Article

FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL

Evaluation of the microbiological safety and sensory quality of a sliced cured-smoked pork product with protective cultures addition and modified atmosphere packaging

Rocío Casquete[1,2], Susana C Fonseca[3,4], Ricardo Pinto[3], Sónia M Castro[2], Svetoslav Todorov[5], Paula Teixeira[2] and Manuela Vaz-Velho[3]

Abstract

The aim of this study was to evaluate the effect of two protective lactic acid bacteria cultures combined with modified atmosphere packaging on the survival/growth of *Listeria innocua* 2030c (as a surrogate for *Listeria monocytogenes*) and on sensory attributes of ready-to-eat 'lombo' over storage time. Sliced 'lombo', a traditional cured-smoked pork loin, was inoculated with *L. innocua* 2030c, *Lactobacillus sakei* ST153 (isolated from 'salpicão') and BLC35 culture (with *Lactobacillus curvatus*, *Staphylococcus xylosus* and *Pediococcus acidilactici*; CHR Hansen) as protective cultures. Samples were packed in two modified atmosphere packaging conditions (20% CO_2/80% N_2 and 40% CO_2/60% N_2) and stored at 5 °C for 124 days. Both cultures led to a reduction of 1–2 log CFU/g of *L. innocua* 2030c after 12 h; however, at the end of storage only *Lb. sakei* ST153 maintained this antilisterial effect, which was more evident at 40% CO_2/60% N_2. The influence of cultures addition and modified atmosphere packaging conditions on the sensory characteristics of the product were not significant. Thus, *Lb. sakei* ST153 combined with modified atmosphere packaging is a strong candidate to be used in a biopreservation strategy maintaining the traditional sensory quality of cured-smoked pork products and increasing their safety with respect to *Listeria* spp.

Keywords

Meat product, bacteriocins, lactic acid bacteria, modified atmosphere packaging, sensory properties, *Listeria monocytogenes*

Date received: 10 December 2017; accepted: 26 December 2018

INTRODUCTION

Cured-smoked pork meat products are much appreciated by south-western European consumers. In addition to the appealing sensory attributes, modern consumers demand convenient foods, without synthetic additives but with an extended shelf-life. Sliced ready-to-eat (RTE) products are of great convenience but have a broad range of emerging microbiological issues

Food Science and Technology International 0(0) 1–10

DOI: 10.1177/1082013219825771
journals.sagepub.com/home/fst
SAGE

[1]Nutrición y Bromatología, Escuela de Ingenierías Agrarias, Universidad de Extremadura, Badajoz, Spain
[2]CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Porto, Portugal
[3]Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viana do Castelo, Viana do Castelo, Portugal
[4]Faculty of Sciences, GreenUPorto, LAQV-REQUIMTE, DGAOT, University of Porto, Porto, Portugal
[5]Departamento de Veterinária, Universidade Federal de Viçosa, Viçosa, Brasil

Corresponding author:
Susana C Fonseca, Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viana do Castelo, Av. Atlântico s/, Viana do Castelo 4900-348, Portugal.
Email: scfonseca@estg.ipvc.pt

regarding food safety (Lucera et al., 2012). The presence of pathogens in RTE meat foods has been previously reported (Borch and Arinder, 2002; Gudbjornsdottir et al., 2004; Melero et al., 2012) and these products have been implicated in foodborne outbreaks caused by, for example, *Listeria monocytogenes* (Duffy et al., 1994; Gudbjornsdottir et al., 2004; Marcos et al., 2008; Nilsson et al., 2000), pathogenic *Escherichia coli* and *Staphylococcus aureus* (Pérez-Rodríguez et al., 2007). *L. monocytogenes* is one of the most important psychrotrophic foodborne pathogens related to anaerobically packed lightly cooked or smoked meat products because of its ability to survive and multiply at refrigerated temperature. Slicing of such products can lead to further contamination with pathogens. Therefore, the prevalence of pathogens in commercial RTE fermented meat products demands improvements in packaging and preservation methods maintaining the freshness, quality and safety of foods (Lucera et al., 2012). Biopreservation or biocontrol, through introduction of natural substances, such as lactic acid bacteria (LAB), is an interesting approach that creates adverse conditions in situ to pathogenic development and consequently assures microbiological safety, and simultaneously allowing chemical additives reduction. However, biopreservation may only be implemented if the appealing sensory attributes of cured-smoked pork meat products maintain throughout shelf-life and do not affect consumer's perception of these products.

Bacteriocin-producing LAB with antilisterial activity naturally occurs on a wide range of RTE meat products (Hugas, 1998). Heterofermentative LAB of the *Carnobacterium*, *Leuconostoc* and *Weissella* genera are usually more involved in meat spoilage than the homofermentative *Lactobacillus* and *Pediococcus*. Therefore, commercially available meat starter cultures for dry-fermented sausage production exclusively belong to the latter two (Kroückel, 2013). *Lactobacillus* species represent the dominant LAB strains currently found in meat starter cultures (Chaillou et al., 2005). The antibacterial spectrum of activity of *Lactobacillus sakei* ST153 has already been studied and results indicated their potential for use in a mixed starter culture for the fermentation of meat products (Todorov et al., 2013). The ability of *Lb. sakei* to compete with other species and grow in the meat environment makes their sakacines highly interesting (Fontana et al., 2012; Jones et al., 2010).

