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MINI REVIEW

Microbial diversity of bivalve shellfish and the use of innovative technologies for preservation, monitoring and shelf-life extension

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Abstract

Bivalve molluscs, comprising oysters and mussels, are important seafood products as they represent over 56% of marine and coastal production and account for 12% of the seafood trade with a value of over USD 34 billion dollars. Bivalve molluscs are increasingly popular among consumers because of their high nutritional value and are considered sustainable seafood products as they do not require feed inputs and can make a significant contribution to food security. As filter feeders, bivalve molluscs can accumulate microorganisms, and improper post-harvesting handling and storage procedures could support the growth of spoilage and pathogenic microorganisms, causing spoilage and potential safety issues. At the same time, there is an increasing demand by consumers for fresh and minimally processed foods. Therefore, understanding the microbial diversity of bivalve molluscs and methods to control microbial growth is of increasing research interest. This review highlighted the recent developments in the understanding of the microbial community of bivalve molluscs and the use of innovative technologies for the preservation and shelf-life extension of seafood.

1. Introduction

Seafood has been described as a sustainable source of protein for the growing world population (FAO, 2020). According to the Food and Agricultural Organisation (FAO) data 2010-2015, marine bivalves' global production exceeds 15 million tonnes annually, making them an important food source (FAO, 2020). Bivalve molluscs represented 56.3% (17.3 million tonnes) of marine and coastal production valued at USD 34.6 billion and accounted for 12% of seafood trade value in 2018 (FAO, 2020). As with other seafood, the production of bivalve molluscs has increased in the past decades from approximately 5 million tonnes in 1990 to 17.3 million tonnes in 2018, primarily from aquaculture. In 2017, bivalve molluscs consumed at 2.6 kg per capita globally (FAO, 2020). Bivalve molluscs such as oysters and mussels are filter feeders and, therefore, accumulate microorganisms from the rearing environment. These microorganisms include bacteria, viruses, and protozoans, and form the microbiota of the organism. The presence of spoilage and pathogenic bacteria could impact the quality and safety of the seafood.

Bivalve molluscs are gathered from the majority of natural growth beds, regardless of pollution levels in those waters. Depuration, a technique that reduces the high-level toxic metals and high bacteria load in shellfish, is not adopted, and they are sold at ambient temperatures in the market, this practice promotes the growth of mesophilic bacteria like coliforms and human

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pathogenic bacteria (Hatha *et al.*, 2005). Bivalve is commonly sold fresh or dried in local marketplaces at the subsistence level of their products in Africa (Ukwo *et al.*, 2020).

Immediately a seafood species dies, spoilage begins because their regular defensive mechanisms fail, and a chain of events occurs that leads to spoilage. Bacteria, chemical action and enzymes all contribute to these alterations. The most common cause of seafood deterioration is bacteria. There are millions of bacteria on the slime surface, the gills, and in the guts of living seafood species. These bacteria or enzymes invade the flesh of seafood species through the blood vessels, directly through the skin, belly cavity lining and the gills. Bacteria thrive and grow in the flesh, creating substances that cause "fishy" flavours and odours, as well as discolourations typical with spoilt seafood (Singh *et al.*, 2011). Their enzymes continue to act, digesting or breaking down the flesh. This softens the flesh and reduces its quality. These enzymes also increase the rate of spoilage by providing more nutrients for bacteria to feed on. Oxygen from the air can oxidize unsaturated oils present in seafood, resulting in rancidity, off-flavours, and off-odours (Prabhakar *et al.*, 2020).

Various methods and technologies used to assess microbial diversity, including pathogens and spoilage bacteria in fish, have been described (Parlapani, 2021). Odeyemi *et al.* (2021) summarized the factors influencing microbial diversity in crustaceans. The use of biofloc technology and neutralized oxidized water at both pre-harvest and post-harvest levels to preserve the quality and shelf-life of crustaceans such as shrimps and prawns was also highlighted (Odeyemi *et al.*, 2021). Some seafood purification procedures used after harvesting to reduce microbial burden have been studied and they include ozone treatment, phage treatment, high-pressure processing, irradiation (Ronholm *et al.*, 2016), aerosolisation and ozonation (Sullivan *et al.*, 2020), modified atmosphere packaging (Sørensen *et al.*, 2020), dielectric barrier discharges (Albertos *et al.*, 2019), hyperbaric storage (Fidalgo *et al.*, 2021), pulsed electric field (Nowosad *et al.*, 2021), sous vide (Cropotova *et al.*, 2019), cold plasma (De Souza Silva *et al.*, 2019). However, none of these studies included or discussed the microbial diversity and shelf-life of bivalve molluscs.

