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CHAPTER 6

Microbial Spoilage of Fish: Quality Evaluation and Measures

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Abstract: Microbial spoilage is a major contributor to fish quality, safety, and shelf life deterioration, which can incur substantial costs associated with post-harvest losses. Specific spoilage organisms (SSOs) like *Shewanella putrefaciens*, pseudomonas species, and lactic acid bacteria are responsible for fish spoiling. These organisms have the ability to reproduce and produce slime, unpleasant odors, and other sensory disturbances. Microbial spoilage rates depend on handling, the fish's microbial load (contaminants) at harvest, and storage temperature. Quality evaluation can be simple sensory evaluation, or total plate count (TVC), Trimethylamine-Nitrogen (TMA-N), Total Volatile Basic Nitrogen (TVB-N),

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peroxide value (PV), Free Fatty Acids (FFAS) and molecular analysis methods like quantitative PCR have also been used to evaluate freshness and spoilage. One of the techniques is to minimize fish spoilage and extend its shelf life by employing the drying method that reduces moisture content to 16%. Addition of ingredients like ginger, garlic, turmeric, onion along with salt may in increase the shelf life of seafood up to 12 months because of antibiotic and anti-microbial compounds in them. Packing the ingredient treated fish in High-Density Polyethylene (HDPE) and metalized film pouch also helps in reduction of spoilage of fish spoilage can be controlled by a number of preservation techniques, including refrigeration, Modified Atmosphere Packaging (MAP), vacuum packaging, and the use of natural antimicrobials (bacteriocins and plant extracts). Advancements in nanotechnology and biopreservation could potentially serve as viable alternatives to synthetic preservatives. It is crucial to have an adequate knowledge of microbial ecology and target SSOs with control actions in order to improve safety and shelf life of fish products, and reduce the economic loss potential associated with the seafood industry.

Keywords: Spoilage, Molds, Bacteria, preservatives, shelf life

Introduction

Fish is an integral part of food that can help in subsisting and dealing with issues of starvation and malnutrition in developing or under-developed countries. Fish is an excellent source of high-quality protein and provides all the essential amino acids that the body cannot generates on its own, thus making it complete protein source and fats including long-chain omega-3 fatty acids like EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) (Hosomi et al., 2012; Barik et al., 2017; Mohanty et al., 2019). More than 20% of the animal protein consumed by about 3.1 billion people comes from fish (Mohanty et al., 2017). During the year 2022-23, global fish production has reached 185.4 million tonnes from capture and culture fisheries. Among this, the capture fish sector contributed 91 million tonnes whereas the culture fish sector contributed about 94.4 million tonnes (FAO 2024). During the year 2022-23, the total fish production of India was 175.45 lakh tonnes, among which marine fish production was 44.32 lakh tonnes and 131.13 lakh tonnes from inland fisheries.

Pre-treatment, proper manipulation, and preservation techniques can improve the quality of aquatic food products, increase their shelf life and prevent deterioration (Ghaly et al., 2010). According to Baird-Parker (2000), chemical deterioration and microbiological spoiling account for 25% of the total primary production in fisheries and agriculture annually. Furthermore, between 25% and 30% of landed fish in the world's food supply are lost due to microbial activity (Amos et al., 2007). Fresh fish can go bad very quickly after being caught. In hot tropical climes, fish will begin to decay (rigor mortis) 12 hours after being caught (Berkel et al., 2004). Adebowale et al., (2008) defined rigor mortis as the loss of fish flexibility induced by the stiffness of fish mussels immediately after death. Fish species deteriorate as a result of oxidation, lipases, digestive enzymes, surface microorganisms, etc. The fish decomposes into its fundamental parts and produces new ones when it spoils. Fish flesh will ultimately change in texture, flavor, and smell due to the new ingredients (Ghaly et al., 2010).

