Control of pathogens in cooked meat products: the beneficial role of lactic acid bacteria

M.P. Castro[1,2], **M.E. Cayré**[1], **N.Z. Palavecino Prpich**[1] and **F.P. Rivas**[1,2]

[1] Laboratorio de Microbiología de Alimentos, Universidad Nacional del Chaco Austral, Comandante Fernández 755, (3700) Pcia. Roque Sáenz Peña, Chaco, Argentina
[2] Consejo Nacional de Investigaciones Científicas y Técnicas CONICET, Argentina

Most of food-borne outbreaks associated with cooked meat products involved pathogenic bacteria such as *Listeria monocytogenes* and *Staphylococcus aureus.* The proteinaceous nature, water activity, salt content and refrigeration temperatures comprising meat products contribute to the selection of these pathogens. From a protective point of view, lactic acid bacteria (LAB) can be added to enhance the safety, to prolong the shelf-life and to preserve the microbial and/or sensorial quality of these products. LAB added as bioprotective cultures have no side-effects on sensorial features due to the low carbohydrate content and the strong buffering capacity of meat. The microbial antagonism of LAB against undesirable microorganisms has been attributed in many cases to metabolic products -organic acids and/or antimicrobial peptides- and changes in the physicochemical environment -pH, CO_2 production- or a combination of these factors. The objective of this chapter is to describe current methods and developing technologies for the biopreservation of meat products, with special emphasis on LAB application. The benefits of some new technologies and their industrial limitations is also presented and discussed.

Keywords: biopreservation; lactic acid bacteria; cooked meat products

1. Introduction

Cooked meat products comprise perishable cured or uncured products that have been subjected to heat treatment -at an internal temperature of 70°C or higher- which are normally commercialized as ready-to-eat products (RTE). They can be regarded as whole pieces of meat (ham, smoked turkey breasts, etc.) or products manufactured with minced secondary pieces of meat, fat, or blood (frankfurters, haggis, black pudding, etc.). Diversity of products arise from: i) ingredients added for flavoring, ii) type and/or shape of stuffing material, iii) final molding of the pieces, and iv) cooking procedure. Once cooked, meat products can be subjected to several interventions at the facility or at the retailing sites, namely peeling, slicing, dicing, packaging, and re-packing in smaller portions. After that, they are kept refrigerated until sold.

Most of cooked meat products share intrinsic characteristics such as pH, water activity (a_w) and sodium chloride concentration. These features are compatible with the growth of pathogenic and spoilage bacteria which can proliferate at refrigeration temperatures during the shelf-life of the product. Although heat treatments kill most viable microorganisms, during the course of post-processing interventions, a variety of unsolicited bacteria from the environment, tools and handlers may reach the product. Several studies have identified cross-contamination (during preparation and sale) and subsequent bacterial growth (during storage) as the main causes of RTE cooked meat contamination and illness [1-3]. Important foodborne pathogens that may contaminate RTE cooked meat products basically include several serovars of *Escherichia (Es.) coli*, including O157:H7, *Salmonella* spp., *Campylobacter jejuni*, *Listeria monocytogenes* and *Staphylococcus aureus* [4]. Among them, *L. monocytogenes* is a major safety concern. It causes listeriosis -a severe disease- especially to elderly, pregnant women and newborns [5]. Moreover, major listeriosis outbreaks have been related to contaminated RTE meat and poultry products [6]. The severity of illness and high case fatality rates (~30%) associated with listeriosis led the United States Department of Agriculture (USDA) to adopt a zero tolerance policy for the presence of *L. monocytogenes* in RTE meat products [7].

Hurdle technology is based on the application of combined preservative factors to achieve microbiological safety and stability of foods [8]. The most important hurdles in food preservation are temperature, water activity, acidity, redox potential, antimicrobials, and competitive microorganisms. For RTE meat products, the most frequently applied hurdles include thermal processing, vacuum packaging, refrigerated storage, and nitrite. However, these hurdles seem insufficient when it comes to *L. monocytogenes* due to its ubiquitous nature, and its ability to survive a wide range of adverse conditions, including acidic pH, low temperatures, and high sodium chloride concentrations [9]. Furthermore, its capacity to form biofilms facilitates its long-term survival in food-processing environment [10]. Therefore, to ensure microbiological safety of RTE meats, additional hurdles are needed. As *L. monocytogenes* contamination in RTE meats is primarily due to post-cooking contamination, post-package decontamination methods such as in-package thermal pasteurization, irradiation, high-pressure processing and the use of antimicrobial additives are common approaches to control this pathogen in RTE meat [11].

© FORMATEX 2015

According to the USDA-FSIS [12], all RTE meat-processing plants must implement post-lethality interventions on RTE food products for the control of *L. monocytogenes*, which may include the use of antimicrobials to inactivate the pathogen, and suppress its growth during refrigerated storage. In this sense, the use of chemical antimicrobials as lactates and sodium diacetate resulted in an effective strategy to control *L. monocytogenes* in RTE meat products [13-15]. However, over the last years, consumer demands for naturally preserved products, has challenged the food industry to focus on the research for alternative, chemical-free preservation methods. Biopreservation has gained increasing attention as a means of naturally controlling the shelf-life and safety of meat products.

Biopreservation refers to the extended shelf-life and improvement of the microbial safety of food products using their natural or controlled microflora [16]. Lactic Acid Bacteria (LAB) are the main tool for biopreservation of meat products since they comprise the normal flora of these goods and because of their ability to give metabolic products with antimicrobial effect against spoilage and pathogenic bacteria. Inhibition by LAB may be due to the effect of one or synergism between several mechanisms, such as competition for nutrients, production of organic acids, hydrogen peroxide and antimicrobial substances as bacteriocins [17].

In general, biological strategy for cooked meat products preservation include the use of different approaches:

1. the inoculation of the food with viable LAB, as protective cultures, with consequent *in situ* production of inhibitory molecules and/or a competitive effect against pathogen and spoilage bacteria,
2. the use of LAB metabolites -in particular bacteriocins- in purified form; i.e. *ex situ* production,
3. the development of innovative bioactive packaging with the direct incorporation of bacteriocin-producing LAB strains into polymeric matrices or enriched with LAB antimicrobial metabolites (mainly bacteriocins).

The objective of this chapter is to describe current methods and developing technologies for the biopreservation of meat products, with special emphasis on LAB application.

