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Review

Role of lactic acid bacteria during meat conditioning and fermentation: Peptides generated as sensorial and hygienic biomarkers

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ABSTRACT

The microbial ecology of meat fermentation is a complex process in which lactic acid bacteria (LAB) and coagulase-negative cocci play a major role. The present work reviews the most significant developments in which LAB are the main characters acting both as starter cultures improving the sensorial quality and as biopreservative agents. New findings about the identification of low molecular weight peptides arisen from protein hydrolysis in dry fermented sausages and their relation with flavor is presented. Also, a brief description of a proteomic approach is detailed in order to exemplify its application as a tool in the search for improved LAB strains that will contribute to food quality and safety. Finally, the most important features of bacteriocinogenic LAB and its bacteriocins in bioprotection of meat and meat products are analyzed.

Contents

1. Introduction

Lactic acid bacteria (LAB) are essential agents during meat fermentation improving hygienic and sensory quality of the final product. Its fermentative metabolism prevents the development of spoilage and pathogenic microflora by acidification of the product, also contributing to its color stabilization and texture improvement. LAB strongly influence the composition of non-volatile and volatile compounds through release/degradation of free amino acids and the oxidation of unsaturated free fatty acids is also prevented (Talon, Leroy-Sétrin, & Fadda, 2002). In addition, certain LAB has also been found to produce bacteriocins being used as bioprotective culture to preserve fresh and processed meat and fish (Katla et al., 2001;

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Castellano, Vignolo, Belfiore, & Fadda, 2008). Most attention has been focused on the development of starter cultures with adequate fermentation characteristics.

The typical flavor of dry cured fermented sausages is the result of a careful balance between volatile (alcohol, ketones, aldehydes and furans) and non-volatile compounds (amino acids, peptides, sugars and nucleotides), these coming from raw materials (meat, spices, nitrites and other additives) or generated from biochemical reactions occurring during fermentation and ripening (Flores, Marcus, Nieto, & Navarro, 1997; Toldrá, 1998; Stahnke, 2002). Non volatile components provide basic tastes, such as sweet, sour, salty and bitter. Recently, umami taste has become widely accepted in Western countries and is considered as the fifth basic taste sensation (Conn, 1992). Umami was first defined as the characteristic taste elicited by glutamates, and has been associated with monosodium glutamate (MSG). This mouthfeel sensation is also provided by disodium salts of the 5′-nucleotides: IMP (disodium 5′-inosine monophosphate), GMP (disodium 5′-guanosine monophosphate) and AMP (disodium 5′-adenosine monophosphate), these compounds being naturally present in many protein-rich foods, such as meat, fish and fungi (Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971).

Many of non-volatile compounds such as peptides and amino acids involved in the characteristic flavor development are produced during meat protein hydrolysis. In this regard, meat LAB have a direct or indirect participation on this phenomenon, so its presence determines to a large extent, the sensory characteristics of the final product.

Recently, proteomic approaches have been applied to correlate proteolytic profiles with technological parameters in view to detect valuable biomarkers to monitor and predict meat quality (Lametsch et al., 2003; te Pas, Jansen, Broekman, Reimert, & Heuven, 2009). Certain peptidic fractions originated from meat have been identified to be related to sensory attributes (Sentandreu et al., 2003, 2007; Mora, Sentandreu, Fraser, Toldrá, & Bramley, 2009). To our knowledge no substantial information about global characteristics of products nor about the exhaustive characterization of generated peptides during meat fermentation are available so far.

Trends in meat industry are focalizing in products with high organoleptic standards, long shelf-life and containing specific nutrients to cover special consumer requirements. In parallel, consumers are increasingly demanding pathogen free foods, with minimal processing and few preservatives and additives. Thus, biopreservation has gained increasing attention as means of naturally controlling the shelf-life and safety of meat products. Some microorganisms commonly associated with meat have proved to be antagonistic towards pathogenic and spoilage bacteria. LAB again stand as the best candidates to be used in biopreservation because of their prevalence during storage and GRAS status.

Based on these considerations, the most significant developments in which LAB are the main protagonists, acting both as starter cultures improving the sensorial quality and as functional starter cultures, are reviewed. New findings on the identification of low molecular weight peptides arisen from protein hydrolysis in Argentinean dry fermented sausages and their correlation with flavor are presented. Also, a proteomic approach is detailed as example of new means by which LAB are studied to obtain improved strains that will contribute to the quality and safety of meat products. Finally, the production of antimicrobial peptides by LAB of meat origin as well as the strategies to improve shelf-life extension of meat products is discussed.

2. Role of LAB in meat protein degradation. An overview

In general, different authors agree about the importance of muscle proteinases in the initial breakdown of sarcoplasmic and myofibrillar proteins, these enzymes being the main agents of proteolysis during the first days of fermentation (Verplaetse, De Bosschere, & Demeyer, 1989; Molly et al., 1997; Kenneally, Fransen, Grau, O'Neill, & Arendt, 1999; Hughes et al., 2002). Although the role of microbial enzymes in this event has been accepted with greater reluctance, LAB are endowed with proteolytic activity; mainly intracellular amino, di and tripeptidases (Montel, Seronine, Talon, & Hebraud, 1995; Sanz & Toldrá, 2002). The intracellular enzymes of *Lactobacillus* were reported to be responsible for the generation of small peptides and amino acids which contribute to the process either as direct flavor enhancers or as precursors of other flavor compounds during the ripening of dry fermented sausages (Fadda et al., 1999a,b; Sanz et al., 1999a,b; Sanz, Sentandreu, & Toldrá, 2002). Contrary to what happen for dairy lactobacilli, no extracellular proteases have been characterized so far from meat-borne lactobacilli (Tsakalidou, Anastasiou, Vandenberghe, van Beeumen, & Kalantzopoulos, 1999; Hebert et al., 2008). In fact, the genome of the meat-borne *Lactobacillus sakei* 23K has not revealed genes coding for any extracellular protease (Chaillou et al., 2005).

To elucidate the origin of meat protein degradation during sausage fermentation several studies were carried out using different model systems, such as sausages added with antibiotic or with muscle protease inhibitors, as well as by using liquid meat-based media (Zeece & Katoh, 1989; Fadda et al., 1999a,b, 1998; Demeyer et al., 2000). In general, data suggest that the addition of protease inhibitors protects myosin, actin and troponin T to a greater extent than antibiotics indicating that in this process endogenous muscle proteases are more important than bacteria (Demeyer, 1992; Demeyer et al., 2000). We evaluated the role of LAB on meat protein degradation by incubating sarcoplasmic or myofibrillar proteins with different meat lactobacilli strains in which whole cells (WC) or cell free extracts (CFE) were used as enzymatic source (Fadda et al. 1999a, b; Sanz et al. 1999a,b). *Lactobacillus plantarum* CRL681 exhibited the strongest hydrolytic profile, sarcoplasmic proteins resulting more susceptible to bacterial hydrolysis (Fig. 1A and B). Data suggested that initial myofibrillar proteins hydrolysis was not significantly affected by LAB, the tissular proteolytic system being responsible for such protein hydrolysis. Similarly, Spaziani, Del Torre, and Stecchini (2009) demonstrated that most of proteolytic activity observed on myofibrillar proteins in traditional low-acid sausages was due to endogenous enzymes, although a contribution of bacterial proteinases to these protein degradation was also determined. In our study, different peptides profiles from sarcoplasmic and myofibrillar proteins were produced in presence of WC or in combination with CFE. Whole cells of *L. plantarum* CRL681 and *Lactobacillus curvatus* CRL705 (formerly *Lactobacillus casei* CRL705) generated hydrophilic peptides from both, sarcoplasmic and myofibrillar proteins while no major changes were detected with only CFE. To exemplify, Fig. 2 shows peptide profiles obtained from sarcoplasmic proteins with *L. plantarum* CRL681. The hydrophilic nature of most of the generated peptides indicates the potential contribution of *L. plantarum* CRL681 and *L. curvatus* CRL705 to the development of desirable cured-meat flavor. When the combination of WC and CFE was present, a net increase of free amino acids (alanine, leucine and glutamic acid) were registered after 4 days, this suggesting that exogenous enzymes addition or peptidases overexpression might be crucial to increase flavor compound generation. In agreement with these findings, other authors (Visessanguan, Benjakul, Riebroy, & Thepkasikul, 2004) showed that Val, Leu, Phe and Lys are mainly liberated from muscle proteins hydrolysis and become dominant during late ripening thus, contributing to the aged flavor of fermented sausages. Di Cagno et al. (2008) have investigated proteolysis from three PDO (protect origin denomination) Italian sausages reporting that microbial peptidase activities play a major role in secondary proteolysis by providing free amino acids. Changes in free amino acids in traditional fermented sausages due to the action of different starter formulations (including *L. curvatus* and *Staphylococcus xylosus*) were also evidenced by Casaburi et al., (2008). In the same work, sarcoplasmic protein hydrolysis was evidenced in samples containing lactobacilli, this indicating that

