The microbiology of meat spoilage: a review

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Abstract

The microorganisms most commonly involved in meat spoilage are *Pseudomonas* spp., the *Enterobacteriaceae* family, lactic acid bacteria and the *Brochothrix thermosphacta*. The factors that are considered to have the main effect on the speed of spoilage and the shelf life of fresh meat are intrinsic factors (initial number of psychrotrophs present on the meat surface, water activity, the pH value and the nutritional content) and extrinsic factors (storage temperature and oxygen availability). Despite our knowledge, uncertainty still exists regarding how and when individual bacterial species or strains are influenced by specific meat storage conditions. The results of studies of spoilage microbiota at both the species and strain level will make it possible to develop storage conditions that prolong the shelf life during which meat quality and safety can be guaranteed.

Pseudomonas, Enterobacteriaceae, Brochothrix thermosphacta, lactic acid bacteria, psychrotrophic Clostridium species

Introduction

Meat is a foodstuff that can be spoiled extremely quickly. Certain species of bacteria multiply easily on fresh meat thanks to its chemical composition, favourable water activity (a_w) value and pH value. Their numbers soon reach levels that cause sensory deviations and lead finally to spoilage of the meat (Doulgeraki et al. 2012). The role played by bacteria in meat spoilage at refrigeration temperatures has been studied since the 1930s (Jay et al. 2003). The initial microbial population on meat depends on the physiological state of the animal at the moment it is slaughtered and on the level of environmental contamination in the slaughterhouse and areas in which subsequent handling of the meat is performed, including the level of hygiene of employees and the tools and equipment used (Nychas et al. 2008; Serraino et al. 2012).

The growth of bacteria that cause food spoilage is influenced by a large number of factors, which can be divided into 4 groups (Bruckner et al. 2012):

1) intrinsic factors, which are an expression of the physical and chemical properties of the foodstuffs themselves (e.g. water activity, content of nutrients, the structure of the foodstuff)
2) extrinsic factors, i.e. storage conditions (e.g. storage temperature, the composition of the atmosphere)
3) processing factors (physical or chemical methods of treating foodstuffs during processing, e.g. cooking)
4) implicit factors, which are a reflection of the synergistic or antagonistic effects between bacteria

Intrinsic factors (the initial content of psychrotrophic bacteria present on the surface of the meat, the water activity, the pH value and the content of nutrients) and extrinsic factors (storage temperature and availability of oxygen) both play a role in fresh meat.

Only some of the bacteria that make up the initial microbial population on the animal carcass after slaughter contribute towards meat spoilage. Borch et al. (1996) state that

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only around 10% of the bacteria present on meat at the end of the slaughter process are capable of growing under refrigeration temperatures. The microbial fraction that is then able to cause meat spoilage is even smaller.

We still do not have sufficient knowledge to understand exactly how and when these bacterial species and strains are influenced by specific conditions in meat storage and what is their impact on the type and speed of spoilage (Doulgeraki et al. 2012). For this, it is necessary to characterise the bacterial microflora contributing to meat spoilage (the spoilage microbiota) at the level of individual species and even individual bacterial strains. It will be possible to develop storage conditions that extend the period of time during which meat quality and safety can be assured on the basis of the results of monitoring the amounts of the given microbial species (Pennacchia et al. 2011).

Temperature and bacterial growth on meat

The shelf life of meat can be extended and the growth of psychrotrophic bacteria limited by reducing the storage temperature (Marshall and Baľa 2001). When meat is stored at 0.2 and 5 °C, its shelf life falls to 70%, 50% and 30% of that of meat stored at –1.5 °C. Kameník et al. (2012) discovered the storage temperature to have a significant influence on the shelf life of meat in a modified atmosphere. While samples of meat stored at 5 °C were unsatisfactory in sensory terms after 9 days, with a microbial population of 8.99 – 9.98 $\log_{10}$ CFU.g^{-1}, meat stored at 3 °C showed no sensory deviations and the bacterial contamination was at least 3 logarithmic orders lower (CFU = colony forming units). These are living bacterial cells that form colonies, i.e. clumps of cells, during microbiological tests as a result of their growth on substrates. Their numbers are given either in absolute values – e.g. 10^7 CFU means 10 000 000, or as $\log_{10}$ CFU in 1 g, in which case 10^7 would be 7 $\log_{10}$ CFU or 1.00E + 07).