2. General features of LAB biopreservation of cooked meat products

Lücke [18] defined protective cultures as those added to meat products in order to inhibit pathogens or extend shelf-life without changing their sensory profile. Compared with starter cultures, protective cultures lack of or have reduced product transformation capabilities. The main focus of protective cultures is pathogen control, especially of *L. monocytogenes*, but also of spoilage organisms such as LAB and *Brochothrix thermosphacta* involved in the spoilage of deli meats. Regardless this function, they can also be used for a number of other applications. As an example, a strain of *Lactococcus (Lc.) lactis*, marketed as Bactoferm® Rubis by Chr.-Hansen A/S, is offered as a protective culture to be used instead of chemicals to preserve/stabilize the normal color of vacuum packed or controlled atmosphere packaged, sliced, cured meat products [19].

Of special interest are LAB strains naturally present in meat and meat products that excrete powerful anti-listerial bacteriocins *in situ.* These strains are especially adapted to the meat environment, and consequently, become more competitive than those isolated from other sources. However, since LAB may contribute to cooked meat spoilage [20], it is essential to evaluate whether a potential protective culture has detrimental effects on food quality. *In vitro* studies should be carried out to survey novel protective cultures for their functional and safety properties prior to their application on food [21]. Especially homofermentative, salt tolerant, psychrotrophic, and adapted to meat substrates lactic acid bacteria, which at the same time have no or only a very weak spoilage potential, have a good prospective to be used for the biopreservation of cooked meat products [22]. Moreover, the selection of a bacteriocinogenic strain should also take into account its ability to grow and produce the bacteriocin *in situ*, the bacteriocin diffusion through the meat [23], its adsorption to food components such as proteins and fats [24], the influence of specific ingredients, namely sodium chloride and nitrite [25], and also those conditions that could destabilize the bacteriocin biological activity [26]. Bacteriocinogenic *Lactobacillus (Lb.) sakei* and *Lb. curvatus* isolates from meat are the most commonly evaluated strains for their potential application in meat preservation [27].

Several authors believe that the effectiveness of protective bacteria in killing *L. monocytogenes* it is mainly due to the production of bacteriocins [28-30]. Nevertheless, competition of protective cultures with potential pathogens is another way to restrict the growth of undesired organisms [5]. Therefore, the notion of protective cultures is broad and it is not specifically related to the production of bacteriocins [31].

Cooked meat can be supplemented with *ex situ* produced LAB bacteriocin preparations to improve quality and safety. Nisin is a class I bacteriocin which has demonstrated antimicrobial activity against pathogens and spoilage flora associated to cooked meat products [32-34]. It is the most commercially important bacteriocin, currently recognized as a safe food preservative in approximately 50 countries. The bacteriocin is incorporated into the product as a dried concentrated powder prepared from a skim-milk derived fermentate. Nisaplin™ (Danisco, Netherlands) is generally considered to be the most commercially available form of nisin for food preservative uses, followed by a product manufactured by Rhodia S.A. (France) along with numerous producers and providers of various antimicrobial products based in China.

In addition, due to their anti-Listeria activity the class IIa group of bacteriocins -which includes pediocin-like peptides- has attracted much of the attention for the biopreservation of meat products. The stability of pediocin PA-1 in foods such as frankfurters, Spanish dry fermented sausages and chicken sausages, has been demonstrated [35]. *In vitro*

© FORMATEX 2015

studies have shown that the pediocin PA-1 produced by *Pediococcus acidilactici* UL5 has strong inhibition activity against a wide variety of *L. monocytogenes* strains [36]. Together with the available commercial preparations of nisin and pediocin PA-1/AcH, other bacteriocins (like for example lacticin 3147, enterocin AS-48 or variacin) also offers promising perspectives. Regarding enterocins, the ability to inhibit growth of *Listeria* spp. is common to most *Enterococcus* bacteriocins [37, 38]; as an example, enterocin 416K1 proved to be an effective inhibitor of *L. monocytogenes* in frankfurters [39].

In order to widen their antimicrobial spectrum, aforementioned bacteriocins can be used in combination with organic acids, lysozyme and EDTA on cooked meat products, providing greater effectiveness even against gram-negative bacteria. Many of these applications had been tested on hot dogs, frankfurters and ham [33, 40]. Besides, the joint use of LAB bacteriocins with post-processing treatments such as High hydrostatic pressure (HHP), irradiation or packaging has to be considered as an attractive approach for the preservation of cooked meat products [40, 41].

3. Bacteriocin-producing LAB: an effective additional hurdle

Bacteriocinogenic *Lactobacillus* strains can be used as protective microbiota in the preservation of meat products mainly directed towards biocontrol of *L. monocytogenes* and other pathogens. Indeed, apart from receiving the status of GRAS (Generally Recognized as Safe) compounds, bacteriocins should be well studied and harmonized with other technological factors in the production -pH, temperature, salt and nitrite- for their direct application and their entry into the full production process [42, 43]. Their implementation has to be addressed as a positive and natural alternative, together with other natural protectors and, undeniably, good hygienic and manufacturing practices [44].

As aforementioned, microbial contamination of cooked meat products occurs during post-processing steps, i.e. handling, packaging, storage, etc.; consequently, the colonization of the food matrix mainly takes place on the surface. The outer layer of the products is the target of the preservation method, thus antimicrobial agents are added to rinsing or spray solutions, protective coatings, or packaging.

