05. Effect of protective cultures and different modified atmosphere packaging on *Listeria innocua* growth and on sensory properties in sliced cured-smoked pork loin

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Abstract

This study aims to evaluate the antimicrobial effect of two protective cultures combined with different modified atmosphere packaging (MAP) systems on *Listeria innocua* growth in sliced ready-to-eat pork loin, a Portuguese traditional cured-smoked product (Lombo). Two protective lactic acid (LAB) cultures - *Lactobacillus sakei* ST153 and BLC35 (CHR Hansen) were tested for their ability against *L. innocua* 20130c growth (as a surrogate for *L. monocytogenes*) in sliced "lombo" packed in two MAP conditions, (20%CO_2/80%N_2 and 40%CO_2/60%N_2) and stored at 5ºC. The influence of MAP and protective cultures in the sensory characteristics of the product was also evaluated by semi-trained panel of fifteen judges. The MAP affected the growth of *L. innocua*, the *Listeria* population decreasing 3 log CFU/g after 120 days of storage at 5ºC. In samples containing protective cultures a reduction of 1–2 log CFU/g in counts of *L. innocua* was observed after 12 hours. At the end of storage results indicated that *L. sakei* ST 153 was more efficient than BLC35 culture on inhibiting *L. innocua* growth and this inhibition was enhanced by MA (40%CO_2/60%N_2). Results of sensory evaluation showed that oiliness, hardness, succulence, and characteristic taste attributes of "lombo" decreased during storage whereas the bitter taste increased in both LAB applications and no significant differences between LAB cultures or MAP conditions were found.

Introduction

Listeria monocytogenes is one of the most important psychrotrophic foodborne pathogens related to anaerobically packed lightly cooked or cured-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 ready-to-eat fermented meats products requires improvements in packaging and preservation methods maintaining the freshness, quality and safety of foods *Lactobacillus* species represent the dominant LAB strains currently found in meat starter cultures (Chaillou et al., 2005). One of the most efficient technologies used for product preservation is Modified Atmosphere Packaging (MAP) combined with refrigeration. Several studies have shown that MAP of many types of meat products interferes with the survival and growth of *L. monocytogenes* (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). Lactic acid bacteria naturally dominate the 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).

The aims of this study were to evaluate the antimicrobial effect of two protective cultures combined with different MAP conditions enriched in CO_2 on survival of *Listeria innocua* 2030c (as a surrogate for *L. monocytogenes*), and on the sensory properties of sliced ready-to-eat "lombo", a Portuguese cured-smoked pork loin.

Material and Methods

"Lombo" was produced and sliced by an industrial meat company and subsequently transferred to the laboratory under refrigerated conditions. *Lactobacillus sakei* ST153Ch was sub-cultured twice (1% v/v inoculum; 24 h at 37 °C) in 10 mL MRS broth and *L. innocua* in TSB broth. The cells were harvested under aseptic conditions by centrifugation (6000 x g for 10 min) at room temperature. The cell pastes obtained were washed twice by centrifugations, and re-suspended in sterilized water before being inoculated onto the product slices. BLC35 culture was used as recommended by the manufacturers (commercial mixed starter culture including strains of *Lb. curvatus*, *S. xylosus* and *P. acidilactici*; CHR Hansen). L. innocua (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 (10^9 CFU/mL) were inoculated onto the slices by immersion with a subsequent air drying stage. Slices were placed in trays (AERpack, B22- 50, COOPBOX Hispania SLU, Spain), covered with a high barrier covering film (OPEX 55 AB PA/EVOHBarrier/PE, Boulanger SAS, France) and heat sealed (Oceania Jolly 20/40, Yang C.R.L., Italy). Twelve batches were obtained by combining the two modified atmospheres (20%CO_2/80%N_2 and 40%CO_2/60%N_2) with the six treatments: (1) uninoculated slices as control (C), (2) slices inoculated with *L. innocua* (C+L), (3) slices inoculated with BLC35 (BLC35), (4) slices inoculated with BCL35 plus *L. innocua* (BCL35+L), (5) slices inoculated with *Lb. sakei* ST153Ch (ST 153), and (6) slices inoculated with *Lb. sakei* ST153Ch plus *L. innocua* (ST153+L). Samples were taken from each batch at the following times of storage: 0 and 12 h, and after 15, 30, 90 and 120 days of storage. Each trial was performed at least in duplicate. For sensory evaluation a sheet with nine attributes ("meat colour", "oiliness", "characteristic odour", "off-odour", "hardness", "succulence", "characteristic flavour", "acid taste", "bitter taste"), each one with a discrete 13-point scale, was established and given to the panelists (semi-trained panel of 15 elements) that evaluated samples at days 5, 33, 94 and 124 of storage (3 to 5 days after the corresponding microbiological sampling times).

Results

The results of microbiological analysis are showed in Figure 1. In the presence of LAB strains (Lb. sakei ST153Ch and BCL35 commercial sample), the *L. innocua* levels decreased between 1-2 Log CFU/g at the beginning of storage. At the end of storage, the combination *Lb. sakei* ST153Ch and MAP conditions of 40%CO_2/60%N_2, was the most effective on controlling *L. innocua* growth in sliced "lombo".

