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## REVIEW

## Meat spoilage: a critical review of a neglected alteration due to ropy slime producing bacteria

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### Abstract

The shelf-life of a product is the period of time during which the food retains its qualitative characteristics. Bacteria associated with meat spoilage produce unattractive odours and flavours, discolouration, gas and slime. There are several neglected alterations that deserve more attention from food business operators and competent authorities. Ropy slime is a typical alteration of the surface of vacuum and modified atmosphere packed cooked meat products, that causes major economic losses due to the increasingly sophisticated consumer requirements. This is a review article that aims at raising awareness of an old problem of new concern, in the light of new advances and trends for understanding the aetiology of the phenomenon, the origins of contamination and the prevention measures.

### Introduction

Food is a complex, dynamic ecosystem, in which every component is continuously changing. It is essential to recognise these changes to minimize unwanted development, such as food spoilage, which is a naturally occurring process leading to undesirable modifications in sensory characteristics (appearance, texture, odour and flavour) and the absence of acceptable qualities. This phenomenon determines not only economic losses, but also the lack of consumable foods. In fact, an excessive amount of food is wasted due to spoilage, even with modern preservation techniques (Gram *et al.*, 2002; Remenant *et al.*, 2015).

The Food and Agriculture Organization (FAO) of the United Nations (UN) and the World Health Organization (WHO) declare that one third of the food produced for human consumption is wasted each year (FAO, 2011).

Food rejection is mainly associated with spoilage and is characterized by any change which determines unacceptable products for the consumer (Koutsoumanis, 2009). The causes of the loss of adequate qualities may be physical damage, chemical reactions, insect and rodent infestation and microbial growth (Gram *et al.*, 2002; Ray and Bhunia, 2013). Despite refrigeration chains, chemical preservatives and the application of recent techniques, it has been estimated that 25% of all food produced globally is wasted *post harvest* or *post slaughter* due to microbial spoilage, so that this is actually the most common cause of alterations in food quality (Gram *et al.*, 2002; Cenci-Goga *et al.*, 2014).

Compared to a multitude of foodstuffs, meat represents one of the most perishable (Doulgeraki *et al.*, 2012): first, for the presence of chemical and enzymatic activities, and second, because it constitutes a perfect *pabulum* for the growth of a wide variety of microorganisms, especially as a result of its nutrient composition, high water content and moderate pH (Dave and Ghaly, 2011). Microbial growth, oxidation and enzymatic autolysis are the three basic mechanisms responsible for the spoilage of meat. In addition to lipid oxidation and enzyme reactions, meat spoilage is almost always caused by microbial growth. The breakdown of fat, protein and carbohydrates in meat results in the development of off-odours, off-flavours and slime formation, which determine disagreeable meat for human consumption (Ercolini *et al.*, 2006; Nychas *et al.*, 2008; Casaburi *et al.*, 2015). The scientific community became interested in meat microbiology when meat products began to be shipped over long distances and when the spread of supermarkets in the 1950s changed consumers' habits (Nychas *et al.*, 2008). Nowadays, products have been directed from local markets to international trade and *ready-to-eat* meat products have unequivocally become part of modern diets. This new food culture requires high food quality and safety standards to be guaranteed for the entire commercial life of the product, with additional strict requisites to comply with in order to be accepted for international trade. The stability of meat characteristics becomes the first essential step for food producers to prevent undesirable modifications during the storage period. Many studies have been conducted so far. However, some alterations on meat, such as ropy slime-formation on the surface of cooked meat products, are still persistent (Iulietto *et al.*, 2014). Ropy filaments were found in vacuum packs and reported in Finland at the end of '80s and the cause was identified as the growth of certain psy-

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chrotrophic strains of lactic acid bacteria (LAB) (Korkeala *et al.*, 1988). Even though several decades have passed, slime is still occasionally evident before the sell-by date, and consumers reject the products, as they find the appearance of the food unacceptable (Aymerich *et al.*, 2002). To confirm the topicality of the problem, Pothakos *et al.* (2014) underlined the current spread of psychrotrophic LAB in Belgian food processing environments, which led to unexpected spoilage in all kinds of packed and refrigerated foodstuffs in Northern Europe. Furthermore, as is easily understandable, ropy slime-forming bacteria determine huge financial losses for food producers in many countries (Korkeala *et al.*, 1988; Aymerich *et al.*, 2002).

Starting from the description of the general aspects of meat spoilage, the aim of this paper is to focus specifically on the particular aspects of meat alterations due to ropy slime-producing bacteria, from contamination sources to prevention strategies, in order to raise awareness to provide an effective answer for preventing the formation of ropy filaments on cooked meat products.

## Shelf-life and microbial meat spoilage

The shelf-life of meat and meat products is the period of time during which storage is possible and food retains its qualitative characteristics until the arrival of spoilage phenomena. The shelf-life of products is strongly linked to their deterioration, creating a borderline between an acceptable and an unacceptable bacterial concentration, which determines off-odours, off-flavours and an undesirable appearance. These sensorial modifications are related to the number and types of micro-organisms initially present and their subsequent growth. For meat products, the starting total microbiota is approximately  $10^2$ - $10^3$  cfu  $gr^{-1}$ , consisting of a huge variety of species (Ray and Bhunia, 2013).

The environmental conditions of the meat during the different steps in its production and trade create a specific ecological niche, which favours some microbial strains initially present in the meat or introduced by cross-contamination, whereas other strains are disadvantaged (Castellano *et al.*, 2008; Nychas *et al.*, 2008). The prevalence of a particular microbial strain depends on factors which persist during processing, transportation and storage. Storage at refrigeration temperatures limits the growth of only 10% of the total microbiota and, when applicable, heat treatments remove the majority of vegetative cells. Therefore, shelf life may vary from days to several months and is strictly linked to post-processing recontamination. During storage, the dominant microbiota can cause the deterioration and release of volatile compounds or slime formation; as a consequence, the product becomes unacceptable for human consumption (Gram *et al.*, 2002; Kreyenschmidt *et al.*, 2010).

