Lec.8

Food Microbiology

Spoilage of Specific Food Groups

Milk and Milk Products:

Raw milk contains many types of microorganisms coming from different sources. The average composition of cow's milk is protein 3.2%, carbohydrates 4.8%, lipids 3.9%, and minerals 0.9%. Besides casein and lactalbumin, it has free amino acids, which can provide a good N source. As the main carbohydrate is lactose, those microorganisms with lactose-hydrolyzing enzymes (phospho-b-galactosidase or b-galactosidase) have an advantage over those unable to metabolize lactose. Milk fat can be hydrolyzed by microbial lipases, with the release of small-molecular volatile fatty acids (butyric, capric, and caproic acids).

Control of Microorganisms in Milk

A. Refrigeration

Milk handling and processing strategies are designed to reduce and control bacterial numbers in processed products to protect milk quality and milk safety. The first of preventive measures involves efficient cooling of milk to 4°C immediately following milking. Reduced temperatures inhibit growth of mesophiles and thermophiles and reduce the activity of degradative enzymes.

B. Heat Treatment

Heat treatment plays a critical role in controlling bacterial numbers in processed milk products. The three basic approaches to heat treatment of raw milk, pasteurization, ultrapasteurization and Ultra Hight Temperature(UHT).

Pasteurization aims to eliminate the non–spore-forming pathogen most resistant o thermal destruction, currently recognized as being *Coxiella burnetii*, and reduce nonpathogenic bacterial numbers in milk. Pasteurization include low-temperature long-time (LTLT) and high-temperature short-time (HTST). In LTLT, which is commonly used for milk intended for manufactured products such as cheese and yogurt, milk is held at a minimum of 63°C for 30 min. In HTST pasteurization is most commonly used for fluid milk products, milk is held at a minimum of 72°C for 15 s. Typical shelf lives for heat-treated fluid milk are 14–21 days.

Ultrapasteurization adds the additional goal of increasing product shelf life through further reduction in total bacterial numbers, milk is held at a minimum of 138°C for at least 2 s. Typical shelf lives for heat-treated fluid milk 40–60 days.

UHT processing aims to achieve microbial sterility to create a shelf-stable fluid milk product, milk is held at $140-150^{\circ}$ C for a few seconds. Typical shelf lives up to 6 months.

HTST and ultrapasteurized products require refrigeration at $4^{\circ}C$ or less during storage, UHT products can be stored at $25^{\circ}C$.

C. Centrifugation

Two techniques known as clarification and bactofugation. Centrifugation of milk causes bacteria, dirt particles, somatic cells, animal hairs, and bacterial spores to migrate outward, whereas lighter fat globules and casein micelles migrate inward.Clarification is primarily designed to remove dirt particles, somatic cells, and animal hairs, whereas bactofugation is specially designed to remove bacterial spores from milk. Using high-force centrifugation, the spore load of raw milk can be reduced by greater than 99%.

D. Filtration

Microfiltration and ultrafiltration utilize the larger relative size of bacterial cells to separate out microbial contaminants. Filters with very small pores allow milk components to pass through while blocking bacteria, thus separating contaminants. Typically rated in terms of pore diameter, microfiltration filters range from 0.2 to 5.0 μ m. Using microfiltration, lactose, minerals, and small proteins pass through into the permeate, whereas fat, very large proteins, and bacteria are retained.Ultrafiltration filters range from $10^3 to 10^5$ D. Using ultrafiltration, minerals and lactose pass through into the permeate, whereas proteins, fats, and bacteria are retained. Although filtration can not remove all microorganisms, it can achieve a 99.99% reduction of the total bacterial count and a 99.95% reduction in the total spore count .Milk with higher fat percentages causes membrane fouling, making this technique most useful for treating skim milk.

E. Additional Microbial Control Methods

Several less commonly utilized techniques exist for controlling microbial growth in milk. Addition of carbon dioxide to milk at 10–30 mm/L inhibits growth of the common spoilage organism *P. fluorescens*. This technique has been reported to extend the shelf life of refrigerated milk by several days. The use of the natural antibiotic nisin to inhibit gram-positive bacterial growth in milk. Addition of lactic acid starter cultures to raw milk has been shown to inhibit growth of psychrotrophs. Although the lactic acid bacteria do not multiply at refrigeration temperatures, their metabolism results in a pH decrease to below 6 and possible organoleptic changes.

