

MICROBIOLOGY OF MEAT, POULTRY AND SEA FOODS

Introduction

The microbiological profile of meat products presented to the consumers is the sum total of the slaughtered animal health, conditions under which it was reared, quality of slaughtering, processing, packaging and conditions under which the meat was stored. Meat pathogens can cause self-limiting human enteric diseases or systemic and fatal infections of the immunocompromised, the elderly and the young. Meat can act as an ideal substrate for microbial proliferation. Major meat associated pathogenic bacteria include *Clostridium perfringens*, *Staphylococcus aureus*, *Salmonella spp*, pathogenic strains of *Escherichia coli*, *Campylobacter spp*, *Yersinia enterocolitica*, *Listeria monocytogenes* and *Aeromonas hydrophila*

Microorganisms Associated with Meat During Processing

Meat spoilages indicate (a) color changes (b) textural changes and (c) development of off-flavour or off-odor or slime as a result of microbial growth. *Salmonella* is the primary microbial challenge for poultry. The primary microbial to the beef industry is *Escherichia coli* O157: H7. *Listeria*, which is an adulterant with zero tolerance, is the major problem for ready to eat meat products. Treatment with organic acids, hot water steam pasteurization and steam vacuuming, trisodium phosphate, acidified sodium chlorite, chlorine dioxide, lactoferrins, peroxyacetic acid, sodium lactate, sodium acetate and sodium diacetate, ozone and radiation have been used as microbial decontaminants during meat processing operations. Current regulatory policies and inspection in the meat industry include the HACCP (Hazard Analysis Critical Control Point) food safety system with an objective to provide safe food for consumption and prevent chemical, physical and biological hazards.

Gram-negative bacteria

Neisseriaceae: Psychrobacter immobilis, P. phenylpyruvica, Acinetobacter spp., A. twoffii, A. Johnsonii,

Pseudomonadaceae: Pseudomonas fluorescens, P. lundensis, P. fragi, P. putida

Gram-positive bacteria: *Brochothrix thermosphacta, Kurthia zophii, Staphylococcus spp., Clostridium estertheticum, Clostridium frigidicarnis, Clostridium casigenes, Clostridium algidixylanolyticum sp. nov.*

Spoilage

Fresh Meat

In contrast to fruits and vegetables, meats are composed mainly of protein and fats rather than carbohydrates. Muscles of healthy animals do not contain any bacteria or fungi but as soon as animals are slaughtered, meat is exposed to contaminants and good sanitation practices are essential to produce high quality meats. The number of spoilage organisms on meat just after slaughter is a critical factor in determining shelf life. Chopping and grinding of meats can increase the microbial load as more surface area is exposed and more water and nutrients become available. A large variety of microbes are commonly found on fresh meat, but different microbes become dominant during spoilage of different meats depending on pH, composition and texture of processed meats, temperature and packaging atmosphere. *Pseudomonas* spp. is the predominant spoilage bacteria in aerobically stored raw meat and poultry. Once the initial low levels of glucose are depleted by various microbes, *Pseudomonas* has an advantage because it can catabolize gluconates and amino acids more readily than other microbes. Break down of these compounds results in production of malodorous sulfides, ammonia, and amines, including the biogenic amines putrescine and cadaverine. Dark, firm and dry meat

with a relatively high pH of 6.0 spoils more rapidly because deamination of amino acids starts earlier. *Shewanella putrefaciens* does not grow on meat at pH<6.0 but can produce sulfides and ammonia even when glucose is still available. These sulfides not only smell bad but also cause color changes in meat, and therefore *Shewanella* has a high spoilage potential on fresh, high pH meats stored aerobically even when it is not a dominant microbe. *Brochothrix thermosphacta* is often a significant spoilage organism on fresh meat stored aerobically at refrigeration temperatures. *Enterobacteriaceae*, particularly species of *Serratia*, *Enterobacter*, and *Hafnia*, are major causes of spoilage in vacuum-packed, high pH fresh meats. These organisms are facultative anaerobes that produce organic acids, hydrogen sulfide and greening of meats.