## Factors influencing shelf-life and spoilage of meat and meat products

The micro-organisms' ability to grow in food

is closely related to many factors, some of which are intrinsic in the substratum. Others are extrinsic, but all of them influence the development of the ecological environment (Cenci-Goga, 2012). The main factors, which affect the shelf-life of meat products and favour some bacterial strains rather than others, are: packaging (aerobically, vacuum or modified atmosphere), storage temperature, the composition of the products (presence of fat, NaCl content, nitrites,  $a_w$ , pH) and other factors, such as antibacterial substances or biopreservatives (Nychas *et al.*, 2008; Remenant *et al.*, 2015) (Table 1).

### Intrinsic factors

#### Composition and antimicrobial hurdles

Meat represents a natural ecosystem in which the advantageous or disadvantageous conditions determine the survival and growth of some specific strains. Micro-organisms need energy for their metabolism, essential substances which they cannot synthesize and components for the constitution of cells; all these necessary elements are collected from the surrounding food environment and their presence allow the effective survival of foodborne strains during the lag phase (Cenci-Goga, 2012). In general, meat is rich in protein, lipids, minerals and vitamins, but poor in carbohydrates; this composition provides an opportunity for some species instead of others with different nutrient requirements. After microbial death, intracellular enzymes can catalyse some food nutrients to simpler forms, which can be exploited by other species. The presence of growth factors and natural or chemical inhibitors (additives such as nitrite) further select specific strains (Ray and Bhunia, 2013). All food substances which do not occur naturally or are environmental contaminants are generally regarded as *added*. There are several categories within the broad class of *added* food constituents. However, a practical definition considers all the sub-

stances deliberately put into foods as intentional substances and those which may get in by accident during processing as incidental. Among the first category of additives, antimicrobial agents are added to prevent bacterial contamination of food, thus avoiding spoilage and poisoning processes caused by pathogens or their toxins (Cenci-Goga *et al.*, 1996). The relatively recent increase in the interest in *green* consumerism has actually encouraged a renewal of scientific interest in natural approaches, such as the addition of bioprotective cultures and natural antimicrobial compounds (essential oils, enzymes, bacteriocins) to meat products, in order to delay the growth of spoilage micro-organisms without interfering with the typical characteristics of the product (Burt, 2004). Plant-derived essential oils (EOs) are aromatic, oily liquids, obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) which have shown remarkable antimicrobial activity against spoilage and pathogenic microorganisms in meat and meat products. Essential oils originating from oregano, thyme, basil, marjoram, lemongrass, ginger and clove were investigated *in vitro* (Barbosa *et al.*, 2009) and on meat products (Burt, 2004; Fratianni *et al.*, 2010) and found capable of affecting the growth and metabolic activity of foodborne microbiota (Skandamis and Nychas, 2001). Bacteriocins are microbial heat-stable peptides, active towards other bacteria (Gálvez *et al.*, 2007); they are added as biopreservatives to improve the microbial stability and safety of chill-stored fresh and cooked meat (Samelis *et al.*, 2006). In the EU, nisin (E234), a polypeptide produced by *Lactococcus lactis*, and natamycin (E235), produced by *Streptomyces natalensis*, are currently the only commercially available bacteriocins (Ercolini *et al.*, 2010; Doulgeraki *et al.*, 2012).

#### Buffer capacity and pH

Meat pH also affects the selection of bacte-

**Table 1. Factors affecting the shelf-life of meat.**

| Intrinsic factors   | Extrinsic factors                     |
|---|---------------------------------------|
| Species, breed, age and feeding of the animal of origin                           | Quality management system             |
| Initial microbiota  | Packaging system                      |
| Chemical properties (pH, $a_w$ , redox potential, peroxide value)                 | Temperature control                   |
| Product composition   | Processing conditions and hygiene     |
| Antimicrobial components  | Storage types                         |
| Biopreservation systems (bacteriocinogenic LAB cultures and/or their bacteriocin) | Relative humidity                     |
|   | Atmospheric gas composition and ratio |

Based on (McDonald and Sun, 1999; Dave and Ghaly, 2011; Cenci-Goga, 2012; Kalschne *et al.*, 2014).

ria; each species has an optimum and a range of pH for growth. During post slaughtering, muscle pH, normally decreases to 5.4-5.8, while pH is >6 in meat coming from stressed animals (defined as *dark, firm, dry meat*) and in cooked meat products, such as sliced ham (Aymerich et al., 2002). The presence of adipose tissue and a high pH in meat determines a more rapid spoilage process due to a more rapid bacterial growth and consumption of nutrient (Ray and Bhunia, 2013).

#### Redox potential

The oxidation-reduction potential is a function of the pH, gaseous atmosphere and presence of reductants. It measures the potential difference, in a system generated by a coupled reaction, in which one substance is oxidized and a second substance is reduced simultaneously, in electrical units of millivolts (mV). The redox potential of a food is related to its chemical composition, processing treatments and storage. Raw meat has an *Eh* (i.e., redox potential) of -200 mV, ground raw meat has an *Eh* of +225 mV and cooked meat a range of +90mV to -50mV (Cenci-Goga, 2012).

