Diversity of microflora in meat and meat products

Cukon[1], N., Ž. Cvrtila Fleck[2], M. Bratulić[3], L. Kozačinski[2], B. Njari[2]

professional paper

Summary

The term meat on the market or in processing implies muscles (muscle tissue) with skin or without it, depending on the kind of cattle, poultry or game, with belonging adipose or connective tissue, bones and cartilages, blood and lymphatic vessels, lymph nodes and nerves in a natural connection. Considering the chemical content, meat is a very favorable medium for growth and reproduction of different microbes. The main sources of primary (initial) microflora of meat are the animals themselves, workers who manipulate with meat, and production environment. Gram- negative bacteria, including intestinal bacteria Escherichia coli and Salmonella spp., and Pseudomonas spp., prevail in primary microflora, while out of gram– positive bacteria, lactobacilli and enterococci are most frequently found. On a fresh carcass there could be expected a high mold count, of which Penicillium, Mucor and Cladosporium prevail, and Candida and Rhodotorula genera of yeasts. The survival of primary microflora of meat depends on the degree of contamination (total microbial count), type of microorganisms, chemical content and pH value of meat, then temperature and the presence of oxygen.

On the one hand microorganisms are the causative agents of meat decay, but on the other, they contribute to ripening of meat, as well as to the final color, taste and odor of meat and meat products. Being familiar with optimal conditions of growth, development and reproduction of individual microbes enables managing the quality and sustainability of meat and meat products, as well as creating technological procedures of processing and methods of preservation. It is also a basis for a functional use of microbes in the production of meat products.

Keywords: *meat, microorganisms, microflora*

Introduction

As an irreplaceable source of protein, fat, vitamins, minerals and other valuable nutrients, meat has always been an important factor of survival and a social standard criterion. The meat on the market or in processing implies muscles (or muscle tissue) with or without skin depending on the kind of cattle, poultry or game, with the belonging adipose or connective tissue, bones and cartilages, blood and lymphatic vessels, lymph nodes and nerves in natural connection (Anon., 2007). Due to its chemical composition (Table 1), meat is a very favorable medium for growth and propagation of microbes. Meat is a very perishable food and if it is not stored, processed or packed well, it becomes "dangerous" for consumption very soon (Lambaša- Belak et al., 2005). But, sarcolemma, which envelops a muscle tissue, protects it from a breakthrough of microorganisms at the same time. Proteolytic enzymes (of endogenous and microbial origin) decompose sarcolemma in the process of ripening of meat, "releasing" the content of a muscle cell along the way. The activity of proteolytic bacteria in this sense mainly depends on their count, i.e. on the quantity of proteolytic enzymes which are produced by the aforementioned bacteria. The degree of microbial contamination of meat largely depends on health condition of the animal immediately before the slaughter, as well as on the application of appropriate hygienic measures in processes of slaughter, handling and storage of meat. The combination and interaction of internal and external factors of contamination determine the microbiology of meat (McDonald and Sun, 1999). The population of microorganisms which colonizes a certain area is called a microbial community or microflora. Microorganisms of meat, as well as of other food, can be divided into three basic groups: useful, harmful and pathogenic. Useful microorganisms take part in processes of processing and ripening of products by their metabolism (e.g. lactic acid bacteria). Harmful microorganisms lead to rotting of meat and cause degradation of aroma, texture and color of meat by their growth and enzymatic reactions. Pathogenic microorganisms cause different diseases which can be more or less life threatening (Šumić, 2009).

[1] **Andrijana Kegalj, BSc, Marina Krvavica, PhD, Iva Ljubičić, DVM, univ. mag. med. vet.**, University of Applied Sciences "Marko Marulić" Knin, Kralja Petra Krešimira IV 30, 22300, Knin, Croatia; tel: ++385 (0)22 668 123; e-mail:akegalj@veleknin.hr

