FRI BRIEFINGS

Microbial Food Spoilage — Losses and Control Strategies

A Brief Review of the Literature

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INTRODUCTION

Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics. Spoiled foods may be safe to eat, i.e. they may not cause illness because there are no pathogens or toxins present, but changes in texture, smell, taste, or appearance cause them to be rejected. Some ecologists have suggested these noxious smells are produced by microbes to repulse large animals, thereby keeping the food resource for themselves (*21;144*)!

Food loss, from farm to fork, causes considerable environmental and economic effects. The USDA Economic Research Service estimated that more than ninety-six billion pounds of food in the U.S. were lost by retailers, foodservice and consumers in 1995. Fresh produce and fluid milk each accounted for nearly 20% of this loss while lower percentages were accounted for by grain products (15.2%), caloric sweeteners (12.4%), processed fruits and vegetables (8.6%), meat, poultry and fish (8.5%), and fat and oils (7.1%) (*79*). Some of this food would have been considered still edible but was discarded because it was perishable, past its sell-by date, or in excess of needs. There are also environmental and resource costs associated with food spoilage and loss. If 20%

of a crop is lost, then 20% of the fertilizer and irrigation water used to grow that crop was also lost.

Shelf life of a food is the time during which it remains stable and retains its desired qualities. Some spoilage is inevitable, and a variety of factors cause deterioration of foods:

- ♦ endogenous enzymes in plants oxidizing phenolic compounds (browning) or degrading pectins (softening);
- ♦ insects infesting foods and rodents chewing on foods;
- ♦ parasites, when visible for example in meat or fish, rendering food undesirable;
- ♦ microbes (bacteria, molds, yeasts) growing on and metabolizing foods;
- ♦ light causing degradation of pigments, fats, and proteins (off-flavors and odors) or stimulating pigment production (greening of potatoes);
- ♦ temperature; both excessive heat and freezing physically affecting texture of foods and breaking emulsions;
- ♦ air, particularly oxygen, oxidizing lipids producing strong off-odors and flavors;
- ♦ moisture: too little causing cracking, crumbling, or crystallization whereas excess causes sogginess, stickiness, or lumping.

These factors are interrelated, as certain temperatures and oxygen and moisture levels increase the activities of endogenous enzymes and of microbes. Rodent and insect damage may provide an entry point for microbial growth.

Food spoilage is a broad topic that cannot be completely addressed in one review article. This paper will emphasize spoilage caused by microorganisms and will consider spoilage of foods that people purchase or consume. For example, spoilage of bread will be considered but not deterioration of wheat plants in the fields or wheat grains in storage. Non-microbial spoilage such as loss of water (shriveling of greens or carrots) or changes induced by degradative enzymes in plants (yellowing of broccoli) will not be covered. Pathogenic organisms, e.g. *Listeria* that cause human illness, will not be considered even though they may also cause some spoilage.

DETECTION OF SPOILAGE

Spoilage is manifested by a variety of sensory cues such as off-colors, off-odors, softening of vegetables and fruits, and slime. However, even before it becomes obvious, microbes have begun the process of breaking down food molecules for their own metabolic needs. Sugars and easily digested carbohydrates are used first, plant pectins are degraded. Then proteins are attacked, producing volatile compounds with characteristic smells such as ammonia, amines, and sulfides. These odors start to develop in meat when there are about 10^7 cfu of bacteria/cm^2 of meat surface and are usually recognizable at populations of 10^8 cfu/cm^2 (*46*).

Early detection of spoilage would be advantageous in reducing food loss because there may be interventions that could halt or delay deterioration, and on the other hand food that had reached the end of its designated shelf life but was not spoiled could still be used. Numerous methods for detection of spoilage have been devised with the goals of determining concentrations of spoilage microbes or volatile compounds produced by these microbes. However, many of these methods are considered inadequate because they are time-consuming, labor-intensive, and/or do not reliably give consistent results. Some representative papers using different methods are described below.

