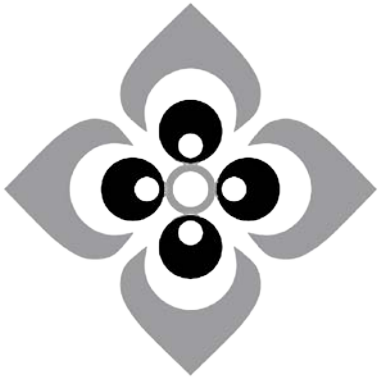

Consortium for Educational Communication

Module
on
Microbial Spoilage Of Foods

By

BASIT AMIN SHAH

Department of Biotechnology
University of Kashmir
Email: basitaminshah@gmail.com
Phone: 9596000715

TEXT

Introduction

Food spoilage can be considered as any change which renders a product unacceptable for human consumption. Foods are not only nutritious to consumers, but are also excellent source of nutrients for microbial growth. Microorganisms are the most important cause but not the only cause of spoilage of foods. Microorganisms can be used to transform raw foods into fermented delights, including yoghurt, cheese, sausages, pickles and other alcoholic products. On the other hand, microorganisms can degrade food quality and lead to spoilage. Importantly, foods also can act as vehicles for disease transmission, and thus detection and control of pathogens and spoilage organisms are important areas of food microbiology. During the entire sequence of food handling from the producer to the final consumer, microorganisms can affect food quality and human health.

Growth of microorganisms in foods

Foods, because they are nutrient-rich are excellent environments for the growth of microorganisms. Microbial growth is controlled by factors related to the food itself, called **intrinsic factors**, and also to the environment where the food is stored, described as **extrinsic factors.** The intrinsic or food-related factors include pH, moisture content, water availability, oxidation- reduction potential, physical structure of the food, available nutrients, and the possible presence of natural antimicrobial agents. Extrinsic factors include temperature, relative humidity, gases (CO2, O2) present, and the types and numbers of microorganisms present in the food. Since foods are of plant & animal origin, so it is worthwhile to consider those characteristics of plant & animal tissues that affect microbial growth. The plants and animals that serve as food sources have all evolved mechanisms of defense against the invasion and proliferation of microorganisms, and some of these remain in effect in fresh foods. Knowledge of sources of microorganisms and factors influencing microbial growth is necessary for food microbiologists because desirable growth conditions are needed for applicability of microorganisms for fermentation & single cell protein production and understanding of undesirable conditions are used for food preservation. The various sources through which microorganisms gain entry into the foods are:

Primary sources of microorganisms found in foods

Sources
Micro flora present in soil and water
Micro flora present in air
Micro flora present on plant and plant products
Micro flora present on Food utensils and equipment's
Micro flora present in animal feeds
Micro flora present on animal hides
Micro flora present in Intestinal tracts of humans and animals
Food Handlers

Microorganisms and Food Spoilage

Food spoilage can be defined as:

Any changes in the visual, smell and texture of food that makes it unacceptable for consumption.

Or

The process in which food deteriorates to the points it is not edible to humans or its quality of edibility becomes reduced.

Food spoilage can be the result of:

- Insect damage
- Physical injury
- Enzymatic degradation

- Microbial activity

Classification of Foods by ease of Spoilage:

Foods are classified into three groups based on ease of their spoilage:

- **Relatively stable or nonperishable foods:** Foods that do not spoil unless handle carelessly. Example: grains, flour, sugar, pulses etc.

- **Protectable or semi perishable foods:** Foods those remain unspoiled for a fairly long period if properly handled and stored. Example: potatoes, apples, onions etc.

- **Perishable foods:** Foods that spoil readily unless special preservative methods are used. E.g. milk, eggs, meat, fish, poultry, most fruits and vegetables.

Types of Food Spoilage

Foods are classified into three groups based on the type of their spoilage:

- **Physical spoilage:** Spoilage due to the loss or gain of moisture.
- **Chemical spoilage:** Foods that are spoiled due to some chemical reactions of foods. Example: Oxidation of fats, Browning of fruits and vegetables etc.
- **Microbial spoilage:** Spoilage due to the growth and activity of microorganisms. The microorganisms responsible for food spoilage are moulds, yeast and bacteria. **(Figure 5)**

Moulds:

Moulds are in the form of threads developed on perishable foods and are easily visible to the eye. They contain spores which can spread through the air and start new mould plants. When these moulds find a favorable environment, they germinate and produce a fluffy growth, often white or grey but sometimes bluish green, red, orange or some other color, depending upon the variety of the mould. Most moulds are not harmful. A relatively small proportion of the moulds, found on foods are capable of producing toxic materials known as mycotoxins of which aflatoxins is an example.

