



A peptidomic approach of meat protein degradation in a low-sodium fermented sausage model using autochthonous starter cultures



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ABSTRACT

Fermented sausage technology is currently compromised in decreasing the addition of NaCl. Use of starter cultures with peptidogenic potential could be a valuable strategy that can mask or hide off flavors produced by the use of NaCl substitutes. In the present work, the peptidogenic potential of four lactic acid bacteria species was evaluated in a low-sodium beaker sausage (BS) model. Using a peptidomic approach, a total of 86 low molecular weight (LMW) peptides were accurately identified, mostly derived from myofibrillar proteins, especially actin, which generated 53 peptides. The BS inoculated with *L. curvatus* CRL705 generated 56 LMW peptides, followed by *Enterococcus (E.) mundtii* CRL35 with 43 peptides. In addition, BS inoculated with *Lactobacillus (L.) plantarum* and with *L. sakei* produced higher amino acid amounts over time as compared to the rest of BS models, highlighting the importance of both, time and sample effect on the overall free amino acid generation. The presence of each LAB strain in BS models generated a unique profile of small peptides and amino acids that could serve as a distinctive biochemical trait to differentiate specific fermented products. According to these results, *E. mundtii* and *L. sakei*, which are compatible between them, are proposed as the most efficiently adapted to low-sodium conditions. The use of selected strains during the processing of low-sodium fermented sausages could have a positive effect on the production of small peptides and free amino acids.

1. Introduction

Fermented sausages can be defined as meat products consisting of a mixture of mainly lean meat, fat, salt, curing agents and spices stuffed in casings, fermented and dried (ripening) (Cocolin, Urso, Rantsiou, Cantoni, & Comi, 2006). In the traditional method of spontaneous fermentation without the addition of starter cultures, the bacteria conducting the fermentation processes originate from the indigenous microbiota from raw materials and environment (Fadda & Vignolo, 2014; Leroy, Lebert, & Talon, 2014). Different species of lactic acid bacteria (LAB) and Gram-positive, coagulase-negative cocci (GCC), are the microorganisms primarily responsible for sausage fermentation. LAB, in particular lactobacilli, contribute to the hygienic and sensory quality mainly through their carbohydrate and protein catabolism resulting in sugar depletion, pH reduction, production of antimicrobial agents and generation of taste and aroma compounds (Talon et al., 2008). GCC participate in colour development and stabilization through a nitrate reductase activity (Mainar, Stavropoulou, & Leroy, 2017).

On the other hand, cured fermented meat products are an important source of sodium. In fact NaCl concentration reaches values between 3.0 and 5.0% at the end of the ripening (Toldrá, 2012). NaCl has many important functions during sausage ripening; it enhances taste, promotes water release and consequently texture, inhibits undesirable microbial growth and enzyme activity at high concentrations (Andrés, Barat, Grau, & Fito, 2008). However high sodium intakes have been linked to health problems such as increase in blood pressure levels, risk of cardiovascular and cerebral vascular accidents or stroke. The drug-dependence of these diseases has been related with NaCl consumption (Mente et al., 2014). Thus, an intense debate has emerged about excessive sodium ingestion directly associated with public health implications (Dötsch et al., 2009). In view of this situation, together with current general exigencies of consumers for safe and healthier foods, fermented sausage technology is compromised in decreasing the addition of NaCl in cured meats (Toldrá & Barat, 2014). However this practice could produce some negative effects related to the sensorial, safety and technological aspects (Desmond, 2006; Ruusunen &

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Puolanne, 2005; Yotsuyanagi et al., 2016). One of the strategies applied by fermented sausage technology is the replacement of sodium chloride by other non-sodium salts such as magnesium, calcium or potassium chloride, although those compounds usually entail unpleasant tastes (bitter, metallic, astringent) and could also affect texture properties, resulting in consumers rejection of the product (Almeida de et al., 2016; Kilcast and Den Ridder, 2007).

During the ripening of fermented sausages, many of non-volatile compounds such as peptides and amino acids, involved in taste, are produced during meat protein degradation (Dashdorj, Amna, & Hwang, 2015). The meat protein degradation involves the sequential action of endopeptidases and exopeptidases; proteins are first degraded by endopeptidases and the resulting fragments are further hydrolyzed by several types of exopeptidases (Fadda, López, & Vignolo, 2010). Proteolysis of dry cured meat products has been attributed to either endogenous and/or exogenous enzymes from microorganisms (Mauriello, Casaburi, & Villani, 2002; Nishimura, 2002; Spaziani, Torre, & Steccini, 2009). The low molecular weight (LMW) peptide fractions (< 3 kDa) have been related to taste (Sentandreu et al., 2003) and proposed as biomarkers for a specific applied technology (Fadda, Sentandreu, & Sentandreu, 2018; Sentandreu & Sentandreu, 2011). In this regard, as mentioned before, due to its metabolism, 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 (Chen, Liu, Sun, Kong, & Xiong, 2015). In fact, LAB are endowed with proteolytic activity, mainly intracellular amino-, di- and tripeptidyl-peptidase activities (Liu, Bayjanov, Renckens, Nauta, & Siezen, 2010). However, the proteolysis pattern in fermented sausages is influenced by different variables such as product formulation, processing conditions and starter cultures (Hughes et al., 2002). In a previous study, sarcoplasmic and myofibrillar protein degradation by *Lactobacillus curvatus* CRL705 and *Staphylococcus vitulinus* GV318, an autochthonous starter culture, was evaluated in a beaker sausage model, and a wide diversity of LMW peptides and protein targets were identified (López, Sentandreu, Vignolo, & Fadda, 2015a, 2015b). The knowledge of the proteolytic potential of LAB through the analysis of its peptidogenic activity allows, as a first strategy, the selection of better combinations of starter cultures to improve flavour development of fermented meat products (Vignolo, Fontana, & Fadda, 2010).

On this basis, the objective of this study was to undertake a first biochemical approach for analyzing the production of small peptides and free amino acids by different LAB strains in a sausage model with reduced sodium contents. In a next stage, the sensory evaluation of the final product will be addressed.

2. Materials and methods

2.1. Strains and culture conditions

Four different species of LAB and one GCC strain belonging to the CERELA-CONICET collection (Centro de Referencia para Lactobacilos, Tucumán, Argentina) were used in this study. *Lactobacillus (L.) plantarum* CRL681, *L. curvatus* CRL705, *L. sakei* CRL1862 were isolated from artisanal fermented sausages (Tucumán, Argentina), and the *Enterococcus (E.) mundtii* CRL35 strain was from dairy origin (isolated from an artisanal cheese -Táffí cheese-, Tucumán, Argentina) (Farfás et al., 1996). The GCC selected for this work was *Staphylococcus (S.) vitulinus* GV318, from fermented sausage origin (Tucumán, Argentina).

Frozen LAB cells (−20 °C) (*L. plantarum*, *L. sakei* and *L. curvatus*) were activated in liquid culture medium using De Mann Rogosa and Sharp (MRS) or LAPTG broth medium for *E. mundtii* CRL35 and incubated at 30 °C for 16 h prior to use in the meat model system. *S. vitulinus* GV318 to be used in combination with each LAB was activated in Brain Heart Infusion (BHI) broth (Britania, Argentina) for 16 h at 30 °C under agitation (150 rpm).

2.2. Test of compatibility among strains

Compatibility between LAB and *S. vitulinus* was tested in agar plate by the spot method (Schillinger & Lucke, 1989). Briefly, 5 µL of cell free supernatant culture of the active LAB or GCC strain were spotted onto an indicator lawn of the GCC or LAB to be evaluated and incubated for 16–18 h at 30 °C. The indicator lawn was prepared by adding 10 µL of an overnight culture of the strain to be tested to 10 mL of BHI for GCC or MRS for LAB agar (0.7% at 42 °C). If inhibition halos are observed after the incubation time, the existence of incompatibility between the tested strains can be confirmed.

2.3. Beaker sausage models (BS)

All operations were carried out in a laminar flow hood. Pork *Longissimus dorsi* (70%) and bovine *Semimembranosus* (30%) cooled carcasses were aseptically sampled by removing the surface using sterile knives. Next, the meats were minced in a home processor machine under aseptic conditions (López et al., 2015a; Vignolo, Cuozzo, De Kairuz, Holgado, & Oliver, 1996). One kilogram of aseptic minced meat was thoroughly mixed with the curing additives: 1.0% NaCl, 0.25% KCl, 0.25% CaCl₂, 0.75% sucrose and 0.75% glucose, previously autoclaved at 121 °C for 15 min and 0.015% Na nitrite, 0.015% Na nitrate and 0.05% Na erythorbate previously filtered-sterilized (0.22 µm) (Millipore, Billerica, USA).

