MICROBIAL FOOD SPOILAGE OF ANIMAL BASED PRODUCTS AND PRESERVATION BY USING DIVERSE METHODS

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Abstract
Meat, fish, dairy and its products provide favorable conditions for the growth of microbes. These microbes cause changes in meat, fish and dairy products. The breakdown of nutritional components like Carbohydrates, lipids, proteins and fats leads to the physical as well as chemical changes by enzymatic activities in them which is the main cause of their rejection to consume. To increase their shelf life and decrease the growth of microbes in them various methods and techniques have been introduced to preserve them. Most commonly food preservation is done from the time of their harvesting to reach the consumers. This is done to create a variety and for some additional nutritional components in different food stuffs. A comprehensive study is done on the preservation methods of these products to prevent them from spoilage. Previous data about their preservation reveals that salting, drying, freezing, fermentation, chilling and canning are better methods to prevent meat and fish spoilage and for milk, pasteurization is one of the best methods to be used. But nowadays due to the advanced technologies in the field of science, various techniques like irradiations, UV radiations, osmotic pressure, and natural as well as synthetic preservatives are also available. After all these Techniques, there is still a big challenge to preserve these products in their natural state and other physical characters.

Key words: Favorable conditions, enzymatic activities, fermentation and irradiation
INTRODUCTION

Many people in the world consume meat as animal protein which is their first choice (Heinz and Hautzinger, 2007; Geiker et al., 2021). Meat utilization in Canada was assumed at a total of 36.6 kg per capita (including beef, chicken, turkey, pork and lamb (SC, 2009). In the USA, the total consumption of meat was assumed as 101 kg. The consumption of meat has increased day by day in the world from 10-26 kg from 1960 to 2000, and it is assumed that it will reach 37 kg by 2030 (Heinz and Hautzinger, 2007) but from this, a very high quantity of meat and its products are getting spoiled. Near about 3.5 billion kg of meat is assumed to be spoiled before reaching the consumers, which causes huge ecological and economic problems (Kantor et al., 1997). Much of this spoilage is caused by microbes. Since meat has a high nutritious content, it is necessary to include meat and meat products in human diets. Regrettably, there are problems associated with the production and consumption of meat, including ethical dilemmas, public health problems, and ecological effects (Boukid et al., 2021, Tóth et al., 2021; Liu, et al., 2023). Only 5% of the preservation of meat is required out of total spoiled meat to meet the demand of 320,000 people (Cerveny et al., 2009).

The conversion of meat from an animal requires many essential steps like truanting the animals to their farms, picking and loading the animals to their slaughterhouse and poor slaughtering operations lead to serious injury and cause major microbial attacks (Chambers and Grandin, 2001), that’s why proper hygienic conditions are very important (FAO, 1991). To increase the reservation and storing of meat expertise and hygienic handling of meat is required during slaughter. Many techniques like the removal of blood, skin and removal of internal eye contents, presence of acidic compounds in meat have a great effect on its spoilage for example muscles of beef take time to spoil than that of the liver (FAO, 1990). Vegan meats and other plant-based meat replacements, sometimes known as plant-based meat analogues, are gaining popularity. Because the quality of the things that are accessible is always rising, so is the amount that is consumed. Meat analogues' main function is to substitute the meat component in meals while also offering the proper nutritious content and hedonic value. There has been less research done on the food safety elements of these recently developed food products (Tóth et al., 2021).

About 25% of plants and fish products are lost every year due to microbes and chemical degradation (Baird-Parker, 2000). Just because of improper techniques used for the storage of fish and shrimps about 4-5 million tons are destroyed (Unklesbay, 1992). The spoilage of fish is
very fast just after it is caught because muscular stiffening occurs after 12 h at tropical temperatures (Berkel et al., 2004). Many marine or freshwater species die because of their own enzymes and microbes that are present on their surface (AMEC, 2003). Certain new compounds are formed during the spoilage of fish which causes physical changes as well as their taste to unfit to eat. Storage at very low temperatures and the use of better and advanced preserving methods can prevent the spoilage of fish (Mahmoud et al., 2006). Combinations of synthetic as well as natural preservatives can overcome the compound’s degradation and oxidation in a better way (Bagamboula et al., 2004).