The strain *Lb. curvatus* ACU-1, isolated from artisanal dry sausages manufactured in Argentina, produces the bacteriocin sakacin Q which is active against *L. innocua* ATCC 33090 and several strains of *Staphylococcus aureus* [45]. Bacterial growth and sakacin Q production kinetics showed a growth associated production similar to most of bacteriocinogenic LAB strains. Sakacin Q showed to be heat stable, effective after refrigerated storage and freeze/thaw cycles and even active against pathogens when produced under refrigeration at 3 % NaCl concentration. Thereby, its application in meat products as a protective culture was evaluated on cooked pork meat surface against *L. innocua* ATCC 33090 by researchers from the same group [46]. A piece of pork meat (~1 kg) was cooked/sterilized at 121 °C for 15 min, cooled at room temperature in aseptic conditions and uniformly sliced with a sharp blade. Then, pork meat slices were immersed during 5 min in different suspensions (pH 6.5) containing the bacteriocin-producing strain, the cell-free supernatant (CFS) of the bacteriocin-producing strain and a combination of the latter. Then, every slice was inoculated with a suspension of *L. innocua* (~1 $\times 10^3$ cfu ml^{-1}). *In situ* bacteriocin production (800 AU ml^{-1}) significantly reduced listerial counts (approximately 5 log cycles at the end of the trial). System which comprised the CFS of the bacteriocin-producing strain, also inhibited *Listeria* growth, but the reduction decreased after 3 weeks, being 3 log cycles higher than the one observed for *in situ* production. However, a more pronounced listericidal effect up to the third week was verified for the combination of the producer cell or the CFS in comparison with the solely use of each of them. For this reason, authors postulated that the combination of *in situ* and *ex situ* bacteriocin production could be a highly promising way of bacteriocin supply. This strategy is based in optimizing bacteriocin production by its inclusion as stimuli. Moreover, a freeze-dried reconstituted CFS was assessed as the most effective one against *Listeria* on meat surface, reducing its population to undetectable levels. Taking into consideration bacteriocin concentration of this system (3200 AU ml^{-1}), it can be presumed that microbial inhibition was concentration-dependent. This fact evidenced that possible bacteriocin adsorption to the food matrix might have been counteracted by the increase in bacteriocin concentration. The antilisterial effect which could have been attributed to LAB metabolic activity was ruled out since pH value of the systems had not shown statistically significant differences ($p>0.05$) throughout the study. This fact was in keeping with Kouakou and co-workers [47] who tested antilisterial activity of bacteriocin-producing *Lb. curvatus* strain CWBI-B28 in lean pork meat co-cultured with *L. monocytogenes* at 4 °C.

Incorporation of the bacteriocin-producing strain into the package where the piece of meat is to be commercialized offers an additional barrier to post-processing contamination of the product. As a relevant example, *Lb. sakei* 10A, isolated by Vermeiren and co-workers [48] from cooked turkey fillet, offered opportunities as a biopreservative since this protective culture showed to be effective for the safety of cooked meat products by inhibiting the growth of contaminating *L. monocytogenes* cells. The antagonistic interaction of *Lb. sakei* 10A towards *L. monocytogenes* was tested on a model cooked ham product at refrigeration temperatures (4 and 7 ºC) and under both vacuum-packaging and modified atmosphere packaging. The combination of *Lb. sakei* 10A and a storage temperature of 4 ºC or the biopreservative plus a modified atmosphere containing 50 % of CO_2 fully prevented growth of the pathogen. In addition, the tested LAB strain had no impact on the sensory quality of the model cooked ham, increasing its potential application for the control of *L. monocytogenes* growth in ready-to-eat meat products.

 © FORMATEX 2015

As direct addition of crude bacteriocin preparations to a complex food environment may result in the complete loss or partial reduction in the antimicrobial activity, the development of packaging films having antimicrobial properties has been intensively studied in the last two decades [49-51]. Antimicrobial substances can be either simply coated on the surface of films or incorporated in the matrix of the films. Regardless the long list of antimicrobials which have been tested in the preparation of antimicrobial films, bacteriocins are the most promising as safe and natural antimicrobial compounds [43]. Bacteriocin-containing films avoid the risks of negative interaction with the food components and can provide longer preservation compared with the conventional addition of bacteriocins directly into food. Definitely, nisin is the most frequently tested antimicrobial in the preparation of antimicrobial films [52]. The use of other bacteriocins rather than nisin, have also been investigated for the preparation of such films. Enterocins strong anti-listerial properties give them advantage over nisin and other bacteriocins, especially in meat products where nisin is not an effective preservative [53-55].

Biodegradable films (alginate, zein and polyvinyl alcohol) containing enterocins were investigated for their antimicrobial effect against *L. monocytogenes* [56]. Survival of the pathogen was studied by means of challenge tests performed at 6 °C during 8 and 29 days, for air-packed and vacuum-packed sliced cooked ham, respectively. Air packaging was tested with two concentrations of enterocins (200 and 2000 AU cm^{-2}). Packaging with antimicrobial films effectively slowed down the pathogen's growth, leading to final counts lower than in control lots. Air-packaging with alginate films containing 2000 AU cm^{-2} of enterocins effectively controlled *L. monocytogenes* for 8 days. Vacuum packaging with films containing enterocins (2000 AU cm^{-2}) also delayed the growth of the pathogen. The most effective treatment for controlling the pathogen during 6 °C storage was vacuum-packaging of sliced cooked ham with alginate films containing 2000 AU cm^{-2} of enterocins. These results showed that antimicrobial packaging containing LAB bacteriocins can improve the safety of sliced cooked ham by delaying and reducing the growth of *L. monocytogenes*.

Furthermore, in recent years the direct incorporation of bacteriocin-producing strains into polymeric matrices has been investigated [57, 17, 58]. This new approach to control pathogen growth opens the lines of research on the possibility of using polymers as a support for viable pathogen antagonists, such as LAB, and could lead to an alternative method of food preservation which could be particularly developed for ready-to-eat meat products.

On the other hand, effectiveness of preservation methods such as irradiation -which mainly acts at a surface level of the product- can be enhanced by the presence of bacteriocins. According to Grant and Patterson [59], *L. monocytogenes* is more resistant to irradiation than *Es. coli*, *Yersinia enterocolitica* and *S. aureus*; hence, the combination of radiation treatment and bacteriocins could increase the effect on elimination of *L. monocytogenes* in cooked meat products. Turgis and co-workers [60] observed an increase of the radiosensitivity of *L. monocytogenes* in meat in the presence of the bacteriocin MT 104b produced by *E. faecium* and nisin. Thereby showing a synergic antimicrobial effect on controlling the growth of *L. monocytogenes* in meat when ɣ-irradiation was combined with nisin and MT 104b. These combinations of bacteriocins and ɣ-irradiation can be used to maintain the safety of meat products.

3.1 Combination of LAB bacteriocins with other preservatives

The most widely used compounds for controlling *L. monocytogenes* in a variety of meat products are sodium lactate and sodium diacetate, applied individually or in combination [61]. Other salts have been used to control the growth of pathogenic microflora in these products while contributing to several technological features; i.e. phosphates, nitrates and/or nitrites, sorbates and benzoates, among many others. The joint use of these chemical preservatives and LAB bacteriocins has been investigated in last decades as a feasible approach to help decrease the concentration of chemical substances; some examples of successful application are summarized herein.