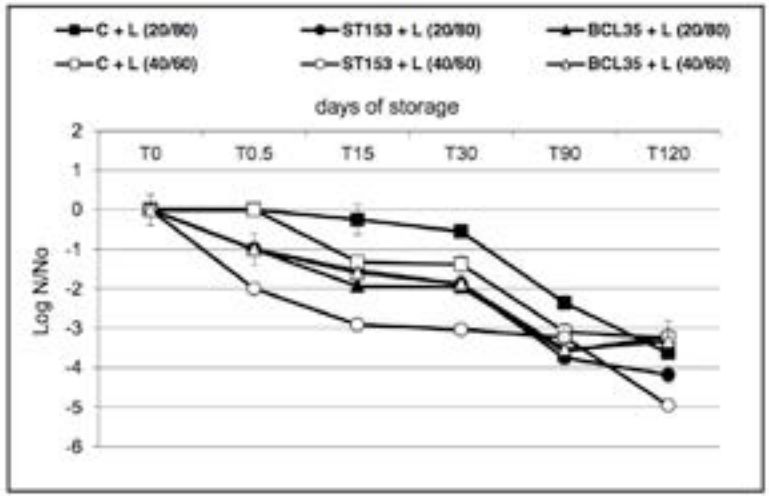

Figure 1. Effect of LAB cultures (*Lb. sakei* ST153Ch and BCL35) and MAP (20%CO_2/80%N_2 and 40%CO_2/60%N_2) on *L. innocua* numbers in sliced lombo during 120 days of storage at 5°C

Sensory evaluation results are presented in Figure 2. The reference value of control (commercial sample) for each attribute, corresponding to a score of 7, is also noted in Figure 2.

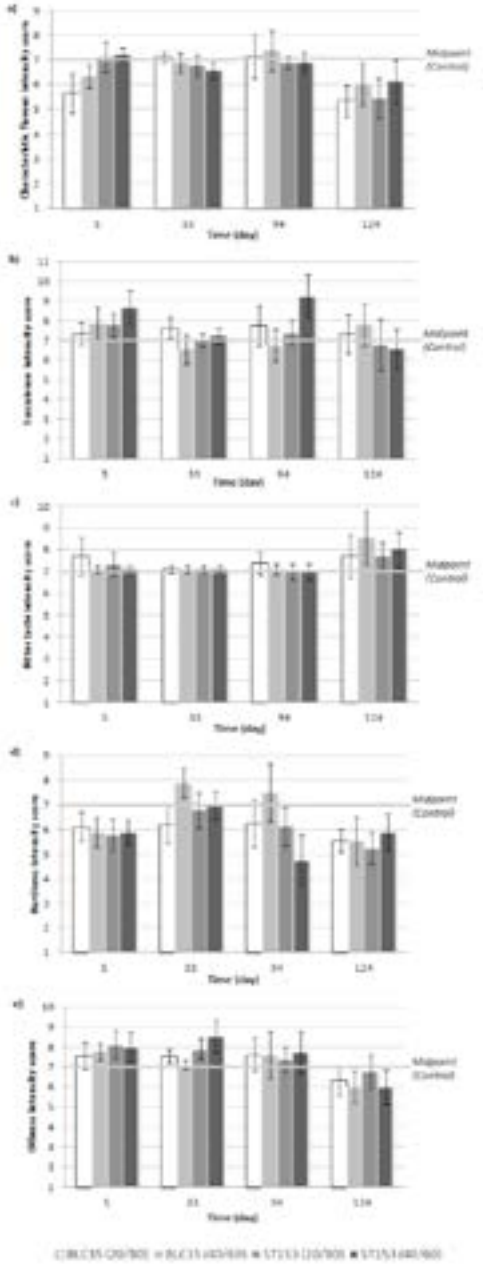

Figure 2- Sensory evaluation of a) characteristic flavour, b) succulence, c) bitter taste, d) hardness and e) oiliness of sliced "lombo" with protective LAB strains addition (BLC35 or ST153) packed under MAP (20%CO_2/80%N_2 or 40%CO_2/60%N_2) during 124 days of storage at 5°C.

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 panelists did not notice this compared to the control commercial samples. Results showed that, during storage, oiliness,

hardness, succulence, and characteristic flavour attributes decreased whereas the bitter taste increased in both LAB applications, this effect of LAB application being significant at the end of storage time ($p < 0.05$). In general, samples with commercial culture BLC35 addition were considered harder and less succulent than those with *Lb. sakei* ST153Ch addition, but these differences were not statistically significant. A conformity evaluation of the samples, using a 5- point hedonic scale that allowed perceiving potential defects that were not expressed in the attributes (data not shown) scored all samples above the conformity level (>3) during the 124 days of storage.

Discussion

The antibacterial spectrum of activity of *Lb. sakei* ST153Ch has been previously studied and results indicated their potential for use in a mixed starter culture for the fermentation of meat products (Todorov et. al., 2013). In the present study the best strain with respect to *Listeria* safety was *Lb. sakei* ST153Ch. Another study, using the same strains but added to another cured-smoked pork product, reported samples containing BLC35 being harder and less succulent than the ones containing *Lb. sakei* ST153Ch although the "conformity" attribute was not influenced by the type of starter culture and MAP conditions during 120 days of storage. (Jácome et al., 2014).

Conclusion

Lactobacillus sakei ST153Ch, an autochthonous strain of Portuguese cured-smoked pork products, combined with MAP, can be regarded as an effective tool for increasing safety in ready-to-eat sliced cured-smoked products with respect to *Listeria* spp.

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