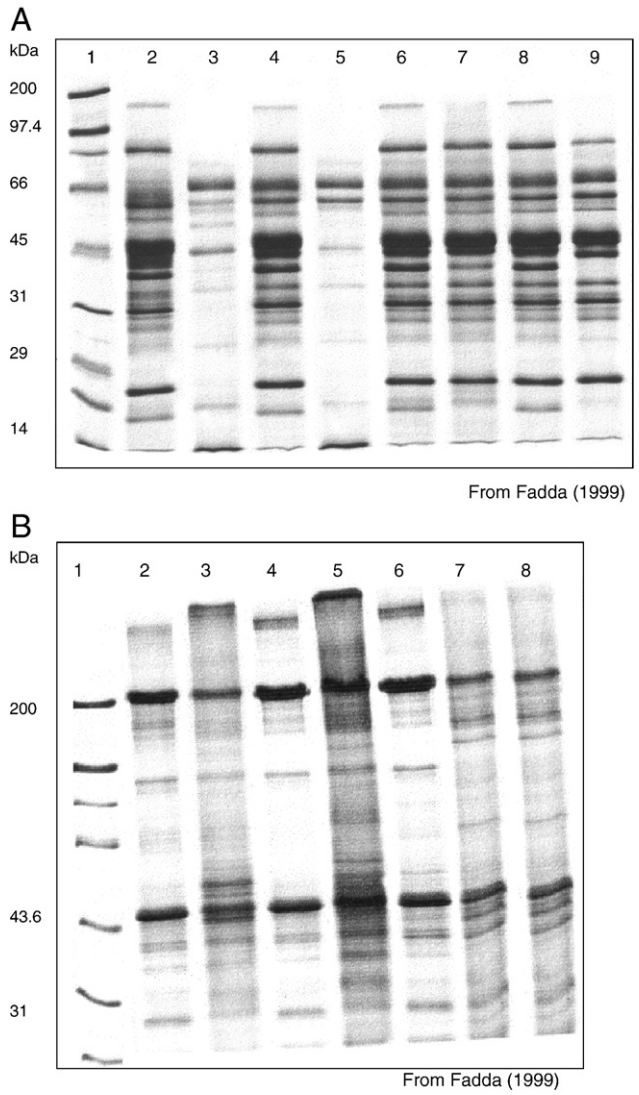

Fig. 1. A. SDS-PAGE patterns of sarcoplasmic protein hydrolysis of different LAB strains using whole cells (WC) + cell free extracts (CFE) as enzymatic source. Lane 1: Molecular weight marquers; lane 2: *L. plantarum* CRL 681, T0; lane 3: *L. plantarum* CRL 681, T 96 h; lane 4: *L. curvatus* CRL705, T0; lane 5: *L. curvatus* CRL705, T96h; lane 6: *L. sakei* CTC4808, T0; lane 7: *L. sakei* CTC4808, T96; lane 8: *L. curvatus* CTC904, T0; lane 9: *L. curvatus* CTC904, T96h (from Fadda, 1999). B. SDS-PAGE pattern of myofibrillar protein hydrolysis of different LAB strains of using whole cells (WC) + cell free extracts (CFE) as enzymatic source. Lane 1: Molecular weight marquers; lane 2: *L. plantarum* CRL 681, T0; lane 3: *L. plantarum* CRL 681, T 96 h; lane 4: *L. curvatus* CRL705, T0; lane 5: *L. curvatus* CRL705, T96h; lane 6: *L. sakei* CTC4808, T0; lane 7: *L. sakei* CTC4808, T96; lane 8: *L. curvatus* CTC904, T0; lane 9: *L. curvatus* CTC904, T96h (from Fadda, 1999).

proteolysis was carried out by both, microbial activity and endogenous proteases activated by pH decrease. In fact, acid production due to the acidogenic metabolism of LAB during sausage fermentation cannot be excluded from the global proteolytic event, which could overlap bacterial hydrolytic activity. In a recent work we analyzed the effect of bacterial inoculation independently from the lactic acid effect on sarcoplasmic protein degradation (Fadda, Vildoza, & Vignolo, in press). Meat fermentation was carried out in a meat model system under controlled pH conditions (pHs 4.0 and 6.0) at 30 °C for 96 h. Results confirmed the ability of the strain to grow and degrade soluble meat proteins at both assayed pHs. In addition, our findings strongly suggest that sarcoplasmic protein degradation recorded at pH 4.0, as occurring during sausage fermentation, can be ascribed to the synergistic action of acidic meat endogenous proteases, *L. plantarum* CRL681 proteolytic activity and acid-induced changes arising from the bacterial fermentative metabolism, while the slighter hydrolytic changes observed at pH 6.0 may be attributed mainly to bacterial proteolysis (Fig. 3A and B) (Fadda et al., in press). Accordingly, other authors observed that acidification stimulates myofibrillar protein degradation by activation of acid muscle proteinases and suggested proteolysis and denaturation events to explain proteolytic SDS-PAGE profiles of meat samples treated with lactic acid (Saunders, 1994; Syed Ziauddin, Rao, & Amla, 1995).

2.1. Proteolytic compounds generated during sausage fermentation

Argentinean dry fermented sausages are produced in different regions using local artisan techniques, these having a strong Spanish and Italian influence. The most common dry fermented sausages are *salamis*, *fuet* and *milano*-type sausages which include beef and/or pork meat, pork fat, salt, and different spices (Fadda & Vignolo, 2007). Even when starter cultures have been applied by local fermented sausage industries, lack of uniformity in the products, inadequate raw materials as well as the need for quick economical incomes, lead to a lower quality products comparing to the European ones. Thus, quality improvement is a challenge that will allow not only increasing standards of local products but to enable a successful competition in international market.

2.1.1. Analysis of Argentinean fermented sausages according to their proteolytic profiles

As described above, even when muscle enzymes are greatly involved in meat protein degradation, bacterial proteolytic activity leads to a richer composition of small peptides and amino acids which contribute to the ripening process either as direct flavor enhancers or as precursors of other flavor compounds. An attempt to characterize Argentinean fermented sausages and their potential flavor compounds using different methodologies and focusing on proteolytic products such as peptides is described: Ten different dry fermented sausages were selected from local supermarkets and a general description of them is shown in Table 1.

2.1.2. Electrophoretic profiles

Tricine-SDS-PAGE ($T = 17\%$) (Shaegger & von Jagow, 1987) was performed to analyze and compare proteolytic patterns generated of sarcoplasmic and myofibrillar proteins extracted from dry sausages and fresh meat (control), further stained by Sypro Ruby gel stain. When myofibrillar profiles (Fig. 4A) were analyzed, differences among the major proteins were observed. The 45 kDa band, probably corresponding to actin, did not show significant intensity variation during sausage technological process, with one exception (sample 9) exhibiting a complete degradation of actin (45 kDa) and myosin light chain-2 (20 kDa). In addition, some proteins not detected in other samples are present in sample 9 pattern, these corresponding to hydrolytic products or solubilized proteins arisen during the ripening period (Fig. 4A). On the other hand, bands of 24 and 20 kDa, probably corresponding to myosin light chain-1 and -2 respectively, showed slight differences in all analyzed samples when compared to fresh meat. According to SDS-PAGE results, it may be suggested that both proteolytic systems (endogenous and microbial) displayed a mild effect towards myofibrillar proteins in the range of 70–10 kDa, producing only slight changes in most of the analyzed products. However an intense proteolysis could eventually occur (sample 9), which may be related to a longer ripening period or to a different technology applied.

As previously reported (Fadda et al., 1999b; Spaziani et al., 2009), fermentation and ripening exert more significant modifications on sarcoplasmic than on myofibrillar proteins (Fig. 4B). Accordingly, among sarcoplasmic proteins, myoglobin (17 kDa) evidenced a complete degradation in all fermented sausages and the 42 kDa band assigned to creatine kinase-M type, an important skeletal muscle enzyme, presented a slight intensity decrease in samples 3, 5, 8 and 9. In addition, peptides of lower molecular mass (21 and 18 kDa) absent on fresh meat,

