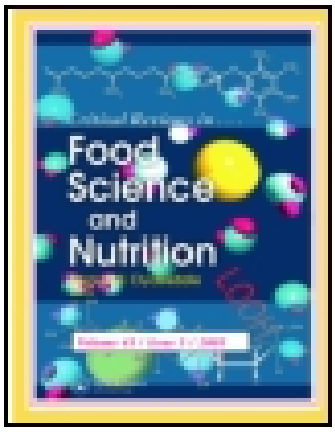

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SHELF-LIFE REDUCTION AS AN EMERGING PROBLEM IN COOKED HAMS UNDERLINES THE NEED FOR IMPROVED PRESERVATION STRATEGIES

Charalampos Vasilopoulos [a] , Luc De Vuyst [a] & Frédéric Leroy [a]
[a] Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bio-engineering Sciences , Vrije Universiteit Brussel , Pleinlaan 2, Brussels , B-1050 , Belgium
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Charalampos Vasilopoulos, Luc De Vuyst, Frédéric Leroy *

Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bio-engineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium.

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* Correspondent footnote

Mailing address:

Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bio-engineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium.

Phone: +32 2 6293612

Fax: +32 2 6292720

e-mail: fleroy@vub.ac.be

Abstract

Cooked hams have gained an important position within the delicatessen market. Nowadays, consumers not only demand superior sensory properties but also request low levels of sodium and fat and the absence of conventional chemicals and preservatives used for the increase of the technological yield and shelf-life of the products. As a result, products that apply strict quality certificates or 'clean' labels become increasingly important. However, such cooked hams suffer from a limited shelf-life. Besides some physicochemical effects, this is mainly due to microbial impact, despite the application of modified-atmosphere-packaging and chilling. Microbial spoilage is mostly due to the metabolic manifestation of lactic acid bacteria and *Brochothrix thermosphacta*, although Enterobacteriaceae and yeasts may occur too. Several preservation strategies have been developed to prolong the shelf-life of such vulnerable cooked meat products by targeting the microbial communities, with different rates of success. Whereas high-pressure treatments do not always pose a straightforward solution, a promising strategy relates to the use of bioprotective cultures containing lactic acid bacteria. The latter consist of strains that are deliberately added to the ham to outcompete undesirable microorganisms. Spoilage problems seem, however, to be specific for each product and processing line, underlining the importance of tailor-made solutions.

Keywords: cooked meat, spoilage, bioprotective cultures, packaging

1. Introduction

Cooked hams are nutritionally, gastronomically, and economically important products. On average, cooked hams constitute over one fourth by volume of all delicatessen products sold in Europe (Casiraghi *et al.*, 2007). They can be defined as products obtained from the application of heat and curing agents on muscle. Their quality depends on both the raw materials and ingredients used and the processing applied (Delahunty *et al.*, 1997). Overall, the production process transforming the raw meat into cooked ham encompasses two main steps, which are each affecting microbial safety and shelf-life (Fig. 1).

In a first step, the raw meat is tumbled and blended with the rest of the ingredients, injected with brine, and compacted into ham logs. During tumbling, the brine is distributed throughout the meat mass and causes a number of desirable effects via its major constituents, *i.e.*, sodium chloride and sodium nitrite (Katsaras and Budras, 1993; Casiraghi *et al.*, 2007). Sodium chloride leads to textural changes, perceived saltiness, and the prolongation of the shelf-life of the end-product. Nitrite yields the typical cured properties of cooked ham, in particular the characteristic pink colour caused by nitrosomyochromogen. Although the latter compound is a stable pigment, it may eventually fade out due to microbial action and light exposure (Kerry *et al.*, 2006; Munk *et al.*, 2010). Nitrite also leads to curing flavour, has an antioxidant potential, inhibits spore-forming anaerobic bacteria such as *Clostridium botulinum*, and negatively affects survival of pathogens such as *Listeria monocytogenes* (Sebranek, 2009). Besides sodium chloride and sodium nitrite, other brine ingredients are frequently added to offer specific advantages. Ascorbate, for instance, acts as an acidulant, promotes the formation of nitric acid,

and acts as an antioxidant (Sebranek, 2009). Phosphates are added to improve colour and flavour of the end-product and to increase the water holding capacity. As phosphates are not essential brine ingredients and are mainly added for economical reasons, high-quality cooked hams are usually produced without them (Casiraghi *et al.*, 2007). Carbohydrates may be added to the brine as well, to finalise flavour by masking undesirable pungent and salt effects from other ingredients and, indirectly, to contribute to the texture of the product (Feiner, 2006).

The second step in the manufacturing process relates to the cooking process of the formed ham logs, followed by cooling. Cooking affects the shelf-life and safety by killing spoilage and pathogenic bacteria, respectively (Franz and von Holy, 1996a; Björkroth *et al,* 1998). In addition, it leads to aroma development, colour formation, denaturation of proteins, and loss of water (Katsaras and Budras, 1993; Tornberg, 2005). Subsequent protein gelation makes the ham cohesive and sliceable (Katsaras and Budras, 1993; Casiraghi *et al.*, 2007). During cooking, temperature control is the key point for the quality of these meat products. Whereas fast treatments can be obtained at high temperatures, these also causes high cooking losses, low texture quality, inferior organoleptic quality, and colour deviations (Hedrick *et al.*, 1990; Bejerholm and Aaslyng, 2004). Common manufacturing practices include heating of the products up to a core temperature of 65 to 75 °C for a specific period of time, usually for 2 to 4 h. Three main ways of heat treatment are usually applied, *i.e.*, immersion in a water bath, dry-air cooking, and wet-air cooking. Small deviations herein may result in large sensory differences (Cheng and Sun, 2004). Several advantages have been attributed to water cooking, such as an increase of tenderness and cooking yield, improvement of the microbiological safety through efficient heat penetration, easily available equipment, precise control over the degree of

doneness, low running costs, and less working space (Cheng and Sun, 2007). The way of cooking affects also the subsequent cooling speed, since water evaporation is affected by microstructure differences on the surface and altered porosity (McDonald and Sun, 2001; Cheng and Sun 2004). To keep a good hygiene and manufacturing status and to comply with food safety objectives, it is essential to keep the cooling of the ham logs as short as possible. However, cooling is intrinsically a slow process due to the low thermal conductivity of meat (Desmond *et al*., 2002). If desired, slicing and vacuum- or modified-atmosphere-packaging (MAP) may be done prior to distribution. Additional steps may be carried out, such as post-pasteurisation of the ham logs to reduce microbial cross-contamination and smoking to influence flavour.

2. Limited shelf-life of cooked hams: an emerging problem

Inadequacies in shelf-life of cooked hams are frequently observed and may in some cases be linked to the quality standards of the end-products. Although quality differentiation of cooked hams does not exist as in the case of dry-cured ham, certain countries such as Belgium, France, and Italy provide a regulatory framework and allocate labels. This is based on multifactorial schemes that take into account raw meat ingredients, moisture levels, technological practices, and additives used (Moretti *et al.*, 2009). The adoption of quality labels beneficially affects the consumers' appreciation but may also compromise shelf-life. The Belgian cooked ham quality label, *Meesterlycke*, for instance, requires the absence of chemical additives and a limitation of the sodium chloride level to a maximum of 2% (wt/wt), frequently resulting in unstable products and severe bacterial spoilage before consumption (Vasilopoulos *et al*., 2008). In addition, shelf-

life limitations of cooked meat products become increasingly important due to changes in storage processes, habits, global trade, and social aspects (Quested *et al.*, 2010).

Shelf-life limitations generally lead to a fast deterioration of the sensory properties of cooked ham, *i.e.,* appearance, taste, odour, tenderness, hardness, springiness, cohesiveness, gumminess, chewiness, juiciness, and colour. These properties are largely dependent on the quality of the meat used as raw material, which can be considered as a system of different, interdependent chemical constituents, *i.e.*, water, protein, fat, and sodium chloride and other minerals (Válková *et al.*, 2007). Quality deterioration of cooked hams ultimately leads to the rejection of the products by the consumers and consequent economic losses. It is a complex process that may be perceived as spoilage, combining the effects of physical damage, enzymatic reactions, and microbial action (Borch *et al.,* 1996; Gram *et al.,* 2002). Two types of spoilage of cooked ham can be distinguished, *i.e.,* physicochemical deterioration and microbial spoilage (Fig. 1).

In principle, it should be possible to monitor spoilage via specific criteria and biomarkers. Such indices could be used to measure and even ultimately predict the spoilage onset, bypassing laborious and time-consuming microbiological and sensory tests (Dainty, 1996; Huis in't Veld, 1996). Thus, positive correlations between bacterial growth and metabolite generation would enable real-time evaluation of the spoilage process, although a definite establishment of quality indicators with high predictive power remains difficult (Stolzenbach *et al.,* 2009). Whereas the nutrients supporting microbial growth are relatively limited, the variety of potential end-metabolites is much larger, hindering a proper selection of potential indices. Also, difficulties have been ascribed to the effects of packaging, different product types, sample heterogeneity,

and numerous bacterial variations and interactions (Dainty, 1996). In addition, there are cases where the emergence of metabolic compounds is poorly understood (Huis in't Veld, 1996).