The bacteriocin AS-48, named after its producer *E. faecalis* S-48, has high stability in a wide range of temperature and pH values, presents sensitivity to digestive proteases and a broad bactericidal activity against most of the Gram-positive bacteria and some Gram-negative bacteria [62]. The efficacy of enterocin AS-48 by itself and in combinations with the chemical preservatives currently licensed in meat products and/or heat to control the food-borne pathogens *L. monocytogenes* and *S.aureus* in a cooked ham meat model was assesed by Ananou and co-workers [63]. This type of product was chosen since it is a ready-to-eat food greatly prone to post-processing contamination due to its high water activity and neutral pH. AS-48 inhibited *Listeria* in cooked ham in a concentration-dependent way at 5 °C and 15 °C. Nevertheless, even at the higher concentration used (60 µg g^{-1}) it was not possible to avoid the regrowth of listeria after 15–30 storage days at 5 °C. This regrowth of the surviving bacteria was attributed to an unusual decrease in bacteriocin levels after day 7. Nevertheless, when tested combined treatments of AS-48 and different chemical preservatives (pentasodium tripolyphosphate 0.5 %, sodium nitrate/nitrite 0.015 % or 0.007 %, sodium pyrophosphate 0.15 %, sodium acetate 0.2 %, sodium lactate 2 %, potasium benzoate 0.1 %, potasium sorbate 1 %) the most effective combination was AS-48-nitrite/nitrate (0.007 %), which reduced listeria below detection level from the first sampling. To a lesser extent, other combinations of AS-48 (40 µg g^{-1} with other preservatives as pentasodium tripolyphosphate - STPP, sodium benzoate or potassium sorbate) were also effective in reducing *Listeria* during storage at 5 °C, while the combination of the chemical with a higher AS-48 concentration of 60 µg g^{-1} decreased listeria counts below detection level, thus indicating that it is possible to achieve a complete inactivation of *Listeria* with other more effective combination by increasing enterocin concentration. A synergistic effect in the antilisterial action between AS-48 and heat was also observed when applied a sub-lethal heat treatment (60 °C- 2 min). This fact has technological relevance in

cooked products in which AS-48 could represent an additional hurdle to prevent failures in the homogeneous distribution of heat through the whole meat mixture. The bacteriocin alone had a slight inhibitory effect on *S. aureus;* even when it was applied to the highest concentration (60 µg g^{-1}) no *staphylococci* removal was detected. A remarkable fact from this research is that neither of the combined treatments of AS-48 plus the chemical preservatives achieved the complete inactivation of the target pathogens, but most of them (nitrite/nitrate, STPP, sodium lactate and sodium acetate) improved the inhibitory effect of enterocin. This synergism can be explained by the fact that bacterial cells sub-lethally injured by different stressing conditions become sensitive to different physical and chemical agents to which healthy cells are resistant [64].

Moreover, an exahustive study conducted by Geornaras and co-workers [65] evaluated post-processing chemical solutions in combination with nisin for their antilisterial effects on commercial smoked sausage. Target *L. monocytogenes* -prepared under various conditions- were used to contaminate sausages (approximately 3–4 log cfu cm^{-2}). Inoculated samples were left untreated, or were immersed in solutions of acetic acid (2.5 %), lactic acid (2.5 %), potassium benzoate (5 %) or Nisaplin® (0.5 %, i.e. 5000 IU ml^{-1} of nisin) alone, and in sequence (nisin followed by acetic acid, lactic acid or potassium benzoate), before vacuum packaging and storage at 10 °C (48 days). Acetic acid, lactic acid or potassium benzoate applied alone reduced initial *L. monocytogenes* populations by 0.4–1.5 log cfu cm^{-2}, while treatments including nisin caused reductions of 2.1–3.3 log cfu cm^{-2}. Post-processing antimicrobial treatments that included the bacteriocin had substantial bactericidal effects on initial contamination levels of *L. monocytogenes*. The antilisterial effects of the immersion treatments tested in this study showed to be enhanced when they were applied to smoked sausage formulated with potassium lactate–sodium diacetate. Thus, according to these results, immersion of sausage formulated with potassium lactate–sodium diacetate in Nisaplin® followed by acetic or lactic acid conducts to substantial reductions of initial contamination levels and subsequent inhibition of growth during storage.

3.2 High pressure processing + LAB bacteriocins

High-pressure processing (HPP) is an attractive preservation technology which has a good potential for the meat industry in particular. It has proved very successful for the preservation of meat products when pressures of 100 to 600 MPa are applied. It is mild for food yet eliminates pathogenic and spoilage microorganisms. This technique has proved very useful to control *Salmonella* spp., *Es. coli*, *L. monocytogenes* and other meat pathogens. The resistance of the microorganisms is variable depending on the strain and the meat matrix to be treated [66]. As a consequence, antimicrobial agents are used together with HPP in ready-to-eat (RTE) meat products so as to contribute with their preservation, by killing the sub lethally injured cells [67, 68]. The combined effect of HPP and LAB bacteriocins was studied against *L. monocytogenes* inoculated in cooked ham. The bacteriocins applied were enterocins A and B, sakacin K and nisin. The storage was done at 6 °C for three months. It was found that the addition of enterocin at the rate of 2000 AU cm^{-2} combined with high pressure treatment at 400 MPa gave the best results in terms of controlling *L. monocytogenes* for three months followed by a combination of sakacin K and high pressure [69]. In a following study, Jofré and co-workers [70] studied the behaviour of *L. monocytogenes* and *S. aureus* in sliced cooked ham formulated either with nisin, lactate or both, together with a HPP to 600 MPa and storage at 1 and 6 °C for 3 months. Diferentes batches of vacuum packed sliced cooked ham was prepared with 800 AU g^{-1} of nisin, 1.8 % lactate, 800 AU g^{-1} of nisin + 1.8 % lactate and the non-antimicrobial containing control. In nisin and lactate ham batches, *L. monocytogenes* did not grow for 75 days of storage and increases of 1.8 and 0.5 log cfu g^{-1}, respectively, were observed for the last 15 days of storage at 1 °C. Moreover, the nisin-lactate batch was able to reduce the counts 1.8 log cfu g^{-1} during the storage, being 5.8 log units lower than the control at the end of the storage. The combination of the bacteriolytic nisin and the bacteriostatic lactate was very effective and inhibited the growth of *L. monocytogenes* for 75 days at 1 and 6 °C. The HPP had little effect on *S. aureus,* but significant differences were found 1 day after pressurization among the antimicrobial-containing batches and the control. During storage, the levels of *S. aureus* did not increase in any of the batches but significantly decreased in nisin and nisin-lactate at both 1 and 6 °C. The storage temperature did not affect the control, lactate and nisin-lactate batches, but nisin turned out to be more effective when ham was stored at 6 °C than at 1 °C. After 3-months, nisin batch showed the lowest counts of *S. aureus* either at 1 or 6 °C. Sliced cooked ham containing nisin, lactate or both did not have demonstrated noticeable reduction of *S. aureus* at temperatures at which their growth is inhibited. Nevertheless, the pathogen is not capable of growing at refrigeration temperatures and the dose of toxin necessary to elicit symptoms requires populations exceeding 10^5 per gram; thus, *S. aureus* would not be a major concern if these food products are properly refrigerated.