This review, therefore, focused on the microbial diversity and shelf-life of shellfish and the use of innovative technologies for preservation, monitoring and shelf-life extension. In the first section of this review, the storage conditions and microbial diversity of the most widely consumed bivalves (mussels and oysters) were briefly discussed. The second section of the review focused on the innovative technologies for preservation, monitoring and shelf-life extension of shellfish.

2. Microbial diversity of oysters

Oysters are a group of rough-shelled water bivalve molluscs that in 2018 accounted for over 33% of aquaculture production, making them one of the largest farmed seafood both in quantity and value (Wijsman *et al.*, 2019; Botta *et al.*, 2020; Parlapani *et al.*, 2020). Oysters are members of the family *Ostreidae*, and commercially valuable species mainly belong to the genera *Ostrea*, *Crassostrea*, and *Saccostrea* (Vilanova, 2014).

Oysters are filter feeders and commonly consumed raw, thus, the food safety risks associated with them are of increasing interest (Araújo *et al.*, 2019). Culture environment contributes to sources of microbial contamination of seafood and this should be well considered (Chen *et al.*, 2019). The normal microbiota of oysters consists of many phyla representatives, including *Firmicutes, Proteobacteria, Cyanobacteria, Spirochaetes, Planctomycetes, Verrucomicrobia, Fusobacteria, Tenericutes,* and *Bacteroidetes*. Others include a wide diversity of bacterial genera such as *Lactococcus, Lactobacillus, Enterobacter, Pseudomonas, Acinetobacter, Vibrio, Photobacterium, Moraxella, Aeromonas, Micrococcus*, and *Bacillus* (Clerissi *et al.*, 2020). Yu *et al.* (2021) reported that *Lactococcus*, *Vibrio*, *Shewanella*, *Clostridium*, *Mycoplasma*, *Acinetobacter,* and *Aeromonas* were the most prevalent genera in pacific oysters. The microbial diversity of oysters depends on different factors such as the growing environment, temperature, and storage conditions (Chen *et al.*, 2019). Bacterial communities in oysters change after harvest, especially during refrigeration (Wang *et al.*, 2014). Prapaiwong *et al.* (2009) found that the total aerobic bacteria count was over 10^7 CFU/g in autumn after one week of storage at 4°C. According to Scanes *et al.* (2021), oysters are vulnerable to climate change resulting in different microbial diversity. Moreover, elevated temperatures and CO_2 can reduce the immunity of oysters making them susceptible to infection. Wang *et al.* (2014) established that the highest bacterial diversity in oysters appeared in autumn and the bacterial diversity in gills was higher than that in digestive glands and other tissues. A higher bacterial load in oysters is responsible for spoilage and, hence, the unacceptable quality, which results in a visible liquefied appearance (Chen *et al.*, 2019).

Early studies characterising the microbial populations present in oysters by culture-dependent methods revealed the dominance of *Vibrio* and *Pseudomonas* spp. (Cao *et al.*, 2009; Fernandez-Piquer *et*

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al., 2012). However, more recent investigations on the microbial profiles of oysters using molecular techniques such as denaturing gradient gel electrophoresis (DGGE) and next-generation sequencing have revealed diverse bacterial species including spoilage and pathogenic bacteria (Table 1).

Important spoilage bacteria in oysters include *Pseudoalteromonas* and *Vibrio* (Madigan *et al.*, 2014). However, some of the bacterial genera represented in oysters may also include pathogens that are natural inhabitants in cultured water such as *Vibrio parahaemolyticus* and *Vibrio vulnificus*, whereas other species such as *Vibrio cholerae*, *Salmonella*, *Escherichia coli*, *Shigella*, *Campylobacter fasci,* and *Yersinia enterocolitica* are associated with faecal contamination. These pathogenic bacteria represent a public health risk after oyster consumption, but they can also cause the mortality of farmed oysters (Horodesky *et al.*, 2020).

