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Review

Preserving Raw Oysters with High Hydrostatic Pressure and Irradiation Technology

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Abstract: Refrigerated raw oysters, including Pacific oysters (*Magallana gigas*), eastern oysters (*Crassostrea virginica*), and European flat oysters (*Ostrea edulis*), are popular seafood products. Pathogenic contamination and spoilage during storage and transport limit their shelf life. High hydrostatic pressure (HHP) and irradiation effectively reduce pathogens and spoilage microorganisms in raw oysters while preserving their taste and texture. This review article presents a comprehensive analysis of the use of HHP and irradiation as sanitation methods for raw oysters, incorporating findings from geographical distribution, mathematical modeling, and radiation quality's impact on sterilization efficacy. The results demonstrate that untreated eastern oysters can maintain a total bacterial count below the recommended limit of 10^7 CFU/g for only 2–3 weeks at 5 °C, and are at risk of harboring pathogens such as *Vibrio* spp. and norovirus. HHP treatment at 600 MPa and irradiation treatment at 2 kGy can effectively reduce the pathogen load in raw oysters. However, supplementary measures such as additional cleaning or lower temperatures are required to prolong the shelf life of treated raw oysters to 2–3 weeks. Taken together, the application of HHP and irradiation to raw oyster sanitation represents a promising approach for enhancing the safety and quality of this beloved seafood delicacy.

Keywords: raw oyster; high hydrostatic pressure (HHP); irradiation

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1. Introduction

Raw oyster products are commonly distributed, stored, and sold using two main methods: one involves storing whole-shell oysters in a low-temperature, humid environment and the other involves storing shucked oyster meat in refrigerated water. Both methods are unable to reduce the pathogens present in raw oyster meat [1]. The presence of pathogens in oysters poses significant risks to public health and international trade [2]. Oysters are a common source of contamination and have been associated with outbreaks of gastroenteritis in humans [3]. Pathogenic microorganisms such as *Vibrio parahaemolyticus*, *V. vulnificus*, *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* can be found in oysters due to their interaction with the surrounding environment [4,5]. Despite this risk, raw oysters continue to be consumed due to consumer preferences [6].

Vibriosis, caused by consuming raw oysters contaminated with *V. parahaemolyticus*, is a significant concern [7]. Recent reports from the CDC have shown an increase in Vibriosis outbreaks [8]. *V. parahaemolyticus* naturally exists in the marine environment, where oysters are harvested [9,10]. Another pathogen, *V. vulnificus*, is commonly found in warm environments such as Gulf Coast waters and can be fatal, especially for individuals with underlying health conditions [2]. These bacteria require specific environmental conditions to thrive [11]. High salinity and temperatures between 15 °C and 35 °C promote the growth of *Vibrio* species. They are halophilic—thriving in environments with high salt concentrations—and are typically found in marine and estuarine waters.

Human norovirus (HuNoV) and Hepatitis A Virus (HAV) are recognized as significant viruses that can infect people through ingestion and fecal transmission [12]. These highly

infectious viruses have led to widespread outbreaks, causing symptoms such as diarrhea, vomiting, and fever [13]. Oysters can accumulate HuNoV through the filtration of seawater, and outbreaks have been associated with oysters harvested from contaminated waters [14–18]. With respect to the number of outbreaks and people affected, HuNoV and HAV are recognized as two of the most impactful viruses that can infect people after ingestion and then be transmitted through stool [19]. Controlling the prevalence and distribution of these viruses in shellfish waters is crucial to preventing outbreaks.

Bacterial proliferation in oysters can lead to protein breakdown and off-flavors [20]. Healthy oysters with tightly closed shells can survive out of water for weeks under cool and wet conditions, maintaining acceptable microbiological quality with an aerobic plate count below the suggested limit of 10^7 CFU/g [21]. Oysters possess immune cells such as hemocytes that can phagocytose bacteria, and they also produce antimicrobial peptides and reactive oxygen species (ROS) that exhibit antimicrobial properties [22,23]. These natural defense mechanisms contribute to maintaining the microbiological quality of oysters and reducing the risk of foodborne illnesses. These antimicrobial peptides act as natural defense agents, targeting the cell membranes or intracellular components of pathogens, while ROS molecules, such as superoxide anions and hydrogen peroxide, exert oxidative stress on bacteria, leading to their inactivation or destruction.

