

**FARM MICROBIOLOGY 2008**  
**PART 4: FOOD MICROBIOLOGY – GENERAL PRINCIPLES**  
INCLUDING SPOILAGE, PRESERVATION, AND SOME EXAMPLES  
OF FOOD FERMENTATIONS (INCLUDING SILAGE)

**I. Importance of microorganisms in foods.**

**A. Spoilage of foods.** Problem of contaminating organisms causing undesirable changes in food that may be improperly kept. (In lab we will be determining the contamination level in hamburger by the dilution plating method. Also, when we make sausage we will be purposely contaminating one batch with a “greening organism.”)

**B. Production of some foods.** Use of bacteria and other microorganisms to cause desirable changes in a raw product such that the finished product benefits by longer shelf-life and improved taste and appearance. (In lab we will make **sausage** and compare batches made with and without **starter cultures**.)

**C. Food-borne illnesses.** Pathogenic organisms transmitted via food and also toxins produced by microorganisms in food which may be ingested. (These will be treated in more depth when we discuss diseases in the lecture.)

**D. Indicator organisms.** For example, finding coliforms may indicate a contamination problem; *E. coli* would indicate fecal contamination. (This topic will be covered when we discuss water quality in the lecture. We will also do a “coliform test” on hamburger in the lab.)

**II. Food spoilage.**

**A. Proteins, fats and carbohydrates.** Excellent substrates for a variety of microorganisms. Meats, fish, poultry, eggs and milk are examples of high-protein foods. Milk, fruits and vegetables contain sugars which can be fermented.

**B. Foods containing very high levels of sugar.** High concentrations of sugars can be inhibitory to a variety of organisms, but certain yeasts and molds may require such a high concentration in order to grow. Such organisms are called **osmophiles** and may contaminate jams, jellies, honey, etc. over a period of time.

**C. Dry foods.** Generally resistant to microbial activities. What little H<sub>2</sub>O there may be can be saturated with solutes and thus be inhibitory. With increasing H<sub>2</sub>O such as high humidity, molds tend to be the initial spoilage organisms.

**III. Food preservation – i.e., prevention of spoilage.**

**A. Principles.**

1. Keep microorganisms out.
2. Inhibit microbial growth and activity.
3. Remove microorganisms.
4. Kill microorganisms.
5. Inactivate enzymes in foods.

**B. Methods to control microbial growth in foods.**

1. **Keeping organisms out.** Keeping utensils and food preparation areas clean. Preventing cross-contamination between raw and cooked food. As complete a separation as possible of highly contaminated parts of animals (skin, intestines) from meat in slaughterhouses. Cleaning vegetables and fruits effectively. (Etc.)

2. **Removing organisms.** Filtration of liquid foods.
3. **Low temperatures.** Tend to inhibit microbial activity. Cold temperatures (including freezing) are not sterilization methods by any means.
4. **High temperatures.** Choice of a suitable time/temperature combination to kill pathogenic and spoilage organisms – depends on type of food, expected concentrations of organisms. Also – when serving food – keeping hot so as to prevent growth of organisms.
5. **Drying.** Remember that H<sub>2</sub>O is essential for microorganisms in that nutrients are taken in as a solution and all enzymatic activities take place in an aqueous environment. When H<sub>2</sub>O is removed during the drying process, the concentration of solutes rises such that H<sub>2</sub>O becomes no longer "available" to microorganisms, and the solutes come out of solution eventually.
6. **Inhibitory chemicals.**

a. **Overview, showing multiple functions for various inhibitors:**

	<b>CHEMICALS</b>	<b>FUNCTIONS</b>
	NaCl (added)	inhibitory, taste, help in extracting water
	Nitrite (added)	inhibitory, color (with heat)
	Anaerobic conditions	inhibitory, help fermentation
Sugar (some- times added)	bacterial fermentation-----> Acid	inhibitory, taste, "tighten up" the protein
	yeast fermentation-----> Ethanol	inhibitory, taste

b. **Inhibitors inherent to the food product itself.** Many foods contain anti-microbial agents – e.g., benzoic acid in cranberries.

c. **Inhibitors added to the recipe.** Salt (NaCl) is an often-used inhibitory ingredient.

d. **Inhibitors added by microorganisms.** Acids are the most significant inhibitory agents – sometimes inhibitory to those that produce the acids!