Primary microflora of meat

Primary microflora of meat is primarily the result of the methods and conditions of slaughter and carcass processing and it is primarily connected to transferring microorganisms from the skin of an animal to carcass during slaughter and processing. The main sources of primary microflora of meat are the animals, workers who manipulate with meat, tools and equipment, as well as the production environment in a slaughterhouse. The most frequent sources of primary microflora of meat are external surface of the body (skin, fleece, feathers, hair, hooves, etc.) and digestive and respiratory organs. Therefore, microbiological quality of a processed carcass primarily depends on a health condition and external microflora of an animal, then on hygienic conditions of slaughter and processing. As the consequence of applying necessary procedures in slaughterhouse processing (especially skinning and evisceration) and due to the contamination from the "outside", there progressively grows the microbial count on the carcass in the process of slaughter and processing of animals, whereas the later growth of microbial count is connected to a further reproduction of the existent microflora (Mead, 2007). The listed reproduction implies several phases. During the initial so-called lag phase microorganisms adjust to a new environment gradually, there is no reproduction, i.e. a decline in their count is possible. There follows a logarithmic or exponential phase where microorganisms are reproducing at a maximum and constant rate. After nutrient medium is depleted by microorganisms, cells stop dividing (stationary phase), after which there follows the so-called extinction phase (Aleksić et al., 2010).

Muscle tissue and internal organs of healthy animals which do not have a direct contact with the environment are considered to be sterile. But, during the process of slaughter and carcass processing, microorganisms can be "transmitted" to blood, tissue and internal organs. By slaughtering sick or tired animals and due to sepsis or a bacterial infection of an animal during slaughter, muscle tissue and internal organs can contain specific pathogens, causative agents of diseases (Gracey et al., 1999). In primary microflora of meat there prevail gram-negative bacteria including intestinal bacteria Escherichia coli and Salmonella spp., and Pseudomonas spp., while out of gram– positive bacteria, lactobacilli and enterococci are most frequently found. Due to a wide prevalence in the environment, on a fresh carcass there could be expected a high mold count, of which Penicillium, Mucor and Cladosporium prevail, and Candida and Rhodotorula as the most frequent genera of yeasts (Jay et al., 2005). Pathogenic microorganisms which the animal itself is the source of can also be found in primary microflora, but their count should be insignificant. Majority of main causative agents of food poisoning (*Salmonella* spp., *Clostridium perfringens*, *Escherichia coli* i *Campylobacter jejuni)* originate from a fecal contamination of carcasses. Other causative agents, such as *Listeria monocytogenes* can originate from an animal, but they are most frequently transmitted from a production environment. The microbial count which can be found on a carcass also depends on the part of carcass where the samples are taken from. So, for example, a larger microbial count can be found on ribs, hindquarter and neck (Mead, 2007). *The Regulation on microbiological criteria for foodstuffs* (Anon, 2008) prescribed the position of the sampling site on a carcass, as well as maximum allowable aerobic mesophilic bacteria count (to 10^4 CFU/g).

Primary microflora of red meat (before the storage, i.e. cooling) consists of different kinds of mostly mesophilic microorganisms (sorted by frequency): *Micrococcus*, *Pseudomonas*, *Moraxella/Acinetobacter, Lactobacillus, Flavobacterium coryneforms,* yeasts, enterobacteria, *Staphylococcus*, *Kurthia*, *Streptococcus, Bacillus* and *Brochothrix thermosphacta.* But, primary microflora can also contain some psychrotrophs (*Pseudomonas* spp., *Moraxella* spp.*)* because low temperature favors them and they can cause rotting of meat. The share of psychrotrophs present in primary microflora shows seasonal variation and ranges from about 1% in summer months to 10% in winter. Generally, yeasts and molds don't affect the rotting of meat, except the fact that they can, in certain conditions, slow or prevent the growth of bacteria (Fernandes, 2009). The survival and growth of the primary microflora depend on a number of factors:

- the total count of present microorganisms,
- the kind of microorganisms,
- temperature,
- pH-value of meat,
- the presence of nutrients and
- the presence of oxygen (Gracey et al., 1999).

Immediately after the slaughter, on a processed carcass there prevail gram- negative proteolytic bacteria which decompose proteins and create an unpleasant smell (rotten eggs) and taste along the way (Anon, 2011), whereas saprophytic gram- positive bacteria (e.g. *Kurthia* and non – toxic staphylococci) are far less frequent. Pathogenic and toxic bacteria from intestines of a healthy animal or organs of a diseased animal can contaminate the carcass during processing or the contamination can appear crosswise from the hands of the staff or animal's skin (*Staphylococcus aureus*, *Listeria monocytogenes*, *Group A streptococci*, *Clostridium* spp.). Considering the fact that the presence of these bacteria can represent a health hazard to consumers, the possibility of controlling their growth should be taken into account when determining practical hygienic measures (Holzapfel, 1998). When the count of live bacteria grows to about 10^7 CFU/cm^2 of meat, there appears an unpleasant smell, whereas mucus appears when their count grows to about 10^8 CFU/cm^2 (Olofsson et al., 2007).

