Microbiological Considerations in Food Packaging: Effects of Different Atmospheres and Preservation Methods

Toms C. Joseph
Microbiology Fermentation and Biotechnology Division
ICAR- Central Institute of Fisheries Technology, Cochin
tomscjoseph@gmail.com

Introduction

In the world of food production and distribution, the significance of food packaging cannot be overstated. It serves as a vital barrier between the external environment and the food products, protecting them from various contaminants, including microbial pathogens. Microbial contamination poses a considerable threat to food safety, as it can lead to spoilage, reduced shelf life, and even foodborne illnesses. To ensure the safety and quality of food products, it is essential to understand the microbiological considerations associated with different atmospheres in food packaging and preservation methods.

Raw foods naturally contain a diverse range of microorganisms, but not all of them are capable of colonizing the food and proliferating to significant levels. Some microorganisms have the ability to dominate and grow in particular conditions, leading to the formation of a specific microbial community known as a spoilage association. The exact mechanism through which one group of bacteria outcompetes another closely related group is not always fully understood. It can depend on various factors such as the initial microbial load, the availability of nutrients, the pH level, temperature, and other environmental conditions. Additionally, the interactions between different microorganisms within the food can also play a role in determining the composition of the spoilage association. Changes in the processing and packaging of fish products, even minor ones, can have a significant impact on the development and composition of spoilage associations. Different processing techniques and packaging methods can create different environmental conditions that favour the growth of specific microorganisms. As a result, the type of spoilage that occurs in fish products can vary drastically based on these factors. Furthermore, spoilage development can vary even for the same type of product, depending on factors such as geographical origin and other unknown variables. Microbial populations can be influenced by regional variations in environmental conditions, the presence of specific microorganisms in the surrounding environment, and local processing and handling practices. These factors can interact with the microbial development and contribute to the differences observed in spoilage patterns.

1. Packaging Methods

1.1. Reduced Oxygen Packaging

The reduction of oxygen levels within a package effectively inhibits the growth of aerobic spoilage bacteria, as these bacteria require oxygen for respiration. This technique is commonly employed in vacuum-sealed packages to preserve the quality of the contents. However, it is important to note that lowering oxygen levels may create an anaerobic environment, which is conducive to the growth of anaerobic bacteria, including *Clostridium botulinum*. This particular bacterium is a spore-forming pathogen that produces the highly potent botulinum toxin. Examples of ROP techniques are vacuum packaging, modified atmosphere packaging, controlled atmosphere packaging and sous-vide packaging.

1.1.1. **Modified Atmosphere Packaging** (MAP)/ Use of Gas Mixtures

Modified Atmosphere Packaging (MAP) involves altering the composition of gases surrounding the food product within a sealed package to extend its shelf life. The key gases used in MAP are carbon dioxide (CO_2), nitrogen (N_2), and oxygen (O_2). The effects of different gases on microbial growth include:

a. *Carbon Dioxide* (CO_2): CO_2 acts as a natural inhibitor of spoilage and pathogenic microorganisms. By creating an unfavourable environment, it impedes the growth of these microorganisms. One of the ways CO_2 achieves this is by lowering the pH and making it difficult for microbes to thrive. This preservation method is commonly used in the food industry to extend the shelf life of various perishable products. The presence of CO_2 helps to maintain the visual appeal of fresh produce. Fruits and vegetables tend to undergo enzymatic browning due to the activity of polyphenol oxidase enzymes. CO_2 slows down this enzymatic reaction, preserving the natural colour of the product for a longer period. Similarly, CO_2 helps to retain the desired texture, preventing softening and wilting of fruits and vegetables. CO_2 possesses inherent antimicrobial properties, which contribute to its effectiveness in food preservation. It exhibits bacteriostatic and fungistatic effects and it thus inhibits the growth and reproduction of bacteria and fungi without completely eliminating them. This property is particularly beneficial in inhibiting the growth of spoilage microorganisms and extending the freshness of perishable foods. While CO_2 has a broad-spectrum antimicrobial effect, it is essential to note that some microorganisms exhibit resistance to its inhibitory effects. Certain yeasts and molds, for instance, have developed mechanisms to tolerate high levels of CO_2. These microorganisms can still proliferate even in the presence of elevated CO_2 concentrations. Therefore, alternative preservation methods may be necessary to control their growth effectively.

