

MICROBIOLOGY OF THERMALLY PROCESSED COMMERCIALY STERILE AND SHELF-STABLE MEAT AND POULTRY PRODUCTS

To understand the microbiology of thermally processed commercially sterile and shelf-stable meat and poultry products you must first be familiar with the microbiology of meat and poultry, as well as some basics of processed food microbiology in general. In this section you will learn about microorganisms of significance in thermally processed commercially sterile and shelf-stable products, the sources of these microorganisms, the conditions affecting their growth and inactivation, and the specific microbiology of acidified and low-acid “canned” (commercially sterile) foods. You will learn more about the specific microbiology of other shelf-stable meat and poultry products such as dry and acidified/fermented products in the section on the Microbiology of Shelf-Stable Dried Meats.

The objectives of this section are for you to be able to:

1. Recognize pathogens of concern for thermally processed commercially sterile and shelf-stable products and distinguish them from spoilage organisms.
2. Identify conditions affecting microbial growth and related control methods.
3. Identify causes of canned food spoilage.

Introduction to the Microbiology of Food Processing

All raw foods normally contain microorganisms that will eventually cause spoilage unless they are controlled or destroyed. Food preservation is a competition between the human species and microorganisms – we attempt to preserve the food, the microorganisms attempt to destroy it (by breaking down the food for its own consumption) in order to survive. Many of the thousands of microorganisms that have been discovered and identified perform some useful function. Without microorganisms, we would not have some of the tasty foods we enjoy, such as breads, cheese, wine, beer, sauerkraut and other fermented foods, including many sausages. Vanilla, olives, tea and chocolate are other products that include a microbial fermentation step. In addition, these microorganisms are needed to make products useful to industry and medicine, such as enzymes, antibiotics, glycerol and other alcohols. Still other types of microorganisms have the ability to break down organic matter and return it to the earth in a recycling process to form food for plants, which in turn provide food for animals. Without such microorganisms, the earth would accumulate dead animals, leaves and other non-decayed matter – all of which would have eliminated life on earth long ago.

However, it is also true that most diseases of humans, animals or plants are caused by specific microorganisms. The microorganisms that can cause illness are known as pathogens. The pathogen, or the substances it produces, must invade the human, animal or plant body to cause illness. Fortunately, comparatively few of the tens of thousands of known microorganisms are harmful to humans. While many diseases can be transmitted from person to person or from animals to humans, only a few can be transmitted through foods. Although it is becoming recognized that the vast majority of cases of food borne illnesses are caused by viruses such as hepatitis A and noroviruses (67% of all cases of food borne illness, according to one analysis), bacterial agents, such as *Salmonella* and *Campylobacter*, are most frequently identified as the cause of illness because we have a much greater understanding of how to isolate and identify them.

Significant Microorganisms in Food Processing

There are many different ways to classify and group microorganisms, including groupings based on microscopic appearance, the materials they can use as foods, the byproducts resulting from the breakdown of these foods, their tolerance to oxygen, their growth temperatures, and their resistance to such destructive agents as heat and chemicals. The microorganisms of primary concern to the food processor are generally molds, yeasts and bacteria, because they can grow in the food and cause spoilage. Processors are also concerned about viruses and parasites in foods, although they are not capable of growing in the food and do not cause spoilage. Bacteria, viruses and parasites are of particular concern because a number of them cause illness. A brief review of the significant groups of microorganisms in food processing follows.

► Molds

Molds exhibit some of the characteristics of the higher plants. They are multiple cell organisms forming tubular filaments. Molds demonstrate branching and reproduce by means of fruiting bodies, called spores, which are borne in or on aerial structures. Their mycelia, or intertwined filaments, may resemble roots. They are many times larger than bacteria and somewhat longer than yeasts.

Molds are widely distributed in nature, both in the soil and in the dust carried by air. Under suitable conditions of moisture, air and temperature, molds will grow on almost any food. The black or green discoloration that appears on moldy bread is familiar evidence of such growth. Molds are also able to survive on a wide variety of substances not normally thought suitable for the support of life. These include concentrated solutions of some acids and water containing minute quantities of certain salts, as well as on building structures. Molds grow readily on the walls and ceilings of buildings where there is high humidity and

considerable moisture condensation. Mold growth can even occur in refrigerators, because molds are much more tolerant to cold than to heat. Molds can grow at reduced water activities (a_w) and can be a problem in improperly processed dry and semi-dry fermented products, as discussed later.

Molds are capable of consuming acids, thereby raising the pH of products. Their growth in foods has, on very rare occasions (and never in meat or poultry products), removed the acid conditions that inhibit growth of *Clostridium botulinum*, a food borne pathogen discussed later in this section.

Most molds have little heat resistance and cannot survive the thermal processes for low-acid canned foods. Some molds produce a type of spore (ascospore) that is more resistant to heat, but these spores are much less resistant than the spores that are the target of processes for low-acid and acidified canned foods; these heat-resistant molds have not caused problems in meat and poultry products. Therefore, molds are present in canned meat and poultry products only as a result of gross under-processing or as a post-processing contaminant. Since molds must have oxygen to grow, only slight growth can occur unless the food container has an opening to the outside environment.

Mold growth in thermally processed commercially sterile and shelf-stable foods has to date not been shown to present a public health problem. In fact, mold is used in the ripening process of some sausages, as will be discussed in other sections.

► Yeasts

Another microorganism of importance to food preservation/spoilage is yeast. Yeasts are single cell, microscopic living bodies, usually egg-shaped. They are smaller than molds, but larger than bacteria. Their greatest thickness is about 1/2,000 of an inch. Yeasts reproduce mainly by budding. A small bud forms on the parent yeast cell and gradually enlarges and breaks off into another yeast cell. A few varieties reproduce by forming spores within a special cell; later these spores may form new yeast cells.

Yeasts are widely found in nature and are particularly associated with liquid foods containing sugars and acids. They are quite adaptive to adverse conditions such as acidity and dehydration. Like molds, yeasts are more tolerant to cold than to heat. Compared to bacterial spores, yeasts and their spores possess little resistance to heat. Most yeast forms are destroyed on heating to 170°F (77°C). Spoilage may result from the presence of yeast in canned food, but if this happens, gross under-processing or leakage must be suspected. Usually the growth of yeasts results in the production of alcohol and large

amounts of carbon dioxide gas, which swells the container.

Yeast growth in processed foods does not present a public health problem.

► Viruses

Virus particles are so small they cannot be seen by the standard light microscopes used in laboratories – a special electron microscope is needed to see these microorganisms. A virus particle is composed of either RNA or DNA enclosed in a coat of protein, sometimes with an outer envelope containing lipids (fats). (Deoxyribonucleic acid (DNA) is a nucleic acid which carries genetic instructions for the biological development of all cellular forms of life and many viruses. Ribonucleic acid (RNA) transmits genetic information from DNA to proteins, and carries the genetic instructions for many viruses.) Viruses lack the enzymes and other components needed to replicate. Thus, viruses cannot multiply in food – they can only replicate themselves in suitable living host cells. Viruses transmitted by food are produced in the human body and shed in the feces. Of particular concern for foods are the hepatitis viruses and noroviruses. These latter viruses have been mentioned in many news stories in recent years for causing large outbreaks of vomiting illnesses at large gatherings and on cruise ships. Viruses get into food through contaminated water and infected food handlers with poor hygienic practices.

Viruses are not heat resistant, with most having resistance similar to non-spore forming bacteria (see below). Hepatitis A virus is somewhat more resistant, but is still inactivated at 185°F (85°C). Avian influenza virus, which can infect chickens, turkeys, pheasants, quail, ducks, geese, and guinea fowl, as well as a wide variety of other birds, has been known to infect humans, but it is not transmitted through foods, nor is exotic Newcastle disease virus, which also causes a highly contagious poultry disease. Heating to at least to 161.6°F (72°C) internal temperature is considered adequate to inactivate both these viruses.

The Human Immunodeficiency Virus (HIV) which causes the disease AIDS (Acquired Immune Deficiency Syndrome) is a severe public health problem. AIDS has never been shown to be transmitted by food or drink. Individuals who are known to be infected with the virus can handle food safely if they observe basic sanitation precautions for food handling and take care to avoid injury when preparing food. As with any food handler, should an injury occur, food contaminated with blood should be discarded for aesthetic as well as safety reasons. Employees should be restricted from handling food if they have evidence of infection or illness that would otherwise require that they not handle food.