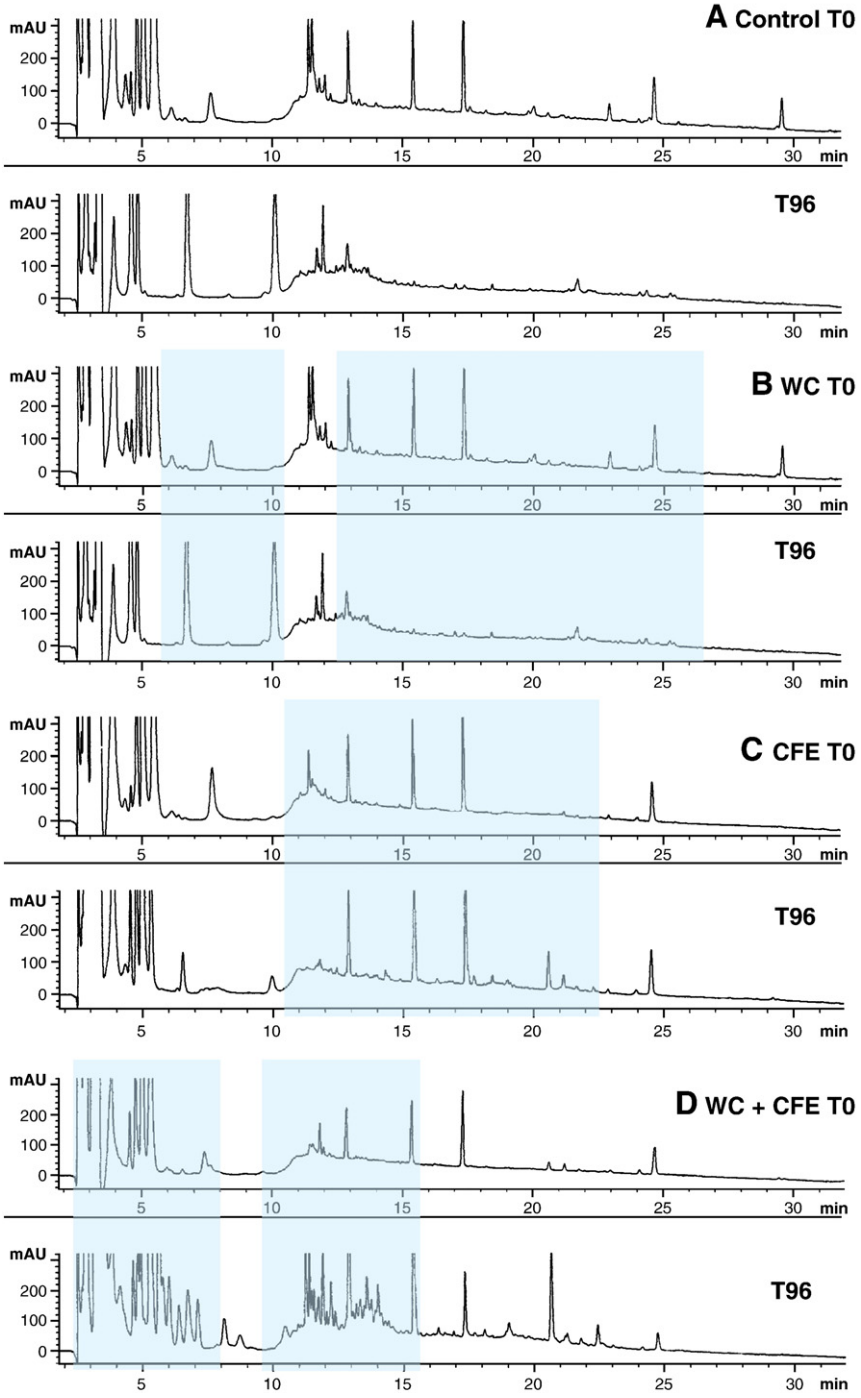

Fig. 2. Reverse-phase HPLC chromatograms of peptides contained in meat extracts treated with *L. plantarum* CRL 681 at 0 and 96 h of incubation. (A) Control samples; (B) samples containing WC; (C) samples containing CFE and (D) samples containing WC + CFE.

are present in fermented samples, possibly as consequence of their solubilization on high ionic strengths or due to protein hydrolysis during the ripening process (Fig. 4B). *Fuet* type sausages (samples 2 and 6), exhibited a different proteolytic profile when compared to other fermented samples; their patterns being similar to fresh meat. This result may indicate the occurrence of a slight sarcoplasmic proteolysis due to milder or shorter ripening process.

2.1.3. Isolation and characterization of low molecular weight peptides with flavor potential

Five out of ten Argentinean fermented sausages were selected in a next step according to their sensorial attributes (assigned by a non trained panel), physicochemical features (pH, a_w) and safety criteria (absence of spoilage or pathogen microflora). Protein extracts of selected samples were submitted to ultrafiltration and the fraction containing peptides with molecular weight lower than 3000 Da, which are more related to flavor (Nishimura, 2002; Claeys, De Smet, Balcaen, Raes, & Demeyer, 2004) was separated. The >3000 Da fraction was analyzed by rp-HPLC and peaks showing changes among samples were collected and submitted to mass spectrometry with further fragmentation by Maldi-ToF-ToF. Detailed methodologies, analyses and findings will be presented in forthcoming papers.

The obtained rp-HPLC chromatograms are shown in Fig. 5. The peptidic profiles of fermented sausages presented a more complex

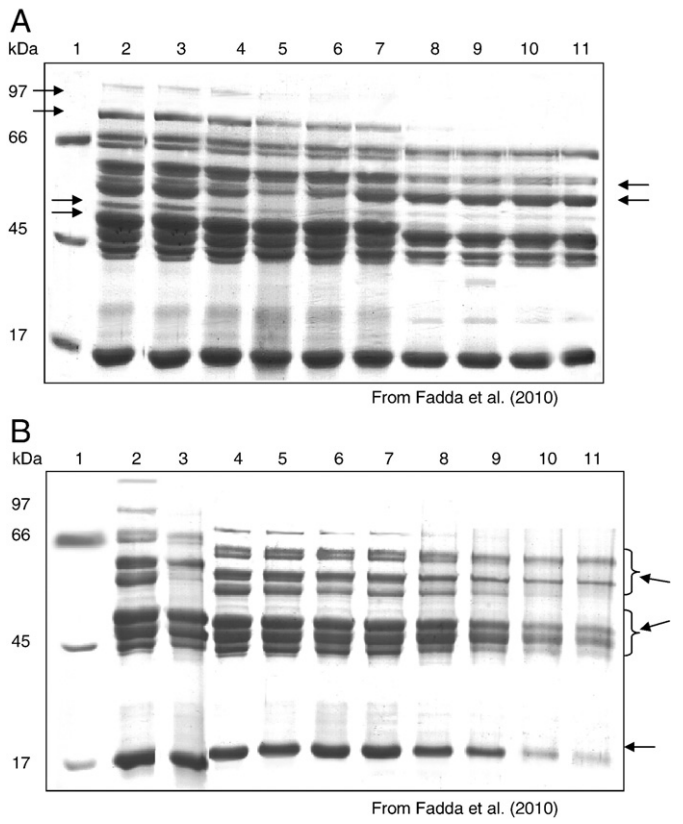

Fig. 3. A. SDS-PAGE pattern of sarcoplasmic protein hydrolysis by *L. plantarum* CRL681 incubated at controlled pH 6.0 for 96 h at 30 °C. Lane 1: Molecular weight markers; lane 2: Control, 0 h; lane 3: Control, 96 h; lane 4: *L. plantarum* CRL681, 0 h; lane 5: *L. plantarum* CRL681, 4 h; lane 6: *L. plantarum* CRL681, 8 h; lane 7: *L. plantarum* CRL681, 12 h; lane 8: *L. plantarum* CRL681, 24 h; lane 9: *L. plantarum* CRL681, 48 h; lane 10: *L. plantarum* CRL681, 72 h; lane 11: *L. plantarum* CRL681, 96 h. B. SDS-PAGE pattern of sarcoplasmic protein hydrolysis by *L. plantarum* CRL681 incubated at controlled pH 4.0 for 96 h at 30 °C. Lane 1: Molecular weight markers; lane 2: Control, 0 h; lane 3: Control, 96 h; lane 4: *L. plantarum* CRL681, 0 h; lane 5: *L. plantarum* CRL681, 4 h; lane 6: *L. plantarum* CRL681, 8 h; lane 7: *L. plantarum* CRL681, 12 h; lane 8: *L. plantarum* CRL681, 24 h; lane 9: *L. plantarum* CRL681, 48 h; lane 10: *L. plantarum* CRL681, 72 h; lane 11: *L. plantarum* CRL681, 96 h.

composition and higher peak concentration than fresh meat (control). A common peak for fermented products which is absent in fresh meat was detected at the 14th min (G6), this compound being associated with proteolytic changes occurred during fermentation and ripening rather than to specific differences among the assayed sausages. In samples 2 and 6 (*fuet* type) two peaks with similar concentration (17.8 and 18.2 min) were observed while two other peaks present in fresh meat (11.75 and 13 min; G1 and G2 respectively) were fully hydrolyzed in fermented sausage samples. Fractions presenting more significant differences (G1, G2, G3, G4, G5, G6, G7 and G8), were

Table 1
General characteristics of the analyzed Argentinean fermented sausages.

Dry fermented sausage	Comminution degree	Industrial brands	Artisan brands	Consumer preference
Sample 1	High	X		Lowest
Sample 2[a]	High	X		Highest
Sample 3	Low		X	
Sample 4	Low		X	Lowest
Sample 5	Low		X	Highest
Sample 6[a]	High	X		
Sample 7	High	X		
Sample 8	High		X	
Sample 9	Low		X	
Sample 10	Low	X		

[a] *Fuet* type sausage.

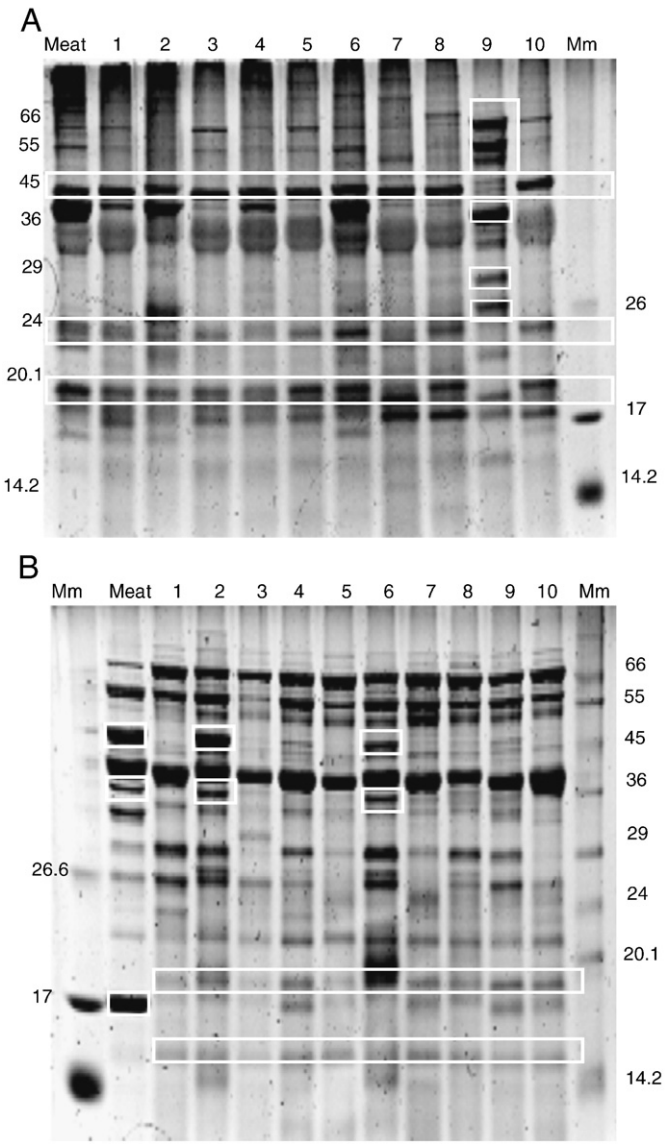

Fig. 4. A. Myofibrillar proteins profiles of Argentinean dry fermented sausages (named as 1 to 10) and fresh meat. Interesting differences are boxed. B. Sarcoplasmic proteins profiles of Argentinean dry fermented sausages (named as 1 to 10) and fresh meat. Main band changes are boxed.

submitted to tandem mass spectrometry (Fig. 5). The peptidic sequences obtained after fragmentation were compared using BLASTP against *Bos taurus* data from non redundant protein sequences (http://blast.ncbi.nlm.nih.gov) and the exact position of peptides on the parental protein was achieved (Tables 2 and 3), the fragmentation being successful in five out of eight selected peaks.