b. *Nitrogen* (N_2): Nitrogen, a widely utilized inert gas, plays a crucial role in the process of MAP. Its primary function is to displace oxygen, creating an environment devoid of oxygen that inhibits the growth of spoilage organisms that thrive in aerobic conditions. While nitrogen effectively prevents the proliferation of aerobic spoilage organisms, it does not directly impact the growth of anaerobic bacteria or spore-forming organisms.

c. *Oxygen* (O_2): In MAP, the existence of oxygen can facilitate the proliferation of harmful microorganisms and lead to the deterioration of food items through oxidation. Thus, it is imperative to lower the oxygen content or establish a barrier against oxygen to ensure the preservation of food quality and prolong its shelf life.

Effects on Microorganisms:

a. *Aerobic Microorganisms*: In MAP, the reduction of oxygen levels effectively hinders the proliferation of aerobic microorganisms, including molds and certain bacteria. These microorganisms rely on oxygen for their survival, and by limiting their presence, the shelf life of oxygen-sensitive products can be extended.

b. *Anaerobic Microorganisms*: In MAP, elevated carbon dioxide levels play a crucial role in inhibiting the development of anaerobic microorganisms.

1.1.2. Vacuum Packaging

Vacuum packaging is a preservation technique that involves the removal of air from the package before sealing, resulting in a vacuum environment. This method of preservation offers numerous advantages for packaged products.

a. *Microbial Growth Inhibition*: Vacuum packaging creates an environment devoid of oxygen, which effectively suppresses the growth of aerobic spoilage bacteria. By eliminating the presence of oxygen, this method inhibits the proliferation of bacteria that rely on oxygen for their survival.

b. *Prevention of Oxidative Reactions*: Vacuum packaging plays a vital role in preventing oxidative reactions in food products by removing oxygen. These reactions, if allowed to occur, can cause undesirable colour changes, rancidity, and nutrient degradation. Because of the absence of oxygen, vacuum packaging effectively safeguards the quality and freshness of the packaged food.

Effects on Microorganisms:

a. *Aerobic Microorganisms*: The absence of oxygen limits the growth of aerobic microorganisms, leading to extended shelf life of the product and a reduced risk of spoilage. Vacuum packaging effectively hinders the proliferation of aerobic microorganisms, contributing to the preservation and freshness of the product.

b. *Anaerobic Microorganisms*: Vacuum packaging does not directly inhibit the growth of anaerobic microorganisms, such as *Clostridium botulinum*. Therefore, it is vital to implement additional measures, including temperature control and other factors, to prevent their growth and ensure product safety.

Specific Spoilage Organisms and Reduced Oxygen Packaging

Specific spoilage organisms (SSOs) are microorganisms that have a high affinity for particular food types. They possess unique metabolic capabilities that allow them to thrive and proliferate under specific environmental conditions. These organisms have a detrimental impact on the sensory attributes, appearance, and overall quality of food products. Different food products are susceptible to specific spoilage organisms. For instance, *Pseudomonas spp.* are commonly associated with the spoilage of seafood, while yeasts like *Candida* and *Debaryomyces* are prevalent in fruit and bakery products. Lactic acid bacteria, such as *Lactobacillus spp*., are responsible for the spoilage of dairy and fermented foods.

Microbial Spoilage Flora/Specific spoilage organisms in Aerobically Packaged Chilled Stored Fish

Shewanella putrefaciens and *Pseudomonas spp*. are the specific spoilage bacteria of iced fresh fish regardless of the origin of the fish. Modified atmosphere stored marine fish from temperate waters are spoiled by the CO_2 resistant *Photobacterium phosphoreum* whereas Gram-positive bacteria are likely spoilers of CO_2 packed fish from fresh or tropical waters.

Pseudomonas spp.: *Pseudomonas spp*. are gram-negative bacteria commonly found in water and soil. They are capable of utilizing various carbon sources. During aerobic storage, *Pseudomonas* produce enzymes that break down proteins and lipids, producing off-flavours and odours. These bacteria thrive in the presence of oxygen and can cause sliminess and discoloration of the fish.

Shewanella spp.: Among the H_2S-producing bacteria, *Shewanella putrefaciens* is considered a specific spoilage organism in fish from temperate and tropical waters. It can grow under aerobic conditions. *Shewanella spp*. are psychrotrophic bacteria that thrive at low temperatures. *Shewanella spp*. are often associated with the spoilage of refrigerated fish. They produce hydrogen sulfide, resulting in the characteristic "rotten egg" smell.