Viruses are not a concern in thermally processed commercially sterile and shelf-stable meat and poultry products.

► Parasites

The parasites of concern in the production of meat and poultry products include worms and protozoa. Some of them are large enough to be seen with the naked eye, whereas others are microscopic. Parasites cannot multiply in food, only in a host cell, and they are not heat resistant.

Parasitic worms of public health importance are the beef and pork tapeworms (*Taenia saginata* and *Taenia solium*, respectively) and the roundworm that causes trichinosis (*Trichinella spiralis*, also referred to as trichinae) found in pork.

These small cysticerci (referred to as *Cysticercus cellulosae*) are approximately 6-18mm wide by 4 - 6mm in length when found in the muscles or subcutaneous tissues (the normal sites for the larvae of this parasite). The cysticerci may however be found in other tissues, such as those of the central nervous system, where they may grow much larger up to several cm in diameter.

Muscle and organs of animals with severe tapeworm infection are usually visually detected by government inspection personnel or by plant employees through evidence of the immature stages (larval stage in a cyst known as a cysticercus) of tapeworms, which are 6- 18 mm wide by 4 – 6 mm in length when found in the muscles. Such product cannot be further processed for human consumption. When the cysts are less severe or evident, infected meat may enter the human food chain, however illness will not occur if meat is properly cooked. Humans consuming undercooked meat infected with these tapeworms become ill with taeniasis generally after the mature stages of the tapeworms, which develop from the cysticercus, invade the intestinal tract. Most cases of infection with adult worms are without symptoms. Some persons may experience abdominal pain, weight loss, digestive disturbances, and possible intestinal obstruction. Taeniasis may last many years without medical treatment. However, people can get a more serious illness called cysticercosis by consuming food or water contaminated with the eggs of *T. solium* (pork tapeworm). Worm eggs hatch and the larvae then migrate to various parts of the body and form cysts (cysticerci). This can be a serious or fatal disease if it involves organs such as the central nervous system, heart, or eyes. Symptoms may vary depending on the organ or organ system involved. For example, an individual with cysticercosis involving the central nervous system (neurocysticercosis) may exhibit neurological symptoms such as psychiatric problems or epileptic seizures. Death is common.

Trichinella spiralis is an intestinal worm that produces larvae that migrate to and encyst in muscles of a number of animals, particularly swine. Humans consuming infected pork that is undercooked get ill from the cysts, which then live in the muscles of the human hosts. The first symptoms are nausea, diarrhea, vomiting, fever, and abdominal pain, followed by headaches, eye swelling, aching joints and muscles, weakness, and itchy skin. In severe

infections, persons may experience difficulty with coordination and have heart and breathing problems. Death may occur in severe cases.

Parasitic protozoa of concern in meat processing include *Cryptosporidium parvum* and *Toxoplasma gondii*. *Cryptosporidium* is typically transmitted to humans from fecal material of animals, primarily cattle, via contaminated water or occasionally, food. The organism is destroyed by boiling water. *Toxoplasma gondii* is carried by cats but can infect many warm-blooded animals. A form known as the oocyst is shed and can sporulate and survive in soil and other environments for extended times; the sporulated oocyst is infectious to all warm-blooded hosts. When ingested, the sporulated oocysts go through several forms, eventually forming cysts in tissue such as muscle. These cysts are infective if ingested. *Toxoplasma* can cross the placenta and affect the fetus, resulting in blindness and more serious effects in the brain.

Parasites are readily destroyed at cooking temperature and are not a major concern in thermally processed commercially sterile meat and poultry products since they are subjected to temperatures well in excess of what is needed to destroy parasites. Parasites are a concern with respect to shelf-stable products that are not cooked. For example, trichinae are a concern with respect to shelf-stable products, such as dried sausages, containing pork. We'll discuss this further in the module for the microbiology of shelf-stable dried meats.

► Bacteria

Bacteria are the most important and troublesome of all the microorganisms for the food processor. Bacteria are single-celled living bodies so small that individually they can be seen only with the aid of a microscope. They are among the smallest living creatures known. The cells of bacteria vary in length from 1/25,000 to 1/1,000 of an inch. The number of these tiny microorganisms that could be placed on the head of a pin would equal the population of New York City! Viewed with a microscope, bacteria appear in several shapes or forms, but are primarily either round in shape (called "cocci") or rod-shaped (called "rods").

Reproduction of bacterial cells

Bacteria reproduce by division, which microbiologists call fission. When a bacterial cell is ready to divide, the cell material gradually increases until its volume is almost doubled. The cocci shapes become oval while rod shapes stretch to nearly twice their length. The cell then constricts in the middle. This constriction deepens until the cell contents are held in two distinct compartments separated by a wall. These two compartments finally separate to form two new

cells, which are duplicates of the former cell and each other. Since the reproduction of bacteria increases the numbers, it is often referred to as “growth.”

Experiments conducted to determine the growth rate of bacteria under favorable conditions have found that each cell divides, on the average, about every 20 or 30 minutes. At this rate of cell division, each single cell will produce four cells at the end of the first hour. At the end of two hours, each cell will have produced 16 new cells. After 15 hours, each parent cell will have produced 1,000,000,000 (one billion) cells identical to the original. For example, if there were 75,000 bacteria per square inch on a conveyor belt, by the end of one hour there could be 300,000 bacteria per square inch of that belt. At the end of a three-hour shift, the bacteria count per square inch of belt surface could be 4,800,000.

Bacterial growth becomes limited without a constant supply of available fresh food. Also, large numbers of bacteria result in an accumulation of substances that are byproducts of bacterial growth and that also inhibit growth. With cessation of growth due to pollution of their environment, the cells may die. However, if the microorganism is a type that forms resistant but dormant spores, these cells can remain alive under conditions that kill other cells.

Sporeforming and non-sporeforming bacteria

Bacteria can be divided into two groups based on their ability or inability to form spores. Practically all of the round-shaped bacteria (cocci) and many of the rod-shaped bacteria cannot form spores; thus they are classified as non-sporeformers. However, a number of the rod-shaped bacteria have the ability to produce a spore within the cell (endospore). Spores are a dormant stage in the normal growth cycle of these organisms. They have the ability to survive a wide range of unfavorable conditions. The primary function of most spores is to ensure the survival of the organism through periods of environmental stress. Spores have been compared to plant seeds because they will germinate and grow when conditions are suitable. The major sporeforming bacteria are species of *Clostridium* and *Bacillus*. Cells of non-sporeformers and the cells of sporeformers that have not formed spores are referred to as “vegetative cells.” These cells generally have little resistance to heat, drying and other unfavorable conditions.

When formed in yeasts and molds, spores represent reproductive bodies, but bacterial spores are a resting stage in the growth cycle of these organisms. When a bacterial spore germinates, it is simply the same organism continuing its growth process.

Resistance of spores to the environment

In general, bacterial spores are extremely resistant to heat, cold and chemical agents. Some bacterial spores can survive in boiling water – 212°F (100°C) – for more than 16 hours. The same organisms in the vegetative state and the non-sporeforming bacteria will not survive heating in boiling water.

As a general rule, spores that successfully resist heat are also highly resistant to destruction by chemicals. There are bacterial spores that can survive more than three hours in sanitizing solutions normally used in a food processing plant. On the other hand, vegetative cells (non-sporeforming bacteria and the vegetative cell form of sporeformers) are readily destroyed by these sanitizing agents. The purpose of sanitizing is not to sterilize surfaces (to remove all bacteria), so the survival of spores under appropriate sanitation practices is not a concern – they will be present in low numbers and will be inactivated or controlled in the final product.