7. **Ultraviolet and ionizing irradiation.**

8. **Combination of methods and the "Barrier Concept."**

a. **Concept.** There is the need to inhibit spoilage and disease organisms and still maintain the character of the food, so a combination of moderate methods can be utilized rather than a drastic method. Any agent may not be in high enough concentration to effect inhibition by itself (and it may cause some discomfort to the consumer if it were in such a high amount), but a combination of agents in lower concentrations may do the job. Called "barrier" or "hurdle" concept using the analogy of many small hurdles eventually wearing out a runner as would just one that is high enough.

**b. Example.** Here's an example (and it is probably not the best one but you can get the general idea): One can sterilize milk in a pressure cooker and it will last a long time, but the taste and color of the milk may be altered to an unacceptable degree. Pasteurization and keeping the milk cool will have it keep reasonably well and the character of the milk will be retained.

#### **IV. Production of some foods with the aid of microorganisms. (Silage and fermented dairy products are considered separately below.)**

**A. Use of starter cultures vs. wild fermentation.** Starter cultures are added to effect a reliable fermentation in certain foods such as fermented milk products and many types of sausages. Some foods are best prepared by not adding any microorganisms – just letting the organisms that are expected to be in the raw food product do the job. This is a "wild fermentation" and is employed in the production of sauerkraut and some sausages.

#### **B. Bread.**

**1. Functions of yeast.** Ferment carbohydrates to CO<sub>2</sub> and ethanol. CO<sub>2</sub> necessary for the rising of the dough. Ethanol burns off in the baking process.

**2. Sources of flavor.** Yeast cells and their products can impart considerable flavor. (Nucleotide component of yeast is used as a flavoring agent.)

**3. Spoilage.** Chiefly molds.

**C. Alcoholic beverages.** Sugars fermented by yeasts with production of CO<sub>2</sub> and ethanol. Different strains of yeasts have their use for certain varieties of alcoholic beverages. The ability to produce an abundance of gas would be important for a champagne yeast.

**D. Vinegar.** Basically an alcoholic fermentation which has been allowed to oxidize – i.e., ethanol is oxidized to acetic acid by certain bacteria such as *Acetobacter*. Use of the word "vinegar" implies product made from fermented apples (also called apple vinegar or cider vinegar) unless modified otherwise – such as "wine vinegar" which is made from fermented grapes. Other substrates for fermentation and subsequent oxidation include various fruits, cereals, molasses, honey and coconut.\_

#### **E. Fermented sausage.**

(See <http://www.jlindquist.net/generalmicro/324sausage.html>.)

**1. Manufacture.** Meat is mixed with NaCl, sugar and a small amount of nitrite. NaCl and nitrite inhibitory to a number of spoilage and disease-causing organisms – nitrite effective against *Clostridium botulinum*. Nitrite also helps in ultimate development of desirable color. Sugar provides substrate for fermenting bacteria to produce acid.

#### **2. Fermentation.**

**a. Use of starter culture – *Lactobacillus* or *Pediococcus*.** Both are lactic acid bacteria that are inoculated to produce a reliable fermentation in which one expects considerable acid to be produced.

**b. Multiple importance of acid.** Acid important in preservation, taste and "tightening up" the meat protein. – resulting in a relatively hard product. Acid also helps (with nitrite) in ultimate development of desirable color.

### 3. Defects.

**a. If too much nitrite.** Too much nitrite can eventually cause a green discoloration. This can happen if nitrate is added and there is too much nitrate reduction to nitrite by indigenous microorganisms.

**b. If not enough fermentation.** Also, if starter culture is not added, there may not be enough indigenous lactic acid bacteria to carry on fermentation; less acid leads to more spoilage potential.

## F. Sauerkraut. (Also applicable to cucumber pickles.) (See <http://www.jlindquist.net/generalmicro/324sauerkraut.html>.)

1. **Process involves addition or creation of inhibitory/preservative agents.** Namely, NaCl, anaerobic conditions and acid.

