Emerging methods for fresh-keeping and spoilage detection of aquatic products

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Abstract: Increasing demand for healthy and safety aquatic products has prompted new technical methods applied to food refreshing, as well as microorganism analysis. As is known, aquatic products are commonly consumed among various food markets. While these products are highly perishable as within pathogenic bacteria will grow gradually and spoil the product finally. It has been thought to be paradoxical to both maintain the taste and keep sterilization of aquatic products in the market place. More importantly, using informative approaches to detect the quality and potential spoilage or pathogens of aquatic products on trade or sale will much improve quality of life for consumers. Here we review commonly and newly reported methods and treatments to further investigate aquatic food preservation and storage，and demonstrate typical and practical methods for spoilage detection that have recently emerged. The former includes cryogenic preservation, chemical preservation, physical preservation and biological preservation, while the latter focuses on culture dependent and independent techniques reported recently for food inspection. This article aims to highlight effective skills and provide information for future researchers to make much further contributions to food safety and public health.

1. Introduction

In modern life, consumers have seen more and more commercial products as the technology developed rapidly, which has improved their quality of life dramatically and surprisingly. On the other hand, the convenient transportation system has facilitated commodity transfer across different regions, making it possible for customers to purchase fresh food produced in distant places. However, seafood quality is highly influenced by autolysis, contamination by and growth of microorganism, and loss of protein functionality. Compare with agricultural and livestock products, seafood is more likely to suffer from spoilage, because it has high water content, less fat and more fragile muscle tissue[1, 2]. After life ceased in the seafood, the value of pH will decline and bacteria can reproduce in large quantities. Besides, most seafood's viscera will not be cleaned in time after fishing and the viscera are easily corrupt[2, 3]. So the seafood will corrupt very quickly if we don't use the preservation technology. Therefore, fresh-keeping has been long thought to be serious trouble for manufacturers. More importantly, as many aquatic products such as cold-smoked salmon are consumed without heat-treatment, the presence of undefined bacteria in high numbers is a pathogenic factor that should be considered in public health assessments. Which brings another question, that is, how to detect and evaluate the number and species of microorganism inside aquatic products efficiently and practically? Although the effectiveness of various techniques and methods for their abilities to enhance and analyze the quality of aquatic products have been reported by numerous researchers, no comprehensive review has been published yet. Therefore, in this article, we summarize the scientific reports and practical applications of different methods.

2. Technical methods for aquatic products refreshing.

Food preservation and refreshing refers to a complicated technology that uses physical, chemical and biological methods to delay the spoilage of fresh food and ensure its quality will not decline during the process of circulation and production. In this section, we would like to focus on cryogenic preservation, chemical preservation, physical preservation and biological preservation

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containing traditional and essential techniques widely used to maintain the quality of aquatic products.

2.1 Cryogenic preservation

In general, the most common method is cryogenic preservation. This method is very easy to achieve and cheaper than any other techniques. It also includes ice storage, cold storage, frozen storage, superchilled storage, vacuum freeze-drying technology, etc[4].

2.1.1 Ice storage

This method includes ice preservation and ice free preservation. Ice preservation puts the seafood on ice and ensuring that the temperature of production is near to freezing point. The temperature is always maintained at 0~3℃ and lasts 7~12h[4]. Ice free preservation uses ice in water to reduce the water temperature to freezing point at first, then spray the water on productions. This method is very convenient and cheap, so most people use this method to extend the shelf-life of seafood.

2.1.2 Cold storage

Cold storage is a refrigerated room with seafood and the temperature is between 10℃ and freezing point. This method can inhibit the activity of spoilage bacteria in a certain range but the effect of fresh-keeping is not good, only for short-term storage[5].

2.1.3 Frozen storage

Frozen storage is putting the production in a room in which temperature is below -18℃[6]. In this temperature, the water contained in seafood will be frozen and can also inhibit the activity of most spoilage bacteria. To some extent, it can extend the shelf-life of seafood[6]. Beyond that, low temperature can also inhibit enzymes in seafoods, thus reducing the chemical reaction rate in the production. And this method also can be divided into quick freezing and slow freezing. Quick freezing will quickly freeze the moisture in aquatic products and form a lot of tiny ice crystals, which will not damage cells during thawing, and can better maintain the flavor and quality of productions. In contrast, slow freezing will form larger ice crystal and stab the cell membrane and result in serious loss of juice[7]. In a conclusion, the quick freezing technology is widely used in the preservation of aquatic products.

