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Effect of Lactic Acid Bacteria on Quality and Safety of Ready-to-eat Sliced Cured/Smoked Meat Products

Samuel L. Jácome[a], Susana Fonseca[a,b], Rita Pinheiro[a], Svetoslav Dimitrov Todorov[c], Lucia Noronha[d], Joana Silva[d], Ana Gomes[d], Manuela Pintado[d], Alcina M. M. B. Morais[d], Paula Teixeira[d], Manuela Vaz-Velho[a*]

[a] Escola Superior de Tecnologia e Gestão – Instituto Politécnico de Viana do Castelo, Avenida do Atlântico s/n, 4900-348 Viana do Castelo, Portugal;
[b] REQUIMTE, Universidade do Porto, Portugal;
[c] Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo – SP, Brasil;
[d] CBQF-Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Portugal
mvazvelho@estg.ipvc.pt

The bioprotective properties of native lactic acid cultures and their bacteriocins may be used for increasing the microbiological safety and stability of traditional sliced smoked pork products. It is expected that this addition would not affect consumer's perception and, consequently, the acceptability of these traditional smoked meat products. The effect of two different LAB cultures, two different application methods and two different modified atmosphere packaging conditions (MAP) on growth control of *Listeria monocytogenes* and on sensory properties of "Chouriço", a sliced cured/smoked pork product, were studied.
Lactobacillus sakei ST153 (autochthonous bacteria) and BLC35 (commercial mixed starter culture including strains of *Lactobacillus curvatus, Staphylococcus xylosus and Pediococcus acidilactici*; CHR Hansen) both with bacteriocinogenic activity against *Listeria monocytogenes*, were applied by immersion or spray on smoked pork slices that were produced at industrial scale and packed under MAP (8 % or 12 % v/v CO_2) and stored at 5 ^{0}C. Previously to the incorporation of LAB cultures, *L. monocytogenes* was gently spread with a sterile cotton swab into the slices. The enumeration of LAB and *L. monocytogenes* was performed during the storage at 5 ºC.
A quantitative descriptive sensory test was performed by a sensory trained panel at 30, 90 and 120 days, involving previous sessions for main descriptors definition, their scale limits as well as verbal anchors by panel consensus. A final sheet with nine descriptors (meat colour, greasiness, characteristic odour, off-odour, hardness, succulence, characteristic taste, acid taste, bitter taste), each one with a 13-point scale, was validated.
Listeria monocytogenes decreased to values <100 CFU/g in smoked pork slices of both application techniques. Meat colour, succulence and characteristic taste were the common attributes for both LAB cultures that varied over the shelf life. The application method had no significant effect on any of the sensorial attributes analyzed. MAP influence was only noticed in terms of greasiness and hardness. The samples containing BLC35 addition were categorized as harder and as less succulent than the ones containing *L. sakei* ST153.

1. Introduction

Cured/smoked pork products play an important role in traditional Portuguese meals. The appealing sensory attributes of these traditional products must fit currently consumers demand for convenience products, thus leading to an increased interest in sliced products with an extended shelf-life. Thinly sliced and ready-to-eat

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food items are very appealing to a large market segment, but these features make products more susceptible to quality alterations (Lucera et al., 2012).
Emergent preservation techniques like biopreservation or biocontrol, through introduction of natural substances, are interesting technologies to create adverse conditions for microbial growth (Lucera et al, 2012). The use of bioprotective cultures of lactic acid bacteria (LAB) and their bacteriocins in the production and preservation of ready-to-eat meat products, is a methodology that has been studied as an alternative to chemical additives for assuring food safety (Gálvez et al., 2007). These bacteria can be isolated from the very same product and reintroduced into the process, having two simultaneous actions: controlling the growth of undesirable bacteria as well as contributing to the improvement of sensory characteristics (Bello et al., 2010). This biological control also allows to reduce the amount of salt, nitrite and other additives required to effectively preserve food (Gálvez et al., 2007)
Modified atmosphere packaging (MAP) is a well-known preservation technique for meat and meat-based products, due to the reduction/exclusion of oxygen (O_2) and the increase of carbon dioxide (CO_2) content (Sebranek and Houser, 2006). MAP is defined as "the packaging of a perishable product in an atmosphere which has been modified so that its composition is other than that of air" (Hintlian and Hotchkiss, 1986). MAP consists of a barrier-film package, where air is removed, and the package is filled with a predetermined gas (or mixture of gases), with a composition different than air, followed by sealing of the package (Rao and Sachindra, 2002).
The general objective of the present study was to obtain an effective antilisterial agent which doesn't affect consumer's perception leading to the acceptability of these traditional cured meat products.