MICROBIOLOGY OF MILK AND MILK PRODUCTS

A. Raw Milk

Microbial spoilage of raw milk can occur from the metabolism of lactose, proteinaceous compounds, fatty acids and the hydrolysis of triglycerides. If the

milk is refrigerated immediately following milking and stored for days, the spoilage will be predominantly caused by Gram-negative psychrotrophic rods, such as *Pseudomonas*,*Alcaligenes*,*Flavobacterium* spp., and some coliforms. *Pseudomonas* and related species, being lactose negative, metabolize proteinaceous compounds to change the normal flavor of milk to bitter, fruity, or unclean. They also produce heat-stable lipases (producing rancid flavor).

The growth of lactose-positive coliforms produces lactic acid, acetic acid, formic acid, CO2, and H (by mixed-acid fermentation) and causes curdling, foaming, and souring of milk. Some *Alcaligenes* spp. and coliforms can also cause ropiness (sliminess) by producing viscous exopolysaccharides. However, if the raw milk is not refrigerated soon, growth of mesophiles, such as species of *Lactococcus, Lactobacillus, Enterococcus, Micrococcus, Bacillus, Clostridium,* and coliforms, along with *Pseudomonas, Proteus*, and others, predominates. But lactose-hydrolyzing species, such as *Lactococcus* spp. and *Lactobacillus* spp., generally predominate, producing enough acid to lower the pH and prevent or reduce growth of others. Yeast and mold growth, under normal conditions, is generally not expected.

B. Pasteurized Milk

Raw milk is pasteurized before it is sold for consumption as liquid milk. Thermoduric bacteria (*Micrococcus, Enterococcus,* some *Lactobacillus, Streptococcus, Corynebacterium,* and spores of *Bacillus* and *Clostridium*) survive the process. In addition, coliforms, *Pseudomonas, Alcaligenes, Flavobacterium,* and similar types can enter as postpasteurization contaminants. Pasteurized milk, under refrigerated storage, has alimited shelf life, mainly due to growth of these psychrotrophic contaminants.

C. Concentrated Liquid Products

Evaporated milk, condensed milk, and sweetened condensed milk are the principal types of concentrated dairy products susceptible to limited microbial spoilage during storage. All these products are subjected to sufficient heat treatments to kill vegetative microorganisms as well as spores of molds and some bacteria. Evaporated milk is condensed whole milk with 7.5% milk fat and 25% total solids. It is packaged in hermetically sealed cans and heated to obtain commercial sterility. Under proper processing conditions, only thermophilic spores of spoilage bacteria can survive, and exposure to high storage temperature (43C or higher) can trigger their germination and subsequent growth. Under such conditions, *Bacillus* species, such as *B. coagulans*, can cause coagulation of milk (flakes, clots, or a solid curd).

Condensed milk is generally condensed whole milk and has 10 to 12% fat and 36% total solids. The milk is initially given a low-heat treatment, close to pasteurization temperature, and then subjected to evaporation under partial vacuum (at 50C). Thus, it can have thermoduric microorganisms that subsequently can grow and cause spoilage. Other microorganisms can also get into the product during the condensing process.

Sweetened condensed milk contains 8.5% fat, 28% total solids, and 42% sucrose. The whole milk is initially heated to a high temperature (80 to 100C) and then condensed at 60C under vacuum and put into containers. Because of a low *aw*, it is susceptible to spoilage from the growth of osmophilic yeasts , causing gas formation. If the containers have enough headspace and oxygen, molds (e.g., *Penicillium* and *Aspergillus*) can grow on the surface.

D. Flavored Milks

The microbiology of flavored milk differs from that of unflavored milk in that conventionally pasteurized chocolate milk typically spoils faster than conventionally pasteurized unflavored milk. After 14 days at 6°C, chocolate milk samples had higher standard plate counts and higher psychrotrophic plate counts than unflavored milk samples from the same raw milk. The chocolate powder, and not the additional sucrose, contributed to the increased bacterial growth. The chocolate powder did not introduce additional microbes into the milk. Rather microbes already present in the raw milk grew faster owing to the presence of the chocolate powder.

E. Butter

Butter contains 80% milk fat and can be salted or unsalted. The microbiological quality of butter depends on the quality of cream and the sanitary conditions used in the processing. Growth of bacteria (*Pseudomonas* spp.), yeasts (*Candida* spp.), and molds (*Geotrichum candidum*) on the surface causes flavor defects (putrid, rancid, or fishy) and surface discoloration. In unsalted butter, coliforms, *Enterococcus*, and *Pseudomonas* can grow and produce flavor defects.

