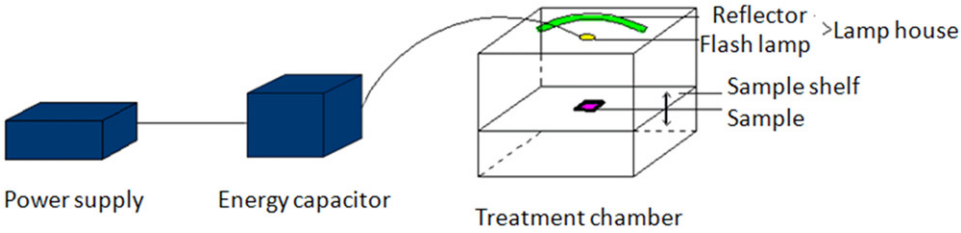

Figure 2. Schematic representation of pulsed light equipment at laboratory scale.

Food and Drug Administration (USFDA) after being evaluated as safe and effective (Federal Register, 1999). Some limitations of the use of PL on food have been reported, such as changes in food protein structure (Shriver and Yang 2011) and cell membrane damage (Gómez López et al. 2007). Sample heating is also a major limiting factor of PL and some detrimental effects on food quality have been described (Gomez-Lopez et al. 2007; Ozer and Demirci 2006).

The common PL equipment in laboratory mainly includes a high-voltage power supply, an energy capacitor and a treatment chamber (Figure 2). The power supply offers electric energy to the capacitor, and then it is released to the lamp within nanosecond or milliseconds. The control system allows the modulation of frequency, peak power and pulse width. The treatment chamber is made of stainless steel, which have high-reflecting capacity to enable the light to be reflected to the product, resulting in a high efficacy. Inside the treatment chamber, there is a lamp house made up of a reflector and one or two xenon flash lamps, and a sample shelf to hold the sample which can be regulated vertically to change the distance between the light and the targeted samples.

Photochemical, photothermal and photophysical mechanisms are thought to be responsible for microbial inactivation by PL (Gomez-Lopez et al. 2007). It's probable that all the mechanisms coexist at the same time, but when it comes to which one is more important, it mainly depends on the target microorganisms and the fluence. The photochemical mechanism mainly results from the UV component of the wavelength. UV illumination is lethal for most kinds of microorganisms because it can cause modification or damage to genomic material (DNA/RNA). The photothermal mechanism is about the temperature increment due to the absorption of pulsed radiation. Photophysical mechanism is related to structural damages, such as cell wall damage, expanded vacuoles, membrane distortions, and other cell morphological changes.

The factors that affect germicidal efficacy of PL mainly includes process parameters (fluence, electrical power, distance between target and light source, type of lamp, and pulse duration), food properties, such as thickness and composition, and microbial characteristics, being gram-negative bacteria more sensitive to PL treatments, followed by gram-positive bacteria and fungal spores (Gomez-Lopez et al. 2007).

Applications in fish industry

Table 4 lists different studies about the microbial inactivation achieved on fish by PL treatments. All the results showed that higher power, closer distance and longer treatment time resulted in better microbial reduction, but also followed by different extent of quality degradation, therefore, finding optimal treatment conditions is necessary for fish safety and sensory quality. Ozer and Demirci (2006) treated raw salmon fillets by PL at 5.6 J cm^{-2} at distances of 3, 5, 8 cm for different times and found that the surface temperature of some fish samples increased up to 100 °C at the distances of 3 and 5 cm within 60 s, which led to significant changes in color and quality, although good microbial reduction was obtained. However, when fillets were treated for 60 s at 8 cm distance, about one log reduction of *Escherichia coli* O157:H7 or *L. monocytogenes* Scott A was achieved without compromising the quality. Hierro et al. (2012) observed an increase of lightness (L^*) and a decrease of redness (a^*) when fluences of 8.4 J/cm^2 or higher were applied on tuna carpaccio. Yellowness (b^*) was more sensitive to PL and decreased significantly when fluences was above 0.7 J/cm^2. The results showed that treatment of 2.1 J/cm^2 could improve the microbial quality and sensory properties of tuna carpaccio samples stored for one week.

