MICROBIOLOGY - SHELF-STABLE DRIED MEATS

Dried meat and poultry products can be classified as either fermented or non-fermented. If they are fermented, generally they are dry and semi-dry sausages. Semi-dried meats are simply dried to a different level (15% weight loss during drying, compared to 30% for dried products). Non-fermented dried sausages, and, more commonly, jerky and dried whole muscle meats (such as dried ham), are also shelf-stable.

This section discusses two important aspects of the microbiology of shelf-stable dried meat and poultry products – pathogens and spoilage organisms of concern and starter cultures utilized in fermented dried meat and poultry products.

This module will

1. Review the types of microorganisms associated with these products, including pathogens of concern and microorganisms used to control the pathogens.
2. Distinguish pathogens of concern from spoilage organisms associated with shelf-stable meat products.
3. Describe the role starter cultures play in fermentation.
4. Identify the factors that control the safety of these products.
5. Identify factors that impact the fermentation process.

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

1. Recognize pathogens of concern specific to dry and acidified/fermented products and distinguish them from spoilage organisms.
2. Describe the functions of starter cultures and their use.

Meat Microbiology

Although unaware of the process, early sausage makers knew that once the animal was killed, it was a race between external preservation techniques and the decomposition of the raw meats to decide the ultimate fate of the tissue. We now realize that once the inherent defense mechanisms of the live animal are destroyed, the meat tissue is subjected to rapid decay. The slaughtering process affords extensive contamination of the sterile tissue with primarily Gram-negative microorganisms. This includes enteric bacteria from animal intestines (*Enterobacteriaceae*, including *E. coli* and *Salmonella*), as well as contaminants such as *Pseudomonas* and Gram-positive lactic acid bacteria and staphylococci associated with humans, animals and the environment. As a result, fresh meat spoilage is usually associated with Gram-negative, proteolytic bacteria which literally decompose the protein with the production of offensive odors (putrid, rotten egg) and flavors.

Salting, curing and drying of the fresh meat have proven to be effective means to control the fresh meat microflora and thus preserve the tissue for later consumption. This is possible since the microbiology of salted meats is entirely different from that of fresh meats. The curing salts (sodium chloride, sodium nitrite, sodium nitrate) and subsequent proper handling methods favor the growth of Gram-positive bacteria while inhibiting the proliferation of Gram-negative bacteria. This “microbial inversion,” occurring during the salting and curing process, provided the “accidental” origin of fermentation cultures for all salted meats and fermented sausages.

Once the raw meat was salted and spiced, the mixture was stuffed tightly into sausage casings or skins, which excluded air and held the sausage during aging. The casing also provided a convenient medium to hang the mixture for smoking and drying. Excluding air also prevented mold growth, except on the casing surface. Consequently, a microenvironment was provided for those microorganisms that were not only salt tolerant, but which also could grow in the absence of air. These Gram-positive, fermentative types of microorganisms included the lactic acid bacteria, staphylococci, micrococci, and yeasts.

As the product dried, a fermentation ensued whereby residual meat sugar, or added carbohydrates, was fermented to a variety of end products, including various organic acids, carbon dioxide, alcohols, etc., that contributed to a variety of flavors and textural properties. The primary fermentation product, lactic acid, served to lower the pH and contributed to the stability of these sausages against food-borne pathogens and other undesirable microorganisms. The lower pH also aids in drying since the meat proteins are less able to bind water under acidic conditions (as will be discussed later). Although actually a spoilage condition, the “souring” of the product could be tolerated to a much greater extent than the fresh meat type of proteolytic spoilage, and this served to preserve the meat. If properly controlled, the resulting flavor could make the meat more acceptable to the palate, through a “tangy” sensation in combination with the salt, spices, smoke, and other imparted formulation and processing characteristics. Lactic acid resulting from fermentation has a tendency to enhance the “salty” and “smoky” flavors. Lactic acid is non-toxic to man and moderately pleasant to the palate, but it is inhibitory to most undesirable microorganisms, including most pathogenic microorganisms, which tend to die off in the final product during storage. Similar lactic-acid fermented foods include cheeses, sauerkraut, yogurt and pickles.