To ensure the safety and quality of oysters, it is crucial to have reliable methods to control disease risks and preserve the products, considering the significant economic impact of the oyster industry [24]. In addition to established methods, emerging techniques such as ultrasounds (US), non-thermal atmospheric plasma (NTAP), pulsed electric fields (PEF), and electrolyzed water (EW) offer alternative approaches to conventional heat treatments for oyster sanitation [25]. These innovative methods show promise in reducing pathogens and spoilage microorganisms while preserving the sensory attributes of oysters. Recent studies and reviews in the field provide further insights into the application and effectiveness of these novel techniques.

While high hydrostatic pressure (HHP) and irradiation have been effective in reducing pathogens in oysters, additional measures are required to maintain microbiological safety and quality during storage and distribution. HHP treatment can reduce bacterial counts but may not eliminate all pathogens, and the microbiota can still grow during refrigerated storage [26–28]. Similarly, irradiation treatment can reduce *V. vulnificus*, but microbial populations can rebound during storage, indicating the need for supplementary treatments [29]. Moreover, HHP treatment alone may not completely prevent the growth of spoilage bacteria, including both pathogenic and non-pathogenic strains, during storage [30]. Studies have shown that an HHP-based hurdle approach can have a synergistic effect when combined with other treatments, such as mild heat, in enhancing the safety and quality of raw oysters [31]. It was demonstrated that combining HHP with mild heat treatment inhibited the growth of pathogens in raw oysters and extended their shelf life. It is important to note that these additional treatments may alter the texture and flavor of raw oysters. Further research in this area might contribute to the development of effective preservation strategies for ensuring the safety and maintaining the desired sensory properties of raw oysters. Implementing supplementary key parameters that can act in parallel or with a synergistic action, such as proper temperature control, packaging techniques, and regular microbiological testing, is essential to ensure the safety of raw oysters and prevent microbial growth and contamination.

This review provides an overview of the effectiveness of sanitation methods for raw oysters in inactivating pathogens and reducing spoilage bacteria without compromising the raw state of the food. It is based on academic research and industrial experience, emphasizing the need for comprehensive approaches to maintain the safety and quality of oysters throughout the production and distribution process.

2. High Hydrostatic Pressure Treatment

HHP is a non-thermal processing technique that has gained significant attention in the food industry for its ability to inactivate microorganisms and extend the shelf life of various food products, including raw oysters. HHP involves subjecting food to elevated pressure levels, typically ranging from 100 to 600 megapascals (MPa), which leads to the destruction of bacteria, viruses, molds, and yeasts [32]. It operates at ambient or moderately low temperatures, minimizing the negative impact on the sensory attributes of raw oysters, such as taste, texture, and color. HHP treatment does not require the use of chemicals or additives, making it a clean and environmentally friendly technology.