The meat components, curing salts and other ingredients were thoroughly mixed and the sausage batter was then divided into five portions and inoculated or not with microorganisms as follows:

- (1) BS-Control: added with antibiotics (20.000 UI/Kg penicillin, 20 mg/Kg streptomycin, 50 mg/Kg amphotericin B and 0.01% Na azide) (Gibco, Grand Island, USA), having the objective to exclusively evaluate proteolysis due to endogenous meat enzymes.
- (2) BS-Curv: inoculated with *L. curvatus* CRL705 (7–8 log CFU/g) + *S. vitulinus* GV318 (7–8 log CFU/g),
- (3) BS-Plant: inoculated with *L. plantarum* CRL681 (7–8 log CFU/g) + *S. vitulinus* GV318 (7–8 log CFU/g),
- (4) BS-Sakei: inoculated with *L. sakei* CRL1862 (7–8 log CFU/g) + *S. vitulinus* GV318 (7–8 log CFU/g),
- (5) BS-Ent: inoculated with *E. mundtii* CRL35 (7–8 log CFU/g) + *S. vitulinus* GV318 (7–8 log CFU/g).

Sterile polyethylene tubes containing different BS models were incubated at 22 °C and samples were collected at 0, 3, 6, and 10 days (d) of fermentation and ripening. Three independent replicates were performed for each BS model.

2.4. Bacterial counts and pH measurement

Ten grams of each beaker sausage sample were mixed with 90 mL of sterile 0.1% peptone water (Britania, Buenos Aires, Argentina) and homogenized for 8 min in a stomacher machine (Lab blender 400, Seward medical, London, U.K.). Appropriate decimal dilutions of the samples were prepared and plated in duplicate in selective media to enumerate the microbiota. The following media and conditions were used: MRS agar (Britania, Argentina) at 30 °C during 48 h for LAB and Mannitol Salt Agar at 37 °C for 48 h for GCC. The microbiological quality of the Control was followed by plating on Plate Count Agar (30 °C, 48 h) for total aerobic mesophilic microorganisms. The pH values were obtained by directly inserting the tip of the probe (Meat pHmeter, Hanna Instruments Argentina, Buenos Aires, Argentina) into different portions of BS samples.

2.5. Extraction of amino acids

Samples of 2.5 g from each BS sample were homogenized in stomacher during 8 min with 12.5 mL of 0.1 N HCl (Minkiewicz et al., 2008; Panchaud, Affolter, & Kussmann, 2012). This extract was

transferred to tubes and centrifuged for 15 min at 13,500 $\times g$ at 4 °C. The supernatant was filtered using Whatman paper, then aliquoted and frozen (–20 °C) until further analysis.

2.6. Analysis of free amino acids by RP-HPLC

A Shimadzu HPLC equipped with a 5 μ m Gemini column (150 \times 4.6 mm) and a pre-column with pre-column filter was used for these analyses. Mobile phases were: Phase A, NaH₂PO₄ (40 mM) pH 6.4 and phase B was CH₃CN/MeOH/H₂O (45:45:10). Analytical conditions were: flow rate 1.0 mL/min, injection volume 1 μ L, T° 40 °C at an initial pressure of 9 MPa and Fluorimetric detector ($\lambda_{\text{ex}} = 340$ nm; $\lambda_{\text{em}} = 460$ nm). Samples were derivatized (1:1) for 60 s prior to injection with o-faldialdehyde (OPA). The derivatizing reagent composition was: Methanol (MeOH) (1.25 mL), borate buffer pH 10 (1.25 mL), OPA (20 mg) and 3-Mercaptopropionic acid (3-MPA). Frozen samples were diluted 50 times and injected in duplicate. This chromatographic method allowed detection of the following amino acids: aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), histidine (His), glutamine (Gln), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala), gamma-aminobutyric acid (GABA), tyrosine (Tyr), valine (Val), methionine (Met), tryptophan (Trp), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), ornithine (Orn) and lysine (Lys). Concentrations of these amino acids, expressed as mg/100 g of BS, were determined at 0 h, 3, 6 and 10 days of incubation.

2.7. Peptide extraction

Samples of 2.5 g from each BS sample at 0 and 10 days of incubation were homogenized in a stomacher during 8 min with 12.5 mL of 0.1 N HCl (Sentandreu et al., 2003). The slurries were then centrifuged (13,500 $\times g$ at 4 °C for 20 min) and supernatants submitted to ultra-

filtration in an Amicon Ultra-4 Centrifugal Filter Unit with Ultracel-3 membrane (Millipore, Billerica, USA). The obtained filtrate containing peptides of molecular weight lower than 3 kDa was frozen at –20 °C during 24 h and then freeze-dried until further analysis.

2.8. Peptide sequence identification by LC-ESI-MS/MS

Freeze-dried samples corresponding to peptides lower than 3 kDa were re-dissolved in 1 mL of 0.1% trifluoroacetic acid (TFA). Twenty-five microliters of this solution were injected into a Surveyor LC system directly coupled to a LCQ Advantage Ion trap MS instrument (Thermo Scientific, San Jose, CA). Separation of peptides was carried out on a Jupiter Proteo reverse phase column (150 \times 0.5 mm; Phenomenex, Torrance, CA), using the following conditions: linear gradient from 0 to 40% Acetonitrile in 0.1% formic acid for 140 min at a flow rate of 30 μ L/min. Operating parameters of the ion trap detector were the following: electrospray ionization in the positive mode, capillary temperature 250 °C, collision energy normalized to 35%, spray voltage 4.5 kV, and capillary voltage 33.0 V. First scan event was full MS detection for m/z values in the range 400 to 2000. The second event was a dependent MS/MS scan of the most intense ions having charges from +2 to +4, enabling dynamic exclusion after three scans of the most intense ion for a period of 5 min. The minimum ion intensity for triggering a MS/MS scan was 5×10^5 . Data acquisition was done using the Xcalibur v2.0 software. Peptide identification was done from the information contained in the generated MS/MS spectral data using an in-house version of the Mascot search engine v2.3 (www.matrixscience.com) against the Uniprot KB protein database (www.uniprot.org). We defined the following search parameters: Enzyme: “none”; no fixed modifications, variable modifications: “Deamidation (NQ)” and “Oxidation (M)”. Mass accuracy was set to 1.2 and 0.6 Da for MS and MS/MS mode, respectively. The option “Mammalia” was selected as

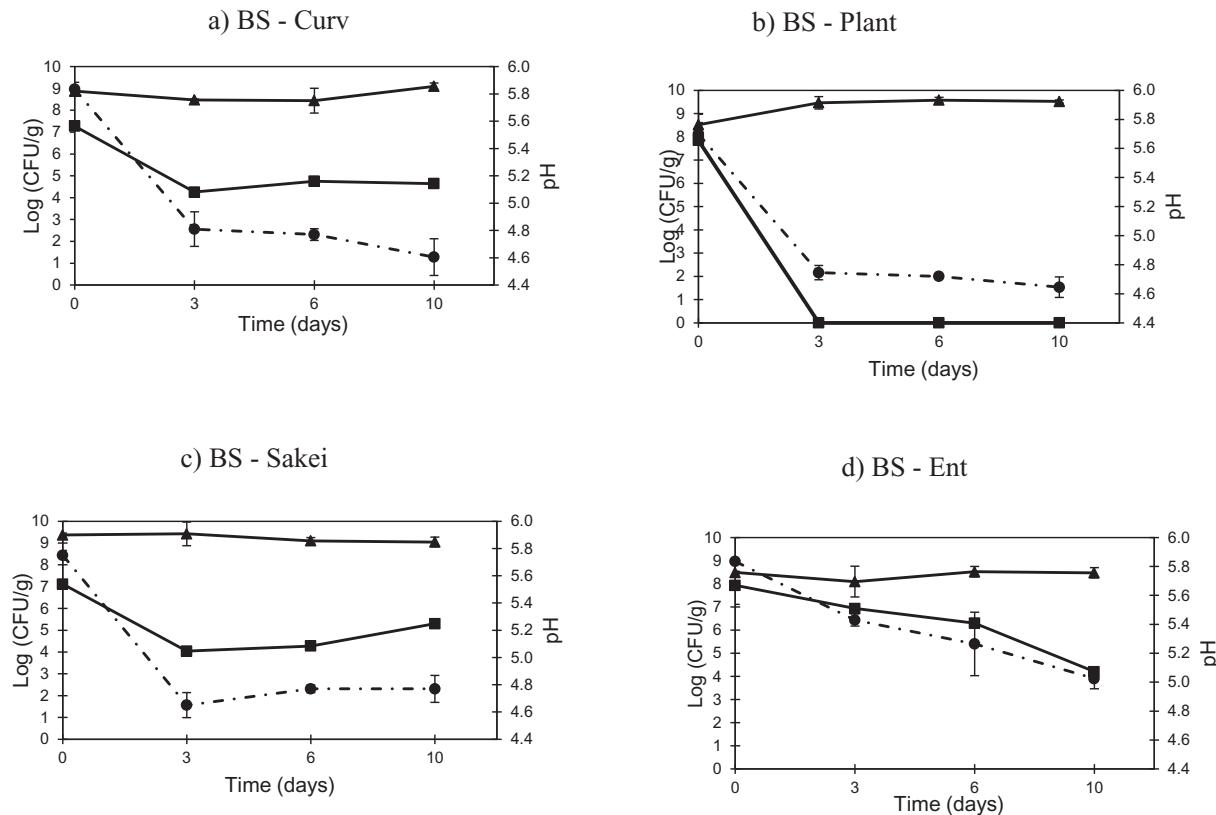


Fig. 1. Microbial counts and pH of beaker sausage models (BS) during 10 days of incubation at 22 °C. LAB (▲), GCC (■) and pH (dash and dot). (a) BS – Curv: Beaker sausage model (BS) inoculated with *L. curvatus* CRL705 + *S. vitulinus* GV318; (b) BS – Plant: BS inoculated with *L. plantarum* CRL681 + *S. vitulinus* GV318; (c) BS – Sakei: BS inoculated with *L. sakei* CRL1862 + *S. vitulinus* GV318; (d) BS – Ent: BS inoculated with *E. mundtii* CRL35 + *S. vitulinus* GV318.

