Meat Science 85 (2010) 394–401

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Protective action of *Lactobacillus curvatus* CRL705 on vacuum-packaged raw beef. Effect on sensory and structural characteristics

P. Castellano [a,*], C. González [b,c], F. Carduza [b], G. Vignolo [a]

[a] *Centro de Referencia para Lactobacilos, CERELA, CONICET, Chacabuco 145, 4000 Tucumán, Argentina*
[b] *Instituto de Tecnología de Alimentos, CIA, INTA. CC 77, B1708WAB, Morón, Buenos Aires, Argentina*
[c] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

ARTICLE INFO

Article history:
Received 15 December 2008
Received in revised form 26 January 2010
Accepted 8 February 2010

Keywords:
Biopreservation
Lactobacillus curvatus
Vacuum-packaged beef
Microstructure
Sensory properties

ABSTRACT

Lactobacillus curvatus CRL705 was examined for its effectiveness as protective culture in the biopreservation of vacuum-packaged fresh beef stored during 60 days at 2 °C. For this purpose, *L. curvatus* CRL705, producer of lactocin 705 and lactocin AL705, was inoculated on the meat surface (10^6 cfu g^{-1}). This microorganism became the dominating population throughout the storage period controlling the growth of *Brochothrix thermosphacta* and spoilage lactic acid bacteria naturally present on the meat. When the microstructural characteristics of the meat were evaluated using light microscopy, beef samples inoculated with the bioprotective culture showed a 10 days delay for the appearance of tissue degradation signs. Sensory analysis demonstrated that beef samples treated with *L. curvatus* CRL705 only developed an "acid" off-flavor after 60 days of refrigerated storage, and no undesirable off-odors were found. Therefore, inoculation with this bacteriocinogenic strain would provide an additional hurdle to improve storage life of refrigerated vacuum-packaged beef without affecting its sensory and structural characteristics.

1. Introduction

Modern food processing is dependent on a number of preservative technologies to ensure an acceptable level of quality from manufacture to consumption. Globalization of the commerce, the gradual increase in world population and changes in the lifestyle has resulted in consumer demands for safety oriented to foods of animal origin. Particularly, meat is an excellent substrate for bacterial growth so that if restriction methods are not used it becomes easily contaminated. The extrinsic factors often manipulated for meat shelf-life extension are mainly related to storage and processing conditions, temperature and environment being considered as the principal hurdles for bacterial growth during meat conditioning. Systems for distribution of retailed meat are principally based on vacuum-packaging of meat cuts using low gas permeability films and refrigeration, CO_2 concentration in the pack being critical for microbial inhibition (Jeyamkondan, Jayas, & Holley, 2000; Phillips, 1996). Microbial antagonisms are also exploited by manipulating environmental conditions to achieve controlled competition and favor the growth of specific organisms, especially lactic acid bacteria (LAB), so that a succession of predictable sequence and composition is produced.

LAB has been involved in the preservation of foods since ancient times through the production of a range of antimicrobial metabolites including bacteriocins, which have been intensively investigated and their potential for biopreservation is well documented (Castellano, Belfiore, Fadda, & Vignolo, 2008). LAB, due to its GRAS status, has a major potential for use in biopreservation because they are safe for human consumption and, during the current storage conditions of fresh meat, they are the prevalent microbiota (Ercolini, Russo, Torrieri, Mais, & Villani, 2006; Fontana, Cocconcelli, & Vignolo, 2006). At refrigeration temperatures, LAB were reported to exert antagonistic actions against the growth of spoilage bacteria and pathogens in fresh beef, pork, poultry and fish (Castellano et al., 2008; Katla et al., 2002; Scannell, Ross, Hill, & Arendt, 2000; Senne & Gilliland, 2003; Yamazaki, Suzuki, Kawai, Inoue, & Montville, 2003). The use of selected LAB strains as bioprotective cultures to extend the shelf life of chill stored vacuum-packaged fresh meat, implies the growth and acid production of the bacteriocinogenic strain on the meat. Organoleptic changes related to its metabolism could be perceived by consumers as sour or cheesy off-odors or off-flavors, color changes and the presence of superficial slime. In addition, *Lactobacillus sakei* and *Lactobacillus curvatus*, currently isolated from meat, showed the ability to hydrolyze muscle sarcoplasmic and myofibrillar proteins generating small peptides and amino acids (Fadda et al., 1999; Fadda, Vignolo, Ruiz Holgado, & Oliver, 1998). The metabolic events carried out by the bioprotective culture may also affect the microstructural characteristics of muscular tissue conducting to alterations of meat tenderness and visual appearance. The use of light and electron microcopy has been considered an interesting tool to evaluate

* Corresponding author. Tel./fax: +54 381 4311720.
E-mail address: patricia@cerela.org.ar (P. Castellano).

0309-1740/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2010.02.007

the effect of incorporated additives and/or processing parameters on the micro- and ultra-structure of raw and cooked whole-muscle meat. In previous work, it was used to analyze the effect of whey protein concentrate and NaCl injection together with different tumbling procedures in *Semitendinosus* beef muscle (Szerman et al., 2007).

The characterization of two different antibacterial substances produced by *Lactobacillus curvatus* CRL705; lactocin 705 a class IIb bacteriocin inhibitory against other LAB and *Brochothrix thermosphacta* (Castellano, Raya, & Vignolo, 2003; Cuozzo, Castellano, Sesma, Vignolo, & Raya, 2003; Cuozzo, Sesma, Palacios, Ruiz Holgado, & Raya, 2000), and lactocin AL705 an anti-*Listeria*, class IIa bacteriocin (Castellano, Holzapfel, & Vignolo, 2004) was reported. Even more, *L. curvatus* CRL705 proved to be able to grow and produce bacteriocins in vacuum-packaged fresh meat stored at chill temperatures (2–8 °C) with a negligible effect on the meat pH (Castellano & Vignolo, 2006). On these bases, the aim of the present work was to evaluate whether *L. curvatus* CRL705 is able to assure microbiological quality of fresh meat during refrigerating storage without affecting microstructural and sensory characteristics of the muscular tissue.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Lactobacillus curvatus CRL705, lactocin 705 and lactocin AL705 producer and *Lactobacillus plantarum* CRL691 used as an indicator of lactocin 705 were isolated from dry cured sausages (Vignolo, Suriani, Ruiz Holgado, & Oliver, 1993). *Listeria innocua* 7 used as an indicator of lactocin AL705 was obtained from the Unité de Recherches Laitieres et Genetique Appliqué, INRA (France) and grown at 30 °C in trypticase soy broth (TSB; BBL, Cockeysville, MD) with 0.5% added yeast extract (YE).