Results showed that low molecular mass peptides (between 1000 and 2100 Da) were arisen from both type of muscle proteins indicating that myofibrillar and sarcoplasmic proteins were affected during fermentation and ripening (Tables 2 and 3). Although by the elution time peptides may be considered to be hydrophilic (all eluted before 20 min), sequence analysis showed a complex amino acid composition presenting hydrophilic and hydrophobic areas, thus they could not be surely related to "good taste" compounds. The wide variety of cleavage sites deduced from their positions on the parental protein suggested the complexity of proteolytic systems involved in their production. Four peptides originated from actin were obtained; they corresponding to the N-terminal and central region of the protein. However, the three identified myosin-derived peptides arise from the N-terminal region and sheared a common sequence (EAAPYLRK) (Table 2). In accordance

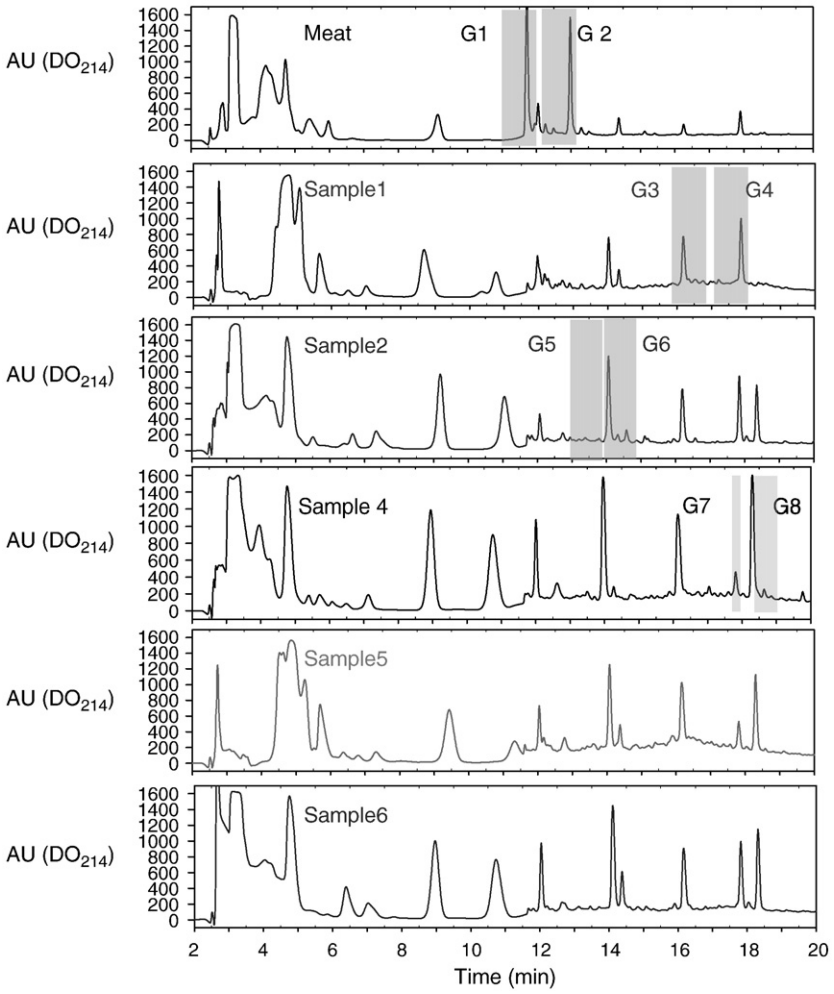

Fig. 5. rp-HPLC chromatograms of less than 3000 Da peptide fractions from fresh meat and five selected Argentinean dry fermented sausages. Sample 1: high comminution degree; sample 2: *fuet* type sausage; sample 4: low comminution degree; sample 5: low comminution degree; sample 6: *fuet* type sausage.

with previous findings (Sanz et al., 1999a; Fadda et al., 1999b; Spaziani et al., 2009), the results (Table 3) confirmed that sarcoplasmic proteins are more susceptible to degradation, as its peptide profile resulted with more abundant peaks.

As a result of this study, the changes on protein profiles of Argentinean dry sausages allows to highlight that (i) sarcoplasmic proteins were the most susceptible to proteolysis, this being observed with both applied techniques (SDS-PAGE and rp-HPLC); (ii) major differences were detected between fresh and fermented meat samples; (iii) although some differences have been observed among sausages, no specific biomarkers were detected able to differentiate fermented sausage brands; however *fuet* type (samples 2 and 6) and artisan long ripened sausage (sample 9) presented unique/different profiles; (iv) rp-HPLC resulted more sensitive than Tricine-SDS-PAGE to detect changes on the lighter fraction of peptides. Tandem MS analyses enable us to obtain the sequence of the peptide as well as its position and cleavage site on the parental protein, this indicating that rp-HPLC coupled to MS analyses is an accurate method to characterize proteolytic metabolites from this kind of products.

3. LAB strategies to cope, survive and grow in meat environments. A proteomic approach

Nowadays, it is accepted that an appropriate starter culture have to be selected from indigenous populations present in a food product in order to be more competitive, well-adapted, and with high metabolic

Table 2
Peptide sequences of selected rp-HPLC fractions from Argentinean fermented sausages originated from myofibrillar protein hydrolysis. Identified sequence with cleavage sites, molecular mass (*Z*), calculated score of MS-tag software, origin of peptides, total protein residues and position of identified peptides on the parental protein are shown.

Peptide number	RP fraction	Sequence	*Z* calculated (Da)	Score mascot	Original protein and accession number	Total protein residues	Position
1	G8	(G)FAGDDAPRAVFPS(I)	1349.65	23.4	Actin, alpha skeletal muscle; P68138	377	23–35
2	G7	(A)VFPSIVGRPRHQG(V)	1449.81	19.1	Actin, alpha skeletal muscle; P68138	377	32–44
3	G7	(M)EKIWHHTF(Y)	1097.24	39.3	Actin, alpha skeletal muscle; P68138	377	85–92
4	G4 G7	(L)RVAPEEHPTL(L)	1148.61	38.0	Actin, alpha skeletal muscle; P68138	377	97–106
5	G3	(G)AGQHPARASSSEAEDGCGSP(K)	1970.83	10.7	Capz-interacting protein; Q3ZBT0	381	253–272
6	G3	(F)GEAAPYLRK[a]SEKERIEAQN(K)	2189.13	64.5	Myosin-1; Q9BE40	1938	11–29
7	G3	(G)EAAPYLRKSEKERIEAQN(K)	2132.11	58.4	Myosin-1; Q9BE40	1938	12–29
8	G3	(F)GEAAPYLRK(S)	1004.55	17.0	Myosin-1; Q9BE40	1938	11–18

[a] Common sequence.

Table 3
Peptide sequences of selected rp-HPLC fractions from Argentinean fermented sausages originated from sarcoplasmic protein hydrolysis. Identified sequence with cleavage sites, molecular mass (*Z*), calculated score of MS-tag software, origin of peptides, total protein residues and position of identified peptides on the parental protein are shown.

