CHR. HANSEN
Improving food & health

back cover

Application & Technology Centers

Denmark
Chr. Hansen A/S
Boege Allé 10-12
DK-2970 Hoersholm
Denmark
Phone: +45 45 74 74 74
Fax: +45 45 74 89 94

USA
Chr. Hansen, Inc.
9015 West Maple Street
Milwaukee, WI 53214
USA
Phone: +1 414 607 5700
Fax: +1 414 607 5959

Brazil
Chr. Hansen Indústria e Comércio Ltda.
Caixa Postal 371
CEP 13276-970 Valinhos, SP
Brazil
Phone +55 19 3881 8300
Fax: +55 19 3881 8253

Germany
Chr. Hansen GmbH
Giessener Strasse 94
D-35415 Pohlheim
Germany
Phone: +49 6403 950 10
Fax: +49 6403 950 130

Italy
Chr. Hansen Italia spa
via Cicerone 2/4
IT-43100 Parma
Italy
Phone: +39 0521 497 211
Fax: +39 0521 497 251

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www.chr-hansen.com

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Bactoferm™ Meat Manual vol. 1

Fermented sausages with Chr. Hansen starter cultures

inside cover

Preface

With this booklet Chr. Hansen describes the advantages of using starter cultures in the production of fermented sausages. The booklet is a supplement to the Bactoferm™ product information sheets and is primarily directed towards customers and distributors.

The intention of the booklet is to give the reader an overview of fermented sausage processing and to answer some of the theoretical and practical questions that may arise. The booklet covers a general description of the production of fermented sausages, the cultures and processing parameters involved, a guideline to application of cultures and a troubleshooting guide.

In addition to the Bactoferm™ Meat Manual Chr. Hansen has created a range of other information material that covers specific topics within sausage fermentation and application of cultures. For further information please feel free to contact the addresses on the back cover. Chr. Hansen's worldwide facilities and the personnel of our Application and Technology Centers are at your disposal with assistance, instructions and guidance for your choice of culture and other needs in relation to your process and products. For more information on Chr. Hansen and our products please visit www.chr-hansen.com or contact meat@chr-hansen.com

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Introduction

Sausage fermentation has always been based on the presence of lactic acid bacteria and *Micrococcaceae* species in the meat and in the environment of the sausage factory. Before starter cultures were available the fermentation relied on the indigenous bacterial flora that was favored by pre-salting, by the mincing and stuffing procedure and by the climatic conditions during maturation. The method was not always successful, though. Sometimes it resulted in an unfermented sausage with too high pH, growth of spoilage bacteria and, in the worst-case, growth of pathogenic bacteria. Also growth of undesirable lactic acid bacteria was sometimes detected, for example lactic acid bacteria with the ability of producing gas (hetero-fermentative strains).

As early as 1919 patents were registered for the use of microorganisms for meat fermentation, but it was not until the beginning of the sixties that the first cultures were placed on the market. As part of the general improvement of hygiene and production methods, the application of starter cultures is now widespread.

Today a great variety of cultures from several manufacturers are available, in most cases including the microorganisms that predominate in the traditional fermented products. Thus, a uniform fermentation with the right homo-fermentative lactic acid bacteria is secured, and the flavor development, color formation and color stability are improved by adding an additional flora of species from the Micrococcaceae family; primarily staphylococci, but also other species. Additionally, prevention of mycotoxin formation in mold-fermented sausages is ensured by application of well-defined *Penicillium* cultures.

This booklet gives a theoretical as well as a practical overview of the subjects of relevance for fermented sausage production. At first, a general description is given on the processing steps involved in the production of dried sausages, followed by a description of the applied microorganisms and their performance with respect to processing parameters. Finally, a guideline is given on how to choose and apply the correct culture for a specific product, succeeded by a troubleshooting guide to the problems that may be encountered. Persons already familiar with the practices and theories behind sausage fermentation are welcomed to jump directly to the guideline chapters.

In order to make this booklet a very practical guide to dried sausage production, it only gives a superficial overview of some of the more theoretical aspects of sausage fermentation. For the more interested reader a list of references to excellent reviews and books is presented in the end of the booklet together with a list of other relevant Chr. Hansen material.

Production of fermented dried sausages

Introduction

The process of fermenting and drying sausages is believed to be one of the oldest techniques for preserving meat. Production started in the Mediterranean countries and was well known in the Roman Empire. Since then the concept was spread all over Europe and later on to other parts of the world.

A fermented dried sausage consists of a mince of raw, comminuted meat, fat and curing agents that is stuffed into casings, fermented and dried. During the fermentation period sugars in the mince are converted into lactic acid by lactic acid bacteria and water is migrating to the surface where it evaporates. Those processes result in a sausage with a lowered pH and a reduced water activity that makes the final sausage stable with long shelf-life even if not being subjected to heat treatment.

Over time sausage manufacture has given rise to a large variety of local, but closely related products. This diversity makes classification difficult. In the United States fermented sausages are divided into two categories, dry and semi-dry sausages, based on the products protein/moisture ratio or water activity - dry sausages have a final a_w below 0.90, semidry an a_w below 0.95 - whereas in Europe fermented sausages are usually divided according to the length of the ripening period. Additionally, there is a differentiation according to the treatment of the sausage exterior which may be untreated, mold covered, smoked, or both.

In this booklet it has been chosen to categorize fermented sausages according to the typical characteristics used by sausage manufacturers in today's industrial production. Thus, in the following chapters fermented sausage production is divided into the north and south European and US style processes and the application of cultures described accordingly. Variations within those three categories will also be touched upon.

The definitions are:

North European style
The mince has no added nitrate and the sausages are fermented at temperatures between 22–26°C (70–80°F) to a pH around 4.5–4.8. The time to pH=5.3 is less than 30 hours (=fast fermentation). The sausages are smoked and the water activity is above 0.90 (=semidry). The production time is less than 3 weeks. Typical products: German Mettwurst, Danish salami.

South European style
The mince has added nitrate or a mixture of nitrite and nitrate. Fermentation temperature is between 18–24°C (60–75°F) and pH does not go below 5.0 at any time. The time to pH=5.3 is longer than 40 hours (=traditional fermentation). The sausages are typically covered with mold and the water activity is below 0.90 (=dry). The production time is 3 weeks or longer. Typical products: French saucisson, Naples salami, Salame Milano, Italian pepperoni, Spanish Chorizo.

US style
The mince has no added nitrate and the sausages are fermented at temperatures above 32°C (90°F) to a pH below 4.8. The time to pH=5.3 is less than 15 hours (=very fast fermentation). The water activity is above 0.90 (=semidry) and the production time 2–3 weeks. The final product is cooked and smoked, but often this process takes place right after fermentation. Typical products: American pepperoni, Summer sausage, Lebanon Bologna.

Production procedure

By and large the production process for fermented sausages is very similar in most countries. The differences in the various national sausage types depend first and foremost on the composition of recipes, the degree of mincing of the meat, and the fermentation and drying procedures as outlined above.

Mince ingredients

The sausage mince basically consists of meat, fat, curing salts, carbohydrates, spices and in modern sausage productions, starter cultures of various kinds. Very often the mince is also added sodium ascorbate and other additives.

Raw materials
The raw materials of meat and fat may derive from all kinds of livestock; national traditions play a decisive role in this matter. Pork is the meat most commonly used, particularly in Southern European and in Far Eastern products. Other meats, principally beef, are used in North America, in Northern Europe and in countries where religious beliefs preclude the consumption of pork. In Germany, fermented sausages usually consist of pork and beef in equal amounts, while Italian, Spanish and Hungarian sausages mostly are manufactured with pork only. In the US turkey and chicken sausages are very common. Mutton and horse meats are frequently used in countries where those meats serve part of a normal diet.

The fatty part of a fermented sausage comprises 25–55%. Pork back fat is most frequently used, but for ethical reasons beef and sheep fat are applied instead in certain products. One example is the Turkish Soudjouk sausage that contains sheep fat, giving a specific mutton flavor of great popularity in Turkey. In general, use of soft fatty tissue is not recommended as it may cause color and flavor defects due to a higher content of unsaturated fatty acids with higher susceptibility to chemical oxidation resulting in rancidity and color stability problems.

Curing salts
The basic curing agents include common salt (NaCl), sodium nitrite ($NaNO_2$) and/or sodium- or potassium nitrate ($NaNO_3$ or KNO_3). Sometimes other salts such sodium ascorbate or erythrobate are added as well in order to accelerate and stabilize cured color formation (see colour formation paragraph). Lately, there has also been a growing interest for using KCl as a substitution for part of the NaCl.

Usually 2.0–3.5% common salt (NaCl) is added to the sausage mince giving an initial water activity around 0.97–0.96 and a 'salt-in-water' level around 5–7% depending on the amount of fat. Nitrite and nitrate are added to the sausage mince in order to produce the characteristic cured color and to inhibit growth of undesired bacteria. Traditionally, nitrate was added as the only curing agent besides salt, but today it is more common to add nitrate together with nitrite, if any at all. Usually, the amount of added sodium nitrite lies in the range of 50–200 mg/kg and nitrate in the range of 200 to 600 mg KNO_3/kg, but some products are traditionally made with much more nitrate, e.g. Hungarian salami and Lebanon bologna that may contain up to 1700–1900 mg/kg. Nitrite promotes the establishment of the indigenous lactic acid bacteria and Micrococcaceae species, but can inhibit lactic acid bacteria if added in excessive amounts.

Sodium ascorbate or erythrobate are commonly added in levels of 200–600 mg/kg, but even higher amounts are also used in certain products.