Whereas the effort of establishing quality indices in fresh meat and fish matrices is well documented, work on cooked cured meat products is somewhat limited. Spoilage of sliced cooked ham by lactic acid bacteria has been correlated with changes in pH and in glucose, lactic acid, and acetic acid levels (Mataragas *et al.*, 2007). Still, lactic acid is not considered as a proper quality index, not only due to its variable initial presence in muscle but also becuase of the buffering capacity of cooked ham products. Spoilage can be apparent from 150 to 400 mg of lactic production per 100 g of meat (Metaxopoulos *et al.*, 2002; Vermeiren *et al*, 2005; Vasilopoulos *et al.*, 2008). Other candidate compounds that display positive correlations with spoilage encompass metabolites such as 2- and 3-methyl-1-butanal, 2- and 3-methyl-1-butanol, acetoin, and diacetyl, although their acceptability may be considerably product-dependent (Leroy *et al.*, 2009; Holm *et al.*, 2012). Up till now, no metabolites have been definitely identified as collective spoilage indicators. Nevertheless, the emergence of meta-metabolomics and the improved use of databases and statistical and computational methods offer perspectives.

3. Spoilage through physicochemical deterioration

3.1. Oxidation effects

Physicochemical deterioration of cooked hams includes discolouration and the formation of off-odours, mostly due to oxidation involving free fatty acids during heating and light exposure.

Lipid oxidation is a major cause of physicochemical deterioration and occurs in either the triglyceride or the phospholipid fractions of cooked ham. Phospholipids contain more unsaturated fatty acids than triglycerides and are therefore particularly prone to oxidative degradation. Lipolysis is responsible for the release of carboxylic acids, which can be more easily oxidised to odour-active compounds (Gandemer, 2002). Although slight oxidation of unsaturated fatty acids during the cooking process contributes to the characteristic aromatic profile of the end-products, it may cause rancidity and colour deterioration during storage (Freybler *et al.,* 1993). The thin line separating desirable aromas from off-odours results from a subtle equilibrium between the products from lipid oxidation and those from Maillard reaction formed during heating. Several hundreds of volatile compounds derived from lipid degradation have been found in cooked meat products, including aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids, and esters (Dransfield, 2008). Prolonged storage leads to rancid and unpleasant flavours due to the presence of such compounds in elevated concentrations (Morrissey *et al.,* 1998). Aldehydes, such as hexanal and nonenal, seem to play a major role in the rejection of cooked hams (Zanardi *et al.*, 2002; Dransfield, 2008). Also, auto-oxidation of unsaturated fatty acids is known to form peroxides and hydroperoxides that lead to an enhancement of the rancid aftertaste of the products, with fatty, waxy, pungent, and green odours (McMillin, 2008; Brewer, 2009).

Unfortunately, even at low oxygen levels, exposure of nitrosomyochromogen to light promotes its oxidation and imposes a dull greyness on the product surface (Walsh and Rose, 1956; Andersen and Skibsted, 1992; Kerry *et al*., 2006). This colour change generally proceeds

in parallel to rancidity. It is further hypothesised that lipid oxidation derivatives have the ability to promote discolouration of the meat (Munk *et al*., 2010).

3.2. Influence of the brine solution

Physicochemical deterioration is not only linked to heating and light but also to the ingredients used, in particular the brine solution. Sodium chloride is known to display prooxidative potential and to linearly promote lipid oxidation, even in the range of conventional brine solutions containing 2.5 to 5.0% (wt/vol) of sodium chloride (Rhee and Ziprin, 2001). In contrast, nitrites are the main factor delaying lipid oxidation in cooked hams (Walsh *et al*., 1998). It has been observed that nitrosomyochromogen acts as bioactive reservoir, leading to stabilization of the lipid fraction and preventing the development of oxidative rancidity (Freybler *et al*., 1993; Carlsen *et al*., 2005). Likewise, the brine component ascorbate is known for its antioxidant potential (Honikel, 2008). However, high concentrations of ascorbate are required to activate its antioxidant mechanism, since lipid oxidation is actually promoted when used in low concentrations (Decker and Xu, 1998).

3.3. Effect of packaging

The rate of physicochemical deterioration of cooked hams largely depends on the effects of the distribution chain, in particular the packaging of the end-products and the temperature at which the packed products are stored (Munk *et al*., 2010). Packaging is to be considered as an effective

manner to limit physicochemical defects and many attempts have been made to optimize this technology, in particular with respect to oxygen levels, for instance by applying vacuum-packaging or MAP (Labadie, 1999; McMillin, 2008). Retail packaging under the latter conditions effectively delays rancidity development (Honikel, 2008). As the environment in the package is not controlled, the initial volume and gas composition alters virtually immediately after packaging. This change is attributed to diffusion of gases into the cooked meat, transmission of oxygen through the package, and bacterial respiration. Oxygen diffusion in the package enhances the risk on product deterioration and the residual oxygen concentration in the headspace is a critical parameter for discolouration and oxidation processes (Møller *et al*., 2000; Nannerup *et al*., 2004).

4. Spoilage due to bacterial effects

4.1. Microbial spoilage criteria

Based on the observed defects in colour, pH, and sensory characteristics, maximal microbial spoilage levels for MAP cooked meat products have been suggested. Such levels are generally set to 6 log cfu of viable counts per gram of cooked ham product (Samelis *et al*., 1998, Stekelenburg and Kant-Muermans, 2001; Stolzenbach *et al*., 2009), 7 log cfu g^{-1} (Korkeala *et al*., 1987; Vermeiren *et al*., 2005; Slongo *et al*., 2009; Kreyenschmidt *et al*., 2010), or may even be as high as 8 log cfu g^{-1} of viable counts (Mataragas *et al*., 2006). This suggests that it is not the growth rate and final concentrations of spoilage-related microorganisms that determine microbial

spoilage as such, but rather their metabolic activities, both type and intensity, resulting from growth (Pin *et al*., 2002). To complicate things further, the production of some metabolites of spoilage bacteria in cooked meat products tends to be more pronounced during their stationary growth phase (Borch *et al*., 1996; Huis in't Veld, 1996; Mataragas *et al*., 2006; Stolzenbach *et al*., 2009).

The growth and activity of spoilage microorganisms in cooked meat products is determined by the available substrates and the prevailing physicochemical conditions, such as temperature, pH, water activity, and atmosphere. These parameters will not only select the dominating spoilage-associated microorganisms but also define their interactions and metabolic behaviour (Borch *et al*., 1996; Gram *et al*., 2002; Comi and Iacumin, 2012). The perceived spoilage through the microbial production of metabolites, such as organic acids, carbon dioxide, amines, sulphur compounds, dextrans, alcohols, aldehydes, and ketones, is often described as a function of external factors, such as overall processing, storage temperature, packaging environment, and properties intrinsic to the nature of the cooked ham products.

4.2. Intrinsic effects of the ham matrix

The ham matrix plays an essential role in the selection for the dominant microbiota due to its intrinsic characteristics, including the presence of brine ingredients such as sodium chloride, nitrite, and carbohydrates. The curing salt present in the brine only slightly decreases the water activity of the cooked ham matrix, to levels at which bacterial growth cannot be effectively inhibited (Mataragas *et al.* 2006). Although the use of nitrite in concentrations as low as 50 ppm

is enough to protect the cooked ham products against spore-forming pathogens, most notably *C. botulinum*, it hardly affects the growth of other spoilage-associated bacteria (Sebranek, 2009). Salts of organic acids, frequently added to the brine mixture, have various effects on spoilage-related bacteria. Such salts mainly enhance the safety of the final products, due to their antibacterial effect against pathogens commonly associated with cooked cured meat products, such as *L. monocytogenes* (Simpson and Sofos, 2009). With respect to the availability of carbohydrates, glucose is the main one present in ham. Ribose and ribonucleotide compounds are largely reduced in concentration, due to their reactions with cysteine and phospholipids (Mottram, 1998; Xu *et al*., 2008). Glucose levels are depending on the nature of the raw meat, the brine recipe, and the amount of brine that is injected into the meat mass. Final amounts of glucose range from values as low as 0.1% up to 7% (wt/wt) (Bautista *et al*., 2000; Stekelenburg and Kant-Muermans, 2001; Vermeiren *et al*., 2006d). The applied glucose concentrations, however, have little effect on the dominance of spoilage-associated microorganisms (Vermeiren *et al*., 2006d). Occasionally, other carbohydrates than glucose may be used in the brine to enhance flavour, mask pungent aromas, or stabilize colour. Maltodextrins and other starch derivatives, lactose, and sucrose are most commonly used for bulking, as texture agents, or for fat-mimicking, but their effect on microbial selection is minimal (Jolly and Anderstein, 2009). However, they may be responsible for other spoilage phenomena, such as ropiness during growth of slime-forming lactic acid bacteria on sucrose (Aymerich *et al*., 2002). Other intrinsic properties of cooked hams, in particular water activity, pH, and buffering capacity, will affect the diffusion of carbon dioxide into the water and fat phases of the cooked ham matrix, selecting for specific microbiota (Sivertsvik and Jensen, 2005).