Traditional methods of estimating bacterial populations do not provide results quickly enough to allow for interventions. Microbial population levels can be measured by real time PCR in liquids, such as *Gluconobacter* in an electrolyte replacement drink (*56*) and yeasts in wine and fruit juices (*23;71;145*). Other methods for detecting bacteria include ATP bioluminescence and electrical impedance assays but some food matrices may contain interfering substances (*46*).

Detection of volatile compounds produced by spoilage bacteria can be a less invasive and more rapid means for monitoring spoilage. Biogenic amines (putrescine, cadaverine, histamine, and tyramine) are commonly produced during spoilage of high protein foods and can attain levels that cause illness, particularly in spoiled fish. HPLC methods have been used to quantitate different amines in fish (*9*), chicken (*10*), and cheese (*75*). Combined concentrations of these amines are expressed as a biogenic amine index that is related to the extent of food spoilage and to the concentrations of spoilage organisms.

Electronic noses were first developed about twenty years ago and have undergone many refinements since (*24*). They consist of a set of sensors that react with different volatile chemicals and produce an electrical signal. An odor profile can be analyzed by using pattern recognition files. Such systems have been used to detect spoilage in beef (*12;121*), bakery products (*103;104*), fish (*2;70*), and milk (*69*). Other techniques being developed to detect microbes or chemicals associated with spoilage include: (a) FT-IR (Fourier Transform-Infrared Spectroscopy) used with beef (*45*) and apple juice (*95*); (b) visible and short-wavelength near-infrared spectroscopy to detect microbial load in chicken by diffuse reflectance (*94*); (c) ion mobility spectrometry for detecting trimethylamine in meat (*14*); and (d) gas chromatography-mass spectrometry for analyses of fish. It is expected that some advances in nanotechnology will improve the portability, sensitivity, and speed of detection systems.

SPOILAGE ORGANISMS

Chemical reactions that cause offensive sensory changes in foods are mediated by a variety of microbes that use food as a carbon and energy source. These organisms include prokaryotes (bacteria), single-celled organisms lacking defined nuclei and other organelles, and eukaryotes, single-celled (yeasts) and multicellular (molds) organisms with nuclei and other organelles. Some microbes are commonly found in many types of spoiled foods while others are more selective in the foods they consume; multiple species are often identified in a single spoiled food item but there may be one species (a specific spoilage organism, SSO) primarily responsible for production of the compounds causing off-odors and flavors. Within a spoiling food, there is often a succession of different populations that rise and fall as different nutrients become available or are exhausted. Some microbes, such as lactic acid bacteria and molds, secrete compounds that inhibit competitors (*62*).

Spoilage microbes are often common inhabitants of soil, water, or the intestinal tracts of animals and may be dispersed through the air and water and by the activities of small animals, particularly insects. It should be noted that with the development of new molecular typing methods, the scientific names of some spoilage organisms, particularly the bacteria, have changed in recent years and some older names are no longer in use. Many insects and small mammals also cause deterioration of food but these will not be considered here.

Yeasts

Yeasts are a subset of a large group of organisms called fungi that also includes molds and mushrooms. They are generally single-celled organisms that are adapted for life in specialized, usually liquid, environments and, unlike some molds and mushrooms, do not produce toxic secondary metabolites. Yeasts can grow with or without oxygen (facultative) and are well known for their beneficial fermentations that produce bread and alcoholic drinks. They often colonize foods with a high sugar or salt content and contribute to spoilage of maple syrup, pickles, and sauerkraut. Fruits and juices with a low pH are another target, and there are some yeasts that grow on the surfaces of meat and cheese (*84;129;150*). There are four main groups of spoilage yeasts:

Zygosaccharomyces and related genera tolerate high sugar and high salt concentrations and are the usual spoilage organisms in foods such as honey, dried fruit, jams and soy sauce. They usually grow slowly, producing off-odors and flavors and carbon dioxide that may cause food containers to swell and burst. *Debaryomyces hansenii* can grow at salt concentrations as high as 24%, accounting for its frequent isolation from salt brines used for cured meats, cheeses, and olives. This group also includes the most important spoilage organisms in salad dressings (*26;105;106;111;142*).