2.1. HHP Operation Steps and Research Findings

HHP operation involves several steps. First, the oysters are carefully inspected and sorted to ensure their quality. Then, they are loaded into the HHP machine by vessel. The machine applies intense pressure to the oysters [32]. The oysters are held under pressure for a specific duration, allowing the pressure to penetrate and neutralize pathogens and bacteria. After the holding time, the pressure is gradually released, and the oysters undergo quality control measures. Table 1 summarizes research on the inactivation of internalized pathogens by HHP with different operating conditions, conducted on various oyster species in different areas. A study conducted by Ma and Su demonstrated the effectiveness of HHP in reducing *V. parahaemolyticus* in Pacific oysters. The research showed a reduction of over 3.52 logs of *V. parahaemolyticus* using HHP treatment [33]. The study also reported that the shelf life of the treated oysters was 6–8 days when stored at 5 °C or 16–18 days when stored in ice [33]. A study conducted in Virginia investigated the impact of high-pressure processing (HPP) on *V. parahaemolyticus* and *V. vulnificus* in whole eastern oysters. The results showed that at 586 MPa, both *V. parahaemolyticus* and *V. vulnificus* were reduced to non-detectable levels during the 7–8 min pressure come-up times [34]. The sensitivity of suspended *V. vulnificus* and *V. parahaemolyticus* to high-pressure processing (HPP) in relation to whole eastern oysters was investigated in Mississippi. The oysters were exposed to pressures ranging from 207 to 379 MPa for 1 to 20 min. The study reported that the sensitivity to HPP varied among bacterial populations, indicating that not all strains exhibited the same response to HPP treatment [35]. A 5-log MPN/g reduction of *V. parahaemolyticus* and the complete inactivation of *V. vulnificus* were achieved at 300 MPa for 2 min in Delaware oysters, and treatment temperatures did not significantly affect the pressure inactivation of *V. parahaemolyticus* or *V. vulnificus* [36]. Overall, while HHP has been shown to effectively reduce *Vibrio* populations in oysters, it is not sufficient to keep raw oysters free from spoilage for extended periods without additional treatment. In combination with HHP, additional treatments such as mild heat are used to optimize pathogen reduction in raw oysters [31].

2.2. Inactivation of Norovirus, Hepatitis A Virus (HAV), and Microbial Models

The inactivation of 6.85 log10 PFU of internalized mouse norovirus 1 (MNV-1, a surrogate for human norovirus) was achieved in seeded eastern oysters harvested in Mobile Bay, AL, USA by 450 MPa HHP treatment [37]. The authors demonstrated that norovirus can be inactivated by high pressure and suggested that propagable strains of human norovirus may also be inactivated in oysters using HHP. The authors further noted that the rate of inactivation decreased over time, which was consistent with Weibull inactivation kinetics, and suggested that there are at least two groups of MNV-1 with different susceptibility to HHP. While the first-order inactivation kinetic model is the most famous expression of microbial inactivation and is still commonly used in the thermal processing industry, the Baranyi and bi-phasic models have demonstrated that there are two or more susceptible groups of microorganisms in oysters to HHP treatments. The modeling concept for high hydrostatic pressure-induced microbial inactivation has been described previously [38], and these previous reports have shown that the two-stage model is effective

for predicting the inactivation of foodborne pathogens such as *E. coli*, *V. vulnificus*, and *V. parahaemolyticus* by HHP.

Table 1. Inactivation of the internalized pathogen by HHP with various operating conditions.

Pathogen	Species/Region	HHP Condition	Results	Reference
V. parahaemolyticus	Pacific oysters/ Oregon	293 MPa for 90, 120, 150, 180, or 210 s at 8 ± 1 °C	3.52-log reductions at 120 s	[30]
V. vulnificus and *V. parahaemolyticus*	Eastern oysters/ Virginia	241, 276, 310, 345, 379, and 586 MPa for 0 to 10 min at 5–10 °C	D-values; linear regression	[31]
V. vulnificus and *V. parahaemolyticus*	Eastern oysters/ Mississippi	from 207 to 379 MPa for 1 to 20 min	Weibull distribution provided the best fit for vibrio's inactivation.	[32]
V. vulnificus and *V. parahaemolyticus*	Eastern oysters/ Delaware	from 225 to 300 MPa for 2 min at 4, 21, or 35 °C	5-log MPN/g reduction of *V. parahaemolyticus* and complete inactivation *V. vulnificus*	[33]
MNV-1	Eastern oysters/ Alabama	from 350 to 450 MPa for 5 min at 20 °C	450-MPa treatment sufficient to inactivate 6.85 $\log_{10}$ PFU of MNV-1	[34]
HuNoV	Eastern oysters/ Rhode Island	400 MPa at 25 °C, 600 MPa at 6 °C, or 400 MPa at 6 °C for 5 min	complete inactivation of HuNoV at 600 MPa in seeded oysters	[36]
HAV	Eastern oysters/ Alabama	400 MPa for 5 min at 17–22 °C	2.56 $\log_{10}$ inactivation of HAV	[37]