Sugars
The glucose content of fresh, post-rigor beef and pork is in the order of 0.08–0.1% which is not enough to produce significant amounts of lactic acid. Therefore, various sugars such as glucose, sucrose, lactose, corn syrups (containing fructose, dextrose, maltose and other carbohydrates) etc. are added to the sausage mince as fermentation substrate for the lactic acid bacteria. Depending on sugar type, up to 2% carbohydrate is added to the sausage mince but usually 0.3–0.8% sucrose or glucose prove sufficient. Some traditional long-ripened sausages obtaining very low water activities are not added sugars at all.

Spices
Several different spices are used for fermented sausages, both as intact spices or as oleoresins. Ground pepper is usually present in all types of sausages, others are characteristic for certain sausage styles, e.g. hot pepper and cayenne pepper in pepperoni and huge amounts of paprika in the Spanish chorizo. In general, south European sausages are more heavily spiced than north European sausages.

Starter cultures
In modern sausage productions starter cultures are added to the sausage mince in a total level of approx. 107 CFU/g of mince in order to ensure a reproducible lactic acid formation and thereby creating a safe and uniform product. The cultures consist typically of blends of lactic acid bacteria and *Staphylococcus* species and less commonly, of different yeast species. South European sausage types are also applied with molds on the surface. For more details on starter cultures see *Cultures in the production of fermented sausages*, pg 18.

Additives
In sausage productions where speed is of more importance than quality, chemical acidulants such as glucono-delta-lactone (GdL) or encapsulated citric acid is sometimes used instead of applying lactic acid bacteria and sugars. GdL is an oxidized form of glucose and is immediately hydrolyzed to gluconic acid after being added to the mince. This results in a rapid pH decrease, but may create drawbacks such as metallic off-flavor and crumbling texture. Up to 0.8% GdL is used in the manufacture of fermented sausages, but smaller amounts are also applicable in blends with lactic acid bacteria.

Processing procedure

Figure 1 shows the general steps in the processing of fermented sausages. The major steps will be described in more details below.

Figure 1. General flow diagram of sausage production

Mince production
The meat and fat is pre-ground (Wolfed) before mixing or is directly chopped in the bowl-chopper simultaneously with the mixing of the other ingredients. Starter cultures, sugars, spices and additives are normally added in the beginning of the mixing process and salt and the (pre-chopped and) still frozen fat in the end. The late addition of salt prevents too high extraction of the soluble proteins from the meat that otherwise would enhance the water binding properties of the mince and impede the drying process later on. The late addition of fat makes sure that a sharp differentiation between the fat and meat particles is maintained. The temperature of the meat mixture during the whole operation should not exceed 0–2°C in order to prevent smearing of fat onto the meat particles.

Stuffing
The sausages are stuffed into synthetic, collagen or natural casings. The latter is more often used in Southern Europe. During stuffing it is important to maintain a low temperature in the mince as this prevents smearing. Pronounced smearing on the inside of the sausage casing will impede the drying loss of the fermented sausage.

To minimize the presence of oxygen and cavities inside the final product the sausages are generally stuffed under vacuum. For the same reason vacuum is also often applied during the mincing procedure. Entrapped oxygen may result in gray spots in the final sausage and increase rancidity of the fats due to autoxidative processes (chemical oxidation).

After stuffing the sausages hang at room temperature for a few hours to allow for temperature equilibration. Thus, the formation of condensed water on the surface is avoided which would otherwise take place when the very cold sausages meet the warm humid air in the climate chamber. If the temperature is not allowed to rise and equilibrate, surface discoloration could occur.

Surface inoculation
In Southern and Eastern Europe it is common practice to let the indigenous mold from the house-flora develop on the surface of the sausages during ripening or to treat the surface with a starter mold culture. The purpose of the mold coverage is to give the sausage an appetizing white or grayish appearance and to create a characteristic flavor.

Sausages are inoculated with the mold culture immediately after stuffing by dipping into a suspension of mold spores or by spraying the suspension onto the surface. Hereafter the processing continues as for non-molded fermented sausages. The mold coating reduces the rate of drying and the risk of case hardening, rancidity or discoloration.

Smoking
Since ancient times smoking has been used as a mean of surface preservation. Today it is still applied as such in the sausage production, especially in Northern Europe and in the US. However, nowadays smoke treatment tends to be more important for its flavor properties than for its preservative effect.

Smoke contains compounds that are harmful to the surface microorganisms. By giving a light smoke treatment early in the process undesirable growth of yeast and mold may be avoided on the surface of the sausages. At this time during the manufacturing process the surface is still too wet for the flavor components to be absorbed by the sausages. Therefore, smoking is repeated later in the process. The smoking time is primarily based on the required flavor development. However, deposition of harmful carbonyls, acids, phenols and in particularly 3,4-benzpyrene must be kept as low as possible and high smoke generation temperatures of the timber (open flames) should therefore be avoided. Friction generated smoke is recommended.

Fermentation
The fermentation step includes the period in the sausage production where pH decreases to its lowest value. The fermentation time lasts from less than 12 hours to several days depending on the sausage style. See introduction on page 4.

In order to achieve optimal acidification the fermentation parameters are carefully chosen. Quite a number of factors have an influence on the fermentation process, the most important being:

- lactic acid bacteria culture
- temperature
- salt concentration and water activity
- sugars
- initial pH
- level of inoculation
- microbial contamination of raw materials
- sausage diameter
- spices
- nitrite concentration

The importance of the various factors on the fermentation process has primarily to do with their influence on the performance of the lactic acid bacteria; this will be described further in *Influence of processing parameters on culture performance*, pg 24.

From the more practically side, the temperature, humidity and air speed in the modern climate chambers are automatically controlled during the fermentation and subsequent drying periods in order to make a uniform production. The temperature of the sausages should increase to the required fermentation temperature as fast as possible to ensure that growth of the added starter culture is on-set before the indigenous bacteria have a chance to multiply. As out-lined on page 4 the fermentation temperature is characteristic for different processing styles.

In order to make sure not to initiate the drying of the sausages too fast in the fermentation period the relative humidity (RH) within the chamber is kept at around 95–90% and high air velocities are avoided. Ideally, the relative humidity during fermentation should be 2–4 RH% lower than the water activity (*100) of the sausage. It is very important not to decrease the relative humidity below this level, since case hardening may occur.

Drying
The drying stage is defined as the period from the end of the fermentation cycle to the point where the sausage has achieved the desired weight loss and water activity for microbial stability, and the desired maturity has been obtained. The right texture and firmness of the fermented dried sausage is completed during the drying step due to removal of water and denaturation of proteins, (see *Texture formation*, pg 12). However, for some semi-dry sausages with very short production times and weight losses of only 10–15%, most of the drying may have been accomplished during the fermentation cycle. On the other hand, some traditional Italian and Hungarian sausages are dried for up to 3–6 months.

To achieve uniform drying it is very important that the rate of water evaporation from the surface of the sausage does not exceed the rate of which moisture migrates from within. Otherwise the sausage may exhibit case hardening and dry rim, and obstruction of water diffusion from the interior of the sausage may be the result. Also the flavor and appearance may be affected. As mentioned above the rate of drying is mainly determined by the relative humidity and the air velocity in the drying chamber, but also to a large extent by the pH reached during the preceding fermentation process. Therefore, drying rate is indirectly influenced by factors of importance to acidification, see *Influence of processing parameters on culture performance*, pg 24.

Drying is normally accomplished at low temperatures, 12–18°C, with a relative humidity decreasing from around 85% to as low as 65% in some cases. Ideally the relative humidity should be 5–10 RH% lower than the water activity (*100) within the sausage and air should be ventilated in the chamber at 0.1–0.5 m/s, but this depends greatly on the specific product and climate chamber.

Sensory quality

During sausage fermentation, drying and ripening a number of microbial, biochemical and physico-chemical reactions take place in the sausage mince converting the raw meat mixture into a firm, sliceable product. The sensory quality of the fermented dried sausage is determined by its appearance, texture and flavor and those criteria may be further divided into specific parameters that are commonly sought when discussing sausage quality in the industry.

Sausage appearance is largely covered by its color, but descriptors such as sausage structure, particle size, glistening of fat, appearance of tendons etc. are also of major part of the appearance. However, those parameters are primarily determined by the mince production procedure and not so much by the changes that go on during the conversion of mince into dried sausage.

Color formation

The overall color of a fermented sausage is determined by the color tone and intensity of the meat and fat particles. The color of the meat particles is partly determined by the meat type (chicken is lighter than pork and beef, and horse is very dark) and partly by the color forming reactions going on in the meat during the production process. The color of the fat is primarily a result of the quality of the raw materials.

The color of fresh meat is caused by the content of myoglobin and oxymyoglobin that have purple and bright red color tones, but are not very stable. During sausage production myoglobin and oxymyoglobin are transformed via a number of reactions, including nitrite, into the more stable nitrosylmyoglobin that is dark red and gives the sausage the typical reddish-brown appearance.

During preparation of the sausage mince the added nitrite acts as a very reactive oxidant and is rapidly reduced into nitric oxide (NO) parallel to the oxidative formation of metmyoglobin (the iron atom in the hem group of the molecule is oxidized from the ferrous (Fe^{2+}) to the ferric state (Fe^{3+}). This results in an immediate grayish discoloration of the mince. Later on in the process NO reacts with metmyoglobin and myoglobin to form nitrosylmyoglobin, simultaneously converting the grayish color to reddish. This reaction is promoted at reducing conditions as the iron atom in metmyoglobin must be reduced to Fe^{2+}.
Figure 2 shows a simplified reaction scheme.