The fact that survival and growth of microorganisms depend on the composition of nutrient medium (water activity and share of nutrients), pH value, the presence of oxygen, temperature and the presence of other microorganisms (antagonism and synergism), is very well known. The optimum pH for most bacteria is 6.5 – 7.0, even though most microorganisms can grow in a wide range of pH values (4.0 to 9.5) and acidophilic bacteria can even survive <pH 4.0. The reproduction rate of microorganisms also depends on water content in meat which is accessible for biochemical reactions and is expressed as water activity (a_w). The water activity of fresh meat is greater than 0.97 and optimum a_w for most bacteria is from 0.93 to 0.99 (Table 2). The growth of most bacteria is limited at a_w <0.90, so in such medium fungi prevail (Table 2). The lag phase of microbial growth is extended by decreasing a_w of meat.

Psychrotrophic *Pseudomonas* spp., which dominates in microbial population of fresh and unprocessed meat, grows under aerobic conditions and cooling temperature below 0°C. In anaerobic conditions it can substitute oxygen by nitrite, as the final electron acceptor. When their count increases to 10^7 - 10^8 CFU/cm^2, an unpleasant smell and mucus appear. The unpleasant smell is the first sign of meat rotting and it appears during fermentation process, which is called fetid ripening. Fruity smells appear after the initial cheesy and buttery smells, and after *Pseudomonas* spp. spends glucose, it moves over to metabolizing nitrogen compounds such as amino acids (cystine, cysteine, methionine) and sulfur compounds smells appear. Later, the production of ammonia and amines grows with the growth of pH and there appears visible mucus on the surface (Krznarić et al., 2006). Many species synthesize soluble yellow-green pigment which fluoresces in UV area. *Pseudomonas phosphorescence* causes shimmering, i.e. luminescence already after 7 to 8 hours on meat and it is an important indicator of the absence of rotting bacteria because those photo-bacteria are their antagonists (Živković, 1986).

At the end of microbiological shelf life of meat, only one or several microbial species dominate in microflora. The shelf life of raw meat is under the influence of a couple of main factors:

- the initial microbial count (especially of those with rotting potential),
- species/genus of present microorganisms,
- storage temperature ("lower is better" – meat can be cooled to about -2°C befor being frozen),
- gaseous atmosphere (Mead, 2007).

The proven ways for controlling microflora of fresh meat, and so for preserving meat for a later consumption are the methods of salting and dry- curing. Salting is one of the oldest forms of food preserving. Salts like sodium chloride, sodium nitrite and sodium nitrate inhibit proliferation of gram- negative bacteria and improve the growth and reproduction of gram- positive bacteria. This "microbial inversion" which appears during salting and dry- curing enables "accidental" original fermentation cultures for all salted meats and fermented meat products (Anon., 2011). Salt deprives water from microorganisms and the consequences are the stopping of growth and dying of bacteria. Most halophilic bacteria are not pathogen for humans, except for some representatives of *Vibrio, Salmonella, Bacillus, Pseudomonas, Staphylococcus* and *Clostridium* genera. Micrococci, enterococci and lactobacilli prevail in cured meat (Mioković and Zdolec, 2004).

Lactic acid bacteria

Most gram- positive bacteria found in meat and meat products phylogenetically belong to the so- called *"Clostridium"* branch which is characterized by a low content of guanine and cytosine in DNA (<54%). Within clostridium branch there are lactic acid bacteria (LAB), then aerobic and facultative anaerobic bacteria: *Bacillus*, *Staphylococcus*, *Listeria* and anaerobic bacteria *Clostridium*, *Peptococcus*, *Ruminococcus*. Along with some exceptions, LAB don't synthesize catalase, even though in a medium with a low sugar concentration they can synthesize some pseudo-catalase (Holzapfel, 1998). Lactic acid bacteria comprise groups of gram- positive cocci, coccobacilli or bacilli which ferment carbohydrates to ATP (energy) and lactic acid. They don't contain cytochrome system and they can't synthesize ATP by a respiratory chain but only through fermentation (Khalid, 2011). They are mostly mesophilic, but they can also grow at temperatures lower than 5°C or higher than 45°C. For their growth they need amino acids, nitrogenous bases and vitamin B complex. Lactic acid bacteria are very heterogeneous. There are more than 60 genetically and physiologically different species which are divided physiologically into two groups, depending on the metabolic pathway of glucose degradation:

- homofermentative bacteria (e.g.