4. Non-bacteriocin producing LAB as bioprotective cultures

Alves and co-workers [5] studied the protective effect of producing and non-producing LAB bacteriocins in cooked ham against *L. monocytogenes*. They express that despite the detection of bacteriocin production by *L. sakei* 1 when it was co-inoculated with *L. monocytogenes* 4b, there was no significant difference in inhibition pattern of both *L. monocytogenes* strains by the bac^+LAB compared to the bac^-LAB (p>0.015). Thus, these results indicate that either bac^+LAB or bac^-LAB reduced the proliferation of the pathogenic bacterium. Since inhibition was not due to bacteriocin

© FORMATEX 2015

production, it could be attributed mainly to competition for nutrients, acid production [71] or other antilisterial metabolites [72]. Similar results were found by Nilsson and co-workers [73], who demonstrated the efficiency of the competitive microbiota (*Carnobacterium piscicola* bacteriocin-producing and non-bacteriocin producing strains), in inhibiting *L. monocytogenes* in cold-smoked salmon, kept at 5 ºC. Those authors concluded that the inhibition was due to mechanisms other than bacteriocin production, such as competitive advantage of LAB in colonization of the product. Bredholt and co-workers [74] verified the efficiency of a LAB strain (*Lb. sakei*) isolated from commercial cooked ham in inhibiting *L. monocytogenes* and *Es. coli* O157:H7 multiplication in cooked ham and in sliced, vacuum-packaged servelat sausage. That LAB strain did not produce bacteriocin, but it grew faster under refrigeration (8 ºC) than the pathogenic bacterium. According to Buchanan and Klawitter [75], bacteriocin production in solid food matrices plays an important role in inhibition of undesired bacteria, if the antibacterial compound reaches high concentrations locally. However, problems with inconsistent bacteriocin production may be observed because not all the bacteriocinogenic strains are able to produce significant amounts of bacteriocin in solid foods. Also, even though bacteriocin is produced, its efficacy may be affected by factors, such as NaCl concentrations, pH, storage temperature and nitrite levels [74, 76].

In addition, the results presented by Jacobsen and co-workers [30] had shown that the living cells of *Leuconostoc carnosum* 4010 were more effective than leucocins 4010 alone for inhibition of growth of *L. monocytogenes* in cooked meat products. To conclude, they found that an even distribution of the protective culture on all surfaces of the meat product was necessary to prevent growth of *L. monocytogenes*.

5. Concluding remarks

Microbiological safety of RTE meats needs the use of additional hurdles to control the growth of pathogens after processing. Although it is known that biopreservation do not provide the magic bullet for the inhibition of spoilage and pathogenic microorganisms, it is used as one stress factor within the hurdle technology. Both types of biopreservation, bacteriocins and protective cultures, have their own advantages and disadvantages. As bacteriocins are not heat sensitive as live cultures they can be added to products without significant modification of manufacturing methods. Besides, they are easily handled and stored. The main disadvantage of bacteriocins is that, like any other chemical preservative, they have to be used at effective MIC regardless of the future storage conditions of a product (whether it is temperature abused or not). Furthermore, only those bacteriocins regarded as GRAS can be applied to food products, which restricts its use to few additives commercialized worldwide. On the other hand, live protective cultures application does not require special equipment. The most practical way of delivery is in a freeze-dried form; freeze-drying does not affect the growth and bacteriocin production [77]. When protective cultures are used for preservation, the correct inoculum and its viability become production parameters and should be treated as critical limits in HACCP programs. Moreover, two major practical limitations have to be faced when applying living cultures: first is that a culture cannot survive in hot products, second is possible spoilage of the product during chilled storage.

Ready-to-eat meat products with protective cultures will be different from products without protective cultures. However, providing this difference is only revealed in a minor sour taste, this kind of sensory deviation may be a minor drawback to overcome for an increased food safety with less and/or without chemical preservatives, especially with respect to *L. monocytogenes.* Furthermore, the control of more conspicuous spoilage organisms, e.g. such as *Brochothrix thermosphacta,* can also be addressed using biopreservation methods. Food preferences are changing, many consumers tend to prefer products free of chemical preservatives while others simply accept the products as long as they are safe and affordable. Consequently, protective cultures may be interesting for health and wellness-oriented consumers in countries with higher living standards. But less developed countries could also benefit, especially where cold-chain management is difficult and high-tech processing aids are not readily available [19]. Regarding cooked meat products, the challenge can be simply confined to find the right LAB cultures for each particular product.

Acknowledgements The financial support of Agencia Nacional de Promoción Científica (ANPyCT) and Universidad Nacional del Chaco Austral from Argentina is gratefully acknowledged.

References

[1] Vorst KL, Todd ECD, Ryser ET. Transfer of *Listeria monocytogenes* during slicing of turkey, bologna, and salami with simulated kitchen knives. Journal of Food Protection. 2006; 69, 619−26.

[2] Pérez-Rodríguez F, Valero A, Todd ECD, Carrasco E, Carcía-Gimeno RM, Zurera G. Modeling transfer of *Escherichia coli* O157:H7 and *Staphylococcus aureus* during slicing of a cooked meat product. Meat Science. 2007; 76, 692−99.