MEAT PRODUCTS

A. Raw Meat

Meats are the most perishable of all important foods. Meats contain a large quantity of all nutrients required for the growth of bacteria, yeasts, and molds. Fresh meats from food animals and birds contain a large group of potential spoilage bacteria that include species of *Pseudomonas, Acinetobacter, Moraxella, Shewanella, Alcaligenes, Aeromonas, Escherichia, Enterobacter, Serratia, Hafnia,*

Proteus, Brochothrix, Micrococcus, Enterococcus, Lactobacillus, Leuconostoc, Carnobacterium, and Clostridium, as well as yeasts and molds.

The predominant spoilage flora in a meat is determined by nutrient availability, oxygen availability, storage temperature, pH, storage time of the product, and generation time of the microorganisms present in a given environment. To delay microbial spoilage, fresh meats are stored at refrigerated temperature. Thus, normally psychrotrophic bacteria are the most predominant types in raw meat spoilage. Under aerobic storage at low temperature, growth of psychrotrophic aerobes and facultative anaerobes is favored.

In retail-cut meats, because of a shorter generation time, *Pseudomonas* spp. grows rapidly, using glucose first and then amino acids; the metabolism of amino acids is accompanied by the production of malodorous methyl sulfides, esters, and acids. In meats with high pH or low glucose content, or both, *Acinetobacter* and *Morexella*, which metabolize amino acids instead of glucose, can grow rapidly and produce undesirable odors. Spoilage by these strict aerobes in the form of off odor and slime .

Along with offensive odors, some strains also produce H_2S in small amounts to cause greening of the meat (H_2S oxidizes myoglobin to a form of met myoglobin, causing a green discoloration). Facultative anaerobic *Enterobacter, Serratia, Proteus*, and *Hafnia* species metabolize amino acids while growing in meat to produce amines, ammonia, methylsulfides, and mercaptans, and cause putrefaction. Because amines and ammonia are produced, the pH of the meat usually changes to alkaline range, and meat can have a pinkish to red color.

Yeasts may grow under aerobic conditions on the meats and causes sliminess, lipolysis, off odors and tastes, discolorations (white, cream, pink or brown, due to pigments in yeasts).

Aerobic growth of molds may cause the following:

1-Stickiness:Growth of molds makes the surface of the meat sticky.

2- discoloration : Black spot: Usually caused by *Cladosporium*.

White spot: Caused by *Geotrichum*. Green patches: Caused by *Penicillium* spp.

3- Decomposition of Fats: Many molds have lipases an cause hydrolysis of fats. Molds also help in the oxidation of fats.

4-Off odour and off tastes: Molds give a musty flavour to meat.

To reduce spoilage of fresh meats, initial microbial level should be reduced. In addition, storage at low temperatures (close to 0 to -1C), modified atmosphere packaging, and vacuum packaging should be done. Several other methods to reduce initial microbial load and slow growth rate of Gram-negative rods are being either used or tested. These include the addition of small amounts of organic acids

to lower the pH of meat (slightly above pH 5.0), drying of meat surfaces (to reduce a_w), and a combination of the factors given , including lower storage temperature.

B. Processed Meat Products

This group includes high-heat-processed and low-heat-processed uncured and cured meat products. High-heat-processed cured and uncured meats are given heat treatment to make them commercially sterile. Thus, they may only have some thermophilic spores surviving, which will not germinate unless the products are temperature abused. Low-heat-processed uncured meats, such as roasts, are given heat treatment at an internal temperature of (60 to 71C). Generally, the surface of the meats (and thus most of the microorganisms) is exposed to the final temperature for 1 h or more, depending on the size of the meat. Under this condition, only the spores of Bacillus and Clostridium spp. and some thermoduric vegetative species (some Enterococcus, Micrococcus, lactobacillus inside the product) can survive. Many types of microorganisms can enter as post heat contaminants into the products from equipment, personnel, water, and air. In some situations, spices and other ingredients are added to the products after heating, which, in turn, can be the source of microbial contamination of the products. Some products are sliced before vacuum packaging, which increases the chances of contamination on the surface area of the product from the equipment and environment. Psychrotrophic facultative anaerobic and anaerobic bacteria have been implicated in the spoilage of these products.