Lactococcus and *Streptococcus*) convert glucose to two molecules of lactate, i.e. up to 90% of lactate appears by their metabolism, and

- heterofermentative bacteria (*Leuconostoc* and *Weissella*) which metabolize glucose to lactate, ethanol and carbon dioxide, i.e. a smaller quantity of lactate appears by their metabolism (about 50%) (Beasley, 2004; König and Fröhlich, 2009).

According to Jin et al. (2009), four main genera belong to Lactic acid bacteria: *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* but, according to a newer revision of taxonomic classification, the following genera of LAB were added to them: *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Globicatella*, *Lactococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella*.

On the one hand, lactic acid bacteria are the most frequent causative agents of meat rotting, but at the same time, they are used increasingly for preserving of meat, mostly through fermentation. If there is a high moisture on the surface of the meat and a relatively high temperature of meat storage, the growth and development of LAB can lead to the appearance of mucus which becomes visible when their count exceeds 3-5x10^7 CFU/cm^2 (Živković, 1986; Krznarić et al., 2006). Except for the appearance of mucus, LAB can lead to acid fermentation which causes an unpleasant smell and taste of meat, grey color at the cross section and softness of meat. Acid fermentation can also be a consequence of improper bleeding or of postponing the process of cooling. During a spontanous fermentation of meat, LAB produce lactic acid from the raw material and lead to a decrease in pH value of meat (to 5.9 – 4.6). Due to increased acidity, muscle proteins coagulate which leads to the decrease in elasticity and hydration ability of muscle protein. On the other hand, a decrease of pH prevents the growth and development of many bacteria, including spoilage ones.

Today, contemporary meat industry must ensure high quality, decrease variability and improve organoleptic traits of fermented meat products, which cannot be achieved by using a spontaneous fermentation. In the last 60 years starter cultures which shorten the fermentation time have developed, whereby a low content of nitrite and nitrate in end product is ensured, as well as the standardization of organoleptic traits. Most commercially available starter cultures are mixtures of lactic acid bacteria and staphylococci and/or micrococci (Hugas and Monfort, 1997). Researches on antimicrobial activity of LAB have been intensified for the last couple of years. Except for organic acids, hydrogen peroxide and carbon dioxide, they produce and excrete reuterin and bacteriocins. Organic acids and reuterin inhibit the growth of most microorganisms, whereas bacteriocins primarily have bactericidal activity. Bacteriocins are synthesized on ribosomes of bacteria and have a great potential for practical application in food preserving, but also for prevention and treating of bacterial infections (Mallesha et al., 2010). Most LAB which take part in fermentation of meat synthesize bacteriocins which act against other LAB and

Table 1 **Chemical (nutritional) composition of 100 g of lean red meat**

	Beef	Lamb
Moisture (g)	73.1	72.9
Proteins (g)	23.2	21.9
Fat (g)	2.8	4.7
Energy (kJ)	498	546
Cholesterol (mg)	50	66
Thiamine (mg)	0.04	0.12
Riboflavin (mg)	0.18	0.23
Vitamin B6 (mg)	0.52	0.12
Vitamin B12 (μg)	2.5	0.10
Vitamin A (μg)	< 5	8.6
β carotene (μg)	10	< 5
Sodium (mg)	51	69
Potassium (mg)	363	344
Iron (mg)	1.8	2.0
Zinc (mg)	4.6	4.5
Magnesium (mg)	25	28

Source: Williams, P. G., 2007

Table 2 **Minimum a_w for growth of some microbial groups**

Kind of microorganisms	Minimum a_w
gram-negative bacteria	0,97
gram-positive bacteria	0,90
most yeasts	0,88
most molds	0,80
halophilic bacteria	0,75
osmophilic yeasts	0,61

Source: Rovira et al.,2006

toward the main pathogens in meat such as *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium perfringens*. Inhibitory activity of bacteriocin is based on forming pores in cytoplasmic membrane of cells and disturbing the synthesis of DNA and proteins (Olusegun and Iniobong, 2011). Bacteriociogenic strains which are a part of natural microflora of fermented sausages are *Lactobacillus curvatus* and *Lactobacillus sakei* which can take larger concentrations of salts and nitrites. Due to these characteristics, these two kinds are the dominant microflora of raw fermented sausages and pasteurized emulsified meat products such as frankfurters and cooked sausages. They are also an important part of microflora of meat and meat products which are stored in modified atmosphere and vacuum packaging at the temperature less than 5°C. Sulfide- producing strains *Lb. sakei* are frequent causative agents of spoilage of vacuum- packed meat which is stored at low temperatures. These two species of lactobacilli are used today as starter cultures in the industrial production of fermented sausages. *Lb. sakei* synthesizes bacteriocins which act inhibitory to *Listeria monocytogenes*, so it is used as protective culture against *Listeria* genus (Medić et al., 2009).