Apart from being produced during metmyoglobin formation, NO is also formed by microbial reduction of nitrite or chemically from nitrous acid, in particular if the sausages are added ascorbate. Thus ascorbate speed up color formation (Figure 3). It is not certain which reactions predominate as the color formation mechanisms are not fully elucidated. However, as mentioned above low redox potential will in general promote and stabilize color. That is, lack of oxygen and other

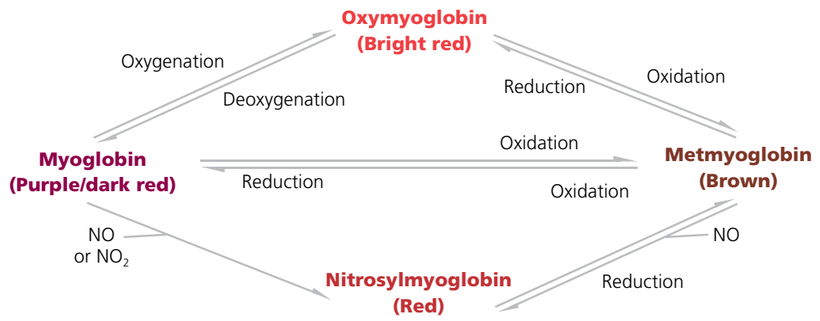

Figure 2. Simplified scheme of the reactions responsible for color formation. NO = nitric oxide, NO_2- = nitrite

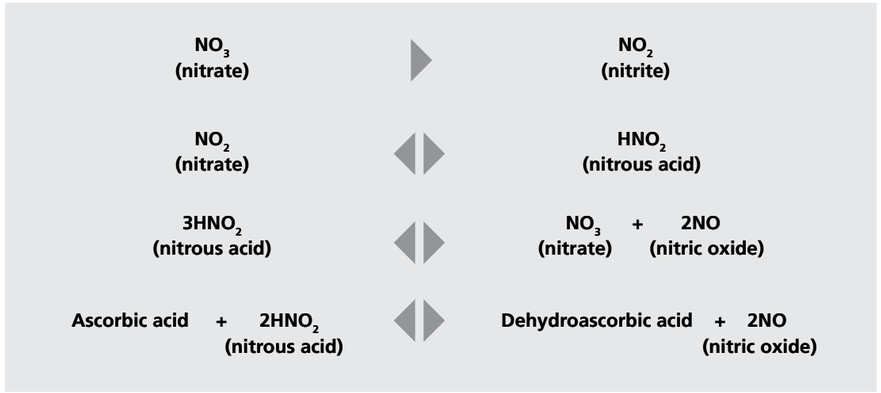

Figure 3. Nitrate reduction and formation of nitric oxide (NB! The equations are not balanced)

oxidative compounds in the mince and the presence of anti-oxidative components such as sodium ascorbate, alpha-tocopherols (vitamin E), phenolic compounds from added spices etc.

When nitrate is used instead of nitrite as the color forming agent, the nitrate molecule must be reduced to nitrite before the color forming reactions can take place (Figure 3). This conversion is performed by *Micrococcaceae* species producing nitrate reductases during growth in the mince. Inevitable, this means that the color forming process will be more dependent on the activity of the *Micrococcaceae* species and that color formation will take longer than in sausages added nitrite. Since *Micrococcaceae* species are inhibited at low pH, sausages relying on nitrate reduction must be fermented by a traditional process (see page 4).

Nitrate is still used by many sausage manufactures because nitrate serves as a long time reservoir of nitrite, but it has also been reported that sausages cured with nitrate have a better flavor than when cured with nitrite, see *Flavor formation*, pg 13.

Color stability

During storage of the finished dried sausage, in particular if sliced, the sausage color is prone to fading and becoming grayish. This is caused by oxidation of the hem group of the nitrosylmyoglobin molecule as the ferrous iron is oxidized to the ferric state.

In general, the susceptibility of nitrosylmyoglobin to oxidize is tightly linked to lipid oxidation and redox potential and increases with decreasing pH. Parameters such as atmospheric oxygen, oxidized (rancid) fat containing large amounts of peroxides and free radicals, and hydrogen peroxide producing microorganisms growing in the sausage or on the surface of the slices will have a negative impact.

In order to avoid pigment oxidation to take place, anti-oxidative compounds are added to the sausage mince as mentioned above, and the sausages are packed in air-tight or modified atmosphere packages. Growth of *Micrococcaceae* species in the sausage and their ability to produce catalase will reduce the redox potential and peroxide accumulation, respectively.

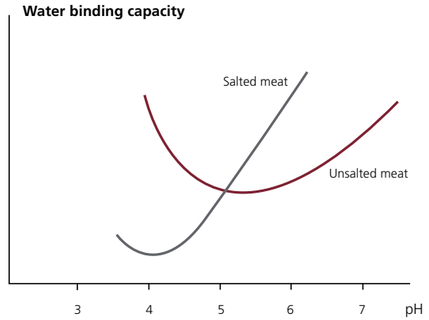

Figure 4. Water-binding capacity of salted and un-salted meat in relation to pH in a fresh meat model.

Texture formation

Formation of the correct texture is an important part of the overall quality of fermented dried sausages and commonly texture is described by the attributes hardness, firmness, fattiness, juiciness, stickiness, tenderness, softness, granularity etc. In general, Southern European style sausages are much firmer than the Northern European and US style sausages that tend to be more soft, elastic and rubbery-like

Sausage texture is a result of the physico-chemical reactions taking place in the sausage mince during the fermentation and drying cycles and is influenced by the mince ingredients as well as the processing procedure. Simplified, the texture formation process can be divided into three steps: extraction of proteins during and after meat mincing, formation of a protein gel during fermentation and release of water during drying.

During mincing the added salt solubilizes and extracts proteins (primarily myosin) from the meat myofibrils forming a sticky protein film around the mince particles. In the succeeding fermentation process, pH decreases, coagulating the solubilized proteins, forming a firm gel that embeds and binds the fat and meat particles closely together.

Coagulation by acidulation is associated with the release of water and this water is removed continuously in the beginning of the drying process. As the drying process continues the more tightly bound water will be released as well, but at a slower rate.

Depending on the processing parameters and the drying time the resulting texture will exhibit different properties. The protein extraction during the mincing procedure is directly influenced by the intensity of chopping and salt concentration. High protein extraction will result in a sausage with a more elastic texture, on the other hand high protein extraction could induce too high water binding capacity of the mince impeding the drying process.

Additionally, salt interacts with the myofibrillar proteins, decreasing their iso-electric point from approximately pH 5.3 down to as low as pH 4.3 depending on the salt concentration as shown in Figure 4. This has a drastic effect on the water-binding capacity of the proteins since the intermolecular spaces for retaining water is at a minimum at the iso-electric point. Thus as the pH value approaches the iso-electric point during the fermentation cycle, the release of water is increased. However, since pH-lowering will also induce coagulation of the meat proteins and this process begins around pH 5.3, the gellification process and the partly entrapment of water will start at pH below 5.3, opposing the water release that otherwise could have taken place. In fact, practical experience shows that sausage recipes with normal salt quantities show an optimal iso-electrical range from 4.8 to 5.3; in general acidification to pH below 4.8 will not increase the rate of water loss.

As described above, the fermentation process is of utmost importance for the texture formation in fermented dried sausages. In fact, texture development during fermentation is determined by the drop in pH, primarily, whereas the further texture development during drying is determined by the loss of water. Hardness increases sharply when sausage pH reaches 5.3 and is further increased until pH 4.8. If pH is not lowered below 5.3, it is necessary to reduce the aw to below 0.90 in the drying process to ensure formation of sausage texture, but the texture may still not become optimal. In order to control texture development it is therefore essential to control the fermentation process. The parameters that influence fermentation is further described in chapter 4. Other parameters of importance for the drying process were described in *Processing procedure*, pg 7.

Flavor formation

Flavor is a sensorially perceived quality involving complex interactions between taste and aroma, and is also influenced by texture and other sensory sensations arising during the eating process. With regard to dried sausage, this involves as an example the burning and stinging sensations from spices such as cayenne pepper or hot peppers that are added to the mince. However, in the following, the description of flavor formation will be focused on the characteristic taste and aroma formation that takes place during the sausage production and which is determined by the processing procedure.

Cabbage,sulfur, putrid
Garlic, onion, salami
Cooked meat, potato, gravy, vitamins
Vomit, sweaty socks, wet dog
Butter, sweetish, fruity, candy
Sourdough, chutney, olives
Vinegar, sourish
Fresh air, seaside
Green, cut leaves, cucumber
Popcorn, crackers
Deep-fried, chips
Pelargonium, dried flower
Mushrooms, earthy
Rose, honey, orange
Clean laundry, soap
Paint, glue, plastic, rubber
Phenolic, leather, horse, library

Table 1. Odor notes detected in fermented dried sausages of various origin

In fermented dried sausages the formation of aroma plays a more important role than the formation of taste for obtaining the characteristic sausage flavor. This is due to the high sensitivity of the nasal receptors for the volatile aroma components released during chewing of the sausage and due to the very complex volatile profile. It has been shown that the taste fraction of dried sausages are just composed of broth-like, sour, salty and bitter tastes of no similarity to the sensory experience when eating dried sausage, whereas the aroma fraction consists of an immense number of aroma notes with different characteristics that when mixed together create the dried sausage odor (table 1).

The volatile compounds responsible for the odor notes in table 1 cover a wide variety of compounds such as aldehydes, acids, ketones, esters, sulfides, thiols, O-heterocycles and more. Many of those compounds are formed in the product by enzymatic and chemical reactions during production, whereas others arise from added spices, surface smoking (in north European style sausages), and from the raw materials of the meat and fat (e.g. boar and mutton taints). However, the compounds formed during the production process are by far the most important compounds for creating the typical dried sausage flavor.