One of the most efficient technologies used for product preservation is modified atmosphere packaging (MAP) combined with refrigeration (Baracat et al., 2005). Active MAP relies on modification of the atmosphere inside the package (complete removal of the original atmosphere inside package, replacing it with the desired atmosphere rich in CO_2 and absent in O_2, by injection), controlled by a high barrier packaging material. CO_2 is the most important antimicrobial component in the normally applied gas mixtures (Farber, 1991) and N_2 is used as filler (Sørheim et al., 1999). Several studies have shown that MAP of many types of meat products interferes with the survival and growth of *L. monocytogenes* (García de Fernando et al., 1995; Hudson et al., 1994; Hugas et al., 1998). Research on antimicrobial substances, mainly bacteriocins, produced by LAB, has led to consideration of their use as natural preservatives in meat products (Castellano et al., 2008; Fadda et al., 2010). LAB naturally dominate the natural microflora of meat products that are stored under vacuum or in an environment enriched with CO_2 and their use as protective cultures has been studied as an alternative to chemical additives for assuring food safety (Holzapfel et al., 1995; Stiles, 1996). Nilsson et al. (2000) reported that nisin and an atmosphere rich on CO_2 acted synergistically on the cytoplasmic membrane of *L. monocytogenes* by enhancing membrane permeabilization.

The aim of this study was to evaluate the effect of two protective cultures combined with different MAP conditions enriched in CO_2 on survival and growth of *Listeria innocua* 2030c (as a surrogate for *L. monocytogenes*), and on the sensory properties of sliced RTE 'lombo', a cured-smoked pork product.

MATERIALS AND METHODS

Bacterial strains and media

Lb. sakei ST153, isolated from 'salpicão', a Portuguese cured-smoked pork product, BLC35 culture (commercial mixed starter culture including strains of *Lactobacillus curvatus, Staphylococcus xylosus* and *Pediococcus acidilactici*; CHR Hansen), both with antilisterial activity tested in 'chouriço', another type of cured-smoked product (Jácome et al., 2014), and *L. innocua* 2030c PHLS (Public Health Laboratory Service, Colindale, London), already tested as surrogate for *L. monocytogenes* (Albano et al., 2007; Vaz-Velho et al., 2001), were used in the assays. *Lb. sakei* ST153 was grown in de Man, Rogosa Sharpe (MRS) broth (Lab M, Bury, UK) at 37 °C for 24 h and *L. innocua* 2030c was grown in Tryptone Soy Broth with Yeast Extract (0.6% w/v) (TSB-YE; Lab M) at 37 °C for 24 h. Both strains were stored at −20 °C in broth supplemented with 30% (v/v) glycerol.

Samples' preparation

One batch of 'lombo' was produced and sliced by an industrial meat company and subsequently transferred to the laboratory under refrigerated conditions. *Lb. sakei* ST153 was sub-cultured twice (1% v/v inoculum; 24 h at 37 °C) in 10 ml MRS broth and *L. innocua*

2030c in TSB-YE broth. The cells were harvested under aseptic conditions by centrifugation ($6000 \times g$ for 10 min) at room temperature. The obtained cells were washed twice with sterilized water by centrifugations and re-suspended in sterilized water before being inoculated onto the product slices. BLC35 culture was used as recommended by the manufacturers (CHR Hansen). *L. innocua* 2030c (10^7 CFU/ml) was spread onto the slices using a sterile cotton swab, prior to the inoculation of the LAB cultures. The LAB cultures (10^8 CFU/ml) were inoculated onto the slices by immersion with a subsequent air-drying stage. Slices (20-25) were placed in trays (AERpack, B22-50, COOPBOX Hispania SLU, Spain), heat sealed (Oceania Jolly 20/40, Yang C.R.L., Italy) with a high barrier covering film (OPEX 55 AB PA/EVOH Barrier/PE, Boulanger SAS, France), after modified atmosphere injection. Twenty-four tray samples were obtained by combining the two MA (20% CO_2/80% N_2 and 40% CO_2/60% N_2) with the five treatments and corresponding controls: (1) uninoculated slices as control (C), (2) slices inoculated with *L. innocua* 2030c (C+L), (3) slices inoculated with BLC35 (BLC35), (4) slices inoculated with BCL35 plus *L. innocua* 2030c (BCL35+L), (5) slices inoculated with *Lb.* sakei ST153 (ST153) and (6) slices inoculated with *Lb.* sakei ST153 plus *L. innocua* 2030c (ST153+L). Each treatment/control was performed in duplicated trays each one with 20–25 slices. All samples were stored at 5 °C for 124 days. Monitoring of CO_2 content inside the packs was measured at days 0, 33, 94 and 124 (PBI DanSensor Checkmate II Ringsted, Denmark).

Microbiological analysis

Aliquots were taken from each sample at 0, 12 h, and after 15, 30, 90 and 120 days of storage. Twenty-five grams of each sample was weighed aseptically, added to 225 ml of sterile Ringer's solution (Lab M) and homogenized in a stomacher for 2 min. Appropriate decimal dilutions were prepared in sterile Ringer's solution (Lab M) for enumeration of LAB and *L. innocua* 2030c: 20 μl samples of the appropriate dilutions were spotted, in duplicate, on selective agar plates – MRS Agar and ALOA Agar (Bio-Rad), respectively. Counting was performed after incubation at 37 °C for 48 h under microaerophilic conditions for LAB and at 37 °C for 24 h for *L. innocua* 2030c.