2. Materials and methods

2.1 Samples preparation

A ready-to-eat sliced cured/smoked pork product, known as "Chouriço", was produced by an industrial meat company and subsequently transferred to the laboratory under refrigerated conditions.
Lactobacillus sakei ST153, isolated from a cured/smoked pork product, was sub-cultured twice (1 % v/v inoculum; 24 h at 30 ºC) in 10 mL MRS broth (Pronadisa) and *L. monocytogenes* in TSB broth (Lab M). The cellular suspensions were then washed twice and resuspended in sterilized water before being inoculated in the product. BLC35 culture (commercial mixed starter culture including strains of *Lactobacillus curvatus, Staphylococcus xylosus* and *Pediococcus acidilactici*; CHR Hansen) was used as recommended by the manufacturers.
Listeria monocytogenes (10^4 CFU/mL), also isolated from a cured/smoked meat product, 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 two methods: spray and immersion, with a subsequent air drying stage.
Samples were stored at 5 ºC for 120 days. After inoculation (day 0) and after 15, 30, 60 and 120 days of storage, samples were analysed for growth of the inoculated strains. The experimental conditions were: (1) uninoculated slices as control, (2) slices inoculated with *L. monocytogenes*, (3) slices inoculated with BLC35, (4) slices inoculated with ST153, (5) slices inoculated with *L. monocytogenes* and BLC35 and (6) slices inoculated with *L. monocytogenes* and ST153. Each trial was performed at least in duplicate.

2.2 Microbiological sampling

Twenty-five gram of each trial sample were added to 225 mL of sterile buffered peptone water (Merck), and homogenized in a stomacher for 1 min. Appropriate decimal dilutions were prepared in sterile Ringer's solution (Lab M) for LAB and *L. monocytogenes* enumeration: 20 µL samples of the appropriate dilutions were spotted, in duplicate, on selective agar plates; MRSA (Pronadisa) and ALOA Agar (Biorad), respectively. Counting was performed after incubation at 30 ºC for 72 h under microaerophilic conditions for LAB, and at 30 ºC for 72 h for *L. monocytogenes*.

2.3 Sensory analysis

A quantitative descriptive analysis (QDA) was performed by a sensory trained panel with 9 elements at 30, 90 and 120 days, involving previous sessions for main descriptors definition, their scale limits as well as verbal anchors, using the very same type of commercial "Chouriço". A final sheet with 9 attributes (meat colour, greasiness, characteristic odour, off-odour, hardness, succulence, characteristic taste, acid taste, bitter taste), each one with a 13-point scale, was established. It was consensual that the reference value for each attribute corresponds to a score of 7, except for "off-odour " that was set as score 1. The effect of the two LAB treatments on the sensory profile of "Chouriço" packed under MAP was compared to the standard

commercial product. A general attribute of conformity was set using a 5-point hedonic scale that allowed perceiving potential defects that were not expressed in the attributes. A score 1-3 was assigned, depending on the severity of the defect, and 4 or 5 if the product was good or very good, respectively. All samples were packed under MAP (8 % or 12 % v/v CO_2) and analysed at 30, 90 and 120 days of storage at 5 ^{0}C. The results were subjected to analysis of variance (two-way ANOVA) using STATISTICA 7 and Microsoft® Office Excel 2007 tools.