Simplified, proteins in the mince are hydrolyzed into smaller proteins and peptides by endogenous enzymes of the meat, and those peptides are further hydrolyzed into amino acids by microbial enzymes. Hereafter, the amino acids are degraded into a wide variety of volatile compounds such as aldehydes, acids, thiols and sulfides with very low sensory threshold values. Lipids are hydrolyzed both by adipose and microbial enzymes, releasing unsaturated and saturated free fatty acids that are oxidized into aldehydes and ketones by both chemical (autoxidative) and microbial oxidation. Sugars are degraded into primarily lactic acid, which is a taste compound, but also into side products such as acetic acid and diacetyl, contributing with vinegar and buttery aroma notes.

Regarding taste compounds formed during production, primarily lactic and acetic acids contribute to the sour taste, and free amino acids and small peptides to the broth-like and bitter taste. NaCl added to the mince will of course contribute to saltiness whereas nucleotides are not considered being of importance for the taste.

The microorganisms responsible for producing the aroma compounds are primarily *Micrococcaceae* species of the genera *Staphylococcus* and *Kocuria*, but also lactic acid bacteria, yeast and, in molded sausages, the fungi growing on the surface. The diverse activities of those microorganisms will be further described in the next chapter.

When comparing the flavor profiles of typical South European sausages versus North European types it has been shown that except for relatively few compounds, the volatile compounds are much the same but the relative proportions different. South European sausages contain typically higher levels of lipid oxidations products, fruity esters and specific aldehydes from amino acid degradation, whereas North European sausages in general contain higher amounts of lactic and acetic acids. Regarding specific differences, molded sausages contain a very characteristic popcorn-smelling compound with an extremely low sensory threshold value.

Those differences are readily reflected in the sensory perceived aroma: South European sausages are rated as having the most complex flavor being more rancid, fatty, oily, porky, nutty, cheesy, flowery and ammonia-like than the North European style sausages, which on the other hand are more acidic tasting.

The differences between US, North and South European sausages are not so much a result of differences in the mince ingredients as to the starter culture and the processing procedure. The major cause for differences in the aroma profiles is the milder acidification in the South European technology that enables better growth of the *Micrococcaceae* species within the starter culture and also the longer ripening period and mold coverage. However, it has been shown that sausages with added nitrate instead of nitrite contain higher levels of many essential aroma compounds from amino acid degradation and that the softer fat often used in South European technologies is more prone to chemical oxidation and rancidity.

Food safety

By nature the raw materials of meat and fat used in dried sausage production are contaminated with a characteristic microbial flora to which new microorganisms are added during the production cycle with the addition of ingredients, in particular spices, and by contamination from the processing equipment and the handling procedures. Therefore, the indigenous flora of a sausage mince consist of a wide variety of bacteria, yeast and mold spores, including typical spoilage bacteria and, in unfortunate circumstances, also pathogenic bacteria and molds.

During the mince production the application of salt lowers the water activity depending on the amount of salt and fatty tissue in the recipe. The lowered water activity inhibits growth of the spoilage flora normally associated with meats and, together with the effect of nitrite, the mince conditions will be selective for microorganisms such as lactic acid bacteria and *Micrococcaceae* species. However, undesired microorganisms such as the pathogenic *Staphylococcus aureus*, *Listeria monocytogenes, Salmonella* and *E. coli O*:157 are still able to grow.

After the tight stuffing (often under vacuum) of the mince into the casing, oxygen diffusion into the sausage is hindered, available oxygen used by the microorganisms and low redox potential induced. This promotes growth of lactic acid bacteria. During the succeeding fermentation period lactic acid bacteria converts sugars in the mince into lactic acid, lowering pH and promoting lactic acid bacteria growth even more. The acid formation inhibits the spoilage bacteria and to some extends the pathogens as well, in particular *S. aureus*, depending on the pH-decrease. During the following drying period water evaporates from the sausage, decreasing the water activity to even lower levels. This process further inhibits growth of unwanted bacteria and in most instances pathogenic bacteria will disappear during prolonged drying.

Although acid production and drying are the primary mechanisms for inhibiting unwanted bacteria, some lactic acid bacteria naturally present in the mince will also inhibit unwanted bacteria by producing bacteriocins that damage their cell membranes thus reducing their numbers. Bacteriocins are small peptides excreted by the bacteria. The antagonistic effects are particular directed towards other lactic acid bacteria and Gram positive pathogens, i.e. *Listeria monocytogenes*.

The high temperature employed for sausage fermentation in North European and US technologies will inevitably promote the growth of mesophilic pathogenic microorganisms such as *S. aureus* unless the pH-drop is relatively sudden and well-controlled. Therefore, high concentrations of starter cultures of lactic acid bacteria are applied to control the on-set of fermentation and to control the fermentation profile. Apart from diminishing the risk of pathogenic growth, addition of high amounts of competing flora will also out-number the less stable growing natural lactic acid bacteria flora.

South European molded sausages are frequently inoculated by natural colonization with the mold spores from the house-flora. However, colonization by the house-flora may lead to sausages covered with mycotoxin producing species and therefore it is becoming more and more common to inoculate the sausages with standardized fungal cultures. Also, the formation of a presentable uniform moldy layer is enhanced and the correct drying process favored.

Cultures in the production of fermented sausages

Introduction

As already described in the previous chapter sausage mince possesses a natural selectivity favoring the development of a desired micro flora of lactic acid bacteria and *Micrococcaceae*. However, production time can be reduced and product uniformity enhanced by the addition of an appropriate inoculum of starter culture. A traditional way of achieving this is done by 'back-slopping'. This method is not altogether reliable, though, since undesirable as well as desirable microorganisms could be selected, or the properties of the microorganisms could change over time. Today, 'back-slopping' is only used for minor traditional productions and sausage mince is inoculated with standardized starter cultures.

Table 2 shows the microorganisms used in starter cultures offered by Chr. Hansen.

At Chr. Hansen the microorganisms in table 2 are categorized into four groups: acidifying bacteria, microorganisms with color and/or flavor forming activities, microorganisms for surface coverage and bacteria for bio-protection. Depending on the physical form of the cultures and the processing conditions during sausage production the performance of the cultures will vary. In the following paragraphs a brief introduction is given to the cultures and their characteristics, whereas the influence of processing conditions will be described in *Influence of processing parameters on culture performance*, pg 24.

Microorganism	Genus	Species
Bacteria	*Lactobacillus*	*L. pentosus, L. sakei, L. plantarum, L. curvatus*
	Pediococcus	*P. pentosaceus, P. acidilactici*
	Staphylococcus	*S. carnosus, S. xylosus*
Mold	*Penicillium*	*P. nalgiovense, P. candidum*
Yeast	*Debaryomyces*	*D. hansenii*

Table 2. Typical microorganisms used as meat starter cultures offered by Chr. hansen

Acidifying cultures

Lactobacillus

Lactobacillus species are the predominant lactic acid bacteria in most meat products that are fermented by the indigenous flora and the most robust lactobacilli have been selected and incorporated into commercial starter cultures. All *Lactobacillus* used as starter cultures in dried sausages are homo-fermentative, micro-aerophilic rods, i.e. they grow best at low redox potential and their main fermentation product from sugar is lactic acid. The properties of the major *Lactobacillus* species available at Chr. Hansen are summarized in table 3. The Chr. Hansen strains are all able to ferment the simple sugars glucose and fructose while producing either L-lactic acid or a mixture of D- and L-lactic acid. The salt-limit for proper growth is between 9–13% salt-in-water and the temperature optimum is between 30–37°C (86–99°F).

Culture	*L. pentosus*	*L. sakei*	*L. plantarum*	*L. curvatus*
Optimum growth temperature (°C / °F)	35 / 95	30 / 86	30 / 86	30 or 37* 86 or 99*
Salt limit (% salt-in-water)	9	9	13	10
Lactic acid formation	DL(-/+)	L(+)	DL(-/+)	L(+)
Fermentable sugars				
Glucose (dextrose)	+	+	+	Strain dependent, please refer to product sheets
Fructose	+	+	+	
Maltose	+	-	+	
Lactose	+	-	+	
Saccharose (sucrose)	+	-	+	
Starch	-	-	-	

Table 3. Typical microorganisms used as meat starter cultures offered by Chr. hansen

Pediococcus

Pediococcus species are occasional found as minor components of the micro flora in indigenous fermented sausages, in particular in the US-style sausages where a high fermentation temperature is used. Today they are widely used as starter cultures in all kind of dried sausages.

Pediococcus species are homo-fermentative, micro-aerophilic cocci. Some of the more important properties of the Chr. Hansen pediococci are compiled in table 4. Compared to the *Lactobacillus* strains the *Pediococcus* strains in general grow at higher temperatures and have a broader sugar fermentation pattern.

Culture	*P. pentosaceus*	*P. acidilactici*
Optimum growth temperature (°C / °F)	35 / 95	40 / 104
Salt limit (% salt-in-water)	**7**	**10**
Lactic acid formation	**DL(-/+)**	**DL(-/+)**
Fermentable sugars		
Glucose (dextrose)	+	+
Fructose	+	+
Maltose	+	+
Lactose	(+)	(+)
Saccharose (sucrose)	+	(+)
Starch	-	-

Table 4. Acidifying Pediococcus *species offered by Chr. Hansen*

Color and flavor forming cultures

Staphylococcus

The *Micrococcaceae* species most often encountered in the largest numbers in indigenous fermented sausages are different species of *Staphylococcus*, more specifically *S. xylosus, S. saprophyticus* and occasionally *S. carnosus. Staphylococcus* species are thus clearly dominant compared to the *Kocuria* species sometimes offered, due to their higher salt tolerance and lower oxygen requirements.