[3] Pérez-Rodríguez F, Castro R, Posada-Izquierdo GD, Valero A, Carrasco E, García-Gimeno RM, Zurera G. Evaluation of hygiene practices and microbiological quality of cooked meat products during slicing and handling at retail. Meat Science. 2010; 86:479-85.

[4] Cabeza MC, Cambero MI, Núñez M, Medina M, de la Hoz L, Ordóñez JA. Lack of growth of *Listeria monocytogenes* and *Staphylococcus aureus* in temperature abuse of E-beam treated ready-to-eat (RTE) cooked ham. Food Microbiology. (2010); 27:777- 82.

© FORMATEX 2015

[5] Alves VF, Martinez RCR, Lavrador MAS, De Martinis ECP. Antilisterial activity of lactic acid bacteria inoculated on cooked ham. Meat Science. 2006; 74: 623–27.

[6] Georgaras I, Panagiotis N, Skandamis N, Belk KE, Scanga JA, Kendall PA, Smith GC, Sofos JN. Post-processing application of chemical solutions for control of *Listeria monocytogenes*, cultured under different conditions on commercial smoked sausage formulated with and without lactate-sodium diacetate. Food Microbiology. 2006; 23: 762-71.

[7] USFSIS. Revised policy for controlling *Listeria monocytogenes*. Food safety and inspection service. Washington, DC: US Dept. of Agriculture. Federal Register 54. 1989; 22345-46.

[8] Leistner L. Hurdle effect and energy saving. In: Downey WK, editor. Food quality and nutrition. London: Applied Science Publishers; 1978. p. 309–29.

[9] Farber JM, Peterkin PI. Listeria monocytogenes, a food-borne pathogen. Microbiological Reviews. 1991; 55: 476–511.

[10] Fatemi P, Frank JF. Inactivation of *Listeria monocytogenes*/*Pseudomonas fluorescens* biofilms by peracid sanitizers. Journal of Food Protection. 1999; 62, 761–65.

[11] Zhu M, Du M, Cordray J, Ahn DU. Control of Listeria monocytogenes Contamination in Ready-to-Eat MeatProducts. Comprehensive Reviews in Food Science and Food Safety. 2005; 4: 34-42.

[12] USFSIS, U.S. Food Safety and Inspection Service. Compliance guidelines to control *Listeria monocytogenes* in post-lethality exposed ready-to-eat meat and poultry products. 2012. Available from: http://www.fsis.usda.gov/shared/PDF/Controlling_LM_RTE guideline_0912.pdf.USDA-FSIS.

[13] Barmpalia IM, Koutsoumanis KP, Geornaras I, Belk KE, Scanga, JA, Kendall PA, Smith GC, Sofos JN. Effect of antimicrobials as ingredients of pork bologna for *Listeria monocytogenes* control during storage at 4 or 10 1C. Food Microbiology. 2005; 22: 205–11.

[14] Geornaras I, Belk KE, Scanga JA, Kendall PA, Smith GC, Sofos JN. Postprocessing antimicrobial treatments to control *Listeria monocytogenes* in commercial vacuum-packaged bologna and ham stored at 10 1C. Journal of Food Protection. 2005; 68:991–98.

[15] Stopforth JD, Visser D, Zumbrink R, van Dick L, Bontenbal EW. Control of *Listeria monocytogenes* on cooked ham by formulation with a lactate-diacetate blend and surface treatment with lauroc arginate. Journal of Food Protection. 2010; 73: 552-55.

[16] Ananou S, Maqueda M, Martínez-Bueno M and Valdivia E. Biopreservation, an ecological approach to improve the safety and shelf-life of foods. In: A. Méndez-Vilas, editor. Communicating Current Research and Educational Topics and Trends in Applied Microbiology. Badajoz: Formatex, 2007. P. 475-86.

[17] Gialamas H, Zinoviadou K, Biliaderis C, Koutsoumanis K. Development of a novel bioactive packaging based on the incorporation of *Lactobacillus sakei* into sodium-caseinate films for controlling *Listeria monocytogenes* in foods. Food Research International. 2010; 43(10): 2402-8.

[18] Lücke FK. Utilization of microbes to process and preserve meat. Meat Science. 2000; 56: 105-15.

[19] Kröckel L. The Role of Lactic Acid Bacteria in Safety and Flavour Development of Meat and Meat Products. In: Marcelino Kongo, editor. Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes [Internet]. Publisher: InTech, Chapters published January 30, 2013. Available from: DOI: 10.5772/51117.

[20] Borch E, Kant-Muermans M, Blixt Y. Bacterial spoilage of meat and cured meat products. International Journal of Food Microbiology. 1996; 33; 103-20.

[21] Maragkoudakis PA , Mountzouris KC , Psyrras D , Cremonese S, Fischer J , Cantor MD, Tsakalidou E. Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella enteritidis*. International Journal of Food Microbiology. 2009; 130: 219–26.

[22] Vermeiren L, Devlieghere F, Debevere J. Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. International Journal of Food Microbiology. 2004; 96: 149– 64.

[23] Dicks LMT, Mellett FD, Hoffman LC. Use of bacteriocin-producing starter cultures of *Lactobacillus plantarum* and *Lactobacillus curvatus* in production of ostrich meat salami. Meat Science, 2004; 66: 703–8.

[24] Aasen IM, Markussen S, Møretrø T, Katla T, et al. Interactions of the bacteriocins sakacin P and nisin with food constituents. International Journal of Food Microbiology. 2003; 87: 35–43.

[25] Verluyten J, Messens W, De Vuyst L. The curing agent sodium nitrite used in the production of fermented sausages, is less inhibiting to the bacteriocin-producing meat starter culture *Lactobacillus curvatus* LTH 1174 under anaerobic conditions. Applied and Environmental Microbiology. 2003; 69: 3833–9.

[26] Sarantinopoulos P, Leroy F, Leontopoulou E, Georgalaki MD, et al. Bacteriocin production by *Enterococcus faecium* FAIR-E 198 in view of its application as adjunct starter in Greek Feta cheese making. International Journal of Food Microbiology. 2002; 72: 125–36.