Pediococcus spp. is a homofermentative species which can handle higher salt concentrations (to 5.5%). As starter cultures there are used *Pediococcus acidilactici*, and *Pediococcus pentosaceus,* which has a lower optimum temperature than *P. acidilactici,* so it produces lactic acid more slowly and at lower fermentation temperatures from 15 to 27°C (Nežak et al., 2011). *Pediococcus cerevisiae* is used most often as starter culture in fermented sausages. *Tetragenococcus halophilus* (before *Pediococcus chalophilus*) grows in extremely salty conditions (NaCl concentration up to 18%) and it can be found in cured meats and cured bacon.

Leuconostoc spp. prevails in processed meat because they have a capability of fermenting carbohydrates at low temperatures and reduced redox potential. *Leuconostoc* spp. produces bacteriocins which destroy *Listeria* genus (Stiles and Holzapfel, 1997). According to Kozačinski et al. (2008), in naturally fermented sausages out of LAB there prevail *Lactobacillus plantarum*, *Lactobacillus curvatus*, *Lactobacillus sakei,* as well as *Pediococcus spp*. and *Leuconostoc spp.* There was also found a significant species count of *Staphylococcus* and *Kocuria* genera.

Family *Micrococcaceae*

Except for LAB, two species of *Micrococcaceae* family - *Micrococcus* and *Staphylococcus* are present in fermented meat products as natural starter cultures.

Micrococcus spp. contains nitrate reductase which reduces nitrates to nitrites that stabilize red color in meat products. Except for that, they are catalase- positive and contribute to the stabilization of color and taste of meat products by decomposing hydrogen peroxide which is a strong oxidation means. Some species, like *Kocuria varians* (before *Micrococcus varians*) are used as starter culture in salamis due to their contribution in the development of color and taste, and they also prevent the development of rancidity by improving the decomposition of hydrogen peroxide (Liu, 2011). *Micrococcus* spp. usually appears in meat products with longer ripening period (Frece et al., 2010).

Coagulase- negative staphylococci are very tolerant toward a salty environment and they can grow with or without the presence of oxygen. The most frequent strains in meat belong to the species *Staphylococcus carnosusi* and *Staphylococcus xylosus*. Coagulase- negative staphylococci reduce nitrates and nitrites whereby nitrosyl myoglobin appears, which is responsible for a characteristic color of meat products. Without the reduction of nitrates to nitrites, the meat wouldn't "transform" into a characteristic cured meat product. The main characteristic of staphylococci is their lipolytic and proteolytic activity during which there appear compounds of low molecular weight such as amino acids, peptides, free fatty acids, aldehydes and amines, which contribute to the development of a specific aroma of fermented meat products (Frece et al., 2010a; Rantsiou and Cocolin, 2006). These bacteria are mostly nonpathogenic and do not represent a hazard for consumers' health. *Kocuria* and staphylococci are more tolerant toward the increased concentrations of salts than LAB, which is the reason why they survive and develop in an environment with a significantly lower water activity, as it is the case with very salty products. But, these microorganisms are sensitive to acids and they have the tendency to die at lower pH values during and after the process of fermentation. Because of that, reduction of nitrates to nitrites takes place before fermentation, i.e. before LAB become the dominant microflora of meat. As LAB produce lactic acid, so staphylococci gradually die out (Anon., 2011).

The kind of microflora which prevails also depends on the time of ripening of fermented sausages. The sausages with a short period of ripening have more lactobacilli and a "sour" taste prevails in them, especially if the ripening period is shorter than two weeks. By a prolonged ripening there prevail coagulase- negative staphylococci and yeasts which lead to accumulation of different volatile compounds by their activity (Rantsiou and Cocolin, 2006). According to the research by García et al. (1995), there was determined that bacteria of Micrococcaceae family prevail in the microflora of sausages. Out of 159 isolates from Micrococcaceae family, 81% of them belong to *Staphylococcus* genus including *S. equorum* (43 %), *S. xylosus* (29 %), *S. saprophyticus* (11 %) and *S. simulans* (8 %). The rest of 11% are the bacteria of *Micrococcus* genus, and 8% were unknown species. They also determined that the share of *Staphylococcus* spp. and *Micrococcus* spp. changes depending on the concentration of salt and a_w.