Yeasts:

Yeasts are tiny organisms which are not visible to the naked eye, but which can be seen through the microscope. They multiply very fast and cause fermentation by acting on certain components of the perishable foods like fruit juices, syrups etc. During yeast fermentation, the sugars present in the food are broken up to form alcohol and carbon dioxide. Foods liable to be spoiled by yeasts are fruit juices, syrups, molasses, honey, jams and jellies.

Bacteria:

Bacteria are unicellular organism and are much smaller in size than either yeasts or moulds. They occur in different sizes and shapes and are classified as coccus (spheroidal), bacilli (cylindrical) or spirillae (spirillar) on the basis of their shape as seen under the microscope. They also vary in their requirement for food, moisture, acidity, temperature and oxygen. Bacteria can grow and develop rapidly between 20°C and 53°C. Bacteria are classified according to the temperature ranges that they need for growth:

1. A higher temperature than 45°C are known as thermopile, (e.g. in canning industry and milk processing plants).

2. Temperatures between 20-25°C are called Mesophiles.

3. Temperature less than 20°C are called psychrophiles (e.g. in Refrigerator and in cold storages).

Spoilage of different types of food

1. Meat Spoilage

Sources of contamination

- Cutting board contamination
- Conveyor belts

- Temperature
- Delay between storage and distribution
- Fecal contamination from intestines

Storage temperature is the single most important control factor for meat spoilage. Several genera of moulds grow on the surface of meat and can cause spoilage like ***Penicillium, Mucor, Cladosporium, Alternaria*****,** but cannot grow on meat stored below 5^{o}C. Meat spoilage is characterized by the appearance of off odors and slime, which are manifest when surface loads exceed 10^{7}cfu/cm^{2}. The slime is due to the accumulation of bacterial cells. Interestingly, meat spoilage (including poultry and fish) occurs without any significant breakdown of the primary protein structure. Instead, spoilage bacteria utilize glucose, free amino acids or other simple nitrogenous compounds to attain population of about 10^{8}cfu/cm^{2}, at which point the organoleptic quality of the meat will clearly reveal it is spoiled.

Fresh Meats:

- Chemical composition:
- 75% water
- 18% protein
- 3% fat, 1% ash, traces of CHO, vitamins, etc.

a. Whole Meats: The microflora of fresh whole meat is primarily composed of**:**

1. Gram negative aerobic rods such as :
    - *Pseudomonas* spp,
        - *P. fragi, P. lundensis and P. fluorescens*
    - *Acinetobacter*
    - *Psychrobacter immobilis*

2. *Bacillus* and *clostridia* (e.g. *C. perfringens*) are also common on all types of meat.

Although subsurface portions of meat are generally sterile, some parts such as lymph nodes may be heavily contaminated. Mechanical disruption of the tissue during processing can distribute microorganisms from the meat surface throughout the product.

b. Ground Meats: contain same microorganisms as whole meats, but always have higher microbial loads, because it has:

- Greater surface area which gives microbes better access to the food and also traps air to favor the growth of gram negative, aerobic bacteria like *Pseudomonas* spp.
- Every handling or processing (storage utensils, cutting knives, grinders) step can contribute additional contamination to the final product.
- One heavily contaminated piece (e.g. a lymph node) can contaminate an entire lot when they are ground together.

c. Vacuum packaged meats

During vacuum packaging, it has been found that not all the O2 is removed, which is eventually consumed by aerobic microorganisms and the tissue itself. The consumed O2 results in increased CO2 levels and thus vacuum packaged meat gets a longer shelf life. The microflora shifts from predominantly Gram negative aerobes to Gram positive anaerobes and micro-aerophilic lactic acid bacteria (LAB) like ***Lactobacillus, Carnobacterium*** **and** ***Leuconostoc***. If nitrites have been added to the vacuum packaged meat (e.g. to inhibit ***C. botulinum*** *in* hams, bacon), LAB domination is even more pronounced.