Bacterial hazards of concern in meat and poultry

Of the microbiological hazards of concern in meat and poultry, the most important are bacteria. Illness from meat and poultry is primarily caused by bacterial pathogens. The pathogens that are most likely to be found in livestock (cattle, sheep, and swine) and poultry (chicken and turkey) include *Salmonella*, *Campylobacter*, and *Listeria monocytogenes*. *Listeria monocytogenes* also is widespread in the environment. *Escherichia coli* is also found in livestock and poultry, but most strains are not pathogenic; the pathogenic *E. coli* of primary concern is known as *E. coli* O157:H7 and is found in beef. (Although the organism has occasionally been found in chickens and pigs, it has not been known to cause illness from those animals.) *Yersinia enterocolitica* is a pathogen most commonly associated with pork; only certain serotypes (strains) are pathogenic. *Clostridium perfringens* can also be found in meat and poultry; the spores may survive cooking and grow to high numbers in foods due to temperature abuse. *Clostridium botulinum* is rare in meats. When present, it is there in very low numbers (estimates are 0.1 spore to 7 spores per kg meat). *Bacillus cereus* is another sporeformer of concern in meat and poultry products, especially those containing spices, which is a common source of the spores.

All of these pathogens have been implicated in food borne disease outbreaks associated with the consumption of meat and poultry products in which these hazards were not properly controlled. Proper cooking or thermal processing, fermentation, cooling, and storage of food can destroy and/or prevent growth of these bacteria.

Sources of Microorganisms

Raw materials and ingredients are the primary sources of microorganisms that must be addressed in the production of thermally processed commercially sterile and shelf-stable products. Although muscle tissue is generally considered to be sterile, raw meat and poultry become contaminated during slaughter and further processing. The ultimate source for pathogens in raw meat and poultry is apparently-healthy animals that may shed these bacteria in their feces. While dressing the carcasses during the slaughter process, these bacteria may be transferred from the hide, skin, feathers, gastrointestinal tract and other offal to the carcass, causing contamination. This is also a major source of spoilage microorganisms.

Soil or water can be a common source of food borne microorganisms and spores. Vegetables become contaminated during production, with those that grow close to or through the soil usually having high numbers of bacteria and bacterial spores, including spores of *C. botulinum*. Contaminated water used for irrigation has also been a source of pathogens on vegetables. Dried herbs and spices can be a primary source of sporeformers, since the spores will survive for extended times in the dehydrated product. Soy and milk protein ingredients can also be sources of spores.

Contamination can also come from the processing environment. Utensils such as knives used in slaughter and fabrication, workers hands and gloves, equipment, and occasionally aerosols with dust and other particles carrying microorganisms can all contribute to the microbial load of products. Contaminants may be present on containers and other packaging materials, although this is generally not a likely source of pathogens. Proper sanitation of the environment and protecting containers from environmental contamination can prevent these from being major sources of contamination such that they will negatively impact thermally processed commercially sterile and shelf-stable products.

Conditions Affecting Microbial Growth

The following information focuses on bacteria, since we are most concerned with bacterial pathogens; however, much of it is applicable to yeasts and molds as well. It is not applicable to parasites and viruses, since they do not grow in food.

► Nutrient Requirements

A suitable food supply is the most important condition affecting growth of bacteria. Every living cell requires certain nutrients to multiply. These include solutions of sugars or other carbohydrates, proteins and small amounts of other materials such as phosphates, chlorides and calcium. If the food supply is removed, bacteria will not multiply.

► Moisture Requirements

The concentration of moisture and its availability in a food (referred to as water activity, or a_w) are important factors to prevent microbial growth. The bacterial cell has no mouth, and therefore its food must be in a soluble form to enter the cell through the cell wall. Without sufficient available moisture, the inflow of food and the outflow of food residues and cell body fluids would be impossible. Later we will discuss how bacterial growth can be prevented by controlling the amount of moisture available to the bacteria.

► Oxygen Requirements

Some bacteria – called aerobes – require free oxygen in order to survive. For others, called anaerobes, the reverse is the case – the smallest quantity of free oxygen prevents their growth. The majority of bacteria – called facultative anaerobes – are neither strict aerobes nor strict anaerobes, but can tolerate to some degree either the presence or absence of oxygen.

► pH Requirements

The term pH designates the acidity or alkalinity of an aqueous solution. Scientifically, pH is the negative logarithm of the hydrogen ion concentration. The pH scale ranges from 0 to 14, with pH 7 being neutral. Numbers smaller than 7 indicate an increase in hydrogen ion (more acid) and numbers greater than 7 indicate a decrease in hydrogen ion concentration (more basic, or alkaline). All bacteria have an optimum (most favorable) pH range for growth (generally around neutral pH), as well as a minimum below which the organism will not grow (and where the organism may die) and a maximum above which it cannot grow. The pH of foods can be adjusted to help control microbial growth, as will be described later.

► Temperature Requirements

As with pH, all bacteria have an optimum temperature range for growth. Temperatures below and above the optimum for each group adversely affect the growth of the organism; all bacteria have a minimum and a maximum temperature below or above which the organism cannot grow. Bacterial groups bear names that indicate their relationships to temperature – psychrophile, psychrotroph, mesophile, thermophile.

The psychrophilic and psychrotrophic group

The terms psychrophile and psychrotroph are sometimes used interchangeably, but the groups are distinguished by their optimum growth temperatures and temperature ranges. Both psychrophiles and psychrotrophs grow over the temperature range of subzero to 68°F (20°C). True psychrophilic bacteria (“psychro” for “cold,” “phile” for “loving”) have an optimum temperature of 59°F (15°C) and cannot grow above 77°F (25°C). Psychrotrophic bacteria generally grow best at around 77°F (25°C), or even mesophilic temperatures (see below), but can grow slowly in or on food at refrigerator temperatures (around 40°F (4°C)). These organisms are primarily responsible for spoilage of refrigerated foods. *L. monocytogenes* and some strains of *C. botulinum* (*C. botulinum* type E and non-proteolytic strains of type B and F) are considered to be psychrotrophs. None of these bacteria – except perhaps the strains of *C. botulinum* – is of concern to low-acid or acidified canned foods.

The mesophilic group

Mesophilic bacteria grow best at temperatures of 86°F to 104°F (30°C to 40°C) (the normal range of warehouse temperatures, depending on geographic locations), although some mesophiles grow well at higher temperatures such as 116°F (46.7°C). All of the bacteria that affect food safety grow within this mesophilic temperature range, although some may be considered psychrotrophic as well. The sporeforming organism *C. botulinum* is a member of this group, although some strains are considered psychrotrophs (see above).

The thermophilic group

Thermophiles (“thermo” for heat, “phile” for loving) are bacteria that grow at high temperatures. Thermophilic bacteria are found in soil, manure, compost piles, and even hot springs. Many are sporeforming bacteria and are divided into two groups based on the temperature at which the spores will germinate and grow. If the spores will not germinate and grow below 122°F (50°C), the bacteria are

called obligate thermophiles, i.e., the high growth temperature is an absolute requirement. If growth occurs at thermophilic temperatures of 122° to 150°F (50°C to 66°C) and at lower temperatures – e.g., about 100°F (38°C) – the bacteria are called facultative thermophiles, meaning they have the ability to grow at both temperature ranges.

Some of the obligate thermophiles can grow at temperatures up to 170°F (77°C). Laboratory tests have indicated that the spores of these bacteria are so heat-resistant that they can survive for more than 60 minutes at temperatures of 250°F (121°C). Thermophilic bacteria are not pathogenic and do not produce toxins during spoilage of foods; therefore, they do not affect food safety.

► Interaction of Factors

The level of a single growth-limiting factor to inhibit a microorganism is usually determined under conditions under which other factors that could influence growth are optimal. When other factors are not optimal, the organism will not be able to grow at the minimum or maximum level of another factor. For example, when the water activity is lower, the pH range at which an organism can grow is more limited. When the pH is lower, the water activity that limits growth will be higher. The presence of preservatives can affect the pH at which an organism grows; growth may take longer at lower temperatures when preservatives are present. With that in mind, let's look at some of the control methods for microorganisms in foods.

Control Methods for Microorganisms

► Control of Bacteria by Temperature

Bacterial growth can be controlled by keeping food at temperatures below the minimum or above the maximum for the organism to grow. Thus, refrigeration, freezing and hot holding can be used to control growth. However, the most effective use of temperature to control microorganisms is to kill them with heat. The amount of heat needed to inactivate the microorganisms of concern will be dependent on the specific microorganism (the species and whether or not it is in the spore form), the number of microorganisms to be inactivated, and the food product in which the microorganism is heated (and factors of the food such as its pH, a_w , the presence of preservatives such as nitrite).