In 2011, a randomized, double-blinded clinical trial was conducted on 44 healthy, positive-secretor adults in Rhode Island to assess the effect of HHP on HuNoV inactivation in seeded eastern oysters [39]. The complete inactivation of HuNoV in seeded oysters was achieved at 600 MPa of HHP, resulting in no HuNoV infection among the subjects and no positive results in detecting HuNoV RNA in their stool or vomitus samples using reverse transcription polymerase chain reaction. HHP at 350, 375, and 400 MPa was also applied for five-minute treatments at 20 °C to inactivate bioaccumulated HAV within oysters under simulated natural conditions to levels >10^5 PFU/oyster [40]. On average, a 2.56 $\log_{10}$ inactivation of HAV was achieved after a 400-MPa treatment for whole-shell oysters. Commercial HHP processing of whole-in-shell oysters was proven capable of inactivating HAV pathogens. However, whether in an academic research experiment or commercial run, opening of the oyster occurred after the oyster was treated with HHP pressure because the adductor muscle was separated from the shell. As open oysters do not meet market demand for whole-shell oysters, producers must use rubber bands to secure the oysters before HHP treatment to prevent them from opening.

2.3. Microbial Resistance and Shelf Life Extension

Some research examples of microbial resistance and shelf life extension of oysters treated with HHP are presented in Table 2. Some microorganisms are resistant to prolonged HHP pressure treatments of up to five minutes under pressures of 400–600 MPa. In a study conducted in Spain, the effect of high-pressure treatment was investigated on European flat oysters (*Ostrea edulis*) [41]. The results showed that after treatment at 400 MPa for 10 min, the total microbial count only exhibited a slight reduction, from 3.13 to 2.72 log CFU/g. In another study, researchers reported that the total aerobic count of class A water-farmed Pacific oysters was reduced from 800 to less than 200 CFU/g after treatment at 260 MPa for 5 min, and to below the detection limit after treatment at 400 or 600 MPa [42]. These findings highlight the influence of water quality and oyster species on the effectiveness of HHP in microbial inactivation. Furthermore, a study observed a reduction in the total aerobic count of raw eastern oysters from 10^5 to 10^4 CFU/g after treatment at 400 MPa for 3 min [43]. The authors concluded this reduction by assessing bacterial loads and microbial composition during oyster storage. The study provided insights into the impact of HHP treatment on the main spoilage bacteria present in oysters. These studies demonstrate

that increased pressure and exposure time of HHP can reduce the total bacterial count in oysters, thereby prolonging their shelf life.

Table 2. Microbiological changes in raw oysters after being processed by HHP.

Microbiological Quality	Species/Region	HHP Condition	Results	Reference
microbial flora, total volatile bases, pH, and texture	European flat oyster/Spain	400 MPa for 10 min at 7 °C	reduced from 3.13 to 2.72	[38]
bacterial loads during storage	Eastern oysters/Louisiana	from 250 to 400 MPa for 1–3 min	bacterial loads of treated oysters reached 10^8 CFU/g at 14 days	[40]
total microflora during storage (2 °C)	Pacific oysters/Ireland	260, 400, and 600 MPa for 5 min at 20 °C	reduced to below the detection limit after treatment at 400 and 600 MPa	[41]
total microflora during storage (2 °C)	Pacific oysters/Ireland	260 MPa for 3 min at 20 °C	reduced to <200/g	[39]
		500 and 800 MPa for 5 min at 20 °C	reduced to below the detection limit	
total microflora during storage in ice	Pacific oysters/Oregon	293 MPa for 90, 120, 150, 180, or 210 s at 8 ± 1 °C	processed oysters had a shelf life of 8 days at 5 °C, shorter than the 18-day shelf life of live oysters stored under the same conditions	[30]