In general, vacuum packaged meats are considered very safe foods and free from most pathogenic species of bacteria, with the possible exception of ***S. aureus*** and ***Y. enterocolitica***.

Spoilage in vacuum packaged meats is manifest by:

- Slime development due to overgrowth of microbe.

- Greening caused by microbial production H2O2 or H2S. H2O2 production in meat has been associated with several types of lactic acid bacteria (primarily *Lactobacillus*).The oxidant (H2O2) reacts with nitrosohemochrome (cured meat color compound) to form a green porphyrin compound. H_2S reacts with myoglobin to form sulphmyoglobin in meats with a pH above 6.0. H_2S is mainly produced by ***Shewanella putrefaciens*** and ***Pseudomonas spp.***

- Off odors which result from:

    - the release of short chain fatty acids

    - the production of volatile compounds like acetoin, diacetyl and H_2S.

The type of spoilage bacteria that will dominate is influenced by several factors that include:

1. Is the meat product raw or cooked?

- **Cooked products** have a higher pH (>6.0) which may allow growth of Gram negative facultative anaerobic pathogens like ***Yersinia enterocolitica*.**
- **Raw products** have a pH of about 5.6 which favors lactic acid bacteria, esp. ***Lactobacillus, Carnobacterium,* and *Leuconostoc.***

2. Nitrite concentration in meat.

- High nitrite concentration favors lactic acid bacteria.
- Low nitrite levels may allow growth of ***Brochothrix thermosphacta*** (gram positive rod, facultative anaerobic, growth @ 0-30°C from pH 5.0 - 9.0).
- ***B. thermosphacta*** is an important spoilage bacterium in anaerobically stored meats kept at low temperature, but the bacterium is inhibited by nitrite.

d. Processed meats

These products are composed of a variety of blended ingredients, any of which can contribute microorganisms to the food. Yeasts and bacteria are the most common causes of spoilage, which is usually apparent in 3 ways:

- **Slimy spoilage:** Like other meat products, this occurs on the surface and is caused by the buildup of cells by yeasts, lactobacilli, enterococci or ***Brochothrix thermosphacta***. Washing the slime off with hot water can restore the product quality.

- **Sour spoilage:** Results from the growth of lactic acid bacteria (which originate from contaminated ingredients like milk solids) under the casing. These organisms ferment lactose and other CHOs in the product and produce organic acids. Taste is adversely affected but the product is not harmful if eaten.

- **Greening due to H_2O_2 or H_2S production:** Because greening indicates more extensive product breakdown, it is not recommend eating green wieners.

2. Poultry and Egg Spoilage

- general trends are the same as other fresh meats.

- whole birds have lower counts than cut-up parts.

- additional processing steps add to the microbial load.

When poultry is in the advanced stages of spoilage, the skin will often fluoresce under UV because so many **fluorescent pseudomonas** are present. **Off odors** generally appear before sliminess develops. The same bacteria can produce visceral taint, a condition manifest by off odors in the abdominal cavity or poultry.

Point to remember: During the initial stages of spoilage, the skin supports bacterial growth better than does the tissue (which remains essentially free of bacteria for some time). Thus, the skin can sometimes be removed to salvage the food.

Eggs:

Eggs have several intrinsic parameters which help to protect the nutrient-rich yolk from microbial attack. These include the shell and associated membranes, as well as **lysozyme** and a high pH (>9.0) in the white. Freshly laid eggs are generally sterile, but soon become contaminated with numerous genera of bacteria.

Eventually, these MO will penetrate the eggshell and spoilage will occur. ***Pseudomonas*** are common spoilage agents, but molds like ***Penicillium*** and ***Cladosporium*** **sometimes grow in the air sac and spoil the egg.**

3. Fish Spoilage

- Fish of both fresh-water and salt-water contain high levels of proteins and nitrogenous constituent with low fat content and practically no carbohydrates.

- The microbial quality of fish is heavily influenced by the quality of the water from which they were harvested.
- Fresh fish and iced fish are spoiled by bacteria while salted and dried fish are spoiled

by fungi. Bacteria on fresh fish are concentrated on the outer slime, gills and intestine.

- Most susceptible part of the fish to spoilage is the gill region, and the best way to detect spoilage in fresh fish is to sniff this area for off odors produced by *Pseudomonas and* ***Acinetobacter -Moraxella*** bacteria. The odors include **ammonia, tri ethylamine, H_2S** and other compounds.