Molds and yeasts in meat and meat products

Fungi are eukaryotic, nonphotosyntetic microorganisms which can be single- celled (yeasts) or multicellular (molds). Molds appear in the form of clusters of fibrous cells (hyphae) which are responsible for spider- like appearance of mold colonies. Yeasts are single- celled fungi whose colonies are smooth. Fungi are very adaptable to the environment and they can survive at places where most microorganisms can't. The manner of contamination by yeasts and molds is similar to the bacterial one. Immediately after the slaughter, the count of yeasts and molds is irrelevant and unimportant, especially due to their slow growth. According to Fernandes (2009), in the initial microflora of beef, the share of yeasts and molds amounts only 2.6% at 20°C. The most frequent genera of mold that can be expected on fresh meat are *Penicillium*, *Mucor* and *Cladosporium*, and *Candida* and *Rhodotorula* genera of yeasts.

Molds predominate on bovine carcasses when the surface is too dry for bacterial growth or in the case when beef cattle have been treated with antibiotics. In other words, molds almost never develop on meat if bacteria are free to grow. The reason for that is the fact that bacteria grow faster than molds, therefore they use available quantities of oxygen and nutrients which are needed by molds for their growth (Jay et al., 2005). At the temperature lower than 1°C, molds compete with bacteria. The optimal a_w for most molds is about 0.90, whereas some osmophilic molds grow at a_w of 0.60. Molds prevail if the surface of the meat is dry and a_w is below 0.90. Along with the growth above 0.90, bacteria and yeasts grow faster at the expense of molds. Except for that, molds can endure bigger changes in pH values than bacteria and yeasts, and they often tolerate higher temperature differences. Although molds progress the best when the pH is about 7.0, they can develop in the pH range from 2.0 to 8.0 (Šumić, 2009).

Molds from *Penicillium* genus are the most widespread on meat and meat products exactly because they can grow on lower temperatures and low a_w. *Penicillium camemberti* is white mold which is responsible for the characteristic aroma of Camembert and Brie cheeses, but also for the aroma of fermented sausages (Bruna et al., 2003). These molds act favorably in sausages in four ways:

- they have an antioxidative effect, protect from rancidity and influence the development and maintaining the color through the activity of catalases,
- they enable the development of a favorable microclimate on the surface of sausages, so the surface can't become sticky or mucous, i.e. they protect from a spontaneous colonization of unwanted molds, yeasts or bacteria,
- due to a lipolytic, proteolytic effect and decomposition of amino acids, they take part in the development of a characteristic taste and odor,
- they give sausages a typical appearance (López-Díaz et al., 2001; Martinović and Vesković – Moračanin, 2006).

Except for the positive effects during ripening of sausages, molds can also cause different negative effects, such as the appearance of green, brown or black spots which are unacceptable to most consumers, and some molds can have a negative influence to taste and odor of sausages (Sunesen and Stahnke, 2003). By contaminating meat with spores of different molds from the walls and/ or ceilings of cold storage, packaging and from the content of the gastrointestinal tract, molds can develop, which is mostly a superficial appearance. Molds cause lipolytic and proteolytic changes and are in favor of the development of spoilage bacteria (Živković, 1986). Molds of *Aspergillus* genus lead to the appearance of green spots on meat and most frequently they appear on moist surfaces of cooled meat. Grayish, spider- like *Mucor* mold appears on the surface of the meat stored at 1°C. Black mold *Cladosporum herborumi* can appear on frozen meat (Krznarić et al., 2006).

Many *Penicillium* spp. have the ability to produce antibiotic penicillin, which can increase the risk of an allergic reaction to that antibiotic. The most dangerous effect of some molds is a synthesis of mycotoxins which are toxic for humans and animals in small concentrations (Sunesen and Stahnke, 2003). According to the same authors, mycotoxins in meat products are located in the first 5 mm below the surface.