**Microbial Spoilage of Meat**

Meat and meat products are the most favorable media for microbial growth. All these microbes are not beneficial but some of them are also pathogens (Jay et al., 2005). Bacterial species mainly include *Bacillus*, *Clostridium*, *Escherichia*, *Lactobacillus*, *Pseudomonas*, *Micrococcus*, *Streptococcus*, *Sarcina*, and *Salmonella* (Nychas and Tassou, 1997; Arnaut-Rollier et al., 1999; Lin et al., 2004). Major filamentous fungal species found in the meat are *Cladosporium*, *Geotrichum*, *Mucor*, *Penicillium* and *Sporotrichum* (Garcia-Lopez et al., 1998). The source of these microflora is normally the skin and GIT of an animal and their composition is mainly controlled by user handling, animal age, husbandry practices, packaging, slaughtering and preservation methods (Cerveny et al., 2009). Hayes and his coworkers studied 981 samples of different meat types and the most prevalent bacteria in collected samples was *Enterococcus* spp. (Hayes et al., 2003). *Acinetobacter* spp., *Moraxella* spp., *Pseudomonas* spp and *Psychrobacter* spp. were also recorded in high numbers, the number and type of microbes in meat are controlled by many intrinsic and extrinsic factors (Cerveny et al., 2009). Members of Family Enterobacteriaceae are mostly present in the meat stored in the refrigerator. In salted cured products lactic acid bacteria, micrococci and enterococci were observed because of their resistance against curing salts. Yeast was also found abundantly in raw and salted-cured products. Enterobacteriaceae and *Pseudomonas* were still growing on pork meat when it was packed in a vacuum or it is stored at 5°C (Garcia-Lopez et al., 1998). At 0°C *Pseudomonas* spp. grows slowly, but fast at 2°C and the shelf life of meat affects it. In the same way, *Salmonella* growth rate is good above 7°C but slow below 7°C and it is also affected by the shell life of meat (Sentence, 1991). The study conducted by Bruckner et al. (2012) revealed that the growth of *Pseudomonas* species is directly influenced by temperature, with higher temperatures leading
to faster growth rates, as has been documented in previous literature. The study found that fresh poultry had a significantly shorter shelf life at constant temperatures between 2 and 15C, as *Pseudomonas* spp. grew on them more quickly than on fresh pork. This finding is supported by Figure 1.1. Additionally, the study showed that pork and chicken had significantly different pH values at every sample point, with the latter having higher pH values. It is important to note that higher pH values have been found to be a contributing factor to the microbiological deterioration of meat.

![Figure 1: Comparison of microbial shelf life in Pork and Poultry meat at various temperatures.](image)

Solutions are required to buck the existing trend in meat production and consumption because they are not environmentally sustainable. Increasing the intake of different protein sources, such as insects, cultured meat, or plant-based proteins, could be one way to solve the problem. Products that mimic meat but are plant-based strengthen the latter argument. Even though there is a growing consumer demand for these products, food processors still struggle to make high-quality products in the accurate numbers. As a result, products need to be developed further with consideration for food safety (Tóth *et al*., 2021).