***Saccharomyces* spp.** are best known for their role in production of bread and wine but some strains also spoil wines and other alcoholic beverages by producing gassiness, turbidity and off-flavors associated with hydrogen sulfide and acetic acid. Some species grow on fruits, including yogurt containing fruit, and some are resistant to heat processing (*42;98;135;145;159*).

***Candida* and related genera** are a heterogeneous group of yeasts, some of which also cause human infections. They are involved in spoilage of fruits, some vegetables and dairy products (*25;52*).

Dekkera/Brettanomyces are principally involved in spoilage of fermented foods, including alcoholic beverages and some dairy products. They can produce volatile phenolic compounds responsible for off-flavors (*34;98;128*).

Molds

Molds are filamentous fungi that do not produce large fruiting bodies like mushrooms. Molds are very important for recycling dead plant and animal remains in nature but also attack a wide variety of foods and other materials useful to humans. They are well adapted for growth on and through solid substrates, generally produce airborne spores, and require oxygen for their metabolic processes. Most molds grow at a pH range of 3 to 8 and some can grow at very low water activity levels (0.7–0.8) on dried foods. Spores can tolerate harsh environmental conditions but most are sensitive to heat treatment. An exception is *Byssochlammys*, whose spores have a D value of 1–12 minutes at 90ºC. Different mold species have different optimal growth temperatures, with some able to grow in refrigerators. They have a diverse secondary metabolism producing a number of toxic and carcinogenic mycotoxins. Some spoilage molds are toxigenic while others are not (*129*). Spoilage molds can be categorized into four main groups:

Zygomycetes are considered relatively primitive fungi but are widespread in nature, growing rapidly on simple carbon sources in soil and plant debris, and their spores are commonly present in indoor air. Generally they require high water activities for growth and are notorious for causing rots in a variety of stored fruits and vegetables, including strawberries and sweet potatoes. Some common bread molds also are zygomycetes. Some zygomycetes are also utilized for production of fermented soy products, enzymes, and organic chemicals. The most common spoilage species are *Mucor* and *Rhizopus*. Zygomycetes are not known for producing mycotoxins but there are some reports of toxic compounds produced by a few species.

Penicillium and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions. They are distinguished by their reproductive structures that produce chains of conidia. Although they can be useful to humans in producing antibiotics and blue cheese, many species are important spoilage organisms, and some produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem). *Penicillium* spp. cause visible rots on citrus, pear, and apple fruits and cause enormous losses in these crops. They also spoil other fruits and vegetables, including cereals. Some species can attack refrigerated and processed foods such as jams and margarine. A related genus, *Byssochlamys*, is the most important organism causing spoilage of pasteurized juices because of the high heat resistance of its spores.

Aspergillus and related molds generally grow faster and are more resistant to high temperatures and low water activity than *Penicillium* spp. and tend to dominate spoilage in warmer climates. Many aspergilli produce mycotoxins: aflatoxins, ochratoxin, territrems, cyclopiazonic acid. Aspergilli spoil a wide variety of food and non-food items (paper, leather, etc.) but are probably best known for spoilage of grains, dried beans, peanuts, tree nuts, and some spices.

Other molds, belonging to several genera, have been isolated from spoiled food. These generally are not major causes of spoilage but can be a problem for some foods. *Fusarium* spp. cause plant diseases and produce several important mycotoxins but are not important spoilage organisms. However, their mycotoxins may be present in harvested grains and pose a health risk.