#### Water activity

Water activity ( $a_w$ ) is the measure of the amount of water in a food which is available for the growth of micro-organisms, including pathogens. It identifies the water available for carrying out enzymatic reactions, synthesizes cellular materials and takes part in other biochemical reactions. Raw meat has  $a_w$  values of 0.98-0.99 and cooked meat approximately 0.94; those values allow the growth of most micro-organisms (Aymerich et al., 2002). Dried products are usually considered shelf stable and are, therefore, often stored and distributed unrefrigerated. The characteristic of dried foods which makes them shelf stable is their low water activity. A water activity of 0.85 or below will prevent the growth and toxin production of pathogens, including

*Staphylococcus aureus* and *Clostridium botulinum*. *S. aureus* grows at a lower water activity than other pathogens, and should, therefore, be considered the target pathogen for drying. Control of the drying process to prevent the growth and toxin production of pathogens, including *S. aureus*, in the finished product is critical to product safety if the product is distributed or stored unrefrigerated. Similarly, drying may not be critical for the safety of dried stored, refrigerated products, since refrigeration may be sufficient to prevent pathogen growth. Controlling pathogen growth and toxin formation by drying is best accomplished by: i) scientifically establishing a drying process that reduces the water activity to 0.85 or below; ii) designing and operating the drying equipment, so that every unit of product receives at least the established minimum process (Leonard, 2011).

#### Extrinsic factors

##### Packaging and gaseous atmosphere

Packaging conditions and the gaseous composition of the atmosphere surrounding the meat greatly influence the composition of spoilage flora (Borch et al., 1996; Sechi et al., 2014; Rossaint et al., 2015). Aerobic storage conditions promote, above all, the growth of Pseudomonads (Rossaint et al., 2015). *Pseudomonas* spp., *Acinetobacter* spp., *Moraxella* spp. are considered the major source of meat deterioration in aerobically stored meat products at different temperatures from -1 to 25°C. Members of the *P. fluorescens* group, together with the psychrotrophic *P. fragi*, *P. ludensis* and *P. putida*, are the most commonly isolates in aerobically packed, spoiled meat (Ercolini et al., 2006; Ercolini et al., 2010). The population of Pseudomonads at the arbitrary level of  $10^7$  CFU  $g^{-1}$ , has been attributed to the formation of slime and off-odours, especially when the metabolism of nitrogenous compounds prevails over the fermentation of carbohydrates. *Shewanella* spp.

is a genus closely related to *Pseudomonas* spp. and contributes significantly to spoiling food: *S. putrefaciens* is one of the predominant spoilers in chill-stored, vacuum-packed (VP) meat and high pH VP meat (Doulgeraki et al., 2012).

Packaging of meat under vacuum or CO<sub>2</sub> modified atmosphere has resulted in extended shelf-life compared to traditional packaging conditions (Yost and Nattress, 2002). The use of CO<sub>2</sub> and N<sub>2</sub> extends the lag phase of aerobic microorganisms and promotes the growth of facultative and strict anaerobic species. This change in packaging conditions determines a shift from aerobic bacteria, such as *Pseudomonas* spp., to facultative anaerobic species, such as *Brochotrix thermosphacta* (Nychas et al., 2008) and lactic acid bacteria (Doulgeraki et al., 2012) (Table 2). Lactic acid bacteria are the predominant microflora of vacuum or CO<sub>2</sub>-modified atmosphere packed products, representing dominant spoilage-causing bacteria (Yost and Nattress, 2000; Arvanitoyannis and Stratakos, 2012). In fact, the combination of micro-aerophilic conditions and a reduced  $a_w$  inhibits gram-negative spoilage flora and favours the proliferation of LAB (Borch et al., 1996; Korkeala and Björkroth, 1997; Samelis et al., 2000b; Audenaert et al., 2010).

In addition, Modified Atmosphere Packaging (MAP) meats are affected by dynamics changes of headspace gases (headspace being the space in the package between the inside of the lid and the top of the food): CO<sub>2</sub> concentration changes during storage in relation with meat absorption or evolution of CO<sub>2</sub>, depending on initial headspace CO<sub>2</sub>, temperature, packaging configuration and meat characteristics. CO<sub>2</sub> would be adsorbed by the muscle and fat tissue until saturation and its absorption determines a decrease in headspace volume in MAP until packages collapse (Zhao et al., 1995; Ercolini et al., 2006). Among Enterobacteriaceae, *Serratia* spp. is the most common genus isolated from MAP meat (Doulgeraki et al., 2012).

**Table 2. Expected shelf-life of cooked meat products under refrigerated storage and dominant microbiota.**

| Storage conditions            | Gas composition                          | Expected shelf-life | Dominant microbiota   |
|-------------------------------|--|---------------------|---|
| Aerobic                       | Air                                      | Days                | <i>Pseudomonas</i> spp.   |
| Modified atmosphere packaging | >50% CO <sub>2</sub> with O <sub>2</sub> | Weeks               | <i>B. thermosphacta</i>   |
|                               | 50% CO <sub>2</sub>                      | Weeks               | Enterobacteriaceae  |
|                               | <50% CO <sub>2</sub> with O <sub>2</sub> | Weeks               | <i>B. thermosphacta</i>   |
| Vacuum packaging              | 100% CO <sub>2</sub>                     | Weeks               | Lactic acid bacteria  |
|                               | no gas                                   | Months              | <i>B. thermosphacta</i> , <i>S. putrefaciens</i> , lactic acid bacteria |

Based on (Borch et al., 1996; Nychas et al., 2008; Doulgeraki et al., 2012).

### Storage temperature

Storage temperature affects the duration of the lag phase, the maximum specific growth rate and the final cell number (Doulgeraki *et al.*, 2012). Lower refrigeration temperatures decrease bacterial growth and modify the composition of the microbiota present on meat: psychrotrophic bacteria could grow, either Gram-positive, such as LAB, or Gram-negative, such as *Pseudomonas* spp. (Doulgeraki *et al.*, 2012), at chill temperature. In MAP and vacuum packed meat products, the dominance of lactic acid bacteria is also maintained under refrigerated conditions. However, the growth rate is affected: *Carnobacterium* spp. prevails in a vacuum at -1.5°C, whereas homofermentative *Lactobacillus* spp. dominate at 4°C and 7°C (Ray and Bhunia, 2013). Among the Enterobacteriaceae, *Hafnia alvei* dominates at 4°C, and *S. liquefaciens* predominates at 1.5°C (Borch *et al.*, 1996). Psychrophilic *Clostridium* spp. could be detected in vacuum-packed, chilled meat (Doulgeraki *et al.*, 2012). Storage temperatures above 10°C are not unusual and a shift in microbial populations can be observed. Temperature abuse determines the growth of Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp (Koutsoumanis *et al.*, 2006).