2.2. Experimental design

Six *Semimembranosus* muscles (right and left muscles were selected at random) were dissected from British breed steer carcasses 48 h post-slaughter. Slaughter procedure was performed in a commercial meat packing and processing plant with Good Manufacture Practices, licensed for exporting to the UE. Fifty-four steaks of 15 cm diameter × 2.5 cm thick were aseptically obtained from the centre of each muscle. Before slicing, the dissection area and knives were cleaned and disinfected with absolute ethanol in order to avoid meat contamination. Nine of the 54 slices, randomly assigned to each sampling period were collected immediately after inoculation (day 0) and at 20, 30, 40, 50 and 60 days storage at 2 °C. Three of these slices were submitted to one of the following treatments: protective culture spraying (CRL705), saline solution spraying (SS) and non-treated samples (C) for comparison purposes. The described experiment was performed in duplicate.

The three steaks assigned to each treatment were evaluated for: A (1 steak): Warner Bratzler shear force (WBSF); B (1 steak): sensory analysis; C (1 steak): pH, microbiological and microestructural analysis. Steaks assigned to Warner Bratzler shear and sensory analysis were frozen at −18 °C until assay.

2.3. Preparation and inoculation of vacuum-packaged steak meat

L. curvatus CRL705 cells diluted in saline solution (0.85% NaCl) were sprayed onto the surface of the raw meat using a hand-operated spraying bottle to obtain a final concentration of 10^6 cfu g^{-1} (CRL705). A similar set of steaks was sprayed with saline solution (SS), and another set was used as a control (C). All steaks were vacuum-packaged using a film BB4 (Cryovac, Argentina) with a diffusion coefficient of 6/14 $cm^3\ m^{-2}\ atm^{-1}\ 24\ h^{-1}$ to oxygen at 25 °C and 75% RH. The packages were sealed at a final vacuum of 99% using a TURBOVAC 320 ST vacuum packaging machine (Howden Food Equipment, Holland).

2.4. Microbiological analysis and pH determination

A 10 g portion of each steak was homogenized in 90 ml of saline solution (NaCl 0.85%) in a Stomacher Lab Blender (model 400, A.J. Seward Lab. London, England) during 1 min. Decimal dilutions were prepared, and the following analyses were carried out on agar plates: total viable counts on plate count agar (48 h at 30 °C); LAB on MRS agar (48 h at 30 °C); *Listeria* spp., on PALCAM selective agar (Difco Laboratories, Inc., Detroit, Mich.) (48 h at 30 °C); *Brochothrix thermosphacta* on STAA (Gardner, 1966) (48 h at 25 °C); presumptive *Pseudomonas* on Cetrimide agar (48 h at 37 °C); and coliforms on MacConkey agar (48 h at 37 °C). Unless otherwise specified, all media and ingredients were obtained from Britania (Argentina). The pH was measured on the original meat homogenates using a Metrohn 692 pH/Ion Meter.

2.5. Antimicrobial activity

A semiquantitative diffusion assay (Vignolo et al., 1993) was used to determine bacteriocin activity (lactocin 705 and lactocin AL705). Ten grams of each steak were stomached for 8 min in the presence of saline solution (1:2) and twofold serial dilutions were prepared. Five microlitre of each dilution was placed spot in a semi-solid MRS and TSB plates overlay inoculated with *L. plantarum* CRL691 and *L. innocua* 7 used as indicator for lactocin 705 and lactocin AL705, respectively. Positive bacteriocin activity was evidenced as a zone of inhibition on the indicator organism lawn.

2.6. Evaluation of microstructural changes

Three samples of 1.0 cm^3 each were taken from the central area of each beef steak from different sampling periods and treatments and were subjected to light microscopy analysis according to Romeis (1928). Briefly, all samples were immediately immersed in buffered formaldehyde solution (10% v/v), followed by dehydration in ethanol solution (15 min each) at increased concentrations (50–100% v/v). Subsequently, they were immersed in ethanol:xylol solution (50:50) for 15 min at 4 °C, and finally included in paraffin. Transversal sections, 10 μm thick were cut and allowed to air-dry. Sections were stained with hematoxilin–eosin. Microscopy evaluation was performed with a Wild Heerbrugg model M20 Microscope equipped with a MEL 13 camera. A linear magnification of approximately 10 and 40× was used (details in each slide). All samples were analysed in duplicate.

2.7. Warner–Bratzler shear force determination

Beef steaks were thawed (for 12–18 h at 4 °C), weighed and cooked in an electric grill until a final internal temperature of 71 °C was reached. Cooking temperature was monitored by iron/constantan thermocouples inserted into the geometric centre of the sample. After cooked, steaks were weighed and cooled to <10 °C, and the percent of cooking loss was determined. Eight core samples (1.3 cm-diameter) obtained from each steak (paralleling muscle fiber orientation) and kept at 8 °C during approximately one hour were sheared with a Warner Bratzler Shear machine (Model 1955; GRE Manufacturing, Manhattan, KS). The shear force value was the mean of the maximum force required to shear each set of core samples.