Table 1. Preferred temperatures for the growth of microbial groups (Feiner 2006)

Microbial group	Minimum temperature (°C)	Optimal temperatures (°C)	Maximum temperature (°C)
psychrophilic	-12	5 – 15	20
psychrotrophic	-8	20 – 25	35
mesophilic	5	30 – 45	50
thermophilic	35	45 – 60	70
extremely thermophilic	70	85 – 90	100

Temperature is seen to be the most important factor influencing spoilage and meat safety (Nychas et al. 2008). Transport from the moment of the product purchase by the consumer to the home is a weakness part in the distribution of meat. This phase is practically impossible to control and may involve fluctuations in temperature depending on the temperature of the external environment. The result may be a shortening of the shelf life given by the producer, who can guarantee low storage temperatures only during the phase of preparation, packing and transport to the retail.

All bacteria that cause meat spoilage use soluble compounds contained in muscle tissue for their growth, particularly glucose and amino acids. The preferred substrate is generally glucose. If it is present in the surface layers of the meat, no significant degradation of other substances will occur. When glucose ceases to be available, however, the bacteria begin to attack amino acids and a large quantity of ammonia and a lesser amount of organic sulphides and amines causing an unpleasant smell are released (Bell 2001).

The content of glucose in meat is a critical factor determining the relationship between the development of microflora causing meat spoilage and the time at which incipient spoilage appears. The concentration of glucose in meat with a normal pH value ranges from 100 to 1 000 $\mu g.g^{-1}$. Sensory deviations in meat (off-odour) begin to appear when the bacterial population reaches a density of around 10^8 CFU cm^{-2} in the case of an initial level of glucose in the muscle of approximately 100 $\mu g.g^{-1}$. If the glucose concentration is higher (1 000 $\mu g.g^{-1}$), then changes accompanying spoilage (off odour, slime) do not occur until the bacterial population reaches 10^9 CFU cm^{-2}. When the pH is high, the level of glucose will be low, and spoilage will occur when bacterial growth reaches a level of 10^6 CFU cm^{-2} (Bell 2001). Kameník et al. (2012) discovered sensory deviations accompanying spoilage of pork meat packed in a modified atmosphere (80% O_2; 20% CO_2) at values of 6.62 log CFU.g^{-1} of meat.

Bacterial groups on fresh unpacked meat

The most frequent bacteria to occur on fresh meat are bacteria of the genera *Acinetobacter, Pseudomonas, Brochothrix, Flavobacterium, Psychrobacter, Moraxella, Staphylococcus* and *Micrococcus*, lactic acid bacteria and various genera of the *Enterobacteriaceae* family. The survival and growth of these microbes is influenced, to a great extent, by the composition of the atmosphere surrounding the meat. Meat spoilage is, according to Pennacchia et al. (2011), most frequently caused by the following groups of bacteria:

1) *Pseudomonas* spp.
2) *Enterobacteriaceae*
3) *Brochothrix thermosphacta*
4) Lactic acid bacteria

It is common knowledge that meat can be spoiled quickly under aerobic conditions (in the presence of an atmosphere comprised of air). This is caused by the rapid growth of pseudomonades. Psychrotrophic species such as *Pseudomonas fragi, P. lundensis, P. putida* and *P. fluorescens* can be isolated from unpacked meat showing signs of spoilage (see Plate I, Fig. 1). *P. fluorescens* occurs more frequently on fresh meat, though during longer periods of storage *P. fragi* becomes dominant. Higher concentrations of CO_2 (10%) inhibit the growth of both *P. fluorescens* and *P. fragi* on red meat. *P. fragi* plays a significant role in meat spoilage; meat is even considered the ecological niche for this species. *P. fragi* represents the dominant species among the pseudomonades regardless of the packaging of the meat. All other species occur primarily on unpacked meat, i.e. under aerobic conditions. Bruckner et al. (2012) discovered *P. putida* accounting for the greater proportion on fresh pork and poultry meat under aerobic conditions (around 90% of *Pseudomonas* spp.), with *P. fluorescens* occurring less frequently. The population of pseudomonades attained values of 9 – 10 $\log_{10}$ CFU.g^{-1} after several days depending on the storage temperature. Doulgeraki and Nychas (2013) demonstrated *P. fragi* and *P. putida* as the principal species in minced beef.