Bacteria

Spore-forming bacteria are usually associated with spoilage of heat-treated foods because their spores can survive high processing temperatures. These Gram-positive bacteria may be strict anaerobes or facultative (capable of growth with or without oxygen). Some spore-formers are thermophilic, preferring growth at high temperatures (as high as 55ºC). Some anaerobic thermophiles produce hydrogen sulfide (*Desulfotomaculum*) and others produce hydrogen and carbon dioxide (*Thermoanaerobacterium*) during growth on canned/hermetically sealed foods kept at high temperatures, for example, soups sold in vending machines. Other thermophiles (*Bacillus* and *Geobacillus* spp.) cause a flat sour spoilage of high or low pH canned foods with little or no gas production, and one species causes ropiness in bread held at high ambient temperatures (*126*). Mesophilic anaerobes, growing at ambient temperatures, cause several types of spoilage of vegetables (*Bacillus* spp.); putrefaction of canned products, early blowing of cheeses, and butyric acid production in canned vegetables and fruits (*Clostridium* spp.); and "medicinal" flavors in canned low-acid foods (*Alicyclobacillus*) (*29*). Psychrotolerant sporeformers produce gas and sickly odors in chilled meats and brine-cured hams (*Clostridium* spp.) while others produce off-odors and gas in vacuum-packed, chilled foods and milk (*Bacillus* spp.).

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria, including species of *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Oenococcus*, some of which are useful in producing fermented foods such as yogurt and pickles. However, under low oxygen, low temperature, and acidic conditions, these bacteria become the predominant spoilage organisms on a variety of foods. Undesirable changes caused by LAB include greening of meat and gas formation in cheeses (blowing), pickles (bloater damage), and canned or packaged meat and vegetables. Off-flavors described as mousy, cheesy, malty, acidic, buttery or liver-like may be detected in wine, meats, milk, or juices spoiled by these bacteria. LAB may also produce large amounts of an exopolysaccharide that causes slime on meats and ropy spoilage in some beverages.

Pseudomonas and related genera are aerobic, Gram-negative soil bacteria, some of which can degrade a wide variety of unusual compounds. They generally require a high water activity for growth (0.95 or higher) and are inhibited by pH values less than 5.4. Some species grow at refrigeration temperatures (psychrophilic) while other are adapted for growth at warmer, ambient temperatures. Four species of *Pseudomonas* (*P. fluorescens*, *P. fragi*, *P. lundensis*, and *P. viridiflava*), *Shewanella putrefaciens*, and *Xanthomonas campestris* are the main food spoilage organisms in this group. Soft rots of plant-derived foods occur when pectins that hold adjacent plant cells together are degraded by pectic lyase enzymes secreted by *X. campestris, P. fluorescens* and *P. viridiflava*. These two species of *Pseudomonas* comprise up to 40% of the naturally occurring bacteria on the surface of fruits and vegetables and cause nearly half of post-harvest rot of fresh produce stored at cold temperatures. *P. fluorescens, P. fragi, P. lundensis,* and *S. putrefaciens* cause spoilage of animal-derived foods (meat, fish, milk) by secreting lipases and proteases that cause formation of sulfides and trimethylamine (off-odors) and by forming biofilms (slime) on surfaces (*55;73*). Some strains are adapted for growth at cold temperatures and spoil these foods in the refrigerator.

Enterobacteriaceae are Gram-negative, facultatively anaerobic bacteria that include a number of human pathogens (*Salmonella, E. coli, Shigella, Yersinia*) and also a large number of spoilage organisms. These bacteria are widespread in nature in soil, on plant surfaces and in digestive tracts of animals and are therefore present in many foods. *Erwinia carotovora* is one of the most important bacteria causing soft rot of vegetables in the field or stored at ambient temperatures. Biogenic amines are produced in meat and fish by several members of this group while others produce off-odors or colors in beer (*Obesumbacterium*), bacon and other cured meats (*Proteus, Serratia*), cheeses (several genera), cole slaw (*Klebsiella*), and shell eggs (*Proteus, Enterobacter, Serratia*). Temperature, salt concentration, and pH are the most important factors determining which, if any, of these microbes spoil foods.

Many Gram-negative bacteria, including pseudomonads and enterobacteriaceae, secrete acyl homoserine lactones (AHLs) to regulate the expression of certain genes, such as virulence factors, as a function of cell density. These AHL quorum-sensing signals may regulate proteolytic enzyme production and iron chelation during spoilage of some foods (*134*) although the role of these signals in other spoilage systems is not clear (*20;97*).