3. Results and discussion

The aim of the present study was to compare the effect of the addition of two cultures of LAB, on the growth of *L. monocytogenes* and on the sensory properties of ready-to-eat sliced cured/smoked meat-products. In this study, the bacteriocin producer *Lactobacillus sakei* ST153, isolated from "Chouriço", and a commercial starter culture were investigated. The enumeration of LAB and *L. monocytogenes* in sliced "Chouriço" is illustrated in tables 1 and 2. In the absence of the bacteriocinogenic strains, *L. monocytogenes* was able to maintain initial levels until the end of storage. No influence in LAB populations was observed when inoculated or not with *L. monocytogenes (p<0.001).*

Both methods, spray and immersion, had a positive effect on the decrease of *L. monocytogenes* in the product (values lower than 100 CFU/g). Both *Lb. sakei* ST153 and BLC35 showed bacteriocinogenic activity against *L. monocytogenes*. The bacteriocin ST153 has been described as having a narrow spectrum of activity, do not adsorbing to the surface of the producer cell and being produced at higher levels during the stationary phase of fermentation. This bacteriocin is heat resistant, and stable between pH 2.0 and 10.0 (Todorov et al., 2013). This LAB strain was also tested in previous experiments in "Alheira", a sausage-like product (Vaz-Velho et al., 2013).

Table 1: Enumeration of Lb. sakei ST153 and L. monocytogenes immediately after the production (t=0 days) and after 30 days of storage at 5 ºC.

	Sample	Bacteria Enumeration	Day 0 (log CFU/g)	Day 30 (log CFU/g)
Immersion	**Control**	*L. monocytogenes*	4.5 ± 0.0	4.0 ± 0.1
	Lb. sakei* ST153 and *L. monocytogenes	*Lb. sakei* ST153	7.7± 0.2	8.3 ± 0.1
	Lb. sakei* ST153 and *L. monocytogenes	*L. monocytogenes*	4.3 ± 0.0	<1.8± 0.0
	Control	*Lb. sakei ST153*	7.8 ± 0.0	7.8 ± 0.1
Spray	**Control**	*L. monocytogenes*	4.5 ± 0.0	4.1 ± 0.1
	Lb. sakei* ST153 and *L. monocytogenes	*Lb. sakei* ST153	9.3 ± 0.4	8.3 ± 0.1
	Lb. sakei* ST153 and *L. monocytogenes	*L. monocytogenes*	4.1 ± 0.0	<1.8± 0.0
	Control	*Lb. sakei ST153*	8.1 ± 0.0	8.3± 0.1

In the presence of *Lb. sakei* ST153, irrespective of the inoculation method, *L. monocytogenes* decreased to acceptable values (<100 CFU/g) after 30 days of storage at 5 ºC (Table 1). The same tendency was observed when the BLC35 culture was inoculated by immersion (Table 2). When the culture was added by spray, *L. monocytogenes* decreased to values <1.8 CFU/g at sampling point taken at day 45 (data not shown). These results differ from those reported by Albano et al. (2007), who demonstrated that the inhibition of Listeria occurred early after production. This was probably due to the presence of chemical compounds in "Chouriço", which probably reduced the rate of bacteriocin production. Albano et al. (2007) worked with "Alheira", a fermented sausage produced without the addition of any chemical preservative.

Results showed that “meat colour”, “succulence” and “characteristic taste” were the common attributes for both LAB cultures, which varied significantly over the shelf life ($p < 0.05$). For the attributes “meat colour” and “succulence”, the sample with *L. sakei* ST153 was more similar to the control in the three sampling periods. The “characteristic taste” was more similar for sample with BLC35 addition, compared to “Chouriço” control. The results revealed a decrease on “meat colour” over shelf life for both LAB conditions.

Table 2: Enumeration of BLC35 and L. monocytogenes immediately after the production (t=0 days) and after 30 days of storage at 5 ºC.