4.3. Effects of packaging

It is common practice to distribute cooked ham products in an oxygen-free environment obtained through packaging under vacuum or modified atmosphere (Devlieghere *et al*., 2000). As an immediate effect, aerobic spoilage bacteria fail to dominate the microbiota of the packed product. Bacteria typically associated with aerobically stored meat include *Pseudomonas* spp., *Acinetobacter* spp., *Psychrobacter* spp., and *Moraxella* spp. (Borch *et al.*, 1996). Besides the absence or low availability of oxygen, these bacteria are also inhibited by the carbon dioxide present in modified atmospheres. Indeed, volumes of carbon dioxide above 20% (vol/vol) have a bacteriostatic effect not only on psychrotolerant aerobic bacteria (Coma, 2008) but also on lactic acid bacteria (Holley, 1997; Devlieghere *et al.*, 1998). Too high concentrations of carbon dioxide may lead to discolouration, drip losses, and collapse of the package due to the diffusion of the carbon dioxide into the cooked ham matrix (Møller *et al*., 2005). The antimicrobial effects of carbon dioxide are due to a number of mechanisms, including a decrease of intracellular pH, interference with enzyme synthesis, and disruption of the cell membrane because of changes in its permeability and rigidity and specific protein–lipid interactions (Phillips, 1996).

4.4. Effects of chilling

The major effect of the chilling of meat products is retardation of the growth and metabolism of the meat-associated microbiota. The direct effect of low temperature on the maximum specific

growth rate of these microorganisms explicitly denotes that a progressive slowdown of their growth is obtained, depending on the microbiota and their environment (Russell and Fukunaga, 1990). This mainly occurs through a continuous decrease in cytoplasmic membrane fluidity (Nedwell, 1999). As a result, adaptation of the microbiota to chilled conditions has been described, leading to a dominance of psychrophilic bacteria. Cold-shock proteins are produced and modifications are introduced in the cell membrane (Kim *et al.,* 1998; Russell, 2002). Such effects have been described for a number of meat-associated psychrophilic lactic acid bacteria (Marceau *et al.*, 2004), explaining their dominance in MAP cooked meat products. Usually, temperature fluctuations are seen during chilled distribution and storage of MAP cooked hams. Temperature history will eventually determine the microbial consortium dominating and spoiling cooked meat products prior to consumption. Temperature deviations have a more significant impact on the shelf-life and final quality of cooked meat products than initial contamination levels prior to distribution (Mataragas and Drosinos, 2007).

4.5. Effects of processing

During processing of brined raw ham logs, cooking plays inarguably the largest role in bacterial selection (Borch *et al.,* 1996). The effect of cooking is often measured by monitoring the core temperature of the treated ham logs, which determines the application of the appropriate temperature and time combinations. However, deviations from proper cooking practices frequently occur (Samelis *et al.,* 2000). Moreover, sanitation of the processing line and cooking, even intensive, are not always effective against handling-related post-contamination or against

thermotolerant bacteria (Peirson *et al.*, 2003a; Perez-Chabela *et al*., 2008). The process hygiene and the technological parameters that are associated with the final pH and water activity of the products, in combination with the house microbiota, eventually dictate the type and extent of microbial contamination and, subsequently, the type of spoilage (Samelis *et al.*, 2000; Vasilopoulos *et al.*, 2010a).

Unfortunately, there is a lack of studies dealing with the microbiota present in different stages of the ham production line, in comparison to the amount of studies dealing with the microbial association of the final MAP cooked ham products. As a consequence, little is reported on the origin and development of microbial consortia throughout production. The debate on the origin of the contamination focuses on the cooking process of hams (Björkroth *et al*., 1998; Metaxopoulos *et al*., 2003; Samelis *et al.*, 2006). It is not clear yet whether microorganisms are coming from the meat cuts introduced to the factory or from post-contamination occurring after cooking and during slicing of the products. It seems, however, that the main processing area of the meat products is of major importance (Goto *et al*., 2004; Byrne *et al.*, 2008; Vasilopoulos *et al.*, 2010a). Microorganisms that reside on the skin or in the intestines of the animals can contaminate meat during slaughtering, carcass skinning, and trimming, and can be subsequently transferred onto surfaces of the meat processing equipment. *Carnobacterium* spp., *Lactobacillus sakei, Brochothrix thermosphacta*, enterococci, and Enterobacteriaceae have been linked to the raw meat mass, whereas sanitation schemes in the processing area have been correlated with the presence of *B. thermosphacta* and Enterobacteriaceae in the final products (Nielsen, 1983; Holley and McKellar, 1996; Samelis *et al*., 2000; Vasilopoulos *et al*., 2010a). The presence of leuconostocs has been associated with their frequent occurrence in the air and on utensils rather

than on the meat itself (Mäkelä *et al.,* 1992b; Goto *et al.*, 2004; Vihavainen and Björkroth, 2009). Whether the microbiota are originating from the raw meat (Björkroth *et al*., 1998; Vasilopoulos *et al.,* 2010a) or should be considered as contaminants of the processing area, they are specific for the production facility and its products (Franz and von Holy, 1996b; Björkroth and Korkeala, 1997a; Samelis *et al*., 1998; Metaxopoulos *et al*., 2002, Vasilopoulos *et al.*, 2010a). As a result, *Leuconostoc* spp. are more frequently isolated from facilities that are producing only cooked meats than from facilities that are also producing fermented meat products in the same area. In the latter case, the isolation of lactobacilli, such as *Lb. sakei*, is more common (Ammor *et al.,* 2005; Chevallier *et al., 2*006). Also, lactobacilli are favoured in manufacturing sites that apply smoking and high cooking temperatures (Samelis *et al.,* 1998).

During raw meat and product handling, microorganisms are carried over and can potentially adhere to several surfaces in the process facility, forming biofilms (Brightwell *et al.,* 2006; Badel *et al.,* 2008). In food-processing environments, microbial attachment is additionally affected by food matrix constituents. Residues from ready-to-eat meat products, such as small amounts of juice extracted from the meat or pork fat, initially reduce biofilm formation by *L. monocytogenes*, but with time support increased biofilm cell numbers and prolonged survival on a variety of materials, including stainless steel, conveyor belt rubber, and wall and floor materials (Somers and Wong, 2004; Shi and Zhu, 2009). This may also lead to increased tolerance to different hygiene techniques (Jessen and Lammert, 2003; Brooks and Flint, 2008).

Interventions throughout the production line also interfere with microbial development. Cleaning techniques play a significant role in microbial selection. For example, an acid cleaning step may delay growth of carnobacteria and aerococci but will eventually select more acid-

tolerant lactic acid bacteria (Peirson *et al*., 2003b). In addition, cooking and bag handling determine the bacterial contamination rate of and survival on whole-cooked hams (Cheng and Sun, 2007).

5. A detailed look at the microbiota of MAP cooked ham

5.1. Lactic acid bacteria

Lactic acid bateria are Gram-positive, facultative anaerobic bacteria that produce lactic acid from carbohydrate fermentation (Axelsson, 2004). As they are favoured by oxygen-restrained conditions and are not inhibited extensively by the presence of carbon dioxide, they have been found as specific spoilage bacteria in several MAP cooked meat products. The low temperatures used during distribution and storage of MAP cooked meat products are known to favour the development of psychrotolerant lactic acid bacteria. Both homofermentative and heterofermentative lactic acid bacteria use glucose, the sole carbohydrate of cooked ham, to mainly produce lactic acid. In addition, they may produce acetic acid, formic acid, ethanol, and carbon dioxide. These metabolic end-products lead to acidity and/or package bulging. Spoilage by lactic acid bacteria has been attributed to the production of volatile compounds resulting from the metabolism of amino acids or other substrates during oxygen-free storage (Dainty, 1996). As a result, metabolic activity of lactic acid bacteria may not only result in acidification but also in putrefactive off-flavours and off-odours. Also, other types of spoilage appear in cooked meat products, such as slime formation, greening, and hydrogen sulphide production (Ahvenainen *et*

al., 1989; Björkroth and Korkeala, 1997b; Samelis *et al*., 1998, 2006), as well as the production of biogenic amines (Ruiz-Capillas *et al*., 2007).