Other bacteria are associated with spoilage of chilled, high protein foods such as meat, fish, and dairy products. They may not be the predominant spoilage organisms but contribute to the breakdown of food components and may produce off-odors. Most species are aerobic although some grow at low oxygen levels and may survive vacuum packaging, and one (*Brochothrix*) is a facultative anaerobe. Some examples include:

- *Acinetobacter* and *Psychrobacter*, which are predominant bacteria on poultry carcasses on the processing line and have been isolated from a variety of spoiled meat and fish. *Acinetobacter* grows at a pH as low as 3.3 and has been detected in spoiled soft drinks. These two genera do not produce extracellular lipases, hydrogen sulfide, or trimethylamine (fishy odor) and so are considered to have a low spoilage potential.
- *Alcaligenes* is a potential contaminant of dairy products and meat and has been isolated from rancid butter and milk with an off-odor. These bacteria occur naturally in the digestive tract of some animals and also in soil and water.
- *Flavobacterium* is found widely in the environment and in chilled foods, particularly dairy products, fish, and meat. It uses both lipases and proteases to produce disagreeable odors in butter, margarine, cheese, cream, and other products with dairy ingredients.
- *Moraxella* and *Photobacterium* are important constituents of the microflora on the surface of fish. *Photobacterium* can grow and produce trimethylamine in ice-stored, vacuum-packaged fish.
- *Brochothrix* has been isolated from meat, fish, dairy products and frozen vegetables. During spoilage, it produces odors described as sour, musty, and sweaty (*139*).

MODELING SPOILAGE

Several physical factors determine whether spoilage microbes will be successful in utilizing the nutrients in a food. These include water activity and types of solutes, pH, temperature (storage and processing), oxygen and carbon dioxide levels, solid or liquid state of food, available nutrients, and preservatives. Models for microbial spoilage of different foods and for spoilage by specific organisms examine the effects of these factors—singly and in combination—to predict the initiation and course of the spoilage process. These models are based on and validated with actual experimental data and can provide useful information for product development and modification, shelf-life estimates, processing requirements, and quality assurance programs.

Food spoilage is a complex process involving a variety of organisms, food preservatives and additives, and food matrices in addition to temperature, pH, and water activity, the most important determinants of microbial growth. Depending on their objective, models are constructed to focus on probability of growth/no growth, time required to initiate growth, growth rate, or survival of spoilage organisms under a particular set of parameters. Inactivation and destruction of microbes exposed to different preservatives or preservation techniques can also be modeled. However, models cannot incorporate every factor that may affect the spoilage process and processors should validate models for their own products to account for different variables. Examples of some recent models are described below:

Growth/no growth was modeled for yeast in a simulated fruit-based or alcoholic food or drink as a function of temperature, pH, and sucrose, sorbitol, and alcohol levels. The model could predict the likelihood of growth and the time to growth (*49*).

Enzyme activity of lipases from five spoilage bacteria were modeled as a function of temperature, pH, and water activity (*15*).

Inactivation/destruction of *Saccharomyces cerevisiae* in orange juice by high intensity pulsed electric fields was modeled as a function of field strength and time of exposure (*44*).

Growth rate models:

- *Pseudomonas fluorescens* at 7ºC as a function of different levels of oxygen (5–75%), carbon dioxide (0–15%) and nitrogen (10–80%) validated on cut lettuce (*58*).
- *Leuconostoc mesenteroides* under aerobic and anaerobic conditions as a function of temperature, pH, and sodium chloride and sodium nitrite levels (*164*).
- *Alicyclobacillus* in orange juice as a function of temperature, pH, soluble solids concentration (ºBrix) and nisin levels (*125*).
- *Brochothrix* as a function of temperature, pH, and water activity (*18*).
- Cocktail of *Pseudomonas*, *Shewanella*, and *Acinetobacter* as a function of temperature, pH, and water activity (*16*).
- Cocktail of several strains of Enterobacteriaceae as a function of temperature, pH, and water activity (*19*).
- Spoilage bacteria on ground meat as a function of temperature and pH (*82*).
- *Aspergillus niger*, a mold, as a function of temperature and water activity (*122*).
- *Penicillium italicum*, a mold, as a function of temperature, water activity and solute levels (glycerol, sorbitol, glucose, NaCl) (*85*).
- Molds (*Eurotium*, *Aspergillus*, *Penicillium*) on sponge cake as a function of pH, water activity, and carbon dioxide levels (*64*) and in fermented bakery product analogues as a function of pH, water activity, and potassium sorbate levels (*66*).
- Yeast, *Pichia anomala*, associated with olive fermentation, as a function of temperature, pH, and NaCl (*8*).
- Yeast, *Zygosaccharomyces bailii*, as a function of pH and benzoic acid levels (*133*).
- Cocktail of three spoilage yeasts (*Yarrowia, Pichia, and Zygosaccharomyces*) as a function of temperature, pH, and water activity (*17*).