►Lactic Acid Bacteria (LAB)

The most common lactic acid microorganisms found in fermented meats are various strains of lactobacilli, leuconostocs, pediococci, and streptococci. *Lactobacillus plantarum* is mostly found in sausages fermented at higher temperatures (86°F, 30°C) and *L. sake* and *L. curvatus* dominate the flora at mild temperatures (68-75°F, 20-24°C) more typical of European processes. Pediococci involved include *P. acidilactici* at higher temperatures (>85°F, 29°C) and *P. pentosaceus* at lower temperatures (<85°F, 29°C). These harmless, beneficial types of microbes are ubiquitous in the environment

and are highly competitive. They are fastidious microorganisms, requiring many nutrients for growth (which meats can provide). They primarily convert various sugars to lactic acid and other end products. They can grow with or without air, but are very rapid acid producers in the absence of air, since the fermentation process is very inefficient for energy production and large quantities of acid must be produced for growth. This anaerobic (no air) environment is the characteristic condition within the internal portion of the sausage. The lactic acid microorganisms are also very salt tolerant and thrive within the typical sausage formulation. Typically, the initial counts of LAB in raw meat mixes, depending upon the raw materials and the environment, are around 1,000-10,000 cells/g. During fermentation, those numbers increase to 10,000,000-100,000,000 cells/g.

Lactic acid type microorganisms are commonly used commercially today as starter cultures to produce a variety of fermented food products, including cheese, yogurt, sour cream, pickles, and olives, as well as dry salami, pepperoni, summer sausage, and fermented meat snacks. They are also used for human and animal nutrition, establishing a beneficial intestinal microflora (i.e., probiotic cultures).

►*Micrococcaceae* and *Staphylococcaceae*

Other types of common microbes in salted meats are members of the *Micrococcaceae* and *Staphylococcaceae* families, including *Kocuria* and *Staphylococcus*, respectively. The predominant types are coagulase-negative staphylococci that are very salt tolerant and can also grow with or without oxygen. By far, the most common strains belong to the species *Staphylococcus carnosus*, *S. xylosus*, and *K. varians*. Previously, many of these strains were classified as *Micrococcus* species. These bacteria generally are harmless and do not represent a microbial hazard. However, *Staphylococcus aureus* is a known food pathogen (as will be discussed later). The *Kocuria* and staphylococci are more salt tolerant than the lactic acid microorganisms, and thus survive and grow in much lower water activity environments, as is the case with highly salted products and as the sausage loses water. Fortunately, these microorganisms are not very acid tolerant and tend to die off at lower pH during and after the fermentation process. They produce catalase, which reduces hydrogen peroxide, and some strains contribute to flavors as a result of lipolytic and proteolytic activity. A characteristic of the micrococci and staphylococci is that they can reduce nitrate (NO_3) to nitrite (NO_2), which provides the active component (nitric oxide, NO) that initiates the typical meat curing reactions. This characteristic was very important in the early days of meat processing before the availability of commercial cures, since the nitrate was found as a natural contaminant in the salt and/or saltpeter (KNO_3) that was added. Without this nitrate reduction to nitrite, the meat will not show the characteristics of cured meat. Since the staphylococci are sensitive to acid, this conversion would occur early in the process (prior to fermentation) before the lactic acid microorganisms became the dominant microflora of the meat mix. With “microbial succession,” the staphylococci would gradually die off as the lactic acid microorganisms produced lactic acid. Today, most processors, particularly in the United States, just add the nitrite directly to the meat mix, thus eliminating the need for nitrate

reduction by these microbial types. Initially, these strains are present at 100-1000 cells per gram, increasing to 1-10 million cells per gram in some fermentations.

►Undesirable Microorganisms

Not all the lactic acid bacteria that can occur in meat are desirable. For example, *L. viridescens* can cause greening of the meat due to the production of hydrogen peroxide. *L. brevis* and *L. mesenteroides* can cause gas production and unacceptable souring. *Brochrothrix* can cause souring, off flavors and odors.

Yeasts and molds can also be present in salted meats and can survive and grow at lower pH. Both prefer to grow under aerobic conditions at the outer edge and external surface of the meat product (although yeast can grow anaerobically). Yeast can result in gas production and fruity flavors. Some molds can attack proteins and produce ammonia, which raises the pH on the sausage surface. Others may be lipolytic, attacking fats, or cellulytic, attacking casings. There is also the potential for production of mycotoxins, although this has not yet been shown to be a problem in sausage production. Although these microorganisms are often undesirable, they can be controlled via fermentation and proper drying.

Many pathogenic microorganisms can also be present in the raw sausage mix, including *S. aureus, E. coli* O157:H7*, Listeria monocytogenes*, *Salmonella*, and clostridia. Fortunately these pathogens also are not very competitive with the lactic acid microorganisms and are inhibited by low pH, nitrite and lower water activity. The parasite *Trichinella spiralis* may also be present in pork.

Staphylococcus aureus

Staphylococcus aureus is associated with mucous membranes (nose and throat) and is commonly found on the skin and hair of healthy humans and animals. It is also present in infected wounds, lesions and boils from both humans and animals. It is easily spread though the air via coughing and sneezing, and can contaminate meat from the animal skin or tissue during slaughter. After slaughter and after cooking, the meat can be contaminated from handling by individuals carrying the organism.