3. Microbial diversity of mussels

Mussels refer to numerous bivalve mollusc species inhabiting both marine (*Mytilidae* family) and freshwater (*Unionidae* family) habitats. Commercially important mussels are predominantly marine and include blue (*Mytilus edilus*), Mediterranean (*M. galloprovincialis*), green (*Perna viridis*), Chilean (*M. chilensis*), South American rock (*P. perna*) and green-lipped (*P. canaliculus*) mussels (Duncan, 2003). Similar to other seafood products, global production of mussels by aquaculture has increased significantly over the last 70 years reaching 2 million tonnes in 2016, with a trade value of 3.8 billion USD (FAO, 2019).

Mussels are considered highly nutritious and a rich source of polyunsaturated fatty acids, proteins, minerals, and vitamins (Cherifi *et al.*, 2018; López *et al.*, 2018; Khan and Liu, 2019). Beyond their traditional role in the diet, mussels are important actors in aquatic ecosystems. Mussels purify water by filtering out particulate matter, which then serves as a source of nutrients for other organisms (van der Schatte Olivier *et al.*, 2020). Mussels also contribute to nutrient cycling and enrich biodiversity by acting as a habitat for other organisms. They are also used as bio-monitors for environmental contaminants such as microplastics (Catarino *et al.*, 2018; Vaughn, 2018; Weingarten *et al.*, 2019).

Mussels are highly perishable with a shelf-life of only a few days (Odeyemi *et al.*, 2018; Tosun *et al.*, 2018; Xin *et al.*, 2021). Mussels are lightly cooked before consumption and coupled with increasing consumer demand for fresher and more minimally processed seafood with preserved nutritional content, there is increasing interest in unravelling the microbial community associated with mussels (Bongiorno *et al.*, 2018; Jeon *et al.*, 2020). It has been suggested that local sources such as water and sediment make a significant contribution to the microbiota of mussels (Mathai *et al.*, 2020). As filter feeders, mussels can accumulate microorganisms from their environment. In addition, improper practices during processing can introduce both spoilage and pathogenic microorganisms (Odeyemi *et al.*, 2019).

Some studies examining the microbial diversity of mussels are summarised in Table 2. There is scarce information about the natural microbiota of mussels, as most studies focus on spoilage or pathogenic bacteria (Odeyemi *et al.*, 2019). The gut microbiome of *M. chilensis* was dominated by species of *Vibrio*, *Psychrilyobacter*, *Mycoplasma,* and *Psychromonas* (Santibañez *et al.*, 2022). Utermann *et al*. (2018)

Table 1. Microbial diversity of oysters

Oyster	Bacterial communities	Reference
Pacific oyster (*Crassostrea gigas*)	*Proteobacteria, Actinobacteria, Bacteroidetes, Fusobacteria, Acidobateria, Firmicutes, Nitrospirae, Verrucomicrobia*	Wang *et al*. (2014)
Pacific oysters (*Crassostrea gigas*)	*Prosthecomicrobium, Mycoplasma, Helicobacter, Terasakiella, Vibrio, Arcobacter, Pseudoalteromonas*	Madigan *et al*. (2014)
Pacific oysters (*Crassostrea gigas*)	*Vibrio, Shewanella, Pseudoalteromonas*	Rong *et al*. (2018)
Pacific oysters (*Crassostrea gigas*)	*Borrelia, Colwellia, Arcobacter, Sphingomonas*	Chen *et al*. (2019)
Sydney rock oysters (*Saccostrea glomerata*)	*Mycoplasma, Spirochaeta, Haloplasma, Pseudoalteromonas, Vibrio, Colwellia*	Madigan *et al*. (2014)
Eastern oysters (*Crassostrea virginica*)	*Marinifilum, Arcobacter, Spirochaeta, Sphingomonas, Bradyrhizobium, Caulobacter, Pelomonas, Psychrobacter, Pseudomonas, Bryobacter*	Chen *et al*. (2019)
Cortez oyster (*Crassostrea corteziensis*) and Kumamoto oyster (*Crassostrea sikamea*)	*Vibrionaceae, Bacillaceae, Brucellaceae, Micrococcaceae, Pseudoalteromonaceae, Rhodobactereceae, Shewanellaceae and Staphylococcaceae*	Luis-Villaseñor *et al*. (2018)