2.1.4 Superchilled storage

Superchilled storage is a kind of frozen or cooling storage technology, the temperature is generally controlled at -3℃. This method not only can prevent the losing of juice in seafood, structure damage and weight loss but also can inhibit the activity of spoilage bacteria and enzymes, so as to prolong the shelf-life of seafood[8]. Besides, it can also reduce the energy consumption. Some research indicates that, compared with the cold storage technology, the shelf-life can be significantly prolonged[4]. This method needs to control the temperature accurately, accordingly the cost is very high, so it is not widely used.

2.1.5 Vacuum freeze-drying

Vacuum freeze-drying means that the water in the food is completely frozen, and then the moisture in the food is sublimated directly from the solid to the gaseous state under low pressure. After that the food exhibits a dry state and the water activity is very low, most microbial life activities are inhibited, but the nutritional components and sensory quality of the products will hardly deteriorate. The porous structure formed by freeze-drying also ensures good rehydration performance of food. This technology is applied to dry seafood, such as sea cucumber and abalone. It can not only extend the shelf-life of products but also lighten the weight and volume of products. This method can also enable the seafood to sell in non-coastal areas, allowing inland consumers to taste aquatic products with various kinds and high nutritional value. But this technology has high demand for drying equipment, high cost of production and relatively high price of products.

2.2 Chemical preservation

Chemical preservation refers to the addition of one or more chemical materials or preservatives to aquatic products. It can maintain production quality during storage, transportation and sale, then extend shelf life of products.

2.2.1 Preservatives

The advantage of this technology is that it can delay the spoilage of aquatic production only by adding preservatives according to the regulations and requirements of products qualitatively and quantitatively. So this method is very simple, efficient, economical and practical. Recently, the problem of antibiotics or preservatives in many aquatic products has caused a certain impact on human health[9, 10]. So the natural, non-toxic and harmless biological preservatives have become a hot research topic in the field of food preservation. Some research shows that nisin can be a most effective preservative in processed cheese[11-13]. It can delay and prevent the growth and formation of toxin by some spoilage bacteria such as Clostridial and inoculated Cl botulinum strains and make sure the cheese can be stored in a warm condition[14]. Similarly, nisin has been widely used in seafood storage, such as cold-smoked salmon[15], rainbow trout[16] and shrimps[16] combining with another approaches. Besides, chitosan is also a chemical preservative used in food industry. It has been proved that it improves the stability of perishable foods because chitosan has antimicrobial activity[17].

2.2.2 Ozone preservation

Ozone has a bacteriostasis effect, which acts on the cell membrane of the bacteria, causing the permeability of the membrane to increase or even rupture and the content will flow out[18]. In addition, ozone can also destroy the genetic material in the cytoplasm and the activity of various enzymes. Finally, the bacteria will be killed. The advantages of this method are killing ordinary bacteria and even inactivating spore. Moreover, ozone will automatically decompose into oxygen after processing food. It will not remain like chemical preservation and also not cause any negative effects on human health. And the ozone preservation can be carried out at normal temperature which can maximize the preservation of the nutrients in the food and prevent the decline of the quality of the products. In addition, ozone is also very easy to prepare and inexpensive. So this method is very convenient and more practical. Some research shows that sterile and ozonized water can stabilize the quality of stored refrigerated fresh fish and the fish can keep more longer than those kept in sea water[19]. Because the ozone can significantly delay the growth of bacteria in fish muscle. The ozone combine with Modified atmosphere packaging (MAP) can also extend the shelf-life of striped red mullet[20].