2.8. Sensory analysis

Steaks for sensory analysis were thawed and then cooked as in 2.7. After cooking, each steak was trimmed of fat and connective tissue, cut into cubes 1 cm^3 and immediately served to an eight-member trained panel. Panellists were selected on their experience in sensory analysis of meat, and trained according to AMSA (1995) guidelines. The eating quality of treated and control beef samples was evaluated using an 8-point scale for flavor and odor intensity, initial and sustained tenderness, juiciness and connective tissue amount (1: extremely bland, extremely tough, extremely dry, and too much to 8 = extremely intense, extremely tender, extremely juicy, and none). Score sheets were also provided of specific scales for off-odor and off-flavor intensity and a table for the description of these attributes characteristics. All tests were conducted in individual booths under color neutralizing lights. Each sampling period was submitted independently to a sensory session.

2.9. Statistical analysis

Two separate replications of each experiment were carried out. The microbial counts and pH values were analysis using two-way ANOVA and Tukey post-test for multiple comparisons. Data of sensory analysis, tenderness and cooking loss were analysed as a completely randomized design (each sampling period was analysed separately) using the General Linear Models Procedure of SAS (SAS Institute, Inc., Cary, NC) for analysis of variance (Church & Parsons, 2000). Significant (p value <0.05) treatment effect means were separated using Tukey's multiple range test.

3. Results

In the present research, the protective action of *L. curvatus* CRL705 on vacuum-packaged raw beef during 60 days of refrigerated storage and its effect on the meat sensory quality and microstructure, was evaluated. Total viable counts in C and SS samples were 6.38 and 6.89 log cfu g^{-1} at the end of chilled (2 °C) vacuum storage, while CRL705 inoculated samples showed a final value of 7.40 log cfu g^{-1}, this count including bioprotective culture cells (Table 1). The growth of LAB in samples inoculated with the bacteriocinogenic strain exhibited an increase from 6.10 to 8.40 log cfu g^{-1} during the storage period; LAB numbers resulting higher than total viable counts since MRS is a more favorable medium to support their growth. On the other hand, indigenous LAB reached a final population of 6.10 and 6.64 log cfu g^{-1} on C and SS samples, respectively; these counts were significantly lower than those obtained for samples inoculated with the protective culture (Table 1).

Results of total coliforms and presumptive *Pseudomonas* sp. counts are shown in Fig. 1a and b. Meat steak samples (C, SS and CRL705) presented an initial coliforms contamination in the range of 1.60–1.70 log cfu g^{-1}, with C and SS samples reaching a final cell count of 4.68 and 4.98 log cfu g^{-1}, respectively, at the end of storage time. Instead, samples inoculated with *L. curvatus* CRL705 attained a maximum coliform numbers of 4.50 log cfu g^{-1} at 60 days of chill storage. Regarding presumptive *Pseudomonas* sp., increases from 1.80–2.00 to 3.30–3.50 log cfu g^{-1} in C, SS and CRL705 samples were observed, not significant effect of the bioprotective culture being found (Fig. 1b). On the contrary, *B. thermosphacta* showed to be effectively inhibited on beef steaks when *L. curvatus* CRL705 was added as bioprotective culture, while an increase of approximately 2.5 log cycles in C and SS samples at 60 days was evidenced (Fig. 1c). When the presence of *Listeria* was evaluated during the same period, none of the samples contained detectable levels (data not shown). The inhibitory activity and the pH values of meat samples during storage at 2 °C are shown in Tables 2 and 3, respectively. Results indicated that lactocin 705 and AL705 were produced by *L. curvatus* CRL705 throughout the storage period. A pH tendency to decrease from 5.55 (0 day) to 5.45 (60 days) was produced in beef steaks due to the metabolic activity of *L. curvatus* CRL705. In addition, significant differences in pH values between inoculated and non-inoculated series (C and SS samples) were observed at 60 days of storage (Table 3).

In order to assess the effect of *L. curvatus* CRL705 growth on tissue microstructure a light microcopy study was conducted. Uninoculated control samples on day 0 showed fibers with typical coloration, size and shape and an intact sarcolema (Fig. 2a). After 20 days of storage at 2 °C the onset of a structural disorder was observed, these including fiber fragmentation and incipient retraction as well as a slightly nucleus displacement (Fig. 2b, 40×). After 30 days of storage (Fig. 2c), fibers deformation, separation and rupture of the sarcolema, and nucleus displacement (fiber retraction) were evidenced as well. Control samples after 50 days of storage were not suitable for analysis due to a complete deterioration of the tissue. On the other hand, SS samples showed more noticeable alterations, presenting at the end of storage period discoloration patches, hydrolysis, disorder and fragmentation of tissue (data not shown). When samples sprayed with *L. curvatus* CRL705 and stored at 2 °C were analysed, the tissue at 20 days (Fig. 3a) was still well conserved and comparable to C samples picture at 0 day (Fig. 2a) in which fibers presented typical size, shape and color as well as interfiber spaces. A dry aspect with a smaller interfiber separation together with cellular retraction and abundant fiber fragmentation was induced at 30 days (Fig. 3b). On day 50th, more cell retraction and sarcolema separation/rupture were detected (Fig. 3c), these signs being less pronounced than in control samples after 40 days of storage (data not shown).

Results of sensory analysis of C, SS and *L. curvatus* CRL705 inoculated beef steaks are summarized in Table 4. No differences in flavor between treated and control samples until 30 days of storage were found. After that, flavor punctuation for C and SS samples was reduced; the presence of "acid" and "liver" (day 40) and "rancid" and strong "acid" (day 50) off-flavors was detected. On day 60th, C samples were only suitable for odor evaluation due to their

Table 1
TVC and LAB (log CFU g^{-1} ± SD) in vacuum-packaged fresh beef stored during 60 days at 2 °C.