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Table 2. Microbial diversity of mussels

Mussels	Bacterial communities	Reference
Zebra mussels (*Dreissena polymorpha*)	*Aeromonas, Microbacteriacece, Midichloriaceae, Enterobacteriaceae, Mycoplasmataceae, Arcobacter, Bacteroides, Chitinibacter, Deefgea, Vogesella, Enterobacter, Methyloglobulus, Acinetobacter, Cutibacterium, Pseudomonas, Pseudorhodobacter, Dechloromonas, Shewanella, Chryseobacterium, Cloacibacterium, Arenimonas, Thermomonas*	Mathai *et al*. (2020)
Blue mussels (*Mytilus edulis*)	*Flavobacteriales, Fusobacteriales, Pirellulales, Rhodobacterales, Microtrichales, Campylobacterales, Bacteroidales, Vibrionales, Alteromonadales*	Li *et al*. (2020)
Brachidontes mussels	*Proteobacteria, Firmicutes, Tenericutes, Alphaproteobacteria, Betaproteobacteria, Clostridia, Clostridia, Epsilonproteobacteria, Gammaproteobacteria, Mollicutes, Saprospirae, Burkholderiales, Campylobacterales, Marinicellales, Mycoplasmatales, Oceanospirillales, Rhizobiales, Rhodobacterales, Sphingomonadales*	Cleary *et al*. (2015)
Mediterranean mussel (*Mytilus galloprovincialis*)	*Ruegeria, Desulfovibrio, Microbulbifer, Pseudomonas, Spongiibacter, Acinetobacter, Vibrio, Escherichia, Microcella, Propionigenium*	Cappello *et al*. (2015)
Cyclonaias asperata, Fusconaia cerina, Lampsilis ornata, Obovaria unicolor	*Clostridium, Methylocystis, Romboutsia, Flavobacterium, Staphylococcus, Streptococcus, Pseudomonas, Corynebacterium, Bradyrhizobium, Sediminibacterium*	Weingarten *et al*. (2019)
Chilean mussel (*Mytilus chilensis*)	*Vibrio, Psychrilyobacter, Mycoplasma, Psychromonas*	Santibañez *et al.* (2022)

investigated the microbial diversity associated with farmed *Mytilus* spp. from the Baltic Sea. Predominant bacteria orders identified included *Pseudomonadeles, Alteromonadales, Vibrionales,* and *Actinomycetales* while predominant fungal orders were Eurotiales and Mucorales. Vezzulli *et al.* (2018) reported the dominance of *Vibrio* and *Pseudoalteromonas* in the haemolymph of farmed *Mytilus galloprovincialis*.

Spoilage organisms associated with mussels have been reported to include *Shewanella*, *Acidaminococcus*, *Psychromonas*, and *Acinetobacter* (Odeyemi *et al.*, 2019). These studies confirmed that the composition of spoilage microbiota is influenced by the environment in which the mussels are caught, pre-packaging processes like depuration and storage conditions, including atmosphere and temperature (Odeyemi *et al.*, 2018; Odeyemi *et al.*, 2019).

There have been several reports of the presence of bacterial and viral pathogens in mussels. Viruses including Hepatitis A, Hepatitis E and norovirus have been identified at all levels of the supply chain (Erol *et al.*, 2016; López-Cabo *et al.*, 2020; O'Hara *et al.*, 2018). Bacterial pathogens such as *Clostridium difficile* (Pasquale *et al.*, 2012), *Vibrio* spp. (Lamon *et al.*, 2019; Lorenzoni *et al.*, 2021), and *Salmonella* (Lamon *et al.*, 2020) have also been observed in mussels. However, more studies on the microbial ecology of mussels, particularly those utilising next-generation sequencing techniques to determine the non-culturable community are required.