The consortium of lactic acid bacteria that will ultimately prevail in a specific cooked meat product depends on the house microbiota of the producing facility and the characteristics of the applied meat, ingredients, and technologies (Vasilopoulos *et al.,* 2010a). For instance, outgrowth of leuconostocs and carnobacteria is favoured by a high pH of the product and by specific curing conditions, such as the presence of curing salts (Samelis *et al.,* 1998). Although only a limited number of lactic acid bacteria species have been associated with ham spoilage, findings on species level are diverse and the heterogeneity within a species should not be overlooked. For example, through ribotyping it has been observed that various *Lb. sakei* strains found in a processing facility have spoilage potential, but this is not the case for all of them (Björkroth *et al.,* 1996). Also, not all *Leuconostoc carnosum* strains prevailing in the end-product have the ability to lead to cooked ham rejection (Björkroth *et al.,* 1998; Samelis *et al.,* 2006). Similar variability exists within the species *Leuconostoc gasicomitatum* (Vihavainen and Björkroth, 2009).

Concerning slime formation, a direct link with the action of certain homo- or heterofermentative lactic acid bacteria belonging to *Lactobacillus* and *Leuconostoc* species has been found. Slime is strongly correlated with the addition of sucrose to the brine. Sucrose is a common ingredient in cooked hams produced and distributed in Northern-European markets to provide a round and sweet palate, a smooth texture, and an increased cooking yield. However, it is the responsible agent for slime formation and its use in brine is not recommended (Deibel and Niven, 1959). Interestingly, strains of dextran-producing lactic acid bacteria may be more heat-

tolerant than other strains of the same species, which may favour spoilage when insufficient heating is applied (Aymerich *et al*., 2002).

Hydrogen peroxide produced by some lactic acid bacteria can react with (di)nitrosohemochrome, formed because of the effect of cooking temperature on nitrosomyoglobin, to give the meat a green or grey colour appearance (Grant *et al.*, 1988). This discolouration is due to the oxidation of nitrosohaemochrome to choleomyoglobin, which results in a green pigment. Alternatively, green discolouration is caused by oxidation of porphyrins (Holley *et al.*, 2002). As the presence of oxygen is obligatory for hydrogen peroxide production, greening is only a problem in MAP cooked hams that have been opened prior to consumption. Subsequently, lactic acid bacteria that have been associated with greening may deteriorate the product, in particular *Leuconostoc* spp., *Carnobacterium divergens*, *Enterococcus* spp., and *Pediococcus* spp. (Grant *et al*., 1988; Borch and Molin, 1989). Surface greening may also be caused by heat-tolerant lactic acid bacteria that contaminate meat products after cooking. The species *Weissella viridescens*, for instance, is able to survive regular heat processing at 68 °C for more than 40 min during frankfurter-type sausage production (Niven and Evans, 1957; Borch *et al*., 1996). Besides greening, *W. viridescens* may also produce cavities (Comi and Iacumin, 2012). Peirson *et al*. (2003a) have shown that *Aerococcus viridans*, *Carnobacterium viridans*, and *W. viridescens* can cause green discolouration in cooked cured Bologna. They have disputed previous connections of greening with *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp. According to others, leuconostocs on cooked ham that have been exposed to atmospheric oxygen are the main bacteria responsible for greening (Samelis *et al*. 2000, 2006; Anifantaki *et al.*, 2002).

With regard to acid-type spoilage, the most frequently encountered lactic acid bacteria from packed cooked meat products are *Lb. sakei*, *Lactobacillus curvatus*, and *Leuconostoc mesenteroides* (Korkeala *et al*., 1987; Borch and Molin, 1988; Dykes *et al*., 1995; Samelis *et al*, 1998, Drosinos *et al*., 2006; Hu *et al*., 2009; Comi and Iacumin, 2012). They can, however, not always be pinpointed as the actual cause due to possible strain and product specificities. The buffering capacity of the cooked meat products may, to a certain extent, limit the perception of acidification in the final products, at least if the prevailing bacterial communities consist of weak acidifiers. Generally, heterofermentative lactic acid bacteria such as leuconostocs are known to be poorer acidifiers than homofermentative and/or facultative heterofermentative ones such as *Lb. sakei* (Vermeiren *et al.*, 2005). During the last decades, *Lb. sakei* and *Lb. curvatus* have been considered as the major spoilage-associated bacteria of packed cooked meat products. This has been ascribed to the competitive advantage that *Lb. sakei* possesses in adapting to meat matrices (Chaillou *et al.*, 2005; Rimaux *et al*., 2011a,b) and low temperatures (Marceau *et al.*, 2004). Raw meat mass has been identified as a source of *Lb. sakei*, introducing it into the processing area (Vasilopoulos *et al.*, 2010a). Further handling and cross-contamination will eventually be the main factors of *Lb. sakei* occurrence in the final packed cooked meat products (Samelis *et al.*, 1998; Vasilopoulos *et al.*, 2010a). Also, the high prevalence of *Lb. sakei* has been associated with smoking of certain cooked cured meat products (von Holy *et al.*, 1991; Zhang and Holley, 1999), most probably because of its ability to grow on surfaces with lower water activity values.

Leuconostocs are a major concern in MAP cooked meat products, in particular *Leuconostoc mesenteroides* subsp. *mesenteroides* and *Leuc. carnosum* (Vasilopoulos *et al.*, 2008; Audenaert *et al*., 2010; Comi and Iacumin, 2012). *Leuc. gasicomitatum* is a lactic acid bacterium

species that has recently emerged as a spoilage microorganism in different meat matrices distributed under MAP (Björkroth *et al.*, 2000; Susiluoto *et al*., 2003; Vihavainen and Björkroth, 2009). Leuconostocs are related to product rejection because of the production of heterofermentative end-products, mainly acetic acid and ethanol, as a result of glucose consumption. Also, they may produce volatile compounds derived from amino acid catabolism, such as aldehydes, phenols, alcohols, and ammonia, in meat matrices (Dainty, 1996; Björkroth *et al.*, 2000; Metaxopoulos *et al.*, 2002; Diez *et al.*, 2009a). Moreover, leuconostocs are known dextran producers, leading to slime formation (De Vuyst and Degeest, 1999). Also, they may produce gas because of glucose metabolism and in rare cases because of amino acid decarboxylation (Björkroth *et al.*, 2000). Spoilage due to gas and slime formation by leuconostocs has been found in, for instance, Vienna sausages (Dykes *et al.*, 1994) and cooked hams (Hamasaki *et al.*, 2003; Samelis *et al*, 2006). Ropy slime-type of spoilage in MAP cooked meat products has also been attributed to consortia of lactic acid bacteria, for instance *Leuc. mesenteroides, Leuconostoc amelibiosum*, and *Lb. sakei* (Mäkelä *et al*., 1992a). Dominance of leuconostocs over the *Lb. sakei/curvatus* group is influenced by the manufacturing process (Samelis *et al*., 1998). In non-smoked cooked meats, leuconostocs seem to have an advantage (Shaw and Harding, 1989, Björkroth *et al*., 1998; Samelis *et al*., 2000, 2006). The dominance and adaptation advantage that leuconostocs have in these products has been highlighted by molecular methods. A large genotypic variability has been found in a cooked ham matrix through pulsed-field gel electrophoresis (Björkroth *et al.*, 1996). Also, through $(GTG)_5$-PCR-fingerprinting, two different types of *Leuc. carnosum* have been shown to dominate a cooked ham product depending on the storage temperature (Vasilopoulos *et al.*, 2008).