taxonomy restriction parameter. For selection of the MS/MS identifications obtained with Mascot, only top ranking significant peptides were considered, taking a reference peptide score threshold of 25 for considering a true sequence. Selected identifications following these criteria were further verified by manually checking the assignation of the identified masses to *b* and *y* series ions. Peptide identifications were not considered in those cases where there was not a good correlative identification of several *b* and/or *y* ions (at least in part of the whole peptide sequence) or in those cases in which the MS/MS spectrum was of poor quality (too low peak information or too low signal-to-noise ratio).

2.9. Statistical analysis

Three independent replicates were performed for each BS model. Residual normal distribution of total amino acid content was checked by the Shapiro-Wilk test, then compared by ANOVA (Source of Variation: Sample, Time, Sample * Time) and Tukey test at 5% of significance using the R language version 3.3.1 (R Core Team, 2017). From RP-HPLC, the amino acid concentrations were obtained by PeakSimple software based on the concentration of standards for each amino acid. The representation of free amino acids over time in all samples using Principal Components Analysis (PCA) was performed at 0, 3, 6, and 10 days using XLSTAT 2015 software (Addinsoft, New York, USA), as described by Saldaña et al. (2018). The Pearson's correlation matrix of the average amount of the free amino acids at each sample and assayed time was calculated and eigenvalues and eigenvectors were obtained. Finally, BS samples and free amino acids were displayed in a biplot maintaining > 80% of inertia of the original data.

3. Results

3.1. Performance of selected strains in BS models

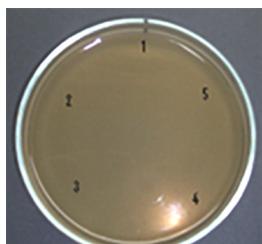
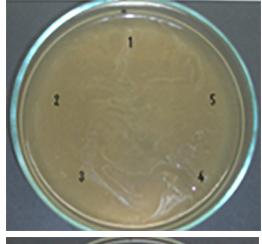
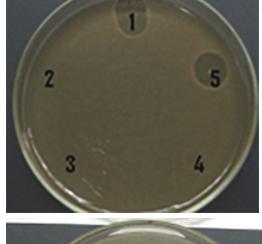
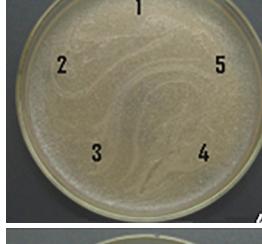
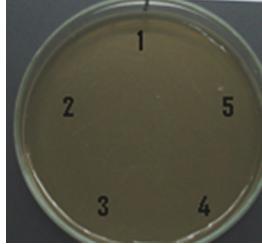
When BS models were inoculated with the different LAB + GCC combinations a satisfactory LAB growth and adequate pH decrease was observed in all inoculated models (Fig. 1). In general, pH values reached around 4.7–5.1 at three days and 4.7–4.8 at the end of fermentation. However, BS-Ent showed lower pH decrease compared with other batches, this moderate acidification was expected according to the lower acidification potential of *E. mundtii* CRL35 (Orihuel et al., 2018). Regarding the GCC counts, all BS models were inoculated with around 7–8 log CFU/g, as is depicted in Fig. 1. A similar performance of GCC, was observed for BS-Curv and BS-Sakei batches, viable cocci decreased during the first days while a slight cell recovery was registered towards the end of the incubation period. In BS-Ent, constant decrease in GCC population occurred throughout the time. In the batch inoculated with *L. plantarum* CRL681, GCC were registered only at initial time (7 log CFU/g), then it being dramatically inhibited by *L. plantarum* CRL681 (Fig. 1). It should be noted that Control batches (non inoculated and supplemented with antibiotics) kept without pH changes and minimal bacterial content at < 2 log CFU/g all over the time, while the batches without antibiotics and non inoculated with the starters became rapidly contaminated, which produced colour and aroma decomposition of the meat system (data not shown). This fact allows confirming that added LAB + GCC have effectively dominated the fermentation process in the inoculated BS models. Most of assayed strains resulted to be compatible among each other with exception of *L. curvatus* CRL705 which presented ability to inhibit *L. sakei* CRL1862 and *E. mundtii* CRL35 (Table 1).

3.2. Low molecular-weight peptides in low sodium fermented sausage models identified by LC-ESI-MS/MS analyses

High amino acid sequence homologies (70–100%) of the isolated peptides with known muscle proteins from various mammalian sources

Table 1

Compatibility between LAB and *S. vitulinus* tested in agar plate by the spot on lawn method.

Lawn	Spot	Results ^a
Plate n°1: <i>L. curvatus</i> CRL 705	(1) <i>L. plantarum</i> CRL 681; (2) <i>L. sakei</i> CRL1862; (3) <i>S. vitulinus</i> GV 318; (4) <i>E. mundtii</i> CRL35; (5) <i>L. curvatus</i> CRL 705.	
Plate n° 2: <i>L. plantarum</i> CRL 681	(1) <i>L. curvatus</i> CRL 705; (2) <i>L. sakei</i> CRL1862; (3) <i>S. vitulinus</i> GV 318; (4) <i>E. mundtii</i> CRL35; (5) <i>L. curvatus</i> CRL 705.	
Plate n° 3: <i>L. sakei</i> CRL1862	(1) <i>L. curvatus</i> CRL 705; (2) <i>L. plantarum</i> CRL 681; (3) <i>S. vitulinus</i> GV 318; (4) <i>E. mundtii</i> CRL35; (5) <i>L. curvatus</i> CRL 705.	
Plate n° 4: <i>S. vitulinus</i> GV 318	(1) <i>L. curvatus</i> CRL 705; (2) <i>L. plantarum</i> CRL 681; (3) <i>L. sakei</i> CRL1862; (4) <i>E. mundtii</i> CRL35; (5) <i>L. curvatus</i> CRL 705	
Plate n° 5: <i>E. mundtii</i> CRL35	(1) <i>L. curvatus</i> CRL 705; (2) <i>L. plantarum</i> CRL 681; (3) <i>L. sakei</i> CRL1862; (4) <i>S. vitulinus</i> GV 318; (5) <i>L. curvatus</i> CRL 705.	
Plate n° 6: <i>L. curvatus</i> CRL 705	(1) <i>L. curvatus</i> CRL 705; (2) <i>L. plantarum</i> CRL 681; (3) <i>L. sakei</i> CRL1862; (4) <i>S. vitulinus</i> GV 318; (5) <i>E. mundtii</i> CRL35.	

^a Inhibition halos confirms the existence of incompatibility between the tested strains

Table 2

Identified LMW peptides produced by BS models during fermentation at 22 °C during 10 days.