Staphylococcus species are facultative anaerobic cocci capable of reducing both nitrate and nitrite, and possessing diverse enzymatic activities of major importance for the flavor formation of the fermented dried sausage. More specifically: catalase activity, lipolytic and proteolytic activity, and high capability of degrading amino acids and fatty acids into a wide variety of aroma compounds.

Staphylococci prefers availability of oxygen during growth but are capable of using nitrate as an electron acceptor instead of oxygen during respiration, which will increase their survival in the sausage mince. Otherwise *Staphylococcus* inoculated into the mince will only grow little or not at all during ripening, and mostly in the outer parts. However, the desired activities of the added *Staphylococcus* are still achievable if a sufficient number is added (10^6–10^7 CFU/g) since the organisms are still metabolically active even if not growing. It is important, though to restrict the rate of lactic acid formation because their survival is sensitive to low pH. Table 5 shows some typical properties of the *Staphylococcus* species offered by Chr. Hansen for fermented sausages.

Culture	*S. carnosus*	*S. xylosus*	*Micrococcaceae spp.*
Optimum growth temperature (°C / °F)	30 / 86	30 / 86	30 / 86
Salt limit (% salt-in-water)	*16*	*15*	*16*
Fermentable sugars			
Glucose (dextrose)	+	+	+
Fructose	+	+	+
Maltose	-	+	-
Lactose	+	+	+
Saccharose (sucrose)	-	+	-
Starch	-	-	-

Table 5. Micrococcaceae *species offered by Chr. Hansen*

Debaryomyces

Yeasts are often associated with indigenous fermented sausages, but species from the genus *Debaryomyces*, and in particular *Debaryomyces hansenii*, predominate due to their high salt tolerance.

D. hansenii does not reduce nitrate, but decomposes peroxides and consumes both lactic and acetic acids in the sausage thereby increasing pH during the ripening period. Additionally, *D. hansenii* produces ammonia, which also increases sausage pH, and possesses lipolytic and proteolytic activities of importance to flavor development. *D. hansenii* only grows near to the surface of the ripening sausage as it needs oxygen for growth.

Cultures for surface coverage

The predominant molds isolated from spontaneous colonized dried sausages are different *Penicillium* species, but *Scopulariopsis, Aspergillus* and other fungi may also be present. As mentioned in an earlier paragraph the non-starter molds arise from spores of the house-flora.

Chr. Hansen offers three different strains of *Penicillium nalgiovense* giving the sausage surface a whitish-grayish appearance. As with yeasts, molds oxidize lactic acid and other acids, and produce ammonia thereby increasing pH. Since molds form a coating over the surface, use oxygen and produce catalase, they reduce chemical lipid oxidation and thereby rancidity. Additionally, *P. nalgiovense* affects flavor formation due to diverse metabolic activities such as lipolytic and proteolytic activity.

In addition to *Penicillium* species, yeast strains are also used for surface treatment of South European style dried sausages.

Bio-protective cultures

As mentioned in *The formation of sensory quality*, pg 10, it is a risk that certain pathogenic bacteria may survive the fermented dried sausage production despite the strong hurdles of acidification and drying; the most obvious reason being an unusually high initial pathogenic load of the raw materials. Chr. Hansen offers strains from two different species of lactic acid bacteria with bio-protective properties in fermented sausages, specifically against *Listeria monocytogenes.* Strains of *Pediococcus acidilactici* and *Lactobacillus curvatus* produce pediocin and sakacin, respectively that destroys the cell membrane of *Listeria monocytogenes* and reduce their numbers.

Influence of processing parameters on culture performance

Introduction

During processing the microorganisms in the sausage mince are subjected to a large number of parameters that will influence their performance. In the preceding chapters this subject has already been touched upon and a number of factors identified (see *Processing procedure*, pg 7). The effect of the more important factors – fermentation temperature, sugar concentration and sugar type, salt concentration, microbial contamination and pH of the raw materials, physical form of the starter culture – will be further described in the following paragraphs, specifically with relation to the Chr. Hansen cultures.

Fermentation temperature

Temperature is the factor mostly influencing the fermentation process. An increase in temperature increases the rate of pH-lowering if below or close to the optimum growth temperature for the specific lactic acid bacteria. In general it has been found that a 5°C increase in temperature approximately doubles the rate of acid formation. The increase in acid formation is due to an accelerated flux through the enzymatic pathway converting sugar to lactic acid. Figures 5 and 6 show typical profiles for the pH-decrease brought about by four different Chr. Hansen cultures, containing either fast or traditional *P. pentosaceus*

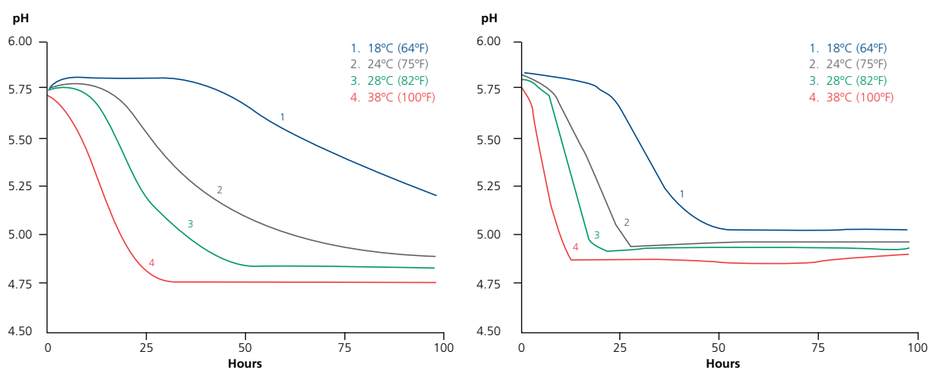

Figure 5. Influence of fermentation temperature on the pH-decrease induced by the traditional fermenting culture T-SPX (left) and the fast fermenting culture F-1 (right), both containing a P. pentosaceus *strain. Sausage mince contains 0.5% glucose.*

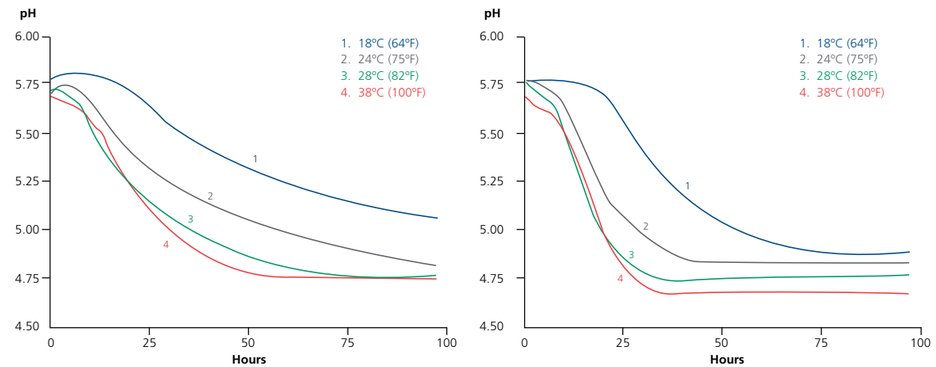

Figure 6. Influence of fermentation temperature on the pH-decrease induced by the traditional fermenting culture T-SC-150 (left) and the fast fermenting culture F-SC-111 (right), both containing an L. sakei *strain. Sausage mince contains 0.5% glucose.*

and *L. sakei* strains. It is clearly seen that increasing temperature from 18 to 38°C (64 to 100°F) increases acid formation by all cultures, but that the effect of increasing temperature is less in the fast cultures than in the traditional cultures. Also, it is evident that *P. pentosaceus* is faster than *L. sakei* at the highest fermentation temperature, but has a similar or a lower speed at the lower temperatures. This corresponds to *P. pentosaceus* having an optimum growth temperature around 35°C (95°F), whereas the optimum temperature for L. sakei is approximately 30°C (86°F) (see tables 3 and 4). In general, higher fermentation speed will result in a lower pH even if the added amount of sugar is the same. Details on the temperature sensitivity of other Chr. Hansen cultures are available on request.

The growth of *Staphylococcus, Debaryomyces* and *Penicillium* strains added besides the lactic acid bacteria will in general proliferate from increased temperatures. The most important effect of temperature is probably related to the influence that temperature has on the acidifying culture and the resulting pH-profile. As mentioned previously, *Staphylococcus* strains are very sensitive to low pH, and at pH below 5.0 the nitrate reductase activity starts becoming hampered (see colour formation paragraph, pg 10). In order to ensure a fast on-set of Penicillium growth on the sausage surface both temperature and humidity must be high; above 20°C (68°F) and 90% relative humidity, respectively.

Sugars

The type and amount of sugar directly affect the pH decrease and the time to achieve the lowest pH. Simple sugars, such as glucose, are readily utilized by all lactic acid bacteria, whereas other more complex sugars, such as lactose or maltose, are less easily fermented (see sugar specifications for different species in tables 3 and 4). Table 6 compiles the amount of lactic acid produced from different sugars by *Lactobacillus pentosus* at optimum temperature conditions. The data distinctly show why it is recommendable to add the most simple sugars to the sausage mince if a fast pH-drop is needed and how it is possible to control the rate and the extend of the pH-drop in a more subtle way. By using a mixture of fast and slowly fermentable sugars, a rapid but small pH decrease can be achieved at the beginning of the fermentation period and a slower rate to the final pH can be achieved in the end of the fermentation period. This is recommendable in sausages added nitrate in order to inhibit unwanted bacteria in the beginning of the cycle, without suppressing *Staphylococcus*, nitrate reduction and color formation by a too fast, extensive pH-drop.