Staphylococcus aureus is not a good competitor with other microorganisms and, thus, usually is not a problem in raw foods. This organism becomes a problem when competitive microbes are removed by cooking or inhibited by high salt levels. In salted meats, where many microorganisms are inhibited, *S. aureus* can flourish without the proper controls. Even though *S. aureus* can grow with or without the presence of air, it prefers to grow aerobically; thus its presence in sausage usually occurs at the product surface and outer 1/8”.

Staphylococcal food poisoning is caused by the consumption of a heat stable enterotoxin produced as a byproduct during the growth of certain strains of *S. aureus*. Toxin production requires considerable growth by the microbe and is normally not present until the total cell numbers reach 1 million per gram of meat. Since the microorganism is readily destroyed by heat but the toxin is heat stable, total counts of *S. aureus* may not indicate if the toxin is present. Proper control is required in the early stages of the sausage production when the pH is high. Proper sanitation and personnel practices reduce product contamination, while temperature control reduces potential growth prior to fermentation. Using a commercial starter culture assures microbial dominance of fermentation strains over any potential pathogens and a controlled reduction of pH to 5.3 to inhibit potential *S. aureus* growth during fermentation. Most companies monitor the rate of pH drop to determine that fermentation is proceeding as expected.

Some companies may test batches for *S. aureus*. This may be done routinely for all products, for certain products known to be more prone to contamination with *S. aureus,* or when a batch does not ferment properly. Monitoring any *S. aureus* numbers should be done immediately after fermentation and before any cooking. If a product has received a heat treatment that could have reduced the number of *S. aureus*, then the lot should be tested for enterotoxin. Thus analyses of final product should be for enterotoxin and not *S. aureus*. All analyzes should be done on the outer 1/8” of the product.

There have been no reported cases of staphylococcal foodborne illness from fermented meats produced in the U.S. for over 20 years. This is due in large part to the widespread used of commercial starter cultures and education of producers by starter culture suppliers and trade associations in best practices for production of fermented meats.

Escherichia coli O157:H7

Escherichia coli O157:H7 is associated with the cheeks, mouth, hide and intestinal tract of animals, particularly cattle, providing opportunity for contamination of the meat at slaughter. The pathogen is shed in the saliva and feces of infected animals, resulting in contamination on the hides of animals that come in contact with the saliva and feces. *E. coli* O157:H7 can be transferred from contaminated hides or the intestines of infected animals during the slaughter process. Although not a good competitor, *E. coli* O157:H7 can survive under refrigerated and frozen conditions. It is acid resistant, and thus it presents a potential problem by its survival in fermented meats.

Even very low numbers of *E coli* O157:H7 are capable of causing infection, thus the microorganism must be completely destroyed during the process. Control principles for *E. coli* O157:H7 include minimizing the presence of the organism in the raw meats and proper fermentation and heating of final product. In most fermented sausages, a combination of low pH and intermediate heat treatment can effectively eliminate high

numbers of *E. coli* O157:H7. For non-heated meat products, reduced water activity (combined with other factors such as salt level, time, and temperature) has been an effective means to reduce numbers of *E. coli* O157:H7.

Following an outbreak of illness from *E. coli* O157:H7 in dry fermented salami, FSIS and industry agreed that processors would validate the manufacturing process for dry and semi-dry fermented sausages to demonstrate an effective 5-log or greater reduction in *E. coli* O157:H7 and prevent recontamination. Ultimately five options were developed -

- Use a heating step in 9 CFR 318.17 or 9 CFR 318.23.
- Apply a validated heat treatment equivalent to at least a 5-log inactivation.
- Hold and test finished products using standard sampling plan protocols (e.g., ICMSF (International Commission on Microbiological Specifications for Foods) lot acceptance criteria; 15 or 30 samples tested, depending on use of product).
- Apply a validated minimum 5-log reduction or process that results in <1 *E. coli* O157:H7/100g (treatments shown to be effective in combination).
- Sample raw ingredients (mix) to demonstrate there is <1 *E. coli* O157:H7/g and apply a 2-log lethality treatment.

A Blue Ribbon Task Force of the National Cattlemen's Beef Association developed option 5 above. The task force also developed several processing procedures that achieved the 5-log reduction, which FSIS considers to be validated processes meeting option 2. These processes involve various combinations of fermentation temperature, pH at the end of fermentation, holding times and temperatures, drying, and cooking.

Salmonella

Salmonella is an enteric microorganism associated with the intestinal tract of many animals and thus is potentially present in most raw meats. *Salmonella* is recognized as a potential problem in salted, dried meats. Illness is usually caused by ingestion of sufficient microorganisms to survive digestion and reproduce in the human intestinal tract.