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Quality of Fresh Aquatic Food Products

Because of the increasing need for consumption, aquatic food products play a significant role in international trade (Pal et al., 2016). In addition to other elements required for a balanced diet, they are high in protein and omega-3 fatty acids. The greatest risks to human health, however, are undercooked, uncooked, and improperly processed fish and fish products (Pal et al., 2016). Infectious or toxic illnesses can arise from eating dead, non-fresh fish or fish products (Adebayo-Tayo et al., 2012). After harvesting and before consumption, aquatic goods are usually evaluated for freshness or deterioration using the senses (Lougovois & Kyrana, 2005). The subtle flavor and aroma of fresh fish and fishery products are difficult to distinguish in less fresh dishes. Espejo-Hermes (2004) adds that aquatic items should be prepared and handled carefully to prevent spoiling and losses due to damage right after harvest.

Types of microbial spoilage

Molds as an agent of Spoilage

Molds are fungi that form chains and branching structures. Their cells range in size from 30 to 100 microns. Once they have branched, molds may be seen with the naked eye and occur in a variety of sizes, forms, and colors (Singh & Anderson, 2004). They have adapted to grow on and through solid surfaces, producing spores either asexually or sexually and using oxygen as a metabolic fuel. Temperature, relative humidity, moisture, water activity, nutrients, pH level, and salt content are the primary causes of mold growth during storage (Sivaraman & Siva, 2015). Molds normally have a pH range of 3 to 8, and some kinds of mold, such as the mold on dried food, can grow well in conditions with little water activity (Sahu & Bala, 2017).

While spores can endure challenging environmental conditions, heat treatments are the most sensitive to them. While some molds can withstand temperatures of 90˚c for 12 minutes or more, others can thrive in the cold environment of a refrigerator. As a natural process of recycling, mold infests various foods and other items that humans find valuable (Rawat, 2015). Molds have a wide range of secondary metabolism, which includes the production of harmful secondary metabolites and carcinogenic mycotoxins with a molecular weight of B700 DA. Diseases can result from Aspergillus strains that produce aflatoxin and other fungi that induce spoiling (Pal et al., 2016; Sahu & Bala, 2017). Despite being widespread, only a few types of mold are dangerous for food spoiling. According to many scholars, common molds that might contaminate food and drinks include Mucor spp., Fusarium spp., Aspergillus, Rhizopus, and Penicillium (Singh & Anderson, 2004).

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Table 1: Frequently identified mold species in fish and fishery products

Fish and Fishery Products	Molds Species	References
Fresh Fish (*Tilapia nilotica*)	*Penicillium* spp.	Hassan et al., (2011)
Smoked Fish sardine (*Sardinella aurita*)	*Aspergillus flavus*, *Aspergillus fumigatus*	Dutta et al., (2018)
Smoked Fish (*Tilapia nilotica*)	*Penicillium citrinum* and *P. expansum*	Hassan et al. (2011)
Salted Fish	*Sporendonema* spp., *Oospora* spp., and *Aspergillus flavus*	Galaviz-Silva et al., (2008), Hassan et al., (2011)
Fermented Fish	*Aspergillus niger*, and *Aspergillus sydowii*	Biango-Daniels and Hodge (2018)
Dried Fish	*Wallemia sebi*, *Eurotium repens*, *E. rubrum*, *E. amstelodami*, *Aspergillus wentii*	Sivaraman and Siva (2015)

The most prevalent spoilage molds in these regions are usually Aspergilli and Penicilli molds because they grow more quickly in warm temperatures (Petruzzi et al., 2017). By generating mycotoxins, Aspergilli mostly contaminate peanuts, tree nuts, cereals, dried beans, and several spices. *Penicillium* spp., which are present in Antarctic and tropical soils and natural environmental waste, are one of the primary mold spoiling organisms for temperate zones. Penicillium species are essential spoiling organisms because they produce strong mycotoxins. Fruits and vegetables can be attacked and spoiled by a variety of Penicillium species. Certain Penicillium species can target processed or chilled foods, such as margarine and jam.

Grain storage may eventually be attacked by Fusarium species. Apart from plant problems, Fusarium species can create mycotoxins that are significant for human health. Among the most prevalent molds in bread are Rhizopus and Mucor. Both of these species usually flourish in high water conditions (Rawat, 2015). From the perspectives of agronomy and public health, the most important mycotoxins include ergot alkaloids, trichothecenes, ochratoxin A, patulin, tremorgenic, and aflatoxin (Petruzzi et al., 2017).