It has been found that increasing glucose concentration above 0.15% does not change the rate of lactic acid formation, in general (i.e. the slope of the pH-curve), but only the extend of the pH-decrease. However, the final pH will also depend on the actual lactic acid bacteria and its sensitivity to low pH and the decline in water activity obtained during the fermentation period. Figures 7 and 8 show the pH-profiles made by four different Chr. Hansen cultures containing either fast or traditional *P. pentosaceus* and *L. sakei* strains. Increasing glucose content from 0.3 to 1.0% in general lowers the final pH, but the effect of changing glucose concentration is strongest for the fast fermenting cultures. Also, it is evident that *P. pentosaceus* in the traditional culture is more affected at high temperature (38ºC /100ºF) than *L. sakei* in the traditional culture. This is a reflection of *P. pentosaceus* having an optimum growth temperature around 35ºC (95ºF), whereas the optimum temperature for *L. sakei* is approximately 30ºC (86ºF). Additionally, the plots show that glucose concentrations as low as 0.3% is enough for reaching a pH below 5.3 and thereby on-setting texture formation and drying-out (see *Texture formation*, pg 12.).

Carbohydrates (1%)	Lactic acid produced (%)	Final pH
Glucose	0.98	4.08
Saccharose	0.86	4.04
Maltose	0.72	4.24
Maltodextrin	0.54	4.54
Galactose	0.31	4.83
Raffinose	0.08	6.10

Table 6. Lactic acis production and final pH achieved by Lactobacillus pentosus *during growth in MRS-broth at 30ºC for 12 hours*

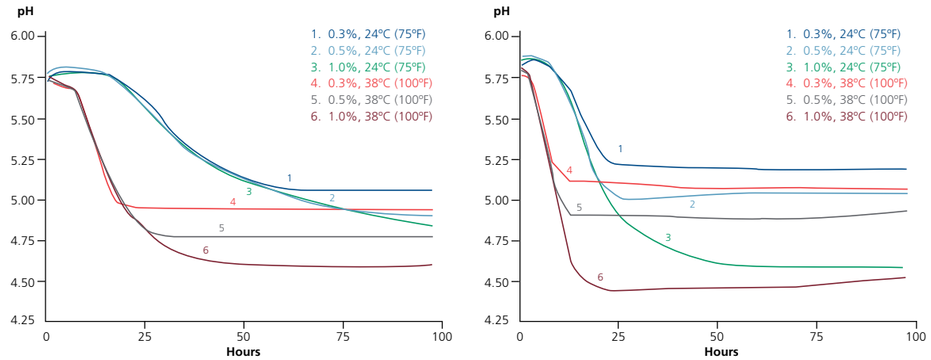

FIgure 7. Influence of amount of glucose on the pH-decrease induced by the traditional fermenting culture T-SPX (left) and the fast fermenting culture F-1 (right), both containing a P. pentosaceus *strain. Fermentation temperature was either 24ºC or 38ºC (75ºF or 100ºF).*

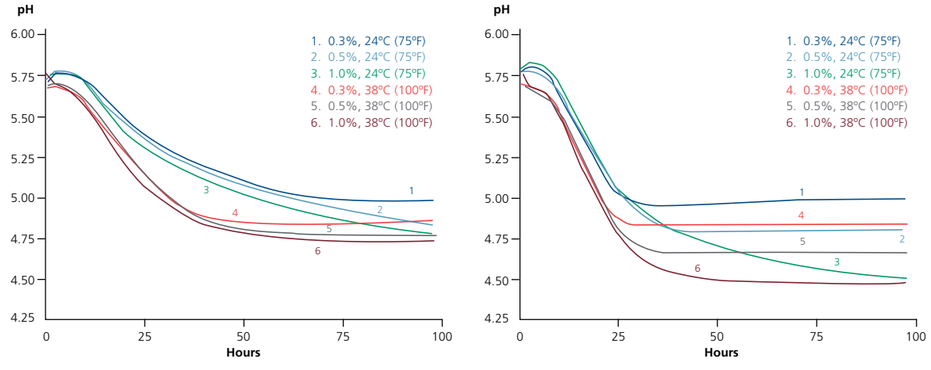

Figure 8. Influence of amount of glucose on the pH-decrease induced by the traditional fermenting culture T-SC-150 (left) and the fast fermenting culture F-SC-111 (right), both containing an L. sakei *strain. Fermentation temperature was either 24ºC or 38ºC (75ºF or 100ºF).*

Salt concentration and water activity

The acidifying bacteria of the sausage mince are only active at water activities above a certain level. Decreasing the water activity of the mince by adding salt or high amounts of fat will prolong the lag phase of the culture, resulting in a prolongation of the total fermentation time. The inhibition brought about depends on the involved species and their sensitivity to the salt-in-water level – a measure often used instead of the more correct measure, water activity, but less difficult to determine. Water activity is determined as the ratio between the vapor pressure of the water in the sausage and the vapor pressure of pure water, whereas salt-in-water level is the amount of salt in percentage of the total water content (see tables 3 and 4). Figure 9 shows the pH-profile of the Chr. Hansen culture T-SL containing *Lactobacillus pentosus*. At 9% salt-in-water, which is the upper limit for this strain, the initiation of the pH-decrease is delayed by 72 hours compared to at 5.7% salt-in-water.

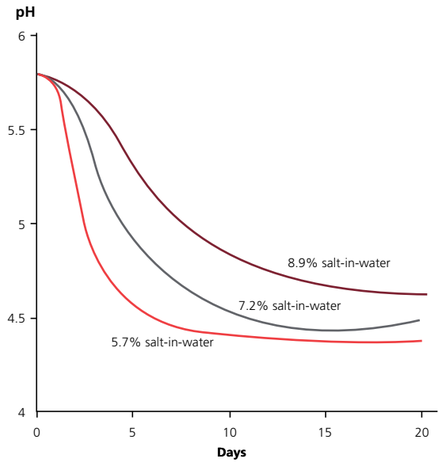

Figure 9. The influence of salt concentration on the fermentation profile of a sausage added T-SL culture

Decreasing the water activity of the sausage during drying will inevitable reduce the activity of the cultures over time. To achieve maximum acidification it is therefore important to regulate the amount of salt in the mince and the drying rate correspondingly, keeping in mind the salt-in-water limit of the specific acidifying bacteria.

Staphylococcus and *Debaryomyces* strains added to sausage mince are much less sensitive to high salt concentrations than lactic acid bacteria (see table 5). The water activities of most dried sausages do not reach levels that totally inactive those species, though their growth and metabolic activity will be partly hampered. The optimum salt concentration for growth of many *Staphylococcus* species is close to the salt-in-water content of the fresh mince.

Raw material quality

The indigenous bacterial flora of meat consists of microorganisms unintentionally added during butchering and preparation of the meat. The composition of this bacterial flora is influenced by the treatment and storage of the meat before it is used for sausage production.

Traditionally, meat was pre-salted in order to favor a bacterial flora consisting mainly of lactic acid bacteria. The method often resulted in fermentation faults as the composition of the lactic acid bacterial flora was always random. Typical fermentation faults included gas formation, bitterness and sourness, the latter especially as a consequence of the formation of acetic acid. The use of pre-salting will in most cases result in the dominance of the starter culture organism and the desired pH-development. However, sometimes the indigenous lactic acid bacteria are so dominating that they control the fermentation process and this may cause problems. This may also be the case if vacuum-packed meat is used. Therefore, it is of utmost importance that the bacterial load of the raw materials, even if just lactic acid bacteria, is as low as possible, preferably lower than 10^5 CFU/g.

Meat that has been left in production rooms before freezing or kept in cold storage for a long time at aerobic conditions contains high numbers of spoilage bacteria. Those organisms are characterized by their ability to break down fat and protein and produce putrid off-flavors. At the same time pH of the meat is slightly raised. When a starter culture is used with such meat, the lag phase of the lactic fermentation may increase. The small pH-rise caused by the spoilage bacteria increases the buffer capacity of the meat and in order to obtain the same pH-drop more acid must be produced by the starter culture. It may even be necessary to add extra sugar to produce the desired amount of acid. All in all, the total processing time is prolonged.

In some cases it is possible to shorten the lag phase of a freeze-dried culture by making a suspension of the culture in tap water (chlorine free) before application to the mince. But, the method is not altogether reliable and is not recommendable. Also, one should be aware that this step will not make up for the difference between a frozen and a freeze-dried culture. If a shorter lag phase is needed, it is recommended to increase inoculation level, change culture or change processing procedure. Mold spores for surface inoculation should always be re-activated in water before use, but in order to avoid contamination with other microorganisms and loss of activity the re-activation time should be kept as short as possible.

Physical form of the starter culture

Starter cultures can be applied as frozen liquid cultures, frozen pellets or as freeze-dried cultures. In general the physical form has no great influence on the fermentation pattern, except that the lag phase of the frozen cultures is somewhat shorter. Only for the very fast fermentation procedures, such as for the US style technology, this delay is of significant importance. Figure 10 shows the pH-profile for *Lactobacillus pentosus* in a frozen liquid and a freeze-dried traditional T-SL culture. In this case the difference due to the delayed on-set of fermentation is approximately 5 hours.