Besides lactobacilli and leuconostocs, carnobacteria are a major group of lactic acid bacteria prevailing in MAP cooked ham products (Laursen *et al.,* 2005). Carnobacteria exploit a number of traits that give them a competitive advantage over other microorganisms in MAP cooked meats. They are psychrophilic and prefer non-aciduric environments. Although they are adapted to low-temperature environments, they are characterised by a tolerance to high temperatures, therefore giving them the chance of surviving heat treatments (Peirson *et al.,* 2003b). This is especially the case if they contaminate the raw ham logs in high numbers. In the past, culture limitations frequently overlooked their presence (Leisner *et al.,* 2007; Vasilopoulos *et al.,* 2008). Originally, they have been described as 'atypical lactobacilli' (Collins *et al.,* 1987), but they are quite distinct from lactobacilli on the basis of 16S rRNA gene identity. Their isolation frequency and subsequent characterisation has been hindered by the widespread use of de Man-Rogosa-Sharpe (MRS) medium for the enumeration of presumptive lactic acid bacteria in various matrices (de Man *et al.,* 1960). Carnobacteria fail to outcompete other lactic acid bacteria when grown in MRS medium, mainly because of the presence of acetate in this medium. It has, therefore, been suggested to omit acetate from MRS, to buffer the medium to a high pH value, and to omit other ingredients such as Tween 80 too, taking advantage of the less fastidious character of carnobacteria as compared to other lactic acid bacteria (Borch and Molin, 1989). Alternatively, other media such as nitrite actidion polymyxin (NAP) or nitrite polymyxin (NP) medium may be used (Paludan-Müller, 1998; Laursen *et al.,* 2005). The habitat of carnobacteria is not yet known and they have not been isolated from the gastrointestinal tract of birds or mammals. However, they seem to be associated with the gastrointestinal tract of fish (Leisner *et al.,* 2007). Of the nine species that comprise the *Carnobacterium* genus, *C. divergens* and *C.*

maltaromaticum are the two most common encountered species in MAP cooked meat products (Chenoll *et al.*, 2003; Macián *et al.*, 2004; Vasilopoulos *et al.*, 2008; Audenaert *et al.*, 2010), but *C. viridans* has been isolated too (Holley *et al.*, 2002). However, carnobacteria species exhibit over 96% identity in their 16S rRNA gene sequences (Kabadjova *et al.*, 2002). In particular, phylogenetic studies based on 16S rRNA gene sequence comparisons have revealed that *C. divergens, C. gallinarum,* and *C. maltaromaticum* are closely related species (Chenoll *et al.*, 2003). However, carnobacteria display a rich phenotypic variation on strain level (Leisner *et al.*, 2007). This makes the formulation of conclusions concerning the spoilage potential of carnobacteria a difficult task (Kabadjova *et al.*, 2002; Laursen *et al.*, 2005). Generation of branched alcohols and aldehydes (2-methyl-1-butanal, 2-methyl-1-butanol, 3-methyl-1-butanal, 3-methyl-1-butanol, 2-methylpropanal, and 2-methylpropanol) by transamination, decarboxylation, and reduction of the branched-chain amino acids valine, leucine, and isoleucine appears to be particularly important for spoilage manifestation in cooked hams, especially with strains of *C. maltaromaticum* (Budde *et al.*, 2003). However, the spoilage potential of these lactic acid bacteria has been questioned in various meat and fish matrices (Palludan-Müller *et al.*, 1998; Joffraud *et al.*, 2001; Leisner *et al.*, 2007). In fermented sausage, added *C. maltaromaticum* may even contribute to aroma development (Larrouture-Thiveyrat *et al.*, 2003). Alternatively, a butter smell has been reported in meat products (Borch *et al.*, 1996), attributed to the formation of diacetyl and 2,3-pentanedione by *Carnobacterium* spp. (Joffraud *et al.*, 2001). An interesting interaction between *C. divergens, C. maltaromaticum, Carnobacterium mobile,* and *B. thermosphacta* has been found in cooked MAP shrimps (Laursen *et al.*, 2006). Not only do specific off-flavours occur (*in casu* a strong 'wet dog' off-flavour), but also the ability of

carnobacteria to catabolise amino acids and of *B. thermosphacta* to produce diacetyl is influenced by the metabolic activity of mixed communities of these bacteria. Therefore, it can be assumed that the spoilage potential of carnobacteria is substrate- and consortium-related.

There are instances of cooked hams where spoilage has been attributed to the dominant presence of enterococci (Houben and Tjeerdsma-van Bokhoven, 2004). Although *Enterococcus* spp. are frequently isolated from fermented meat products (Leroy *et al*, 2006), they also appear as a spoilage concern in some cooked meat products (Vasilopoulos *et al*., 2008). The main explanation for their presence in the latter products is their thermotolerant character (Sörqvist, 2003), which gives them a numerical advantage over other bacteria upon the first contamination steps during slicing of cooked meat products (Fernández *et al*., 2009). For instance, *Enterococcus faecalis* and *E. faecium* have been implicated in the spoilage of frankfurters because of their survival after cooking to 68 °C for 30 min (Gordon and Ahmad, 1991). In addition, they are frequent cross-contaminating agents because of their ubiquitous occurrence in different environments, ranging from the gastrointestinal tracts of animals to soil and plants (Giraffa, 2002). Cross-contamination of industrial environments is also facilitated by the ability of enterococci to adhere onto surfaces and to form biofilms (Franz *et al*., 1999). Abiding by good manufacturing practices is of critical importance to maintain the risk of enterococcal contamination as low as possible. Also, low temperatures are able to suppress their growth (Vasilopoulos *et al.*, 2008). Spoilage of cooked meat products due to enterococci is mainly attributed to a sour taste because of acid production. Reports on the presence of dominant enterococci in cooked hams focus on *E. faecalis* and *E. faecium* (Ben Omar *et al*., 2004). These two species may also be responsible for greening of sliced cooked hams exposed to atmospheric

oxygen, due to hydrogen peroxide production (Borch *et al.*, 1996). Nevertheless, other enterococcal species can occur in MAP meat products too, such as *Enterococcus casseliflavus, Enterococcus durans, Enterococcus hermanniensis,* and *Enterococcus hirae* (Peters *et al*., 2003; Koort *et al.*, 2004).

5.2. Brochothrix thermosphacta

Spoilage of meat and meat products is frequently associated with the outgrowth of *B. thermosphacta*, a Gram-positive, psychrophilic, facultative anaerobic bacterium, closely related to the genus *Listeria* (Lopez-Caballero *et al.*, 1999). Its morphology is changing during growth, altering the initial coccobacillus shape to long rods during exponential growth and short rods during the stationary phase (Rattanasomboon *et al*., 1999). The exact metabolic pattern of *B. thermosphacta* has not yet been clarified, although it has been suggested that it resembles that of *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* (Blickstad and Molin, 1984; Pin *et al*., 2002). Glucose metabolism leads mostly - if not completely - to lactic acid under oxygen-free conditions. When oxygen is present, the metabolism of this bacterium shifts towards the production of lactic acid, acetic acid, ethanol, and diacetyl. Further conversion of diacetyl to acetoin has been noticed *in vitro* but not in cooked ham products (Blickstad, 1983). In addition to these compounds, *B. thermosphacta* can degrade valine, leucine, and isoleucine to produce methyl-butyric acids and alcohols (Dainty *et al.*, 1985). Accumulation of these metabolites may lead to the characteristic sweet, malty, and buttery types of spoiled cooked meat products, which has been associated with *B. thermosphacta* domination (Stanley *et al.*, 1981). Detection of high

amounts of 2-heptanone and 2-hexanone, which results in a blue-cheese odour, has been attributed to the presence of *B. thermosphacta* too (Joffraud *et al.*, 2001). Although slime formation has been associated with the growth of *B. thermosphacta in vitro* (Talon *et al.*, 1988), such findings have not been validated in cooked ham products. The spoilage potential of *B. thermosphacta* can be very high and lead to rapid product rejection depending on the intrinsic properties of the cooked ham. For instance, *B. thermosphacta* may cause sensory deviations at lower cell numbers than is the case for lactic acid bacteria (Vermeiren *et al.*, 2005). Also, they can cause early spoilage in cooked hams (Holley and McKellar, 1996). The rather stable environment of cooked cured meat products in terms of intrinsic properties, such as pH and water activity, is a crucial factor for the prevalence of *B. thermosphacta*. Even minor environmental changes can cause large changes in the growth ability of this bacterium (Masana and Baranyi, 2000). This partially explains the competitive disadvantage against lactic acid bacteria in fermented meat products, whereas in raw meats and cooked hams *B. thermosphacta* and lactic acid bacteria often co-exist, depending on initial contamination levels and inherent strain competitiveness (Cayre *et al.*, 2005; Russo *et al.*, 2006). Interestingly, slicing of cooked hams and fermented meat products on the same line has contributed to the absence of *B. thermosphacta* on the surface of cooked ham slices, in favour of *Lb. sakei* (Holley, 1997).

5.3. Enterobacteriaceae

Maintaining poor processing practices and hygiene standards may result in an increased contamination of cooked hams with communities of Enterobacteriaceae, Gram-negative bacteria

that frequently reside in the gastrointestinal tract of animals (Héquet *et al.*, 2009) and that can be omnipresent in the meat processing environment (Vasilopoulos et al., 2010a). Even if Enterobacteriaceae do not possess the potential to grow fast to high numbers, it seems that their presence drastically reduces the shelf-life of cooked meat products. Also, their contribution to deterioration of cooked meat products is enhanced after their slicing (Holley and McKellar, 1996). In raw meat, package blowing has been ascribed to Enterobacteriaceae, *i.e.*, species of *Enterobacter, Ewingella, Hafnia, Pantoea, Rahnella*, *Serratia*, and *Yersinia* (Brightwell *et al.*, 2007). *Hafnia, Enterobacter*, *Pantoea*, and *Serratia* are rather psychrophilic bacteria that manage to grow on meat tissues at temperatures as low as 1 °C (Ridell and Korkeala, 1997). The aforementioned bacteria are common colonisers of meat processing areas and can proliferate on the surface of meat products, leading to premature product rejection (Stiles and Ng, 1981; Vasilopoulos *et al.*, 2010a).