Table 4. Microbial inactivation by PL in fish products.

Products	Target microorganisms	Treatment conditions	Microbial reduction (log_{10} CFU/g)	Reference
Raw salmon fillets	*L. monocytogenes*, TVC	UV-C (254 nm) (8cm distance for 5 min)+US (45 KHZ, 200 W for 1 min)	0.79, 0.59	Mikš-Krajnik et al. (2017)
Raw salmon	Aerobic flora *P. fluorescens*	Fluencies of 30 J/cm^2 at 3 cm	0.8, 1.0, respectively	Nicorescu et al. (2014)
Shrimp, Salmon and Flatfish fillets	*L. monocytogenes*	Total fluence of 6.3 J/cm^2 for 720 s. Total fluence of 12.1 J/cm^2 for 1380 s	2.2, 1.9 and 1.7 respectively; 2.4, 2.1 and 1.9 respectively	Cheigh, Hwang, and Chung (2013)
Tuna carpaccio	*V. parahaemolyticus*, *L. monocytogenes*	11.9 J/cm^2	Reduction of 1.0 and 0.7, respectively	Hierro et al. (2012)
Salmon fillets	*E. coli* O157:H7, *L. monocytogenes*	5.6 J/cm^2 for 15, 30, 45 and 60 s at 3, 5 and 8 cm from the lamp	Maximum reduction of 1.09, 1.02 on muscle side, maximum reduction of 0.86, 0.74 on skin side for the two bacteria, respectively	Ozer and Demirci (2006)

However, 8.4 J/cm^2 and 11.9 J/cm^2 affected sensory quality of the product negatively. Cheigh, Hwang, and Chung (2013) investigated the effect of intense pulsed light (IPL) on color parameter of shrimp, salmon and flatfish fillets and no observable color changes of tested seafood were reported. Nicorescu et al. (2014) detected that PL treatment at 30 J/cm^2 had the highest effect on lipid oxidation in raw salmon compared to 3 and 10 J/cm^2, suggesting the employment of moderate fluences in order to obtain high product quality while maintaining microbiological safety. Similarly, a decrease of redness (a^*) and yellowness (b^*) and an increase of brightness indicators (L^*) were observed by Mikš-Krajnik et al. (2017), in which raw salmon fillets were treated with UV-C (254 nm) for 5 min at a distance of 8 cm combined with US (45 KHZ, 200 W for 1 min). Lower sensory scores were reported in the treated samples.

Cold plasma (CP)

Fundamentals

Plasma is the fourth state of matter apart from solid, liquid and gas. It is a neutral system in gaseous form where ions, free electrons, neutral particles (atoms, molecules), activated and metastable species (NO_x^-), free radicals like reactive oxygen species (ROS), reactive nitrogen species (RNS) and photons are mixed (Mir et al., 2016; Ekezie et al., 2017). According to the temperature of electrons, plasma can be divided into two categories: thermal plasma and cold plasma (CP). For thermal plasma, electron temperature is almost the same as neutral ions and the whole gas. However, in CP, the temperature of neutral ions and the overall gas is lower than electrons. CP can be produced by different electrical discharges in a gas at different pressure levels. The commonly used gases are air, O_2, N_2, CO_2 and noble gases, including helium, argon, and also the combinations of different gases and gas with humidity. The applications of cold plasma in food decontamination has been investigated in numerous food products, which include fresh beef (Bauer et al. 2017), pork (Fröhling et al. 2012), chicken (Noriega et al. 2011), ready-to-eat meat (Rød et al. 2012), fruit and vegetables (Ramazzina et al. 2015; Misra et al. 2014a; Misra et al. 2014b), and food packaging materials (Pankaj et al. 2014; Lee, Puligundla, and Mok 2015).