2.3 Physical preservation

2.3.1 Modified atmosphere packaging (MAP)

The main components of air conditioning are nitrogen, oxygen and carbon dioxide, which are filled into the packaging bags of aquatic products in a certain proportion[21]. The aim of this method is creating a good environment for the preservation of seafood, slow down or inhibit microbial growth, prevent nutrients loss and the decline of quality, and as far as possible to prolong the shelf-life of seafood[22]. Carbon monoxide has been used to stabilize the color of fish muscle during frozen storage and distribution[23]. Research also shows that Carbon dioxide can inhibit the respiration and growth of meat spoilage bacteria[24],and if keeping the tuna fish samples which packed in vacuum packaging at 4℃ for 3 days, the samples will be a little decreased in redness but can be accepted by consumers. While the fillets which stored in exposed air or kept in 100% Oxygen atmosphere will turn brown very quickly[25].

2.3.2 Ultra-high pressure (UHP)

Ultra-high pressure preservation technology uses a sealed container of water or other liquid as pressure medium, adding 100-1000Mpa to packaged seafood. This method can destroy the non-covalent bond in the protein of spoilage bacteria in food and damage the advanced structure of protein, then inactivate enzymes and coagulate proteins of aquatic products[2]. Beyond that, the cell membrane of the spoilage bacteria can also be damaged, causing the outflow of the contents. These reasons will all cause the microbial death, thus prolonging the shelf-life of the aquatic products. Because this method can effectively kill microorganisms at normal temperature or low temperature and will not lose nutrients and flavor as a result of high temperature sterilization. It has maintained relatively good sensory characteristics of products[26].

2.3.3 Irradiation

Irradiation preservation refers to using gamma rays emitted by 60Co or 137Cs, as well as acceleration of electrons and X-rays to irradiate food. During irradiation, food will produce two kinds of chemical reactions, direct or indirect[27]. The direct reaction is the ionization and chemical effect of the microbiological interstitial cells irradiated by high energy electron radiation. The indirect effect is that the water in products is ionized after irradiation and all kinds of free radicals and hydrogen peroxide are generated. Then it will interact with other substances in the cell to form new compounds. Both of these two reactions can effectively inhibit the biological activities of microbes, thus achieving the purpose of prolonging the shelf-life. The advantage of this method is that it can effectively kill the spoilage bacteria and the common intestinal pathogens in aquatic products[28]. Gamma irradiation studies showing that the maximum dose with dose of 3.0 kGy did not kill the oysters or affect any of their sensory attributes[29].

2.4 Biological preservation

Biological preservation means that some natural substances with bacteriostatic or bactericidal activity can be formulated into a suitable concentration of solution, and can be used for aquatic products by soaking, spraying or spreading, so as to achieve the effect of antisepsis and freshness preservation. This natural substance can effectively inhibit or kill microorganisms in food and isolate the contact between food and air in order to postpone oxidation. This method of preservation has the characteristics of natural, efficient, safe and simple. At present, more researches focus on the use of microbes or their metabolites and biological extracts as preservatives, such as nisin[30], lysozyme[31], chitosan[17], etc. or these compound biological preservatives as main components. Recently, edible coatings have been applied to improve the quality of fresh and frozen aquatic products by functioning as a carrier of food additives[32].

3. Microorganisms detection and analysis.

In the last decades, bacteria culture-independent technical methods have considerably developed in food microbiology [33, 34].

3.1 Bacteria culture

Bacteria culture has been applied into food microbiology study for quite a long time as one of the traditional and basic approaches to detect the pathogens or potential microbial populations. Culture-based analysis needs to purify and separate the pathogenic bacteria in food and observe the morphological characteristics of bacterial strain. Then refer to the book of identification of bacteria like “Bergey’s Manual of Determinative Bacteriology”. So this method is very useful but slower than some other tools. In spite of low sensitivity and unstable results, bacterial culture could solidly be applied to estimate the given virulent microorganisms[35].