5.4. Yeasts

Yeasts do not pose an immediate threat for MAP cooked meat products, because of their slower growth rate in these foods compared with psychrophilic meatborne bacteria. However, the increasing consumer demand for more 'natural' foods that imply the elimination of traditional chemical food preservatives and the use of new bacteriostatic or bacteriocidal preservation techniques may lead to yeast outgrowth (Nielsen *et al*., 2008). The most important yeast isolates of cooked meat products belong to the phyla of the Basidiomycota and the Ascomycota (Nielsen *et al*., 2008; Sanz *et al.*, 2005). Isolates have been assigned to *Candida sake*, *Candida*

zeylanoides, *Cryptococcus carnescens*, *Cryptococcus victoriae*, *Debaryomyces hansenii*, *Kluyveromyces marxianus*, and *Yarrowia lipolytica*. Though scarce, there might be a possibility of yeast outgrowth, especially in environments characterised by oxygen absence. As a result, an increase in malty and vinegar off-odours, especially at elevated storage temperatures, has been found, in association with phenomena such as gas accumulation in the package, slime formation, and formation of white, yellow, or red pigments on the surface of the products (Dillon, 1998; Samelis and Sofos, 2003).

6. Delaying spoilage manifestation in chilled MAP cooked ham products

6.1. Use of common organic acids and their salts

Sodium, calcium, and potassium salts of organic acids, such as acetic acid, propionic acid, citric acid, lactic acid, benzoic acid, and sorbic acid, have been used as antimicrobial compounds to extend the shelf-life of raw meats and meat products and to prevent the outgrowth of pathogens (Houtsma *et al.*, 1993; Devlieghere *et al*., 2000; Samelis *et al*., 2001; Islam *et al*., 2002; Glass *et al*., 2007). Although research is mainly targeting pathogenic bacteria, their role in the inhibition of spoilage by slime-forming psychrophilic bacteria has also been described (Diez *et al.*, 2009b). According to the European legislation, these preservatives fall into the *quantum satis* principle, meaning that no maximum level is specified and that the additives should be used in accordance with good manufacturing practices, at a level not higher than necessary and provided that they do not mislead the consumer, as initially defined by Directive 95/2/EC and furthermore in 2010 by

Regulation 1333/2008. However, consumers perceive even the slightest use of additives as 'unnatural' and, therefore, undesirable.

Differences in activity spectrum and technological activity appear when comparing different organic acids and their salts. For instance, sodium acetate exhibits higher antimicrobial activity than sodium lactate, probably due to its more lipophilic nature and the higher number of undissociated molecules at a given pH (Drosinos *et al*., 2006). The exact mode of action of these components is still not fully understood. The classical 'weak acid' theory proposes that undissociated acid molecules pass through the cytoplasmic membrane of the microbial cell, dissociate inside the bacterial cytoplasm causing acidification and, thereby, inhibiting bacterial growth. However, at the pH range of cooked hams (around pH 6.0), most of these acids are fully dissociated (Devlieghere *et al*., 2000). Therefore, organic acids and their salts are expected to act via several mechanisms, including direct action on the cell membrane and facilitation of its permeability, inhibition of nutrient transport, and lowering of the water activity. The lowering of the water activity in cured meat products has been attributed to denaturation of the meat proteins and subsequent reduction of their water-holding capacity (Simpson and Sofos, 2009). However, conventional concentrations of these additives are not sufficient to significantly lower the water activity of meat products and higher concentrations may lead to sensory and texture changes (Bloukas *et al.,* 1997; Blom *et al*., 1997; Stekelenburg and Kant-Muermans, 2001; Barmpalia *et al.,* 2005). Lipophilic organic acids such as sorbic acid and propionic acid tend to loose their antimicrobial activity in cooked meat products due to the presence of fat. The main quality side-effects by the use of these additives encompass products that are saltier, drier, sourer than untreated ones, and in some cases the texture and colour intensity of the products is described as

not optimal or even irregular. Alternatively, it has been demonstrated that the use of salts of organic acids in brine increases the heat tolerance of bacterial cells (Peirson *et al.*, 2003b; Fernández *et al.*, 2009).

The effective use of organic acids and their salts depends on product composition, target microorganisms, storage temperature, and the presence of different types of salts. The temperature plays a major role, since it has been demonstrated that a mixture of sodium lactate and sodium acetate does not significantly alter the growth of lactic acid bacteria at 9 and 10 °C but it does so at 4 °C (Blom *et al.*, 1997; Barmpalia *et al.*, 2005). Also, synergistic effects with other technological parameters, such as carbon dioxide, have been obtained (Devlieghere *et al.*, 2000; Martínez *et al.*, 2006). Indeed, carbon dioxide from the headspace of the package may dissolve into the cooked ham tissue and locally decrease the pH, which consequently increases the undissociated form of sodium lactate. Furthermore, lactate and diacetate salts are more effective in combination, especially in meat products that are dominated by acid-tolerant lactic acid bacteria such as *Lb. sakei* (Devlieghere *et al.*, 2000; Barmpalia *et al.*, 2005), although exceptions occur (Stekelenburg and Kant-Muermans, 2001; Diez *et al.*, 2008a). Among all bacteria that are of concern in MAP cooked meat products, lactobacilli are the most tolerant to the action of organic acids, whereas *B. thermosphacta,* leuconostocs, *Weissella* spp., and *C. maltaromaticum* seem to be considerably more sensitive (Ouattara *et al.*, 1997; Samelis *et al.*, 2006; Drosinos *et al.*, 2006). However, inhibition of these bacteria *in vitro* by sodium and/or potassium lactate (Sameshima *et al.*, 1997) is not always successful *in situ* (Peirson *et al.*, 2003b, Diez *et al.*, 2009b). Several lactic acid bacteria are not affected by organic acids within a concentration range that still allows acceptance of the product. Stekelenburg and Kant-

Muermans (2001) have found that up to 0.2% of sodium diacetate does not inhibit *Lb. curvatus* dominating a cooked ham. Drosinos *et al.* (2006) have noted that up to 0.15% of potassium sorbate has no effect against lactic acid bacteria in cooked cured meat products when applied as single antimicrobial.

6.2. Mild preservation

The successful development of a preservation strategy should rely on the combined effects of mild multitarget preservative agents rather than on their individual inhibitory actions (Leistner, 2000). In the case of cooked cured meat products, several intrinsic properties that may add to their preservation, such as salt and nitrite, have been seriously questioned because of their contribution to adverse health effects (Demeyer *et al.*, 2008; Taormina, 2010). In addition, current market trends increasingly demand 'natural' products, without chemical additives. As a result, producers are forced to change recipes, thereby rendering them more susceptible to spoilage. Alternative routes for maintaining and/or prolonging the shelf-life of cooked hams need to be adopted and applied on industrial scale. Such routes need to be safe and cost-effective, without deteriorating the sensory properties of the cooked meat products. The most cited example of mild preservation techniques is high-pressure processing. Another possibility is the application of irradiation (Benedito *et al.*, 2011), which however suffers from the low consumers' acceptability and has practically disappeared from the European meat market industry (Aymerich *et al.*, 2008).

High-pressure processing is a preservation technology that offers opportunities to reduce pathogenic and spoilage microorganisms from treated food products (Chefter and Culioli, 1997; Slongo *et al.*, 2009; Vercammen *et al.*, 2011). At low or moderate temperatures, this technique causes inactivation of certain enzymes and the destruction of microbial cells without changing, in general, the sensory attributes of the food product (Considine *et al.*, 2008). The main action site of high-pressure treatments is the bacterial cell membrane that suffers from disruption and loss of intracellular material (Garriga *et al.*, 2004). Besides this effect, which mainly occurs during harsh high-pressure processing, bacterial cells are inactivated because of destabilisation of the membrane's permeability. Also, enzymes vital for cell homeostasis, such as ATPases, are inactivated and protein biosynthesis is inhibited. In general, Gram-negative bacteria are more sensitive to pressure than Gram-positive ones (Hugas *et al.*, 2002). Han *et al.* (2011) have also found differences in sensitivity between lactic acid bacterium species, with *Lb. sakei* and *Lb. curvatus* being much more vulnerable to a high-pressure treatment of 600 MPa for 10 min in comparison with *W. viridescens* and *Leuc. mesenteroides.*

Besides high investment costs and a risk on defects in ham texture and colour when the applied pressure is too high, another main drawback of the technique is its inefficacy towards spores and the potential germinating effect on dormant spores (Devlieghere *et al.*, 2004). Moreover, the efficacy of the treatment depends on the achieved pressure, the exposure time, and the tolerance of the target microorganisms. Besides the pressure tolerance of *Carnobacterium* spp. and *Weissella* spp., there are also strains belonging to *Escherichia coli*, *Listeria* spp., and *E. faecium* that may be difficult to inactivate (Jofré *et al*., 2007; Diez *et al*., 2008b).