**Microbial Spoilage of Fish**

Microbial flora found in fish caught depends mainly upon the water in which fish lives. *Alcaligenes, Micrococcus, Pseudomonas, Serratia* and *Vibrio* are most prominent bacterial species of fish microflora (Gram and Huss, 2000). Fish spoilage occurs due to two main factors i. Microbial growth ii. Microbial metabolism. During metabolism amines and especially
biogenic amines are produced which caused unpleasant and unacceptable flavor (Gram and Dalgaard, 2002; Emborg et al., 2005; Dalgaard et al., 2006). Chilled fish is spoiled due to Gram-negative bacteria such as *Shewanella* and *Pseudomonas* spp. which are psychrotolerant while unpreserved fish is spoiled due to the metabolism and growth of Gram-negative bacteria like *Vibrionaceae* which produce alcohol because they are fermentative (Gram and Huss, 2000). Not all bacteria contribute to spoil food so it is important to differentiate between them (Huss, 1995). Many compounds are formed which participate in spoilage of food. Level of trimethylamine (TMA) showed microbial deterioration which leads towards spoiling of fish. Trimethylamine Oxide (TMAO) is an osmoregulation to save fish from dehydration in hypertonic environment and tissue waterlogging in hypotonic environment. *Shewanella putrefaciens*, *Aeromonas* spp., psychrotolerant Enterobacteriaceae, *P. phosphoreum* and *Vibrio* spp. are the bacterial species which can use TMAO for the production of energy for their need and then TMAO is reduced to TMA (Gram and Dalgaard, 2002). At the start of the spoilage, *Pseudomonas putrifaciens* fluorescent pseudomonads and other bacteria have high activity and grow fast (Hui, 1992). These spoilage bacteria produce proteolytic enzymes (Shewan, 1961). Proteases can degrade proteins into peptides and aminoacids. Lipases are also produced which degrade lipids into glycerol and fatty acids (Liston, 1980). There are four phases of fish spoilage, in first two phases enzymes are responsible and in last two phases microbes spoil the food or fish to such level that it is not able to eat (Abbas et al., 2009). Olafsdottir et al. (2006) checked the growth and metabolism of specific micro-organisms in haddock fillets refrigerated at 0, 7 and 15°C and they knew *Photobacterium phosphoreum* as one of the most dominant species of bacteria which become the cause of food spoilage. *Pseudomonas* spp. was producing sweet and fruity smell contributing in spoilage while *Shewanella putrefaciens* was producing hydrogen sulfide. Nuin et al. (2008) also checked the growth of bacteria responsible for spoilage at 0°C and 15°C on farmed turbot and found *Pseudomonas* spp. growing fast the both temperatures and becoming the cause of turbot spoilage. The phenotypic characteristics and enzymatic spoiling activities of sixty-six suspected *Pseudomonas* strains isolated from various food matrices (ready-to-eat vegetables, meat, milk, and dairy products) were investigated. Protease-activity was confirmed both quantitatively and subjectively. There was a significant difference in the proteolytic activity of the isolates that tested positive for protease in UHT-milk. The isolates with the highest activity were those tentatively identified as *P. gessardii*-like. Five distinct groups and two sub-groups were shown using an aprX gene-based phylogenetic dendrogram (Caldera et al., 2016).
Microbial Spoilage of Dairy Product

Psychrotrophic bacteria in milk are much percent and *Pseudomonas* are about 70% of total isolated psychrotrophic bacteria (García, Sanz, Garcia-Collia, & Ordonez, *et al.*, 1989; Griffiths, Phillips, & Muir, 1987). Psychrotrophic coliforms are also present and they can reduce diacetyl which is the component of sour cream and buttermilk and change the flavor (Wang & Frank, 1981). Lactic acid producing bacteria also become cause of yogurt like flavor of buttermilk and sour cream also increase their viscosity (Hogarty & Frank, 1982). Yeast can also grow well at acidic PH and reduce diacetyl and produce yeasty or yogurt like flavor to buttermilk and sour cream (Wang & Frank, 1981). Spore forming bacteria are also present in raw milk and products and their count rarely exceeds 5000/ml before pasteurization (Mikolajcik & Simon, 1978); however, milk processing can contaminate it (Griffiths & Phillips, 1990). Enzymes like proteases and lipases as well as phospholipases are produced by microbes present in dairy products is an indirect cause of spoilage of dairy products and these enzymes remain active longer than the life of microbes. Psychrotrophs can produce enough extracellular enzymes which can spoil the milk that can be checked by sensory tests (Fairbairn & Law, 1987). These psychrotrophs can produce proteases and their count is 70–90% in raw milk and they become active when they heated at 149°C for more than 10 sec (Adams, Barach, and Speck, 1975) and it was verified later (Griffiths, Phillips, & Muir, 1981). Products of milk are also affected largely by action of extracellular proteases which produce bitter peptides. Thermally stable proteases work at high temperatures (Shah, 1994; Sørhaug & Stepaniak, 1991).