2. **Manufacture (including use of NaCl).** Cabbage leaves are shredded (discarding the core) and **NaCl** is added to a 2.5% total concentration and thoroughly mixed in. The NaCl creates an osmotic imbalance that will draw water and dissolved nutrients out of the cabbage tissue. Considering that the cabbage leaves are about 90% H<sub>2</sub>O and the NaCl is dissolved only in the H<sub>2</sub>O (and microbial activity is only in the H<sub>2</sub>O), the liquid component actually has about **2.8% NaCl** which is inhibitory to many spoilage and disease-producing organisms. Mixture is placed in a container that will not admit air (oxygen). Eventual **anaerobic conditions** are also inhibitory to many organisms but not the desired fermenters which will function effectively in the 2.8% NaCl concentration.

3. **Obtaining anaerobic conditions.** This occurs when the oxygen is used up by respiration by the cabbage leaves and respiring microorganisms. This can take just a very short time – probably less than a day.

4. **Fermentation – involves floral succession to produce acid.** Sugar in the leaves is utilized as bacteria undergo fermentation, producing **acid** – the 3rd major inhibitory agent and the one most responsible for preservation of the product. (Acid and NaCl also contribute significantly to the taste.) When various kinds of bacteria participate at different stages in the process – a certain organism predominating at a given stage – the term **floral succession** applies. Three groups of bacteria come and go during the sauerkraut fermentation.

**a. Coliforms** During first few days, **coliforms** predominate and produce acid and gas; they then decrease in numbers as the acid increases. (“Non-fecal” coliforms are common soil organisms. Instructor will get into the ecology of coliforms and what it means when they are found in food – such as the implication of fecal contamination if *E. coli* is isolated from any sample.)

b. *Leuconostoc* (in lactic acid bacteria group) is the "second wave," producing more acid (and gas) over the next several days – eventually becoming inhibited by the increasing acid.

c. *Lactobacillus* (in lactic acid bacteria group) continues acid production (lactic acid with no gas) which may drive pH below 4.

5. **Defects.** Sometimes may get a slime-forming strain of *Lactobacillus*. If not anaerobic, molds will readily grow on the surface – easily tolerating the high amount of acid. Sometimes may get a yeast which may impart some off flavors. Another yeast can cause a pink color.

## V. The Silage Fermentation.

### A. Phases of the fermentation/manufacturing process.

1. **Overview.** Process is similar to that of sauerkraut. A useful web page on the subject can be found here: <http://www.ag.ndsu.edu/pubs/ansci/dairy/as1254w.htm>

2. **Aerobic phase.** After the chopped forage material is thoroughly packed into the silo – which is then sealed – the O<sub>2</sub> is used up by microbial and plant respiration with the release of CO<sub>2</sub> and heat.

3. **Lag phase.** During this period, plant cell membranes break down, releasing sugars and other materials usable as nutrients for microorganisms.

4. **Acetic acid phase.** Ideally, bacteria which produce acetic acid start off the fermentation process.

5. **Lactic acid phase.** At about the third day, lactic acid bacteria (usually *Lactobacillus*) take over the fermentation.

6. **Stable phase.** When so much acid has been produced that microbial activity ceases – usually after 3-4 weeks – this phase is achieved and the silage remains stable barring the entrance of oxygen.

### B. Defects.

1. **Due to too much oxygen.** Excess heat may be produced along with too much degradation of plant tissue with loss of nutrients. Very high temperatures cause browning and undesirable taste and texture.

2. **Due to silage being too wet. (Including problem of *Clostridium*.)** Anaerobic conditions are achieved too soon, before desirable temperature for acetic acid-producing bacteria is reached. Organisms such as certain species of *Clostridium* which produce butyric acid can take over, resulting in repulsive taste and odor.

3. **Due to excess contamination by soil.** Enough *Clostridium* (a common soil organism) may be present such that the undesirable butyric acid fermentation can take place.