[27] Todorov SD, Vaz-Velho M, Gombossy de Melo Franco BD, Holzapfel WH. Partial characterization of bacteriocins produced by three strains of *Lactobacillus sakei*, isolated from salpicao, a fermented meat product from North-West of Portugal. Food Control. 2013; 30: 111-21.

[28] Campanini M, Pedrazzoni I, Barbuti S, Baldini P. Behaviour of *Listeria monocytogenes* during the maturation of naturally and artificially contaminated salami: effect of lactic-acid bacteria starter cultures. International Journal of Food Microbiology. 1993; 20:169–75.

[29] Katla T, Moretro T, Sveen I, Aasen IM, Axelsson L, Rorvik LM, Naterstad K. Inhibition of *Listeria monocytogenes* in chicken cold cuts by addition of sakacin P and sakacin P-producing *Lactobacillus sakei*. Journal of Applied Microbiology. 2002; 93:191–96.

[30] Jacobsen T, Budde BB, Koch AG. Application of *Leuconostoc carnosum* for biopreservation of cooked meat products. Journal of Applied Microbiology. 2003; 95:242–49.

[31] Rodgers S. Novel applications of live bacteria in food services: probiotics and protective cultures. Trends in Food Science & Technology. 2008; 19:188-97.

 © FORMATEX 2015

[32] Davies EA, Milne CF, Bevis HE, Potter RW, Harris JM, Willams GC, Thomas LV, Delves-Broughton J. Effective use of nisin to control lactic acid bacterial spoilage in vacuum-packed bologna-style sausage. Journal of Food Protection.1999; 62:1004–10.
[33] Gill AO, Holley RA. Inhibition of bacterial growth on ham and bologna bylysozyme, nisin, and EDTA. Food Research International. 2000; 33:83–90.
[34] Kouakou P, Ghalfi H, Destin J, Dubois-Dauphin R, Evrard P, Thonart P. Enhancing the antilisterial effect of *Lactobacillus curvatus* CWBI-B28 in pork meat and cocultures by limiting bacteriocin degradation. Meat Science. 2008; 80: 640–8.
[35] Nieto-Lozano JC, Reguera-Useros JI, Peláez-Martínez MC, Sacristán-Pérez-Minayo G, Gutiérrez-Fernández AJ, Hardisson de la Torre A. The effect of the pediocin PA-1 produced by *Pediococcus acidilactici* against *Listeria monocytogenes* and *Clostridium perfringens* in Spanish dry-fermented sausages and frankfurters. Food Control. 2010; 21: 679–85.
[36] Naghmouchi K, Drider D, Kheadr E, Lacroix C, Prévost H, Fliss I. Multiple characterizations of *Listeria monocytogenes* sensitive and insensitive variants to divergicin M35, a new pediocin-like bacteriocin. Journal of Applied Microbiology. 2006; 10: 29–39.
[37] Campos CA, Rodríguez O, Calo-Mata P, Prado M, Barros-Velázquez J. Preliminary characterization of bacteriocins from *Lactococcus lactis*, *Enterococcus faecium* and *Enterococcus mundtii* strains isolated from turbot (Psetta maxima). Food Research International. 2006; 39: 356–64.
[38] Pinto AL, Fernandes M, Pinto C, Albano H, Castilho F, Teixeira P, Gibbs PA. Characterization of anti-Listeria bacteriocins isolated from shellfish: Potential antimicrobials to control non-fermented seafood. International Journal of Food Microbiology. 2009; 129: 50–8.
[39] Iseppi R, Pilati F, Marini M, Toselli M, de Niederhäusern S, Guerrieri E, Messi P, Sabia C, Manicardi G, Anacarso I, Bondi M. Anti-listerial activity of a polymeric film coated with hybrid coatings doped with enterocin 416K1 for use as bioactive food packaging. International Journal of Food Microbiology. 2008; 123: 281–7.
[40] Gálvez A, López RL, Abriouel H, Valdivia E, Ben Omar N. Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. Critical Reviews in Biotechnology. 2008; 28: 125–52.
[41] Aymerich T, Picouet PA, Monfort JM. Decontamination technologies for meat products. Meat Science. 2008; 78(1-2): 114-29.
[42] Veskovic S. Andrejevic Publisher. Lactic Acid Bacteria Bacteriocins - Possibilities of Application in Production of Fermented Sausages. Belgrade. Serbia, in Serbian; 2009.
[43] Cleveland J, Montville TJ, Nes IK, Chikindas ML. Bacteriocins: Safe, natural antimicrobials for food preservation. International Journal Food Microbiology. 2001; 71: 1-20.
[44] Veskovic Moracanin S, Turubatovié L, Skrinjar M, Obradovic D. Antilisterial Activity of Bacteriocin Isolated from *Leuconostoc mesenteroides* ssp. *mesenteroides* IMAU: 10231 in the Production of Sremska Sausages: Lactic Acid Bacteria Isolation, Bacteriocin Identifícation and Meat Application Experiments. Food Technology and Biotechnology. 2013; 53: 247-56.
[45] Castro MP, Palavecino NZ, Herman C, Garro OA, Campos CA. Lactic acid bacteria isolated from artisanal dry sausages: Characterization of antibacterial compounds and study of the factors affecting bacteriocin production. Meat Science. 2011; 87: 321–9.
[46] Rivas FP, Castro MP, Vallejo M, Marguet E, Campos CA. Sakacin Q produced by *Lactobacillus curvatus* ACU-1: Functionality characterization and antilisterial activity on cooked meat surface. Meat Science. 2014; 97: 475–9.
[47] Kouakou P, Ghalfi H, Destain J, Dubois-Dauphin R, Evrard P, Thonart P. Effects of curing sodium nitrite additive and natural meat fat on growth control of *Listeria monocytogenes* by the bacteriocin-producing *Lactobacillus curvatus* strain CWBI-B28. Food Microbiology. 2009; 26: 623–8.
[48] Vermeiren L, Devlieghere F, Vandekinderen I, Debevere J. The interaction of the non-bacteriocinogenic *Lactobacillus sakei* 10A and lactocin S producing *Lactobacillus sakei* 148 towards *Listeria monocytogenes* on a model cooked ham. Food Microbiology. 2006; 23: 511–8.
[49] Mauriello G, Ercolini D, La Storia A, Casaburi A, Villani F. Development of polythene films for food packaging activated with an antilisterial bacteriocin from *Lactobacillus curvatus* 32Y. Journal of Applied Microbiology. 2004; 97: 314–22.
[50] Quintavalla S, Vicini L. Antimicrobial food packaging in meat industry. Meat Science. 2002; 62: 373–80.
[51] Suppakul P, Miltz J, Sonneveld K, Bigger SW. Active packaging technologies with an emphasis on antimicrobial packaging and its applications. Journal of Food Science. 2003; 68: 408–20.
[52] Joerger RD. Antimicrobial films for food applications: a quantitative analysis of their effectiveness. Packaging Technology and Science. 2007; 20: 231–73.
[53] Cintas LM, Casaus P, Fernández MF, Hernández PE. Comparative antimicrobial activity of enterocin L50, pediocin PA-1, nisin A and lactocin S against spoilage and foodborne pathogenic bacteria. Food Microbiology. 1998; 15: 289–98.
[54] Delves-Broughton J. Nisin and its uses as a food preservative. Food Technology. 1990; 44: 100–17.
[55] Delves-Broughton J. Nisin as a food preservative. Food Australia. 2005; 57: 525–7.
[56] Marcos B, Aymerich T, Monfort JM, Garriga M. Use of antimicrobial biodegradable packaging to control Listeria monocytogenes during storage of cooked ham. International Journal of Food Microbiology. 2007; 120(1-2): 152-8.
[57] Concha-Meyer A, Schöbitz R, Brito C, Fuentes R. Lactic acid bacteria in an alginate film inhibit *Listeria monocytogenes* growth on smoked salmon. Food Control. 2011; 22(3-4): 485-9.
[58] Sánchez-González L, Iván J, Saavedra Q, Chiralt A. Physical properties and antilisterial activity of bioactive edible films containing *Lactobacillus plantarum*. Food Hydrocolloids. 2013; 33(1): 92-8.
[59] Grant IR, Patterson MF. Sensitivity of foodborne pathogens to irradiation in the components of a chilled ready meal. Food Microbiology. 1992; 9: 95–103.
[60] Turgis M, Stotz V, Dupont C, Salmieri S, Khan RA, Lacroix M. Elimination of *Listeria monocytogenes* in sausage meat by combination treatment: Radiation and radiation-resistant bacteriocins. Radiation Physics and Chemistry. 2012; 81: 1185–8.
[61] Tompkin RB. Control of *Listeria monocytogenes* in the food-processing environment. Journal of Food Protection. 2002; 65: 709–25.