Sensory analysis

A sensory descriptive profiling analysis was performed to samples of sliced 'lombo' with the two treatments (BLC35 or ST153) and packed under MA (20% CO_2/80% N_2 or 40% CO_2/60% N_2) by a sensory semi-trained panel of 15 elements (27% male and 73% female, aged between 23 and 45) at days 5, 33, 94 and 124 of storage at 5 °C. In each of the four sessions duplicates of samples were given to panellists. Sessions for main descriptors' definition, their scale limits as well as verbal anchors, using the very same type of commercial 'lombo' were performed previously. A final sheet with nine attributes ('meat colour', 'oiliness', 'characteristic odour', 'off-odour', 'hardness', 'succulence', 'characteristic flavour', 'acid taste' and 'bitter taste'), each one with a discrete 13-point intensity scale, was established. The reference value of control (commercial sample) for each attribute corresponded to a score of 7, except for 'off-odour' that was set as score 1. The sensory evaluation was carried out in a sensory laboratory of the Polytechnic Institute, in individual booths under normal white lighting. Panellists were provided with a porcelain spittoon, a glass of bottled natural water and toasts. Samples were presented to each panellist on white plastic dishes identified by a three-digit random number. In order to compensate for eventual carry-over effects, each panellist received the set of four samples following a monadic sequential order of presentation, with a balanced presentation order. A control sample was maintained the same over the session to compare with. The panellists were asked to score the perceived intensity of the nine selected attributes when comparing samples with the control sample (standard commercial product with the same storage time). A defect detection/conformity sensory test was also performed using a 5-point scale that allowed perceiving potential defects in the product's overall quality. It was assigned a score 1–3 depending on the severity of the defect (1 – extreme alteration, 2 – pronounced alteration, 3 – slight alteration), and 4 or 5 if the product was of good or extreme quality as expected. Samples were considered in conformity with expected when scored higher than 3 (limit of conformity).

Statistical analysis

Statistical analysis of the microbiological data was carried out using SPSS for Windows, 17.0. (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics of the data was determined, and the differences within and between groups were studied by one-way analysis of variance (ANOVA) and separated by Tukey's honest significant differences test ($p < 0.05$).

An ANOVA and a principal component and classification analysis were carried out to assess the effects of storage time, addition of LAB strains and MAP conditions on panel evaluation results, using the software Statistica 7 (Statsoft Inc., Tulsa, USA). The Fisher's least significant difference test was further used to

Table 1. Enumeration of lactic acid bacteria (LAB) in sliced cured-smoked 'lombo' inoculated with protective LAB strains (*Lb. sakei* ST153 and BCL35) and packed under MA (20% CO_2/80% N_2 and 40% CO_2/60% N_2) during storage at 5 °C

MAP	Treatment/control	Days of storage: 0 Log CFU/g SD	15 Log CFU/g SD	30 Log CFU/g SD	90 Log CFU/g SD	120 Log CFU/g SD
20% CO_2/80% N_2	Control	8.00 ± 0.28a,1	8.24 ± 0.09a,1	8.11 ± 0.09a,b,c,1	7.71 ± 0.07b,c,1,2	7.50 ± 0.14c,d,2
	Control + *L. innocua*	8.40 ± 0.11a,1	8.35 ± 0.07a,1	8.24 ± 0.09a,b,1	8.13 ± 0.02a,b,c,1,2	7.55 ± 0.21a,2
	ST153	8.24 ± 0.09a,1	8.57 ± 0.04a,1	8.33 ± 0.21a,b,1	8.26 ± 0.30a,1	8.22 ± 0.11a,1
	ST153 + *L. innocua*	8.30 ± 0.00a,1	8.48 ± 0.43a,1	8.42 ± 0.17a,b,1	7.99 ± 0.02a,b,c,1	8.01 ± 0.01a,b,1
	BCL35	8.24 ± 0.09a,1	8.35 ± 0.07a,1	8.40 ± 0.00a,b,1	8.38 ± 0.10a,1	7.83 ± 0.07b,c,1
	BCL35 + *L. innocua*	8.42 ± 0.17a,1	8.42 ± 0.17a,1	7.94 ± 0.34b,c,1	7.98 ± 0.06a,b,c,1	8.16 ± 0.08a,b,1
40% CO_2/60% N_2	Control	7.90 ± 0.06a,1,2	8.02 ± 0.03a,1	8.36 ± 0.05a,b,1	7.68 ± 0.05c,1,2	7.35 ± 0.07d,2
	Control + *L. innocua*	8.10 ± 0.08a,1	7.94 ± 0.02a,1	7.44 ± 0.06c,1	7.69 ± 0.21c,1	8.00 ± 0.09a,b,1
	ST153	8.20 ± 0.28a,1	8.42 ± 0.17a,1	8.54 ± 0.09a,b,1	7.90 ± 0.00a,b,c,1	8.24 ± 0.09a,1
	ST153 + *L. innocua*	8.29 ± 0.16a,1	8.51 ± 0.47a,1	8.57 ± 0.12a,b,1	8.18 ± 0.05a,b,1	8.09 ± 0.07a,b,1
	BCL35	8.15 ± 0.21a,1,2	8.57 ± 0.04a,1	8.76 ± 0.08a,1	8.01 ± 0.12a,b,c,2	7.97 ± 0.02a,b,2
	BCL35 + *L. innocua*	8.24 ± 0.09a,1	8.39 ± 0.30a,1	8.33 ± 0.46a,b,1	8.31 ± 0.01a,1	7.98 ± 0.03a,b,1

SD: standard deviation.
Values with different letters (a, b, c, d) mean there are significant differences ($p < 0.05$) between treatments.
Values with different numbers (1, 2) mean there are significant differences ($p < 0.05$) between times of storage.

compare means when significant differences ($p < 0.05$) were found in ANOVA.