## C. Addition of NPN (non-protein nitrogen).

- 1. Overview.** These are compounds such as urea and anhydrous ammonia (NH<sub>3</sub>) which are meant to increase the nitrogen content of the silage by becoming nutrients for bacteria whose growth ultimately augments the overall protein content.
- 2. At beginning of silage production process.** The pH starts a bit higher due to the alkaline effect of the NH<sub>3</sub>, but microbial growth is enhanced and the final pH is still low enough to preserve the silage. On the surface, the additional NH<sub>3</sub> can be inhibitory to some microorganisms like molds.
- 3. When fed to livestock.** Rumen bacteria use these compounds, resulting in additional microbial protein ultimately used by the animal.\_

## VI. Examples of cultured dairy products.

### A. Yogurt.

- 1. Preparation of the milk.** Milk is fortified with additional dry milk and heated to partially denature the protein. Denaturation of protein leads to a more even curd and less expression of whey.
- 2. The fermentation process** employs starter cultures such as *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Lactobacillus acidophilus*.) Cooled, "fortified" milk is inoculated with 2 or 3 species of lactic acid bacteria and allowed to incubate for several hours at a relatively high temperature (45°C). Rapid growth of organisms produces substantial acid which coagulates the protein, forming a curd.

### B. Cheeses.

- 1. Solidification of protein accomplished by:**
  - a. Acid from lactic acid fermentation.** One or more lactic acid bacteria allowed to ferment the lactose.
  - b. Addition of coagulant (rennet).**
- 2. Variety of cheeses due to:**
  - a. Variety of organisms used for fermentation and/or flavoring.**
  - b. Sources of milk.**
  - c. Different aging processes.**

### 3. Two examples.

- a. **Swiss cheese** (addition of *Propionibacterium*). This bacterium is responsible for development of propionic acid and holes (from CO<sub>2</sub> bubbles).
- b. **Blue cheese** (addition of *Penicillium roqueforti*). This mold grows along channels in the curd made by piercing needles, resulting in the blue marbled appearance and the flavor of ketones produced by the mold from fatty acids.

**VII. Probiotics.** These are commercially-available nutrient supplements whose aim is to provide the benefits of bacterial cultures to help prevent diseases and alleviate some of the symptoms associated with internal problems and even bad skin. Often these are seen as capsules containing ultra-concentrated yogurt bacteria which tend to crowd out other organisms in the intestinal tract – not giving intestinal pathogens a foothold to cause disease. To provide any real benefit, these capsules have to be taken daily, as their bacteria cannot develop enough of a foothold to remain for any period of time.

### VIII. Some More About Milk.

**A. Definition of milk.** Liquid secreted by mammary glands of female mammals to nourish their young for a period soon after birth. Must not contain **colostrum** which is what appears immediately after birth. In US, the word "milk" implies cow's milk.

**B. Average gross composition of milk.** (Percentages are shown for cow's milk.)

1. **Water.** 87.3%. (By comparison, reindeer milk contains 63.3% water.)
2. **Fat.** 3.7%. (Reindeer milk contains 22.5% fat!) Fat appears as globules which are suspended in what is otherwise a solution. Globules rise to the top and form the **cream layer**. If skim off the cream or otherwise remove it, resulting milk is called **skim milk** if it contains less than 0.5% fat. If it contains 1-2% fat, it is called **low-fat milk**.
3. **Lactose.** 4.9%. Disaccharide composed of glucose and galactose. This sugar is found nowhere else but in milk.
4. **Proteins.** Casein (2.9%) and albumin (0.5%).
5. **Ash.** Minerals remaining after incineration (0.7%). Includes compounds of calcium and potassium.
6. **Minor constituents.** Includes B vitamins and also C and D. Milk is usually fortified with A and D for sale.

**C. Nutritional value.** Enough to satisfy all nutritional needs for infants of the species.

**D. Keeping quality (shelf-life).** Like leaving a bacteriological medium exposed to the air. Will quickly get contaminated with fast-growing organisms.

**E. Sources of microorganisms in milk.**

1. Interior of the udder.
2. Exterior of the udder.
3. Coat of the cow.
4. Utensils.
5. Air.
6. Flies and other insects.
7. Humans. Can serve as source of spoilage and disease-producing microorganisms.