Peptide number	RP fraction	Sequence	*Z* calculated (Da)	Score mascot	Original protein and accession number	Total protein residues	Position
9	G5	(R)RLKSVGIPVL(C)	1081.71	22.30	Abhydrolase domain-containing protein 3; Q0VC00	411	330–339
10	G7	(E)GALRWDLPRVQGGSQLSGLF(Q)	2157.16	17.90	AP-4 complex subunit mu-1; Q29RY8	452	359–378
11	G4	(V)KAGASIVGVNCHFDPTIS(L)	1872.93	23.00	Betaine-homocysteine S-methyltransferase 1; Q5I597	407	207–224
12	G8	(E)IIEQGIDLFT (K)	1148.62	17.10	Brefeldin A-inhibited guanine nucleotide-exchange protein 1; O46382	1849	701–710
13	G3	(L)GASPTPGEAQRHLQTHR(I)	1842.93	20.90	Calmodulin-like protein 4; Q3T0E8	153	41–57
14	G8	(R)GIQRAADIEQQ(A)	1228.63	16.80	Coagulation factor V precursor; Q28107	2211	534–544
15	G5	(T)GAKNCLRDFIEKV(A)	1549.82	14.10	Complement factor B precursor; P81187	761	288–300
16	G8	(D)DVIQTGVDNPGHPF(I)	1495.72	39.90	Creatine kinase M-type; Q9XSC6	381	55–68
17	G4	(M)PFGNTHNKHKLNF(K)	1553.80	21.20	Creatine kinase M-type; Q9XSC6	381	02–14
18	G7	(V)LGVTKDAGDEDL(K)	1232.60	13.90	DnaJ homolog subfamily B member 14; Q0IIE8	379	113–124
19	G7	(Q)ERVGELMDQNA(F)	1261.58	12.10	General transcription factor IIH subunit 5; Q2T9Z5	71	55–65
20	G7	(L)NAWGKVEADVAGHGQE(V)	1667.78	28.80	Myoglobin; P02192	154	13–28
21	G3	(L)HAKHPSDFGADAQAAMSK(A)	1868.87	18.70	Myoglobin; P02192	154	117–134
22	G7	(M)AAQYKVLGFHG(–)	1190.63	48.90	Myoglobin; P02192	154	144–154
23	G4	(T)IGTDSALHRIMEVIDAITTT(A)	2157.12	20.60	6-phosphofructo-kinase, liver type; A1A4J1	780	176–195*
24	G7	(L)AGPRSDPAGPP(G)	1021.5061	19.4	Phosphate carrier protein, mitochondrial precursor; P12234	362	27–37
25	G8	(W)ASHHDINDASRGTLSS(Y)	1667.77	17.80	Poly(A) RNA polymerase GLD2; Q2HJ44	484	315–330
26	G4	(L)DTAIVDRGKNVVS(G)	1373.74	18.30	Proteasomal ATPase-associated factor 1; Q148I1	392	181–193
27	G4	(S)AGAPSVENVKNGIR(T)	1411.77	17.00	Protein phosphatase 1A; AO62829	382	89–102
28	G4	(Q)TAAKLLHAGFKGRV(T)	1468.87	13.20	Prothrombin precursor; P00735	625	491–504
29	G8	(Q)AGKATLFVSNNSRR(A)	1520.83	21.20	Pyridoxal phosphate phosphatase; Q3ZBF9	296	50–63
30	G5	(T)LETAAGEALGQTL(T)	1273.46	18.30	Retinol dehydrogenase 8; Q9N126	312	48–60
31	G3	(L)DSVNAQADRAF(Q)	1193.55	13.10	Testis-specificY-en-coded-like protein 1; Q0P5N2	432	235–245
32	G7	(N)SSTYWEGKSDMET(L)	1520.62	17.10	Threonyl-tRNA synthetase, cytoplasmic; Q0P5N2	723	281–293
33	G8	(Y)SSHAKKATVD(A)	1043.55	15.20	Transcription initiation factor TFIID subunit 9; Q17QQ4	264	58–67
34	G4	(D)PQGALSLEADGHPAAR(R)	1589.80	24.10	Uncharacterized protein KIAA1462 homolog; A2VE02	1305	1268–1283
35	G8	(S)SSLIRHQRT(H)	1097.62	20.40	Zinc finger protein 572; Q32KN0	425	186–194
36	G3	(S)AGPNSPTGGGGGGGSGGTR(M)	1500.68	13.80	Zinc finger SWIM domain-containing protein KIAA0913; A7E305	1413	49–67

capacities to beneficially affect quality and safety while preserving their typicity (Leroy, Verluyten, & De Vuyst, 2006). Indeed, research of this technological potential and its adaptive mechanisms to a particular environment has been of outmost interest for the industry of starter cultures (Aymerich, Martin, Garriga, & Hugas, 2003). Variations in temperature, osmotic conditions, oxidative or acidic environments are situations to which LAB are routinely subjected during its passage through the gastrointestinal tract or during food processing. Thus, different approaches were used to understand adaptation of bacteria to stressful environments. Currently, the proteomic approach can be an invaluable tool for understanding those adaptive mechanisms (Champomier-Vergès, Zagorec, & Fadda, in press). As the next step in the post genomic era, proteomics focuses on the functionality of detected genes contributing to establish the connection between genome sequences and their biological role.

3.1. L. sakei 23K: a case study

L. sakei is recognized as an important component of starter cultures used for fermented sausages in Western Europe. It was also proposed as a good bioprotective candidate as it has been found to produce bacteriocins (Katla et al., 2001; Castellano et al., 2008). The analysis of *L. sakei* 23K genome revealed potential survival strategies as well as metabolic properties enabling effectively compete in the raw-meat environment (Chaillou et al., 2005). Some proteomic studies have been carried out to evaluate its adaptation to the technological stressful conditions such as cold and high NaCl concentrations, oxidative shock, and high hydrostatic pressure (Champomier-Vergès, Maguin, Mistou, Anglade, & Chich, 2002; Marceau, Zagorec, Chaillou, Méra, & Champomier Vergès, 2004; Jofré et al., 2007).

On the other hand, *L. sakei* 23K strategies to grow and survive on Chemically Defined Medium (CDM) containing meat proteins were studied regardless other stressful technological stimuli (details in forthcoming paper). In this study, *L. sakei* 23K showed to have similar growth rates either in non-supplemented CDM (C) or in myofibrillar- (M-CDM) and sarcoplasmic protein- (S-CDM) supplemented medium. However, cell viability experienced a dramatic decrease in control conditions and no survival in sarcoplasmic and myofibrillar protein-containing media was detected after 24 h (Table 4). The absence of viable cells together with OD_{600} decrease registered in M-CDM after 24 h were suggested to be due to cell lysis. Regarding amino acid kinetics, net amino acid consumption was observed during 48 h for C samples in coincidence with *L. sakei* growth (Fig. 6C; Table 4). Different release/consumption patterns were obtained on S-CDM and M-CDM; the amino acid consumption being more evident in the former one (Fig. 6S-CDM and M-CDM). Even when no *L. sakei* survival

Table 4
Growth and pH measurements of *L. sakei* 23K on Chemically Defined Medium (CDM) containing meat extracts at 30 °C during 72 h.

Culture conditions[a]	Time (h)	DO_{600}	Log CFU/ml	pH
C	0	0.10 ± 0.05	7.39 ± 0.07	6.23 ± 0.12
	6	1.27 ± 0.09	8.32 ± 0.05	4.60 ± 0.10
	24	1.80 ± 0.08	7.81 ± 0.04	4.47 ± 0.06
	48	1.74 ± 0.09	3.00 ± 0.04	4.47 ± 0.02
	72	2.03 ± 0.09	1.48 ± 0.02	4.48 ± 0.05
S-CDM	0	0.10 ± 0.07	8.04 ± 0.07	6.21 ± 0.07
	6	1.60 ± 0.09	8.60 ± 0.05	4.58 ± 0.09
	24	1.85 ± 0.07	6.20 ± 0.07	4.43 ± 0.12
	48	1.79 ± 0.09	0.00	4.43 ± 0.10
	72	2.09 ± 0.08	0.00	4.43 ± 0.02
M-CDM	0	0.12 ± 0.09	7.49 ± 0.04	6.11 ± 0.08
	6	0.65 ± 0.09	9.13 ± 0.06	5.82 ± 0.07
	24	1.00 ± 0.08	7.63 ± 0.05	4.89 ± 0.03
	48	0.69 ± 0.08	0.00	4.87 ± 0.02
	72	0.59 ± 0.09	0.00	4.88 ± 0.10

[a] C: CDM without meat proteins; S-CDM: CDM supplemented with sarcoplasmic; M-CDM: CDM supplemented with myofibrillar proteins.

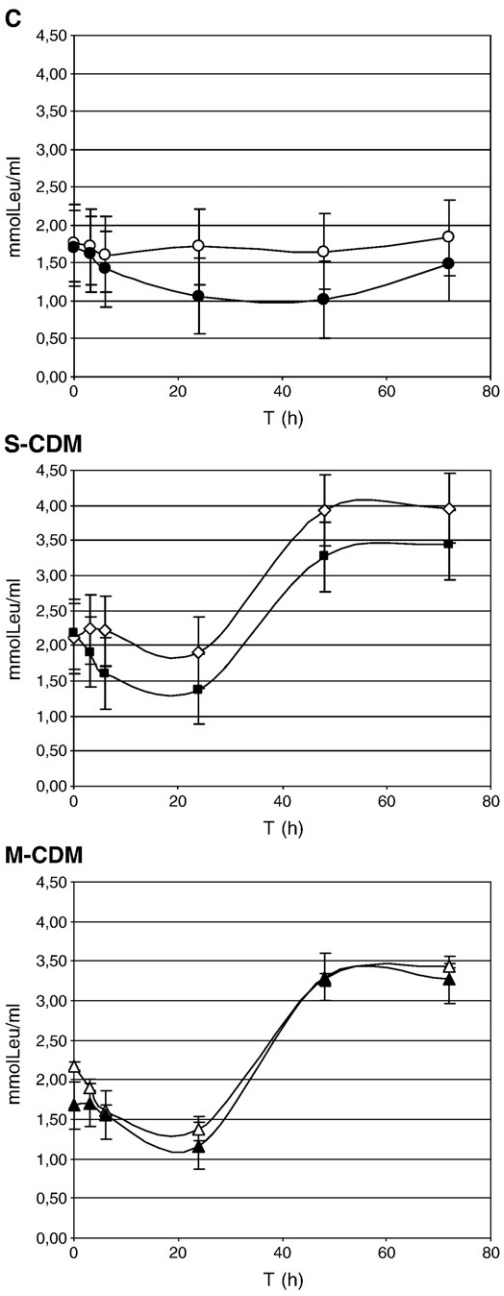

Fig. 6. Free amino acid kinetics of non-inoculated and *L. sakei* 23K inoculated Chemically Defined Medium (CDM) supplemented with meat proteins at 30 °C, 72 h. (C); CDM without meat protein addition; (S-CDM), CDM with sarcoplasmic proteins and (M-CDM), CDM with myofibrillar proteins. Empty symbols: non-inoculated media. Filled symbols: inoculated media.