Fortunately, salmonellae are heat sensitive and easily destroyed with the mild heat treatments for cooking meat. Also, salmonellae are acid sensitive, not surviving well in fermented meats, and are not good competitors, being inhibited by the lactic starter cultures. They are also sensitive to meat curing practices. Salmonellae have not been a problem with fermented sausages if the product is properly fermented using an appropriate starter culture. *Salmonella* not only survives drying but also becomes more heat resistant with drying and is more of an issue in non-fermented dried meats, such as jerky, and whole meat cuts, such as dried hams.

Control mechanisms include proper temperature and sanitation prior to processing, and the use of wet heat early in the heat process to avoid pre-drying. In non-heated meats, the proper use of high salt and proper curing techniques are most effective.

Listeria monocytogenes

Listeria monocytogenes is a common bacterium found in the environment and can be carried by humans and animals. It has been isolated at every level of the meat processing chain, including slaughter and processing plant environments. *L. monocytogenes* can cause an infection after the ingestion of virulent strains. Certain population segments are at high risk of contracting listeriosis, including pregnant women, the elderly and immunocompromised individuals (e.g., transplant patients, persons with cancer), which can result in serious illness and even death. This pathogen is of concern in the production of dried meats, since it is able to grow under both aerobic and anaerobic conditions and can survive dry conditions. It is also salt tolerant and can grow over a wide temperature range (31.3-113°F, -0.4-45°C), which includes refrigeration. It does not grow well in acid conditions, but can survive.

In addition to good sanitation and avoiding cross contamination, *Listeria* can be controlled by a combination of lower pH, high brine concentration, and competitive exclusion (and in some cases bacteriocin production) with lactic acid starter cultures, varying degrees of heat processing, and the drying process.

Trichinella spiralis

Trichinella sprialis (sometimes referred to as trichina, or trichinae in the plural) is a parasitic nematode (roundworm) associated with pork and wild game. It goes through various stages of its life cycle in a single host. Ingestion of cysts in the muscles of infected animals results in release of larvae from the cysts in the intestine; maturation of the larvae into adult worms in the epithelial cells of the small intestine; mating of the worms to produce new larvae; migration of the larvae to the circulatory system, which carries the larvae to muscle where the larvae form cysts. As a result of U.S. regulations, trichinosis from pork is no longer a concern. Recent cases have been attributed to the consumption of improperly cooked wild game, such as bear.

There are a number of effective ways to kill trichinae. These may include heating pork products to a minimum time/temperature combination, freezing for a specified time at a specified temperature, or curing according to specified methods.

Other pathogens

Campylobacter is the most common bacterial cause of diarrheal illness in the U.S. It lives in the intestines of healthy birds and is associated mainly with raw poultry and contaminated water sources. It is a fragile bacterium and is killed with oxygen, freezing,

or drying. Control measures taken for *Salmonella, Listeria*, and *E. coli* O157:H7 will take care of other pathogens such as *Campylobacter* and *Yersinia enterocolitica*, a pathogen associated with pork.

Dried Meat Curing and Microbial Fermentation

Traditionally, dried meat microbiology involves a natural development or "wild" fermentation in which a "microbial succession" occurs. Without a starter culture, initially all types of microorganisms increase. The total microbial count, which is primarily LAB (when starter cultures are used), rises to a high level (between 10^6 and 10^7 CFU/g) and then remains fairly constant. Enterobacteriaceae and psychrotrophs decrease once LAB reach high levels. Micrococcaceae increase more slowly and eventually decrease. As noted previously, Micrococcaceae (Kocuria) and Staphylococcaceae (Staphylococcus) produce nitrate reductases that convert nitrate to nitrite. Lactic acid microorganisms ferment the product and lower pH. The fermentation of the sugars results in lactic acid formation and the acidity of the meat increases.

In whole cuts of meat, the salting process also favors the same types of microorganisms, but the greater amount of surface area and the exposure to oxygen resulted in less fermentation activity and lactic acid production. In these types of dried meats (e.g., prosciutto, basturma), uniform salting over the entire surface is most critical to inhibit pathogens and spoilage microorganisms. Since the internal meat tissue is sterile, the high salt levels applied to the surface retard most microbes as the salt gradually penetrates the tissue, which should be kept cool during penetration/equilibration. These products have higher pH and greater risk of causing illness if the brine content is not sufficient.