The thermal resistance of microorganisms is generally expressed using D- and z-values. The D-value is the time in minutes at a constant temperature to destroy 90% (or 1 log) of the organism present; the z-value is the number of degrees between a 10-fold change (1 log cycle) in an organism's resistance. For

example, if an organism has a D-value of 5 minutes at 140°F and a z-value of 10°F, then the D-value at 130°F will be 50 minutes and the D-value at 150°F will be 0.5 minutes. If there are <100,000 microorganisms present in a sample, then a process that delivered a 5-D reduction (also called a 5-log reduction) to the sample would result in <1 microorganism remaining. We will refer to this log-reduction concept again in the section on thermal processing of commercially sterile products and in the section on the microbiology of shelf-stable dried meats.

In general, low-acid commercially sterile products will require high temperatures that are achieved by processing under pressure to inactivate the organisms of concern. Acidified meat and poultry products can be processed at lower temperatures, since only vegetative cells must be inactivated; the pH prevents germination and outgrowth of the spores. In canned cured products, the presence of nitrite and salt reduces the amount of heat needed to achieve commercial sterility (heat kills the vegetative cells of pathogens; salt and nitrite prevent germination and outgrowth of spores). In most canned products it is not the pathogen of concern that determines the amount of heat needed but rather the spoilage microorganisms, which are more heat resistant than the pathogens. If these organisms are not inactivated, the product will not be shelf-stable. In most instances, this provides a wide margin of safety with respect to survival of pathogens in the product.

► Control of Bacteria by pH

As noted above, microorganisms can be controlled by reducing the pH below the minimum for growth of the organism. An “acidified low acid product” is defined by FSIS as a canned product which has been formulated or treated so that every component of the finished product has a pH of 4.6 or lower within 24 hours after the completion of the thermal process unless data are available from the establishment’s processing authority demonstrating that a longer time period is safe. Proper acidification is necessary to prevent the growth of *C. botulinum*. Unlike processes for low-acid foods that destroy *C. botulinum* spores, processes for acidified foods depend upon the pH of the food to prevent this organism from growing. The final equilibrium pH of an acidified food must be 4.6 or lower to prevent growth of *C. botulinum*. However, since some microorganisms can cause illness at very low levels (e.g., *Escherichia coli* O157:H7), preventing growth alone will not provide a safe product. Reduced pH can be combined with other factors to control the pathogens of concern. For example, a reduced pH, combined with a mild heat treatment, is used to achieve commercial sterility in acidified meat and poultry products such as pasta sauces containing meat. The role of pH in conjunction with other factors in the production of acidified/fermented meat and poultry products will be covered in the sections on Principles of Preservation of Shelf-Stable Dried Meat Products and the

Microbiology of Shelf-Stable Dried Meats. The pH values for representative canned meat and poultry products are shown in Table 1.

Table 1 – pH values of representative canned meat and poultry products

Food	pH
Beans with wieners	5.7
Beef chili	5.6
Beef stew	5.4-5.9
Chicken and dumplings	6.4
Chorizos	5.2
Corned beef	6.2
Corned beef hash	5.0-5.7
Ham	6.0-6.5
Spaghetti and meatballs	5.0
Spaghetti sauce with beef	4.2
Sloppy Joe	4.4
Vienna sausage	6.2-6.5

Acidification procedures

To produce products with a pH of 4.6 or less, acidification must be properly carried out. While there are several methods to obtain properly acidified foods, one commonly used with meat products is direct batch acidification. Ingredients are mixed in a kettle, and acid or, more commonly an acid food, is added directly to the batch. (An elevated temperature may improve the rate of acid penetration into solid particles.) The pH of the batch is checked before the material is sent from the batch kettle to the filler. If the particle size is small enough, the product pH will be below 4.6 at this point. With larger particles, the product may not reach the desired pH until later; however a pH taken at this point can be used to determine that the acidification process is under control. Another method that is used for acidification of meat products is the addition of acidified brine, such as with pickled pigs' feet. Other methods will be discussed in the section on Principles of Thermal Processing.

Determination of pH

The most important factor in the production of acidified foods is the timely attainment and maintenance of a pH level that will inhibit the growth of *C. botulinum* spores. To achieve this goal, it is necessary to measure pH.

Although pH can be measured using colorimetric methods (dye solutions and pH paper) or by titratable acidity, the recommended method for determining pH is the electrometric method using a pH meter. The pH meter measures the electrical potential developed between a glass and a reference electrode when they are immersed in a solution. USDA currently requires the electrometric method to be used any time pH is specified as a critical factor for a scheduled process.

The sensing elements used with pH meters are called electrodes. Combination electrodes contain both a glass and reference electrode in a single probe. They come in a number of different sizes and conformations, which increases the applications of this type of electrode. For example, flat-surface electrodes are useful for measuring the surface pH of a solid sample; long, thin electrodes may be inserted in tubes for measuring pH of small sample volumes or inserted into the process stream for continuous monitoring of pH. Unbreakable electrodes should be used in food processing plants to minimize the chances of contaminating food. For best results, pH meters should be operated in accordance with manufacturer's instructions; the manufacturer's recommendations should be followed for care and maintenance of all pH electrodes.

Once the unit has been turned on and allowed to warm up, the meter should be properly standardized (calibrated) using two buffers to cover the pH range of interest, such as one at pH 4.0 and the other at pH 7.0. The meter should be standardized (1) before any food pH measurements are taken and (2) at least once an hour following that. More frequent standardization and cleaning may be necessary with some products that contain oil, grease or fats.

The sample to be tested must be properly prepared, which is dependent on the type of product to be evaluated. Homogeneous products, such as sauces, require little preparation. Products that consist of solid and liquid components should have the solid components tested separately to determine if they have been properly acidified. One means of sample preparation is to transfer a portion of the solid component to a screen, rinse it with a small volume of distilled water (10-20 ml), and thoroughly blend before taking a pH. Sufficient tests must be made to ensure that the finished equilibrium pH of the product is not higher than 4.6. These tests indicate whether the acidification process is sufficient to bring the product to the appropriate pH.

Electrodes should be rinsed between samples and after use. The purpose of rinsing is to prevent cross-contamination between samples that could result in errors in pH values for products. Rinsing with distilled water is recommended. However, if enough sample is available, rinsing with a portion of the next sample to be measured and throwing away the rinse solution is the best way to prevent cross-contamination. If distilled water is used, the water should be blotted – not

wiped – off the electrodes. If the electrode is rinsed with the next sample, this step is not necessary. Electrodes should not be wiped, because wiping could implant a charge on the electrode causing it to drift. Oil and grease from samples may coat or clog elements; therefore, electrodes should be cleaned with ethyl ether or acetone in accordance with the manufacturer's instructions, and the instrument should be re-standardized frequently. If the primary use is to test high fat/oil products, special electrodes are available.

► Control of Bacteria by Water Activity (a_w)

For thousands of years people have dried fruits, meats and vegetables as a method of preservation. It was also discovered that the addition of sugar would allow preservation of foods such as in the production of candies and jellies. Salt preservation of meat and fish has been extensively practiced over the ages.

As late as 1940, food microbiologists thought that the percentage of water in a food product controlled microbial growth, but gradually they learned that it is the availability of the water that is the most important factor influencing growth. The measure of the availability of water in a food is made by determining the water activity. Water activity is usually designated with the symbol " a_w ."

When substances are dissolved, there is substantial reaction between the substance and the water. A number of the molecules of the water are bound by the molecules of the substances dissolved. All of the substances dissolved in the water reduce the number of unattached water molecules and, in this way, reduce the amount of water available for microbial growth. The extent to which the water activity is lowered depends primarily on the total concentration of all dissolved substances. Thus, if some ingredient – such as sugar, salt, etc. – is added to food, it competes with the bacteria for available water. The water-binding capacity of a particular dissolved ingredient influences the amount of water left for the growth of bacteria.

Meat or poultry containing products with a water activity of 0.85 or less are not covered by the USDA canning regulations (9 CFR 318 Subpart G (meat) and 381 Subpart X (poultry)) even if they are in hermetically sealed containers.

The a_w is the ratio of the vapor pressure of a substance to the vapor pressure of pure water, and is equal to the equilibrium relative humidity divided by 100. Thus, a_w is a fraction between 0 and 1.00, with the a_w of pure water being 1.00.