Treatment		Time (days)						
		0	10	20	30	40	50	60
C	TVC	3.22 ± 0.22a	4.16 ± 0.21c	5.10 ± 0.20e	5.87 ± 0.20g	6.10 ± 0.20i	6.22 ± 0.22j	6.38 ± 0.23k
	LAB	2.30 ± 0.25a	3.60 ± 0.20b	4.26 ± 0.21c	5.00 ± 0.25d	5.89 ± 0.21f	6.00 ± 0.23f	6.10 ± 0.21f
SS	TVC	3.42 ± 0.20b	4.38 ± 0.20d	5.20 ± 0.22e	5.76 ± 0.22g	6.28 ± 0.21j	6.70 ± 0.23m	6.89 ± 0.20n
	LAB	2.10 ± 0.20a	3.40 ± 0.25b	4.20 ± 0.24c	4.90 ± 0.21d	5.60 ± 0.23e	6.40 ± 0.21g	6.64 ± 0.21h
CRL705	TVC	5.10 ± 0.19e	5.35 ± 0.20f	5.89 ± 0.22g	6.00 ± 0.23h	6.50 ± 0.21l	6.70 ± 0.22m	7.40 ± 0.20o
	LAB	6.10 ± 0.25f	6.80 ± 0.20h	7.30 ± 0.20i	7.50 ± 0.25i	7.70 ± 0.20j	8.00 ± 0.24k	8.40 ± 0.23l

TVC: total viable counts; LAB: lactic acid bacteria; SD: standard deviation.
Means with the same letter (a–o) are not significantly different ($p > 0.05$).

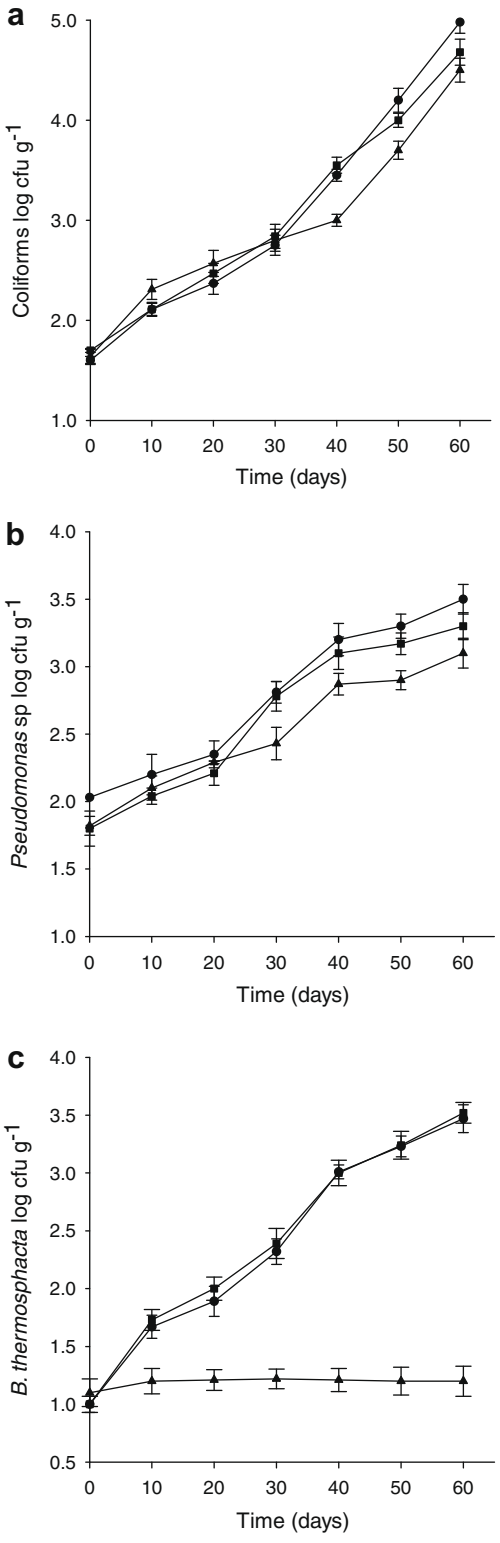

Fig. 1. Growth of coliforms (a), presumptive *Pseudomonas* sp. (b) and *Brochothrix thermosphacta* (c) on vacuum-packaged meat discs stored at 2 °C for 60 days; (■) control (●) saline solution and (▲) CRL705.

putrid appearance, while SS samples were not deteriorated at the same level showing no significant differences for aroma when compared to C samples. In contrast, *L. curvatus* CRL705 treated steaks depicted a "liver" flavor typical of aged meat, which became less intense after 30 days while a slight "rancid" flavor appeared at the end of the study. With regard to odor evaluation, no differences among samples were detected until day 50th (Table 4). On day 60th, C and SS samples showed a significant reduction of their characteristic aroma. C samples showed a typical "putrid meat" off-odor whereas a "perished" off-odor was detected on SS ones. Samples inoculated with *L. curvatus* CRL705 did not show any off-odors and no modification of the original odor was perceived at 60 days. These results point out that C and SS samples were deteriorated in the same level regarding to odor attributes; moreover, C samples were visually spoiled and were not suitable for consumption due to the intensity and type of off flavors. There were no differences among treatments along the refrigeration period in initial and sustained tenderness, connective tissue and juiciness (data not shown). However, a tendency to increase tenderness values in all the treatments was observed.

No differences in cooking weight loss and instrumental tenderness were found among treatments. All samples showed a typical improvement in tenderness due to ageing (data not shown). These values correspond to a "slight tender" to "tender" beef according to category sensory scales.

Table 2
Antimicrobial activity of *L. curvatus* CRL705 on meat vacuum packaged and incubated at 2 °C.

Incubation (days)	Antimicrobial activity	
	Lactocin 705	AL705
0	+*	+**
20	+	+**
30	+	+**
40	+	+**
50	+	+**
60	+	+**

+: Halos of inhibition < at 10 mm of diametric.
* Against *L. plantarum* CRL691.
** Against *L. innocua* 7.

Table 3
Determination of pH value on pieces of meat packaged vacuum and incubated at 2 °C.