A study in Spain analyzed the effect of a 10 min HHP treatment (400 MPa at 7 °C) on the microbial flora, total volatile bases, pH, and texture of purified and unpurified European flat oysters; the counts of the target microorganisms were reduced to as low as 5-log cycles or below the detection threshold [44]. This study found that HHP-treated oysters exhibited better preservation characteristics during storage compared to non-pressurized samples. Specifically, the HHP-treated samples showed less deterioration in terms of microbial flora, total volatile bases, pH, and texture. In another study, untreated Pacific oysters did not reach the limit of acceptability in terms of total viable bacteria during storage on ice at 2 °C for up to 31 days [42]. HHP treatment at 260 MPa for 5 min at 20 °C resulted in a product with counts of <200/g and below the detection limit after treatment at 400 or 600 MPa, but the limit of acceptability in terms of total viable count (TVC) for oysters HHP-treated at 260 MPa was reached after 15 days of storage. The total microflora of oysters in-shell grew after HHP treatment at 260 MPa, 400, or 600 MPa during storage on ice at 2 °C, possibly due to protection against spoilage by the immune system of live oysters. However, HHP processing can still inactivate microorganisms and delay microbial growth in chilled, stored, unpacked, or vacuum-packed oysters, with a criterion set at 5×10^5 CFU/g of oyster tissue [44]. Using this criterion, untreated oysters stored aerobically at 2 °C reached the upper limit of acceptability on day 18. Oysters HHP-treated at 260 MPa stored aerobically or vacuum-packed reached the limit of acceptability in terms of TVC on day 12 or 17, respectively. For oysters HHP-treated at 500 or 800 MPa and stored aerobically at 2 °C, the TVC limit of acceptability was reached at 17 days of storage, while for HP-treated oysters (500 or 800 MPa) stored vacuum-packed, the limit of acceptability was not reached during storage, an appearance of off-flavor was not noticed, and product shelf life exceeded 21 days.

Live oysters can be distributed and sold if they are handled with care and kept moist as their aerobic plate count can be maintained below the suggested limit of 10^7 CFU/g [21,45]. Untreated oysters have a refrigerated shelf life of 18–20 days [21,45]. However, a study reported that HHP-treated oysters stored at 5 °C for 8 days were spoiled by bacteria that survived the pressure treatment [41]. To extend the shelf life of HHP-shucked oysters, alternative strategies include reducing the inherent bacteria number by increasing the pressure and treatment time, washing oysters before HPP, and lowering the storage temperature. A study found that the shelf life of HHP-shucked oysters could be increased to 18 days by lowering the storage temperature from 5 to 2 °C and 17 days by cleaning oysters before HPP and storing them in ice [44]. Additionally, processing oysters at pressures of 293 MPa or higher for a longer period could also extend their shelf life.

2.4. Shucking Process and Quality Improvement

Shucking is the process of removing meat from the shell or detaching it, which is typically done by hand using a knife. Various devices have been developed to reduce the labor involved in hand shucking, but many methods have drawbacks, such as the risk of cooking the meat. HPP has been used to facilitate commercial shucking by detaching the adductor muscle from the shells at lower pressure ranges of 250 to 300 MPa [46]. In Taiwan, it was found that HPP treatments of 250 and 300 MPa for 2 min and 0 min, respectively, resulted in a 100% release of the meat. HPP processing at these pressures also resulted in higher pH and moisture content of the oysters compared to untreated oysters, which improved the quality and acceptability of cooked oysters [47]. Previous studies have shown that HPP-treated oysters have higher lightness (Hunter L) values than untreated oysters, and the lightness values increase with the magnitude of pressure changes [48]. HPP-treated oysters also have a more voluminous and juicy appearance to raw oyster tissue, with increased moisture content and decreased ash and protein contents compared to untreated oysters [48].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a widely used method for separating proteins based on their molecular weight. In this technique, proteins are denatured and then loaded onto a gel matrix containing polyacrylamide. An electric field is then applied, causing the proteins to migrate through the gel according to their molecular weight. The proteins are stained and can be visualized as distinct bands corresponding to their molecular weight. HHP treatment can induce the formation of protein aggregates in oysters' adductor muscles. Disulfide bond formation leads to the aggregation of proteins, which can be observed using techniques such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). [48]. The technique has also been used to identify different shellfish species and analyze their soluble protein profiles, with Pacific and Atlantic oysters displaying distinct protein bands with different molecular weights [49].