Historically, some dry and semi-dry sausage processors would add the salt (and possibly saltpeter), sugars and spices to the meat and hold the resulting meat mix refrigerated for 1- 2 weeks in shallow pans. This process was called "pan curing" or "panning." The salt inhibited Gram-negative bacteria and allowed the lactic acid bacteria, and some micrococci and staphylococci to develop and reduce the naturally occurring nitrate to nitrite. Although rare today, some processors still use a straight "nitrate cure" containing only nitrate – a process that is generally not effective without the presence of the nitrate-reducing microorganisms.

A "mixed cure" of added nitrate and nitrite is very common for dried meats that use a combined starter culture (nitrate-reducing micrococci and LAB) and that are not fully cooked. Nitrate reduction to nitrite provides additional cure color development and the nitrate provides a reservoir for the production of nitrite during the shelf-life of the product.

In the U.S., many processors have converted to a "nitrite cure" containing only added nitrite if they are cooking the product and use a single LAB starter culture.

►Starter Cultures

Use of starter cultures resulted from an understanding of the natural development or "wild" fermentation. Gram-negative bacteria yield to Gram-positive bacteria (micrococci, staphylococci and LAB), resulting in microbial inversion. Micrococci and staphylococci convert nitrates to nitrites and yield to LAB (microbial succession). Fermentation results in lower pH. However, relying on indigenous microorganisms to properly ferment products was a risky business. Thus the "backslop," "back inoculation" or "mother batch" method evolved, which depended on using the batter from a previous batch to inoculate the next one – a potentially uncontrolled starter culture where undesirable, as well as desirable, microorganisms are recycled. However, this method has been successfully used by some processors who have implemented appropriate controls to prevent contamination or loss of the effective fermentation culture. Ultimately this process evolved into controlled development with prepared starter cultures. These cultures resulted in controlled "spoilage" through addition of staphylococci and LAB, if nitrate is added to the batch, or LAB only if nitrite is added. Today, most fermented meat processors either add lactic acid starter cultures and/or harmless staphylococci (mostly outside the U.S.) to the raw meat mix.

The microbial basis for fermentation was first determined about 1900, but the first starter cultures were only patented in the 1940's. Meat starter cultures were first commercialized in 1958, but by 1973 only about 33% of all fermented meat processors in North America used commercial starter cultures. Today, over 95% of all processors of these products use meat starter cultures.

Commercial meat starter cultures are selected microorganisms that have been isolated from the meat, purified, grown to large numbers under controlled conditions, concentrated, and then preserved by freezing or drying prior to use.

Eventually, the level of bacteria in many meat products is the same, whether a culture has been added or not. However, when a bacterial culture is added to meat, the microbial growth is controlled by bacteria with well-known characteristics. The addition of high levels (1-10 million bacterial cells per gram) of appropriate microorganisms such as in an LAB starter culture to the initial fermented sausage mix assures microbial dominance over the potential pathogenic and other undesirable microorganisms that might be present. The lactic acid microbes reduce product pH via fermentation while the staphylococci assure more efficient curing through nitrate/nitrite reduction and oxygen scavenging ability. There are two primary reasons to add such an overpowering population of the desired starter culture. One, this large number instantly becomes the dominant microflora, inhibiting all other microorganisms; and, two, the large number of microbial cells provides a tremendous amount of surface area, which results in high metabolic performance (e.g., desired end products such as lactic acid and/or nitrate reduction to nitrite).

Commercial meat starter cultures are not all the same. They differ in form, strain, purity, activity, and consistency, depending on the manufacturer. The functions of commercial

meat starter cultures are to allow fermentation with acid development and reduced pH, which has preservative effects for safety and shelf-life, and to produce more drying efficiency. Commercial meat starter cultures are also used for flavor development (proteolytic, lipolytic), and for color development and stability (nitrate reduction, oxygen scavenger). There are cultures of molds and yeasts that are used on the surface. Starter cultures can also show antioxidant characteristics.

The commercial cultures used in the U.S. differ from those used internationally. Those that are most common in the U.S. (and the fermentation temperatures used) are *Pediococcus acidilactici* (90-115°F, 32-46°C), *P. pentosaceus* (70-100°F, 21-38°C), *Lactobacillus plantarum* (70-100°F, 21-38°C), and *Staphylococcus carnosus* (formerly classified as *Micrococcus varians or M. halobius*; 70-100°F, 21-38°C). Internationally, the most common are *Staphylococcus carnosus*, *Lactobacillus curvatus*, *L. sake*, *S. xylosus*, *L. plantarum*, *Penicillium* spp. (mold), *P. pentosaceus, P. acidilactici*, and *Debaryomyces* spp. (yeast).

The specific starter cultures used in meat processing and whether a manufacturer uses a single culture or a combination of cultures will depend on the product, process conditions, desired fermentation temperature, final product characteristics desired, and regulatory requirements.