Incubation (days)	pH		
	Control	Saline solution	*L. curvatus* CRL705
0	5.55 ± 0.14bcde	5.57 ± 0.14cde	5.55 ± 0.15abcd
20	5.46 ± 0.16abc	5.50 ± 0.17abcd	5.45 ± 0.21abc
30	5.44 ± 0.20ab	5.50 ± 0.16abcd	5.41 ± 0.16a
40	5.43 ± 0.18ab	5.52 ± 0.20abcd	5.40 ± 0.13a
50	5.50 ± 0.21abcd	5.60 ± 0.18de	5.42 ± 0.19a
60	5.60 ± 0.21de	5.68 ± 0.22e	5.45 ± 0.12abc

Means with the same letters (a,b,c,d,e) are not significantly different ($P > 0.05$).

4. Discussion

L. curvatus CRL705 added as bioprotective culture to vacuum-packaged fresh meat stored at 2 °C was the dominating population throughout the 60 days of storage. This fact would be responsible for the indigenous LAB control in meat, providing a shelf-life extension without compromising the microbiological safety of the product. Indeed, a previous study (Castellano et al., 2004) showed that *L. curvatus* CRL705 was successfully applied to control beef spoilage *Lactobacillus sakei* CRL1424 in meat slurries. Other bacteriocinogenic strains were also reported to control natural LAB microbiota, such as *Lactococcus lactis*, a lacticin 3147 producer, in fresh pork sausages (Scannell et al., 2000) and *L. sakei* TH1 during the commercial production of cooked meat products (Bredholt, Nesbakken, & Holck, 2001). A slight inhibition of coliforms by the bioprotective culture was observed, this effect being explained through competitive inhibition of pathogenic bacteria as it was re-

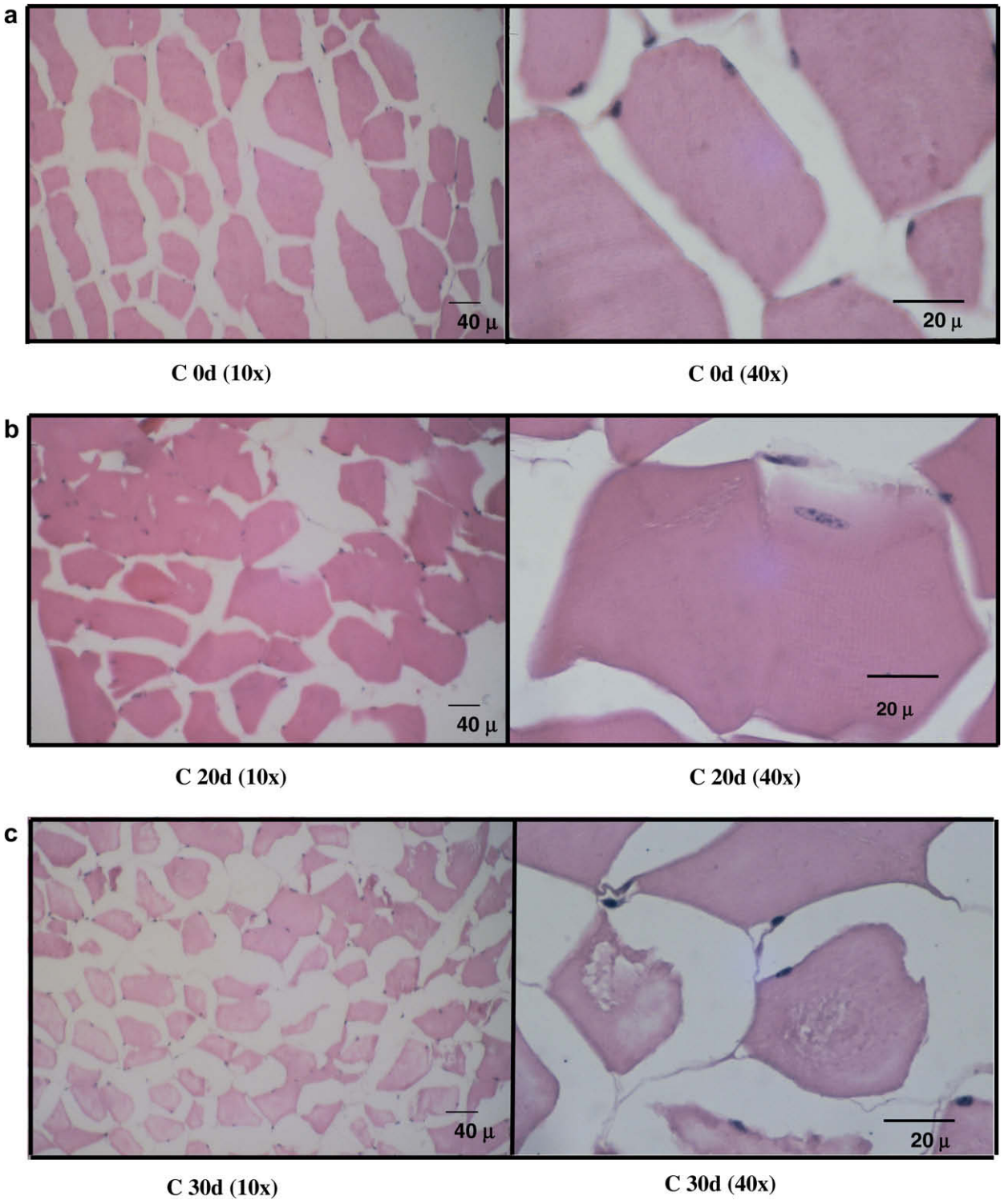

Fig. 2. Light microscopy analysis of control (C) samples stored at 2 °C for 0 (a), 20 (b) and 30 (c) days (10× and 40× magnifications).