N°	Sample*	Sequence	Parental protein	Position**
1	BS-Control 0 h (1)	FNRTPIPWLSSGEPVD.Y	(A) Myozenin 1	267–281
2	R.TIPWLSSGEPVD.Y	Myozenin 1	269–281
3	BS-Control 10 d (2)	M.WITKQEQYDEAGPSIVH.R	(B) Alpha Actin skeletal muscle	358–373
4		T.CVLVSEEDEHAIIVEPE.K	(C) Fructose 1,6 bisphosphatase	93–109
5	BS- Curv 10 d (3)	D.DAPRAVFPSPIVGRPR.H	Alpha Actin skeletal muscle	27–41
6		G.IITNWDDMEKIWHH.T	Alpha Actin skeletal muscle	77–90
7		...HTNWDDMEKIWHH.T	Alpha Actin skeletal muscle	78–90
8		...I.TNWDDMEKIWHH.F	Alpha Actin skeletal muscle	79–91
9		L.DSGDGVTNVPIEYGYALPHAIM.R	Alpha Actin skeletal muscle	156–178
10		SSLEKSYELPDGQVITIGNER.F	Alpha Actin skeletal muscle	237–256
11		..S.LEKSYELPDGQVITIGNER.F	Alpha Actin skeletal muscle	238–256
12		...LEKSYELPDGQVITIGNER.F	Alpha Actin skeletal muscle	239–256
13	E.KSYELPDGQVITIGNER.F	Alpha Actin skeletal muscle	240–256
14	KSYELPDGQVITIGNER.F	Alpha Actin skeletal muscle	241–256
15	S.YELPDGQVITIGNER.F	Alpha Actin skeletal muscle	242–257
16	Y.ELPDGQVITIGNER.F	Alpha Actin skeletal muscle	243–257
17	E.LPDGQVITIGNER.F	Alpha Actin skeletal muscle	244–257
18	BS-Plant-10 d (4)	L.DDVITQTGVVDNP.GHPFI.M	(D) Creatine kinase M-type	54–69
19		T.PSGYTLDCQIQTGVNDNP.GHPFI	(E) Creatine kinase S-type	76–102
20		T.APPIQSPLPVIPH.Q..	(F) LIM domain binding protein 3	93–105
21		T.APPIQSPLPVIPH.Q..	LIM domain binding protein 3	93–106
22	Q.SPLPVIPH..H..	LIM domain binding protein 3	98–104
23	Q.SPLPVIPH.Q	LIM domain binding protein 3	98–105
24		V.MQRDIAAGDFIEHAEFGSGNIYG.T	(G) Guanylate kinase	61–82
25		T.NWDDMEKIWHH	Alpha Actin skeletal muscle	80–89
26		R.DWPDARGIWHN	Creatine kinase M-type	210–219
27	BS-Sakei-10 d (5)	A.AAPAPAPAPAPAPAPAPP.K	(H) Myosin light chain 1/3	14–31
28		N.EMATAASSSSLEKSYELPDGQVITIGNER.F	Alpha actin skeletal muscle	228–256
29	BS -Ent - 10 d (6)	R.LNVKNEELDAMMKMEASGPIN.F	(I) Myosin regulatory light chain 2	61–80
30	V.KNEELDAMMKMEASGPIN.F	Myosin regulatory light chain 2	64–80
31		T.TLVKEDQVFPFMNPP.K	(J) Myosin 2 OS	70–83
32		L.TVKEDQVFPFMNPP.K	Myosin 2 OS	71–83
33		G.DVLRALGTNPTNAEVKKVLGNPSN.E	Myosin light chain 1/3	75–98
34		L.DSGDGVTNVPIEYGYALPHAI.R	Alpha Actin skeletal muscle	156–176
35		E.RNVKMQRQEAKVCLMSPEQLQKKFP.W	(K) FAD-dependent oxidoreductase	173–198
36		L.DFENEMATAASSSSLEKS.Y	Alpha Actin skeletal muscle	224–241
37		A.TAASSSSLEKSYELPDGQVIT.I	Alpha Actin skeletal muscle	231–251
38	A.ASSSSLEKSYELPDGQVIT.I	Alpha Actin skeletal muscle	233–251
39	S.SSLEKSYELPDGQVIT.I	Alpha Actin skeletal muscle	235–251
40	S.SSLEKSYELPDGQVITIGN.E	Alpha Actin skeletal muscle	236–254
41		SSSLEKSYELPDGQVITIGNER.F	Alpha Actin skeletal muscle	236–256
42		N.KMIRKGVFKDQHDFDPNLFMYIEVDK.V	(L) Serine/threonine protein Kinase	911–936
43		F.DPNLNFMYIEVDK.V	Serine/threonine protein Kinase	924–936
44	3;6	M.WAAFPDDVGGNVYD.N	Myosin regulatory light chain 2	141–155
45	3;5	A.AFPDPVGGNV.D.Y	Myosin light chain 1/3	143–153
46	3;4;5;6	A.FPPDV.G	Myosin regulatory light chain 2	144–149
47	3;4;5;6	A.FPPDV.GGNV.D.Y	Myosin regulatory light chain 2	144–153
48	3;5;6	L.DSGDGVTNVPIEYGYALPH.A	Alpha actin skeletal muscle	156–175
49	3;4;5	G.FAGDDAPRAVFP.S.I	Alpha actin skeletal muscle	23–35
50	2;3;4;5;6	G.FAGDDAPRAVFP.SIV.G.R	Alpha actin skeletal muscle	23–38
51	2;3;4;6	G.FAGDDAPRAVFP.SIV.GR.H	Alpha actin skeletal muscle	23–41
52	3;6	...F.AGDDAPRAVFP.SIV.GR.H	Alpha actin skeletal muscle	24–41
53	3;4;5A.GDDAPRAVFP.SIV.G.R	Alpha actin skeletal muscle	25–38
54	3;6A.GDDAPRAVFP.SIV.GR.H	Alpha actin skeletal muscle	25–41
55	2;3;4;5;6G.DDAPRAVFP.SIV.G.R	Alpha actin skeletal muscle	26–38
56	3;4;5;6G.DDAPRAVFP.SIV.GR.H	Alpha actin skeletal muscle	26–41
57	3;5D.DAPRAVFP.SIV.G.R	Alpha actin skeletal muscle	27–38
58	3;6	A.VFPSIVGRPR.H	Alpha actin skeletal muscle	32–41
59	3;4;5;6	V.FPSIV.G.R	Alpha actin skeletal muscle	33–38
60	3;6	A.TAASSSSLEKSYELPDGQVITIGNER.F	Alpha actin skeletal muscle	231–256
61	3;6	SSSLEKSYELPDGQVIT.I	Alpha actin skeletal muscle	236–251
62	3;4;5;6	S.LEKSYELPDGQVIT.I	Alpha actin skeletal muscle	238–251
63	3;6	S.LEKSYELPDGQVITIGN.E	Alpha actin skeletal muscle	238–254
64	3;4;6	L.EKSYELPDGQVIT.I	Alpha actin skeletal muscle	239–251
65	3;4;5;6	L.EKSYELPDGQVITIGN.E	Alpha actin skeletal muscle	239–254
66	3;6	E.KSYELPDGQVITIGN.E	Alpha actin skeletal muscle	240–254
67	3;4;5	K.SYELPDGQVIT.I	Alpha actin skeletal muscle	241–251
68	3;4;5;6	S.YELPDGQVIT.I	Alpha actin skeletal muscle	242–251
69	3;4;5;6	S.YELPDGQVITIGNER.F	Alpha actin skeletal muscle	242–256
70	3;4;5	Y.ELPDGQVITIGNER.F	Alpha actin skeletal muscle	243–256
71	3;4;5	T.LDDVITQTGVVDNP.GHPFI	Creatine kinase M-type	53–68
72	3;4;5	T.LDDVITQTGVVDNP.GHPFI.M	Creatine kinase M-type	53–69
73	1;3;4;5	L.DDVITQTGVVDNP.GHPFI	Creatine kinase M-type	54–68
74	1;3;4;5	D.DVIQTGVVDNP.GHPFI	Creatine kinase M-type	55–68
75	1;3;4;5	D.DVIQTGVVDNP.GHPFI.M	Creatine kinase M-type	55–69
76	3;6	G.RLNVKNEELDAMMKMEASGPIN.F	Myosin regulatory light chain 2	60–80
77	3;4;5;6	L.NVKNEELDAMMKMEASGPIN.F	Myosin regulatory light chain 2	62–80

(continued on next page)

Table 2 (continued)

N°	Sample*	Sequence	Parental protein	Position**
78	3;4;5;6	K-NEELDAMMKAEASGPIN-F	Myosin regulatory light chain 2	65–80
79	3;6	N-EEELDAMMKAEASGPIN-F	Myosin regulatory light chain 2	66–80
80	3;5	LTNWDDMEKIWHH	Alpha actin skeletal muscle	79–89
81	3;4;5;6	I-TNWDDMEKIWHH.T	Alpha actin skeletal muscle	79–90
82	3;4;5;6	T-NWDDMEKIWHH.T	Alpha actin skeletal muscle	80–90
83	3;4;5	T-NWDDMEKIWHHT.F	Alpha actin skeletal muscle	80–91
84	3;4;5	N-WDDMEKIWHH	Alpha actin skeletal muscle	81–89
85	3;4;5;6	N-WDDMEKIWHH.T	Alpha actin skeletal muscle	81–90
86	3;4	N-WDDMEKIWHHT.F	Alpha actin skeletal muscle	81–91

Parental protein information (UniprotKB protein database): (A) MYOZ1_BOVIN: Myozenin-1, *Bos taurus* (Myofibrillar-M); (B) ACTS_BOVIN: Alpha actin skeletal muscle, *Bos taurus* (M); (C) F16P1_BOVIN: Fructose-1,6-bisphosphatase 1, *Bos taurus* (Sarcoplasmic-S); (D) KCRM_BOVIN: Creatine kinase M-type, *Bos taurus* (S); (E) KCRS_BOVIN: Creatine kinase S-type mitochondrial, *Bos taurus* (S); (F) XP_005226590.1: LIM domain-binding protein 3 isoform × 1, *Bos taurus* (S); (G) KGUA_BOVIN: Guanylate kinase, *Bos taurus* (S); (H) MYL1_BOVIN: Myosin light chain 1/3, skeletal muscle isoform, *Bos taurus* (M); (I) MLRS_BOVIN: Myosin regulatory light chain 2, skeletal muscle isoform, *Bos taurus* (M); (J) F1MRC2_BOVIN: Myosin-2, *Bos taurus* (M); (K) FXRD1_BOVIN: FAD-dependent oxidoreductase domain-containing protein 1, *Bos taurus* (S); (L) PRP4B_PONAB: Serine/threonine-protein kinase PRP4 homolog, *Pongo abelii* (S).