- Traditional fermented sausages – European-style, lower temperature, slower fermentations with enhanced color and flavor development
- Fast fermented products – U.S. style, higher temperature, rapid fermentations with emphasis on acid development
- Flavor and color enhancing cultures – strictly for flavor and color development with less emphasis on acid development
- Surface treatment cultures – mold and yeast cultures for surface appearance and flavor
- Whole muscle cultures – whole muscle products with emphasis on color and bioprotection
- Bioprotection cultures – food safety and shelf-life function
- Probiotic cultures – for nutritional purposes

There are different forms of cultures as well. They can consist of frozen liquid or pellets, dry (freeze-dried) cultures, or frozen “syrup.” Frozen pellet cultures are the same as frozen liquid, but are easier to handle. They are a measurable culture, but they must be kept at -40°F (-40°C) or below before use (culture freezer). They are easily customized for specific culture blends. Dry meat cultures are used more worldwide. They are easy to distribute and are used primarily by smaller processors. Their primary use is for slower fermentations at lower temperatures (70-80°F, 21-27°C). These cultures should be kept frozen prior to use and added directly to sausage mix or diluted in water and then added. Cans of frozen liquid culture have been most common in the U.S., but this is changing. Frozen pellets and dry cultures, which are more common worldwide, are becoming more popular in the U.S.

Regardless of the specific starter culture, detailed product specifications should accompany the cultures. These specifications should include:

- Specific microbial strain
- Function of the starter culture
- Purity of the product, including absence of pathogenic microorganisms and maximum number of "non-type" harmless strains
- Minimum number of specific microbial strain per gram in culture
- Minimum activity of the culture in terms of pH reduction and/or color development

Meat cultures are alive and need to be handled appropriately to ensure they do not lose viability and activity. Proper receipt, storage, and stock rotation according to the manufacturer's instructions are essential to maintain optimal performance.

►Fermentation Process

Fermentation results in an increase in acid along with a concomitant decrease in pH due to the fermentation of sugar (usually dextrose). Acid development is monitored by the drop in pH. This phase of the process has historically been referred to as the "holding," "greening," or "dripping of the product." In all cases, this "fermentation phase" is where the conditions are established to effect the most efficient fermentation of added sugars to lactic acid.

In this phase of the process, it is critical to measure product pH. Product pH is the negative logarithm of the hydrogen ion concentration and is indicative of the acid concentration. The product pH can be affected by many factors, including the "buffering capacity" (the resistance to change in pH) of the meat mix. Being a logarithmic measurement, pH is not linear and may not be directly proportional to the acid concentration. Total acidity is a linear measurement (titration) and is directly indicative of the acid concentration (taste or "tang"), but is more difficult to measure in the processing environment. Generally, measuring pH is sufficient as an indicator of the progress of the fermentation phase.

When measuring pH with the direct probe method (i.e., insertion directly in the meat mix), it is very important to routinely clean the probe of protein and fat residue, both of which can result in a false pH reading. Additionally, the pH reading from a direct probe should be routinely correlated with the standard water dilution method (i.e., meat slurry) to assure accuracy. The water used for the dilution should be distilled water of neutral pH and not contain any buffering agents.

Appropriate fermentation rate and final pH in the meat reflect that starter cultures are viable and have been handled correctly. The fermentation rate depends on meat type and condition: pork, beef, poultry, temperature, percentage of fat and moisture, meat age, dominant microflora, and pH.

A salt level >3.0% slows down growth of the starter culture, but can be overcome with higher temperatures and the use of an appropriate culture. The usual added salt for dried sausages is 3.3%. Fermentation depends on the sugar types and levels. In general, dextrose is universally the most fermented carbohydrate, followed by corn syrup, sucrose, lactose, maltodextrins, starches and other more complicated carbohydrates.

In general, increasing sugar levels up to 1% decreases pH proportionately. In specific fermented meat products (e.g., pepperoni), limiting the added sugar to 0.5-0.75% achieves adequate fermentation with no residual carbohydrate present after fermentation. This prevents the “charring” of the product due to the reaction of reducing sugars with protein (i.e., Maillard reaction) during heating. A lower pH is obtained with increasing temperature at the same sugar level.

Spice types can increase or decrease culture activity. This usually is dependent upon the manganese content of the respective spice. For example, black and white pepper increase fermentation rate while the antimicrobial properties of mustard and garlic inhibit fermentation. The following additives can adversely affect the fermentation rate due to microbial inhibition: curing ingredients, antioxidants, phosphates, smoke, liquid smoke, non-fat dry milk, starch, and soy products.