3. Irradiation Treatment

3.1. Ionizing Radiation Mechanisms and Global Application

Ionizing radiation, such as gamma rays, X-rays, and electron beams, are capable of depositing energy gradually as they traverse through matter [50]. As these irradiation photons pass through matter, they deposit energy through three different mechanisms: the photoelectric effect, Compton scattering, and pair production. At low energies, the photoelectric effect is the largest contributor to energy deposition, while Compton scattering dominates at intermediate energies. At high energies, pair production is the principal absorption mechanism for gamma rays, electron beams, and X-rays, but this is less relevant in food irradiation applications.

In 2005, an estimated 405,000 tons of food was irradiated globally [50]. This included 186,000 tons of irradiated spices and dry vegetables for disinfection, 82,000 tons of irradiated grains and fruits, 32,000 tons of irradiated meat and fish, and 88,000 tons of irradiated garlic and potatoes for inhibiting sprouting. An additional 17,000 tons of other food items, including health foods, mushrooms, honey, and more, was also irradiated [51]. China has been at the forefront of using irradiation for food processing, with an estimated total of over 500,000 tons of irradiated foods [52].

3.2. Gamma Radiation and Vibrio Reduction

In the United States, live oysters and blanched shellfish from the Gulf of Mexico are irradiated to reduce the risk of contamination by virulent microorganisms, particularly *Vibrio* spp. [53]. One commercial example of successful seafood irradiation is Gateway America, located in Gulfport, Mississippi, which uses a pool-type irradiator for food sanitation. Despite the potential benefits of food irradiation, there is still some public concern about its safety and efficacy. As a result, it remains a controversial topic in the food industry and among consumers. Gamma radiation exposure can significantly reduce the amount of *V. vulnificus* (MO-624) that is bio-accumulated in live oysters (*Crassostrea virginica*). A dose of 0.75–1.0 kGy gamma radiation exposure was able to reduce

the level of *V. vulnificus* from 10^6 cfu/g oyster meat to nondetectable levels (<3 mpn/g oyster meat) [54]. Similarly, when live oysters were exposed to gamma radiation doses ranging from 0.5 to 3.0 kGy, a dose of 1.0 kGy was found to produce a 6-log reduction in the level of *V. parahaemolyticus*, without affecting the oysters' sensory attributes or survival [55]. The irradiation process for sterilizing oysters involves the use of different equipment depending on the type of radiation. In the case of gamma-ray treatment, a rack system is used instead of a conveyor belt. The oysters are arranged on racks and placed inside the irradiation chamber. The gamma rays emitted by the source effectively sterilize the oysters. After the irradiation process, the oysters are packaged, labeled, and distributed, adhering to regulatory requirements for irradiated food products.

3.3. Optimization and Efficacy of X-ray and E-Beam Irradiation

For X-ray and electron beam (E-beam) treatments, a conveyor belt system is employed. The oysters are carefully prepared and placed in containers, which are then transported on the conveyor belt through the irradiation chamber. The oysters are exposed to the radiation emitted by the source, penetrating their shells and disrupting microbial DNA. Two groups of researchers found that a 0.5 kGy exposure to 60 Co gamma rays or 150 keV X-rays resulted in a 4-log reduction of *V. parahaemolyticus* in pure cultures [56,57]. However, when whole-shell oysters were exposed to these forms of radiation, a 1 kGy dose of gamma radiation resulted in a 6-log reduction of inoculated *V. parahaemolyticus*, while 5 kGy X-rays were needed to achieve a similar reduction [9,56,57]. This suggests that gamma radiation may be more effective than X-rays in inactivating *V. parahaemolyticus* in live oysters.

However, recent studies have shown that the effectiveness of X-ray radiation can be improved by optimizing the energy level and adjusting the irradiation filter and acceleration voltage. For example, an adjustable filter system using 350 keV X-rays was found to be effective in inactivating *V. parahaemolyticus* pure culture without a filter and achieved a higher inactivation than previous reports using gamma radiation or filtered X-rays as sources of irradiation [58]. Furthermore, by optimizing the accelerating voltage and filtering out low-energy X-rays, 350 kV X-rays were found to be as effective as gamma rays in inactivating *V. parahaemolyticus* in live oysters, achieving a 6-log MPN g^{-1} reduction with 1250 Gy.