Spoilage of **crustaceans** (shrimp, lobsters, crabs and crayfish) is similar, but these products have some CHO (0.5%) and more free amino acids, so spoilage can occur more rapidly.

Mollusks (oysters, clams, mussels, squid and scallops) have more CHO (35%) and less nitrogen than either fish or shellfish. Microflora of mollusks can vary a great deal depending on the quality of the water from which they were harvested.

4. Spoilage of Milk and Dairy Products (Figure 15):

Milk: is a very rich medium for microorganisms.

Raw milk flora may include:

- All MO found on the cow hide (which include soil and fecal bacteria), udder, and milking utensils.

- Can include Gram negative, Gram positive, yeasts and molds.

- ***Psychrotropic pseudomonas*** are common in bulk stored raw milk which produce heat stable enzymes that can reduce milk quality and shelf life.

- Pasteurization kills most Gram negative bacteria, yeasts and moulds but some Gram negative enzymes, thermo-tolerant Gram positive bacteria and spores survive.

Pasteurized fluid milk is spoiled by a variety of bacteria, yeasts and moulds.

- Pasteurized milk is more frequently spoiled by aerobic spore formers such as ***Bacillus*****,** whose proteolytic enzymes cause curdling.

- Moulds may grow on the surface of spoiled milk, but the product is usually discarded before this occurs.

Butter has high lipid content and low a_w thus makes it more susceptible to surface mould growth than to bacterial spoilage.

- Some pseudomonas can be a problem; "surface taint"- putrid smell, caused by the production of organic acids (especially from ***P. putrefaciens)***.

- Rancidity due to butter fat lipolysis caused by *P. fragi* are common.

Cottage cheese (Figure 16) can be spoiled by yeasts, molds and bacteria.

- The most common bacterial spoilage is "**slimy curd**" caused by ***Alcaligenes spp***. (Gram negative aerobic rod bound in soil, water, and intestinal tract of vertebrates).

- *Penicillum, Mucor* and other fungi also grow well on cottage cheese and impart stale or

yeasty flavors.

Ripened Cheeses (Figure 17) with their low $a_{w,}$ low pH and high salt inhibit most spoilage microorganisms except surface mold growth.

- Spores of ***C. butyricum, C. sporogenes and others can germinate in cheeses (e.g. Swiss*** Cheese) with intrinsic properties that are less inhibitory (e.g. lower salt, higher pH).

- These organisms may metabolize citrate, lactose, pyruvate or lactic acid and produce butyrate or acetate plus CO_2 ***or*** H_2 ***gas which "blows" the*** cheese.

5. Spoilage of Vegetables and Fruits (Figure 18):

Vegetables are a good substrate for yeasts, molds or bacteria.

Typical composition:

- 88% water.
- 8.6% CHO which includes readily available mono and disaccharides like glucose and maltose, as well as more complex oligosaccharides, which are available to fewer types of

  microorganisms.
- 1.9% protein.
- 0.3% fat.
- 0.84% minerals.
- Also contain fat and water soluble vitamins and nucleic acids (<1%).

- pH of most veggies is around 6.0; within the growth range of many bacteria.

Microflora of vegetables is primarily composed of:

- Gram positive bacteria like lactic acid bacteria (e.g. *leuconostocs, lactobacilli, streptococci, Coryneforms and staphylococci* (the latter coming from the hands of employees during processing. Staphylococci are usually unable to proliferate but cross contamination can introduce them into other foods where growth conditions are more favorable.

It is estimated that 20% of all harvested vegetables for humans are lost to spoilage by these microorganisms. Because bacteria grow more rapidly, they usually outcompete fungi for readily available substrates in vegetables. As a result, bacteria are of greater consequence in the spoilage of vegetables with intrinsic properties that support bacterial growth (favorable pH, Eh).

Soft rot:

One of the most common types of bacterial spoilage.is Soft rot which is caused by *Erwinia carotovora* and sometimes by *Pseudomonas* spp., which grow at 4oC.

Softening can also be caused by endogenous enzymes.

Flavr Savr story:

- Polygalacturonase (PG) hydrolyzes ᾱ (1-4) glycosidic bond in pectin which leads to softening.
- Calgene made antisense RNA to tomato PG, thus delays softening in tomato, which can now be harvested after they are ripe (better flavor).
- It was first commercially available genetically engineered vegetable.