RESULTS AND DISCUSSION

Microbial counts during storage

Enumeration of LAB and *L. innocua* 2030c in sliced 'lombo' with protective LAB strains (*Lb. sakei* ST153 and BCL35) and packed under MA (20% CO_2/80% N_2 and 40% CO_2/60% N_2) conditions during the 120 days of storage at 5 °C is presented in Table 1 and Figure 1, respectively. As shown in Table 1, the different MAP conditions did not influence growth/survival of the inoculated LAB (*Lb. sakei* ST153 and BCL35) in refrigerated 'lombo'. However, the 20% CO_2/80% N_2 MAP condition affected the growth/survival of the indigenous LAB in the control sample. From an initial level of 8.0–8.4 log CFU/g, the number of viable cells decreased to 7.5–7.6 log CFU/g after 120 days. In the control sample, *L. innocua* 2030c was able to maintain initial levels until 12 h of storage (Figure 1). However, in the presence of LAB inoculated (ST153 and BCL35), levels of *L. innocua* 2030c decreased between 1.0 and 2.0 log CFU/g at the beginning of storage. As shown in Figure 1, the combination *Lb. sakei* ST153 and MAP conditions of 40% CO_2/60% N_2, a higher inhibition of *L. innocua* 2030c was observed than in other treatments from the beginning of storage. Previously, Alves et al. (2003) demonstrated that when *Lb. sakei* and *L. monocytogenes* cultures were inoculated in a model meat gravy system, in situ bacteriocin production played an important role in preventing growth of *L. monocytogenes*. Results are like those reported previously by Melero et al. (2012), for fresh chicken burger meat packaged under MAP (50% CO_2/50% O_2), inoculated with a protective culture and stored under refrigerated conditions; LAB counts were constant in MAP treatments. Liserre et al. (2002), working with MAP and a bacteriocinogenic strain, demonstrated that inhibition of *L. monocytogenes* in a Brazilian sausage could not be achieved by the sole application of vacuum. However, the addition of the bacteriocinogenic strain resulted in a greater decrease of levels of *L. monocytogenes* in the MA packaged products. Other authors corroborated these findings, reporting that CO_2 (in MAP) and the use of *Lb. sakei* (bacteriocin negative or positive) have a synergistic inhibitory effect on the growth of *L. monocytogenes* in sliced beef bologna-type sausages (Kaban et al., 2010). Jácome et al. (2014), when working with the same LAB strains used in this study (*Lb. sakei* ST153 and BCL35), and MAP (8% CO_2/92% N_2 or 12% CO_2/88% N_2), also reported inhibition of *L. monocytogenes* in 'chouriço', another cured-smoked meat product, after 120 days of storage.

It is worth mentioning that the content of CO_2 in MAP declined from 20 and 40%, at the time of packaging, to about, respectively, 7.5 and 13% at the end of the storage period (results not shown). Although high barrier films were used, during storage losses of CO_2 occurred by gas diffusion through the film, absorption

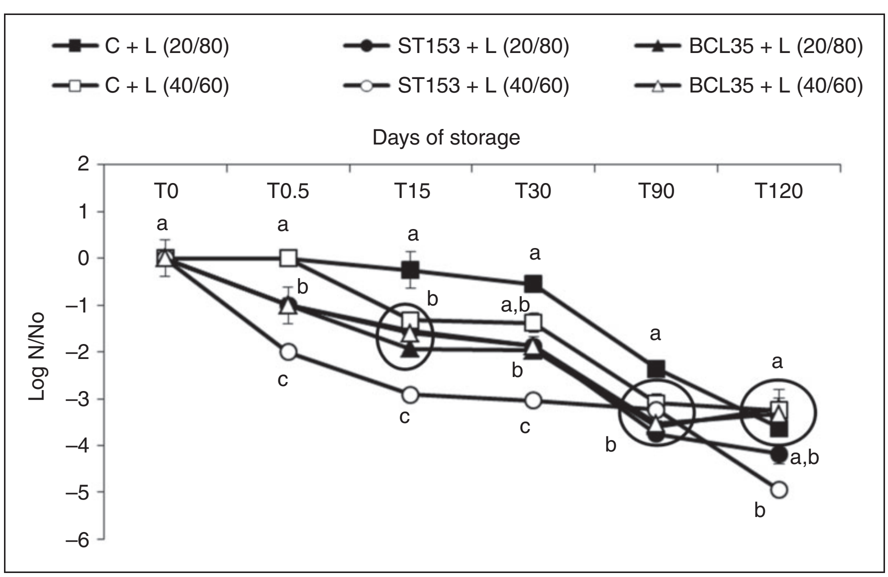

Figure 1. Enumerations of *L. innocua 2030c* in sliced 'lombo' inoculated with protective LAB strains (*Lb. sakei* ST153 or BCL35) and packed under MA (20% CO_2/80% N_2 or 40% CO_2/60% N_2) during 120 days of storage at 5 °C. Points with different letters (a, b, c) mean that there are significant differences ($p < 0.05$) between treatments.

of CO_2 by the products and/or microbial metabolism (Parra et al., 2012; Rubio et al., 2007; Simpson et al., 2009).