ported by Breidt and Fleming (1997). Conversely, no inhibitory effect against presumptive *Pseudomonas* population by *L. curvatus* CRL705 was observed, which is consistent with the fact that bacteriocins from LAB are not active against Gram negative bacteria. Even when the inhibitory action of *L. curvatus* CRL705 against *Listeria* is well documented (Castellano & Vignolo, 2006; Castellano et al., 2004), no *Listeria* was detected in this study because of the application of Good Manufacture Practices during the slaughter procedure and further processing stages. On the other hand, the bacteriostatic effect of *L. curvatus* CRL705 on *B. thermosphacta* growth is in agreement with a previous work (Castellano & Vignolo, 2006). A decrease in *B. thermosphacta* number in vacuum-packaged beef after nisin spray treatments was also reported by Cutter and Siragusa (1998). As well, Metaxopoulos, Mataragas, and Drosinos (2002) found *B. thermosphacta* reductions after the inoculation of the bacteriocin producers *Leuconostoc mesenteroides* L124 and *L. curvatus* L442 in cooked cured meat products stored under vacuum at 4 °C. The production of bacteriocins by *L. curvatus* CRL705 was

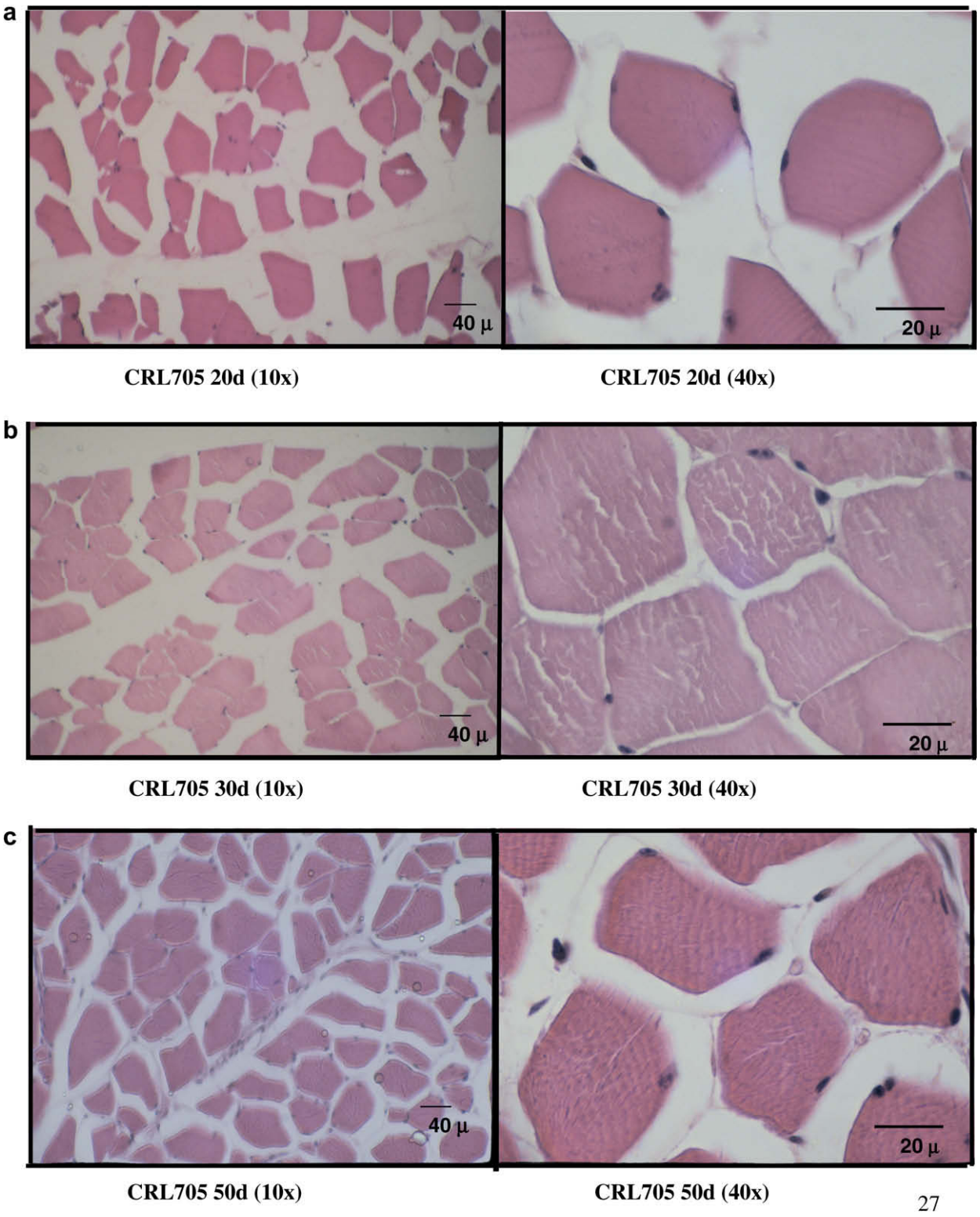

Fig. 3. Light microscopy analysis of samples treated with *L. curvatus* CRL750 (CRL750) 20 (a), 30 (b) and 50 (c) days (10× and 40× magnifications).

stable during the 60 days of incubation at 2 °C and was responsible for the inactivation of naturally occurring psychrotrophic microbiota.

From the point of view of meat structural changes, *L. curvatus* CRL705 treated steaks have a more conserved appearance than C and SS samples during the refrigerated storage. The microstructural changes on meat steaks inoculated with the bioprotective culture showed a 10 days delay in the appearance of tissue degradation signs, this result being attributed not only to the number but to the species of bacteria present on refrigerated vacuum-packaged meat. It has been shown that LAB, *Enterobacteriaceae* as well as *B. thermosphacta* are the dominant populations able to develop in refrigerated raw meat stored under vacuum (Ercolini et al., 2006; Fontana et al., 2006; Sakala, Hayashidani, Kato, Hirata, Mak-

Table 4
Flavor and aroma evaluation by trained sensory panel.