3.2 Polymerase chain reaction based techniques

Polymerase chain reaction is a method of enzymatic synthesis of specific DNA fragments in vitro and it was invented by American scientist Kary Mullis in 1985[36]. The advantages of polymerase chain reaction are faster, easier and don’t need higher purity of sample. With the development of PCR, this tool is also combined with some other methods like quantitative PCR (qPCR), denaturing gradient gel electrophoresis (PCR-DGGE)[34, 37], gene chip technology, Multiplex Super PCR (MS-PCR)[38], Single-Strand Conformation Polymorphism (PCR-SSCP)[39] etc. It can detect the microorganisms in food because this new tool is an easier way to investigate microbial diversity and the specific spoilage organism in food [37, 40, 41].

3.2.1 Quantitative PCR (qPCR)

Quantitative PCR is a method which can be used to quantify the nucleic acids of interest. One of its major advantages is to be faster than conventional culture-based methods. It is, also named real-time quantitative PCR somehow, highly sensitive, specific and enables simultaneous detection of different microorganisms[42]. It has been developed to detect the three major Vibrio spp.

pathogenic for humans in Tunisian seafood products and sediments[43]. This assay has also been applied to study the presence of Anisakis in four types of processed commercial fish derived food with high accuracy and sensitivity[44].

3.2.2 Denaturing gradient gel electrophoresis (DGGE)

DGGE is an electrophoretic method capable of detecting differences between DNA fragments of the same size but with different sequences. When DNA molecules change from a double helix to a local denaturation, the mobility of the electrophoresis decreases, and the gradient required for this change is different for different DNA fragments. So DGGE uses this phenomenon to separate the double stranded DNA fragments with the same length and different nucleotide sequences, then determine the specific spoilage bacteria of the samples according to the brightness of DGGE bands[34].

3.2.3 Gene chip technology

Gene chip technology is a systematic process including that oligonucleotide is spotted on the surface of the chip, DNA samples are amplified by PCR after the preparation of fluorescent probe, then hybridized with oligonucleotide chip, and finally through the quantitative analysis of fluorescence scanner to determine the distribution pattern of samples kept in certain microorganisms. This method is faster and more accurate than other traditional analysis, but the detection fee is high, the standardized program is imperfect and the operation is complex. Some scientists use this method to detect the seven-leading seafood-borne pathogens including Vibrio parahaemolyticus, Vibrio cholera, Vibrio vulnificus, Vibrio mimics, Vibrio alginolyticus, Vibrio anguillarum and Vibrio harveyi. The separated and purified PCR products can be used as template DNA then participated in single base extension labeling reaction[45]. In addition, the PCR products could also be labeled with Cy3 fluorescent dye and hybridized to DNA microarrays[46]. Thus after so many experiments scientists have found that this detection method is very sensitive and the specificity of the detection is nearly 100%[45]. In a conclusion, the method of DNA microarray hybridization combined with multiplex PCR can ensure faster and more accurate detection of potential pathogens in seafood. So customers can also eat more safety seafood products.

3.3 Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay is a detection technique that combines the specific immune response of the antigen and the antibody with the efficient catalysis of the enzyme. And this method has been widely used in the fields of analytical chemistry, biology, dairy products and animal products in food, and has also been applied in the field of aquatic products and managed to detect some specific spoilage organism in it like Salmonella, Listeria monocytogenes, Escherichia coli[38], etc. It has characteristics of high efficiency and specificity, rapid detection and good stability. It was reported that a chicken anti-K. septempunctata antiserum was developed to detect the spores of olive flounder meat by ELISA and immunohistochemical staining, as a foodborne disease associated with consumption of raw olive flounder have increased within last ten years in Japan[47]. But when the samples are fewer, the cost is higher.