A measurement of a_w on a food provides information as to which types of microorganisms are most likely to cause spoilage and how close the a_w is to the safety limits. Most bacteria, yeasts and molds will grow above a water activity of 0.95 (See Table 2), and most foods have a water activity above this level.

Spores of *C. botulinum* are generally inhibited at an a_w of about 0.93 or less. (For more information on the effect of a_w on *C. botulinum*, see the section on salt under Control by Chemicals below.) Therefore if the amount of water available to spores is decreased to a point where they are inhibited and mild heat treatment is applied to destroy the vegetative cells, we have a method of preservation for products whose quality is sensitive to high heat.

Table 2 – Minimum a_w requirements for microorganism growth

Microorganism	Minimum a_w For Growth
Most molds (e.g., <i>Aspergillus</i>)	0.75 ¹
Most yeasts	0.88 ²
<i>C. botulinum</i> ³	0.93
<i>Staphylococcus aureus</i> ⁴	0.85
<i>Salmonella</i>	0.94
<i>Listeria monocytogenes</i>	0.92

¹ some strains – 0.61

² some strains – 0.62

³ Proteolytic strains, 10% NaCl

⁴ Minimum for toxin production is higher.

Examples of foods preserved with mild heat and reduced a_w are some cheese spreads, peanut butter, syrups, jams and jellies, and many meat products. The water activity of some common foods is shown in Table 3.

Table 3 – Water activity of some common foods

Food	a_w
Perishable and canned foods (including meats, vegetables, fish and milk)	0.95-1.00
Liverwurst	0.96
Some cheese spreads	0.95
Some cheeses and cured meats	0.91-0.95
Chorizos	0.92
Many fermented sausages	0.87-0.91
Semi-moist pet food	0.83
Salami	0.82
Chocolate syrups	0.75-0.83
Jams	0.75-0.80
Peanut butter – 15% total moisture	0.70
Jerky	≤0.80

It is apparent that as far as *C. botulinum* is concerned, a water activity of 0.85 provides a large margin of safety. Studies with this organism show that an accurate water activity of 0.93 plus pasteurization will give commercial sterility. However, some questions exist about the precision or accuracy of the instruments and methods used to determine water activity and about some factors that control water activity. Therefore, if water activity between 0.90 and 0.93 plus pasteurization is used to control commercial sterility, data must be obtained and records kept showing that the process yields commercial sterility.

Methods for determining a_w

Several methods exist for determining the water activity of a food. One commonly used method is an electric hygrometer with a sensor to measure equilibrium relative humidity (ERH). As noted above, the equilibrium relative humidity above the food in a closed container divided by 100 is a measure of the available moisture – the water activity. The instrument was actually devised by weathermen, and the sensors are the same as those used to measure relative humidity in air. A dew point instrument is also commonly used to measure a_w . This instrument measures the temperature at which condensation occurs on a cooled mirror in the headspace of the sample chamber. The a_w is computed by converting sample and mirror temperatures to vapor pressures and calculating the ratio, which is the a_w .

In determining the a_w using an electric hygrometer, 30-90 minutes may be required for the water vapor (relative humidity) to reach equilibrium in the headspace above the food in the closed container. A dew point instrument is usually much faster – generally only 5 minutes. Generally the formulation of the product to give the required a_w is predetermined and very accurately controlled at the time of processing. For products that have a reduced a_w due to reduction in moisture (e.g., through a drying process), following proper procedures can be critical. Determination of a_w in a laboratory is often used to verify that the formulation and other steps that reduced product moisture were correctly carried out and the appropriate a_w was achieved. Samples of the final product should be checked as frequently as necessary to ensure that the appropriate water activity is being achieved.

► Control of Bacteria by Chemicals

Chemicals (often called preservatives, antimicrobial compounds, or antimicrobials) may be added to foods to inhibit microbial growth or to kill microorganisms. However, at normal levels of use (which must be approved by regulatory agencies such as FDA and FSIS), most chemicals cause inhibition rather than inactivation. Acids and their salts (e.g., lactic acid, sodium lactate),

nitrites, some phosphates, and sodium chloride (salt) are common chemicals added to meat and poultry. In order to produce a commercially sterile or shelf-stable product, chemicals are usually combined with other factors such as heat or reduced a_w .

Salt

For example, salt has been used to preserve meat products (i.e., salt-cured meats). Salt, which lowers the a_w , is often supplemented with other ingredients, such as nitrites, that aid in spoilage prevention. In all cases the salt is necessary to inhibit the growth of sporeforming bacteria, such as *C. botulinum*, and only enough heat is applied to kill the non-heat resistant vegetative cells. Strains of *C. botulinum* that grow in a suitable food containing 7 percent salt are known. For example, toxin was produced in experimentally produced turkey frankfurters with an a_w of 0.956 (7% NaCl) in 12 days at 27°C (81°F). The growth of these strains, however, is inhibited at a concentration of 10 percent, which is equivalent to a water activity of 0.935, when all other conditions are optimum. If conditions are not optimum for growth (e.g., low pH or temperature) then less NaCl is required to inhibit growth. For example, growth of *C. botulinum* may occur at an a_w of 0.96 (6.5% NaCl) at pH 7.0, but if the pH is reduced to 5.3, growth will be inhibited at an a_w of 0.97 (5% NaCl). The actual salt content of a meat product is not as important in inhibiting *C. botulinum* as the brine concentration (percent of salt in the aqueous portion of the meat). Toxin production is inhibited at a brine level exceeding 9.0%.

Nitrite

When direct addition of nitrite was approved for meats in 1925, it was believed that the sole function was for color development. However, within a few years scientific studies began to demonstrate the antimicrobial effects of this compound. Numerous studies now document the efficacy of nitrite in inhibiting growth and toxin production by *C. botulinum* in meat systems. However, studies also determined that there was little or no effect of nitrite on bacterial growth at or above neutral pH. In spite of large amounts of research, there is still not a complete understanding of how nitrite controls *C. botulinum* in meat products. Nevertheless, it is now recognized that nitrite inhibition is due to a combination of factors, not nitrite alone.

► Control of Bacteria by Combinations of Factors

As has been noted above for chemicals, combinations of inhibitory factors that individually are insufficient to control microorganisms can often be effective. This has sometimes been referred to as the hurdles concept – if enough hurdles or

barriers are included, bacteria will not be able to overcome the hurdles and grow. Commercially sterile, canned cured meats are preserved by thermal destruction of vegetative cells of microorganisms, partial destruction of microbial spores and inhibition of the surviving spores by the effects of salt, nitrite, and possibly other additives such as ascorbate/isoascorbate.

The hurdle approach is used for many fermented meat products – curing chemicals such as nitrite and salt, reduced a_w due to drying, reduced pH due to fermentation, and, in some cases, mild heat processes result in a safe and shelf-stable product. This will be covered in much more depth in the sections on shelf-stable products.

Workshop: Microbiology of Commercially Sterile and Shelf-Stable Products

The following questions are multiple-choice questions. Circle the answer(s) you believe to be correct; some questions have more than one answer.

1. Microorganisms that can grow in food and cause spoilage include _____.
 - a. viruses
 - b. bacteria
 - c. molds
 - d. yeasts
 - e. parasites

2. A psychrotrophic non-sporeforming pathogen that can grow at refrigerator temperatures is _____.
 - a. *Clostridium botulinum*
 - b. *Bacillus cereus*
 - c. *Clostridium perfringens*
 - d. *Listeria monocytogenes*

3. Controlling microorganisms in foods can be achieved by _____.
 - a. temperature
 - b. pH
 - c. acidification
 - d. water activity

4. The pH established to provide safety with respect to *C. botulinum* is _____.
 - a. 4.6
 - b. 4.7
 - c. 4.8
 - d. 4.9

5. Spores of *C. botulinum* are generally inhibited at an a_w of about ____ or less.
 - a. 0.98
 - b. 0.85
 - c. 0.93

6. Reducing the water activity of a food product to 0.85 would have the best potential for inhibiting _____ .
 - a. bacteria
 - b. mold
 - c. yeast

7. Bacteria that can survive adverse conditions caused by heat, cold and chemical agents are _____ .
 - a. psychrotrophic bacteria
 - b. facultative bacteria
 - c. sporeforming bacteria

8. Acidified low-acid foods are products with a pH less than or equal to _____.
 - a. 4.6
 - b. 4.2
 - c. 3.8

9. When low water activity is used to preserve a food, the most important factor controlling microbial growth is
 - a. the amount of available water in the product.
 - b. the total water content.
 - c. a scheduled process approved by a processing authority with supporting data.