A population of pseudomonades of 10^7 – 10^8 CFU.g^{-1} causes slime to form on meat and a bad smell to appear. Both these deviations appear, however, when pseudomonades exhaust the glucose and lactic acid in the meat and begin to metabolise nitrogenous compounds, particularly amino acids (Nychas et al. 2008). When the diffusion gradient of glucose from lower layers of the meat towards the surface no longer serves to cover the needs of a large number of bacteria, the degradation of amino acids and proteins begins, accompanied by the release of ammonia, amines and sulphides

(Koutsoumanis et al. 2008). The characteristic aroma of spoiling meat appears. The proteolytic activity of pseudomonades assists their penetration into the meat. In such case, the capabilities of proteolytic bacteria enable them to gain a competitive advantage over other bacterial groups or species, as they are able to gain access to new sources of nutrients that are not available in this way to microbes with weaker or no proteolytic properties (Nychas et al. 2008). Bruckner et al. (2012) stipulated a level of *Pseudomonas* spp. at which signs of spoilage are evident of 7.5 log CFU.g^{-1} on the basis of the sensory properties of pork and chicken meat. They used the term "microbial shelf life" to indicate the attainment of this value (Table 2).

Table 2. The shelf life of pork and chicken meat at various storage temperatures in aerobic conditions (Bruckner et al. 2012)

The microbial shelf life of meat as the attainment of a population of *Pseudomonas* spp. of 7.5 log CFU g^{-1} (hours)		
Temperature (°C)	Pork (loin)	Chicken (breast)
2	165.8	126.4
4	122.2	98.6
7	92.9	63.9
10	75.4	41.5
15	45.5	27.1

The genus *Shewanella*, which is also frequently present on meat, is a genus of microbes related to *Pseudomonas* spp. The species *Shewanella putrefaciens* releases substances characteristic of meat spoilage and also produces hydrogen sulphide (sulphane). This compound, in combination with muscle pigment, causes greening of meat. *S. putrefaciens* is considered the primary cause of spoilage in vacuum-packed meat stored at refrigerated temperatures, particularly at higher pH values.

Numerous species of the *Enterobacteriaceae* family have been found on beef, lamb, pork and chicken meat and offals. The genera *Serratia, Enterobacter, Pantoea, Klebsiella, Proteus* and *Hafnia* all contribute to spoilage. In view of its potential to spoil meat, the most important in this regard are *Serratia liquefaciens*, *Hafnia alvei* and *Enterobacter* (*Pantoea*) *agglomerans*. The genus *Serratia* is the most frequent genus of the *Enterobacteriaceae* family to be found on meat. *S. liquefaciens*, in particular, is isolated from meat stored in various atmospheres. *S. grimesii* and *S. proteamaculans* are other such species.

The species *Citrobacter freundii* and *Proteus vulgaris* have been detected in minced beef stored in an aerobic or modified atmosphere. *Hafnia alvei* is often found in minced meat in a modified atmosphere (MAP) or in a vacuum. Representatives of the genus *Rahnella*, which also plays a role in the spoilage process, have also been isolated from vacuum-packed beef and beef stored in MAP in the later stages of storage.

The microbiology of packed meat

Vacuum packaging and MAP favour facultative anaerobic bacteria, including lactic acid bacteria and *Brochothrix thermosphacta*. Lactic acid bacteria, in particular, are highly competitive in a modified atmosphere. The genera *Lactobacillus, Carnobacterium* and *Leuconostoc* may be associated with the spoilage of chilled meat. The psychrotrophic lactic acid bacteria occurring most frequently on meat include *Lactobacillus sakei*, which is considered an agent of spoilage of vacuum-packed meat and meat packed in MAP. It is clear from the expert literature that only psychrotrophic lactic acid bacteria, such as the species *Lactobacillus sakei, L. curvatus, L. fuchuensis, Carnobacterium divergens, C. maltaromaticum* and *Leuconostoc* spp., can attain large numbers of cells in meat in MAP and vacuum-packed meat stored at refrigeration temperatures. Species such as *L. sakei* and *L. algidus* have been isolated from vacuum-packed meat at 4 °C. If the temperature falls to 1 °C, *Lactobacillus* spp., *Weissella* spp. and *Leuconostoc mesenteroides* become the dominant species. The effect of temperature on the composition of the microflora under conditions that are otherwise identical is evident in this change (Doulgeraki et al. 2012).