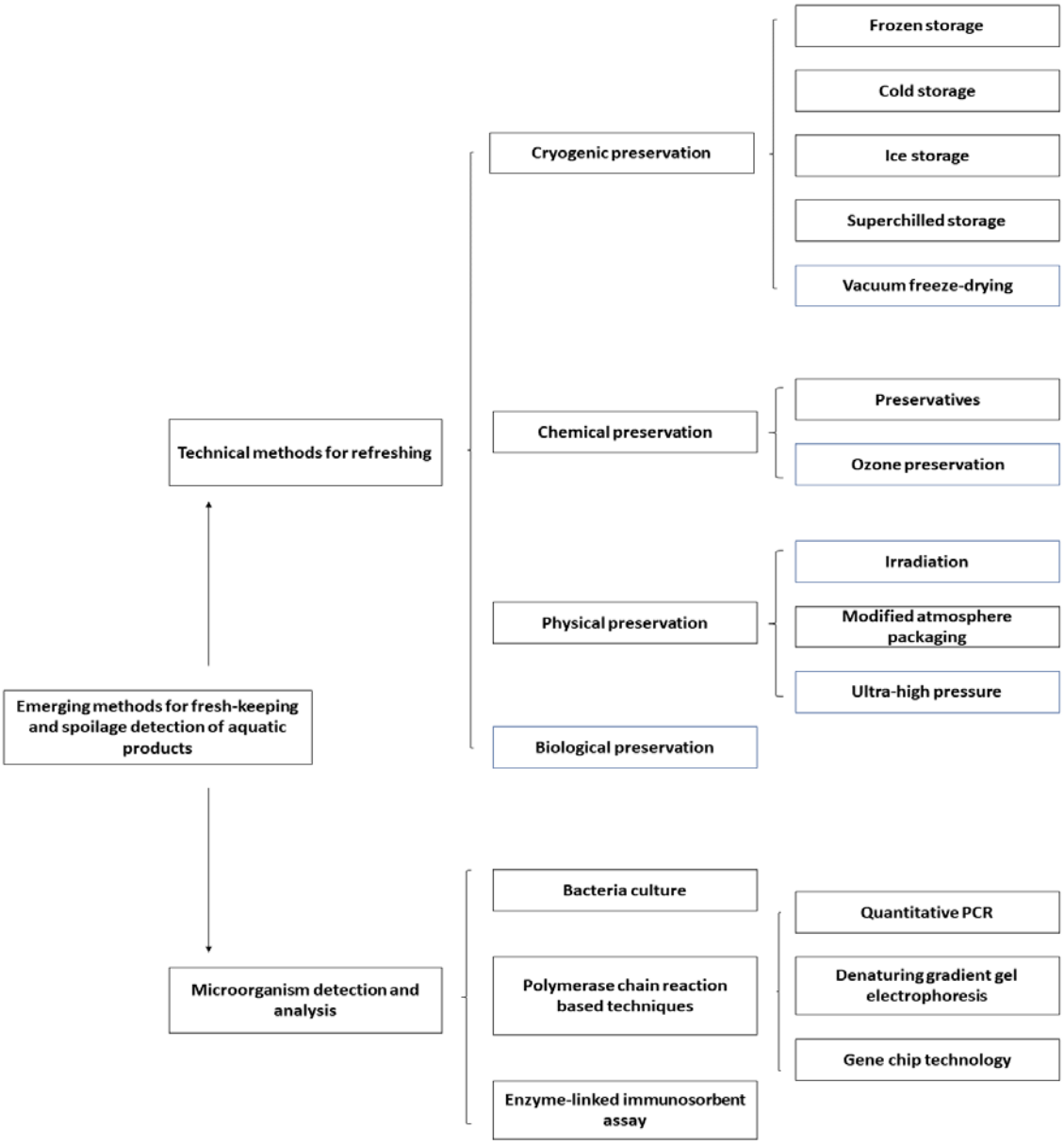

Figure1. Structure diagram of reviewed methods

4. Conclusion

Though plentiful methods and examples are demonstrated in this review, all in all, traditional methods are still the mainstream of food preservation and storage. For microorganism detection, bacteria culture shows obvious shortcomings involving the isolation and enumeration of bacteria from targeted food by means of specific culture media and conditions. Emerging methods combined with PCR and sequencing techniques have extremely improved the efficiency and throughput of detection and analysis. The recent last years have seen the emerging new methods in microbial food ecology and researches have showed that combined techniques will make much more detailed information available in the future[48], allowing scientists to fully comprehend the role and impact of specific microorganisms in defined food sectors. With rapid technology developing, nanotechnology, biotic coatings and next generation sequence are showing great potential in food preservation and spoilage detection[49-51]. It has no doubt that new information will be applied to improve food quality and safety.

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