10. Thermophiles are bacteria that grow best
 - a. at temperatures of about 80°F to 98°F (27°C to 37°C).
 - b. at temperatures of about 122°F to 150°F (50°C to 66°C).
 - c. in acid media.

The Microbiology of Low-Acid Canned Foods

► *Clostridium botulinum*

The pathogen of primary concern for low-acid foods in hermetically sealed containers is not among those typically associated with illness from other meat and poultry products, such as *Salmonella* – it is the sporeforming bacterium *Clostridium botulinum*. The term “*clostridium*” indicates an organism that is able to grow in the absence of air or oxygen and is a sporeformer. The term “*botulinum*” comes from the Latin word “*botulus*,” meaning sausage, because the organism was first isolated from a sausage that had produced the illness now called “botulism.”

Clostridium botulinum (*C. botulinum*) is of great concern to home and commercial canners because (1) when it grows it can produce a potent toxin, (2) it can be isolated from soil or water practically everywhere in the world, (3) it is the pathogen with the greatest heat resistance due to its ability to produce heat resistant spores, and (4) canning foods provides an anaerobic environment favorable to growth of the organism if it has not been destroyed by the process. As noted before, the ability to form spores enables *C. botulinum* to survive a wide range of unfavorable conditions, such as heat and chemicals. The spores survive many heat processes that kill other pathogens of concern in meat and poultry. In fact, certain types of *C. botulinum* spores are able to survive five to 10 hours in boiling water. (As you will learn later, processes to kill *C. botulinum* are on the order of 250°F for 3 minutes, compared to, for example, 157°F for 15 seconds to kill *Salmonella* in certain cooked meat and poultry products.) Oxygen is excluded from sealed containers, thus providing the anaerobic environment for spores to germinate, become vegetative cells, multiply and produce toxin when the foods are stored at temperatures that allow growth. It is important to recognize that it is not the spore that produces the toxin, but the vegetative cell. If spores are present but prevented from forming vegetative cells, as in acidified foods, toxin will not be produced.

Certain strains of *C. botulinum* are called putrefactive because this term describes the odor produced during their growth. These strains require proteins to grow (they are proteolytic) and they grow best at temperatures between 86°F (30°C) and 98°F (37°C), although growth can occur at any temperature between 50°F (10°C) and 104°F (40°C). Other strains, which are non-proteolytic, are more dependent on carbohydrates, such as sugars and starch, for growth and do not produce putrefactive odors. Some of these strains are associated with marine environments; they grow best at 64-77°F (18-25°C) and have a minimum growth temperature of 38°F (3.3°C). Their spores will not withstand heating to 212°F (100°C).

Because *C. botulinum* spores are found everywhere, any raw food may be contaminated with them (although, as was noted before, the organism is rare in meats, and at low levels – 0.1 to 7 spores/kg – when present in meats). However, it is only when the vegetative form of the organism grows in a food that the toxin or poison is produced. Although the spores are heat resistant, the toxin is not. The toxin can be inactivated by boiling temperatures – 212°F (100°C).

Heat processes for low-acid canned meat and poultry are designed, at a minimum, to produce a product that is safe with respect to *C. botulinum*. Microbial inhibitors and pH can impact the processes needed to inactivate *C. botulinum*. Inclusion of sodium nitrite and sodium chloride in meat and poultry products (e.g., commercially-sterile, canned cured meats) can lower the thermal process required to produce a commercially sterile product that is stable at room temperature. For example, processes equivalent to 0.4-0.6 minutes at 250°F (121°C) are common for commercially sterile, cured luncheon meats containing ~ 150 ppm ingoing nitrite and 5.0-5.5% brine strength. These processes may range from 0.1 to 1.5 minutes depending on nitrite, brine strength and other factors (compared to processes of 3.0 minutes for uncured products). (It should be noted that there are both perishable and commercially sterile cured meat and poultry products packed in hermetically sealed containers. If the products are not commercially sterile, they are not subject to the canning regulations.) Reducing the pH can also lower the process required, even when the product is not acidified to a pH that produces an acidified food (see below).

► Other Microorganisms Important in Low-Acid Canned Foods

Although heat processes for thermally processed commercially sterile foods are designed to destroy any microorganisms of public health significance, we are also concerned about other microorganisms that could grow in the product under normal storage conditions and result in adulterated product. As will be discussed when we get to the principles of thermal processing, processes designed to ensure commercial sterility of low-acid canned foods usually target *Clostridium sporogenes* or similar organisms (putrefactive anaerobes). Because spores of *C. sporogenes* have higher heat resistance than those of *C. botulinum*, processes targeted to destroy spores of *C. sporogenes* will also destroy *C. botulinum* spores.

Note that commercial sterility is not the same as absolute sterility – there may be viable microorganisms present in commercially sterile products. Spores of thermophilic bacteria such as *Bacillus stearothermophilus* or *Clostridium thermosaccharolyticum*, if present, can survive processes that achieve commercial sterility. However, these organisms, which are not harmful to humans, cannot grow under normal conditions of storage. If product is properly cooled and stored, generally the spores are not exposed to the high

temperatures they require for germination and growth. Although canned foods are not generally processed to inactivate thermophiles, one exception to this is hot-vended products. Products that will be held hot in vending machines will be exposed to temperatures at which thermophiles can grow and will receive higher processes to ensure thermophilic spoilage does not occur.

The Microbiology of Acidified Canned Foods

Meat and poultry products to which acids or acid products have been added to reduce the pH to 4.6 or below are called acidified foods. Spores of *C. botulinum* do not germinate and grow out in foods that have a pH below 4.8; a pH of 4.6 is set for acidified foods to provide a margin of safety. Heat processes for acidified foods target organisms other than *C. botulinum*, since the pH prevents its growth. The safety of acidified meat and poultry products is achieved by controlling the pH to 4.6 or less and applying a mild heat treatment to inactivate the vegetative cells of pathogens (e.g., *Salmonella*, *E. coli* O157:H7 and *C. botulinum*). However, achieving commercial sterility of these products requires treatments that inactivate yeast, mold and bacteria, including some sporeformers, that could grow in the product. The type of organism to be destroyed by the process will depend on the pH of the product. If the pH is below 4.2, only vegetative cells need be considered; however, if the pH is 4.2-4.6, processes may need to take into account sporeforming organisms such as *Bacillus coagulans* and the butyric acid anaerobes (e.g., *Clostridium pasteurianum* and *Clostridium butyricum*). For more information on these organisms see the later section on Spoilage by Acid-Tolerant Sporeformers.

The Microbiology of Shelf-Stable, Canned, Cured Meats

The safety and stability of shelf-stable, canned, cured meat products (e.g., canned hams and luncheon meats) are related primarily to the concentrations of salt (sodium chloride, NaCl) and nitrite and the thermal process acting synergistically. These products only receive mild heat treatments, relying on NaCl and nitrite to inhibit surviving sporeformers, in particular *C. botulinum*. The inherent low levels of spores in these products also contribute to ensuring safety and shelf stability.

Canned Food Spoilage

► Indications of Microbial Spoilage

Most bacteria produce gas when allowed to grow in a canned food. This gas causes the containers to swell. Exceptions are the flat-sour sporeforming

organisms, which produce acid and sour the food without producing gas, leaving the container ends flat. These organisms are an economic but not a public health problem.

The most obvious indicator of spoilage in processed food is a swollen container – bulging at one or both ends. This implies that the food has possibly undergone spoilage by the action of gas-forming bacteria. Consumers are advised by public health officials, food trade associations and regulatory agencies not to use any container with a bulged end or ends, even though the swelling may be of non-microbial origin.

The appearance and odor of the container contents may also indicate spoilage. If the product is broken down and mushy, or if a normally clear brine or syrup is cloudy, spoilage may be suspected. In jars, a white deposit may sometimes be seen on the bottom or on pieces of food. This is not always a sign of spoilage, as starch sometimes precipitates from certain foods.