From these considerations, it is evident how important an accurate management of time/temperature can be to control not only pathogen growth and toxin formation, but also spoilage micro-organisms. Unwanted bacteria growth and toxin formation as a result of the time/temperature abuse of food products can cause consumer illness. Temperature abuse occurs when the product is allowed to remain a sufficient length of time at temperatures favourable to pathogen growth resulting in unsafe levels of pathogens or their toxins in the product (Cenci-Goga *et al.*, 2005; Leonard,

2011; Cenci-Goga *et al.*, 2014).

### Alterations associated with spoilage

Since microbial survival follows different pathways depending on the many factors which occur, the detectable effects are multiple: visible growth (slime, colonies), textural changes (degradation of polymers) or off-odours and off-flavours (Borch *et al.*, 1996; Gram *et al.*, 2002; Nychas *et al.*, 2008).

The characteristics of meat deteriorations depend on the availability of variable substrates: glucose, lactic acid, nitrogenous compounds and free amino acids present in meat, as the principal precursors of microbial metabolites responsible for spoilage (Nychas *et al.*, 2008). Depending on the microbial species and their oxygen affinity, these compounds will produce different catabolic by-products (Table 3).

### Off odours and off flavours

The volatilome, the volatile fraction of the microbial catabolites, includes: sulphur compounds, ketones, aldehydes, organic acids, volatile fatty acids, ethyl esters, alcohols, ammonia and other metabolites. Depending on their olfactory thresholds and the interaction between the volatile and non-volatile compounds, these molecules will affect the sensory quality of both fresh and cooked meat (Casaburi *et al.*, 2015).

From aerobically stored meat, it is not infrequent to appreciate undesirable odours as putrid, cheesy, sulphuric, sweet and fruity (Borch *et al.*, 1996). Off-odours are perceptible to consumers when the total bacterial count is between 10<sup>7</sup>CFU gr<sup>-1</sup> and 10<sup>7.5</sup>CFU gr<sup>-1</sup>. *Pseudomonas* spp. and *B. thermosphacta* predominantly contribute to foul odours as a result of their metabolism (Nychas *et al.*, 2008). When superficial contamination is

nearly 10<sup>8</sup>CFU gr<sup>-1</sup>, the carbohydrates are depleted and Pseudomonaceae in association with psychrotrophic Gram-negatives, such as *Moraxella* spp., *Alcaligenes* spp, *Aeromonas* spp, *Serratia* spp., *Pantoea* spp., start using amino acids as sources of energy. Nauseating odours are associated with free amino acids and nitrogen compounds (NH<sub>3</sub>, indole, tryptophan). *B. thermosphacta* aerobic metabolism of glucose produces a foul-smelling odour, such as acetoin and acetic acid (Koutsoumanis *et al.*, 2006). Sulphur-containing compounds determine sulphuric odours, originating from hydrogen sulphide formed by Enterobacteriaceae and dimethyl sulphide by *Pseudomonas* spp. Cheesy odours are determined by acetoin/diacetyl and 3-methylbutanol formations produced by Enterobacteriaceae, *B. thermosphacta* and homofermentative *Lactobacillus* spp. (Casaburi *et al.*, 2015).

The off-odour from vacuum and MA-packed meat is less intense and is represented by a sour, acid aroma as a result of the spoilage caused by lactic acid bacteria, associated with the production of lactic- and acetic-acid during the logarithmic and stationary growth phase. The CO<sub>2</sub> and O<sub>2</sub> content affects the rate of consumption of glucose by *B. thermosphacta*. As a consequence, anaerobic metabolism produces less intense odours than aerobic metabolism, so the use of a low concentration of oxygen on modified atmosphere packaging is better for maintaining acceptable qualities (Pin *et al.*, 2002). *Shewanella* spp. produces malodorous compounds, such as H<sub>2</sub>S in vacuum packaged meat (Gram *et al.*, 2002; Doulgeraki *et al.*, 2012).

### Colour alteration

The presence of bacterial patina on the surface of meat products is appreciable when the microbiota are between 10<sup>7.5</sup>-10<sup>8</sup>CFU cm<sup>-2</sup>.

Table 3. Meat spoilage: prevalent alterations detectable.

| Alteration                             | Product  | Aetiology  |
|--|--|--|
| H <sub>2</sub> S production            | Cured meat                                     | <i>Vibrio</i> , Enterobacteriaceae   |
| Sulfide odour                          | Vacuum packaged meat                           | <i>Clostridium</i> spp., <i>Hafnia</i> spp.  |
| H <sub>2</sub> O <sub>2</sub> greening | Meats  | <i>Weissella</i> spp., <i>Leuconostoc</i> spp., <i>Enterococcus</i> spp., <i>Lactobacillus</i> spp.    |
| H <sub>2</sub> S greening              | Vacuum packaged meat                           | <i>Shewanella</i> spp.   |
| Slime production                       | Meats  | <i>Pseudomonas</i> spp., <i>Lactobacillus</i> spp., <i>Leuconostoc</i> spp., <i>Enterococcus</i> spp., |
|  | <i>Weissella</i> spp., <i>Brochothrix</i> spp. |  |
| Blown Pack                             | Vacuum packaged meat                           | <i>Clostridium</i> spp., lactic acid bacteria  |
| Putrefaction                           | Ham  | Enterobacteriaceae, <i>Proteus</i> spp.  |
| Bone taint                             | Meats  | <i>Clostridium</i> spp., <i>Enterococcus</i> spp.  |
| Souring                                | Ham  | Lactic acid bacteria, <i>Enterococcus</i> spp.   |

Based on (Pin *et al.*, 2002; Nychas *et al.*, 2008; Yang *et al.*, 2014).