Mould spoilage (Figure 20):

- In vegetables where bacterial growth is not favored (e.g. low pH), molds are the principal spoilage agents.
- Most molds must invade plant tissue through a surface wound such as a bruise or crack.
- Spores are frequently deposited at these sites by insects like ***Drosophila melanogaster***, the

  common fruit fly.
- Other molds like ***Botrytis cinerea***, which causes grey mole rot on a variety of vegetables, are able to penetrate fruit or vegetable skin on their own.

The microflora of vegetables will reflect:

- the sanitation of processing steps.

- the condition of the original raw product.

Soil borne microorganisms such as *clostridia* are common on raw vegetables, and some species, like ***C. botulinum***, are of such great concern that they are the focus of processing steps designed to destroy microorganisms.

Sources of Contamination

- Surface contamination – Soil, water, air, human pathogens from manure (night soil)
- Harvesting hand picking vs. machines.
    - high damage if crop is ripe ,so harvest before ripe.
    - *Geotrichium candidum* – mold on harvestors.
- Packaging: containers reused.
- Processing plant.
- Markets – handling, cross contamination.

Fruits: Like vegetables, fruits are nutrient rich substrates but the pH of fruits does not favor bacterial growth. As a result, **yeasts and moulds** are more important than bacteria in the spoilage of fruits.

- Several genera of yeasts can be found on fruit.
- Because these organisms grow faster than molds, yeast often initiate fruit spoilage.
- Molds then finish the job by degrading complex polysaccharides in cell walls and rinds.

Average composition:

- 85% water.
- 13% CHO.
- 0.9% protein (a bit low on nitrogen sources).
- 0.5% fat.
- 0.5% ash.
- trace amounts of vitamins, nucleotides, etc.
- less water and more CHO than veggies.
- low pH (1.8-5.6).

Specific Spoilage Organisms:

- Blue rot – *Penicillium,* fruits.
- Downy mildews – *Phytophora*, large masses of mycellium, grapes.
- Black rot – *Aspergillus,* onions.

- Sour rot – *Geotrichum candidum* , grapes.

6. Cereal and Bakery Goods :

These products are characterized by a low a_w which, when stored properly under low humidity, restricts all microorganisms except molds.

- *Rhizopus stolonifer* **(Figure 22)** is the common bread mold, and other species from this genus spoil cereals and other baked goods.

- Refrigerated frozen dough products have more water and can be spoiled by lactic acid bacteria.

7. Fermented Foods and Beverages (Figure 23): The low pH or ethanol content of these products does not allow growth of pathogens, but spoilage can occur.

- Beer and wine (pH 4-5) can be spoiled by yeasts and bacteria.

- Bacteria involved are primarily lactic acid bacteria like *lactobacilli* and *Pediococcus spp*., and (under aerobic conditions) acetic acid bacteria like *Acetobacter* and *Glucono-bacter spp*.

- Acetic acid bacteria convert ethanol to acetic acid in the presence of oxygen.

- The anaerobic bacterium *Megasphaera cerevisiae* can also spoil beer by producing iso-valeric acid and H2S.

Beer:

- Spoilage in packaged beer is often due to growth of the yeast ***Saccharomyces diastaticus*****,** which grows on **dextrins** that brewers yeast cannot utilize.

- In either case, spoilage by yeasts results in the development of turbidity, off flavors and odors.

Wines:

- *Candida valida* is the most important spoilage yeast in wine.

- Can also be spoiled by lactic acid bacteria which are able to convert malic acid to lactic acid **(malolactic fermentation).** This reduces the acidity of the wine and adversely affect wine flavor.

- In some areas (e.g. Northwest), wine grapes have too much malic acid so this fermentation is deliberately used to reduce the acidity of grape juice that will be used for wine.

Fermented vegetables:

Yeasts, molds and lactic acid bacteria can also spoil fermented vegetables such as sauerkraut and pickles, as well as other acid foods like salad dressings and mayonnaise.

- Spoilage in fermented vegetables is often manifest by off odors or changes in the color (chromogenic colony growth) or texture (softening) of the product.
- In mayonnaise or salad dressing, the first signs of spoilage are usually off odors and emulsion separation