Sensory analysis

Sensory evaluation results are presented in Figures 2 to 4. The sensory panel, for each strain application and MAP *conditions*, did not differentiate samples ($p > 0.05$) with respect to the following attributes: meat colour, characteristic odour, off-odour and acid taste (data not shown). The acidic taste attribute was expected to be influenced by LAB additions; however, the panellists did not notice this compared to the control commercial samples. The perception of panellists was corroborated by pH values that were practically constant and equal until day 94, just decreasing on the last sampling day (data not shown). It can be seen in Table 1 that the differences in LAB counts, between the control and inoculated LAB samples, were less than 1.0 log CFU/g, even at the end of storage time. Vermeiren et al. (2006) reported that despite the promising antagonistic effects of *Lb. sakei* 10 A its application to vacuum-packed cooked meat products was in some cases limited by a significant acidification resulting in an acid taste of the product. Other authors reported

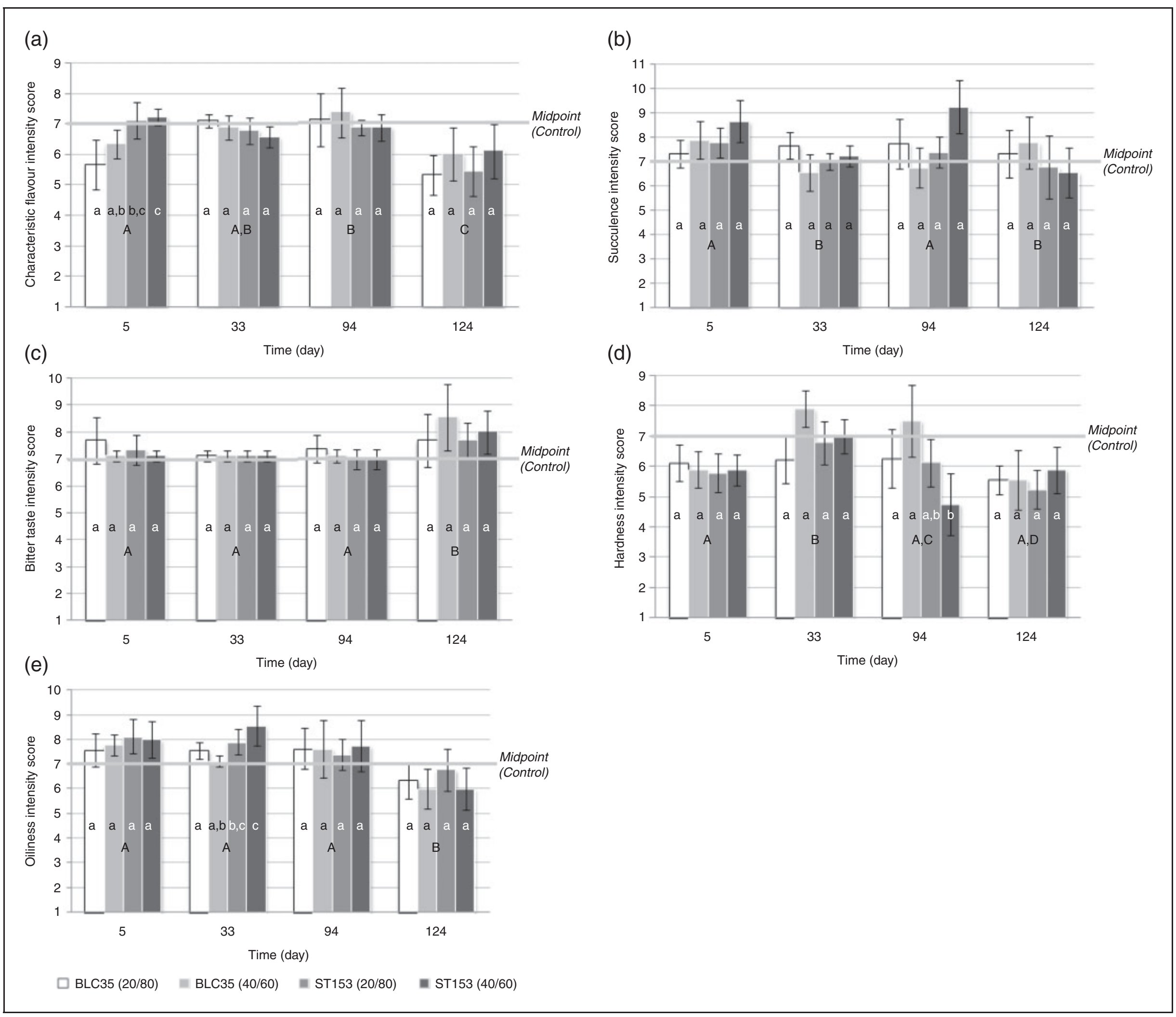

Figure 2. Sensory evaluation of (a) characteristic flavour, (b) succulence, (c) bitter taste, (d) hardness and (e) oiliness of sliced 'lombo' inoculated with protective LAB strains (*Lb. sakei* ST153 or BCL35) and packed under MA (20% CO_2/80% N_2 or 40% CO_2/60% N_2) during 120 days of storage at 5 °C. Columns are 15-panel score average and bars represent the confidence interval of 95%. Scale: a discrete 13-point intensity scale. Columns with different lower case letters (a, b, c) mean there are significant differences ($p < 0.05$) between treatments; columns with different upper case letters (A, B, C, D) mean there are significant differences ($p < 0.05$) between times of storage.

that ham slices MA packaged led to sensory changes caused by low pH due to the presence of CO_2 in the protective atmosphere (Cilla et al., 2006).

The intensity scores of the five attributes that were considered by panellists statistically influenced by LAB strains (oiliness, hardness, succulence, characteristic flavour and bitter taste) are presented in Figure 2. The reference value of control (commercial sample) for each attribute, corresponding to a score of 7, is noted in Figure 2. Results showed that during storage, oiliness, hardness, succulence and characteristic flavour attributes decreased whereas the bitter taste increased in both LAB applications (Figure 2) – this effect of LAB application being more significant at the end of storage time ($p < 0.05$). In general, samples with commercial BLC35 culture addition were considered harder and less succulent than those with *Lb. sakei* ST153 addition, but these differences were not statistically significant. This was in accordance with another study that reported samples containing BLC35 addition were categorized as harder and as less succulent than the ones containing *Lb. sakei* ST153 (Jácome et al., 2014). No significant differences regarding MAP conditions were found ($p < 0.05$).