Hydrogen sulphide, produced by *L. sakei*, *H. alvei*, *S. putrefaciens*, converts the muscle pigment to green sulphomyoglobin and its appearance is a consequence of glucose consumption. Sulphomyoglobin is not formed in anaerobic atmospheres (Borch *et al.*, 1996). *Leuconostoc* spp. and *Leuconostoc*-like microorganisms, such as *Weissella viridescens*, may cause meat products to turn green, due to the formation of hydrogen peroxide, which oxidizes nitrosomyochromogen as the consequence of the exposure of meat to O<sub>2</sub> (Dušková *et al.*, 2013). *S. putrefaciens* may determine green discoloration in vacuum-packed meat (Doulgeraki *et al.*, 2012). In addition, among the factors affecting light-induced oxidative discoloration of cooked meat during the storage, the headspace volume directly influences the total amount of O<sub>2</sub> available for the oxidation (Robertson, 2012).

#### Gas production

*Clostridium* spp. is responsible for the production of a large amount of gases (H<sub>2</sub> and CO<sub>2</sub>): vacuum-packed meat could be affected by *blown pack* spoilage, characterized by deformation of the pack due to the accumulation of a large amount of gases, putrid odours, the presence of exudates, extensive proteolysis, changes in pH and colour. This type of deterioration can occur in chilled, vacuum-packed meat, caused by psychrophilic and psychrotrophic bacteria. Not only *Clostridium* spp. is responsible for *blown pack* (Yang *et al.*, 2014), but LAB also play an important role in the production of the volatile, organic compounds found in the package headspace of spoiled meat (Hernandez-Macedo *et al.*, 2012). CO<sub>2</sub> concentration during the storage of packages is attributed to metabolic by-products of the heterofermentative lactobacilli and leu-

conostocs. It usually determines off-odours as well.

#### Filaments and ropy slime

A high-incidence of ropy slime formation is found in vacuum-packed, cooked meat products, caused by the homofermentative *Lactobacillus* spp. and *Leuconostoc* spp. The stretchy, ropy slime are long, undesirable, polysaccharide ropes between the surface of the products and the casing or between the slices (Figure 1). Slime production gives some bacteria an advantage, since it constitutes a protective layer to keep the bacteria moist (Bjorkroth and Korkeala, 1997a). *W. viridescens* may be the cause of ropy slime formation or meat turning green. After the appearance of individual colonies on a wet surface, a continuous layer of greenish slime is formed (Dušková *et al.*, 2013).

#### Lactic acid bacteria associated with meat spoilage

Lactic acid bacteria are widespread in nature and in the environment of processing plants; they are unavoidably part of the contaminant flora of fresh meat after slaughter, and also of cooked meat. They are generally regarded as safe (GRAS) microorganisms (Nychas *et al.*, 2008; Ogier *et al.*, 2008) with many applications in the food industry; in fact, under specific conditions, they compete efficiently with other microorganisms for nutrients, and achieve substantial, viable counts (Krockel, 2013). In food production, LAB are frequently used for their desired effects, such as their application as a starter in meat to manufacture safe, high quality, fermented sausages or cooked meat products (Cenci-Goga *et al.*, 2008, 2012; Zhao *et al.*, 2014). Protective, bacteriocinogenic cultures estab-

lish a microbial ecosystem, typically associated with MAP and VP cooked meat, which prevents the multiplication of food-borne pathogens (Zhang and Holley, 1999).

Apart from their beneficial effects, some strains of lactic acid bacteria are the major spoilage bacteria in vacuum- and modified atmosphere-packed cooked meat products. In fact, they are indicated as Specific Spoilage Organisms (SSO), determining evident meat spoilage of products stored under packaging conditions with an increased concentration of carbon dioxide (Nychas and Skandamis, 2005; Nychas *et al.*, 2008; Koutsoumanis, 2009; Pothakos *et al.*, 2014b).

The LAB most involved in meat spoilage consist of heterofermentative lactobacilli (*Lactobacillus* spp., mainly *L. curvatus* and *L. sakei*), heterofermentative leuconostocs (*Leuconostoc* spp.), *Carnobacterium* spp. (Hu *et al.*, 2009) and, to a lesser extent, the homofermentative *Lactobacillus* spp. and *Pediococcus* spp. As a result of their metabolism, homofermentative LAB produce almost exclusively lactic acid, which is mild and palatable, whereas heterofermentative LAB produce a significant amount of undesirable catabolites, such as CO<sub>2</sub> gas, ethanol, acetic acid, butanoic acid and acetoin with consequent off-odours and visual effects, such as ropy slime formation and meat discoloration (Krockel, 2013).