6.3. Bioprotective cultures

The need for processed foods with sufficient shelf-life during retail distribution and domestic consumption, and produced with minor addition or in the absence of chemical additives, led towards the exploitation of the antagonistic character of bacteria against each other, in favour of the quality and safety of the final product. The deliberate application of specific bacterial cultures or their metabolic products as an extra hurdle in cooked meat products is framed under the term of 'biopreservation' or 'bioprotection' (Lücke, 2000). The role of bacterial bioprotective cultures in cooked ham is to proliferate in the product, thus suppressing spoilage and pathogenic bacteria, which otherwise would dominate the matrix. The mechanism of action differs according to the bioprotective culture applied, but is mostly ascribed to nutrient competition and the production of inhibitory compounds, in particular organic acids and bacteriocins (Table 1). Bacterial bioprotective cultures usually consist of lactic acid bacteria, due to their generally regarded as safe (GRAS) status, their ability to dominate a plethora of food niches, and their production of lactic acid and other compounds with antimicrobial action (Leroy and De Vuyst, 2004; Corbo *et al.,* 2009; O' Sullivan *et al*., 2009). Unfortunately, too strong accumulation of organic acids beyond the buffering capacity of the cooked ham leads to acid-type spoilage. In addition, other metabolites of lactic acid bacteria have antimicrobial properties provided they are produced in high enough amounts (*e.g*., diacetyl and hydrogen peroxide). Considering the fact that these compounds are spoilage-provoking, even in trace amounts, their role in bioprotection is of little relevance (Gram *et al.,* 2002).

Bacteriocins are often mentioned as one of the key compounds of bioprotective cultures (Gálvez *et al.,* 2007; Castellano *et al.,* 2008). The term bacteriocin comprises a large and diverse group of ribosomally synthesised extracellular antibacterial peptides or proteins, which have a bactericidal or bacteriostatic effect towards Gram-positive target bacteria (De Vuyst and Leroy, 2007). They are not active towards Gram-negative bacteria unless the integrity of the outer membrane has been disrupted, for instance due to the presence of detergents or chelating agents or as a result of osmotic, pulsed electric field, or high-pressure treatments. In general, bacteriocins act at the cytoplasmatic membrane by dissipating its proton motive force through the formation of pores (Héchard and Sahl, 2002). Nisin is the main bacteriocin studied and remains the only bacteriocin commercially available. However, the potential of numerous other bacteriocins has been investigated, as almost every species of lactic acid bacteria may have the ability to produce at least one bacteriocin (Nes and Johnsborg, 2004; Zhu *et al.,* 2009). Rather than using these bacteriocins as additives, the application of bacteriocin-producing strains in the food matrix seems more appropriate (Leroy and De Vuyst, 2004; Alves *et al.,* 2006).

Biopreservation of meat products exploits the beneficial metabolic traits of lactic acid bacteria, for instance bacteriocin production, through either the use of bacteriocin-producing starter or co-cultures or the addition of (semi)-purified bacteriocin preparations (De Vuyst and Leroy, 2007). Common targets of bioprotection are bacteria such as *L. monocytogenes, E. coli,* serovars of *Salmonella enterica,* and *Staphylococcus aureus*. Examples of *in situ* trials include the application of sakacin producers such as *Lb. sakei* CTC 494 to suppress the growth of *L. monocytogenes* in cooked ham (Hugas *et al.,* 1998) and *Lb. sakei* 2512 against *Listeria innocua* that served as a model organism for possible *Listeria* contamination of cooked ham (Héquet *et*

al., 2007). Mataragas *et al.* (2003) have applied the bacteriocin producers *Lb. curvatus* L442 and *Leuc. mesenteroides* L124 to effectively suppress the growth of both *L. monocytogenes* and *L. innocua* on cooked ham, whereas the leucocin-producing *Leuc. carnosum* 4010 has been effectively applied in cooked saveloys against *L. monocytogenes* (Jacobsen *et al.*, 2003). In several cases, *ex situ* trials with several bacteriocins obtained from meat-associated bacteria have been performed with various results. For instance, the combined action of nisin, ethylenediaminetetraacetic acid, and lysozyme reduced the numbers of *E. coli* and *L. monocytogenes* communities in cooked ham and cooked Bologna (Gill and Holley, 2000). Enterocins applied in cooked ham and model cured sausages may help to control the communities of *L. monocytogenes* (Ananou *et al.*, 2005; Marcos *et al.*, 2008). Addition of pediocins bound to heat-killed cells of a *Lb. plantarum* strain to cooked sausages reduced *L. monocytogenes* numbers (Mattila *et al.*, 2003). In another cooked ham experiment, the effectiveness of enterocins, sakacin, and/or nisin has been tested against inoculated *L. monocytogenes*, *S. enterica*, and *St. aureus* (Jofré *et al.*, 2008). Although the bacteriocins tested effectively reduced the numbers of *L. monocytogenes* and *S. enterica*, only nisin managed to suppress the growth of *St. aureus.* In certain cases, non-bacteriocinogenic bacterial strains of *Lb. sakei* have offered a better protective effect against cooked ham spiked with pathogenic bacteria than bacteriocin producers of the same species. Indeed, the non-bacteriocinogenic *Lb. sakei* TH1 strain inhibited the growth of *E. coli* (Bredholt *et al.*, 1999), whereas it was effective against *L. monocytogenes* too (Holck and Berg, 2009). Also, *Lb. sakei* 10A performed better than a bacteriocin-producing *Lb. sakei* in controlling outgrowth of *L. monocytogenes* in cooked ham (Vermeiren *et al.*, 2006c). Apart from *Lb. sakei* strains, other non-bacteriocin producers have

shown antilisterial effects in cooked meat products, such as strains of *Lb. casei, Lb. paracasei,* and *Pediococcus acidilactici* (Amézquita and Brashears, 2002).

Bacteriocin-based strategies have also been applied, albeit on a limited scale, to prolong the shelf-life of cooked meat products by targeting the dominating Gram-positive spoilage bacteria. This can be done by adding bacteriocins directly, as with enterocin AS-48 to inhibit *Lb. sakei*, *B. thermosphacta*, and *Staphylococcus carnosus* (Banos *et al.*, 2012). Alternatively, bacteriocin-producing cultures may be added, as for the application of *Lb. sakei* CTC 494 in cooked meat products to prevent slime formation (Garriga *et al.*, 1998; Aymerich *et al.*, 2002). *Leuconostoc mesenteroides* L124 and *Lb. curvatus* L442 have been successfully used in two different cooked meat products to improve their shelf-life (Metaxopoulos *et al.*, 2002). *Leuconostoc carnosum* 4010 prolonged the shelf-life of saveloys and cooked ham (Budde *et al*., 2003; Jacobsen *et al*., 2003) and *Leuconostoc gelidum* UAL 187 effectively suppressed a spoilage-provoking *Lb. sakei* through bacteriocin action (Leisner *et al.*, 1996). Vermeiren *et al*. (2005, 2006b) have tested *in vitro* and *in situ* the effect of different bacteriocinogenic lactic acid bacteria strains (such as *Lb. sakei* LS5) in cooked meat products with various results. To improve efficacy, bacteriocin-producing bacteria may also be used in combination with added bacteriocins, in particular nisin, as is the case for *Lactococcus lactis* DPC 303-T4 to inhibit spoilage and pathogenic bacteria in a cooked fermented ham (Soriano *et al.*, 2004).

The main reasons for the lack of industrially applied bacteriocin-based strategies are related to technological and product limitations of the cooked meat products. Poor diffusion of bacteriocins into the meat matrix, mostly due to the presence of fat to which bacteriocin molecules easily adsorb, dramatically diminishes their efficacy (Buncic *et al.*, 1997; Aasen *et al.*,

2003). In addition, curing, the high pH of these products, and the low temperatures that are used for their storage, may affect the production of bacteriocins by bacteriocinogenic bacteria (Leroy and De Vuyst, 2005; Kouakou *et al.*, 2009). In the case that bacteriocins are added as additives to the brine, activity losses are to be expected from the long cooking times during pasteurisation. Besides the use of bacteriocin-producing strains, the application of potential bioprotective strains that act through competition for nutrients and niche-related effects may be equally or even more suitable (Perez-Chabela *et al.*, 2008; Héquet *et al.*, 2009; Vasilopoulos *et al.*, 2010b). Bacteria that are highly adapted to the cooked meat matrix and display optimal substrate utilisation have a serious advantage, leading to dominance of this microbiota. Thus, it is essential to select for highly competitive bacteria that do not produce off-flavours or other defects and that are able to grow at refrigerated temperatures. Psychrotolerant, meat-associated, and homofermentative lactobacilli seem to be most appropriate. The first example reported in the literature was a commercialised strain of *Lactobacillus alimentarius*, now reclassified as *Lb. sakei* BJ-33. The strain has been successfully tested in frankfurter-type sausages (Kotzekidou and Bloukas., 1996; Andersen, 1997). However, it has failed to prevent slime formation caused by a *Lb. sakei* strain (Björkroth and Korkeala, 1997a). Other non-bacteriocinogenic *Lb. sakei* strains have been used in cooked meat products, such as *Lb. sakei* TH1 (Bredholt *et al.*, 2001), *Lb. sakei* 10A (Vermeiren *et al.*, 2006a), and *Lb. sakei* B-2 (Hu *et al.*, 2008). However, other lactic acid bacteria may also show potential. For instance, several strains of *Lc. lactis* and *Carnobacterium* spp. inhibit the growth of Enterobacteriaceae (Héquet *et al.*, 2009), whereas application of *Leuc. carnosum* 3M42 may effectively suppress the growth of *B. thermosphacta* and

Enterobacteriaceae resulting in a prolongation of the shelf-life of cooked ham (Vasilopoulos *et al.*, 2010b).