* Samples: BS-Control 0 (1): un-inoculated beaker sausage (BS) model at 0 day, supplemented with ATB (20.000 UI/Kg penicillin, 20 mg/Kg streptomycin, 50 mg/Kg amphotericin B and 0.01% sodium azide); BS-Control 10 (2): un inoculated BS model at 10 days. BS-Curv (3): BS inoculated with *L. curvatus* CRL705 + *S. vitulinus* GV318 at 10 days; BS-Plant (4): BS inoculated with *L. plantarum* CRL681 + *S. vitulinus* GV318 at 10 days; BS-Sakei (5): BS inoculated with *L. sakei* CRL1862 + *S. vitulinus* GV318 at 10 days; BS-Ent (6): BS inoculated with *E. mundtii* CRL35 + *S. vitulinus* GV318 at 10 days.

** Position of the peptide in the parental protein.

were obtained, and 86 peptides were accurately identified (Table 2). In all analyzed models, the largest number of LMW peptides was originated from myofibrillar proteins. In fact, only 17 out of the 86 peptides have derived from sarcoplasmic proteins. Proteins originating the highest number of peptides were alpha actin (82.8%) and different myosin types. In the BS-Control at T0 only two peptides from myozenin-1 (MYOZ1) were observed, probably released by endogenous proteolysis during the post-mortem process. While, after 10 days of incubation also two different peptides, derived from alpha actin skeletal muscle and fructose 1,6 biphosphatase, were identified. The mentioned LMW peptides were originated exclusively in non inoculated models, this indicating the muscle peptidase activity occurring during the ripening time. A significant increment of the peptidogenic activity was registered in the models inoculated with the starter cultures after 10 days of incubation, this evidencing the effect of microorganisms and time in meat proteolysis. Alpha actin proved to be the most important LMW peptide source; a total of 53 peptides were detected to be produced by different BS models from this protein, showing a remarkable action of peptidases and aminopeptidases during the incubation period (Fig. 2). In fact, different regions of alpha actin were affected, especially in positions 221–257, 78–91 and 22–41 (Fig. 2).

The BS inoculated with *L. curvatus* CRL705 + *S. vitulinus* GV318 (BS-Curv 10 d) produced the highest peptide pool with a total of 56 LMW peptides followed by the model containing *E. mundtii* CRL35 (BS-Ent, a total of 43 LMW peptides) after 10 days (Table 2; Table 3; Fig. 3). BS-Curv 10 d registered 13 out of 56 peptides generated exclusively by this model, while BS-Ent generated 15 unique LMW peptides. BS-Plant and BS-Sakei at 10 d produced 38 and 32 total LMW peptides respectively, from those only 9 and 2 respectively, were exclusively produced by each starter culture (Table 3, Fig. 3).

3.2.1. Free amino acid profile

Results obtained from the amino acid analyses are depicted in Table 4 and Fig. 4. ANOVA results showed significant differences ($p < 0.05$) in total free amino acid contents by effect of different batches (sample effect; Table 4) and by effect of time (Fig. 4b). BS-Plant

and BS-Sakei released statistically significant higher amino acids concentration than the non-inoculated Control all over time (Table 4). Tukey's test also indicated that amino acid increase is significantly higher at longer processing times in all analyzed samples. In fact, mean values showed more than a 4 times increase at 10 days with respect to initial time in all analyzed samples (Fig. 4b). When different BS models were compared at 0, 3, 6 and 10 days, it could be observed that also the elapsed time exerted a significant positive effect on most of amino acid profiles (Fig. 4b, Table 4). The PCA biplots show a summary of the most important effects in amino acid variation in relation to samples behavior (Fig. 4a). At the beginning of the incubation period (T0), BS-Ent, BS-Control, and BS-Sakei samples were positioned on the negative side of the first principal component, showing little amount of free amino acids related with the basal content in the meat matrix. After 3 days, considerable changes were observed in the position of both samples and amino acids. As consequence of the overall amino acid increase, a large amount of them moved to the first quadrant of the biplot together with BS-Plant, thus showing its relation with the amino acid increase. BS-Sakei moved towards the positive side of the first principal component, (see Table 4). On the other hand, BS-Control and BS-Ent kept in the same position than that observed at T0, indicating no significant change in amino acid release. After 6 days of incubation, slight changes in the position of samples and free amino acids were observed except for BS-Plant (Fig. 4a). At the end of the incubation period (T10) free amino acids, together with BS-Sakei, BS-Curv and BS-Plant, moved towards the center of the second principal component. The main change in the free amino acid content along time was observed for BS-Sakei (Fig. 4b), being similar to BS-Curv, and BS-Plant in the first principal component, which explained 68.51% of variance.

4. Discussion

In the present work, the proteolytic potential of different LAB strains in a low-sodium meat model was evaluated by a peptidomic approach. The non-inoculated beaker sausage (Control) allowed discriminating the action of muscle from the bacterial enzymes. The

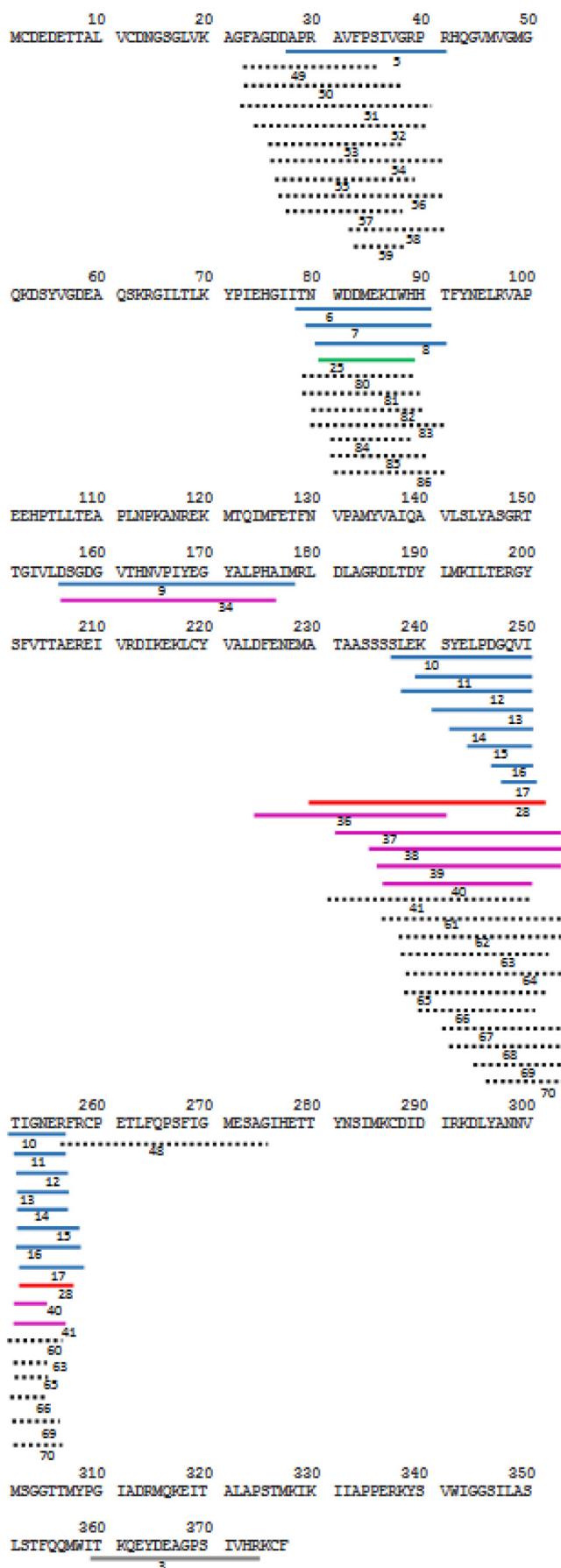


Fig. 2. Map of peptides originated from alpha actin due to the action of different BS models. Identified peptides are represented by lines and its numbers are in coincidence with those shown in Table 2. Blue lines: peptides present in BS-Curv 10 d, red lines: peptides derived from BS-Sakei 10 d; green lines: peptides derived from BS-Plant 10 d; purple lines: peptides generated by BS-Ent 10 d; grey lines: peptides derived from BS-control 10 d and dotted black lines: peptides generated by more than one model. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

Number of LMW peptides produced by each BS model during fermentation at 22 °C during 10 days.