As a consequence, LAB are responsible for some unusual alterations in meat: off-flavours, discoloration, gas production, a decrease in pH and slime formation, determining the spoilage of the products and reduction in shelf-life (Samelis *et al.*, 2000a). Organoleptic modifications produced by LAB become appreciable after they have reached the stationary growth phase (Korkeala and Alanko, 1988; Korkeala *et al.*, 1997b; Samelis *et al.*, 2000b; Yost and Nattress, 2002; Ercolini *et al.*, 2006; Hu *et al.*, 2009; Pothakos *et al.*, 2014b) (Korkeala and Alanko, 1988; Makela *et al.*, 1992a; Bjorkroth *et al.*, 1996; Bjorkroth and Korkeala, 1997a; Samelis *et al.*, 2000b; Aymerich *et al.*, 2002; Hu *et al.*, 2009) (Korkeala and Bjorkroth, 1997) (Borch *et al.*, 1996; Aymerich *et al.*, 2002; Jaaskelainen *et al.*, 2013) (Bjorkroth *et al.*, 1998; Samelis *et al.*, 2006; Nychas *et al.*, 2008; Lyhs *et al.*, 2004) (Pothakos *et al.*, 2014a) (Makela *et al.*, 1992a)

**Table 4. Ropy slime producing bacteria.**

| Origin                             | Packaging | Strains isolated                                  | References   |
|------------------------------------|-----------|---|--|
| Cooked meat products               | Vacuum    | <i>L. mesenteroides</i>                           | (Korkeala <i>et al.</i> , 1988; Bjorkroth and Korkeala, 1997b;                                 |
|                                    | Packaged  | <i>L. mesenteroides</i> subsp. <i>dextranicum</i> | Samelis <i>et al.</i> , 2000b; Yost and Nattress, 2002; Ercolini <i>et al.</i> , 2006;         |
|                                    |           | Homofermentative lactobacilli                     | Hu <i>et al.</i> , 2009; Pothakos <i>et al.</i> , 2014b)                                       |
|                                    |           | <i>L. sakei</i>                                   | (Korkeala and Alanko, 1988; Makela <i>et al.</i> , 1992a; Bjorkroth <i>et al.</i> ,            |
|                                    |           | <i>L. gelidum</i> ,                               | 1996; Bjorkroth and Korkeala, 1997a; Samelis <i>et al.</i> , 2000b;                            |
|                                    |           | <i>L. amelibiosum</i>                             | Aymerich <i>et al.</i> , 2002; Hu <i>et al.</i> , 2009)  |
|                                    |           | <i>L. gelidum</i> subsp. <i>gasicomitatum</i>     | (Korkeala and Bjorkroth, 1997)   |
| Sliced cooked ham                  | Vacuum    | <i>L. carnosum</i>                                | (Borch <i>et al.</i> , 1996; Aymerich <i>et al.</i> , 2002; Jaaskelainen <i>et al.</i> , 2013) |
|                                    | Packaged  | Vasilopoulos <i>et al.</i> , 2008; Kröckel, 2013) | (Bjorkroth <i>et al.</i> , 1998; Samelis <i>et al.</i> , 2006; Nychas <i>et al.</i> , 2008;    |
| Herring                            | Preserve  | <i>L. gelidum</i> subsp. <i>gasicomitatum</i>     | (Lyhs <i>et al.</i> , 2004)  |
| Boiled eggs                        | In brine  | <i>L. gelidum</i>                                 | (Pothakos <i>et al.</i> , 2014a)   |
| Processing rooms<br>at meat plants |           | <i>L. sakei</i>                                   | (Makela <i>et al.</i> , 1992a)   |
|                                    |           | <i>L. amelibiosum</i>                             |  |



cose and rhamnose and homopolysaccharide (HoPS), containing only one type of monosaccharide, either glucose (glucans) or fructose (fructans) (De Vuyst, 2011; Monsan *et al.*, 2001). *Leuconostoc* spp. and some *Lactobacillus* spp. strains synthesize glucans and fructans from sucrose (Monsan, 2011; van Hijum, 2006). However, the formation of ropy slime is not inhibited by the absence of sucrose in the meat product. Many different heteropolysaccharides (HePS) are secreted by LAB, depending on the sugar composition and molecular size (Degeest *et al.*, 2001).

EPS production is associated with the protection of the cell against desiccation, phage attacks, phagocytosis, antibiotics, toxic compounds, predation by protozoans and is involved in osmotic control, adhesion to surfaces and cellular recognition (Dudman, 1977; Ullrich, 2009). Slime production is influenced by the specific conditions of packaging and storage temperature and is linked to biofilm formation, stress resistance and sucrose utilization of responsible strains (Aymerich *et al.*, 2002; Ullrich, 2009).

In late 1980s a Finnish research group (Korkeala and Alanko, 1988; Korkeala *et al.*, 1988) analysed the slime produced by two different, homofermentative lactobacilli and a *Leuconostoc* strain, isolated from different ropy, vacuum-packed meat products: the slime had a molecular weight in the range of 70000-30000, determined by gel permeation chromatography (GPC), and contained glucose and galactose in a ratio of 10:1-10:2.

#### Isolation and identification

The identification of spoilage micro-organisms shows two different approaches: culture dependent and culture independent methods. The first procedure consists of the preliminary isolation and culture of micro-organisms isolated from a food sample and the subsequent identification of a single, colony-forming unit on nutrient and selective media (Figure 2). Culture independent approaches, on the other hand, do not need a preliminary culture, however, strains can be detected directly on the food sample via a DNA and RNA analysis, which is also efficient for strains in a low concentration (Schirone and Visciano, 2014). From the 80s, ropy slime-producing bacteria were identified by means of selective media, and sugar fermentation was investigated with API 50 CHL and the sequencing of 16S ribosomal RNA (Korkeala *et al.*, 1988). In industrial production plants, plate count methods are used in the microbial quality assessment of MAP meat products throughout the processing plant, in order to isolate meat-borne spoilage

LAB strains on Plate Count Agar and de Man Rogosa Sharpe Agar media (Audenaert *et al.*, 2010). For detailed information of the composition or the origin of the microbiota, phenotypic and/or molecular identification and typing of purified colonies is conducted (Audenaert *et al.*, 2010). Molecular techniques in microbial ecology have changed the way of studying microbial diversity. In fact, they allow rapid, reliable identification and typing of microorganisms, usually by means of the detection of DNA polymorphisms between species or strains (Doulgeraki *et al.*, 2012).