Lactic acid bacteria (LAB) grow on meat and poultry packaged under vacuum and modified atmospheres, producing organic acids from glucose by fermentation. This gives rise to aciduric off-odors which may be accompanied by gas and slime formation and greening of meat. However, LAB are weakly proteolytic and so do not produce large amounts of amines or sulfides, and spoilage of meat by LAB is not as offensive. Psychrophilic, anaerobic *Clostridium* spp. are associated with spoilage of vacuum-packaged meats. "Blown pack" meat spoilage is characterized by excessive gas formation with off odors due to formation of butyric acid, butanol and sulfurous compounds. Yeasts and molds grow relatively slowly on fresh meat and do not compete well with bacteria. Therefore, they are a minor component of spoilage flora.

Processed Meat

Addition of sodium chloride, nitrites and/or nitrates, along with various other seasonings, emulsifiers and preservatives to ground or whole muscle meats changes the environment significantly and also the spoilage flora of processed meats. Dried and dry-fermented meats generally do not support microbial growth although process deviations may allow growth of some organisms. Spoilage

organisms can grow on fresh and cooked cured meats, so they are best stored chilled, under a vacuum or modified atmosphere. *Pseudomonas* spp. are not usually important causes of spoilage in processed meats because of their sensitivity to curing salts and heat pasteurization and their inability to grow well in meats packed with a vacuum or high carbon dioxide atmosphere. However, when packages have been opened and there has been insufficient curing, these bacteria may spoil refrigerated processed meats. Some cold- and salt tolerant *Enterobacteriaceae* have been found to cause spoilage in some specific processed meats, such as ham or bacon.

Lactic acid bacteria (LAB) is the group of bacteria primarily associated with spoilage of processed meats. They produce sour off-flavors, gas, slime, and greening, and this spoilage may be more severe than in fresh meat because of the presence of added carbohydrates. Competitive ability of different LAB strains is related to pH and water activity of the meat, cooking and storage temperatures and oxygen and carbon dioxide levels. Sporeformers (*Clostridium* and *Bacillus*) are usually not a spoilage problem in processed meats because of the presence of nitrite and other curing salts. However, faulty cooking/cooling procedures, including long cooling periods and temperature abuse, has allowed growth of these organisms in some cases. Spores of these organisms may be introduced with spices or other ingredients. Yeasts cause some spoilage in processed meats but are generally only important when sulfite is used as a preservative or when meats have been irradiated or are stored aerobically in the cold. Slime may be produced along with vinegary or malty off-odors in some sausages.

Spoilage under aerobic condition

1.) Surface slime, caused by *Pseudomonas acinetobacter*, *Moraxella alcaligenes* *Streptococcus*, *Leuconostuoc*, *Bacillus* and *Micrococcus*.

2.) Change in colour of meat pigment. The red colour of meat may be changed to shades of green, brown or grey by *Lactobacillus* and *Leconostocs* spp.

3.) Changes in fat. The unsaturated fat in meat gets oxidized by lypolitic bacteria which produce off odours due to hydrolysis of fats and production of aldehydes and acids. This type of spoilage is caused by lypolitic *Pseudomonas*, *Achromobacter* and yeast.

4.) Surface color change. The red pigment producing bacteria, *Serratia marcescens*, caused red spots on meat. Blue color surface is caused by *Pseudomonas syncyanea* and yellow color is caused by *Micrococcus* species.

5.) Off odor and off taste. Volatile acid like formic, acetic, butyric and propionic acid produce sour odor and *Actinomycetes* produce musty or earthy flavor. Yeast also cause sliminess discoloration and off odor and taste defects.

6.) Aerobic mold also cause spoilage in meat. These are stickiness, whiskers, black-spot, white-spot, green patches off odor and off taste.

7.) Spoilage under anerobic condition.

i) Souring is caused by production of formic, acetic, butyric, lactic, succinic and propionic acid.

ii) Putrefaction. It is caused by decomposition of proteins under anaerobic condition by *Clostridium* species. The foul smell is due to production of hydrogen sulphide, mercaptans, indol, scatol, ammonia and amines.

Egg

Freshly laid eggs are generally sterile particularly the inner contents. However the shells get contaminated from the environmental sources such as fecal matter of the bird, beddings, by the handlers and wash water and also the packaging materials in which the eggs are packed. There are several extrinsic and intrinsic mechanisms through which the egg protects itself from the microbial invasion. Waxy shell membrane retards the entry of microorganisms. Further, the shell also prevents the entry of microorganisms. The membranes inside the shell behave as mechanical barriers to the entry of microorganisms. Further lysozymes present in the egg white is effective against Gram positive bacteria and avidin in the egg white forms a complex with biotin, thus making it unavailable for the microorganisms. Also high pH (pH 9-10) of albumin inhibits the microbial growth. Binding of riboflavin by the apo protein and chelation of iron by conalbumin further helps in hindering the growth of microorganisms that might have gained entry inside the egg.