Recently, the generation of CP at or near atmospheric pressure has attracted the attention of the food industry since no high temperature or high pressure is required (Misra et al. 2011). Technologies such as corona discharge, dielectric barrier discharges (DBD) or plasma jet and microwave plasma are used to generate plasma at atmospheric pressure, with the DBD and plasma jet being the most explored ones in food research. Apart from the microbial inactivation of CP on solid food, plasma activated water (PAW) has gained increasing attention in the past few years. Among the chemical species in PAW, the combination of H_2O_2, OH· radical, ozone, atomic oxygen are regarded as the main ROS, $NO2^-$, $NO3^-$, and peroxynitrite $ONOO^-$ are the main RNS, which lead to the acidification of water and contribute to antimicrobial effect of PAW. PAW has been applied on fresh products, including the inhibition of total aerobic bacterial counts in sausage (Jung et al. 2015), food-borne pathogen of *S. aureus* on strawberries (Ma et al. 2015), *Agaricus bisporus* on button mushrooms (Xu et al. 2016), and postharvest storage of Chinese bayberries (Ma et al. 2016). The good results indicated that PAW can be a promising strategy in reducing microbial loads and maintaining quality. Gas discharge equipment mainly includes gas container, gas flow rate controller, treatment chamber, and the supporting unit of other parameter controller.

The mechanisms of microbial inactivation by CP are not completely known but include DNA damage by UV radiation produced from plasma, and oxidative damage to membranes or intracellular components, such as proteins and carbohydrates (Liao et al. 2017). The electrostatic forces generated by accumulation of charged particles obtained from plasma are thought to result in the disruption of cell membranes, which leads to cell death (Lunov et al. 2015). Among factors that affect microbial inactivation by CP are processing parameters, such as input power (voltage and frequency), gas composition, gas flow rate, treatment time, type of applications (indirect or direct treatment), food matrix composition, and intrinsic factors of microorganisms such as type, size, growth phase and microbial load.

Applications in fish industry

Chiper et al. (2011) investigated the effect of CP generated by DBD on cold smoked salmon packaged in a modified atmosphere of Ar/CO_2. In the study, slices of salmon were inoculated with two spoilage bacteria (*Lactobacillus sakei* and *Photobacterium phosphoreum*) and one food-borne pathogen (*L. monocytogenes*). Samples were treated with CP for 0, 60 and 120 s, respectively. The results showed that CP for 60 s reduced *P. phosphoreum* by 3 $\log_{10}$ CFU/g in cold smoked salmon packaged with Ar +7% CO_2, while it took 120 s to achieve the same log reduction in the fish spoilage bacteria when salmon was packaged only with Ar. However, no significant effect was detected on *L. sakei* or *L. monocytogenes* when CP was applied for 120 s in Ar or Ar/CO_2 package. In addition, the effect of atmospheric DBD on microbial inactivation and other quality parameters of mackerel fillets was investigated, in which the voltage of 70 and 80 kV, and plasma exposure time of 1, 3 and 5 min were studied. Promising reduction of all the spoilage bacteria (TAP, *Pseudomonas* and LAB) was observed, depending heavily on the voltage and treatment time. Quality results showed that pH and color remained almost the same, but lipid oxidation was significantly increased, suggesting a combination of antioxidants with CP to minimize the negative effect (Albertos et al. 2017).

Choi, Puligundla, and Mok (2016) and Choi, Puligundla, and Mok (2017) investigated the effect of corona discharge plasma jet (CDPJ) on microbial reduction, physico-chemical and sensory characteristics of dried Alaska Pollock shreds and squid shreds. In these two studies, all the spoilage or pathogenic bacteria, molds and yeasts researched were significantly reduced. Except for moisture, TBARS in dried

Table 5. Microbial inactivation by ozone in fish products.