On meat in storage, the genus *Carnobacterium* is represented by the species *C. divergens*and *C. maltaromaticum*. *C. divergens* has been isolated from samples of unpacked meat and vacuum-packed meat. Psychrotrophic *Brochothrix thermosphacta* is an important agent of meat spoilage. This species grows under aerobic conditions and on vacuum-packed meat. While this microbe has been isolated from samples of beef throughout the storage period, it has been isolated from vacuum-packed pork only up to the middle of the period of its shelf life. Lactic acid bacteria then became dominant, which points to their better competitive capabilities.

Nieminen et al. (2011) tested the development of a population of lactic acid bacteria in minced meat at 6 °C and packed in MAP (60% O_2, 25% CO_2 and 10% residual air). During the course of 10-day storage, the number of lactic acid bacteria reached a level of 8.5 – 8.7 log CFU.g^{-1}. Minced meat stored at 4 °C showed a population of lactic acid bacteria of 6.9 – 7.1 log CFU.g^{-1} and *Brochothrix thermosphacta* of 1.7 – 2.5 log CFU.g^{-1} after 11 days. After 19 days, the number of bacteria increased still further – for lactic acid bacteria to 8.6 – 8.8 log CFU.g^{-1} and for *B. thermosphacta* to 7.8 – 7.9 log CFU.g^{-1}. In contrast, the number of representatives of the family *Enterobacteriaceae* did not exceed 4.0 log CFU.g^{-1} even after the 19 days. It proved possible to demonstrate the presence of the species *Lactobacillus algidus, L. sakei, Leuconostoc gasicomitatum, L. carnosum, L. gelidum, L. mesenteroides, Carnobacterium divergens, C. maltaromaticum* and *Enterococcus raffinosus* in the population of lactic acid bacteria.

Pennacchia et al. (2011) analysed 9 samples of beef sirloin of a portion size of 500 g. After initial contamination was determined, the samples were divided into two groups – one was vacuum packed, the other stored under aerobic conditions at 4 °C. At the beginning of the experiment, the bacteria found most frequently on the meat were *Carnobacterium divergens, Brochothrix thermosphacta, Pseudomonas* spp. and *Psychrobacter* spp. After seven days of storage, *B. thermosphacta, Pseudomonas* spp. and *Photobacterium* spp. dominated in the unpacked samples. *Carnobacterium divergens* and *Pseudomonas* spp. were found in the vacuum-packed beef. *B. thermosphacta* was not isolated at all. The results were practically identical after 20 days of storage under aerobic conditions as after 7 days – *Pseudomonas* spp. (though not in all samples), more frequently *B. thermosphacta, C. divergens* and *Photobacterium* spp. The species *C. divergens* was present in all samples of vacuum-packed meat, *Photobacterium* spp. in 8 cases and *Lactobacillus algidus* in 7 cases. The population of *Pseudomonas* spp. reached a level of 5.6 – 8.1 log CFU.g^{-1} in unpacked samples after 20 days of storage. A sensory examination revealed that none of the samples stored under aerobic conditions was eatable after 7 days. Of 9 samples of vacuum-packed beef slices, 5 were acceptable in sensory terms even after 20 days; the remaining 4 displayed an unacceptable aroma and appearance. While the population of lactic acid bacteria reached a level of 4.3 – 6.6 log CFU.g^{-1} in the samples acceptable in sensory terms, lactic acid bacteria attained a population of as much as 8.0 log CFU.g^{-1} in the samples given a negative assessment. The species of the *Enterobacteriaceae* family most frequently isolated were *Serratia grimesii, Hafnia alvei* and *Rahnella*s pp.