Reference	Model	Produced exclusively	Total
(1)	BS-Control 0 h	2	5
(2)	BS-Control 10 d	2	5
(3)	BS- Curv 10 d	13	56
(4)	BS-Plant 10 d	9	38
(5)	BS-Sakei 10 d	2	32
(6)	BS-Ent 10 d	15	43

BS-Control 0 (1): un-inoculated beaker sausage (BS) model at 0 day, supplemented with ATB (20.000 UI/Kg penicillin, 20 mg/Kg streptomycin, 50 mg/Kg amphotericin B and 0.01% sodium azide); BS-Control 10 d (2): uninoculated BS model at 10 days. BS-Curv 10 d (3): BS inoculated with *L. curvatus* CRL705 + *S. vitulinus* GV318 at 10 days; BS-Plant 10 d (4): BS inoculated with *L. plantarum* CRL681 + *S. vitulinus* GV318 at 10 days; BS-Sakei 10 d (5): BS inoculated with *L. sakei* CRL1862 + *S. vitulinus* GV318 at 10 days; BS-Ent 10 d (6): BS inoculated with *E. mundtii* CRL35 + *S. vitulinus* GV318 at 10 days.

growth performance and pH drop of the different LAB strains combined with *S. vitulinus* GV318 evidenced an appropriate adaption of LAB to the low sodium beaker sausage model (Fig. 1). In general, the observed pH values indicated the satisfactory selection of type and concentration of carbohydrates (0.75% glucose and 0.75% sucrose). Sugar fermentation, carried out by LAB, is essential to reach the hygienic properties required in these fermented products. It is well-known the acid sensitivity of GCC (Casaburi, Blaiotta, Mauriello, Pepe, & Villani, 2005; Casquete et al., 2011; Ravyts et al., 2010) which may explain the growth decrease observed during the first hours in the batches inoculated with the acidogenic strains (*L. sakei* CRL1862; *L. curvatus* CRL705; *L. plantarum* CRL681). López et al., 2015a) also registered a decrease of *S. vitulinus* GV318 counts after 24 h in a normal sodium meat model using this GCC strain in combination with *L. curvatus* CRL705. Moreover, *S. vitulinus* GV318 was completely inhibited at 3 days of incubation in the batch inoculated with *L. plantarum* CRL681, maybe as result of additional inhibitory compounds produced exclusively by this LAB strain.

On the other side, > 80 LMW peptides were identified in the low sodium beaker sausages inoculated with LAB + GCC after 10 days at 22 °C, this confirming that meat proteolysis is a highly dynamic process, where the processing time and the presence of microorganisms constitute important variation factors (López et al., 2015a, 2015b; Mora et al., 2015). In fact, the LMW peptide production, mainly arisen from the hydrolysis of myofibrillar proteins, was notably increased along time and in the presence of diverse combinations of starter cultures. It is worth noting the positive contribution of *E. mundtii* CRL35 and *L. curvatus* CRL705 to LMW peptide enrichment under these lower sodium conditions. Creatine kinase and LIM domain-binding protein 3 are among the few sarcoplasmic proteins that generated small peptides. Also other authors working on diverse meat products have reported small peptides coming from these proteins although they are different from those identified herein (Gallego, Mora, Fraser, Aristoy, & Toldrá, 2014; López et al., 2015a; Mora et al., 2009). As the peptide fraction < 3 kDa is frequently related with taste of raw and processed meat (Nishimura, 2002; Sentandreu et al., 2003), the herein identified peptides could possibly exert some influence on taste, although this is the focus of ongoing studies on real low sodium sausages, in which global

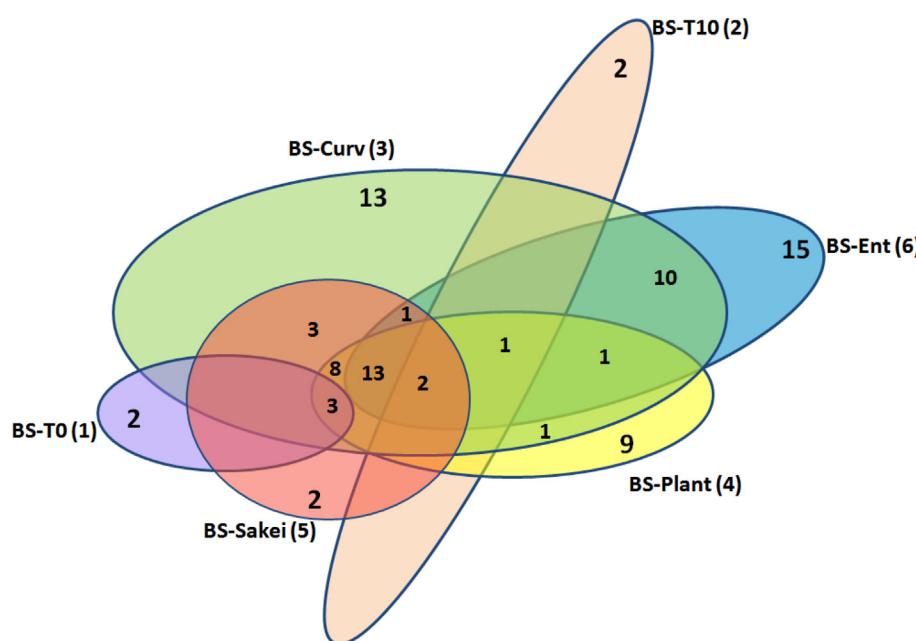


Fig. 3. Venn diagram showing numbers of peptides obtained in the beaker sausage models (BS): BS T0 (1): un-inoculated BS at 0 h; BS T10 (2): un-inoculated BS at 10 days; BS-Curv (3): BS inoculated with *L. curvatus* CRL705 + *S. vitulinus* GV318 at 10 days; BS-Plant (4): BS inoculated with *L. plantarum* CRL681 + *S. vitulinus* GV318 at 10 days; BS-Sakei (5): BS inoculated with *L. sakei* CRL1862 + *S. vitulinus* GV318 at 10 days; BS-Ent (6): BS inoculated with *E. mundtii* CRL35 + *S. vitulinus* GV318 at 10 days.

quality and sensory analysis are envisaged.

It is known that sodium chloride and also sodium substitutors such as KCl and CaCl₂ inhibit endopeptidases, together with dipeptidyl- and amino-peptidases from muscle origin (Armenteros, Aristoy, & Toldra, 2009). Our model contains lower amounts of chloride salts (1% NaCl, 0.25% KCl, 0.25% CaCl₂) compared to normal sodium fermented sausages (2.5–3%), thus increased amounts of proteolysis products could be expected. In fact, fewer amount of LMW peptides from sarcoplasmic proteins were herein registered when comparing to the amount obtained under standard Na chloride concentrations by López et al. (2015a, 2015b), maybe as consequence of their complete hydrolysis towards free amino acid production as explained below. In fact, in the present work only 16 LMW peptides from sarcoplasmic proteins were generated comparing to the approximately 35 reported in that previous work using normal sodium BS model inoculated with *L. curvatus* CRL705 + *S. vitulinus* GV318 (López et al., 2015a, 2015b).

Peptides are produced at an intermediate stage of protein degradation during fermentation and then are subsequently degraded by the action of amino- and/or carboxy-peptidases releasing free amino acids having characteristic basic tastes (sweet, bitter, salted, acid or umami) (Fadda, Vildoza, and Vignolo, 2010). In addition, some other amino acids, such as the branched chain amino acids (valine, leucine and isoleucine) are precursors of aromatic compounds (aldehydes, alcohols and esters) which contribute as well as to enhance the sensory perception of fermented products (Ardö, 2006; Smit, Engels, & Smit, 2009). In the present work, a statistically significant production of free amino acids in BS-Sakei and BS-Plant models with respect to BS-Control was registered, this indicating the intense amino- and/or carboxy-peptidase activity occurring during fermentation. In this sense BS models (BS-Plant; BS-Sakei) with higher total amino acid content presented a lower number LMW peptides at 10 days. On the contrary the models containing *E. mundtii* CRL35 and *L. curvatus* CRL705 accounted for a higher number of LMW peptides produced exclusively and non-exclusively by each model (Table 3, Fig. 3). Our study showed that a lower Na condition allowed the identification of a lesser number of LMW peptides compared to those obtained in normal sodium meat products (López et al., 2015a, 2015b; Mora et al., 2015), being this

indicative of a stronger proteolysis in the low sodium model, promoting a more complete peptide degradation with the concomitant free amino acid release (López et al., 2015b).

The presence of each LAB strain in BS models generates a unique profile of small peptides and amino acids that could serve as a distinctive biochemical trait to differentiate among specific fermented products. According to these results two LAB strains, *L. sakei* CRL1862 and *E. mundtii* CRL35, which are compatible between them, are proposed as the most efficiently adapted to low sodium chloride conditions to constitute the starter culture with *S. vitulinus* GV318. The first strain proved to produce significant increments in free amino acids while *E. mundtii* CRL35, was able to produce the highest quantity of LMW peptides. It is noteworthy that both strains have a known bioprotective activity. In fact, *L. sakei* CRL1862 and *E. mundtii* CRL35 produce bacteriocins with efficient antilisterial activity (Pérez-Ibarreche, Castellano, Leclercq, & Vignolo, 2016; Salvucci, Hebert, Sesma, & Saavedra, 2010), which guarantee safety of the low sodium fermented product. Sensory characteristics of the low sodium product elaborated with the selected starter will be evaluated in the frame of an ongoing project.