Spoilage of egg

Breaks or cracks in egg shell taking place due to transportation or mechanical damage may allow microorganisms to enter in to the egg yolk and cause spoilage on storage. Eggs on storage may lose moisture and, therefore, weight. The white of the egg becomes thinner and more watery on storage. The major changes in the egg take place due to spoilage organisms. In general the spoilage of eggs is caused by bacteria as compared to molds and can be described as green rot due to the growth of *Pseudomonas fluorescens*, colourless rot due to the growth of *Pseudomonas*, *Acinetobacter* and other species; black rots due to *P. roteus*, *Pseudomonas*; red rots due to *Serratia* spp. and custard rots due to *Proteus vulgaris* and *Pseudomonas intermedium*. Growth of *Aeromonas* in the egg yolk turns it to black colour and also there is strong putrid odour due to the formation of hydrogen sulphide (H_2S). Storage of eggs in high humid atmosphere may help in

growth of several molds on the surface of the egg shell. Molds causing spoilage of eggs include species of *Pencillium*, *Mmucor*, *Alterneria* , etc.

Poultry Meat

Poultry meat like meat of other animals is also susceptible to contamination by various sources. Contamination of skin and lining of the body cavity take place during various processing operations. The organisms of great importance in poultry are *Salmonella* spp. and *Campylobacter jejuni*. Several Gram negative psychrotropic bacteria viz., *Pseudomonas*, *Acenitobacter* and *Flavobacterium* have also been isolated from poultry carasses. Ground turkey also may carry fecal streptococci. It is important to freeze the poultry fast in order to keep it in good condition for several months. Freezing further reduces the number of microorganisms in the poultry meat provided the temperature is maintained quite low (-18 ° C or below).

Fish Spoilage

Fish is a very perishable, high-protein food that typically contains a high level of free amino acids. The lipid content of the fish is up to 25%. It has very low content of connective tissue, i.e. approximately 3% of the total weight as compared with around 15% in meat. Fish flesh generally contains 15-20% protein and less than 1% carbohydrate. Non-fatty fish such as teleosts cod, haddock and whiting, the fat levels are only about 0.5%, while in fatty fish such as mackerel and herring, levels can vary between 3 and 25%.

Composition of a fish

Water 65 – 80 %

Fat 1 – 20 %

Protein 14 – 20 %

Microbes metabolize these amino acids, producing ammonia, biogenic amines such as putrescine, histamine, and cadaverine, organic acids, ketones, and sulfur compounds. Degradation of lipids in fatty fish produces rancid odors. In addition, marine fish and some freshwater fish contain trimethylamine oxide that is degraded by several spoilage bacteria to trimethylamine (TMA), the compound responsible for fishy off odors. Iron is a limiting nutrient in fish, and this favors growth of bacteria such as *Pseudomonas* that produce siderophores that bind iron. Spoilage bacteria differ somewhat for freshwater and marine fish and for temperate and tropical water fish. Storage and processing conditions also affect microbial growth. *Pseudomonas* and *Shewanella* are the predominant species on chilled fresh fish under aerobic conditions. Packing under carbon dioxide and addition of low concentrations of sodium chloride favor growth of lactic acid bacteria and *Photobacterium phosphoreum*. Heavily wet-salted fish support growth of yeasts while dried and salted fish are spoiled by molds. Addition of organic acid select for lactic acid bacteria and yeasts. Pasteurization kills vegetative bacteria but spores of *Clostridium* and *Bacillus* survive and may grow, particularly in unsalted fish.

Spoilage of fish and sea foods : Halophilic bacteria like *Serratia*, *Micrococcus*, *Bacillus*, *Alcaligenes* and *Pseudomonas* cause spoilage of salt fish. Shell fish are spoiled by *Acinetobacter*, *Moraxella* and *Vibrio*. Crab meat is spoiled by *Pseudomonas* *Acinetobacter* and *Moraxella* at low temperature and by *Proteus* at high temperature.

