



Effects of *Lactobacillus plantarum* Bacteriocinogenic Culture on Physicochemical, Microbiological, and Sensorial Characteristics of “Chouriço Vinha d’Alhos”, a Traditional Portuguese Sausage

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HIGHLIGHTS

- “Chouriço Vinha d’Alhos” produced with *Lactobacillus plantarum*, fresh or lyophilized, showed similar microbiological characteristics over storage.
- The presence of endogenous *Listeria monocytogenes* was detected until the end of storage in samples not inoculated.
- Fresh or lyophilized *Lb. plantarum* did not result in significant physicochemical differences during storage of the product.

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Acronyms and abbreviations

CFU=Colony Forming Unit
MRS=de Man, Rogosa Sharpe
PC=Principal Component
 a_w =Water Activity

ABSTRACT

Background: “Chouriço Vinha d’Alhos” is a traditional fermented dry meat sausage from North of Portugal. The aim of this work was to evaluate the effects of a fresh and a lyophilized bioprotective *Lactobacillus plantarum* ST153Ch culture, on an industrial scale, on the physicochemical, microbiological, and sensorial characteristics of “Chouriço Vinha d’Alhos”.

Methods: “Chouriço Vinha d’Alhos” added with *Lb. plantarum* ST153Ch (fresh or lyophilized) were analyzed for the physicochemical, microbiological, and sensorial characteristics, over 90 days of storage at 4 °C. All data were statistically analyzed using an ANOVA procedure by IBM SPSS Statistics v. 25.

Results: The results showed that there was no difference in the reduction of *L. monocytogenes*, without or with the addition of *Lb. plantarum* either fresh or lyophilized. There were no significant differences ($p>0.05$) in some analyzed physicochemical parameters of products added with fresh or lyophilized *Lb. plantarum* cultures over the 90 days of storage; but both, fresh and lyophilized cultures, influenced some of the tested physicochemical parameters.

Conclusion: Considering no significant differences between application methodologies (fresh or lyophilized *Lb. plantarum*), industry might be able to choose the most suitable method according to their manufacturing process.

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Introduction

Biocontrol methods have gained considerable attention as natural strategies to extend shelf-life and improve mi-

crobial safety of foods. Bacteriocinogenic bacterial cultures and/or their bacteriocins are relevant bioprotective

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approaches in order to prevent spoilage as well as pathogenic microorganisms (Albano et al., 2007a; Oliveira et al., 2018; Orihuel et al., 2018; Perez et al., 2014; Vaz-Velho et al., 2013). It has been also recommended to use some bacterial strains that are well adapted to the food matrices environment, preferably isolates from similar matrices, for optimal performance and bacteriocin production (Albano et al., 2007a; Mainar et al., 2016). Also, bioprotective culture should not significantly influence the sensory properties of any food, as it might reduce its acceptability (Vaz-Velho et al., 2013).

Lactobacillus plantarum ST153Ch, a bacteriocinogenic lactic acid bacterium, originally identified as *Lb. sakei* but subsequently re-identified as *Lb. plantarum*, is an autochthonous strain isolated from “Salpicão”, a traditional Portuguese salami-like meat product (Todorov et al., 2013). Vaz-Velho et al. (2013) showed the possibility of using this strain as a protective culture to improve the safety of fermented meat sausages with respect to *Listeria monocytogenes*. Todorov et al. (2013) showed that the bacteriocin obtained from *Lb. plantarum* (bacteriocin ST153Ch) is heat resistant which can be produced during the stationary phase of fermentation in the presence of 2% (w/v) D-Glucose. Also, Todorov et al. (2014) showed that it is a safe strain that could contribute to the safety of fermented food products.

“Chouriço Vinha d’Alhos” is a traditional fermented dry meat sausage from North of Portugal. These kind of meat products are mainly considered as microbiologically relatively safe products; this safety assurance is mainly due to antimicrobial impacts of multiple antimicrobial factors according to the so-called “hurdle concept” (Singh and Shalini, 2016). However, in cases of primary bacterial contamination of the raw materials and/or insufficient control of the antimicrobial factors, the safety of these meat products could be endangered. Some studies reported the presence of *L. monocytogenes* in some of these products which indicated that *L. monocytogenes* occurs due to poor hygiene in manufacturing (Ferreira et al., 2007).