Psychrotrophic clostridia in packed meat

Spoilage of vacuum-packed beef may also be caused by psychrotrophic clostridia. This type of spoilage is known as "blown pack spoilage" (BPS), which emphasises the characteristic feature accompanying this kind of spoilage – a strongly inflated package (Silva et al. 2012). Cases of spoilage of this kind have been registered in the USA, Canada, Brazil, New Zealand, South Africa, the United Kingdom and Ireland. The species *Clostridium estertheticum* is considered the most frequent agent of this type of spoilage. This bacterium has been detected in samples of packed beef and lamb meat and in game (Yang et al. 2011). This microbe uses glucose for its growth. Growth diminishes when the glucose has been exhausted, but the bacteria then ferment lactic acid, a process accompanied by the production of a large amount of gas. *C. estertheticum* does not grow at pH values < 5.5. The lactic acid bacteria that comprise the dominant part of the microflora of packed meat and play a part in its spoilage also use glucose for their growth and produce lactic acid, which leads to a drop in pH on the surface of the meat. In this way, lactic acid bacteria compete with *C. estertheticum* bacteria for glucose, and may also inhibit the growth of clostridia by causing pH values to fall. This phenomenon has been confirmed in tests performed by the authors mentioned above (Yang et al. 2011). *C. estertheticum* is not, however, the only species of clostridia to have been isolated from samples of vacuum-packed beef showing signs of BPS. Other representatives of this genus to have been demonstrated include *C. gasigenes, C. frigidicarnis, C. laramiense* and *C. algidixilanolyticum* (Silva et al. 2012).

Meat becomes contaminated with clostridia at the slaughterhouse. The sources of this contamination are particles of soil that remain attached to the skin or excrement. Clostridia get onto the surface of carcasses by means of direct or indirect contact. Psychrotrophic clostridia occur in the slaughterhouse environment and on the surface of meat in the form of spores. It is clear from the literature that even a single spore may cause BPS-type spoilage (Clemens et al. 2010). This highlights the necessity of observing strict hygiene in slaughterhouses. Another unpleasant fact is that clostridia can growth on meat even when refrigeration temperatures are maintained, even at the most extreme temperature used for storing meat in a chilled state, i.e. at –1.5 °C (Adam et al. 2011). During analyses performed by Silva et al. (2012), certain strains of clostridia demonstrated an ability to cause a loss of vacuum and inflation of the package after 6 weeks at 2 °C. The period of 42 days is not an incommensurately long period of time in the case of beef meat. Vacuum packing is used for ageing meat ("wet ageing"), and beef from South America normally has a shelf life of 90 days or even longer. In their tests described above, the authors demonstrated that increasing the temperature from 83 to 87 °C for a period of 3 seconds while the packing foil contracts delayed signs of BPS. The pH of samples showing signs of spoilage attained the value of 6.0 – 7.5, while the negative control retained its initial value of 5.5. Psychrotrophic clostridia were capable of causing an intense putrid aroma, proteolysis and strong gas production (Silva et al. 2012).

Another type of meat spoilage associated with clostridia is known as "bone taint", which is characterised by a putrid smell coming from inside the muscles where they join onto the bone. This smell is the result of the metabolic activity of clostridia capable of growing in the absence of oxygen. The species contributing to this type of spoilage in vacuum-packed pork and lamb meat are *C. algidicarnis* and *C. putrefaciens*.

A rare case of beef spoilage caused by type B *Clostridium novyi* has been described by Eeckhaut et al. (2012). They recorded beef spoilage in one processing plant in Flanders in Belgium in 2010 and 2011. The beef carcasses concerned came from bulls of the Belgian Blue breed fattened on three farms owned by a single owner. The animals were fed vegetable residues (boiled carrots, beans and potatoes) from industrial kitchens. No cases of illness had been recorded among the cattle housed at the farms. After slaughter at the slaughterhouse, the meat was passed as fit for human consumption and the inspectors found no deviations. An unpleasant smell reminiscent of eggs became apparent, however, after several days at 7 °C, and during cutting green meat was found in areas around the hip joint and shoulder, with the most intense changes located around these joints. The areas of altered muscle tissue were sharply demarcated from the normal tissue. Analysis of samples of the spoiled meat revealed gram-positive rods (approximately 2 x 0.5 μm) present in the interstitial connective tissue in the muscle and occasionally between muscle fibres. These tissues showed signs of autolysis. The agent of these changes was identified by PCR as *Clostridium novyi* type B. The greening of meat was caused by the production of sulphane (hydrogen sulphide - H_2S), which reacts with myoglobin to produce green sulphmyoglobin. The authors could not identify the origin of the meat contamination precisely. They considered it extremely unlikely, however, that it occurred post-mortem as only carcasses coming from cattle from the same three farms were affected during the given season of the year. Spores of *Clostridium novyi* type B were clearly present in the tissues of living animals and, for a reason that remained unknown, germination and the growth of vegetative cells occurred in the chilled meat. *Clostridium novyi* spores are known to be transported from the digestive tract to the liver, where they can be isolated even in healthy animals. Anaerobic conditions may induce the growth of these bacteria. No lesions that might indicate pathological changes while the animals were still alive were found in the skeletal muscle of the cattle. This case study first described the spoilage of beef caused by a new agent (Eeckhaut et al. 2012).