Obviously, the starter culture type, activity, handling, and age will affect the culture’s performance. It is critical that the optimum starter culture be used for the desired meat product and process.

The casing diameter will also affect the fermentation rate and final pH by affecting heat penetration and moisture migration in, and then out. Generally, large diameter products ferment slower due to slower heat penetration, but they result in a lower final pH for the same reason and/or slower drying.

As expected, the specific process affects the fermentation rate and final pH. In general, the higher the fermentation process temperature and humidity, the faster the fermentation; however, the fermentation temperature should be at the optimum growth temperature of the added starter culture. Added smoke will sometimes inhibit fermentation at the product surface, but the significance will depend upon the product diameter. The final pH will be affected by the added carbohydrate, the heating temperature after fermentation, and the drying conditions.

Fermentation temperature affects the time to reach pH 5.3, which is critical for control of *S. aureus* in fermented products. At 64.4°F (18°C), it takes 36 hours; at 75.2°F (24°C), it takes 19 hours; at 82.4°F (28°C), it takes 13 hours; and at 100.4°F (38°C), it takes only 7 hours to reach pH 5.3. The importance of reaching pH 5.3 within the appropriate degree-hours will be covered under “Principles of Preservation of Shelf-Stable Dried Meat Products.”

►Fermentation Failures

If the fermentation to a desired pH does not occur within the normal time period, it can be due to a variety of reasons. Generally, if a fermentation problem does occur, it is the result of a total lack of fermentation (the pH does not change from its initial value of 5.6-6.0), a partial fermentation (the pH drops slightly to 5.4-5.6), and/or inconsistent fermentation (variation in fermentation activity from piece to piece or from location to location). The following lists some typical causes for inadequate and/or inconsistent meat fermentations.

No fermentation

- No starter culture added
- No fermentable sugars added
- Excessive salt added
- Starter culture mishandled
    - thawed and refrozen
    - culture premixed with cure, salt, chemicals
- Antimicrobial agents added to formulation
- Antibiotic residues in raw meat

Inconsistent or partial fermentation

- Inadequate distribution of starter culture
- Insufficient fermentable sugars added
- Inconsistent internal product temperature and/or processing temperature and/or humidity
- Reduced fermentative activity of the starter culture
    - out of code product/improper stock rotation
    - mishandled culture
- Antimicrobial agents added to formulation
- Antibiotic residues in raw meat

►New Developments

The sausage manufacturing industry continues to develop new processes and products. High speed fermenting cultures are being developed that result in 4-8 hour processes, compared to multiple-day fermentations in the past. Chemical acidulants are being combined with cultures in new processes. Color enhancing cultures, including oxygen/peroxide scavengers, are sometimes added to enhance cure color development and stability. New mold and yeast cultures are being used to affect appearance, flavor, and oxidative stability of finished products.

There are also developments with respect to *L. monocytogenes* control, such as a culture blend that “kills” and inhibits via competitive inhibition, bacteriocin production, and reduced pH. This type of culture would generally be used in non-heat-processed products or after cooking.

►Non-fermented products

For whole muscle products, high salt tolerance starter cultures are being developed to provide consistent flavor, *Listeria* control, and improved color. These are added directly to the brine or dry rub. There is more attention on whole muscle products because they generally are not fermented, and thus a large pH decrease with a lot of acid development is not desirable. Starter cultures are used more often to control nitrate reduction/color, flavor, and *L. monocytogenes* in non-cooked items, and to develop a little acid to enhance drying.

Starter cultures are generally not used in the production of jerky. Microbial hazards include *Salmonella*, *L. monocytogenes*, *S. aureus*, and, for beef and venison jerky, *E. coli* O157:H7. The product is heat treated and derives its stability from its cooking (lethality) process and drying to low water activity. If the product receives inadequate lethality treatment and is insufficiently dried, *S. aureus* is a potential hazard, since it can grow at lower water activities than most pathogens.

►Application of Bioprotective Cultures

Bioprotection is the application of lactic acid bacteria to a product in order to control the indigenous flora (i.e., starter cultures to control microflora), without significantly altering the sensory properties of the product. (Traditional fermentation can also be considered a type of bioprotection.) The LAB improve quality by delaying growth of spoilage bacteria and increase safety by inhibiting and reducing growth of pathogens. In addition to traditionally dried fermented products, bioprotection is being applied to a variety of traditionally non-fermented products including raw sausages, cooked ham, sliced meats, dried meats, etc. The added starter culture always controls the product microflora (competitive exclusion) and retards pathogen growth, but the specific affect on product quality depends upon the specific culture used and the formulation and final characteristic of the product.