Bacteria as agent of Spoilage

Found on almost every surface of the earth, bacteria are microscopic, unicellular organisms with an almost infinite diversity that are vital to ecosystems (Russell & Gilmore, 2018). Bacteria possess a single DNA loop, which serves as the "control center" for their genetic material. In addition to the main DNA loop, some bacteria possess extra rings of genetic material known as plasmids (Dorman, 2020). The genetic material is located in the plasmid, which is also where the bacteria might gain a competitive advantage over other bacteria. For example, the bacteria may possess a gene that confers resistance to a specific antibiotic (Brito, 2021). Additionally, certain types of bacteria may possess the ability to withstand extreme temperatures and pressures (Sakthipriya et al., 2022). Bacteria range in shape from spheres (cocci), corkscrews (spirochaetes), commas (vibriosis), rods (bacilli), and spirals (spirilla) and are only 1 to 5 µm in size (Pachaiappan et al., 2021). Bacteria can be found almost everywhere on our planet, such as on rocks, in soil, water, the oceans, and even in snow (Azma & Zhang, 2021). Additionally, some are unique in their characteristics, found in both human, animal, and plant bodies (Sheppard et al., 2018). They are commonly found in the inner linings of the digestive tracts and intestines of all living organisms (Johnson et al., 2022). When it comes to bacteria, there are differing opinions, with some considering them beneficial (Popkov et al., 2022). The human body is home to approximately ten times more bacteria than human cells. According to Singh (2023), some of these bacteria are harmless and assist in normal bodily functions. They also contribute to the recycling of nutrients from the environment (Salwan & Sharma, 2022). Furthermore, bacteria play a crucial role and provide numerous benefits in the production of fermented meals (Kumari et al., 2022). While some bacteria provide certain advantages, others can be detrimental and are referred to as pathogenic bacteria since they have the ability to infect humans, animals, and plants. They can also lead to food spoilage, which can cause illness (Sohrabi et al., 2022). Bacterial contamination in food is a significant issue affecting both human health and the safety and quality of the food we consume. While there are 31 pathogens that can lead to food-borne illnesses. *Salmonella*, *Campylobacter*, *L. monocytogenes*, *S. aureus*, and *E. coli* are the most prevalent worldwide (Abebe et al., 2022).

Table 2: Microbial spoilage of fish and seafood products

Aquatic Food Products	Bacterial Type	References
Refrigerated Atlantic Cod (*Gadus morhua*)	*Aeromonas* spp.	Hoel et al., (2022)
Fish burgers made with a combination of sea bass and sea bream meat.	Lactic acid bacteria	Iacumin et al., (2022)
Raw fish, sausages, fried fish, balls, cakes, and others	Enterobacteriaceae spp., *E. coli*, *S. enterica*, *C. freundii*, *Bacillus* spp. and *A. faecalis*	Kyule et al., (2022)
Smoked fish such as *Clarias gariepinus, Oreochromis niloticus*, *Citharinus citharus*	Gram-positive bacteria, *E. coli*	Omojowo and Iluahi (2021)
Shrimp surimi	*Psychrobacte*r spp. and *Brochothrix* spp.	Wang et al. (2021)
Raw and Sun-dried fishes	*E. coli*, *Salmonella* spp., *Staphylococcus* spp., and *Pseudomonas* spp.	Nur et al., (2020)