5. Conclusion

The study using BS models allowed simulating fermentation of meat under low Na chloride conditions. Results demonstrated that the starter combination containing *E. mundtii* CRL35 + *S. vitulinus* GV318 presented the highest peptidogenic potential. On the other hand, *L. sakei* CRL1862 + *S. vitulinus* GV318 showed high influence on the production of amino acids. The low concentration of chloride and the use of proteolytic strains combinations could produce an additional positive effect in terms of the release of amino acids and peptides related to taste. Finally, the use of lower concentrations of sodium in the preparation of fermented sausages in combination with the selected strains could have a further positive effect to the known action on the cardiovascular system, which is related to the production of small peptides and free amino acids during ripening. The sensory evaluation of fermented sausages will confirm, in a future work, the real impact of the selected

Table 4
Changes in free amino acid (FAA) content during fermentation of different BS models at 22 °C during 10 days.

FFA	Incubation time (days)	0						3						6						10					
		Control	Curv	BS Ent	Plant	Sakei	Control	Curv	BS Ent	Plant	Sakei	Control	Curv	BS Ent	Plant	Sakei	Control	Curv	BS Ent	Plant	Sakei				
Asp	3.06 ^{ab}	5.01 ^a	1.69 ^b	1.61 ^b	1.90 ^b	8.05 ^{ab}	13.84 ^{ab}	6.00 ^b	20.13 ^a	17.58 ^{ab}	13.07 ^b	29.71 ^{ab}	8.61 ^b	41.55 ^a	34.81 ^{ab}	24.22 ^b	65.95 ^a	18.50 ^b	73.21 ^a	65.26 ^a	104.76 ^a	119.46 ^a			
Glu	7.68 ^a	12.15 ^a	7.66 ^a	9.06 ^a	9.69 ^a	15.24 ^c	34.78 ^b	18.83 ^c	39.78 ^b	52.61 ^a	26.10 ^b	54.10 ^a	32.34 ^{ab}	74.36 ^a	42.40 ^b	93.48 ^{ab}	53.05 ^b	73.37 ^{ab}	29.06 ^b	45.05 ^{ab}	87.59 ^a	63.48 ^{ab}			
Asn	4.00 ^a	6.76 ^a	4.24 ^a	6.44 ^a	3.79 ^a	10.94 ^c	17.10 ^{ab}	13.89 ^c	26.93 ^a	19.63 ^{ab}	20.27 ^c	33.47 ^{ab}	25.21 ^b	51.09 ^a	35.59 ^{ab}	47.83 ^b	70.26 ^b	48.77 ^b	8.20 ^b	67.21 ^a	0.00 ^c	200.08 ^a			
Ser	12.27 ^b	27.14 ^a	6.97 ^b	22.24 ^{ab}	7.90 ^b	27.89 ^a	26.03 ^a	19.83 ^{ab}	8.02 ^b	27.33 ^a	30.08 ^b	36.50 ^a	29.22 ^{bc}	7.90 ^c	42.58 ^a	183.91 ^a	193.65 ^a	158.45 ^a	171.72 ^a	129.33 ^a	88.17 ^c	200.08 ^a			
His	89.93 ^a	87.27 ^a	90.89 ^a	79.77 ^a	83.34 ^a	123.95 ^a	75.48 ^{ab}	107.20 ^a	89.12 ^{ab}	48.90 ^b	134.17 ^a	98.05 ^b	105.21 ^b	45.91 ^b	59.83 ^a	42.64 ^b	54.39 ^{ab}	54.79 ^{ab}	30.34 ^a	33.17 ^a	24.31 ^a	26.88 ^a			
Gln	4.72 ^b	17.48 ^a	2.04 ^b	12.38 ^{ab}	0.00 ^b	14.76 ^a	13.66 ^a	11.94 ^a	15.10 ^a	26.54 ^a	26.88 ^a	26.35 ^a	24.31 ^a	29.71 ^{ab}	46.94 ^a	44.82 ^a	48.80 ^a	46.04 ^b	44.20 ^b	29.04 ^a	44.20 ^b	70.92 ^{ab}			
Gly	14.42 ^b	29.89 ^a	11.77 ^a	15.62 ^a	12.47 ^b	23.25 ^a	22.26 ^a	18.07 ^a	26.30 ^a	26.49 ^a	26.30 ^a	26.49 ^a	26.30 ^a	29.04 ^a	46.88 ^a	44.20 ^a	31.68 ^a	32.03 ^b	22.14 ^a	18.24 ^a	26.13 ^a	31.68 ^a	53.58 ^a	48.80 ^a	
Thr	3.78 ^b	6.70 ^a	3.36 ^b	5.81 ^{ab}	3.79 ^a	10.38 ^a	14.22 ^a	11.31 ^a	10.45 ^a	12.82 ^a	10.45 ^a	12.82 ^a	10.45 ^a	25.03 ^a	59.73 ^a	29.83 ^a	40.13 ^{ab}	42.58 ^{ab}	2.91 ^c	10.77 ^b	59.36 ^{bc}	86.88 ^{ab}	12.61 ^c	115.79 ^a	
Arg	7.32 ^b	18.72 ^a	5.60 ^b	13.71 ^{ab}	8.64 ^a	22.97 ^{ab}	27.00 ^a	21.10 ^c	27.00 ^a	29.83 ^a	5.29 ^c	40.13 ^{ab}	56.36 ^a	52.80 ^a	66.52 ^a	63.07 ^b	97.73 ^{ab}	67.70 ^b	103.25 ^a	103.25 ^a	103.25 ^a	103.25 ^a	103.25 ^a	103.25 ^a	
Ala	20.35 ^a	24.54 ^a	19.34 ^a	21.16 ^a	19.55 ^a	34.49 ^{ab}	42.10 ^{ab}	32.49 ^b	36.26 ^{ab}	45.63 ^a	42.73 ^b	42.73 ^b	42.73 ^b	1.21 ^b	0.00 ^c	1.81 ^{bc}	9.48 ^a	0.00 ^c	2.70 ^b	0.00 ^b	17.33 ^a	4.39 ^{ab}	6.25 ^b	6.35 ^b	
GABA	3.64 ^{ab}	2.61 ^{ab}	4.36 ^a	1.98 ^{ab}	0.00 ^b	8.11 ^{ab}	0.00 ^c	1.81 ^{bc}	0.00 ^c	1.81 ^{bc}	1.21 ^b	0.00 ^b	0.00 ^b	1.21 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	13.96 ^a	9.57 ^{ab}		
Tyr	8.12 ^b	8.54 ^a	3.53 ^b	5.61 ^{ab}	8.54 ^a	14.48 ^a	17.59 ^a	17.59 ^a	17.59 ^a	19.63 ^a	19.97 ^a	24.28 ^{ab}	24.28 ^{ab}	36.59 ^a	34.45 ^a	36.73 ^a	36.59 ^a	36.59 ^a	36.59 ^a	36.59 ^a	36.59 ^a	36.59 ^a	36.59 ^a	58.47 ^a	
Val	6.28 ^a	9.76 ^a	5.77 ^a	8.01 ^a	5.91 ^a	18.45 ^b	28.60 ^a	22.66 ^b	23.42 ^b	34.76 ^a	35.21 ^b	40.39 ^a	41.37 ^{ab}	39.42 ^{ab}	56.83 ^b	75.56 ^{ab}	41.72 ^a	51.72 ^a	54.92 ^a	41.91 ^b	78.64 ^{ab}	58.20 ^{ab}	89.47 ^a	83.11 ^{ab}	
Met	4.20 ^a	5.87 ^a	3.64 ^a	4.35 ^a	3.60 ^a	13.53 ^c	23.48 ^b	17.82 ^{bc}	31.37 ^a	27.59 ^b	40.35 ^{ab}	40.35 ^{ab}	31.20 ^a	16.08 ^a	16.26 ^a	19.63 ^a	22.73 ^a	28.88 ^a	24.25 ^a	29.16 ^a	10.30 ^{ab}	10.30 ^{ab}	10.30 ^{ab}	10.30 ^{ab}	
Trp	9.04 ^{ab}	11.97 ^{ab}	12.48 ^a	13.94 ^a	7.27 ^b	19.57 ^a	13.33 ^a	14.35 ^a	16.71 ^a	11.45 ^a	16.07 ^a	16.07 ^a	38.54 ^a	43.34 ^a	38.11 ^b	54.95 ^b	42.93 ^b	64.13 ^a	65.07 ^a	58.11 ^b	104.36 ^a	63.66 ^b	72.82 ^a		
Phe	7.90 ^a	10.86 ^a	8.33 ^a	9.93 ^a	7.18 ^a	22.14 ^c	36.59 ^b	22.14 ^c	22.46 ^a	23.84 ^a	27.68 ^a	30.11 ^b	33.12 ^b	67.98 ^{ab}	48.59 ^{ab}	56.01 ^a	43.93 ^b	40.64 ^a	64.86 ^a	48.02 ^a	66.45 ^b	55.36 ^a	71.21 ^{ab}		
Ile	5.95 ^a	7.74 ^a	6.21 ^a	12.52 ^b	13.53 ^{ab}	11.37 ^b	25.97 ^b	43.76 ^a	33.94 ^{ab}	40.76 ^a	43.76 ^a	43.76 ^a	43.76 ^a	67.98 ^{ab}	59.73 ^{ab}	82.42 ^a	86.21 ^a	66.45 ^b	127.26 ^{ab}	98.75 ^{ab}	133.37 ^{ab}	91.11 ^a	0.00 ^c		
Orn	0.00 ^b	6.15 ^{ab}	6.97 ^a	4.07 ^b	4.69 ^{ab}	12.78 ^{ab}	0.00 ^b	20.50 ^{ab}	14.94 ^{ab}	29.70 ^a	0.00 ^b	34.86 ^{ab}	63.43 ^{ab}	43.77 ^a	53.29 ^c	56.83 ^b	78.10 ^a	68.48 ^b	147.76 ^a	119.09 ^{ab}	110.49 ^{ab}	164.10 ^a	1560.28 ^d		
Lys	11.34 ^{ab}	12.35 ^{ab}	14.85 ^a	7.55 ^b	11.22 ^{ab}	14.85 ^a	23.53 ^a	32.03 ^a	44.49 ^a	43.77 ^a	57.59 ^{ab}	644.06 ^b	570.55 ^{ba}	784.36 ^{AB}	646.71 ^{cAB}	932.71 ^{cA}	941.49 ^{cA}	965.42 ^{AB}	1611.82 ^{AB}	1558.61 ^{AA}	1611.88 ^{AA}	AB	AB		
Total	232.75 ^{AB}	327.23 ^{AB}	281.16 ^{AB}	209.13 ^{AB}	471.04 ^{AB}	515.89 ^{AB}	575.97 ^{ba}																		