FACTORS AFFECTING FOOD SPOILAGE AND SHELF LIFE

Foods by their nature are rich in carbohydrates, proteins and lipids that microbes as well as humans find very nutritious. Living plants and animals have structural and chemical defenses to prevent microbial colonization, but once they are dead or in a dormant state these systems deteriorate and become less effective. Many different microbes may potentially be able to use the nutrients in a food but some species have a competitive advantage under certain conditions. Food processors should note that certain spoilage organisms may not grow on particular foods because some nutrient is missing. If the food product is reformulated, then a new ingredient may allow growth of a previously unimportant microbe (*129*). Different food categories present different challenges for inhibition of spoilage organisms.

Dairy Products

Milk is an excellent medium for growth for a variety of bacteria (*13*). Spoilage bacteria may originate on the farm from the environment or milking equipment or in processing plants from equipment, employees, or the air. LAB are usually the predominant microbes in raw milk and proliferate if milk is not cooled adequately. When populations reach about 10^6 cfu/ml, off-flavors develop in milk due to production of lactic acid and other compounds. Refrigeration suppresses growth of LAB and within one day psychrophilic bacteria (*Pseudomonas*, *Enterobacter*, *Alcaligenes* and some spore-formers) grow and can eventually produce rancid odors through the action of lipases and bitter peptides from protease action (*40*). Pasteurization kills the psychrophiles and mesophilic bacteria (LAB), but heat-tolerant species (*Alcaligenes*, *Microbacterium*, and the sporeformers *Bacillus* and *Clostridium*) survive and may later cause spoilage in milk or other dairy products. Immediately following pasteurization, bacterial counts are usually <1000 cfu/ml. However, post-pasteurization contamination of milk, particularly with *Pseudomonas* and some Gram-positive psychrophiles does occur (*109;151*).

Spoilage problems in cheese can sometimes be traced to low quality milk but may also result from unhygienic conditions in the processing plant. Hard and semi-hard cheeses have a low moisture content (<50%) and a pH ~5.0, which limits the growth of some microbes. Some coliforms and *Clostridium* spp. that cause late gas blowing can grow under these conditions as can several species of molds (*32*). Other psychrotrophs produce biogenic amines, particularly tyramine, during storage of cheese (*81*). Soft cheeses with a higher pH of 5.0–6.5 and a moisture content of

50–80% may be spoiled by *Pseudomonas*, *Alcaligenes,* and *Flavobacterium*. *Clostridium sporogenes* has been found in spoiled processed cheese, where it produces gas holes and off-flavors (*100*).

Yeasts and molds are the main spoilage organisms found in cultured milks (yogurt, sour cream and buttermilk) because the higher acidity in these products inhibits many bacteria (*108;159*). *Pseudomonas*, yeasts and molds can spoil butter and "light" butters. Since the light butters have a higher moisture content than butter, they can support more microbial growth. Cream may become rancid when populations of *Pseudomonas* and *Enterobacter* proliferate.

Cereal and Bakery Products

Cereal grains are exposed to a variety of bacteria, molds and yeasts during growth, harvesting, drying and storage. Molds are the most important contaminants because of the low moisture levels in grains, but molds do require some moisture so efficient drying and good storage facilities are necessary to prevent their growth. Microbial populations decrease during milling and storage of grain. Molds cause spoilage by altering the appearance of grains and flours, and some species also synthesize toxic secondary metabolites called mycotoxins.