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Numerous parameters, including temperature, osmotic pressure, and pH, have a significant impact on bacteria's capacity to reproduce (Khan et al., 2022). According to temperature, bacteria can be divided into three types: thermophiles, mesophiles, and psychrophiles. These classes are named after the cold, moderate, and hot temperatures at which they thrive (Zhang et al., 2022). While certain bacteria may withstand high and extremely low pH values, many bacteria thrive at a neutral pH of 7.0 (Takano & Aoyagi, 2022). Bacteria can also be grouped according to their oxygen availability and capacity to live at varying salinity levels. According to Mahon (2023), bacteria can be classified into five groups: facultative (which can grow with or without oxygen), anaerobic (which can grow without oxygen), aerobic (which needs oxygen), microaerophilic (which needs less oxygen than air), and capnophilic (which grows faster with CO_2).The three categories of salt-tolerant bacteria are xerophiles, which can tolerate low NaCl concentrations, severe halophiles, which like concentrations between 15% and 25%, and halophiles, which favor concentrations of 3% or more (Roy et al., 2022). Determining ways to stop these different types of bacteria from contaminating food, including fish and other fishery products, requires an understanding of their traits.

Most common in fish, foodborne bacteria like Escherichia, Bacillus, Clostridium, Micrococcus, Proteus, and others have an ideal growth temperature of around room temperature (Coimbra et al., 2022). Additionally, raw fish can be spoilt by fermentative gram-negative bacteria, commonly known as Vibrionaceae, whereas cold fish can be spoiled by gram-negative psychrotolerant bacteria, such as *Shewanella* spp. and *Pseudomonas* spp. Other facultative anaerobic, aerobic, and/or psychrotrophic gram-negative bacteria may also contribute to spoiling (Inanoglu et al. 2022). Another serious problem that might cause fish to deteriorate bacterially is improper fish handling. The organoleptic features of injured fish are the first and most noticeable indication (Syropoulou et al., 2022). The fish as a whole may experience a slimy skin coating, gill and eye color changes that impact the entire body, and, lastly, the loss of muscle texture as a result of meat proteolysis—basically, the "softening" of tissue. These all are consequences of bacterial multiplication in fish (Torell et al., 2020). According to Abdel-Aziz et al. (2016), bacteria contribute to the putrefaction of proteins, which results in the disagreeable odor of ruined and unclean seafood. The bacteria *Shewanella* spp., *Aeromonas* spp., *Enterobacteriaceae* spp., and *Vibrio* spp. create fishy odors and off tastes that resemble ammonia (Kuley et al., 2017). Moreover, lactic acid-producing bacteria proliferate when organic acid is added to fish (Kuley et al., 2020).

Table 3. Spoilage compounds produced by microorganisms during the storage of fresh fish. (Church 1998; Gram and Huss, 2000).

Spoilage Bacteria	Spoilage Compound(s) Produced
Shewanella putrefaciens	TMA, H_2S, CH_3SH, $(CH_3)_2S$, Hx, and acids
Pseudomonas spp.	CH_3SH, $(CH_3)_2S$, ketones, esters, aldehydes, NH_3, and Hx
Photobacterium phosphoreum	TMA and Hx
Vibrionaceae	TMA and H_2S
Enterobacteriaceae	TMA, H_2S, ketones, esters, aldehydes, NH_3, Hx, and acids

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Lactic acid bacteria	H_2S, ketones, esters, aldehydes, NH_3, and acids
Yeast	Ketones, esters, aldehydes, NH_3, and acids
Aerobic spoilers	NH_3, acetic, butyric, and propionic acids
Anaerobic rods	Ketones, esters, aldehydes, and NH_3

TMA: Trimethylamine; H_2S: Hydrogen sulphide; CH_3SH: Methylmercaptan; $(CH_3)_2S$: Dimethylsulphide; Hx: Hypoxanthine; NH_3: Ammonia.

Methods of preservation of fish

Drying

The process of extracting liquid water from a solid, such as fish muscle, is referred to as drying. There are various techniques for obtaining the water, but the most commonly used method is evaporating the water vapor that forms on the fish's surface. Other techniques involve the use of solvents (such as in the production of fish meal) and freeze drying. By reducing the water supply, drying efficiently extends the shelf life of fish by preventing the proliferation of microorganisms. The growth of spoiling and other microorganisms is hindered when the moisture content of dried fish is reduced or when water is chemically bound to salt or sugars. The fish stays fresh and safe to eat for several days or even weeks, depending on how much it is dried and how well it is packaged. (Gram and Huss, 2000).