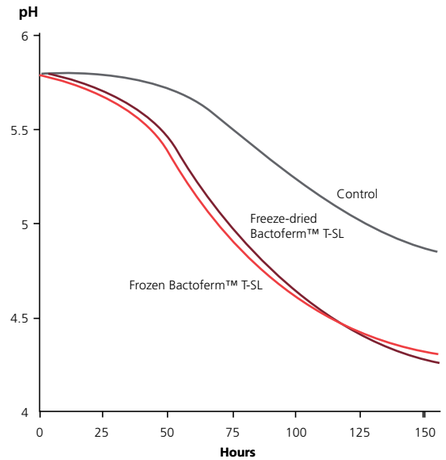

Figure 10. Fermentation profiles for Lactobacillus pentosus *applied as frozen culture or freeze-dried culture. The control is without culture*

Guidelines for choosing Chr. Hansen starter cultures

Starter cultures at Chr. Hansen

In the tables below the standard range of starter cultures offered by Chr. Hansen for fermented dried sausages are compiled and categorized into their primary target groups, taking into account the specific culture needs within each group. Please refer to the sausage style definitions made in the introduction paragraph, pg 4, and to the chapters starting on pages 18 and 24 for details on the involved microorganisms. One should be aware that even if many of the cultures contain the same species, the strains are different and possess different characteristics.

Most of the cultures within the product range contain blends of lactic acid bacteria, *staphylococci* or *Micrococcaceae spp*., in this way simplifying the procedure of applying more than one bacterium. Some of the products also contain yeast.

Starter cultures for traditional fermented sausages

In the production of traditional Southern European style sausages and traditional North European technologies, the fermentation profile must have a short lag phase in order to ensure the growth of the added starter culture at the expense of the background flora. Additionally, the acidification profile must be rather flat not going below pH 4.8–5.0 at any time. This will ensure that the applied staphylococci maintain their activity over a longer period of time; foremost their nitrate reductase and flavor-forming activities. The cultures specified below are specifically selected for traditional fermentation profiles applying fermentation temperatures not higher than 24°C (75°F).

Culture name	Bacteria included	Characteristics
T-SC-150	*Lactobacillus sakei* *Staphylococcus carnosus*	Gives a product flavor which is very typical for German salami such as Westphalia salami type. The acidification leads to a clear lactic acid taste. The used *Lactobacillus sakei* has a very good growth potential and is able to suppress the growth of a lot of indigenous bacteria. The used *Staphylococcus carnosus* gives good color stability and a mild aroma. **Attention: This *Lactobacillus sakei* is sucrose negative.**
TRADI-302	*Lactobacillus sakei* *Staphylococcus xylosus* *Staphylococcus carnosus*	Same features as T-SC-150, but the combination of the two *Staphylococci* leads to a more intensive color formation and a slight milder aroma.
SM-182	*Lactobacillus sakei* *Staphylococcus carnosus* *Debaromyces hansenii*	Same features as T-SC-150, but the yeast *Debaromyces hansenii* on top gives a more "Mediterranean" flavor.
T-SPX	*Pediococcus pentosaceus* *Staphylococcus xylosus*	The high concentration of *Pediococcus pentosaceus* gives a controlled and moderate pH-drop. The acidification gives a mild lactic acid taste. The used *Staphylococcus xylosus* gives good color formation and stability. Furthermore the *Staphylococcus xylosus* gives a very round and mild flavor which is very typical for South European salami types such as Milano.
BFL-T03	*Pediococcus pentosaceus* *Staphylococcus carnosus ssp*.	Same features as T-SPX, but the new developed *Staphylococcus carnosus ssp*. Gives a milder and more "Mediterranean" flavor.
SM-181	*Lactobacillus sakei* *Staphylococcus xylosus*	The sucrose positive *Lactobacillus sake*i gives a moderate pH-drop. This *Lactobacillus sakei* has a very good growth potential and is able to suppress the growth of a lot of indigenous bacteria. The used *Staphylococcus xylosus* gives good color formation and stability. The very high number of *Staphylococcus xylosus* leads to a very round and mild "Mediterranean" flavor.

Starter cultures for fast fermented sausages

In the production of North European and US style sausages the fermentation profile must have a very short lag phase in order to rapidly on-set fermentation and exhibit a fast drop in pH to below 5.3 within 30 hours as a minimum. This ensures an efficient inhibition of background flora and an early on-set of fast drying. Total production time is typically less than 2 weeks.

Staphylococci and *Micrococcaceae spp*. are not added to all cultures, so in order to enhance color formation staphylococci or *Micrococcaceae spp*. Must be added on the side (see *Starter cultures for flavor enhancement and nitrate reduction*, pg 34).

This may be unnecessary in the US style process (fermentation temperatures 35–45°C/100–115°F, very fast pH-drop, very low final pH < 4.8) since staphylococci generally do not survive the fast pH-lowering. In some instances, however, the addition of staphylococci or *Micrococcaceae spp*. has proven beneficial for color stability in the US style process for meat snack sticks.

The *Pediococcus* in F-1 and BFL-F02 has lower salt tolerance than the other fast fermenting strains and are therefore not recommended for sausages with very high salt-in-water levels (>6%) and high fat contents.

Culture name	Bacteria included	Characteristics
F-SC-111	*Lactobacillus sakei* *Staphylococcus carnosus*	Same features as T-SC-150, but faster in pH-drop by different amount and production treatment of the applied *Lactobacillus sakei.* (Faster version of T-SC-150)
FAST-301	*Lactobacillus sakei* *Staphylococcus xylosus* *Staphylococcus carnosus*	This is the fast version of TRADI-302. Acidification features as mentioned under F-SC-111.
F-1	*Pediococcus pentosaceus* *Staphylococcus xylosus*	Same features as T-SPX, but faster in pH-drop by different amount and production treatment of the applied *Pediococcus pentosaceus*. (Faster version of T-SPX)
BFL-F02	*Pediococcus pentosaceus* *Staphylococcus carnosus ssp.*	This is the fast version of BFL-T03. Acidification features as mentioned under F-1.
BFL-F04	*Lactobacillus sakei* *Staphylococcus carnosus ssp.* *Staphylococcus carnosus ssp.*	The sucrose positive *Lactobacillus sakei* shows a very good growth potential and is able to suppress the growth of a lot of indigenous bacteria. The combination of the two new developed *Staphylococci* gives a very good color formation and a more intensive, but mild aroma development. This special combination of the strains shows a fast pH-drop and leads to a firm texture.
BFL-F05	*Lactobacillus sakei* *Staphylococcus carnosus ssp.* *Staphylococcus carnosus ssp.*	Same features as F-SC-111, but this new developed Gives a stronger and more intensive fermentation flavor.
SM-194	*Pediococcus pentosaceus* *Lactobacillus sakei* *Staphylococcus xylosus* *Staphylococcus carnosus* *Debaromyces hansenii*	Multi application culture that combines all positive features of the different strains. *Lactobacillus sakei* with very good growth potential and the ability to suppress the growth of a lot of indigenous bacteria. *Pediococcus pentosaceus* by its mild lactic acid taste and the accelerated pH-drop at higher temperatures. The combination of two different *Staphylococci* for more intensive color formation and mild aroma development. And the yeast *Debaromyces hansenii* on top to obtain a more "Mediterranean" flavor.
LHP	*Pediococcus pentosaceus* *Pediococcus acidilactici*	Extra fast culture targeted for fermentation temperatures 26–38°C (80–110°F).
CSB (pellets)	*Pediococcus acidilactici* *Micrococcaceae ssp.*	Extra fast culture targeted for fermentation temperatures 30–45°C (86–115°F).
HPS (pellets)	*Pediococcus acidilactici*	Very fast culture targeted for fermentation temperatures 32–45°C (90–115°F)

Starter cultures for flavor enhancement and nitrate reduction

Sausages fermented with a chemical acidifier such as GdL or encapsulated acid (see *Mince ingredients*, pg 5.) instead of lactic acid bacteria generally require added staphylococci or *Micrococcaceae spp*. to obtain acceptable flavor and color, see table below. In general, those single strain cultures are recommended in all sausage products in need of extra flavor or nitrate reductase activity. *S. carnosus* is more salt tolerant than *S. xylosus* and convey a more intense flavor in fast fermented products.

Culture name	Bacteria included	Characteristics
CS-300	*Staphylococcus carnosus ssp.* *Staphylococcus carnosus*	The combination of the two different *Staphylococci* leads to intensive color formation and color stability. Furthermore it gives a mild and round aroma. The high concentration of both *Staphylococci* gives high nitrate reductase activity.
S-B-61	*Staphylococcus carnosus*	For a good color formation and color stability and additional flavor development.
S-SX	*Staphylococcus xylosus*	For a good color formation and color stability and additional flavor development. Especially suitable in case of too much and undesired acidification taste.

Starter cultures for surface coverage

South European style sausages covered with mold on the surface will profit from being inoculated with a standardized culture, thus preventing mycotoxin formation by contaminating molds. Additionally, the on-set of mold growth will be faster and a more uniform coverage will be obtained.

The penicillia tabulated below were selected to have toxin free growth features and different appearances under the same conditions.

Culture name	Bacteria included	Characteristics
Mold 500	*Debaromyces hansenii* *Penicillium nalgiovense*	Moderate to medium growth. Sparse, short coverage with marbled appearance. Rich Italian style flavor formation for long ripened, dry, fermented sausages. Moderate suppression of indigenous flora.
Mold 600	*Penicillium nalgiovense*	Fast growing and strong suppression of wild flora. Dense, medium fluffy and uniform coverage. Traditional white coverage. Pronounced mushroom flavor.
Mold 700	*Penicillium nalgiovense ssp.*	Fast growing and suppressing wild flora. Dense, short and very white coverage. Avoids the traditional talcum stage. Neutral flavor.
Mold 800	*Penicillium candidum* *Penicillium nalgiovense*	Fast growing and strong suppression of wild flora. Dense, medium to very fluffy coverage. Generates a fresh camembert aroma / strong mushroom flavor and a typical scent of moss. Good growth potential in dry and unstable growth conditions.
Mold 900	*Penicillium nalgiovense ssp.* *Penicillium nalgiovense*	Early coverage and a very white and powdery look. Suitable for marbled appearance. Nice South European Felino-like aroma. Flexible toward varying growth conditions.