PCR-based, molecular typing methods allow differentiation at the species and intra-species level; the specificity of this approach is based on primer selections and amplification conditions (Randomly Amplified Polymorphic DNA-PCR, Repetitive Extragenic Palindromic-PCR, Amplified Fragment Length Polymorphism) (Yost and Nattress, 2002; Casaburi *et al.*, 2011). Yost and Nattress (2000) defined a systematic approach to identify lactic acid bacteria associated with meat, to detect *Carnobacterium* spp., *L. curvatus*, *L. sakei* and *Leuconostoc* spp by means of specific primers for *Carnobacterium* spp. and *Leuconostoc* spp., created from 16S rRNA oligonucleotide probes and used in combination with species-specific primers for the 16S/23S rRNA spacer region of *L. curvatus* and *L. sakei* in multiplex PCR reactions.

Among the culture-independent approaches, PCR-denaturing gradient gel electrophoresis (PCR-DGGE) is a method to assess the biodiversity and population dynamics of microbiota occurring in different ecosystems, used in food microbiology to investigate bacterial successions in fermented food or the composition of probiotic products (Temmerman *et al.*, 2003; Masco *et al.*, 2005). Among non PCR-based methods, the most promising is Restriction Enzyme Analysis coupled with pulsed-field gel electrophoresis (REA-PFGE), which is used to obtain a strain-specific band pattern for the monitoring of the succession of bacteria in meat during storage. Another method of choice, for taxonomy, is Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), which compares whole-cell protein patterns (Doulgeraki *et al.*, 2012). Restriction Fragment Length Polymorphism (RFLP) technique consists of the digestion of genomic DNA by specific enzymes and separation of obtained fragments on agarose gel. RFLP could be associated with PCR of specific sequences in presence of high interspecific polymorphisms. A specific application of RFLP, the so-called Terminal restriction fragment length polymorphism (T-RFLP) is used to char-

acterize psychrotrophic strains on MAP meat (Nieminen *et al.*, 2011). The identification of causative agents of ropiness is carried out also through *Ribotyping* which is a method based on the analysis of ribosomal RNA where restriction enzymes provoke the formation of specific fragments of rRNA, determining a specific *ribotypes* for each strain (Bjorkroth and Korkeala, 1997a). Pulse Field Gel Electrophoresis (PFGE), finally, is a technique that allows the identification of high molecular weight molecules thanks to the electric field periodically modifying. The final result is a specific pulsotype for each strain (Bjorkroth *et al.*, 1996)

#### Products involved

Nowadays, the consumption of cooked, sliced and packaged meat products, such as cooked ham, chicken and turkey breast, emulsion-style sausages (*e.g.*, frankfurters, luncheon meat) is increasing as a result of consumers' enhanced interest in low-calorie meat products (Hu *et al.*, 2009). The majority of these products are sold under modified atmosphere (MAP) or vacuum-packed conditions and some of them are *ready-to-eat* products (Audenaert *et al.*, 2010). Their storage is under refrigeration with shelf-lives varying from days to several weeks. Modified atmosphere and vacuum packaging conditions prolong the shelf-life of meat and favour the growth of psychrotrophic lactic acid bacteria (Borch *et al.*, 1996; Korkeala and Björkroth, 1997). During slicing and packaging, contamination may occur and psychrotrophic LAB may grow exponentially in the meat product, determining an alteration in the quality of the meat (Krockel, 2013).

The main categories of cooked meat products showing these contaminations are: grilled roast ham, cooked ham, classic cooked ham, roast turkey breast, roast loin of pork. Even though the raw materials have different origins (pork or turkey), they follow a similar production process. Briefly, the main steps are: a careful selection of the meat, trimming, syringing after the preparation of the saline (a mix of water, spices, natural flavourings and additives), churning, cooking in controlled temperature ovens, where the temperature of the product must reach 70°C in the centre, cooling, vacuum-packaging process and pasteurization for several minutes at a temperature of 115°C. Once cooled, they are ready for distribution.

#### Sources of contamination

Since none of the commonly detected LAB species is highly heat-resistant and cooked

meat products are heated to a core temperature of 68°-72°C, the majority of the vegetative cells are killed at the processing plant (Vermeiren *et al.*, 2005). LAB contamination may occur after heat treatment (Makela *et al.*, 1992a; Makela *et al.*, 1992b; Aymerich *et al.*, 2002).

Post-cooking contamination takes place during chilling, handling, slicing and packaging, rather than via natural contaminants initially present on raw meat products determining MAP shelf-life (Borch *et al.*, 1996). The potential contamination sources during the production process of MAP and VP cooked meat products are numerous: salt, spices and raw materials, but also the rooms, where products are stored before packaging. Not only materials collected from the surfaces of the processing rooms, but also air samples from the environment underlined the presence of lactic acid bacteria producing filaments (Makela *et al.*, 1992a, 1992b). The origin of the contamination is also linked to the raw materials used, as confirmed by the isolation of lactic acid bacteria from cooked sausages (Korkeala *et al.*, 1990) and from samples taken from carcasses and raw meat establishments (Makela *et al.*, 1992b). It highlights the fact that lactic acid bacteria can be transmitted through the air, by staff and via tools. Several authors have demonstrated re-contamination after the thermal processes following the handling of products (Mäkelä and Korkeala, 1987; Borch *et al.*, 1988). The environment needs, therefore, to be thoroughly sanitized and a clear separation maintained between raw and cooked products (Mäkelä and Korkeala, 1987).