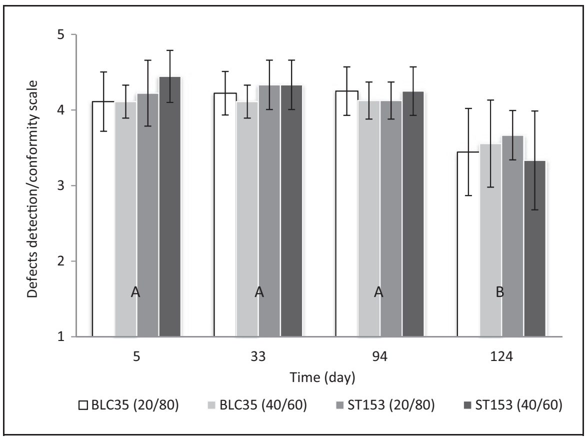

Figure 3. Defects/conformity evaluation of sliced 'lombo' inoculated with protective LAB strains (*Lb. sakei* ST153 or BCL35) packed under MA (20% CO_2/80% N_2 or 40% CO_2/60% N_2) during 120 days of storage at 5 °C. Columns are 15-panel score average and bars represent the confidence interval of 95%. Scale: a discrete 5-point scale with 5 – excellent quality; 4 – good quality; 3 – slight alteration; 2 – pronounced alteration; 1 – extreme alteration. Columns with different letters (A, B) mean there are significant differences ($p < 0.05$) between times of storage.

The results of defect detection and conformity test using a 5-point scale that allowed perceiving potential defects that were not expressed in the attributes are shown in Figure 3. No significant differences in conformity were found with respect to types of LAB and MAP conditions during storage. The effect of time of storage was only significant at the last sampling day (day 124), but nevertheless all samples were scored higher than the limit defined as 3. Studies using the same strain *Lb. sakei* ST153, inoculated in a smoked minced product 'Alheira', compared with the commercial sample without LAB addition and packed under MAP (20% CO_2 for just 15 days), found significant

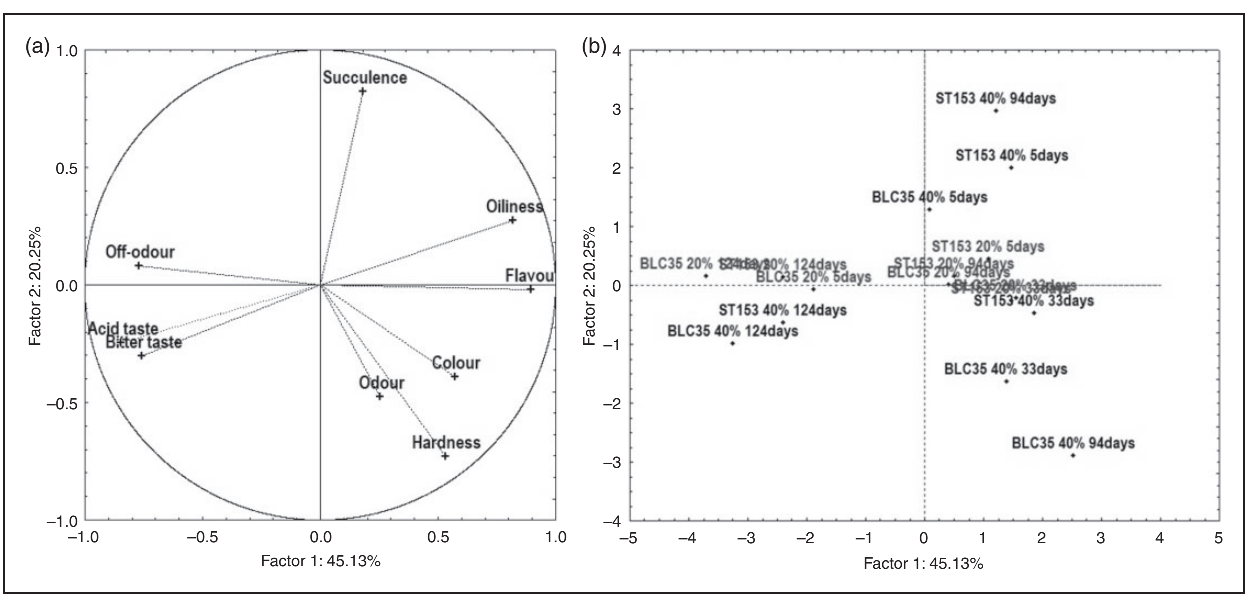

Figure 4. Principal component analysis of sensory evaluation of sliced 'lombo' inoculated with protective LAB strains (*Lb. sakei* ST153 or BCL35) packed under MA (20% CO_2/80% N_2 or 40% CO_2/60% N_2) during 120 days of storage at 5 °C. (a) Projection of variables and (b) projection of cases.

differences only for mass connection, acid taste and atypical taste attributes, but despite that samples were scored above conformity limit (Vaz-Velho et al., 2013). Another study, using the same strains, but added to another product packed under MA (8 or 12% CO_2), showed that conformity was not influenced by the type of starter culture, application methodologies and/or MAP conditions during 120 days of storage (Jácome et al., 2014).