Starter cultures for bio-protection

Contamination of meat products by *Listeria monocytogenes* appears to be an increasing problem. Chr. Hansen has developed several cultures for fresh as well as cooked and cured meat products to lower the general level and/or reduce surface contamination. E.g. F-LC for fermented sausages is a patented culture blend capable of acidification as well as preventing growth of *Listeria*, and it operates in a wide temperature range.

Culture name	Bacteria included	Characteristics
F-LC	*Pediococcus acidilactici* *Lactobacillus curvatus* *Staphylococcus xylosus*	Culture for acidification and prevention of *Listeria*. Applicable at a wide temperature range. *Pediococcus acidilactici* and *Lactobacillus curvatus* give a controlled, moderate pH-drop with a mild acidification flavor. The used *Staphylococcus xylosus* gives good color formation and stability and a very round and mild flavor. Application in: Fermented sausages
B-LC-20	*Pediococcus acidilactici*	Adjunct culture for prevention of Listeria for use on top of existing starter cultures. Application in: Fermented sausages
B-LC-35	*Pediococcus acidilactici* *Lactobacillus curvatus* *Staphylococcus xylosus*	Culture for acidification and prevention of *Listeria*. *Pediococcus acidilactici* and *Lactobacillus curvatus* give a slow, but controlled pH-drop with a mild acidification flavor. The used *Staphylococcus xylosus* gives good color formation and stability and a round and mild flavor. Application in: Fermented sausages
B-LC-48	*Lactobacillus curvatus*	Homofermentative Grows well at temperatures down to 4°C and survives freezing. Removes oxygen, produces bacteriocin and suppresses growth of spoilage and pathogenic bacteria Application in: Sliced cooked hams, Sliced cooked emulsified sausages, Fresh sausages, Cold fermented sausages Spreadable sausages, Fresh meat products
B-2	*Lactobacillus sakei*	Homofermentative Grows well at temperatures down to 2°C and survives freezing. Removes oxygen, produces inhibitory organic acids and suppresses growth of spoilage and pathogenic bacteria Application in: Fresh sausages, Cold fermented sausages Spreadable sausages, Raw cured meat products Fresh meat products, Cooked ham process

How to choose the correct culture

In order to choose the correct culture the following advise may be used as general guidelines:

What style of sausage is produced?
- Traditional South and North European: choose cultures in *Traditional fermented sausages*, pg 30.
- North European fast fermented: choose cultures in *Fast fermented sausages*, pg 32.
- US style: choose the extra fast and very fast cultures in *Fast fermented sausages*, pg 32.

A very short on-set of fermentation is needed
- Choose a frozen culture instead of a freeze-dried culture
- Increase the amount of culture

The salt-in-water percentage in the fresh mince is:
- > 6% : avoid F-1, LHP, BFL-F02, BFL-T03 and T-SPX

The type of sugar is:
- Glucose: all cultures will ferment.
- Sucrose: avoid TRADI-302, T-SC-150, BFL-F05 and F-SC-111

Nitrate is added as a color forming agent to the mince
- Choose cultures in *Traditional fermented sausages*, pg 30 and *Fast fermented sausages*, pg 32, and adjust the process correspondingly to traditional/slow fermentation
- Add extra *staphylococci* or *Micrococcaceae spp*. from *Starter cultures for flavor enhancement*, pg 34, to enhance nitrate reductase activity

A product with an intense flavor
- Choose traditional technology and cultures from *Traditional fermented sausages*, pg 30.
- Add extra *staphylococci* or *Micrococcaceae spp*. from *Flavor enhancement and nitrate reduction*, pg 34, to enhance flavor formation

For more detailed product information, please visit www.chr-hansen.com or contact meat@chr-hansen.com or your local representative.

Troubleshooting guide

pH

Too low final pH

- Failure to monitor acidification.
- Too much sugar has been added.
- The heat procedure used to retard fermentation (US cooking procedure) has been insufficient.

ACIDIFICATION

Too slow acidification

- Frozen culture has been allowed to thaw and subsequently held too long before dispensing into meat. I.e. the culture has exhausted nutrients in the can, reduced pH and partly inactivated itself.
- Temperature during fermentation has been inconsistent with recommended culture optimum – been too low or too high.
- Secondary growth of contaminating microorganisms has outgrown the culture or produced components that buffered the pH-drop.
- Prolonged storage of the mince at cold temperatures (laid down) has resulted in an extended lag phase at the beginning of the fermentation cycle.
- Sausages entering the climate chamber have been colder than normal, resulting in prolonged lag phase of the starter culture.
- Spice formulation adjustment has either decreased acid stimulation or inhibited the culture.
- Excessive salt or cure addition has inhibited the culture.
- Direct contact of culture with salt or curing components has inactivated the culture.
- High fat formulation has reduced the moisture content and thereby the water activity too much.
- Larger diameter product has given slower heat transfer.
- Too rapid moisture loss in the product has decreased water activity too early.
- Insufficient carbohydrate source has been added to the sausage mixture.

Too fast acidification

- Temperature has been higher than normally.
- Spice formulation adjustment has favored the culture.
- Excessive water addition has increased water activity.
- Sausages have been delayed prior to entering the climate chamber resulting in higher initial temperature.
- Leaner product has given higher moisture content and thereby higher water activity.
- Change of meat (from beef to pork) has resulted in lower initial pH.
- Smaller diameter product has been processed at higher humidity than normally
- Initial meat pH has been lower than normally.
- Combination of sugars has been wrong.
- Drying has been slower than normally allowing for longer acidification time.

ACIDIFICATION

Inconsistent acidification from batch to batch

- Inadequate distribution of culture has resulted in "hot" and "cold" spots in the meat mixture.
- Distribution of the culture, salt, cure, spices, dextrose has been inconsistent.
- Stored (laid down) mince has caused some of the mince to dry out.
- Temperatures within the sausages have been diverse.
- Batches have been made with different spice formulations, meat components, casing diameters, pH or water/fat content.
- Temperature/humidity in the fermentation and/or drying room has been uneven from batch to batch.

No acidification

- Culture has not been added.
- Culture has been inactivated by direct contact with salt, cure components, or heavily chlorinated dilution water.
- Culture has been exposed to high temperature during transportation or storage.
- Non-compliance with recommended handling temperatures after thawing the frozen culture.
- Insufficient amount of sugar has been added to the sausage mixture.
- Excessive amount of salt has been added to the mince or the fat content is unusual high.
- Antibacterial agents have unintentionally been added to the meat mixture (preservative, chemical boiler treatments via steam, antibiotics in meat).

MOISTURE

Insufficient moisture loss

- The relative humidity during drying has been too high.
- Excessive air speed and/or too low relative humidity in the beginning of the process has created a dry rim so that moisture cannot pass from the inside to the surface.
- Excessive smoke too early in the process has coagulated the surface proteins thereby retarding moisture migration.
- The pH has been too high, i.e. the acidification has not taken place.
- Smearing has prevented moisture loss.
- Fermentation temperature has been too high; I.e. has commenced fat melting thereby smearing inner side of casing and preventing moisture to pass to the surface.

Too much moisture loss

- The product has been dried excessively – the air velocity has been too high or the relative humidity too low during the drying cycle.
- The acidification has been too fast.
- The applied starter culture has been too fast and thereby the pH too low

FLAVOR

Souring of product, post-processing

- The heat treatment to destroy the microorganisms (US cooking process) has been insufficient.
- Excess residual sugar has permitted secondary fermentation.
- Insufficient drying.
- Temperature has been abused post-packaging.

Off-odor

- Microbial contaminants have either grown during fermentation or post-packaging.
- Spoiled meat raw materials have been used.
- Poor sanitation post-processing.
- Chemical contaminant.

COLOR

Discoloration /green or gray coloration

- *Staphylococci* or *Micrococcaceae* have not been added.
- Meat pigments have been oxidized via microbial contaminants or metal ions in dirty salt.
- The sausages have been exposed to sunlight.
- The sausages have a too high pH.
- Excessive amounts of peroxide-forming bacteria have been present in the sausage
- Too low amount of nitrate/nitrite has been added.
- Acidification has been too fast.
- The raw materials have been spoiled.
- Chemical acidifier has been used.
- There has been too much potassium sorbate in the casing.
- There has been growth of yeast on the surface.
- The smoking temperature has created gray/ brown rim.
- Smearing or dry rim have prevented water loss giving spoiled (gray) center.

TEXTURE

“Mushy” product

- Over-working during mince production.
- Excessive fat extension.
- Insufficient salt level or no salt added.
- Spoiled raw materials.
- Proteolytic microbial contaminant.

‘Slimy, gassy product

- Yeast or heterofermentative lactic acid bacteria contamination in package post-processing.
- Excessive moisture content.
- Inadequate smoke concentration on the surface of the product.

Greasing (fat melting)

- Too high heating rate (US cooking process).
- Too high fermentation temperature.
- Unstable mince, low-binding meats.
- Overworking raw mince.

inside back cover

Reference list and further information

Books, book chapters and journal reviews

Books

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Available from Chr. Hansen on request:

- Product Information sheets and specifications for each culture
- Bulletins on diverse subjects within sausage fermentation
- Recipes within US, North European and South European technologies