From the surface of salamis of different producers, numerous molds that don't produce mycotoxins were isolated, and *Penicillium* and *Scopularopsis* genera prevail among them. They are used most frequently in commercial starter cultures. Although many producers still produce different kinds of fermented sausages with a natural mold culture, using starter cultures of molds of researched functional characteristics is desirable due to a possible harmful activity of some species (Martinović i Vesković - Moračanin, 2006). In Mediterranean area molds (to some extent yeasts too) take an important place in fermentation of sausages (Italian salami, Hungarian salami,…) and ripening of prosciutto. The contribution of these microorganisms to the typical aroma of a product is the result of creating their primary and secondary metabolites. Lipases and proteinases are the most responsible for their synthesis.

The use of the selected strains of yeasts, the most frequently *Debaromyces hansenii* and *Candida famata,* are recommended for the production of fermented meat products that contain a larger quantity of salt. They have a favorable influence to the development of color stability and generally, to sensorial traits of products. Microflora of yeasts in sausages depends on the conditions of sausage production. In the production of fermented sausages yeasts contribute to the final sensorial characteristics of products by their metabolism by increasing the quantity of ammonia, decreasing the quantity of lactic acid, and proteolytic activities (Toldrá, 2008). The same as molds, yeasts also grow slowly, so they are irrelevant in terms of decay of fresh meat. But, due to a higher resistance to low temperatures, a_w and pH, the increased salinity and chemical preservatives, under certain circumstances they can become the dominant microflora of meat. Yeasts are ubiquitous organisms which are easily transferred to a carcass from the environment during slaughter of animals and manipulation with the carcasses. According to Samelis and Sofos (2003), the count and species of yeasts show seasonal variations, so *Rhodotorula* prevails on lamb carcasses in summertime, whereas *Candida* and *Cryptococcus* genera prevail in wintertime (and at storing temperature). According to the same authors, yeasts prevail on the surface of lamb carcasses at -5°C and their population grows from 10 to 10^6 CFU/cm^2 within 20 weeks. Bacterial population dominates by increasing the temperature to 0°C or more, and less than 5% of microflora of fresh meat are yeasts. Yeast population ranges from 10^2 - 10^3 CFU/g on a fresh, cut meat and 10^3 - 10^5 CFU/g in minced meat. The most important role of yeasts in meat rotting is their indirect influence by which they enable the growth of bacteria – causative agents of rotting. Yeasts decrease antimicrobial potential of sulfites and favor the growth of spoilage bacteria. The growth of bacteria is inhibited on pH of meat below 5.5 and yeasts grow. Because of that, a low pH favors the growth of yeasts in fermented meat, such as different salamis. Except for that, yeasts which grow in a wide a_w range, from 0.62 to 0.98, also prevail in cured meats which have a low a_w. Similar to that, yeasts use organic acids (lactic, citric, acetic acids), increase the pH and decrease the concentrations of preservatives, which favor the growth of spoilage bacteria again (Dillon and Board, 1991).

During ripening, yeasts (about 95%) prevail on the surface of the sausages, after two weeks molds and yeasts are present equally, whereas yeasts prevail toward the end of ripening (4 – 8 weeks) (Toldrá, 2008).

Conclusion

The fact that meat and meat products are a good medium for the growth of microorganisms is well known. Some microorganisms contribute to the appearance of desirable and specific organoleptic characteristics of meat and meat products, but at the same time their uncontrolled growth can cause numerous negative effects (deterioration of aroma, texture and color). Microorganisms contribute to the development of specific aroma and color of meat and meat products by their enzymatic activity. Commercial starter cultures are in use today, which ensure high quality, decrease variability and improve organoleptic characteristics of meat and meat products. Except for that, some bacterial species are interesting due to their metabolic activity and the production of bacteriocin. Bacteriocins act in a bactericidal manner, they have a large potential for a practical application in food preserving and prevention and treating bacterial infections.

The sources of microbiological contamination of meat are numerous, both external and internal. During the technological process of meat processing, cutting and chopping, the possibility of contamination increases and increased temperature contributes to the faster reproduction of microflora. In order to prolong meat sustainability, decrease the risk of rotting and prevent the contamination of meat and meat products, a constant control of production is necessary, i.e. taking measures and procedures which are based on principles of the Hazard Analysis Critical Control Point (HACCP) system along with the implementation of a good hygiene practices. Microbiological criterions, i.e. the lack, the presence or the count of microorganisms and/or the quantity of their toxins/metabolites are an extremely significant criterion at determining the acceptability of meat or meat products, i.e. their microbiological safety.