A principal component analysis was further used to ascertain the components (directions) that maximized the variance in the whole data to find the axes with maximum variances (where the data are most spread) (Figure 4). At later storage times it was noticeable that in 40% CO_2/60% N_2 MAP condition the attributes were more extreme and more marked than in 20% CO_2/80% N_2 MAP condition; however, those differences were not significant (Figure 4(a) – projection of variables). Parra et al. (2012) evaluated the effect of different concentrations of CO_2 (20, 30 and 40%) in physicochemical, microbiological and sensorial characteristics of cured ham for 120 days, and no significant differences were found except for colour parameter at 30 and 40% CO_2 concentrations.

It was also noticeable that all positive attributes are grouped in the first and fourth quadrants whereas the negatives attributes are placed in the second and third quadrants; this meaning that the panel had consistently distinguished the product characteristics (Figure 4(a)).

In Figure 4(b) projection of the cases, it is possible to visualize which products were more valorized by the panellists. The points corresponding to the longer storage time and less quoted (124 days) are placed in the second and third quadrants, connoted with negative features and concentred in the axis of factor 2. These results are consistent with defects/conformity evaluation results (Figure 3), and those analyses being independent, it is possible to conclude the panel had consistently valorized products features and their conformity.

CONCLUSIONS

Lb. sakei ST153 combined with MAP conditions of 40% CO_2/60% N_2 reduced *L. innocua* 2030c counts in 5.0 log CFU/g during storage of 'lombo' at 5 °C for 120 days. Despite the reduction of 2.0 log CFU/g induced by the commercial culture (BLC35) at the early stages of storage, at the end of the product shelf-life no significant differences were found compared to the control samples. No differences in conformity were found with respect to LAB addition and MAP during storage and the effect of time of storage was only significant at the last sampling day (day 124) but even at that day all the samples were scored above 3, the considered conformity level. Thus, *Lb. sakei* ST153, an autochthonous strain of cured-smoked pork products, combined with MAP, is a strong candidate to be used in a biopreservation strategy maintaining traditional technological features and sensory quality of cured-smoked pork products and increasing their safety with respect to *Listeria* spp.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by National Funds from FCT – Fundação para a Ciência e a Tecnologia through project ID/Multi/50016/2013 and through project "Biological tools for adding and defending value in key agro-food chains (bio – n2 – value)", n° NORTE-01-0145-FEDER-000030, funded by Fundo Europeu de Desenvolvimento Regional (FEDER), under Programa Operacional Regional do Norte – Norte2020. Additional support was provided by Sistema de Incentivos à Investigação e Desenvolvimento Tecnológico (SI I&DT), Project no. 13338 BIOFUMADOS, promoted by Minhofumeiro, Enchidos artesanais. Dr Rocío Casquete is the beneficiary of a post-doctoral grant (PO12018) funded by the Junta de Extremadura, Consejería de Empleo, Empresa e Innovación, Spain, and co-funded by Fondo Social Europea (FSE).

ORCID ID

Susana C Fonseca http://orcid.org/0000-0003-4793-5965
Ricardo Pinto http://orcid.org/0000-0001-8480-9525

REFERENCES

Albano H, Oliveira M, Aroso R, Cubero N, Hogg T and Teixeira P. (2007). Antilisterial activity of lactic acid bacteria isolated from "Alheiras" (traditional Portuguese fermented sausages): In situ assays. *Meat Science* 76: 796–800.

Alves VF, Lavrador MAS and Martinis ECP. (2003). Bacteriocin exposure and food ingredients influence on growth and virulence of *Listeria monocytogenes* in a model meat gravy system. *Journal of Food Safety* 23: 201–217.

Baracat RS, Luchiari-Filho A, Pereira ASC, Silva SL and Cesar ASM. (2005). Effects of modified atmosphere packaging in preserving portioned bovine meat. In: *Proceedings of the 42nd annual reunion of the Brazilian Society of Animal Science*, Goiânia, Brazil, pp.42.

Borch E and Arinder P. (2002). Bacteriological safety issues in red meat and ready-to-eat meat products, as well as control measures. *Meat Science* 62: 381–390.

Castellano P, Belfiore C, Fadda S and 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.

Chaillou S, Champomier-Verges MC, Cornet M, Crutz-Le Coq AM, Dudez AM, Martin V, et al. (2005). The complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K. *Nature Biotechnology* 23: 1527–1533.

Cilla I, Martínez L, Beltran JA and Roncales P. (2006). Dry-cured ham quality and acceptability as affected by the preservation system used for retail sale. *Meat Science* 73: 581–589.

Duffy LL, Vanderlinde PB and Grau FH. (1994). Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: Effect of pH, aw, nitrite and ascorbate. *International Journal of Food Microbiology* 23: 377–390.

Fadda S, López C and Vignolo G. (2010). Role of lactic acid bacteria during meat conditioning and fermentation: Peptides generated as sensorial and hygienic biomarkers. *Meat Science* 86: 66–79.

Farber JM. (1991). Microbiological aspects of modified-atmosphere packaging technology: A review. *Journal of Food Protection* 54: 58–70.

Fontana C, Fadda S, Cocconcelli PS and Vignolo G. (2012). Lactic acid bacteria in meat fermentations. In: Lahtinen S, Ouwehand AC, Salminen S and von Wright A (eds) *Lactic Acid Bacteria – Microbiological and Functional Aspects*, 4th ed. Boca Raton, London, New York: CRC Press, pp. 247–264.

García de Fernando GD, Nychas GJE, Peck MW and Ordóñez JA. (1995). Growth/survival of psychrotrophic pathogens on meat packaged under modified atmospheres. *International Journal of Food Microbiology* 28: 221–231.

Gudbjornsdottir B, Suihko ML, Gustavsson P, Thorkelsson G, Salo S, Sjoberg AM, et al. (2004). The incidence of *Listeria monocytogenes* in meat, poultry and seafood plants in Nordic countries. *Food Microbiology* 21: 217–225.