	Sample	Bacteria Enumeration	Day 0 (log CFU/g)	Day 30 (log CFU/g)
Immersion	**Control**	*L. monocytogenes*	4.5 ± 0.0	4.0 ± 0.1
	BLC35* and *L. monocytogenes	*BLC35*	7.1± 0.1	7.4 ± 0.1
	BLC35* and *L. monocytogenes	*L. monocytogenes*	4.3 ± 0.0	<1.8± 0.0
	Control	*BLC35*	7.6 ± 0.0	7.4 ± 0.1
Spray	**Control**	*L. monocytogenes*	4.5 ± 0.0	3.0 ± 0.1
	BLC35* and *L. monocytogenes	*BLC35*	7.8 ± 0.4	6.9 ± 0.1
	BLC35* and *L. monocytogenes	*L. monocytogenes*	4.3 ± 0.0	3.0 ± 0.0
	Control	*BLC35*	7.8 ± 0.0	7.0± 0.1

The sensory profiles of “Chouriço”, according to the two starter cultures along the storage period are presented on Figure 1 (a and b). Results showed that “meat colour”, “succulence” and “characteristic taste” were the common attributes for both LAB cultures that significantly varied along storage ($p < 0.05$).
For “meat colour” and “succulence”, the sample with *Lb. sakei* ST153 was more similar to the control sample in the three periods of sampling. The “characteristic taste” was more similar for sample with BLC35 addition, compared to the commercial “Chouriço”. The results showed some decrease on “meat colour” along storage period in both LAB conditions.
Figure 1c) shows the sensory profiles of both LAB regarding the two application methods. No significant effect was detected by the panel for each method on all attributes ($p > 0.05$), therefore the application methodology of LAB in this type of product did not affect the sensory properties. This suggests that the industry may be able to choose the most appropriate technique according to their manufacture process.
MAP had influence only noticed by panellists in terms of “greasiness” and “hardness”, for both LAB cultures ($p < 0.05$) (Figure 1 d). These results showed that both cultures, irrespectively of the type of application, were perceived more similar and distinguished from the control sample in terms of “greasiness” and “hardness“ when packed with 12% of CO_2.
The overall sensory evaluation using the “conformity” attribute (scale1-5) allows the panel to describe some remarkable perception that might not be reflected earlier just with the nine independent attributes. These results are listed on Table 3 (two types of cultures), Table 4 (both cultures under two different application methods) and Table 5 (both cultures under two different MAP conditions).
Results showed that “conformity” attribute was not influenced by the type of starter culture, application methodologies and/or MAP conditions, and also that a good “conformity” was found along the analysed storage period.