**F. Changes in milk resulting from microbial growth.** Note that pasteurized milk can be easily recontaminated. In fact, spoilage is usually caused by organisms recontaminating the milk after pasteurization.\_

1. **Fermentation.**
2. **Proteolysis.**
3. **Alkali production.**
4. **Ropiness or sliminess.**
5. **Changes in butterfat.**
6. **Flavor and aroma changes.**
7. **Color changes.** Examples: *Pseudomonas*, *Serratia*, *Chromobacterium*.
8. **What happens if milk is allowed to spoil – a “floral succession.”**
  - a. **Streptococcus and Lactobacillus** predominate (the latter following the former) and ferment the lactose to produce acid.
  - b. **Yeasts and molds** take off in the acidic conditions and consume some of the acid, raising the pH.
  - c. **Bacillus.** At a more moderate/neutral pH level, *Bacillus* and other protein degraders predominate and the milk becomes watery (less milky in appearance) as the protein is hydrolyzed.\_

**G. Procedures to reduce or minimize changes in milk.**

1. **Asepsis.** Minimizing contamination before and after pasteurization.
2. **Use of low temperatures.** Keeping milk just above freezing is best (0-4°C).
3. **Use of heat.**
  - a. **Pasteurization.** Time and temperature combination necessary to kill pathogens and also significantly reduce spoilage organisms: 63°C for 30 minutes or higher temperatures for shorter times. Nature of milk is maintained which may be altered considerably by attempts to sterilize by using higher temperatures.
  - b. **Boiling and steam under pressure (autoclaving).** Done for very short time, probably not long enough to reduce endospore numbers.

#### 4. **Other methods of preservation.**

- a. **Condensing.** Removing much or most of the water. Microorganisms are inhibited by the increased solute concentration.
- b. **Drying.** Removal of all water with cessation of bacterial metabolism and reproduction.
- c. **Adding preservatives.** As a rule for commercially-available milk, this is not done. Milk is usually shown as consisting only of milk, perhaps with added vitamins which certainly do not act as preservatives.
- d. **Preservation by fermentation.** Practiced for thousands of years, probably originating with attempts to duplicate conditions where a wild fermentation happened to result in a pleasing, long-lasting product. Art became a science in recent centuries.

#### H. **Determining bacteriological quality of milk.**

1. **Standard plate count.** Inoculating petri plates of a standard all-purpose medium (such as "Plate Count Agar") with known amounts of various dilutions of milk samples. Maximum numbers of colony-forming units per ml allowed such as 100,000-300,000 for raw milk and 20,000-30,000 for pasteurized milk. Higher levels allowed for milk used in cheese-making.
2. **Coliform count.** Done with appropriate media that detect these organisms. Coliforms are easily-detectable organisms which, if found, can indicate soil or fecal contamination. (*E. coli* is only associated with intestinal waste and it is a lot easier to test for *E. coli* than it is to test for the various possible intestinal pathogens which may be bacteria, viruses and protozoa.) Maximum allowable number of coliforms per ml in pasteurized milk is very low (10 or less).
3. **Direct microscopic count.** Can count certain number of microscopic fields and then multiply by appropriate factor to get no. of cells per ml.
4. **Dye reduction tests.** Time it takes to reduce (decolorize) an indicator dye such as methylene blue and resazurin decreases with an increased population of organisms. Can relate time to approximate numbers.
5. **Tests for abnormal (mastitic) milk.** Here one is detecting tissue cells from the cow in the milk which result from **mastitis** which is defined as the reaction of milk-secreting tissue to injury produced by bacteria, chemicals or physical force.

#### I. **Presence of foreign chemicals in milk.**

1. **Antibiotics.** From purposeful treatment of mastitis or indiscriminate use not based on careful assessment of need. Antibiotics can cause allergies in consumers. Also, the use of antibiotics leaves antibiotic-resistant microbial cells to continue unaffected while the antibiotic-sensitive cells are inhibited or killed. Problem is minimized by using antibiotics only when appropriate. Let withdraw from animal before milk is taken.
2. **Pesticides of the chlorinated hydrocarbon type.** Pesticides in milk can be traced to treatment of dairy cattle and barns and also ingestion of treated feeds. They accumulate in the fat and tissues and can cause weight loss and other toxic effects in the cow which can be passed along in milk. Problem is minimized by using pesticides only as really needed and allow time between withdrawal of pesticide from animal and availability of milk – but this may take too long.