### Prevention

In order to prevent the presence and growth of ropy slime-producers, there are various different approaches to consider. The rooms and equipment of meat processing plants act as sources of bacterial contamination and disinfection is a necessary procedure to minimize contamination of products with bacteria. Therefore, temporal and spatial separation between raw meat and cooked products decreases the risk of cross-contamination (Audenaert *et al.*, 2010). Not all detergents and sanitizers are effective in eliminating environmental contamination: in particular the use of detergents and sanitizers with a low concentration of hypochlorite is not recommended due to their proved inefficacy towards ropy slime-producing bacteria (Mäkelä *et al.*, 1991). Concerning the use of appropriate products for the in-depth hygiene of meat processing plants, cleaning and sanitizing have to be considered as fundamental procedures not only for

avoiding pathogens contaminations but also for limiting the spoilage due to ropy slime producers. In food industry, detergent and sanitizer are used separately or in association. Detergents contain surfactants that reduce surface tensions between the soil and the surface while sanitizers are made of antimicrobial compounds able to reduce the microbiological contamination to an acceptable level, according to local health regulations. Mäkelä *et al.* (1991) demonstrated that detergent-sanitizer (DS) products with different antimicrobial compounds (Na-dichloroisocyanurate at 0.06%, Na-hypochlorite at 0.017%, cocobenzyl-dimethyl ammonium chloride at 0.027% and Dimethylcoco ammonium betaine at 0.27%) were less effective against ropy-slime producers than sanitizer (S) products used separately. In detail, applied sanitizer compounds were alkyldimethylbenzyl ammonium chloride (0.022% and 0.05%), alkyldimethyl ammonium chloride (0.014%), alkylmethylethylbenzyl ammonium chloride (0.022%), polyhexamethylene biguanide chloride (0.023%), Na-hypochlorite (0.05%), paracetic acid (0.018%) and benzyl-dimethylalkyl ammonium chloride (0.1%). The lower effectiveness of detergent sanitizers was associated to the surface-active compounds which may modify the antimicrobial activity of the product. Consequently, in meat processing plants, it is better to use separately detergent and sanitizers than use combined detergents and sanitizers products. Quaternary ammonium products and acid sanitizer with hydrogen peroxide are reported to be more effective than products containing chlorine compounds and polyhexamethylene biguanide chloride (Makela *et al.*, 1992a, 1992b). The prevention of unwanted meat processes must bear in mind the rising interest of food producers and consumers in healthier food production with fewer added substances. The new technologies of food preservation include non-thermal inactivation, such as ionization radiation, high hydrostatic pressure and pulsed electric fields, active packaging, bio-preservation and natural antimicrobial compounds. The bio-preservation of meat could be the answer to this demand: in fact, it consists of the control of pathogenic and spoilage microbiota by competitive microflora and natural molecules. Bacteriocins, for example, are ribosomally-synthesized, antimicrobial peptides or proteins, which are active towards other bacteria (Gálvez *et al.*, 2007; Castellano *et al.*, 2008). Bacteriocinogenic cultures and specific bacteriocins added to cooked meat are capable of preventing slime production (Aymerich *et al.*, 2002). Nisin is a bacteriocin produced by *L. lactis* subsp. *lactic* and it

inhibits the growth of Gram-positive organisms, including bacterial spores. However, it is not efficient against Gram-negative bacteria, fungi and yeast (Economou *et al.*, 2009). It is not a toxic substance if it is ingested, it does not determine cross-resistance with medical antibiotic molecules and it is degraded by the intestinal tract (Kalschne *et al.*, 2014). Nisin determines a significant inhibition of the growth of *L. sakei* on vacuum-packed sliced ham (Kalschne, 2014) with a shelf-life extension. Aymerich *et al.* (2002) demonstrated that *Enterococcus faecium* and *L. sakei*, bacteriocin producers, prevent ropiness due to *L. sakei*, whereas nisin inhibits the activity of *L. carnosum* in cooked pork loin (Kalschne *et al.*, 2014). In addition, these bacteriocins are heat-stable and resist to pasteurization. It is, therefore, possible to add bacteriocins to the meat before the cooking process. *P. lactis* produces pediocin, a bacteriocin effective against *Listeria* spp. However, novel uses of this strain as a starter culture in some food fermentations also hypothesize the effect on strains of Gram-positive microorganisms (Kalschne *et al.*, 2014). Bacteriocins are also involved in developing active packaging devices, creating an effective surface with antimicrobial effects. Bacteriocin-activated, plastic films for food packaging have been developed for the storage of hamburgers, hot dogs, frankfurters and cooked ham (Ercolini *et al.*, 2010).

An alternative preservation method for the prevention of filaments is High Pressure Processing (HPP) for processed meat and meat products. Most vegetative microorganisms in meat samples are inactivated at a pressure of 400-600 MPa and HPP improves food safety and prolongs the shelf-life of meat products. It could avoid the survival of bacterial strains responsible for ropiness on the surface of the product (Han *et al.*, 2011).

Finally, food preservation through application of Ozone (O<sub>3</sub>) have been investigated, considering the bacterial inactivation determined by the attacks on cellular constituents, avoiding creation of mutants, and leaving no dangerous chemical residuals. The reduction of *L. mesenteroides* in clean water was 5 log count (PPM O<sub>3</sub> per 2 min of application) but direct application of ozone in food processing seems hardly feasible; the application on beef surface, in fact, resulted in low activity towards *Leuconostoc* spp., *Lactobacillus* spp. and *P. fluoresces*, associated with discoloration and odour development (Pirani, 2010).

## Conclusions

Meat spoilage and product shelf-life is an important challenge for all the experts gravitating around this area.

The spoilage due to ropy slime-formation has influenced the marketing of vacuum-packed meat products and the use of this technology. The presence of ropy slime-producing bacteria and their associated sensory abnormalities lead to high direct financial losses (waste product) and indirect (such as product selection, disinfection of contaminated surfaces and non-delivery at destination). Although food security is likely to be guaranteed, the macroscopic appearance of the product at the time of packaging is particularly unpleasant, making it unsuitable for further processing or marketing. Food industries and productions must be supported by research, creating a strong link between discoveries and applications. Nowadays, ropy slime-formation on meat products represents a persistent problem, often ignored. It is, therefore, necessary to provide a basis as a starting point to find a beneficial solution.

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