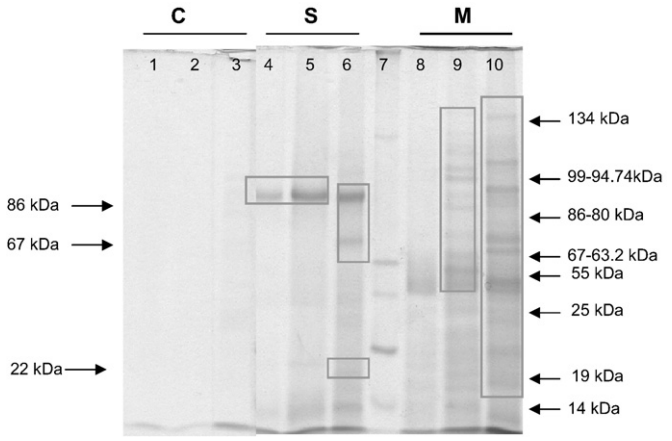

Fig. 7. SDS-PAGE of CDM supernatants (C, S-CDM and M-CDM) inoculated or not with *L. sakei* 23K and incubated during 72 h at 30 °C. Lane 1: C (T0); lane 2: C (T72); lane 3: C + *L. sakei* (T72); lane 4: S-CDM (T0); lane 5: S-CDM (T72); lane 6: S-CDM + *L. sakei* (T72); lane 7: Molecular weight markers; lane 8: M-CDM (T0); lane 9: M-CDM (T72) and lane 10: M-CDM + *L. sakei* (T72).

in S- and M-CDM was registered after 24 h, the amino acid content continued to increase possibly as result of muscle peptidase activity as observed in the non-inoculated media containing meat extracts. The proteolytic activity of *L. sakei* 23K towards added muscle proteins was evaluated by SDS-PAGE on S- and M-CDM supernatants at 72 h (Fig. 7). No bands were observed in non supplemented, non-inoculated CDM (C) at 0 and 72 h (Fig. 7, lanes 1 and 2) while the appearance of faint bands was detected in presence of *L. sakei* 23K at 72 h, probably from bacterial origin (Fig. 7, lane 3). Even when no major changes occurred in non-inoculated sarcoplasmic proteins (Fig. 7, lane 5), new bands of approximately 67 and 22 kDa were generated at 72 h in presence of *L. sakei* 23K (Fig. 7, lane 6). Conversely, new bands between 134 and 42 kDa arisen from myofibrillar proteins were observed in absence of bacterial inoculum (Fig. 7, lanes 8–9), whereas a richer band pattern was registered when *L. sakei* was growing in M-CDM at 72 h (Fig. 7, lane 10). These data suggested not only protein degradation by muscle enzymes but also by bacterial activity, while some additional bands may be attributed to the suggested bacterial lysis. Also, the cytosolic proteins putatively involved in the response of this strain to meat proteins were determined using 2D electrophoresis; the significantly regulated proteins were identified by Maldi ToF-MS analyses. From the 31 differentially expressed spots, 16 occurred in presence of myofibrillar extract while 6 proteins were modulated by the sarcoplasmic extract (Table 5). Two dipeptidases were overexpressed in presence of sarcoplasmic proteins, in correlation to the protein degradation patterns obtained by SDS-PAGE. On myofibrillar extract, *L. sakei* 23K overexpressed proteins related to energy and pyrimidine metabolism as well as ala- and tyr-tRNA synthetases, involved in translation, while others corresponding to general stress response, pyrimidine, vitamins and cofactors biosynthesis resulted down-regulated (Table 5).

Summarizing, from the obtained results, cooperation between muscle endogenous enzymes together with the up-regulated peptidases from *L. sakei* may be proposed. Also, that the supplementary nutrients furnished by meat extracts were responsible for the overexpression of proteins involved in translation, peptide/amino acid metabolism and energy production. Conversely, the under-regulation of stress proteins suggest that sarcoplasmic and myofibrillar extracts could have played an anti-stress role during cell growth. As a whole, meat proteins would not represent a stress environment *per se* for *L. sakei* 23K, constituting a regulated state not subjected to a stress-induced response in contrast to the harsh conditions during meat processing (cold, salt, changing redox potential, etc.). Technological conditions may be the main inducers for bacterial adaptive responses reported so far (Fadda et al., unpublished data).

4. LAB as bioprotective agents during meat conditioning and fermentation

4.1. Contribution of selected LAB to meat conditioning

It is known that meat is an excellent substrate for bacterial growth and if some methods to restrict their presence are not applied, meat becomes contaminated. A widely used practice in meat marketing is vacuum packaging of primal cuts for distribution and extension of shelf-life. As a result of subsequent practices such as removal of meat from their packages, it can become contaminated with pathogens (Vignolo, Fadda, & Castellano, 2008). The new trends of meat industry

Table 5
L. sakei 23K cytoplasmic proteins whose expression was significantly modified during growth on CDM supplemented with sarcoplasmic (S-CDM) or myofibrillar (M-CDM) protein extracts.

Functional category	Spot-putative function[a]	Gene	Accession no.	Mass (kDa)	pI	Protein expression levels relative to C (mean spot ± SEM)[b]			Comments
						C	S-CDM	M-CDM	
Energy metabolism: Glycolysis	23F. 6-phosphofructokinase	*pfk*	Lsa1033	34.35	5.36	1.00 ± 0.09		1.35 ± 0.06	OE[c] in M
Energy metabolism: Methane fixation pentose phosphate pathway, Carbon fixation	16. Putative phosphoketolase	*xpk*	Lsa0289	88.70	5.14	1.00 ± 0.09		0.40 ± 0.08	UE[d] in M
	17. Ribose 5-phosphate epimerase	*rpiA*	Lsa1685	24.97	5.02	1.00 ± 0.08		1.38 ± 0.10	OE in M
Lipid metabolism: Glycerophospholipid metabolism	19 = 28. Glycerol-3-phosphate dehydrogenase	*glpD*	Lsa0650	66.78	5.13	1.00 ± 0.10		0.57 ± 0.08	UE in M
Cofactors and vitamins: pantothenate and CoA biosynthesis	18. 2-dehydropantoate 2-reductase	*panE*	Lsa0041	33.33	5.62	1.00 ± 0.10		0.16 ± 0.05	UE in M
Other cell processes: Zinc ion binding	26. Putative zinc-containing alcohol dehydrogenase (oxidoreductase)	*LSA1395*	Lsa1395	33.64	5.51	1.00 ± 0.10		0.26 ± 0.08	UE in M
Cell wall formation: Glycan biosynthesis; peptidoglycan biosynthesis.	27. UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	*murD*	Lsa0747	49.54	5.07	1.00 ± 0.09		0.031 ± 0.05	UE in M
Pyrimidine metabolism	1 = 15 = 22 = 25. Cytidine triphosphate synthase	*pyrG*	Lsa1629	58.99	5.28	1.00 ± 0.10	0.61 ± 0.05	0.59 ± 0.08	UE in M/S
	24. Aspartate carbamoyltransferase catalytic subunit	*pyrB*	Lsa0952	34.33	5.38	1.00 ± 0.10		2.41 ± 0.11	OE in M
	5. Inosine-5-monophosphate dehydrogenase	*guaB*	Lsa0276	52.30	5.39	1.00 ± 0.10	0.025 ± 0.08		UE in S
Cellular processes and signaling: Membrane and intracellular structural molecules	3. Dipeptidase D-type (U34 family)	*pepD5*	Lsa0897	53.26	5.03	1.00 ± 0.10	2.66 ± 0.08		OE in S
	2. Dipeptidase D-type (U34 family)	*pepD2*	Lsa0196	54.05	4.97	1.00 ± 0.09	1.73 ± 0.10		OE in S
Translation	4. 30 S Ribosomal protein S1	*rpsA*	Lsa1017	43.91	4.61	1.00 ± 0.10	2.67 ± 0.07		OE in S
	23. Elongation factor Tu	*tuf*	Lsa1063	43.28	4.72	1.00 ± 0.09		0.35 ± 0.11	UE in M
Translation	13. Alanyl-tRNA synthetase	alaS	Lsa0387	97.15	4.81	1.00 ± 0.10		1.67 ± 0.11	OE in M
	20.Tyrosyl-tRNA synthetase	tyrS	Lsa769	47.42	5.32	1.00 ± 0.10		2.16 ± 0.08	OE in M
Genetic information processing: Chaperones and folding catalysts; Sorting and Degradation	14. ATP-dependent Hsl protease, ATP-binding subunit HslU	*hslU*	Lsa0984	52.99	5.35	1.00 ± 0.07		0.05 ± 0.02	UE in M
	8. Methionine sulfoxide reductase	*msrA*	Lsa0886	19.56	5.12	1.00 ± 0.05	0.45 ± 0.07		UE in S
General stress	9. ATPase/chaperone ClpE, putative specificity factor for ClpP protease	*clpE*	Lsa1465	79.69	5.25	1.00 ± 0.1		0.123 ± 0.08	UE in M
	21. Similar to universal stress protein, UspA family	*Usp1*	Lsa0042	17.19	5.54	1.00 ± 0.10		0.220 ± 0.10	UE in M
Amino sugar metabolism: Glutamate, Aminosugars	29. Glucosamine-fructose-6-phosphate aminotransferase	*glmS*	Lsa1355	65.97	5.26	1.00 ± 0.10		0.14 ± 0.08	UE in M

[a] The quantities of the spots in each medium are shown as the means ± standard error (SEM). Quantities are scaled to set the average quantity in CDM without meat extracts (C) to 1.00.
[b] Statistical differences were significant at a P value of <0.05.
[c] OE: overexpressed.
[d] UE: underexpressed.

involve the use of a new generation starter cultures with industrial or nutritional important functionalities (Leroy et al., 2006). Functional starter cultures contribute to food safety by producing antimicrobial compounds such as bacteriocins and also provide sensorial, technological, nutritional and/or health advantages. Thus, another goal of our group was to assess whether lactobacilli strains selected as the fittest sausage starter cultures were positive agents in conditioning of chilled meat cuts packed under vacuum.