4. Preservation, monitoring and shelf-life extension: use of innovative and emerging technologies

Seafood spoilage is a complex process involving sensorial, chemical, and biological changes, which begin within hours of being harvested due to the activity of indigenous and microbial enzymes (Dalgaard, 2003; Ghaly *et al.*, 2010; Wang et al., 2019). Altered sensory properties include physical damage, colour change in gills and eyes, and the softness of muscle and smell. Seafood spoilage is an important sustainability issue as it has been estimated that between harvest and consumption, almost 27% of fish is lost, resulting in subsequent loss of income and food availability (Gustavvon, 2011).

Seafood is highly susceptible to spoilage because of intrinsic factors including high nutrient (protein and polyunsaturated fatty acids) and moisture content, a_w > 0.998 and neutral pH. These conditions provide an optimal growth environment for a wide range of microbial species. There is a need to assure consumers of the safety and quality of seafood products. Seafood is often traded over long distances, therefore, appropriate preservative strategies must be undertaken to guarantee product quality and safety. This has necessitated the use of novel packaging technologies to preserve seafood (Gokoglu, 2020). In addition, bivalve molluscs are increasingly recognised as vectors in the transmission of

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viral and bacterial foodborne diseases, further emphasising the need for control measures to minimise the growth of undesirable microorganisms in seafood (López Cabo *et al.*, 2020).

The dynamics of environmental conditions such as the water column, temperature, and growth stages can cause variations in the microbiota of oysters. To extend the shelf-life of seafood, more stringent thermal processes, including glazing, canning, and freezing have been used (Losito *et al.*, 2018; Tosun *et al.*, 2018). Under suitable conditions, harvested oysters can survive for weeks if appropriately handled and stored. However, super-chilled storage extended the shelf-life of ozonated oysters by nine days (López *et al.*, 2018). Other studies have investigated the effect of different treatments on the safety and quality attributes of mussels (Losito *et al.*, 2018; Tosun *et al.*, 2018; Xin *et al.*, 2021). However, there remain significant data gaps. Since the focus of this review is on bivalve molluscs, only novel packaging technologies used in the seafood industry are briefly discussed below.

4.1. Modified atmosphere packaging

Modified atmosphere packaging (MAP) is the most common packaging system that has been used to preserve live, fresh or cooked seafood in the last few decades. The effect of MAP alone or in conjunction with other hurdle methods to prolong the shelf-life of seafood has been studied (Lekjing and Venkatachalam, 2018; Gonçalves and Santos, 2019; Sørensen *et al.*, 2020). Lekjing and Venkatachalam (2018) reported that 75% CO_2 and 25% N_2 in MAP was found to be the optimum condition for pasteurized oyster meat compared with 75% CO_2 and 25% N_2 stored at 4°C. Gonçalves and Santos (2019) demonstrated that adding a pre-treatment stage with cold ozonated water to the fresh shrimp processing packed in MAP (100% CO_2) could prolong the shelf-life by up to 24 days compared with chlorinated water or traditional practice. A combination of MAP (40% CO_2 and 60% N_2) and super chilling storage could extend the shelf-life of Atlantic cod above 32 days (Sørensen *et al.*, 2020). MAP has also been used for the preservation of shrimp (Kimbuathong *et al.*, 2020; Shiekh *et al.*, 2020). Melanosis of white shrimp (*Litopenaeus vannamei*) may be avoided during storage when it is packed in high CO_2 60–80% MAP. Microbial growth in the product was inhibited by the high CO_2 concentration in the packaging as a result, trimethylamine formation during storage could be prevented (Kimbuathong *et al.*, 2020). The combination of interaction between pulse electric field, extract of chamuang (*Garcinia cowa*) and MAP gives hurdles for microorganisms to grow in the shrimp during storage and subsequently, melanosis could be prevented effectively (Shiekh *et al.*, 2020). This synergistic combination delayed the oxidation of fatty acids and inhibited microbial growth during storage. Therefore, the quality of the shrimp can be maintained. Depuration is a bacterial load reduction technique where molluscs are kept in potable water, which has been treated with ozone, chlorine, or UV radiation for a period of a few hours before they are consumed. A study on live mussels packed in MAP (80% O_2 and 20% N_2) showed that adding a depuration step prior to packaging could eliminate spoilage bacteria like *Shewanella* and *Acidaminococcus* during storage (Odeyemi *et al.*, 2019). The depuration was done for 8 h in a tank fixed with an aerator. In conclusion, the combination of MAP and additional hurdles technology prior to or post package could be an alternative solution to extend the shelf-life and preserve the quality of seafood including shellfish.