In addition, phospholipases can be heat stable. Experiments raveled that production of phospholipase in raw milk can be a cause of bitter off-flavors (Fox, Chrisope, & Marshall, 1976; Chrisope & Marshall, 1976). Heat-resistant lipases of bacteria have been linked with the incoming of rancid flavors in UHT milk (Adams & Brawley, 1981). Production of lipases are mostly seen by *Pseudomonas fluorescens* in milk and milk products, not only this bacterial species can do this but Gram-negative psychrotrophic bacteria also can produce lipases which can affect the taste of milk and its products, actually lipases convert lipids into short chain fatty acids of 4 carbons which produce rancid flavor and production of long chains with soapy flavor and transformation of free unsaturated fatty acids into ketones and aldehydes by oxidation results in an oxidized flavor (Deeth & Fitz-Gerald, 1983), lipolysis cause fruity off-flavor of milk and esterification of short chain fatty acids with alcohols by *Pseudomonas fragi* (Reddy, Bills, Lindsey, & Libbey, 1968). Lipases go with fat portion of milk which cream is separated.
from non-fat part of the milk (Downey, 1980; Stead, 1986). The accumulation of fat globules and in them action of lipases enhance the chances of interaction between enzyme and substrate. Extra foaming to butter is provided by churning and lipolysis (Deeth & Fitz-Gerald, 1983). By enhancing the time of churning can enhance the rancidity of butter due to the lipase activity in the milk or butter. Actually, fatty acids with short chain are formed in rancid cream are water soluble that is why lost in buttermilk and are removed on washing of water from butter (Stead, 1986). Lipases that are still present in butter can work even under frozen conditions (Nashif & Nelson, 1953). Lipase activity is decreased at acidic pH in some cheeses. Lipolysis can occur even at near 7 as in Brie and camembert (Dumont, Delespaul, Miquot, & Adda, 1977). To get a desired flavor in cheddar cheese, large number of lipases are required (Law, Sharpe, & Chapman, 1976). Heat resistant bacteria lipases which remain left in non-fat whole milk powder may affect it and to the related lipid portion which is add to these products (Stead, 1986).

**Prevention of food spoilage from microbes**

During preparation, storage and distribution of food products are prevented from spoilage. There is a need to extend the shelf-life for these food products because these are transported to far distant areas from their production. Preservation is the most important aspect of food from microbes to make it fit for use. These includes many preservation processes like heating, addition of antimicrobial compounds and freezing the product to reduce the risk of food poisoning however, these techniques frequently linked with undesirable changes in organoleptic distinctiveness and loss of nutrients. New methods of food preservation have emerged as a result of growing customer demand for tastier, more nourishing, all-natural, and simple-to-handle food products. International trading in perishable goods has become viable because to advancements in the cold supply chain, however refrigeration cannot guarantee the quality and safety of all perishable commodities. The weak organic acids, such as acetic, lactic, benzoic, and sorbic acid, are the most often used traditional preservatives. Sorbic acid has also been shown to prevent the germination and development of bacterial spores. These molecules also prevent the growth of both bacterial and fungal cells. The food sector now faces a greater challenge in successfully achieving this goal due to contemporary consumer preferences and food legislation.

The first is that consumers demand more high-quality, safe, preservative-free, moderately processed foods with long shelf lives. For instance, this would require treating food at mildpasteurization temperatures rather than sterilization ones and preserving it at higher pH levels.
Considering that acidity and sterilization processes are two essential elements in the management of the spread of harmful spore-forming bacteria, such as *Clostridium botulinum*, meeting this consumer demand necessitates the development of novel strategies to assure product preservation. Second, regulation has limited the use and permissible dosages of particular preservatives that are now approved for use in various foods. A growing percentage of consumers favour meals that have been prepared with little processing and no artificial preservatives. According to Arneborg, Jespersen, and Jakobsen (2000), contemporary methods are more focused on the opportunities provided by biological preservation. If the moisture and salt are not distributed equally, psychrotrophic bacteria can develop and create lipases in refrigerated salted butter (Lindow & Brandl, 2003). Concentrated (condensed) milk must be stored in the refrigerator until usage when used in bulk. Adding roughly 44% sucrose and/or glucose will drop the water activity below the point at which live spores would germinate (aw 0.95), allowing for preservation.