Results expressed as mg/100 g of BS and are means of two replicates.

FAA values for different treatments within rows, for each time, followed by different letters (a–c) are significantly different (Tukey's test, $p < 0.05$).

Total FAA values with different lower-case letters in the same row indicate significant differences between BS models according to Tukey test ($p < 0.05$).

BS: Beaker sausage; Control: un inoculated BS; Curv: BS inoculated with *L. curvatus* CRL705 + *S. vitulinus* GV318; Ent: BS inoculated with *E. mundtii* CRL1862 + *S. vitulinus* GV318;

Plant: BS inoculated with *L. plantarum* CRL681 + *S. vitulinus* GV318; Sakei: BS inoculated with *L. sakei* CRL1862 + *S. vitulinus* GV318.

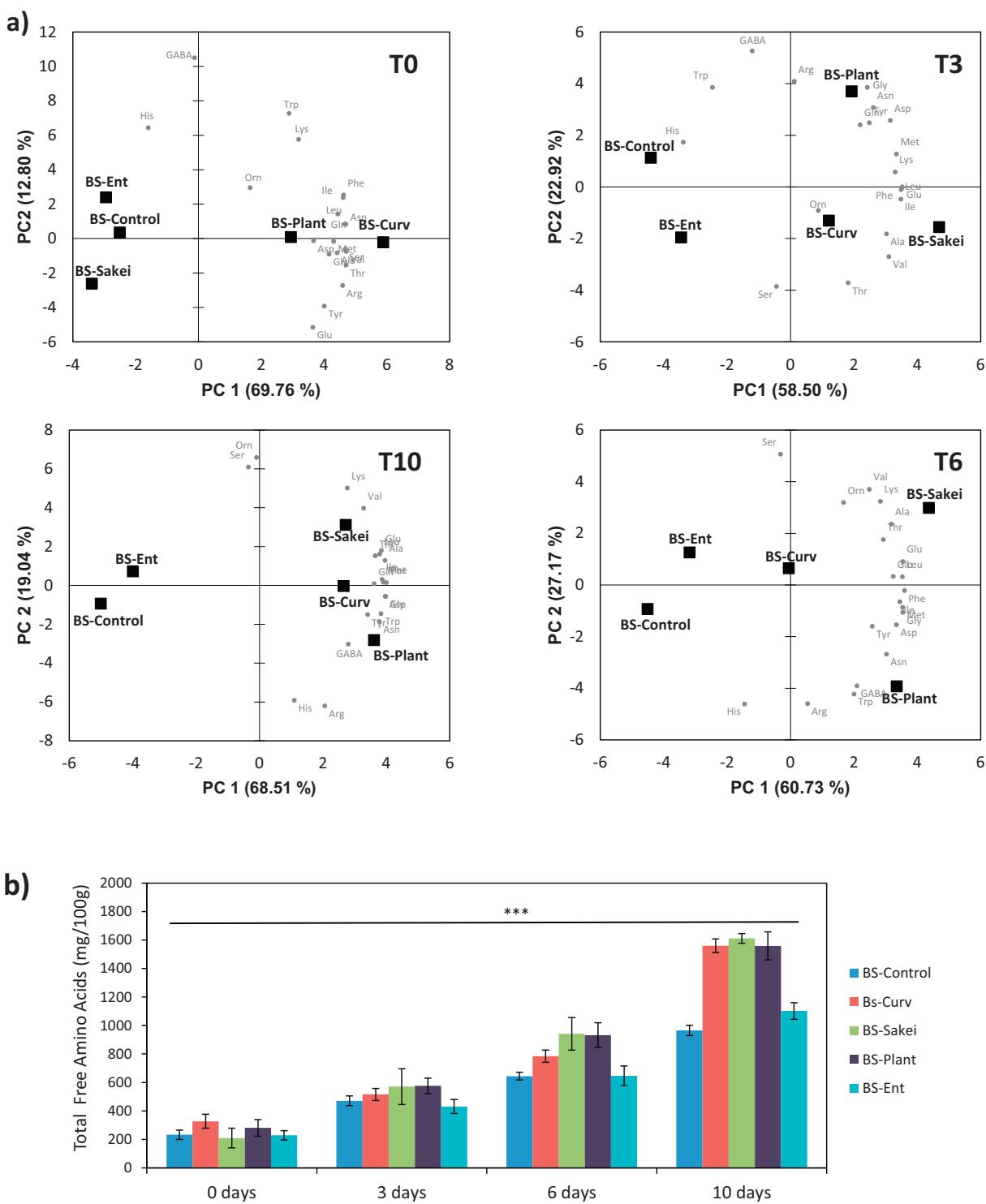


Fig. 4. Free amino acid analysis of the different beaker sausage models (BS) at the different processing times: a) Biplot of samples and free amino acids, considering the first two dimensions of Principal Component Analysis. T0: 0 days of incubation, T3: 3 days, T6: 6 days, T10: 10 days of incubation. b) Evolution of total free amino acids (mg/100 g of BS) in the different BS along time. BS-Control: un-inoculated BS supplemented with antibiotics; BS-Curv: BS inoculated with *L. curvatus* CRL705 + *S. vitulinus* GV318; BS-Plant: BS inoculated with *L. plantarum* CRL681 + *S. vitulinus* GV318; BS-Sakei: BS inoculated with *L. sakei* CRL1862 + *S. vitulinus* GV318; BS-Ent: BS inoculated with *E. mundtii* CRL35 + *S. vitulinus* GV318. Error bars indicate standard deviations (SD). Asterisks indicate statistically different averages across the time by the Tukey test ($p < .05$).

starters on its ability to counterbalance off flavors produced by sodium replacers.

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Conflict of interests

Authors declare no conflict of interest.

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Update

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Corrigendum

Corrigendum to “A peptidomic approach of meat protein degradation in a low-sodium fermented sausage model using autochthonous starter cultures”
 [Food Research International 109 (2018), 368–379]



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The authors regret < to inform you that an involuntary error about the origin of one of the microorganisms of study was made in Materials and Methods section of the present article and we would like to correct it as indicated as follows:

Lactobacillus (L.) plantarum CRL681 and *L. curvatus* CRL705 were isolated from artisanal fermented sausages (Tucumán, Argentina), *L. sakei* CRL1862 was isolated from artisanal cooked sausages (Chaco, Argentina) (Herman 2014) and the *Enterococcus (E.) mundtii* CRL35

strain was from dairy origin (isolated from an artisanal cheese -Taff cheese-, Tucumán, Argentina) (Farías et al., 1996).

Herman, Cristian (2014). Isolation, selection and evaluation of lactic acid bacteria for bioprotection of cooked meat products. National University of the Southern Chaco, Chaco, Argentina. Doctoral Thesis. > .

The authors would like to apologise for any inconvenience caused.

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