4.2. High-pressure processing, time-temperature indicators and emerging technologies

This is a non-thermal cold pasteurization technology used for the long-term preservation of the freshness and quality of seafood by reducing undesirable sensory changes and retaining its the high-nutritional value (Cartagena *et al.*, 2020; Cepero-Betancourt *et al.*, 2020). In a recent study by Cepero-Betancourt *et al.* (2020), it was observed that HPP improved the degree of hydrolysis (DH) of protein digestibility in abalone without negatively affecting the nutritional values. Similarly, Li *et al.* (2021) observed that the DH of clam (*Aloididae aloidi*) was improved using the combination of heat-ultrasound pre-treatment while only heat treatment improved the flavour of the shellfish. Temperature fluctuation during the cold chain impacts the quality of seafood due to the possibility of microbial growth during elevated temperatures. To help solve this problem, time-temperature indicators (TTIs) have been developed with at least twelve TTIs currently available for monitoring seafood products in the last decade. These indicators are effectively used to monitor the real-time history of storage temperature thereby preventing food wastage and improving food safety (Gao *et al.*, 2020). Recently, there has been the emergence of new technologies that help in preserving seafood quality and shelf-life extension. For example, Alamdari *et al.* (2021) reported the development of a low-cost, paper-based, pH -sensitive (colorimetric) meat spoilage detector that was also used to monitor the spoilage of fish. This technology could also be used to monitor the spoilage of shellfish.

4.3 Dielectric barrier discharges

Dielectric barrier discharges (DBD) is one of the electrical discharges used to generate cold plasma (CP).

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According to Choi *et al.* (2020), CP is useful for heat-sensitive products, prevents contamination, is non-toxic, and reduces chemical agents. Applying DBD plasma treatment conditions of 70 kV or 80 kV for 5 mins of treatment time, Albertos *et al.* (2019) showed that the technology significantly reduced the spoilage of fresh herring during storage at 4°C. The same technology was also used with oysters and results showed that it is effective in reducing human norovirus (HuNoV) GII.4 infectivity in fresh oysters (Choi *et al.*, 2020). One of the advantages of DBD treatment is that post-process contamination can be avoided since the treatment can be applied inside a sealed package (Albertos *et al.*, 2019).

4.4 Hyperbaric storage

Hyperbaric storage (HS) consists of preserving foods under moderate pressure (up to ~ 100 MPa), which leads to longer shelf-life and equal/better quality compared to present-day commercial refrigeration (Fidalgo *et al.*, 2021). Using hyperbaric cold storage at 50 MPa, Otero *et al.* (2019) found that microbial counts did not increase in Atlantic mackerel fillets stored after 12 days of storage at 5°C. The level of pressure needed to effectively control microbial growth depends on the storage temperature. For example, Otero *et al.* (2019) showed that a minimum pressure of 50 MPa was needed to prevent microbial growth in razor clams (*Ensis directus*) stored at 5°C, while a minimum of 75 MPa was required at 20°C. HS technology was able to extend the microbial shelf-life of the razor clams to, at least, twice that achieved in conventional refrigeration (Otero *et al.*, 2019).

4.5 Aerosolisation and ozonation

Aerosolisation involves the use of dispersion of a liquid phase into the air in the form of a fine mist and is usually used for sanitary purposes, especially for respiratory medical treatments (Sullivan *et al.*, 2020). According to Sullivan *et al.* (2020), this technology can be applied in combination with natural antimicrobial materials to seafood to deliver antimicrobial coating and enhance the microbiological quality shelf-life extension of these products. The authors obtained promising results with hake fillets aerosolised with chitosan (CS), chitosan nanoparticles (CS NP) or commercially available carnosolic acid nano-solubilisate (CASB) antimicrobial coating solutions since shelf-life was significantly enhanced in comparison to control-treated hake fillets. The ozone molecule is formed by three oxygen atoms and the arrangement of its unpaired electrons with an oxygen nucleus at its centre provides it with a strong reactivity (Pandiselvam *et al.*, 2019). Ozone is an attractive alternative preservative that the food industry needs due to its properties such as quick decomposition and little residual effect during food preservation (Pandiselvam *et al.*, 2019). Using ozone as a pre-treatment to modified atmosphere packaging, Gonçalves and Santos (2019) showed that the shelf-life of whole chilled Pacific white shrimp increased more than twice.