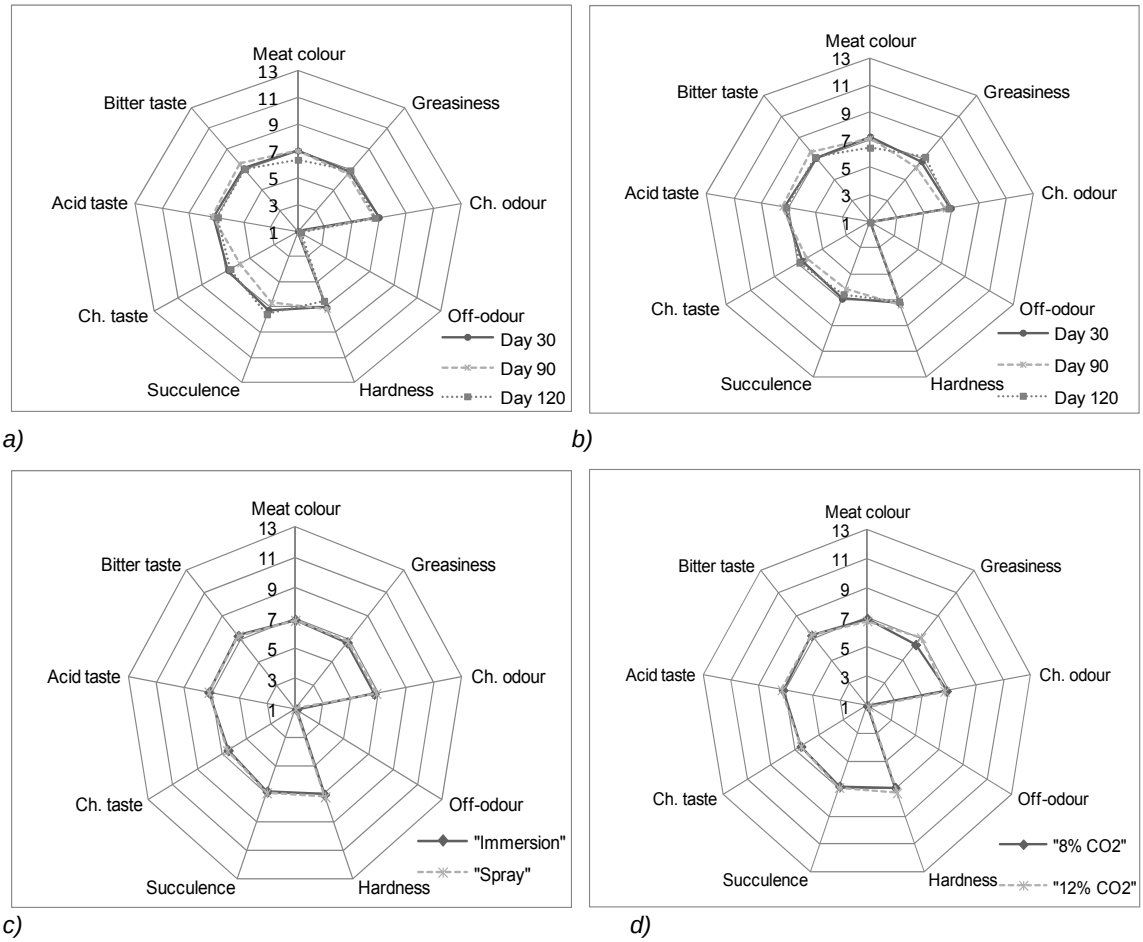

Figure 1: Sensory profile of "Chouriço" during storage at 5 °C in an intensity scale of the main descriptors considered in a QDA for a) "Chouriço" with Lb. sakei ST153 b) Chouriço with BLC35 c) "Chouriço" with both LAB strains for each application methodology (immersion and spray); d) "Chouriço" with both LAB strains under different MAP.

Table 3: Mean panel valorisation (± standard deviation) of conformity attribute for each type of starter culture.

Storage time (days)	Sample + *Lb. sakei* ST153	Sample + BLC35
30	4.2(±0.6)	4.1(±0.7)
90	4.0(±0.5)	4.1(±0.6)
120	4.2(±0.7)	4.3(±0.6)
p-value	**0.064**	**0.559**

Table 4: Mean panel valorisation (± standard deviation)) of conformity attribute, with both starter cultures, for each application methodology.

Storage time (days)	Immersion	Spray
30	4.1±(0.7)	4.2±(0.6)
90	4.1±(0.5)	4.0±(0.6)
120	4.2±(0.7)	4.3±(0.6)
p-value	**0.739**	

Table 5: Mean panel valorisation (± standard deviation) () of conformity attribute, with both starter cultures, for each MAP condition.

Storage time (days)	8 % (v/v) CO_2	12 % (v/v) CO_2
30	4.2±(0.7)	4.2±(0.7)
90	4.1±(0.5)	4.0±(0.6)
120	4.3±(0.5)	4.2±(0.7)
p-value	**0.900**	

4. Conclusions

Concerning the activity of the added cultures against *L. monocytogenes*, a decrease of more than two log cycles in the number of viable cells of the pathogen was observed but only after 30 or 45 days of storage, depending on the culture and method of addition.

The results obtained revealed that the panel only detected differences in a few attributes under MAP conditions, but no differences with respect to the application methods. In general, the ready-to-eat sliced cured/smoked product treated with *Lb. sakei* ST153, and packed with a12 % (v/v) of CO_2, seemed to be the most similar to the commercial product, and also that the application method did not significantly affect the sensory characteristics, in both cultures.

Results showed that “conformity” attribute was not influenced by the type of starter culture, application methodologies and/or MAP conditions, and also that a good “conformity” was found along the storage period, in all analysed samples.

The results of this study are very promising regarding the application of these Lab starters as effective antilisterial agents once they did not affect consumer’s perception the acceptability of these traditional cured meat products not questionable.

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