Molds are also the primary spoilage organisms in baked goods, with *Aspergillus*, *Penicillium*, and *Eurotium* being the most commonly isolated genera. *Penicillium* tends to be the more important in sourdough breads and in breads stored at cooler temperatures. Freshly baked breads do not contain viable molds but soon become contaminated upon exposure to air and surfaces (*149*). *Bacillus* spores are very heat resistant and can survive baking in the interior of bread loaves and then germinate and start growing as the bread cools. Some strains cause a defect called ropiness, a soft sticky texture caused by starch degradation and slimy exopolysaccharides often accompanied by a fruity odor (*126*). Yeasts may also be involved in spoilage of some breads and fruitcakes, causing a chalky appearance on surfaces and off-odors.

High sugar content and low water activity of cakes also favors molds over other spoilage microbes but some species of yeasts and bacteria (*Bacillus* and *Pseudomonas*) may also attack cakes. Bakery products containing cream, custard or fruit fillings are targets of additional spoilage organisms.

Vegetables

Vegetables are another tempting source of nutrients for spoilage organisms because of their near neutral pH and high water activity. Although vegetables are exposed to a multitude of soil microbes, not all of these can attack plants and some spoilage microbes are not common in soil, for example, lactic acid bacteria. Most spoilage losses are not due to microorganisms that cause plant diseases but rather to bacteria and molds that take advantage of mechanical and chilling damage to plant surfaces. Some microbes are found in only a few types of vegetables while others are widespread. *Erwinia carotovora* is the most common spoilage bacterium and has been detected in virtually every kind of vegetable. It can even grow at refrigeration temperatures (*156*).

Bacterial spoilage first causes softening of tissues as pectins are degraded and the whole vegetable may eventually degenerate into a slimy mass. Starches and sugars are metabolized next and unpleasant odors and flavors develop along with lactic acid and ethanol. Besides *E. carotovora*, several *Pseudomonas* spp. and lactic acid bacteria are important spoilage bacteria.

Molds belonging to several genera, including *Rhizopus*, *Alternaria*, and *Botrytis*, cause a number of vegetable rots described by their color, texture, or acidic products. The higher moisture content of vegetables as compared to grains allows different fungi to proliferate, but some species of *Aspergillus* attack onions.

Fresh Meat

In contrast to fruits and vegetables, meats are composed mainly of protein and fats rather than carbohydrates. Water content is 71–76% and therefore moisture is not an issue except for spoilage microbes on cured meats. 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. The surface of beef carcasses may contain anywhere from 10^1 to 10^7 cfu/cm^2, most of which are psychrotrophic bacteria. 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 (*48;92;141*).

Pseudomonas spp. are the predominant spoilage bacteria in aerobically stored raw meat and poultry (*10*). 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. Breakdown 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 (*139*).

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.

Fish

Fish is a very perishable, high-protein food that typically contains a high level of free amino acids. Microbes metabolize these amino acids, producing ammonia, biogenic amines such as putrescine, histamine, and cadaverine, organic acids, ketones, and sulfur compounds (*9;35;47;116*). Degradation of lipids in fatty fish produces rancid odors (*70*). 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 pseudomonads that produce siderophores that bind iron (*61*).

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 (*55;73*). 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 acids selects for lactic acid bacteria and yeasts (*101*). Pasteurization kills vegetative bacteria but spores of *Clostridium* and *Bacillus* survive and may grow, particularly in unsalted fish (*61*).

Fruits and Juices

Intact, healthy fruits have many microbes on their surfaces but can usually inhibit their growth until after harvest. Ripening weakens cell walls and decreases the amounts of antifungal chemicals in fruits, and physical damage during harvesting causes breaks in outer protective layers of fruits that spoilage organisms can exploit. Molds are tolerant of acidic conditions and low water activity and are involved in spoilage of citrus fruits, apples, pears, and other fruits. *Penicillium, Botrytis,* and *Rhizopus* are frequently isolated from spoiled fruits (*23*). Yeasts and some bacteria, including *Erwinia* and *Xanthomonas*, can also spoil some fruits and these may particularly be a problem for fresh cut packaged fruits (*115;135*).