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Verschiedenartigkeit der Mikroflora in Fleisch und Fleischerzeugnissen

Zusammenfassung

Unter dem Begriff Fleisch wird auf dem Markt und in der Verarbeitungsindustrie folgendes verstanden: Muskelgewebe, mit oder ohne Haut, abhängig von Viehsorte, Geflügel oder Wild, mit dazugehörigen Fett- und Bindegewebe, Knochen, Knorpel, Blut- und Lymphgefäßen, Lymphknoten und Nerven in natürlicher Verbindung. In Bezug auf die chemische Zusammensetzung ist Fleisch ein ausdrücklich günstiges Medium für Wachstum und Vermehrung verschiedener Mikroorganismen. Die Hauptquellen der primären (anfänglichen) Mikroflora in Fleisch sind die Tiere selbst, Arbeiter die mit Fleisch manipulieren, sowie die Umgebung, in der die Herstellung stattfindet. In der primären Mikroflora sind die gramnegative Bakterien vorwiegend, einschließlich Darmbakterien Escherichia coli und Salmonella spp, sowie Pseudomonas spp, und von den grampositiven sind Laktobazillen und Enterokokken vorwiegend. An dem frischen Kadaver ist eine große Zahl von Schimmelsubstanzen zu erwarten, vorwiegend aus der Gattung Penicillinum, Mucor und Cladosporium, von den Hefesubstanzen Candida und Rhodotorula. Das Überleben der primären Mikroflora in Fleisch hängt vom Kontaminierungsgrad ab (gesamte Zahl der Mikroorganismen), Art der Mikroorganismen, chemischer Fleischzusammensetzung und pH, sowie Temperatur und Anwesenheit des Sauerstoffes.Die Mikroorganismen sind einerseits die Erreger des Fleischverderbens, andererseits bringen sie mit ihrem Metabolismus dem Fleischreifen, der Finalfarbe, dem Geschmack und dem Geruch der Finalprodukte bei. Das Kennen der optimalen Wuchsbedingungen, der Entwicklung und der Vermehrung von einzelnen Mikroorganismen ermöglicht die Herrschaft über die Qualität und Erhaltbarkeit von Fleisch und Fleischerzeugnissen, sowie die Kreierung der technologischen Verarbeitungsverfahren und Konservierungsmethoden. Sie stellen die Grundlage für die funktionale Nutzung der Mikroorganismen in der Herstellung von Fleischerzeugnissen dar.

Schlüsselwörter: *Fleisch, Mikroorganismen, Mikroflora*

Diversità della microflora nella carne e nei prodotti a base di carne

Sommario

Il termine ‘carne’ sul mercato sottintende il tessuto muscolare, con la pelle o senza (la cosa che dipende del tipo di bestiame, pollame o selvaggina) con l’appartenente tessuto grasso e connettivo, ossa e cartilagine, vasi sanguini e linfatici, nodi linfatici e nervi nella connessione naturale. Per quanto riguarda la sua composizione chimica, la carne è un medio straordinariamente addatto alla crescita e riproduzione di diversi microorganismi. Le fonti principali di microflora primaria (iniziale) della carne sono gli animali stessi, gli operai che manovrano la carne, e l’ambito produttivo. Nella microflora primaria prevalgono i batteri gram-negativi insieme con i batteri intestinali del genere Escherichia coli, Salmonella spp. e Pseudomonas spp. Dei batteri gram-positivi i lattobacilli e gli enterococchi sono i più frequenti. Sulla carcassa fresca è da aspettare un numero grande di muffa, dove prevalgono i rappresentanti dei generi Penicillium, Mucor i Cladosporium, e dai lieviti i generi Candida i Rhodotorula. La sopravvivenza della microflora primaria della carne dipende dal grado di contaminazione (il numero totale di microorganismi), tipo di microorganismi, composizione chimica, pH della carne e la temperatura e presenza di ossigeno.

I microorganismi sono da una parte quelli che causano la decomposizione della carne, e dall’altra contemporaneamente con il suo metabolismo aiutano la maturazione della carne, ed il colore, gusto e aroma di carne e di prodotti a base di carne. La conoscenza di condizioni ottimali per la crescita, sviluppo e riproduzione di certi microorganismi favoriscono la direzione qualità e sostenibilità della carne e di prodotti a base di carne, e la creazione di procedimenti tecnologici di produzione di alimenti e di metodi di conservazione, che sono fondamentali per l’uso di microorganismi nella produzione di prodotti a base di carne.

Parole chiave: *carne, microorganismi, microflora*

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Received: March 9, 2012
Accepted: April 4, 2012