Photobacterium spp.: *Photobacterium spp.* are gram-negative bacteria that are common in marine environments. They are known for their ability to produce histamine, a potent toxin responsible for scombroid fish poisoning. Histamine formation can occur even under reduced oxygen storage conditions.

Microbial Spoilage Flora in Reduced Oxygen Packaged Chilled Stored Fish

Reduced oxygen storage involves controlling the oxygen levels around the fish to inhibit the growth of spoilage microorganisms. By reducing the oxygen concentration, the growth of aerobic bacteria is suppressed, extending the shelf life of the fish.

Lactobacillus: *Lactobacillus* is a genus of bacteria commonly associated with anaerobic spoilage in fish. While LAB is commonly associated with beneficial processes, some species can act as spoilers in fish and other perishable foods. These bacteria are facultative anaerobes, meaning they can switch between aerobic and anaerobic metabolic pathways depending on the oxygen availability. *Lactobacillus* species are known for their ability to produce lactic acid, which contributes to the characteristic souring of spoiled fish.

Brochothrix thermosphacta: It is a facultative anaerobic organism, capable of growing under both aerobic and anaerobic conditions. This bacterium can proliferate even in the absence of oxygen, making it a significant contributor to spoilage in vacuum-packed and modified atmosphere-packaged fish products.

1.2. Aseptic Packaging

Aseptic packaging involves the separate sterilization of both the food product and the packaging material, followed by filling the sterile product into a sterile container under aseptic conditions. This packaging method encompasses several key considerations.

a. *Sterility Maintenance*: Aseptic packaging ensures the product and packaging remain devoid of microbial contamination throughout the filling, sealing, and storage processes. By maintaining a sterile environment, it effectively inhibits the growth of spoilage microorganisms and pathogens.

b. *Barrier Properties*: Aseptic packaging materials offer a protective barrier against microorganisms, light, oxygen, and moisture. This barrier preserves the quality of the food product and extends its shelf life by preventing external factors from compromising its integrity.

Effects on Microorganisms:

a. *Sterilization*: Aseptic packaging employs sterilization technique (heat treatment) to eliminate microorganisms. This process effectively eradicates spoilage organisms, pathogens, and enzymes, thereby increasing the product's shelf life.

b. *Microbial Barrier*: The sterile packaging material acts as a barrier against microbial recontamination during storage and distribution. This barrier is crucial for maintaining product safety and preventing spoilage.

1.3. Active Packaging

Active packaging systems are specifically designed to interact with the food product and its environment, providing additional preservation benefits. These systems incorporate antimicrobial agents or substances that can inhibit microbial growth.

Antibacterial Packaging Materials: The integration of antimicrobial agents into packaging materials creates an additional barrier against microbial contamination. These materials release antimicrobial compounds that effectively hinder the growth of bacteria, fungi, and other microorganisms. By reducing the risk of spoilage and foodborne illnesses, these antibacterial packaging materials contribute to the overall safety and quality of the food product.

Effects on Microorganisms:

a. *Antimicrobial Agents*: Active packaging utilizes antimicrobial agents, such as organic acids, enzymes, or bacteriocins, which exhibit inhibitory effects on specific microorganisms. These agents assist in suppressing the growth of spoilage and pathogenic bacteria, thereby enhancing product safety and extending the shelf life of the food.

b. *Controlled Release Systems*: Certain active packaging systems employ controlled release mechanisms that dispense volatile compounds, such as ethanol or essential oils, possessing antimicrobial properties. These compounds create an unfavourable environment for microorganisms, impeding their growth and extending the shelf life of the product.

2. Preservation Methods

In addition to packaging, various preservation methods can be employed to mitigate microbial growth and prolong shelf life of food. Preservation methods play a crucial role in controlling microorganisms, ensuring food quality and longevity. Here are several ways in which preservation methods achieve these objectives:

2.1. Temperature control: Temperature is vital in controlling microbial growth. Preservation methods such as refrigeration and freezing effectively slow down the growth of bacteria, yeast, and molds. Cold temperatures inhibit their metabolic activities, reproduction, and enzyme production, ultimately extending the shelf life of food products. Freezing is a widely accessible and convenient preservation method. By lowering the temperature, microorganisms' growth is significantly slowed or halted and thus it helps to maintain the quality and freshness of food products.