Proteolytic events and the role of three *Lactobacillus* strains with proved bioprotective and technological potential (Vignolo, Ruiz Holgado, & Oliver, 1988; Fadda et al., 1998; Castellano, Holzapfel, & Vignolo, 2004; Castellano & Vignolo, 2006) were analyzed *in situ* (Fadda, Chambon, Champomier-Verges, Talon, & Vignolo, 2008). Results showed that the studied strains (*L. curvatus* CRL705, *L. plantarum* CRL681 and *L. sakei* 23K) experienced a good adaptation to meat environment, as was previously demonstrated (Fadda et al., 1998; Champomier-Vergès, Zuñiga, Morel-Deville, Pérez-Martínez, & Zagorec, 1999; Castellano et al., 2004). *L. curvatus* CRL705 and *L. sakei* 23K showed the highest competitiveness on meat as substrate during 15 days at 7 °C. As structural and biochemical *postmortem* changes, including proteolysis, have a great role in the ultimate tenderness and palatability of meat (Demeyer et al., 2000; Nishimura, 2002; Kemp, Sensky, Bardsley, Buttery, & Parr, 2010), the release of soluble amino acids and peptides was evaluated. The highest amino acid accumulation was observed on meat discs inoculated with *Lactobacillus* indicating aminopeptidase activity from bacterial origin in the assayed conditions. Among the tested strains, *L. plantarum* CRL681 presented the highest amino acid release probably due to the lesser acidic environment (pH: 5.12) which could prevent enzyme inactivation. These peptidases may contribute to the release of small peptides and free amino acids during conditioning of meat, which will positively impact on meat flavor. Nishimura (2002) also observed that storage of beef at low temperature improves flavor by increases in free amino acids, peptides and inosine 5′-monophosphate. Moreover, a substantial increase in peptide concentration related to taste intensity was also reported in fresh meat with increasing postmortem time (Claeys et al., 2004). In our study the highest hydrolytic profile was observed on sarcoplasmic proteins when *Lactobacillus* strains were present. SDS-PAGE analysis resulted in the disappearance of three protein bands and the generation of a new one as a consequence of *Lactobacillus* growth on meat discs. Even when *L. plantarum* CRL681 accounted for the maximal amino acid release, *L. curvatus* CRL705 and *L. sakei* 23K were found to be more proteolytic (Fig. 8). Even when the acidification produced by LAB metabolism may play a role on protein bands disappearance, an enhancing effect of *Lactobacillus* on meat conditioning could be assumed again. The appearance of troponin I, a

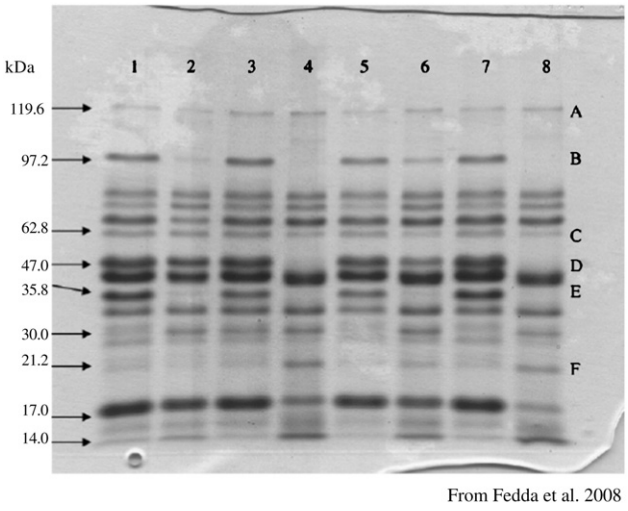

Fig. 8. SDS-PAGE of sarcoplasmic proteins from vacuum-packaged beef discs after 15 days at 7 °C. Lane 1: control, T0; lane 2: control, T15; lane 3: *L. curvatus* CRL705, T0; lane 4: *L. curvatus* CRL705, T15; lane 5: *L. plantarum* CRL681, T0; lane 6: *L. plantarum* CRL681, T15; lane 7: *L. sakei* 23K, T0; lane 8: *L. sakei* 23K, T15. Maldi ToF-MS identified proteins: A: amylo-1,6-glucosidase; B: alpha-1,4-glucan orthophosphate glycosyl transferase; C: glucose-phosphate isomerase; D: B-enolase; E: glyceraldehyde-3-phosphate dehydrogenase; F: troponin I.

myofibrillar protein, on sarcoplasmic extracts after 15 days in presence of *Lactobacillus* strains was a surprising event that could be explained by changes on the protein conformation that facilitate its solubilization on meat extracts of low ionic strength. This study evidences the possible use of the studied strains, specially the bacteriocin-producer *L. curvatus* CRL705 for the improvement of shelf-life of vacuum packaged meat while contributing to meat ageing through the release of small peptides and free amino acids (Fadda et al., 2008). Moreover, a delay in the appearance of meat tissue degradation signs in samples inoculated with this bioprotective culture, without affecting sensory and structural characteristics was recently demonstrated (Castellano, González, Carduza, & Vignolo, 2010).

4.2. LAB peptides involved on safety of meat and meat products

The preservative ability of LAB in foods is attributed to their competition for nutrients and to the production of antimicrobial metabolites including organic acids, hydrogen peroxide, enzymes and bacteriocins. Bacteriocins are a heterogeneous group of peptides and proteins ribosomally produced that kill or inhibit the growth of close related micro organisms such as food pathogens (*Listeria monocytogenes* and *Clostridium*) and other meat spoilage bacteria such as *B. thermosphacta*. The use of bacteriocins in biopreservation is a growing area of research. Numerous bacteriocins have been isolated over the past three decades. They vary in size from small (<3 kDa), heavily post-translationally modified peptides to large heat labile proteins. The latest revised classification scheme divides them into two main categories: the lanthionine-containing, lantibiotics (class I) and the nonlanthionine-containing bacteriocins (class II) while the large; heat-labile murein hydrolases (formerly class III bacteriocins) constitute a separate group called bacteriolysins (Cotter, Hill, & Ross, 2005). At present only nisin is approved as a food additive by both FDA and WHO and consequently widely used in foods.

Bacteriocins generally act by creating pores in the membrane of their target cells which cause a cytotoxic effect due to the disturbance of the cell membranes. Alternatively, bacteriocins can pass the membrane to a target inside the cell causing major damage in cell functions. Different mechanisms of action have been described although the majority, if not all, involve the contact between bacteriocin and susceptible cell (Cleveland, Montville, Nes, & Chikindas, 2001; Castellano, Raya, & Vignolo, 2003). Among the many benefits bacteriocins offer, while extending shelf-life of foods, are: (i) an extra protection during temperature abuse conditions, (ii) decrease the risk for transmission of foodborne pathogens through the food chain, (iv) minimization of the economic losses due to food spoilage, (v) reduction of chemical preservatives application, (vi) possibility of less severe heat treatments application, this implying a better preservation of food nutrients and vitamins, and keeping organoleptic properties of foods, (vii) marketing of "novel" foods (less acidic, with a lower salt content, and with a higher water content) (Gálvez, Abriouel, Lucas López, & Ben Omar, 2007).

Bacteriocins can be introduced into food in at least four different ways: (i) as purified or semi-purified bacteriocins added directly to food; (ii) as bacteriocins produced *in situ* by bacterial cultures that substitute all or part of the starter culture; (iii) as an ingredient based on a fermentate of a bacteriocin-producing strain or (iv) immobilized as constituent of a bioactive food packaging film.

4.2.1. Strategies to improve bioprotective effect in meat processing

The effectiveness of bacteriocins in food systems should consider the adequacy of the environment for bacteriocin stability and/or production. A loss of activity is often detected when bacteriocins are added to meat and meat products, this being a direct consequence of additives (nitrites, NaCl and spices) or meat components, such as proteolytic enzymes, bacteriocin-binding proteins or fat particles (Verluyten, Leroy, & De Vuyst, 2004; Verluyten, Messens & De Vuyst, 2004). However some spices such as black pepper was shown to enhance the inhibitory activity of sakacin K against *L. monocytogenes in vitro* and in model sausages, also a synergistic effect with nitrite, NaCl and Bac^+ starter cultures was observed (Hugas, Garriga, & Monfort, 2002). On the other hand, significant variations in the sensitivity of *Listeria* strains to the same bacteriocin as well as the appearance of spontaneous resistant mutants to individual bacteriocins were reported (Cotter et al., 2005).

Table 6
Bacteriocins and hurdle technology. Combinations to improve the effectiveness in meat systems.