4.6 Irradiation

Food irradiation is a new technology that has the potential to enhance the safety and shelf-life of a wide range of food products. Irradiation has various distinct advantages including the immediate inactivation of microbes in frozen foods (Ronholm *et al.*, 2016). Gamma irradiation and, lately, X-rays are becoming preferred alternatives to heat treatment for eradicating harmful bacteria like vibrios in live oysters (Mahmoud, 2009).

On live oysters, doses of gamma irradiation ranging from 0.5 to 3.0 kGy have been studied, with reports showing that the greatest dose of 3.0 kGy did not inhibit the oysters or change their sensory qualities. Although, on the administration of dosage levels as low as 1.0 kGy, 6-log *V. parahaemolyticus* decreases were reported (Jakabi *et al.*, 2003). X-ray treatments on *V. parahaemolyticus* ready-to-eat shrimp products inoculated in the lab resulted in a 6-log reduction in CFUs at 3 kGy. To get a 6-log drop in *V. vulnificus* in oysters, it took 3.0 kGy for whole shell oysters and 1.0 kGy for half shell oysters (Mahmoud, 2009).

4.7 Phage treatment

The *V. parahaemolyticus* phage VPp1a and the Siphoviridae phage pVp-1 (Jun *et al.*, 2014) have both shown promise in controlling *V. parahaemolyticus* populations in raw oysters (Peng *et al.*, 2013). While depuration is particularly successful in reducing coliform levels, it is not effective against vibrios except if it is done at a low temperature for several days (Phuvasate *et al.*, 2012). On the other hand, in the presence of the phage VPp1a, depuration reduced the concentration of *V. parahaemolyticus* in oysters by 2.35–2.76 log CFU/g over a period of 36-hour at 16°C (Rong *et al.*, 2014).

Guenther *et al.* (2009) investigated the efficacy of the phage pVp-1 in eradicating *V. parahaemolyticus* infection when used directly and as a bath immersion on contaminated oyster meat. Treatment by bath immersion reduced counts of *V. parahaemolyticus* from 8.9×10^6 CFU/g to 14 CFU/g after 72 hours, while direct phage application to contaminated oyster meat almost eliminated contaminants within 12 hours at 18°C with only 1.9 CFU/g in the treated samples and 1.4×10^6 CFU/g in the control (Jun *et al.*, 2014). When applied to in-shell oysters, however, the wide and uneven surface area reduces contact time between bacterial targets and phage particles, posing multiple problems for phage treatment

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(Guenther *et al.*, 2009).

4.8 Pulsed electric field

Pulsed electric field (PEF) processing is a non-thermal technique for preserving foods. PEF is mostly utilized for microbial inactivation in addition to extraction, drying, and other mass transfer operations. PEF technology works by applying short pulses of high electrical currents with a duration of microseconds to milliseconds and an intensity of 10–80 kV/cm to suppress microbial proliferation (Nowosad *et al.*, 2021). PEF method of processing can be utilized in preserving the physicochemical features of the finished product while also achieving desired organoleptic parameters and nutritional and vitamin content (Pourzaki and Mirzaee, 2008). Kontominas *et al.* (2021) reported that treatment by PEF can make the flesh of fish more porous, enhance water retention capacity, and be utilized as a fish drying pre-treatment. These authors, however, found no enhancement in the softness of shellfish gastropod and mollusc goods. This makes it a very good preservation technique for shellfish which are exoskeleton-bearing aquatic organisms.