Bacterial decomposition of thermally processed product may result from one of six causes:

1. Incipient spoilage – growth of bacteria before processing;
2. Contamination after processing – leakage of bacteria into the container;
3. Inadequate heat processing;
4. Growth of thermophilic bacteria in the processed food;
5. Spoilage by acid-tolerant sporeforming bacteria; and
6. Spoilage due to improper curing.

The last two causes of spoilage are specific to products with reduced pH and to cured products, respectively. In addition, there can be non-microbial causes of spoilage.

► Incipient Spoilage (Spoilage Before Processing)

Processed food is sometimes held too long between filling or closing the containers and thermal processing. Such delays may result in growth of bacteria normally present in the food and the initiation of spoilage before the retorting process. This type of spoilage is referred to as “incipient spoilage.” The microorganisms that grow will be killed by the process; with sufficient time, sporeformers may germinate and form vegetative cells that will be killed. Typically incipient spoilage manifests itself as low or no vacuum in the container and a slight change in pH of the product. Generally no viable microorganisms are recovered in subculture media. Although the product presents no risk to public health, if there is sufficient growth, the product may be considered to be adulterated (e.g., if the product characteristics are changed). The degree of

spoilage depends on the specific product and the time and temperature conditions during the delay. For example, if the product has been heat treated (e.g., cooked) prior to container filling, there may be low levels of bacteria present such that holding product for several hours may result in bacterial increases that are not significant. Products that contain inhibitors, those that are held at lower temperatures (e.g., below 70°F) or those that are filled hot (above microbial growth temperatures) may also demonstrate only limited bacterial growth for several hours.

The loss of vacuum that can result from growth of microorganisms in sealed containers held too long prior to retorting may lead to extensive internal pressures in the containers during retorting. The build-up of internal pressure strains the container seams or seals and increases the potential of leaker spoilage. Some containers may actually buckle or rupture, rendering them unusable. Steps should be taken to avoid such a delay before retorting the containers.

► Contamination After Processing (Leakage)

Leaker spoilage – or post-processing contamination with microorganisms – usually shows up rapidly as swollen containers. It may take several weeks until all spoilage has ceased. If many swells are present, a small percentage of flat, spoiled containers (flat-sours) may be expected, and normal appearing cans should be examined with this potential in mind. Leakage is generally due to inadequately formed seams, container damage or cooling water contaminated with large numbers of microorganisms.

While theoretically leakage could result in post-processing contamination with pathogens, this is unlikely in plants operating under good manufacturing practices. Incidents of typhoid fever resulted from consumption of meat products (imported into the UK from Argentina in the 1950s and 1960s) that were contaminated when cans were cooled using river water contaminated with sewage. Recontamination with *C. botulinum* has occurred with seafood, but not meat products. Based on existing reports of food borne disease for which package defects are alleged or proven to have contributed to the problem, the probability of post-process contamination with organisms of public health significance is extremely low. In fact, it was estimated in 1984 that the probability of botulism from container leakage is about one chance in every 260 billion cans of food consumed (or one potential botulism incident about every 9 years). The report concluded that this probability compares well to the risk associated with the minimum acceptable thermal process for low-acid canned foods.

► Inadequate Heat Processing

The term “inadequate heat processing” (also referred to as under-processing) is almost self-explanatory. Heat processes for thermally processed food are designed to destroy any microorganisms of public health, as well as non-health, significance that could grow in the product under normal storage conditions. In many instances, the product receives sufficient heat to inactivate pathogenic sporeformers such as *C. botulinum*, with the more heat resistant spoilage organisms surviving and spoiling the product. However, if the heat process is inadequate to destroy *C. botulinum*, the situation can be very hazardous, since botulinum toxin could be produced and, if the product is consumed, cause botulism in the consumer.

Botulism is the disease caused by ingestion of the toxin produced when *C. botulinum* grows in a food. Symptoms include, dry mouth, dizziness, and weakness, generally within 12-48 hours after consumption of the toxin. Nausea and vomiting may also occur. Neurologic symptoms follow, including blurred or double vision, inability to swallow, difficulty in speech, descending weakness of skeletal muscles and, ultimately, respiratory paralysis. Untreated, this can lead to death.

A heat process may be inadequate for a variety of reasons, including but not limited to the following:

1. If the time and/or temperature (or its equivalent) specified in the scheduled heat process for the particular product in the particular size of container is not used.
2. If the scheduled heat process was not established properly.
3. If the scheduled heat process is not properly applied because of some mechanical or personnel failure.
4. If one or more of the scheduled process critical factors is not met.
5. If the formulation is changed such that the critical factors change.

► Thermophilic Spoilage

Generally, the higher the temperature at which a sporeforming organism can grow, the greater the heat resistance of its spores will be. Thus, the spores of thermophilic bacteria usually have a greater heat resistance than the spores of mesophilic bacteria. The spores of thermophilic bacteria are so resistant to heat that heat processes designed to kill the mesophilic bacteria may not be adequate to destroy thermophilic bacteria. In order to prevent thermophilic spoilage, the product must be properly cooled, preferably below 105°F (41°C), after thermal processing and held below 95°F (35°C). Thermophiles may grow in equipment

that contacts food if the temperature is within their growth range. Consequently, product should always be held at 170°F (77°C) or above or at room temperature or below to prevent the growth of thermophiles.

For meat and poultry products containing ingredients known to be a source of thermophiles (e.g., such as sugar, starch and/or spices) where thermophilic spoilage may be a problem, prudent processors will use ingredients that the supplier guarantees are free of thermophilic bacteria or that meet specifications for thermophiles for canning processes. This is particularly important if the product is to be hot-vended. This is the responsibility of the establishment, as it is a quality issue, not one of safety.

► Spoilage by Acid-Tolerant Sporeformers

As noted above, acidified foods (those products with a pH 4.6 or below) do not require a severe thermal process to assure product safety. Therefore a variety of acid-tolerant sporeformers may survive the process. A thermal process scheduled for acidified foods is designed to inactivate a certain level of these sporeformers. Their survival is typically a result of excessive pre-processing contamination. Sometimes underprocessing, either due to inadequate processing or process deviations, may also result in survival of these acid-tolerant sporeformers. The organisms of spoilage significance are butyric-acid producing anaerobes and aciduric flat sour sporeformers.

The butyric-acid producing anaerobes, such as *Clostridium butyricum* and *Clostridium pasteurianum*, are mesophilic sporeformers that produce butyric acid as well as carbon dioxide and hydrogen. The spores are capable of germination and growth at pH values as low as 4.2-4.4 and consequently are of spoilage significance in acidified foods, particularly if the pH is above 4.2. In products where the pH is not low enough, spoilage by butyric acid anaerobes may be controlled by either further acidification of the product or by increasing the thermal process. Growth of these organisms in foods is characterized by a butyric odor and the production of large quantities of gas. Occasionally strains will be encountered that can grow at a pH lower than 4.2. If these strains are present in high numbers, the heat process may be inadequate and spoilage may occur.

Aciduric “flat sours” are facultative anaerobic sporeformers that seldom produce gas in spoiled products. The ends of spoiled cans remain flat; hence the term “flat sour.” Spoiled products have an off flavor that has been described as “medicinal” or “phenolic.” These organisms have caused spoilage in acid foods such as tomato products (by *Bacillus coagulans*) and could cause problems in meat products with tomato sauces if the sauces are prepared from fresh tomatoes. (The problem is unlikely if the tomato ingredients are previously

processed to inactivate these organisms, as with commercially sterile products). It may be necessary to ensure that the thermal process is adequate to inactivate an expected number of spores, which can be determined through bacteriological surveys. Pinpointing the ingredient that is contributing the most to the total spore load may prove beneficial in process control. For example, proper handling of vegetables prior to use, such as washing and culling, may help to reduce spore loads.

Most food processing operations do not provide anaerobic conditions; therefore, heavy build-up of acid-tolerant anaerobic sporeformers seldom occurs. This is one reason why “dead ends” (“dead legs”) must be avoided in processing lines. However, when this does occur, under-sterilization spoilage can result because of the heavy load of spores in the product. The spoilage pattern within the affected lots is often spotty and scattered, more typical of post-processing spoilage that is due to container leakage, than of the pattern expected from sporeformers that survive a thermal process. Thermal processing records and other processing parameters usually give no indication of any irregularities. In most cases, the problem can be identified only by investigation at the factory, which includes a bacteriological survey, plus the absence of demonstrable leakage and package defects in the spoiled containers.