Bacteriocins combined with	References[a]
✓ Chemical substances and natural antimicobials: NaCl, NO_2^-, organic acids, chelating agents, ethanol, reuterin, essential oils (phenolic compounds)	1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12
✓ Other bacteriocins	13; 14; 15; 16
✓ Enzymes (lysozyme, lactoperoxidase system, lactoferrin)	17; 18; 19
✓ Heat treatments: reduction of heat intensity (cost saving and reduction of heat impact on food)	20; 1; 2
✓ Modified atmosphere packaging (MAP)	21, 22
✓ Pulse Electric Fields (PEF) (in process)	23; 24; 25
✓ High Hydrostatic Pressure (HHP)	26; 27; 28; 29; 30; 31
✓ Other non thermal treatments are in exploration (irradiation, Pulsed Magnetic Fields)	32; 33

[a] 1 Ananou, Valdivia, Martínez Bueno, Gálvez, & Maqueda, 2004, 2 Ananou, Gálvez, Martínez-Bueno, Maqueda, & Valdivia, 2005; 3 Hugas, Neumeyer, Pagés, Garriga, & Hammes, 1996; 4 Vignolo, Fadda, Kairuz, Holgado, & Oliver, 1998; 5 Gill & Holley, 2003; 6 Ukuku & Fett, 2004; 7 Cobo Molinos et al., 2005; 8 Fang & Tsai, 2003; 9 Belfiore et al., 2007; 10 Brewer, Adams, & Park, 2002; 11 Burt, 2004; 12 Grande et al., 2007; 13 Vignolo et al., 2000; 14 Hugas et al., 2002; 15, 16 Gravesen, Jydegaard Axelsen, et al., 2002; Gravesen, Ramnath, et al., 2002; 17 Nattress & Baker, 2003; 18 Boussouel, Mathieu, Revol-Junelles, & Millière, 2000; 19 Branen & Davidson, 2004; 20 Modi, Chikindas, & Montville, 2000; 21, Nilsson, Huss, & Gram, 1997; 22 Nilsson et al., 2000; 23 Heinz, Alvarez, Angersbach, & Knorr, 2002; 24 Sobrino-Lopez & Martin Belloso, 2006; 25 Santi, Cerrutti, Pilosof, & de Huergo, 2003; 26 García-Graells, Valckx, & Michiels, 2000; 27 Kalchayanand, Dunne, Sikes, & Ray, 2004; 28 Garriga, Aymerich, Costa, Monfort, & Hugas, 2002; 29 Aymerich et al., 2005; 30 Arqués, Rodríguez, Gaya, Medina, & Nuñez, 2005; 31 Jofré, Garriga, & Aymerich, 2008; 32 San Martín et al., 2001; 33 Chen, Sebranek, Dickson, & Mendonça, 2004.

In this context, the application of bacteriocins as part of hurdle technology has received great attention in recent years (Leistner, 2000; Chen, & Hoover, 2003; Deegan, Cotter, Hill, & Ross, 2006). The combined use of bacteriocins with other hurdles helps to overcome drawbacks related to bacteriocin inactivation on food matrix or emergences of resistant mutants (Table 6). An example is the combined effect of nisin, lactocin 705 (produced by *L. curvatus* CRL705) and enterocin CRL35 (produced by *Enterococcus mundtii* CRL35) against different *L. monocytogenes* strains in meat slurry that showed no viable counts after incubation. In fact, a mixture containing more than one bacteriocin will be bactericidal to more cells in a sensitive population, since cells resistant to one bacteriocin would be killed by another (Vignolo et al., 2000). However, when the direct addition of enterocin A, sakacin K (produced by *Enterococcus faecium* CTC492 and *L. sakei* CTC494, respectively), Nisaplin (nisin) and ALTA 2351 (pediocin) to frankfurter sausages meat batter was carried out, only the batch with enterocin and sakacin showed significant *Listeria* reduction numbers (Hugas et al., 2002). However, the developed resistance to one bacteriocin may also afford protection against other bacteriocins, and the observed cross-resistance between pediocin-like bacteriocins has been attributed to a general mechanism of resistance (Gravesen, Jydegaard Axelsen, et al., 2002; Gravesen, Ramnath, et al., 2002). Therefore, research should focus on the finding of novel bacteriocins showing no cross-resistance with existing ones.

Other strategy applied as complementary and alternative preservation tool, is the combination of high hydrostatic pressure with nisin and lactate salts to inhibit the growth of *L. monocytogenes* and *Salmonella* on cooked meat products. It seems that the injured survivors after pressure became sensitive to nisin resulting in a viability loss (Aymerich, Jofré, Garriga, & Hugas, 2005). Also, food-grade chelators such as EDTA in combination with bacteriocins were assayed to overcome Gram-negative resistance to bacteriocins. In fact, vacuum-packaged and refrigerated fresh beef treated with nisin combined with EDTA increased its shelf-life by inhibiting the growth of *Escherichia coli* as well as *B. thermosphacta* (Tu & Mustapha, 2002). A recent report, showed the effect of lactocin 705/AL705 and nisin in combination with chelating agents against *E. coli* strains. Results also showed that the direct addition of bacteriocins and chelators was not as effective as compared to the deferred treatments (Belfiore, Castellano, & Vignolo, 2007).

As described before, direct surface application of antibacterial substances onto foods (sprays or dip solutions) had limited benefits since the active substances can become neutralized on contact or diffuse rapidly from the surface to the bulk food (Quintavalla & Vicini, 2002) while incorporation of antimicrobial agents into food formulations may result in partial inactivation of the active substances by food constituents. Thus, other promising and rapidly emerging strategy is the active/smart packaging, in which antimicrobial agents are incorporated into or coated onto food packaging materials (Vermeiren, Devlieghere, & Debevere, 2002). The bacteriocin coating of polyethylene (PE) surface is reported as an effective method to confer antimicrobial properties to food packages (Lee, An, Park, & Lee, 2003; Lee, An, Lee, Park, and Lee, 2004; Grower, Cooksey, & Getty, 2004; Mauriello, Ercolini, La Storia, Casaburi, & Villani, 2004; Mauriello, De Luca, La Storia, Villani, & Ercolini, 2005; Vartiainen, & Shytti, 2004; Ercolini, Storia, Villani, & Mauriello, 2006). Nisin was the antimicrobial most commonly incorporated into films either as the only substance or combined with other antimicrobials (Joerger, 2007).

Particularly, for meat and meat products different antilisterial packaging materials and coatings have been assayed using nisin (Scannell et al., 2000; Franklin, Cooksey, & Getty, 2004; Grower et al., 2004; Guerra, Macías, Agrasar, & Castro, 2005), pediocin (Nielsen, Dickson, & Crouse, 1990; Ming, Weber, Ayres, & Sandine, 1997) and an antilisterial bacteriocin produced by *L. curvatus* 32Y (Mauriello et al., 2004). Also active PE-based films development using lactocin 705 and lactocin AL705 produced by *L. curvatus* CRL705 were evaluated (Blanco Massani, Fernandez, Ariosti, Eisenberg, & Vignolo, 2008). Results clearly demonstrated the retention of antimicrobial activity when it is adsorbed to PE-based films. Particularly the active PE film obtained by contact with lactocin 705 + lactocin AL705 proved to be more effective than nisin inhibiting the growth of *Listeria* without modifying functional properties of the film, this constituting a promising tool for extending the shelf-life of meat products (Blanco Massani et al., 2008). The use of bacteriocin-producing strains that are well adapted to the meat environment will provide an extra hurdle in a multi-hurdle integrated system (Vignolo & Fadda, 2007).

5. Concluding remarks

Lactobacilli play a key role in industrial and artisan food fermentation, including meat products. They contribute to raw-material preservation due to acidification and to a production of antimicrobial compounds such as bacteriocins, but also because of their capacity to contribute to product characteristics such as flavor and texture. Our findings confirm that meat protein degradation occurring during sausage fermentation, may be ascribed to the synergistic action of meat endogenous proteases, LAB proteolytic activity and acid-induced changes arising from the bacterial fermentative metabolism. In addition, the study carried out on Argentinean sausages allowed to detect changes on meat proteins during fermentation and ripening, confirming that sarcoplasmic proteins were the most susceptible to proteolysis. Many low molecular weight peptides which could help to characterize fermented meat were identified. Those peaks coming from sarcoplasmic and myofibrillar proteins will be further analyzed to corroborate their specific function in sausage flavor. However in this first proteomic approach, none specific biomarker has been detected to differentiate sausage brands, those compounds could provide an objective index of flavor quality development. In addition, the analysis of *L. sakei* 23K protein expression has extended our understanding of the molecular responses and growth mechanisms of *L. sakei* 23K in presence of meat proteins.

On the other hand, there is no doubt that the application of bacteriocins in food preservation may serve to satisfy industrial and consumer's demands while extending shelf-life of foods. However, the effectiveness of bacteriocins in food systems should consider the adequacy of the environment for bacteriocin stability and/or production. In this context, it should be borne in mind that bacteriocins are not meant to be used as the sole means of food preservation but as a multi-hurdle integrated system.

The topics covered in this review not only confirms existent knowledge about the importance of LAB in food, but also calls into relevance the importance of its generated peptides such as flavor proteolytic products and bacteriocins on the overall quality of meat and meat products. The mechanisms of production of each type of peptide, different methods for flavor compounds characterization as well as the better strategies to improve bioprotective effect of bacteriocins, were underlined. The proteomic approach may identify candidate molecules, which may support specific bioflavor effects associated with particular *Lactobacillus* strains. In addition, candidate lactobacilli proteins involved on adaptive response to meat environment can then be validated via mutant approaches, which will allow for improved strain selection procedures and molecular science-based quality and safety claims.

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