Another *Vibrio* species, *V. vulnificus*, has also been studied for its response to ionizing radiation. A 3.0 kGy exposure to 150 keV X-rays was found to result in greater than a 6-log reduction of *V. vulnificus* in whole-shell oysters [3]. These findings suggest that ionizing radiation, particularly gamma radiation and optimized X-ray radiation, may be a promising method for reducing the risk of *Vibrio* spp. contamination in live oysters.

The difficulty in growing HuNoV in laboratory settings has led researchers to use surrogate models to investigate the efficacy of various treatments in reducing viral loads in oysters. One such study utilized murine norovirus-1 (MNV-1) as a surrogate model and evaluated the effectiveness of X-ray doses in reducing MNV-1 in half-shell oysters. Plaque assays were used to determine the susceptibility of MNV-1 to X-ray treatments, and the results showed that 4.0 kGy X-ray achieved a reduction of 2.7 log PFU g^{-1} in half-shell oysters [59]. Further experimentation with X-ray treatments showed that 5.0 kGy X-ray reduced MNV-1 from approximately 5.5 log PFU g^{-1} to below the detection limit (<2.0 log PFU g^{-1}) in half-shell oysters. Additionally, X-ray treatments significantly reduced the population of internalized MNV-1 in live oysters. The initial population of 4.3 ± 0.4 log PFU g^{-1} was reduced to 3.6 ± 0.5, 3.2 ± 0.2, 2.8 ± 0.2, and 2.5 ± 0.1 log PFU g^{-1} by 1.0, 2.0, 3.0, and 4.0 kGy X-ray, respectively [21].

Another study evaluated the inactivation of HAV and MNV-1 in oysters (*Crassostrea virginica*) using electron beam (E-beam) irradiation [60]. This study quantified the reduction of potential infection risks for E-beam irradiation technology on raw oysters at various virus contamination levels. The results showed that the E-beam dose required to reduce MNV-1 and HAV titers by 90% (D10 value) in whole oysters was achievable with E-beam treatment. With treatment of 5-kGy E-beam achieved a 26% reduction (from 2.74 to 2.03

out of 10 persons) in HuNoV and a 91% reduction (from 2.1 to 1.93 out of 100 persons) in HAV infection risks. In conclusion, studies have shown that X-ray and E-beam irradiation treatments can effectively reduce the population of pathogenic microorganisms, such as *Vibrio vulnificus* and norovirus, in oysters. These treatments can significantly decrease the risk of viral illness, though complete elimination of the risk cannot be guaranteed.

3.4. Microbial Resistance and Shelf Life Extension

It has been reported that the viability of live eastern oysters is not significantly affected by treatment with 5.0 kGy X-ray, compared to the control, for up to 10 days during storage at 5 °C [45]. The mesophilic counts for the control samples of whole shell oysters were 5.09 ± 0.05, 5.69 ± 0.21, 5.83 ± 0.48, 6.84 ± 1.08, and 6.98 ± 1.27 log CFU g^{-1} on day 0, 5, 10, 15, and 20, respectively, during storage at 5 °C. Treatment with X-ray kept the mesophilic counts lower than the control until day 20. These research findings indicate that ionizing irradiation has the potential to reduce overall bacterial counts, potentially mitigating food spoilage issues. Retailers could sell live oysters treated with cold sterilization by irradiation [21]. Researchers also conducted shucking of Mangrove Oyster (*Crassostrea belcheri*) meat, individually packed it in plastic bags, irradiated it with 1 kGy gamma ray, and stored it in chilled conditions (4 ± 2 °C) [61]. Their results showed that a radiation dose of 1.0 kGy can completely eliminate 5 log CFU g^{-1} of initially inoculated *Salmonella Weltevreden* throughout the 30-day storage time. These irradiated products had an equivalent sensory quality to the non-irradiated ones and had a 3-day extension of shelf life.