**Principles of Prevention of Spoilage**

The exclusion of microbial activity is necessary for food preservation and spoiling avoidance (Figure 2). In theory, this may be accomplished by keeping out or eliminating germs, preventing their growth and activity, or even by killing them.

**A. Physical Preservation Methods**

**High Temperature**

Heating of foods also helps us to pull down nasty microbes that negatively affect our food quality. The basic purpose of heating and boiling is to destroy vegetative cells and spores of microbes (Silliker, Elliott, Bryan, Christian, & Clark, 1980). It is the safest and reliable methods of food products preservation. Steam under pressure is most effective method of high temperature. It can kill all kinds of microbes including endospores. Mostly foods are heated to destroy specific pathogens and food spoiling microbes. Heating may be generated by hot water, dry heat. Pasteurization is a physical technique in which every particle of food is heated up to a specific range of temperature and specified time to demolish spoilage-causing microbes (Ranken, 2012). Method of pasteurization is used commercially including High Temperature Short Time (HTST), a Low Temperature Long Time (LTLT) method. Applications of pasteurization is to preserve different food items. Efficiency of pasteurization depends on the
temperature–time combination. This union depend on the thermal death-time of microbes that is likely to resist microbes (Kutz, 2007). Vat pasteurizer is favorable for milk and requires constant supervision to prevent overheating, over holding, or burning (Rahman, 2007). HTST pasteurization is also known as ‘fash pasteurization’ (Amit, Uddin, Rahman, Islam, & Khan, 2017). Vat and HTST pasteurization are effectively for pathogenic microorganisms. HTST and UHT both treatments are effective to demolish heat resistance non-spore forming pathogenic microorganisms such as Mycobacterium tuberculosis and Coxiella burnetti (Khan, 2015). After heating, items are aseptically packed in sterile containers (Bodyfelt, Drake, & Rankin, 2008). Heat treatment process that completely pull down all the kinds of microbes. In this way, we can increase its shelf life (Rahman, 2007). Final product is comparable in flavor and nutritional quality to pasteurized milk. Drying usually is accomplished by the removal of water by sun, air and heat. Dehydration is due mainly to microbistasis. The main purpose of drying food is to obtain solid material with low water content to reduce the growth of vegetative cells and spores of microbes. It is an ancient method of food preservation (Berk, 2013). Water plays an important role for microbial growth (Jay, Loessner, & Golden, 2008). It has varies advantages such as reduces weight and volume of foods, packing, facilitates foods storage, transportation and cheapest method of preservation (Agrahar-Murugkar & Jha, 2010). Some important compounds like protein, vitamin C, lipid thiamin are lost because of dryness (Salvato, 1992). In 1810, Nicolas Appert and same years Peter Durand described the use of tin containers for food preservation (Barbier, 1994).

**Low Temperatures**

Foods are kept at low temperature in many ways to enhance their shelf life. Modern refrigeration and freezing equipment have made to transport and store foods for long periods of time. Both have improved quality and increased a variety of foods are available. Temperatures between 10 and 20°C are used to lower the survival, development, and metabolic rates of fruits and vegetables. Foods are placed over ice, which has a surface temperature between 0 and 10°C. Fresh fish, shellfish, meats, cut fruits, bagged vegetable salads, several ready-to-eat salad varieties made at the retail location, and salad dressing (high pH, low calorie). Benefits and drawbacks of chilling effective short-term storage is made possible by cooling. According to Amit et al. (2017), cooling reduces the development and metabolic activities of microorganisms.
It might make food products less crisp (Arora, 2007). Over time, the required temperature for food refrigeration has changed. 7°C was previously thought to be a nice temperature. However, because of technology advancements, 4-5°C household refrigeration units are now economically viable. 4°C is thought to be an ideal refrigeration temperature for perishable goods. Perishable goods (such as fresh meat and fish) can be refrigerated at temperatures as low as 1°C in commercial food processing facilities. Along with low temperatures, the relative humidity and the right spacing of the items are also managed for optimal refrigeration in commercial facilities. Most residential refrigerators and freezers operate at a minimum temperature of -20°C, where the majority of food is still frozen.