The cause of blown pack spoilage of packed meat need not necessarily be psychrotrophic clostridia. The literature also contains information about the involvement of psychrotolerant bacteria of the *Enterobacteriaceae* family. Spoilage occurs, however, at higher temperatures than in the case of clostridia. Representatives of the genera *Hafnia, Enterobacter, Serratia, Rahnella* and *Ewingella* have been isolated from samples of vacuum-packed lamb stored at 4 °C showing signs of BPS (Brightwell et al. 2007).

Conclusions

Meat spoilage caused by bacteria is a problem for producers, retailers and consumers alike. Due to its composition, meat is a product that spoils easily. The microflora on meat creates an ecosystem that is affected by a large number of variables, such as temperature, the composition of the surrounding atmosphere, the pH of the meat, etc. The mutual relationships between individual groups and species of microorganisms are also complex. A selection pressure is created during meat storage that provides an advantage to bacteria whose metabolic activity may lead to meat spoilage. The increasing standard of operational hygiene during meat handling and new forms of packaging are influencing the composition of the microflora that develops on meat. Our current knowledge allows us to use means that dramatically restrict the growth of bacteria and extend the shelf life of meat. On the other hand, biology always finds its own way (by the action of psychrotrophic clostridia, for example), and new agents that must be taken into consideration during meat processing and storage are appearing.

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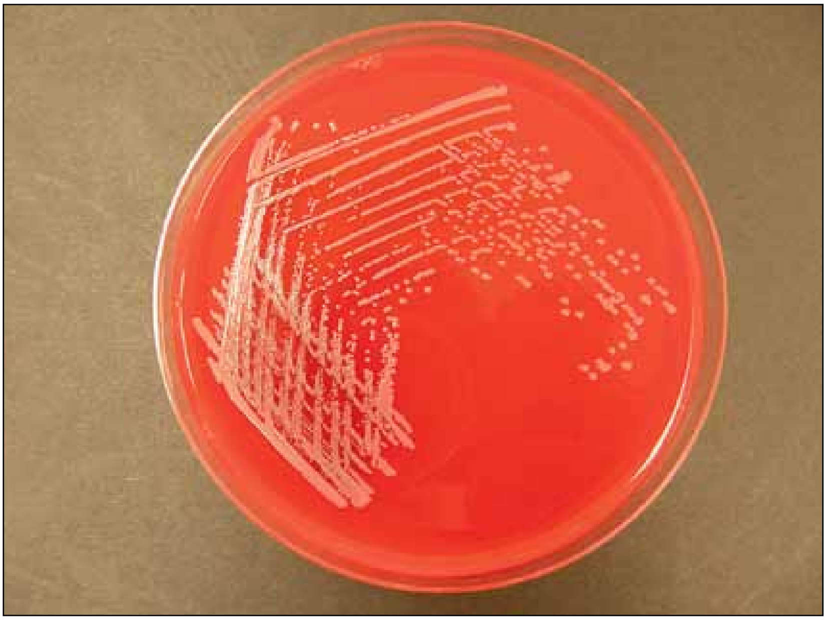

Fig. 1. Colonies of *Pseudomonas fluorescens* on agar (Dušková M)

Fig.2. Colonies of *Proteus vulgaris* on agar (Dušková M)