Trait	Treatment	Time (days)					
		0	20	30	40	50	60
Flavor	C	5.00	5.09	4.97	4.71	4.25	(*)
	SS	4.95	4.94	4.97	5.33	4.72	3.02
	CRL705	5.05	4.64	4.55	4.38	4.94	3.05
Aroma	C	4.83	5.05	4.71	4.43	5.22	2.05a
	SS	5.03	5.14	4.82	5.04	4.61	3.02a
	CRL705	5.05	4.81	4.55	4.48	4.89	5.30b

Means in a column with different letter in the same trait differ ($P < 0.05$); (*) deteriorated samples.

ino, Fukushima, Yamada, Kaneuchi, & Ogawa, 2002). It was histologically demonstrated that proteolytic spoilage bacteria including *B. thermosphacta* can penetrate the deep muscle mass by following the perimysium or endomysium connective tissue (Labadie, 1999; Stackebrandt & Jones, 2006). Since the bacteriocins produced by *L. curvatus* CRL705 demonstrated in this study to be bacteriostatic against *B. thermosphacta*, major microstructural changes in beef steaks were prevented. Instead, the higher counts of this microorganism found in C and SS meat samples may account for the observed tissue deterioration. In addition, more significant degradation observed in SS samples compared to C samples may be associated to the promotion of bacterial growth (total viable and LAB) induced by the addition of saline solution, which increases moisture and nutrients availability in meat substrates.

The sensory evaluation of meat samples inoculated with the bioprotective culture demonstrated that organoleptic quality of beef *Semimembranosus* muscles steaks was not significantly modified by the surface spraying of *L. curvatus* CRL705 during the first 30 days of storage. Evaluation of odor, flavor, presence of off-odors and off-flavors pointed out that *L. curvatus* CRL705 treated samples were able to reach to the end of the study period in better conditions than C and SS samples. CRL705 steaks have no signals of deterioration, only an "acid" off-flavor-characteristic of these microorganisms- was evidenced at 60 days. At this time, a slight but significant pH decrease was observed in CRL705 treated meat compared to C and SS samples, probably accounting for the incipient sensory alteration. In contrast, the sustained deterioration of C and SS samples from day 40 of chilled storage may be related to *B. thermosphacta* counts (3.00 log cfu g^{-1}), since small amounts of acetic and propionic acids in addition to L(+) lactic acid, formate and ethanol were reported to be produced due to anaerobic glucose consumption by this organism (Pin, García de Fernando, & Ordóñez, 2002; Stackebrandt & Jones, 2006). When the WBSF was determined in beef steaks, no differences were detected among treatments during storage by the evaluation panel. Interesting, a tendency to increase tenderness as occur during beef aging (Koohmaraie, 1996) was also observed in *L. curvatus* CRL705 inoculated meat samples, this result confirming that inoculation with the bioprotective culture does not affect aging development.

Results obtained in the microscopy analysis of CRL705 treated samples are in agreement with those of panel evaluation, instrumental tenderness and cooking weight loss, since non significant structural changes were observed until day 50th. A similar correlation for SS and C muscular samples started around 30 days; the tissue was practically deteriorated at the end of the refrigeration period (50–60 days) thus, impeding its proper fixation and microscopy analysis. This observed deterioration rose together with the appearance of off-flavors, and odor changes detected by the panelists. In addition, the structural changes detected between 30 and 50 days, were not extensive enough to affect sample tenderness; sustained tenderness was not modified and instrumental tenderness appeared to increase under refrigeration conditions (this event was not supported statistically). Moreover, due to the observed tissue structural modifications, a decrease in juiciness and an important reduction of cooking weight loss should be expected. Unfortunately, no explanation for the lack of correlation between juiciness and cooking weight loss results and those of microscopy analysis was found. This disagreement may be attributed to different samples marbling score due to their commercial source, this producing a high variability in cooking weight loss values.

The bioprotective effectiveness of *L. curvatus* CRL705 on beef steaks was demonstrated in this study without causing major organoleptic and structural changes. These results are in agreement with Katikou, Ambrosiadis, Georgantelis, Koidis, and Georgakis (2005) who reported that *L. sakei* CECT 4808 used as protective culture on refrigerated vacuum-packaged sliced beef, inhibited spoilage microbial growth without affecting its chemical and sensory quality during 26 days of storage. Similarly, when the bacteriocinogenic *L. sakei* 148 was inoculated in cooked hams, they were not rejected by the sensory panel at the 34th day of the vacuum packaged storage at 7 °C (Vermeiren, Devlieghere, & Debevere, 2004). In conclusion, inoculation with the bacteriocinogenic strain *L. curvatus* CRL705 would provide an additional hurdle to improve storage life of refrigerated vacuum-packaged beef without substantial sensory and structural changes.

Acknowledgements

The authors are grateful to the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) PICT2003 No. 09-13499, Argentina, for financial support of this work. They also want to thank Elena Bru for statistical analysis.

References

American Meat Science Association (AMSA) (1995). *Research guidelines for cookery, sensory evaluation, and instrumental measurement of fresh meat*. Chicago, IL: National Livestock and Meat Board.

Bredholt, S., Nesbakken, T., & Holck, A. (2001). Industrial application of an antilisterial strain of *Lactobacillus sakei* as a protective culture and its effect on the sensory acceptability of cooked, sliced, vacuum-packaged meats. *International Journal of Food Microbiology, 66*, 191–196.

Breidt, F., & Fleming, H. P. (1997). Using lactic acid bacteria to improve the safety of minimally processed fruits and vegetables. *Food Technology, 51*(9), 44–48.

Castellano, P., Belfiore, C., Fadda, S., & Vignolo, G. (2008). A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. *Meat Science, 79*, 483–499.

Castellano, P., Holzapfel, W. H., & Vignolo, G. (2004). The control of *Listeria innocua* and *Lactobacillus sakei* in broth and meat slurry with the bacteriocinogenic strain *Lactobacillus casei* CRL705. *Food Microbiology, 21*, 291–298.

Castellano, P., Raya, R., & Vignolo, G. (2003). Mode of action of lactocin 705, a two-component bacteriocin from *Lactobacillus casei* CRL705. *International Journal of Food Microbiology, 85*, 35–43.

Castellano, P., & Vignolo, G. (2006). Inhibition of *Listeria innocua* and *Brochothrix thermosphacta* in vacuum-packaged meat by addition of bacteriocinogenic *Lactobacillus curvatus* CRL705 and its bacteriocins. *Letters in Applied Microbiology, 43*, 194–199.