► Spoilage Due to Improper Curing

As was noted before, canned cured meat and poultry products are made commercially sterile by the interrelationship of salt, nitrite, heat and low levels of spores. Spoilage due to underprocessing in canned cured meats is rare, and is usually the result of improper curing rather than inadequate heating. The heat processes for canned cured meat and poultry products are not designed to inactivate mesophilic sporeformers, as their outgrowth will be inhibited by salt and nitrite. Reduced levels of salt or nitrite can result in spoilage, as the heat treatment may be inadequate for product containing these lower levels.

► Non-microbial Spoilage

Spoilage in canned foods can sometimes occur as the result of container deterioration; this may at times result from chemical interactions of the food and the container. To avoid this type of spoilage, the packer must be aware of the impact of container, processing, and product chemistry variables on the corrosion shelf life of the container. Container deterioration may result in swollen containers resulting from the production of hydrogen (“hydrogen swells”) or in leaking containers due to pinholes or cracks.

Non-microbial spoilage rarely results in a health hazard. However, in high acid products where detinning of containers has occurred, there have been instances of illness due to high levels of tin (>200 ppm), which can cause acute toxicity (nausea, vomiting, cramps and diarrhea). This has not been a problem in meat and poultry products.

There are four main types of corrosion inside plain tinplate containers: normal corrosion, rapid detinning, pitting corrosion and cosmetic corrosion. The normal corrosion process is slow, even detinning of the tinplate surface. The canned product will have a minimum shelf life of about 2 years. Rapid detinning involves rapid tin dissolution of the tinned surface and pitting corrosion involves rapid dissolution of iron, with or without tin dissolving. These two forms of corrosion lead to either hydrogen swells or perforations. Cosmetic corrosion problems, such as sulfide staining, are not of public health significance, but consumers may reject the pack for aesthetic reasons.

Meat and poultry products are usually packed in enameled cans rather than plain tinplate. Corrosion inside enameled cans is localized at fractures in the coating where the plate is exposed to the product. There are five main manifestations of corrosion in coated cans – 1) normal corrosion, 2) pitting corrosion, 3) under-enamel corrosion and enamel flaking, 4) stress corrosion cracking and 5) sulfide black corrosion. The normal corrosion process involves iron dissolution from small pores, and the corrosion shelf life will exceed 18-24 months. Pitting corrosion involves rapid iron dissolution from the container walls at coating defects. Under-enamel corrosion is detinning or staining through the coating at areas where the coating has lost adhesion. Stress corrosion cracking involves a reaction between the container and stress inducing components in the product. Cracks through the container have been observed in as little as 4 months. Sulfide black corrosion involved rapid iron dissolution through the coating with black deposits forming about 24 hours after processing. Sulfide black discoloration is a type of cosmetic corrosion that is objectionable to the consumer.

External corrosion involves rusting, detinning or staining of the outside walls of the container. It rarely leads to perforations from the outside in. Filiform corrosion involves tunneling through the walls of the can. It occurs at the scratch defects in the coating on the outside of cans. It sometimes leads to pinholes in the container.

► **Determining the Cause of Spoilage**

Analysis of spoilage of commercially sterile canned food products requires expertise to conduct the analysis and to appropriately interpret the results. The isolation of microorganisms from spoiled product does not necessarily mean they

are the cause of spoilage. For example, if acidified foods are cultured in neutral laboratory media, microorganisms that are present in the product but are inhibited by the pH may grow out. These are of no significance since they cannot grow in the product due to its pH. Likewise, if other inhibitors are present in product, culturing in laboratory media may dilute the inhibitors such that microorganisms which are viable in the product but prevented from growing can grow in the media. Again, it is unlikely that these organisms are significant with respect to spoilage of the product. Thus, microbiological examination of products intended to be commercially sterile requires a trained analyst following accepted procedures such as those outlined in the USDA/FSIS Microbiology Laboratory Guidebook for examination of heat processed, hermetically sealed (canned) meat and poultry products or the equivalent.

Workshop: Microbiology of Canned Products

The following questions are multiple-choice questions. Circle the answer(s) you believe to be correct; some questions have more than one answer.

1. Commercial sterility refers to applying a heat treatment to a low-acid canned food designed to destroy pathogens and spoilage organisms capable of growing in the product at normal storage conditions. What is the pathogen of greatest concern in a low-acid canned food?
 - a. *Clostridium botulinum*
 - b. *E. coli* O157:H7
 - c. *Clostridium perfringens*
 - d. *Listeria monocytogenes*

2. Certain types of *Clostridium botulinum* spores
 - a. produce a potent toxin.
 - b. are able to survive for 5 to 10 hours in boiling water.
 - c. produce toxin that cannot be inactivated by boiling at 212°F (100°C).

3. *Clostridium botulinum* is
 - a. an anaerobic, sporeforming bacterium.
 - b. a mesophilic acid-tolerant microorganism.
 - c. a thermophilic microbe.

4. Botulism is an illness that can be caused by
 - a. eating spores of *Clostridium botulinum* or other food spoilage organisms.
 - b. eating vegetative cells of *Clostridium botulinum* which may be found in raw meat and spices.
 - c. eating food in which vegetative cells of *Clostridium botulinum* have grown and produced toxin.

5. Incipient spoilage in canned foods is caused by
 - a. holding closed containers too long before retorting.
 - b. under processing.
 - c. post-process contamination, especially from dirty cooling water.

6. Thermophilic bacterial spores
 - a. are less heat resistant than mesophilic bacterial spores.
 - b. are never present in low-acid canned foods.
 - c. may not be destroyed during the heat process for low-acid foods.

Workshop: Spoilage of Canned Foods Case Study

(**Note:** This is a simplified training example only.)

While walking through the warehouse of Uncle Sam's Canned Goods Company, you and the warehouse supervisor notice several cases of canned beef stew that look wet. The QA manager is called to the warehouse to inspect the problem. Upon opening the cases he finds **several cans that are swollen or leaking**. He pulls out the swollen and leaking cans, along with several of the normal-looking flat cans. He then sends some of the sample cans to Microtesting, Inc., a microbiology laboratory that specializes in analyses of canned food products. You send the remaining samples to the FSIS laboratory in Athens, Georgia. The lot of product is placed on hold pending the laboratory results.

Upon receipt of the samples on Friday afternoon at 4:45pm, the diligent laboratory technician at Microtesting, Inc., begins analysis of the samples. The laboratory sends back preliminary results to the QA manager the following week.

The laboratory results listed below are four possible "what-if" scenarios. Please read the possible scenarios then answer the following questions.

	Microbiological Results	Container Evaluation
Scenario #1	Thermophilic microorganisms were identified.	No container defects were detected.
Scenario #2	Mixed culture of rods and cocci were identified. No sporeformers or heat resistant microorganisms were recovered.	Can exam revealed false seam on packer's end.
Scenario #3	Mesophilic organisms with spores were identified.	No container defects were detected.
Scenario #4	No viable bacteria (sporeformers or non-sporeformers) were recovered.	Hydrogen gas was detected.

► Questions

1. Which of these scenarios, if any, suggests microbiological problems due to potential under-processing? Why?

2. Which of these scenarios, if any, suggest post-process contamination?
Why?

3. Which of the scenarios, if any, is indicative of microbial spoilage that has the potential for an adverse public health consequence?
Why?

4. Which of the scenarios, if any, is indicative of detinning of the can interior?
Why?

5. After receiving the results indicated in Scenario #1, Uncle Sam's decides to sort and remove the swollen and leaking cans from the lot and release the normal cans.

The lot of cans are incubated in an unair-conditioned trailer held at 110-120°F and checked weekly. After 6 weeks there are no additional swollen containers. Since the cause of spoilage was the growth of thermophilic microorganisms, the plant manager believes that it is acceptable to release the flat containers. The plant microbiologist wants to conduct a simple test to determine the pH of random samples before making the decision to release. Why?

6. Is every swollen can an indication of an adverse public health consequence? Why or why not?