Besides the efficacy of the applied bioprotective culture and its adaptation to the product, other factors may be important, such as the initial bacterial load and the combination with other hurdles (Hugas *et al.*, 2002; Rodgers, 2008). To fulfil industrial requirements and to provide a product with a natural hue (Vandendriessche, 2008), a bacterial bioprotective culture should be easy to grow and apply. The most important bottleneck remains obviously the fact that microbial inoculation may lead to negative sensory properties, such as acid off-flavours or other defects (Bredholt *et al.*, 1999; Jacobsen *et al.*, 2003; Vermeiren *et al.*, 2006b; Castellano *et al.*, 2008). Moreover, microbial inoculation will result in higher cell counts, which may exceed threshold levels that are often perceived by the retail sector as being at the borderline of spoilage. Therefore, potential bioprotective strains need to be carefully investigated regarding the effect of the cooked meat environment with respect to growth kinetics, inhibitory potential, acidification, and overall metabolism. Obviously, the production of biogenic amines or any toxic substance and the presence of transferable antibiotic resistance factors should be avoided in any case (Lücke, 2000).

7. Conclusions

Due to the market evolution towards increased importance of cooked ham products with quality labels or with a minimum use of salt and common additives, several shelf-life problems have emerged, mostly of a bacterial nature. As a result, novel preservation strategies are being

investigated but a ready-made solution is not available yet. A particular concern is that spoilage seems to be specific for each product and processing line. Also, adapted microbiological standards and agreements will have to be foreseen if inoculation with bioprotective cultures is to be applied in practice.

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Figure 1. Schematic flowchart of the main steps and technological aspects involved in the production of packed cooked ham, with indication of the critical points prone to microbial contamination and manifestation of spoilage.

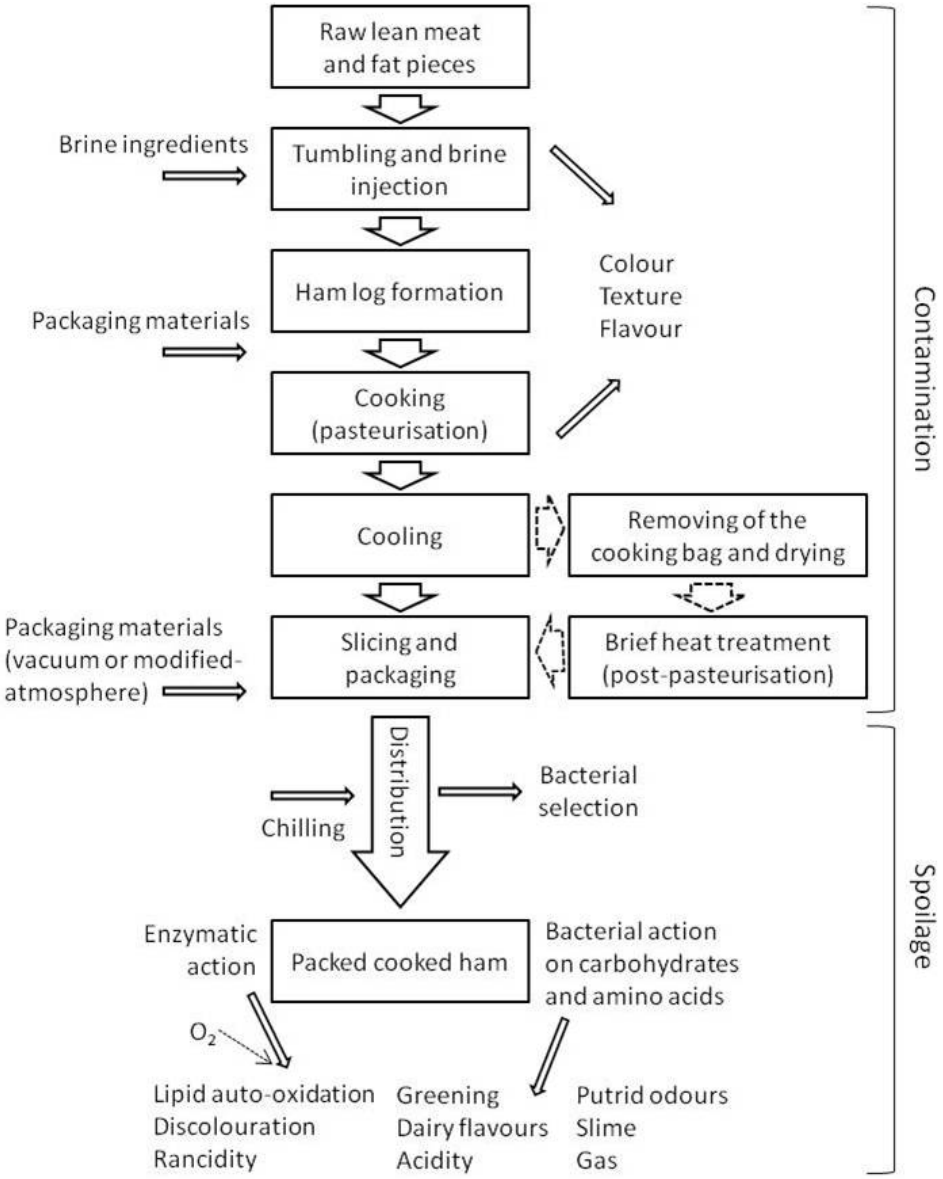

Table 1. Use of bioprotective cultures for the extension of the shelf-life of cooked meat products

Product	Bioprotective culture	Type of action	Reference
Cooked ham	Several lactic acid bacteria	Various types of action, depending on the strain used	Vermeiren *et al.* (2005)
	Lactobacillus sakei subsp. *carnosus* 10A and *Lactobacillus sakei* LS5	Possibly via nutrient competition (10A) and bacteriocin production (LS5)	Vermeiren *et al.* (2006)
	Lactobacillus sakei B-2	Not reported	Hu *et al.* (2008)
	Several lactic acid bacteria (*Lactobacillus sakei*, *Lactococcus lactis*, *Carnobacterium* spp.)	Possibly via acid production	Héquet *et al.* (2009)
	Leuconostoc carnosum 3M42	Possibly via nutrient competition	Vasilopoulos *et al.* (2010b)
Cooked ham and Servelat sausage	*Lactobacillus sakei* TH1	Possibly via nutrient competition	Bredholt *et al.* (2001)
Cooked pork sausage	*Enterococcus faecium* CTC 492 and *Lactobacillus sakei* CTC 494	Bacteriocin production	Aymerich *et al.* (2002)

	Leuconostoc carnosum 4010	Bacteriocin production	Budde *et al.* (2003)
	Several thermotolerant lactic acid bacteria	Not reported	Perez-Chabela *et al.* (2008)
Cooked pork -whole muscle	*Lactococcus lactis* DPC 303-T4	Bacteriocin production	Soriano *et al.* (2004)
Frankfurter-type sausage	*Staphylococcus xylosus* DD-34 and *Lactobacillus sakei* BJ-33	Not reported	Kotzekidou and Bloukas (1996)
	Lactobacillus sakei BJ-33	Not reported	Andersen (1997) Björkroth and Korkeala (1997)
Frankfurter-type sausage and cooked shoulder	*Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442	Bacteriocin production	Metaxopoulos *et al.* (2002)
Saveloys	*Leuconostoc carnosum* 4010	Bacteriocin production	Jacobsen *et al.* (2003)
Several cooked meats	*Lactobacillus sakei* CTC 494	Bacteriocin production	Garriga *et al.* (1998)
Several cooked meats	*Lactobacillus sakei* subsp. *carnosus* 10A	Not reported	Vermeiren *et al.* (2006)