For not- fully-cooked products such as raw sausages, fermented sausages and whole muscle products, the cultures can be added to the raw meat mix, to the added curing mix (if dry cultures are used) or the liquid brine. For cooked meat products such as frankfurters and cooked ham, the bioprotective cultures are added after the lethality process via spraying onto the product surface.

►Hurdle Concept

The safety and storage stability of processed meat products depends on a combination of several hurdles. The hurdles concept involves combining sub-inhibitory levels of factors that limit microbial growth in a manner that effectively inhibits the microorganisms of concern. Biological competition can be considered a hurdle. Competitive exclusion involves use of desirable competitive microorganisms to inhibit undesirable microorganisms. End-product metabolites (e.g., bacteriocins, lactic acid) from specific microorganisms can inhibit and/or kill competitors. Inhibition can also occur through fermentation (lowering pH to a level inhibitory to other microorganisms).

Bacteriocins are produced by some bacteria as a defense mechanism against competitive flora. These proteins, produced during growth, specifically attack competitors. Low concentrations are needed to produce an effect. For example bacteriocin-producing starter cultures can provide a more effective reduction in *L. monocytogenes* than a normal starter culture. Bacteriocin-producing cultures may also be effective in production of non-acidified products such as dry hams. Generally competitive exclusion is viewed as a means of controlling growth of another organism; however, use of competitive exclusion cultures that produce bacteriocins can actually reduce the number of the undesirable microorganism, not just control its growth.

Workshop: Microbiology – Shelf-Stable Dried Meats

The following questions are multiple-choice, matching, or True/False. Circle the answers you believe to be correct; some questions have more than one answer.

1. Match the microorganism(s) associated with each product:

   a. *Staphylococcus aureus*
   b. *Salmonella*
   c. *E.coli* O157:H7
   d. *Listeria monocytogenes*
   e. *Trichinella spiralis*

   Beef Pepperoni _____
   Pork Pepperoni _____
   Country Ham _____
   Beef Jerky _____
   Freeze Dried Beef _____
   Dried Bacon Bits _____

2. Identify which of the following organisms are known pathogens associated with meat or poultry products. Place a (P) next to the pathogens.

Enterobacteriaceae	*L. brevis*
Staphylococcus aureus	Yeast
Pseudomonas	*Listeria monocytogenes*
Lactobacillus viridescens	*Trichinella spiralis*
Salmonella	Molds
E. coli O157:H7	*Campylobacter*
Brochrothrix	

3. Commercial meat starter cultures are used to _____

   a. reduce pH.
   b. provide preservative effects.
   c. increase drying efficiency.
   d. control the microflora.
   e. achieve a desired end product.

4. The primary end product(s) produced in the fermentation by starter cultures is (are)

   a. lactic acid.
   b. carbon dioxide.
   c. alcohols.
   d. diacetyl.
   e. pyruvic acid.

5. Adequacy of fermentation is usually measured by

   a. pH or total acidity.
   b. total acidity.
   c. enumerating the total microbial population.
   d. enumerating pathogens.

6. Salt level >3.0% slows down growth of the starter culture, but can be overcome with higher temperatures and the optimum culture.

   True
   False

7. The fermentation process is impacted by increasing the sugar level up to 1%, increasing pH proportionately.

   True
   False

8. Generally a large casing diameter product ferments faster due to a slower heat penetration and results in a lower pH.

   True
   False

9. What types of microorganisms are used in a meat fermentation process to provide desirable characteristics?

   a. Lactic acid bacteria
   b. Staphylococci
   c. Korcuria/micrococci
   d. Yeast

10. Which of the following is primarily associated with the intestinal tract of cattle?

    a. *Staphylococcus aureus*
    b. *Salmonella*
    c. *E. coli* O157:H7
    d. *Listeria monocytogenes*
    e. *Trichinella spiralis*

11. The primary source of this microorganism is the environment, but it is also present in raw meat.

    a. *Staphylococcus aureus*.
    b. *Salmonella*
    c. *E. coli* O157:H7
    d. *Listeria monocytogenes*
    e. *Trichinella spiralis*

12. This microorganism is associated with pork and wild game.

    a. *Staphylococcus aureus*
    b. *Salmonella*
    c. *E. coli* O157:H7
    d. *Listeria monocytogenes*
    e. *Trichinella spiralis*

13. Which of the following is associated with skin and mucous membranes, wounds, lesions, and boils on humans and animals?

    a. *Staphylococcus aureus*
    b. *Salmonella*
    c. *E. coli* O157:H7
    d. *Listeria monocytogenes*
    e. *Trichinella spiralis*

14. Live fermentation cultures are available as:

    a. room temperature cultures.
    b. frozen liquid or pellets.
    c. freeze dried or frozen “syrup”.
    d. dry cultures.