4. Future Perspectives

Based on available information, it has been observed that on the Gulf Coast, both high hydrostatic pressure (HHP) and irradiation methods have been adopted for microbial inactivation in raw oyster products. These technologies have gained significant commercial utilization in this area. The HHP method involves subjecting oysters to high pressure, which facilitates the natural detachment of oyster meat. The processed meat is then collected and packaged for sale. Another approach is the individual wrapping of oysters with rubber bands prior to HHP treatment, which helps preserve their flavor and maintain the integrity of the whole oyster. In the case of irradiation, food packed in containers is submerged in a pool and treated using a cobalt-60 irradiation source. It is worth noting that this region has seen the establishment of multiple facilities utilizing these technologies for oyster processing. Efforts are also underway to construct additional facilities in Texas and New Jersey to meet the growing demand.

The utilization of high-pressure processing (HPP) treatments and gamma irradiation for microbial inactivation in oysters has been extensively studied. However, the presence of multiple susceptible groups of microorganisms in oysters, as suggested by studies using Baranyi's model and the bi-phasic model, poses a challenge in achieving the complete eradication of bacteria with these methods [37,38]. The inactivation curves observed during HPP treatments exhibit an exponential death phase followed by a tailing effect, further complicating the sterilization process. Additionally, the variation in microbial populations in oysters from different environments adds to the complexity of sterilization outcomes. To address these challenges and enhance microbial inactivation and the preservation of seafood products, alternative methods such as non-thermal atmospheric plasma (NTAP), pulsed electric fields (PEF), and electrolyzed water (EW) have emerged alongside conventional heat treatments [25]. NTAP involves the use of ionized gases to generate reactive species that can effectively destroy microorganisms. PEF utilizes short electric pulses to disrupt cell membranes, leading to microbial inactivation. EW is produced by passing an electric current through a dilute salt water solution, resulting in the generation of antimicrobial agents. Combining these alternative methods with HPP or irradiation treatments presents a promising approach for improving sterilization and preservation techniques. The integration of NTAP, PEF, and EW with HPP or radiation treatment may offer synergistic effects, enhancing microbial inactivation efficiency while preserving the biochemical and

sensory quality of oysters and other seafood products. Further research and development in this area are needed to optimize the combined approaches and ensure their practical application in the seafood industry.

Gamma-ray irradiation has been widely used for food sterilization purposes, but it can be a time-consuming process. However, alternatives such as X-rays and E-beam offer potential solutions by allowing products to be treated while being transported on conveyor belts, thereby streamlining the process. X-ray irradiation has shown promising results as an effective method for microbial inactivation in various food products [62]. The advantage of X-ray irradiation lies in its ability to penetrate deep into the food product, ensuring uniform microbial inactivation throughout. Similarly, E-beam irradiation has gained attention as an efficient and practical technology for food sterilization [63]. The advantage of E-beam irradiation lies in its ability to provide precise control over the irradiation dosage and uniform treatment coverage, resulting in consistent and reliable microbial inactivation. Both X-ray and E-beam irradiation offer the advantage of continuous treatment, as products can be exposed to the radiation source while being transported on a conveyor belt. This eliminates the need for individual batch processing, leading to increased efficiency and reduced processing times.

The promotion of irradiated food still faces consumer acceptance issues [64]. The sterilization of food through irradiation should be accompanied by the scientific promotion of irradiated food, aiming to increase public awareness and provide authoritative information, thus advancing the commercialization of irradiated food.

5. Conclusions

The seafood industry has adopted various techniques to reduce the risks associated with raw oyster consumption. HHP and irradiation are two effective methods that have been commercialized for oyster sterilization while maintaining their raw state. Although a complete sterilization effect like that of canned goods is unlikely to occur due to the complex microbial population in oysters, both HHP and irradiation have been shown to suppress pathogenic and spoilage microorganisms, thereby extending their shelf life. However, it is important to note that the number of microorganisms may increase under refrigerated storage conditions, requiring additional measures to prevent their growth. Overall, the successful implementation of these technologies in the seafood industry depends on their ability to ensure safety without compromising the quality and sensory attributes of the final product. Further research is needed to fully understand the mechanisms of action and potential limitations of HHP and irradiation, as well as to develop comprehensive safety protocols that take into account the entire seafood supply chain.

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