Product	Target microorganism	Treatment conditions	Microbial inactivation outputs (log_{10} cfu/g)	Reference
Nile tilapia (whole and fillets)	Total mesophilic count	0.5, 1.0, 1.5 ppm for 5, 10, and 15 min at 11 °C	58.77%to 88.2% and 25.3 to 79.4% reductions were obtained from low to highest concentration in whole fish and fish fillets, respectively	De Mendonça Silva and Gonçalves (2017)
European anchovy and sardine	TVC, *Pseudomonas* spp, *Bacillus* spp, *Shewanella* spp.	Ozonized slurry ice of 0.3 mg/L with either one-time treatment and or repeated ozone treatments	TVC and *Pseudomonas* spp were lower by repeated treatment than one-time treatment	Bono et al. (2017)
Pacific white shrimp	Aerobic plate count (APC)	Sequential minimal ozone treatment (100mg/h for 60 s)	1 log_{10} cfu/g after 11 days ice-stored	Okpala (2014)
Atlantic salmon fillet	*Listeria innocua* APC	3 spray passes (1 ppm ozone) immediately after treatment 3 spray passes (1.5 ppm ozone) immediately after treatment	1.17 log_{10} cfu/g 1.05 log_{10} cfu/g	Crowe, Bushway, and Davis-Dentici (2012)
Stripped red mullet	Total viable count (TVC)	0.3 ppm ozonated water at 5 °C for 10 min + MAP (50% N_2+ 50% CO_2)	1.2 log_{10} cfu/g; Maximum TVC level of acceptability for marine fish (6 log_{10} cfu/g) achieved at 21 d of storage at 1 °C	Bono and Badalucco (2012)
Farmed blackspot seabream	TVC and psychrotrophic count	0.2 ppm ozone + flow ice (40% ice +60% water) at -1.5 °C	Slow down microbial development (1.00-3.53) compared to flow ice (FI)	Álvarez et al. (2009)
Farmed cod	Psychrotrophic bacteria, APC, H_2S-producing bacteria	Ozonized water (2 ppm ozone) for 30 min + MAP (60% CO_2+40% N_2)	No significant extension of shelf-life at 4 °C	Hovda et al. (2007)
Farmed turbot	TVC Psychrotrophic count	0.2 ppm ozone + slurry ice (40% ice +60% water) at -1.5 °C	1.79 log_{10}cfu/cm^2 after 28 days at 2 °C	Campos et al. (2006)
Sardine	Total aerobic and psychrotrophic count	0.17 ppm ozone + slurry ice (40% ice +60% water) at 1.5 °C	Reduction in anaerobes, psychrotrophic bacteria, proteolytic and lipolytic microorganisms along storage	Campos et al. (2005)

Pollock shreds, and moisture, TBARS, pH in dried squid shreds, all the other tested properties were significantly affected, however, these factors resulted in minimal-to-no effect on quality of the two shreds, and 2 min exposure time was found to be the optimal condition.

Ozone

Fundamentals

Ozone (O_3) is an allotropic form of oxygen (O_2), being one of the most powerful oxidants. Excessive ozone decomposes automatically and rapidly to produce oxygen and thus leaves no residues in foods. The multifunctionality of O_3 makes it a promising agent in food processing. In 1997, ozone received GRAS (Generally Recognized as Safe) status from USFDA, and then USFDA officially approved direct contact of O_3 with food products in 2001 (O'Donnell et al. 2012). Nowadays, ozone has been regarded as a cost-effective, safe and additive-free method in the food industry, and has been shown to be effective against gram-positive and gram-negative bacteria, fungi and yeasts, spores (Restaino et al. 1995; Khadre and Yousef 2001). Ozone has been applied to food disinfection, including fruit and vegetables(Crowe, Bushway, and Davis-Dentici 2012a; Ong et al. 2013; Ushida et al. 2017; Vlassi, Vlachos, and Kornaros 2018), meat products(Cárdenas et al. 2011; Cantalejo, Zouaghi, and Pérez-Arnedo 2016), fish and seafood (Crowe, Bushway, and Davis-Dentici 2012b; Okpala 2014), sanitation of equipment and packing films (Naitou and Takahara 2008), sterilization of drinking water and removal of odors (Guzel-Seydim, Greene, and Seydim 2004).

Ultraviolet radiation and corona discharge methods can be used to trigger the formation of free radical oxygen, thus generating ozone. The equipment of corona discharge method includes a high and a low tension electrode, which are separated by a ceramic dielectric medium and narrow discharge gap. When the electrons obtain enough energy (around 6–7 EV) to dissociate the oxygen molecule, some fraction of these collisions happen, leading to the formation of ozone molecular (Güzel-Seydim, Bever, and Greene 2004).