Table 1: Classification of bacteria based on temperature requirement

Bacteria	Temperature Range	Examples
Psychrophiles	Below 20°C	*Psychrobacter, Flavobacterium*
Psychrotrophs	0°C - 30°C	*Listeria monocytogenes, Pseudomonas*
Mesophiles	20°C - 45°C	*Escherichia coli, Staphylococcus aureus*
Thermophiles	45°C - 80°C	*Thermus aquaticus, Sulfolobus*
Hyperthermophiles	Above 80°C	*Pyrococcus furiosus, Thermococcus*

2.2. Drying/ Dehydration: Drying is a preservation method that removes moisture from food, creating an inhospitable environment for microbial growth. Bacteria, yeast, and molds require moisture to survive. By reducing the moisture content, drying inhibits microbial activity and enzymatic reactions, thereby prolonging the shelf life of food. This preservation method can be achieved through sun drying, air drying, or using specialized equipment like food dehydrators. Dried foods have an extended shelf life and are lightweight, making them ideal for storage and transportation.

The water activity (a_w) of a food refers to the availability of water for microbial growth and chemical reactions. Drying food to lower water activity levels can inhibit microbial growth and enzymatic reactions, thereby extending the shelf life of the food. Water activity reduction during drying depends on factors such as drying method, temperature, and duration. The aim is to reach a water activity level that prevents microbial growth and enzymatic reactions, typically below 0.6 or 0.7. At such low water activity levels, most microorganisms are unable to grow, making the dried food more resistant to spoilage and microbial contamination.

Table 2: Minimum water activity (a_w) values for microbial growth

Microorganism	**Minimum a_w value**
Bacteria	0.91
Yeast	0.86
Mold	0.80
Halophilic Bacteria	0.75
Xerophilic Bacteria	0.65
Osmophilic Bacteria	0.60

2.3. Salting and Sugaring: Salting and sugaring are ancient methods of food preservation. Both techniques work by drawing moisture out of the food, creating an environment where microorganisms cannot thrive. Salted and sugared foods, such as cured meats and jams, can be stored for long periods without spoilage. Salting involves the addition of salt. Salt draws out moisture from the food through osmosis, creating an environment with a high salt concentration that inhibits microbial growth. Additionally, it reduces water activity, making it difficult for bacteria and other microorganisms to survive and spoil the food.

2.4. Low pH and Acidity: Acidic conditions inhibit microbial growth by disrupting their cellular structure. Techniques like pickling and fermenting create an acidic environment that is unfavourable for most harmful microorganisms, while beneficial bacteria thrive and provide preservation.

Fermentation: Fermentation is a preservation method that harnesses the growth of beneficial microorganisms, such as lactic acid bacteria and yeast. These microorganisms produce acids, alcohol, and other compounds that create an acidic or alcoholic environment, inhibiting the growth of harmful bacteria. Foods undergoing fermentation, like yogurt, sauerkraut, and kimchi, have an extended shelf life due to the presence of these beneficial microorganisms.

Pickling: Pickling is a preservation method that involves immersing food items, such as vegetables or fruits, in a brine or vinegar solution. The acidic environment created by the pickling solution inhibits the growth of harmful bacteria, yeasts, and molds, effectively preserving the food. The process of pickling not only extends the shelf life of the food but also imparts a unique tangy flavour and texture. Pickled foods are popular for their versatility and can be enjoyed as condiments, toppings, or standalone snacks.

2.5. Smoking: Smoking is a preservation method that exposes food to the smoke of burning wood or other plant materials. The smoke contains antimicrobial compounds that inhibit bacterial growth and prevent food spoilage. Smoking is commonly used for preserving fish, meat, and cheese.

2.6. Chemical preservatives: Certain chemicals can be used as preservatives to control microbial growth. Common preservatives include organic acids (e.g., citric acid, acetic acid), antioxidants (e.g., vitamin C, tocopherols), and antimicrobial agents (e.g., sodium benzoate, sorbic acid). These substances inhibit the growth of bacteria, yeast, and molds, thereby preventing spoilage and extending the shelf life of food products.