Freezing

The water in the food is frozen after being separated from other food ingredients. As a result, food is shielded from damaging factors like water and temperature. In addition to removing water from the area of activity, the lower temperature slows down the rate of chemical reaction. When food thaws, the water is reabsorbed by the food ingredients, returning the meal to its former state. Gopakumar (2006).

Synergetic effect of natural preservatives and drying

As drying of fish involves salt curing, latest drying methods involves addition of naturally occurring preservatives like ginger, garlic, turmeric, onion and tomato paste have been applied along with salt before drying and later are allowed to dry. During this process the antibiotic, anti-microbial compounds in this preservative are absorbed into fish muscle and improve the flavor and help in reserving the quality of fish and increases the shelf life of fish from 6-8 months. Salting is one of the oldest techniques used by humans to preserve and prolong the shelf life of fish, and it was used long before other methods like smoking, drying, canning, marinating, etc (Fuentes et al., 2007).

Happiness et al., 2024) aimed to optimize the use of spices during the storage of sundried marine sardines. Different combinations of garlic *(Allium sativum)* (0-3%) and turmeric (*Curcuma longa* (0-3%) were used. Hence the study concluded that optimising the use of spices such as garlic increases the quality and shelf life of sun-dried marine sardines, making it a recommended method for seafood preservation.

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Retarding or Delaying Spoilage

Aquatic food products can be processed in a variety of ways to slow down or postpone spoiling. These include the traditional methods of drying, brining, fermentation (including lactic acid fermentation), salting, and smoking (Tahiluddin & Kadak, 2022) as well as the non-traditional methods of chilling, freezing, and canning. Processing aquatic foods with microwave energy has gained popularity in recent years (Viji et al., 2022). It has also been observed and well established that natural preservatives may be used as spoiling inhibitors to help increase the shelf life of aquatic food products (Mei et al., 2019). By reducing moisture content, lowering pH, applying heat, and fermenting fish and fisheries goods, traditional processing methods will delay or prevent spoiling.

Anaerobic conditions, high temperatures, and low temperatures are used in non-traditional processing methods. The main advantage of natural preservation is that natural ingredients have potent antibacterial qualities. All of these techniques are merely meant to slow down or postpone the deterioration of aquatic food items brought on by a wide range of microbes.

Future perspectives

The main objectives of the future of seafood processing technology are to develop and implement innovative, nonthermal, and environmentally friendly methods that ensure the safety and quality of seafood products while increasing their shelf life. Advancements in nonthermal technology are transforming the fish industry by significantly improving the nutritional value, taste, and longevity of fish-based products. The development of new technologies will likely play a vital role in meeting the increasing demand for seafood products that are convenient, safe, and of high quality. They offer an alternative to conventional heat treatments that minimize loss while preserving sensory attributes, which is particularly beneficial for the seafood industry.

Conclusion

Microbial spoilage has a substantial impact on the quality, safety, and shelf life of fish products, posing significant risks to the seafood industry. The speed of decay of fish is primarily due to SSOs (Specific Spoilage Organisms), such as *Pseudomonas* spp., *Shewanella putrefaciens*, and photobacterium (phosphoreum), which can thrive at unsuitable storage temperatures. The mechanisms of spoilage microorganisms eat the components of the fish issue, producing undesirable compound, including ammonia, sulphides, and biogenic amines that result in decline of three sensory attributes of fish products, i.e., odour, texture, and appearance. Microbial spoilage can be reduced by implementing a full chain of preservation practices, including the cooling chain, Modified Atmosphere Packaging (MAP), vacuum packaging, and the use of natural antimicrobials or hurdle methods. Application of ingredients like ginger, garlic, turmeric and onion to fish and later kept for drying also helpful in extending shelf life of fish because of antibiotic compounds in them all of these factors are being advanced further through real time detecting processes and smart packaging to allow for control systems for spoilage detection. In conclusion, a combination of proper hygiene practices, technological advancements, and careful handling will help maintain the quality of fish, ensure food safety, and prevent economic losses in the value chain.

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