Fruits juices generally have relatively high levels of sugar and a low pH and this favors growth of yeasts, molds and some acid-tolerant bacteria. Spoilage may be manifested as surface pellicles or fibrous mats of molds, cloudiness, and off-flavors. Lack of oxygen in bottled and canned drinks limits mold growth. *Saccharomyces* and *Zygosaccharomyces* are resistant to thermal processing and are found in some spoiled juices (*52;146*). *Alicyclobacillus* spp., an acidophilic and thermophilic spore-forming bacteria, has emerged as an important spoilage microbe, causing a smoky taint and other off-flavors in pasteurized juices (*29;31;145*). *Propionibacterium cyclohexanicum*, an acid-tolerant non-sporeforming bacterium also survives heating and grows in a variety of fruit juices (*161*). Lactic acid bacteria can spoil orange and tomato juices, and some pseudomonads and Enterobacteriaceae also spoil juices. These bacteria are not as heat tolerant but may be post-pasteurization contaminants.

CONTROL OF SPOILAGE MICROORGANISMS

Spoilage organisms are not originally an integral part of foods but are widely present in water, soil, air, and other animals. Healthy living plants and animals can ward off bacteria and fungi, but as soon as they are slaughtered or harvested their defenses deteriorate and their tissues become susceptible to spoilage microbes. Good manufacturing practices with strict attention to sanitation and hygiene can prevent colonization by many, but not all, microbes and are the most important first step in delaying the spoilage process.

Microbes require certain conditions for growth, and therefore management of the environment of foods can change these factors and delay spoilage:

- Many, but not all, microbes grow slowly or not at all at low temperatures, and refrigeration can prolong the lag phase and decrease growth rate of microbes.
- Many microbes require a high water activity and therefore keeping foods such as grains and cereal products dry will help to preserve them.
- Some microbes require oxygen, others are killed by oxygen, and still others are facultative. Managing the atmosphere during storage in packaging can retard or prevent the growth of some microbes. Several types of modified atmosphere packaging (MAP) have been developed to retard growth of pathogenic and spoilage organisms (*10;30;35;47;48;64;65;76;96;135; 147;148;157;158*).

However, microbes are endlessly innovative and eventually seem to circumvent the barriers we set up against them. Therefore further strategies and multiple hurdles are utilized to extend shelf life. These procedures must be assessed for compatibility with different foods so that there are no significant organoleptic changes in the foods caused by the treatment or preservative. These methods for food preservation will not be covered in depth here.

Processing technologies, in addition to thermal processing, are being developed to kill spoilage microbes, including:

- high pressure processing of fruits, juices, meat and fish (*72;86;87;113;123;138;154;155*);
- ozone (*41;74;102*);
- irradiation of fruit and meat (*11;51;102;130; 137*);
- pulsed electric fields of juices (*42,43,44;93; 112;143*).

Formulation of processed foods may include compounds that alter the water activity or pH of foods, thereby limiting growth of many organisms.

Antimicrobial compounds may be added to foods or packaging to inhibit growth of many spoilage organisms:

- Organic acids can help control bacteria, molds and yeasts in bakery products, meat, juices, and other foods (*1;49;65;66;67;83; 88;99;110;131; 133;153;162;163*).
- Bacteriocins, including nisin, can help control spoilage bacteria in dairy products, fish, juice, and vegetables (*22;28;36;57;63;77;90;91;102; 117; 118;125*).

♦ Chitosan incorporated into foods or used as a coating for fruits and vegetables inhibits growth of some spoilage bacteria and yeasts (*3;37;59; 60;80;124*).

♦ Many herbs, essential oils, and spices have demonstrated some inhibitory activity against spoilage microbes in a variety of foods. Thyme, oregano, vanillin, and cinnamon are the most commonly mentioned substances in recent papers (*4;5;6;7;27;33;38;39;50;52;53;54;68; 78;89;107; 114;119;120;127;132;136;140; 152;160*).

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