© FORMATEX 2015

[62] Ananou S, Garriga M, Hugas M, Maqueda M, Martínez-Bueno M, Gálvez A., et al. Control of *Listeria monocytogenes* in model sausages by enterocina AS-48. International Journal of Food Microbiology. 2005; 103: 179–90.
[63] Ananou S, Baños A, Maqueda M, Martínez-Bueno M, Gálvez A, Valdivia E. Effect of combined physico-chemical treatments based on enterocin AS-48 on the control of *Listeria monocytogenes* and *Staphylococcus aureus* in a model cooked ham. Food Control. 2010; 21: 478–86.
[64] Kalchayagand N, Hanlin MB, Ray B. Sublethal injury makes gram-negative and resistant gram-positive bacteria sensitive to the bacteriocins, pediocin AcH and nisin. Letters in Applied Microbiology. 1992; 16: 239–43.
[65] Geornaras I, Skandamis PN, Belk KE, Scanga JA, Kendall PA, Smith GC, et al. Post-processing application of chemical solutions for control of *Listeria monocytogenes*, cultured under different conditions, on commercial smoked sausage formulated with and without potassium lactate-sodium diacetate. Food Microbiology. 2006; 23: 762–71.
[66] Garriga M, Aymerich MT, Costa S, Monfort JM, Hugas M. Bactericidal synergism through bacteriocins and high pressure in a meat model system during storage. Food Microbiology. 2002; 19: 509–18.
[67] Hugas M, Pages F, Garriga M, Monfort JM. Application of the bacteriocinogenic *Lactobacillus sakei* CTC494 to prevent growth of *Listeria* in fresh and cooked meat products packed with different atmospheres. Food Microbiology. 1998; 15: 639-50.
[68] López-Caballero ME, Carballo J, Jiménez-Colmenero F. Microbial inactivation in meat products by pressure/temperature processing. Journal of Food Science. 2002; 67: 797–801.
[69] Jofré A, Garriga M, Aymerich T. Inhibition of *Listeria monocytogenes* in cooked ham through active packaging with natural antimicrobials and high-pressure processing. Journal of Food Protection. 2007; 70: 2498–502.
[70] Jofré A, Garriga M, Aymerich T. Inhibition of *Salmonella* sp. *Listeria monocytogenes* and *Staphylococcus aureus* in cooked ham by combining antimicrobials, high hydrostatic pressure and refrigeration. Meat Science. 2008; 78: 53–9.
[71] Amezquita A, Brashears MM. Competitive inhibition of *Listeria monocytogenes* in ready-to-eat meat products by lactic acid bacteria. Journal of Food Protection. 2002: 65: 316–25.
[72] Zhao T, Doyle MP, Zhao P. Control of *Listeria monocytogenes* in a biofilm by competitive-exclusion microorganisms. Applied and Environmental Microbiology. 2004; 70(7): 3996–4003.
[73] Nilsson L, Nielsen MK, Ng Y, Gram L. Role of acetate in production of an autoinducible class IIa bacteriocin in *Carnobacterium piscicola* A9b. Applied and Environmental Microbiology. 2002; 68(5): 2251–60.
[74] Bredholt S, Nesbakken T, Holck A. Protective cultures inhibit growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in cooked, sliced, vacuum- and gas packaged meat. International Journal of Food Microbiology. 1999; 53: 43–52.
[75] Buchanan RL, Klawitter LA. Effectiveness of *Carnobacterium piscicola* LK5 for controlling the growth of *Listeria monocytogenes* Scott A in refrigerated foods. Journal of Food Safety. 1992; 12: 217–34.
[76] Gänzle M, Weber S, Hammes WP. Effect of ecological factors on the inhibitory spectrum and activity of bacteriocins. International Journal of Food Microbiology. 1999; 46: 207–17.
[77] Rodgers S. Potential applications of protective cultures in cook-chill catering. Food Control. 2002; 14: 35-42.

 © FORMATEX 2015