Microbial loads in shrimps, oysters, and clams depend on the quality of the water from which they are harvested. If the sewage is drained to water bodies, the microbial quality deteriorates. During handling, fecal coliforms, fecal streptococci, and *S. aureus* may be incorporated into the product. *Salmonella* also is found in oysters possibly due to contaminated water. Seafood also is the source for *Pseudomonas* spp., *C. perfringens*, *L. monocytogenes*, *Vibrio parahemolyticus*, *Salmonella enterica* serovar *enteritidis* and *typhimurium*, *Campylobacter jejuni*,

Yersinia enterocolitica, and Enteroviruses (Hepatitis A). Smoked salmon and shrimps also are found to carry pathogenic *L. monocytogenes*.

Meat Borne Disease

Food borne microbial hazards have a devastating impact on human suffering. Microbial pathogens of current concern that need to be controlled in the fresh meat include *Salmonella*, *Campylobacter*, enterohaemorrhagic *Escherichia coli* including serotype O157:H7 and other enteric pathogens. Infection due to *Listeria monocytogenes* following consumption of ready to eat meat and poultry products is a major problem in the recent years. Also there are food borne infections caused by *Yersinia enterocolitica*, *Staphylococcus aureus*, *Clostridium botulinum*, *Clostridium perfringens* and *Bacillus cereus*. Prevalence of some food borne pathogens recognized since 1970's include *Vibrio cholerae*, *Vibrio vulnificus*, Noro virus, *Enterobacter sakazakii*, prions and resistant bacteria. In recent years the food borne pathogens associate with animal health pandemics include Avian Influenza (AI) and Foot and Mouth Disease (FMD) viruses. Avian influenza is not of concern to poultry meat safety, because it is inactivated by proper cooking with a temperature of 70°C and more. Also the oral route of transmission is less important than the non food borne route. Presently there is continuous adaptation and development of resistance by pathogenic microorganisms to antibiotics and potentially to traditional food preservation barriers, like low pH, heat, cold temperatures, dryness, low water activity and chemical additives. Development of antibiotic resistance in food borne pathogens is very important from public health view point in present days and in the future.

Control of meat borne pathogens

Effective control of meat borne pathogens and enhancement of meat safety can be achieved by control of latent infections among livestock, animal welfare and humane treatment, application of antimicrobial interventions at the farm, during harvesting, dressing and product processing, improvement in process food hygiene

and potential application of new or novel processing and preservation technologies. Animal stress can damage meat quality and lead to more contamination and increased pathogen shedding. Antimicrobial intervention technologies can be used effectively to improve the microbiological quality of meats. These technologies include reduction of contamination on the raw product, minimization of microbial access to the products, reduction of contamination that has gained access to the product, inactivation of the microbes on the product without cross contamination and prevention and control of the growth of non-inactivated microbes, which have gained access to the meat. An effective pathogen control at pre-harvest, postharvest, processing, storage, distribution, merchandizing, preparation, food-service and consumption of meat include activities employed during pre-harvest or in the field, during post harvest or processing in the plant, at retail and food service and at home. Pre-harvest pathogen control interventions include diet manipulation, use of food additives, antibiotic, bacteriophage therapy, and immunization of the animals, complete exclusion, probiotics and proper animal management practices like pen management, clean feed, clean and chlorinated water, and clean and unstressful transportation. Antimicrobial intervention activities during harvest and post harvesting should be designed to minimize introduction of microbial contaminants and reduce existing contaminant levels through implementation of decontamination and sanitization interventions, processing treatments for partial or complete destruction of contaminants and antimicrobial procedures for inhibition or retardation of microbial growth. Certain inspection regulations should be followed in meat and poultry plants, such as establishment of sanitation standard operating system, operations under the HACCP system and performing HACCP verifications to meet microbiological standards, establishment of good manufacturing practices (GMP) and good hygiene practices (GHP). Antimicrobial interventions used to control pathogens in further processed meat products include physical hurdles (low and high temperature, non thermal process like irradiation and high pressure and packaging treatments), physiochemical interventions (low pH, reduced water activity, modification of oxidation reduction potential through

packaging and application of antimicrobial additives), and biological interventions (microbial competitors, such as lactic acid bacteria and antimicrobial products, such as bacteriocins). Events of the most food borne illness happen due to mishandling of foods in various ways. So, there should be provisions to educate the food handlers and consumers particularly on culinary tips.