2.7. Heat Treatment: Thermal processes, such as pasteurization and canning, are employed to eliminate or reduce the microbial load in food. These methods involve the application of heat to kill or deactivate microorganisms. High temperatures destroy the enzymes and proteins essential for microbial growth, effectively controlling their population.

High-Temperature Short-Time (HTST) Pasteurization: HTST pasteurization involves heating the food product to a specific temperature for a short duration, thereby eliminating or reducing the microbial load. It is commonly used for liquid or semi-liquid food products like juices and sauces before they are packaged.

Low-Temperature Long-Time (LTLT) Pasteurization: LTLT pasteurization utilizes lower temperatures for a longer duration, making it suitable for more delicate food products such as dairy and egg-based items. This process ensures the reduction of pathogens and spoilage microorganisms while minimizing quality degradation.

Canning: Canning is a preservation method that involves heat processing food in sealed containers. The high temperatures applied during canning destroy bacteria, yeasts, and molds that could cause spoilage or foodborne illnesses. The hermetically sealed containers act as a barrier, preventing recontamination and hindering the entry and multiplication of microorganisms.

2.8. Non-thermal preservation methods: Non-thermal food preservation methods offer alternative approaches to controlling the growth of microbes and extending the shelf life of food without relying on high temperatures. These methods utilize various techniques that work synergistically to inhibit microbial activity and maintain the quality of the food.

***High-Pressure Processing** (HPP)*: HPP subjects the food to intense pressure, usually between 100 and 800 megapascals (MPa), which disrupts the cellular structure of microbes. The increased pressure leads to the inactivation of bacteria, yeasts, molds, and enzymes, thereby extending the shelf life of the food while preserving its nutritional value and sensory attributes. HPP helps to ensure food safety while extending shelf life.

***Pulsed electric field** (PEF)*: PEF technology involves exposing the food to short bursts of high-voltage electricity. The electrical pulses create tiny pores in the microbial cell membranes, disrupting their integrity and leading to cell death. This method effectively controls microbial growth while minimizing the impact on the food's organoleptic properties.

Pulsed light technology: The pulse light technique, also known as pulsed light technology, involves the use of intense and short bursts of light energy to eliminate or reduce microbial contamination on various surfaces, including food products. This non-thermal method is particularly effective in targeting bacteria, yeasts, molds, and other pathogens that can compromise food safety. Pulse light technology operates by emitting high-intensity light pulses, typically in the ultraviolet (UV) or visible spectrum range. These light pulses disrupt the DNA or RNA structure of microorganisms, rendering them unable to reproduce or causing their death. The short duration and high energy of the light pulses ensure effective microbial inactivation while minimizing heat transfer to the treated food.

***Ultraviolet** (UV) Treatment*: UV light is a highly effective non-thermal method for microbial control. It disrupts the DNA and RNA of microorganisms, rendering them incapable of growth or reproduction. UV treatment can be applied to surfaces, packaging materials, or directly to food products, providing an additional layer of protection against microbial contamination.

Irradiation: Irradiation is a non-thermal preservation method that uses ionizing radiation, such as gamma rays or electron beams, to destroy microbes. The radiation damages the genetic material of microorganisms, inhibiting their ability to reproduce and causing their demise. Irradiation is a highly regulated process that has been extensively studied and approved for use in certain food products.

Cold plasma technique: Cold plasma technique is known for its remarkable ability to significantly reduce microbial load. By employing this innovative technology, harmful microorganisms can be effectively eliminated, leading to improved safety and hygiene in various applications. Cold plasma works by generating a non-thermal plasma state at low temperatures, which releases a variety of active species such as reactive oxygen species and reactive nitrogen species. These highly reactive species then interact with microbial cells, causing damage to their membranes and genetic material. As a result, the viability and proliferation of microorganisms are greatly hindered, leading to a substantial reduction in their overall population. This technique offers a promising solution for microbial control in diverse fields, including food processing, healthcare, and environmental sanitation.

Conclusion

Microbiological considerations play a crucial role in the realm of food packaging, ensuring both the safety and quality of food products. It is important to note that although these preservation methods and packaging atmospheres aid in controlling microbial growth, they do not provide a complete guarantee of sterilization. Alongside these measures, maintaining proper hygiene practices, controlling temperatures, and ensuring appropriate storage conditions are essential in minimizing the risk of foodborne illnesses and preserving the overall quality of packaged food products.

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