Holzapfel WH, Geisen R and Schillinger U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food grade enzymes. *International Journal Food Microbiology* 24: 343–362.

Hudson JA, Mott SJ and Penney N. (1994). Growth of *Listeria monocytogenes, Aeromonas hydrophila*, and *Yersinia enterocolitica* on vacuum and saturated carbon dioxide controlled atmosphere packaged sliced roast beef. *Journal of Food Protection* 57: 204–208.

Hugas M. (1998). Bacteriocinogenic lactic acid bacteria for the biopreservation of meat and meat products. *Meat Science* 49: 139–150.

Hugas M, Pagés F, Garriga M and Monfort JM. (1998). Application of the bacteriocinogenic *Lactobacillus sakei* CTC494 to prevent growth of *Listeria* in fresh and cooked meat products packaged with different atmospheres. *Food Microbiology* 15: 639–650.

Jácome SL, Fonseca S, Pinheiro R, Todorov SD, Noronha L, Silva J, et al. (2014). Effect of lactic acid bacteria on quality and safety of ready-to-eat sliced cured/smoked meat products. *Chemical Engineering Transactions* 38: 403–408.

Jones RJ, Wiklund E, Zagorec M and Tagg JR. (2010). Evaluation of stored lamb biopreserved using a three-strain cocktail of *Lactobacillus sakei*. *Meat Science* 86: 955–959.

Kaban G, Kaya M and Lucke FK. (2010). The effect of *Lactobacillus sakei* on the behavior of *Listeria monocytogenes* on sliced bologna-type sausages. *Journal of Food Safety* 30: 889–901.

Kroückel L. (2013). The role of lactic acid bacteria in safety and flavour development of meat and meat products. In: Marcelino Kongo J (ed.) *Lactic Acid Bacteria – R&D for Food, Health and Livestock Purposes*. Croatia: Intech, pp. 129–152.

Liserre AM, Landgraf M, Destro MT and Franco BDGM. (2002). Inhibition of *Listeria monocytogenes* by a bacteriocinogenic *Lactobacillus sake* strain in modified atmosphere-packaged Brazilian sausage. *Meat Science* 61: 449–455.

Lucera A, Costa C, Conte A and Del Nobile MA. (2012). Food applications of natural antimicrobial compounds. *Frontiers Microbiology* 3: 287.

Marcos B, Jofré A, Aymerich T, Monfort JM and Garriga M. (2008). Combined effect of natural antimicrobials and high pressure processing to prevent *Listeria monocytogenes* growth after a cold chain break during storage of cooked ham. *Food Control* 19: 76–81.

Melero B, Diez AM, Rajkovic A, Jaime I and Rovira J. (2012). Behaviour of non-stressed and stressed *Listeria monocytogenes* and *Campylobacter jejuni* cells on fresh chicken burger meat packaged under modified atmosphere and inoculated with protective culture. *International Journal of Food Microbiology* 158: 107–112.

Nilsson L, Chen Y, Chikindas ML, Huss HH, Gram L and Montville TJ. (2000). Carbon dioxide and nisin act synergistically on *Listeria monocytogenes*. *Applied and Environmental Microbiology* 66: 769–774.

Parra V, Viguera J, Sanchez J, Peinado J, Esparrago F, Gutierrez JI, et al. (2012). Effect of exposure to light on physico-chemical quality attributes of sliced dry-cured Iberian ham under different packaging systems. *Meat Science* 90: 236–243.

Pérez-Rodríguez F, Valero A, Todd ECD, Carrasco E, García-Gimeno RM and Zurera G. (2007). Modelling transfer of *Escherichia coli* O157:H7 and *Staphylococcus aureus* during slicing of cooked meat product. *Meat Science* 76: 692–699.

Rubio B, Martínez B, González-Fernández C, García-Cachan MD, Rovira J and Jaime I. (2007). Effect of modified atmosphere packaging on the microbiological and sensory quality on a dry cured beef product: "Cecina de leon". *Meat Science* 75: 515–522.

Simpson R, Acevedo C and Almonacid S. (2009). Mass transfer of CO_2 in MAP systems: Advances for non-respiring foods. *Journal of Food Engineering* 92: 233–239.

Sørheim O, Nissen H and Nesbakken T. (1999). The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. *Meat Science* 52: 157–164.

Stiles ME. (1996). Biopreservation by lactic acid bacteria. *Antonie Van Leeuwenhoek* 70: 331–345.

Todorov SD, Vaz Velho M, Franco BDGM and Holzapfel WH. (2013). Partial characterization of bacteriocins produced by three strains of *Lactobacillus sakei*, isolated from *salpicão*, a fermented meat product from North-West of Portugal. *Food Control* 30: 111–121.

Vaz-Velho M, Fonseca F, Silva MV and Gibbs P. (2001). Is *Listeria innocua* 2030c tetracycline resistant, a suitable marker for replacing *Listeria monocytogenes* in challenge studies with Portuguese cold-smoked fish? *Food Control* 12: 361–364.

Vaz-Velho M, Jácome S, Noronha L, Todorov SD, Fonseca S, Pinheiro R, et al. (2013). Comparison of antilisterial effects of two strains of lactic acid bacteria during processing and storage of a Portuguese salami-like product ‘‘alheira’’. *Chemical Engineering Transactions* 32: 1807–1812.

Vermeiren L, Devlieghere F, Vandekinderen I, Rajtak U and Devebere J. (2006). The sensory acceptability of cooked meat products treated with a protective culture depends on glucose content and buffering capacity: A case study with *Lactobacillus sakei* 10A. *Meat Science* 74: 532–545.