Ozone inactivates microorganisms by progressive oxidation of essential cellular constituents. Two major mechanisms about ozone sterilization have been identified (Victorin 1992). The oxidation of proteins, amino acids of enzymes and sulfhydryl groups to shorter peptides has been proposed as one mechanism and the oxidation of polyunsaturated fatty acids to acid peroxides by ozone as the other one. Ozone degradation of the unsaturated lipids leads to cell disruption and subsequent leakage of cellular components. The disinfection and sterilization efficiency of ozone depends on several factors, such as pH, relative humidity, temperature and the presence of organic matter (Kim, Yousef, and Dave 1999).

Applications in the fish industry

The antimicrobial effect of ozone has been studied in many fish species, including salmon, cod, sardine, turbot, red

mullet, sea bream, and shrimp (Table 5). Bono et al. (2017) combined different ozonized slurry-ice with superchilling storage (−1 °C) to detect the effects on two pelagic fish: European anchovy and sardine. The results showed that the combination of two methods at repeated ozone-treated ("Seq-T") produced better disinfection activity than one-time treated ("One-T"), in which TVC and *Pseudomonas* spp. values were lower. De Mendonça Silva and Gonçalves (2017) investigated the efficiency of three concentrations of ozonated water (0.5, 1.0, 1.5 ppm) for different contact time (5, 10, and 15 min) to Nile tilapia samples (whole and fillets). The antimicrobial effect was increased proportionally to increases in ozone concentration and contact time, with 88.25% and 79.49% reduction for whole tilapia and tilapia fillets at 1.5 ppm and contact for 15 min, respectively. Except for a little bit increase in TBARS, pH and color of tilapia fillets were not influenced by ozonated water. Crowe, Bushway, and Davis-Dentici (2012b) firstly studied the application of aqueous ozone sprays at concentrations of 1.0 and 1.5 mg/L to farmed Atlantic salmon fillets with high lipid content stored at 4 °C. In order to simulate closely industrial processing parameters, the number of passes under spray nozzles (1, 2, and 3) was also researched. Effective reduction in original aerobic counts and *L. innocua* were obtained without significantly affecting lipid oxidation, but some increase in TBARS and propanal value were observed. Although the extension of shelf-life in refrigerated salmon was not seen, the inactivation effects of ozone sprays offered an additional way for keeping salmon fillets at frozen storage. Feng et al. (2012) investigated the effects of tea polyphenol (TP) coating in combination with ozone water washing on striped black sea bream that stored for 15 days at 4 °C. Microbial loads, lipid oxidation, and protein decomposition were significantly reduced by TP + O_3 treatment, while improved characteristics in color, texture and sensory were observed compared with the controls (untreated, TP treated, or ozone treated only). All the above studies demonstrated that ozone is promising in the fish industry especially when it is combined with other technologies.

Conclusions

HPP, US, PEF, PL, CP and ozone are novel non-thermal food processing technologies developed in the past decades, and have received much attention in the food industry. These technologies have minor quality degradation compared with traditional thermal pasteurization. This review presents the main results obtained about the applications of the six technologies on microbial inactivation and other sensory quality parameters in the fish industry. Neither HPP nor PEF works on spore inactivation, but spores are sensitive to CP and ozone treatments. The results showed great potential alternatives of the non-thermal technologies to traditional preservation methods. However, some undesirable changes should not be neglected when harsh treatments were applied. The combination of synergistic technologies can increase microbial safety as well as sensory quality. For example, the combination of HPP with high temperature is effective in inactivating spores, which is also known as pressure-assisted thermal sterilization (PATS). Studies available also demonstrated that the combination of these novel technologies could obtain better microbial safety than single usage. In addition, the combination with modified atmospheric packing (MAP), the bacteriostatic nanophotocatalyst compositions (e.g. Fe_2O_3+ ZnO) on food packing films, oregano or rosemary on functional edible films, and tea polyphenol coating have been showed effective in minimizing the negative effects on sensory quality parameters of fish with improved microbial safety. Last but not the least, as fish are perishable products, it is important to ensure low temperature during supply-chain and storage. Moreover, a better understanding of mechanisms involved in the antimicrobial effect of HPP, US, PEF, PL, CP and ozone is required. Some challenges for industrial implementation also need to be solved since the majority of research is still at laboratory stage with limited large trials at industrial setting.

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