Bacteriocin production is not always accomplished in complex food matrices such as fermented meat sausages. Some researchers have previously indicated the causes that may affect both bacteriocin production as well as activity in food matrices (Kouakou et al., 2016; Verluyten et al., 2004). Important aspects include i) the capacity of the strain in growing and producing bacteriocin *in situ*; ii) bacteriocin diffusion in meat; iii) hydrophobic content of the matrix; and iv) salt and nitrates concentration in meat (Kouakou et al., 2016; Verluyten et al., 2004).

The aim of this work was to evaluate the effects of a fresh and a lyophilized bioprotective *Lb. plantarum* ST153Ch culture, on an industrial scale, on the physico-

chemical, microbiological, and sensorial characteristics of “Chouriço Vinha d’Alhos”.

Materials and methods

Manufacture of “Chouriço Vinha d’Alhos” and sampling procedures

“Chouriço Vinha d’Alhos” samples were manufactured in a meat plant according to Portuguese traditional recipes and techniques. The used ingredients were mainly as follows: red meat and fat (89%), garlic, salt, spices, emulsifiers, antioxidant, and preservatives. The sausage mixture, before stuffing, was divided into three batches: one batch was inoculated with *Lb. plantarum* ST153Ch fresh (S2BLAF), another batch with *Lb. plantarum* ST153Ch lyophilized (S2BLA), and the other one was non-inoculated (S2BC) to act as a control. *Lb. plantarum* ST153Ch, fresh or lyophilized (prepared as indicated in section “preparation of fresh and lyophilized culture”) was added before stuffing, in order to reach $\sim 10^6$ Colony Forming Unit (CFU)/g in the final product (Vaz-Velho et al., 2013). Lyophilized culture was suspended in 3 L water and 6% sucrose (added to activate the culture) and then added to the fresh sausage mixture. Fresh culture was directly added to the sausage mixture. Sausages were smoked by natural convection at 12 °C for 15 days. Then, “Chouriço Vinha d’Alhos” samples were packed under modified atmosphere (20% CO₂ and 80% N₂) according to Vaz Velho et al. (2013); and the packs were stored at 4 °C for 90 days.

Microbiological procedures

Microbiological analyses were performed before smoking (-1 day), immediately after smoking (day 0) and at times 3, 7, 15, 21, 30, 60, and 90 days. For the other analytical procedures, sampling was performed at 0, 15, 30, 45, 60, 75, and 90 days of storage.

Microorganisms and growth conditions

Lb. plantarum ST153Ch was grown in de Man, Rogosa Sharpe (MRS) broth (Bury, UK) at 30 °C for 48 h. *L. monocytogenes* Scott A and *L. monocytogenes* 3701 (isolated from a fermented meat sausage) were included in the cocktail of *L. monocytogenes* used in the assay, grown in Tryptone Soy Broth (TSB; Biokar Diagnostics, Beauvais, France) supplemented with 0.6% (w/v) of yeast extract (TSBYE; LabM, Bury, UK) at 37 °C for 18 -22 h (Briers et al., 2011).

All bacterial strains were subcultured twice under appropriate conditions before use in experiments. All strains were stored at 20 °C in the presence of 30% (v/v) glycerol.

-Preparation of fresh and lyophilized culture

One L of MRS broth was inoculated (1% v/v) with *Lb. plantarum* ST153C and after 24 h at 30 °C (in order to obtain their maximum AU/ml); cells were centrifuged at 8000 rpm for 10 min at 4 °C. Cells were resuspended in 100 ml of sterilized deionized water, in order to have 10⁹ CFU/ml. For fresh culture, cells were kept at 4 °C until the inoculation procedure. It should be noted that the fresh culture was prepared in the same day of inoculation (Vaz-Velho et al., 2013).

For preparation of lyophilized culture, the method used was adapted from Barbosa et al. (2015): cells were initially frozen at -80 °C overnight, and then desiccated under vacuum (2 ATM) for 4 days in a freeze-drier (SB4 Armfield, UK) at room temperature; and the condenser was cooled at -48 °C.

-Antilisterial activity during growth of *Lb. plantarum* ST153Ch

One hundred ml of MRS broth was inoculated with 1% (v/v) of an overnight culture and incubated at 30 °C for 39 h. Samples were collected every 3 h for 39 h and *Lb. plantarum* ST153Ch were counted on MRS agar, incubated at 30 °C for 48 h. Changes in pH were also recorded every hour for 39 h. The antilisterial activity (AU/ml) was verified in the cell-free supernatant every 3 h during 39 h, as described by Van Reenen et al. (1998). *L. monocytogenes* Scott A and *L. monocytogenes* 3701 were used as target strains.