Other methods of freezing include the usage of dry ice (-78°C) and liquid nitrogen (-196°C), which are utilized for quick and fast freezing. At -20°C, microorganisms cannot develop in frozen foods; instead, they perish during the freezing process. When an organism is lyophilized, it is severely dehydrated while it is frozen and then vacuum-sealed. Quick freezing is a freezing technique that preserves the integrity of food cells by using temperatures of -32°C or below. These goods include fruit juices, papaya pulp, mango pulp, and other liquid, pulpy, or semiliquid products. For the rapid freezing process, a larger investment is needed (Pruthi, 1999).

Non-Thermal Processing

Exposure of food items to ionizing radiation (cathode rays, X rays and gamma rays) have high-energy electrons that are lethal for microbes. It is mostly used in industry for food processing. It is not effective to viruses, and is only useful for food of high initial quality (Organization, 1988). It is a best sterilizing agent and destroy endospores and vegetative cells. Irradiation can effective for pathogens (E. coli, Staphylococcus aureus and Campylobacter jejuni). Recently it is being used to treat fruit, beef, poultry, vegetables and species (Prescott, Harley, & Klein). Food irradiation is used in the term ‘cold pasteurization’. UV has very limited penetration power. UV irradiation is limited to control microbes on surface, thin and clear layer of liquid. Mostly used in food industry and bakery products.

B. Chemical Preservation Methods

Food additives are included to stop the chemical degradation of food products (Ray, 1992). Only a small number of compounds, such as lactic, sorbic, acetic, benzoic, and propionic acids, as well as their salts, are permitted by law for use as food preservatives. Green olives of various
sorts are combined with lactic and acetic acids. Water-soluble benzoic acid salt prevents juice fermentation. Apple cider vinegar and pickles both include acetic acids in the form of vinegar. Yeast and bacteria respond best to it.

**High Osmotic Pressure**

High osmotic pressure also inhibits the bacterial growth. Sugar has a tendency to plasmolyze water from the microorganisms. Due to the high sugar content, microorganisms seldom impact jams and jellies. Food won't spoil due to microbes (Msagati, 2012). It is used to preserve fruits such as apples, pears, peaches, apricots, and plums. It is also used to preserve fruit in crystallized form, which involves cooking the preserved material in sugar until it becomes crystallized and then storing the finished product dry. Mould and yeast are resistant to osmotic pressure changes. Brine (which is heavy in salt), vinegar, alcohol, and vegetable oils—particularly olive oil but also a variety of other oils—are pickling agents. In many chemical pickling procedures, heating or boiling is used (Lee & Kang, 2004). In fermentation pickling, the food generates the preservative on its own, often through a lactic acid-producing process.

![Classification of various food preservation methods](image)

**Figure 2:** Classification of various food preservation methods.
Biological Processing

The fermentation process is essential for food preservation. By microbes (bacteria, yeasts, and moulds), carbohydrates are broken down in this technique. The nutritional value and digestibility of food products are improved by fermentation (Lewin, 2021). When aerobic microbes (acetobacter) are present, alcohol fermentation takes place. Hexose, a type of simple sugar, turns into alcohol and carbon dioxide (Dagoon, 1989). In the presence of too much oxygen, Acetobacter transforms alcohol to acetic acid (Amit et al., 2017). Alcohol is oxidized during vinegar fermentation, resulting in acetic acid and water (Battcock, 1998). Desirable fermentation products like pickles and olives are mostly produced by lactic acid bacteria.

Conclusion

It is concluded that activities cause by microbes and their enzymes can be controlled by proper handling of meat, fish and their products. They should be stored at low temperature of about (5°C) in the absence of light. Pasteurization is the best method approved for the preservation of milk and milk products. Microbial activity was also reduced by the help of antimycotics likes ascorbic acid and natamycin for milk. While certain chemicals like TBHQ, EDTA and ascorbic acids were better methods to preserve meat and fish. Internal and external environmental factors are taken in under consideration for the preservation of meat and fish.

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