4.9 Sous vide

Sous vide cooking is a new cooking method that involves cooking foods in vacuum pouches at specific temperatures while ensuring even heat dispersion. Wan *et al.* (2019) noted that the sous vide method of cooking might be employed as a healthy option because it is effective in preserving the quality of seafood. However, careful monitoring of operational technological parameters is essential to protect nutritional and sensory quality during the thermal processing of seafood. In seafood items, both cooking time and temperature have been demonstrated to impact lipid oxidation (Cropotova *et al.*, 2019). Furthermore, greater temperatures cause a variety of metabolic events, protein aggregations, and forms in the muscles of seafood, all of which alter the tissue gaps. Vacuum pre-treatment, on the other hand, can be used to isolate oxygen and, thus, avoid metabolic reactions that require oxygen, as well as to decrease lipid damage during the heating process (Wan *et al.*, 2019). According to Bongiorno *et al.* (2018), sous vide processing of fresh *Mytilus galloprovincialis* mussels was found to preserve product quality while also extending shelf-life and improving product safety. The mesophiles had a population of > 5 log CFU/g, total volatile basic nitrogen was less than 35 mg/100 g, and the mussels had scores of less than 7. Mussels cooked traditionally (90°C for 10 mins) had a shelf-life of around 14 days, whereas mussels that are sous vide cooked and cooled had a shelf-life of about 21 days, with a shelf-life of about 30 days when brine was added, corresponding to the parameters (85°C for 10 mins) used by Bongiorno *et al.* (2018).

To preserve the quality and safety of seafood products, sous vide is usually used in combination with other processing techniques like different packaging technologies. It is possible to store sous vide cooked marine species in modified atmosphere packaging to achieve a longer shelf-life and lower temperatures for storage (DeWitt and Oliveira, 2016).

4.10 Cold plasma

Cold plasma preservation technique is a non-thermal technology for food processing which involves utilizing energetic, reactive gases in killing bacteria in food products. A carrier gas, such as air, oxygen helium or nitrogen is used alongside electricity in this versatile sanitizing procedure. Antibacterial chemical compounds are not required. UV radiation and cold plasma ionization process reactive chemical products are the primary mechanisms of action (Sunil *et al.*, 2018).

De Souza Silva *et al.* (2019) employed an atmospheric cold plasma generator with a dielectric barrier discharge configuration and phenolic coplanar plates, which generates reactive oxygen and nitrogen species, UV radiation, and an intense electric field in atmospheric air (saturated with nitrogen and oxygen in standard concentrations). De Souza Silva *et al.* (2019) demonstrated the effectiveness of cold plasma in preserving the quality of white shrimp (*Litopenaeus vannamei*) increasing their shelf-life and delaying the process of melanosis in white shrimp. The study found that the cold plasma technique improved the shrimp samples' physicochemical features such as stabilizing pH, increasing water holding capacity, lowering weight losses after cooking and reducing colour change during storage. Cold plasma, also lowered the microbial burden over time, demonstrating the cold plasma technique's promising potential in extending the shelf-life of shellfish.

5. Conclusion

Bivalve molluscs have become a major category of seafood products and with immense potential for improving food security as a source of nourishment and income. To meet growing consumer demand, the supply chain for these highly perishable products has become global. This has focused interest on the microbial communities associated with bivalve molluscs, particularly spoilage and pathogenic bacteria. The accumulation of bacteria, viruses and toxins in bivalves means that the microbiota is largely dependent on the local environment and that their food safety requires

https://doi.org/10.26656/fr.2017.7(2).998

continual monitoring.

Some genera and species of the microbial community present in bivalve molluscs differ due to different cultural environments, pre- and post-harvest handling, and storage conditions. The spoilage prevention measures to be taken will depend on the scale of production of the seafood. For example, a commercial scale will require the combination of different technologies to ensure premium products while maintaining the cost of production and maximizing profits. The seafood could be produced using a biofloc technology which has been stated to reduce spoilage bacteria in shrimps. The harvested seafood could be treated with neutral electrolysed water to further reduce spoilers. At the post-harvest level, the products could be packaged and processed using high-pressure processing, packaged using MAP while TTIs can then be used to monitor the products during transportation. The combination of these technologies will help to reduce spoilage and waste of seafood including bivalve molluscs without compromising quality. However, there is a need to study the economic feasibility of combining these technologies.

Conflict of interest

The authors declare no conflict of interest.

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