Church, I. J., & Parsons, A. L. (2000). The sensory quality of chicken and potato products prepared using cook-chill and sous vide methods. *International Journal of Food Science and Technology, 35*, 1–8.

Cuozzo, S., Castellano, P., Sesma, F., Vignolo, G., & Raya, R. (2003). Differential roles of the two-component peptides of lactocin 705 in antimicrobial activity. *Current Microbiology, 46*, 180–183.

Cuozzo, S., Sesma, F., Palacios, J., Ruiz Holgado, A. P., & Raya, R. (2000). Identification and nucleotide sequence of genes involved in the synthesis of lactocin 705, a two-peptide bacteriocin from *Lactobacillus casei* CRL705. *FEMS Microbiology Letters, 185*, 157–161.

Cutter, C. N., & Siragusa, G. R. (1998). Incorporation of nisin into a meat binding system to inhibit bacteria on beef surfaces. *Letters Applied Microbiology, 27*, 19–23.

Ercolini, D., Russo, F., Torrieri, E., Mais, P., & Villani, F. (2006). Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. *Applied Environmental Microbiology, 72*, 4663–4671.

Fadda, S., Sanz, Y., Aristoy, M., Vignolo, G., Oliver, G., & Toldrá, F. (1999). Hydrolysis of pork muscle sarcoplasmic proteins by *Lactobacillus curvatus* and *Lactobacillus sake*. *Applied and Environmental Microbiology, 65*(2), 578–584.

Fadda, S., Vignolo, G., Ruiz Holgado, A. P., & Oliver, G. (1998). Proteolytic activity of *Lactobacillus* strains isolated from dry-fermented sausages on sarcoplasmic muscle proteins. *Meat Science, 49*, 11–18.

Fontana, C., Cocconcelli, P. S., & Vignolo, G. (2006). Direct molecular approach to monitoring bacterial colonization on vacuum-packaged beef. *Applied Environmental Microbiology, 72*, 5618–5622.

Gardner, G. A. (1966). A selective medium for the enumeration of *Microbacterium thermosphactum* in meat and meat products. *Journal of Applied Bacteriology, 29*, 455–460.

Jeyamkondan, S., Jayas, D. S., & Holley, R. A. (2000). Review of centralized packaging systems for distribution of retail-ready met. *Journal of Food Protection, 63*, 796–804.

Katikou, P., Ambrosiadis, I., Georgantelis, D., Koidis, P., & Georgakis, S. A. (2005). Effect of *Lactobacillus*-protective cultures with bacteriocin-like inhibitory substances producing ability on microbiological, chemical and sensory changes during storage of refrigerated vacuum-packaged sliced beef. *Journal of Applied Microbiology, 99*, 1303–1313.

Katla, T., Møretrø, T., Sveen, I., Aasen, I. M., Axelsson, L., & Rørvik, L. M. (2002). Inhibition of *Listeria monocytogenes* in chicken cold cuts by addition of sakacin P and sakacin P-producing *Lactobacillus sakei*. *Journal of Applied Microbiology, 93*, 191–196.

Koohmaraie, M. (1996). Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Science, 43*, S193–S201.

Labadie, J. (1999). Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Science, 52*, 299–305.

Metaxopoulos, J., Mataragas, M., & Drosinos, E. H. (2002). Microbial interaction in cooked cured meat products under vacuum or modified atmosphere at 4 °C. *Journal of Applied Microbiology, 93*, 363–373.

Phillips, C. A. (1996). Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science and Technology, 31*, 463–479.

Pin, C., García de Fernando, G. D., & Ordóñez, J. A. (2002). Effect of modified atmosphere composition on the metabolism of glucose by *Brochothrix thermosphacta*. *Journal of Applied Microbiology, 68*, 4441–4447.

Romeis, B. (1928). *Guía formulario de técnica histológica*. Barcelona: Labor (pp. 171–176).

Sakala, R. M., Hayashidani, H., Kato, Y., Hirata, T., Makino, Y., & Fukushima, A. (2002). Change in the composition of the microflora on vacuum-packaged beef during chiller storage. *International Journal of Food Microbiology, 74*, 87–99.

Scannell, A. G., Ross, R. P., Hill, C., & Arendt, E. K. (2000). An effective lacticin biopreservative in fresh pork sausage. *Journal of Food Protection, 63*, 370–375.

Senne, M. M., & Gilliland, S. E. (2003). Antagonism action of cells of *Lactobacillus delbrueckii* subsp. *Lactis* against pathogenic and spoilage microorganisms in fresh meat systems. *Journal of Food Protection, 66*, 418–425.

Stackebrandt, E., & Jones, D. (2006). The Genus *Brochothrix*. In M. Dworkin, S. Falkow, E. Rosenberg, K. Heinz Schleifer, & E. Stackebrandt (Eds.), *The prokaryotes (3rd ed., pp. 483–487)*. New York: Springer.

Szerman, N., Gonzalez, C. B., Sancho, A. M., Grigioni, G., Carduza, F., & Vaudagna, S. R. (2007). Effect of whey protein concentrate and sodium chloride addition plus tumbling procedures on technological parameters, physical properties and visual appearance of sous vide cooked beef. *Meat Science, 76*, 463–473.

Vermeiren, L., Devlieghere, F., & Debevere, J. (2004). Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. *International Journal of Food Microbiology, 96*, 149–164.

Vignolo, G. M., Suriani, F., Ruiz Holgado, A. P., & Oliver, G. (1993). Antibacterial activity of *Lactobacillus* strains isolated from dry fermented sausages. *Journal of Applied Bacteriology, 75*, 344–349.

Yamazaki, K., Suzuki, M., Kawai, Y., Inoue, N., & Montville, T. J. (2003). Inhibition of *Listeria monocytogenes* in cold-smoked salmon by *Carnobacterium psicicola* CS526 isolated from frozen surimi. *Journal of Food Protection, 66*, 1420–1425.