-Antilisterial activity of bacteriocinogenic *Lb. plantarum* ST153 culture in “Chouriço Vinha d’Alhos”

The antagonistic effect of the bacteriocinogenic *Lb. plantarum* ST153Ch strain (fresh or lyophilized) was studied against a cocktail of *L. monocytogenes* in an industry pilot scale. *L. monocytogenes* strains were subcultured twice (24 h at 37 °C) in TSB broth using a 1% v/v inoculums. Each culture was centrifuged and mixed in the same Ringer’s solution. An aliquot (300 µl) of the cocktail of *L. monocytogenes* suspension (10⁷ CFU/ml for each strain of *L. monocytogenes*) was inoculated with a sterilized syringe in 300 g of each sample of “Chouriço de Vinha d’Alhos”. This procedure was done after smoking, in the microbiology laboratory, in order to reach 10⁵ CFU/g of sample of “Chouriço Vinha d’Alhos”: fresh *Lb. plantarum* ST153Ch inoculated with cocktail of *L. monocytogenes* (S2BLAF+LM); another batch with lyophilized *Lb. plantarum* ST153Ch inoculated with cocktail of *L. monocytogenes* (S2BLA+LM) and the other batch, control one, was inoculated with cocktail of

L. monocytogenes (S2BC+LM).

-Determination of microorganisms

In this step, 225 ml of sterile buffered peptone water (Biokar Diagnostics, Beauvais, France) were added to 25 g of each sample, and homogenized in a stomacher for 2 min. For microbial enumeration, decimal dilutions were prepared in Ringer’s solution, according to ISO standards: LAB on de Man, Rogosa and Sharpe agar (MRS; Biokar Diagnostics; Beauvais, France (ISO, 1998) incubated at 30 °C for 72 h; Enterobacteriaceae on RAPID[®] Enterobacteriaceae medium (Bio-Rad, CA, USA; ISO 16140, 2016) and *Staphylococcus aureus* on Baird Parker agar (Bio-Rad, CA, USA; ISO 6888-1, 1999), both incubated at 37 °C for 48 h; *Escherichia coli* on Tryptone Bile X-glucuronide agar (Bio-Rad Diagnostics, Beauvais, France; ISO 16649-2, 2001) incubated at 44 °C for 24 h; *Bacillus cereus* on Mannitol Egg Yolk Polymyxin agar (VWR International, Pennsylvania, USA; ISO 7932, 2004), and yeasts and molds on Rose-bengal Chloramphenicol agar (Oxoid, Hampshire, UK; ISO 11133, 2014) incubated at 25 °C for 5 days. Also the detection of some agents was performed: *L. monocytogenes* on pre-enrichment Half Fraser broth (Merck, Germany; ISO 11290-1, 2017a) and incubated at 30 °C for 24 h; *Salmonella* spp. on pre-enrichment Buffered Peptone Water (BPW; Biokar, France; ISO 6579, 2017b) and incubated at 37 °C for 24 h and sulfite- reducing *Clostridium* spores according to ISO 15213-1 (2003). Detection of *Yersinia* was enriched on Peptone Sorbitol Bile broth (HiMedia, Laboratories, Mumbai, India), for 2 days at 25 °C followed by potassium hydroxide treatment. After treatment, samples were plated onto Cefsulodin-Irgasan-Novobiocin agar (CIN; Becton Dickinson GmbH, Germany) at 30 °C for 48 h (ISO 10273, 2017c). Every analysis was performed in duplicate.

Physicochemical analysis

-Moisture, pH, water activity (aw), peroxide index, and acidity index

Moisture content was determined by oven drying according to ISO 1441 (1997), *a_w* was measured in a Pawkit (Decagon, Hopkins Court, USA) apparatus. The pH was measured directly using a pH meter (model CRISON pH 25+, Barcelona, Spain). The peroxide index was determined according to ISO 3960 (2017d) and values were expressed as milliequivalents/kg of extracted fat. The acidity index of the products was determined in accordance with ISO 660 (2009) and results were expressed as % acidity.

-Instrumental textural profile and color

Texture evaluation was carried out using a texture analyzer (TA-XTplus Texture Analyzer, Stable Micro Systems, Vienna Court, UK). Textural parameters were measured using a Cylinder Probe P/10 (10 mm diameter) of surface contact. Sausage pieces of 1×1×2.5 cm (height×width×length) were compressed at a crosshead speed of 60 mm/min. Compression measurements were recorded with regard to hardness (expressed in N) and the adhesiveness (expressed in N/sec) force using the computer software (Texture Exponent 32, Stable Micro Systems, Vienna Court, UK).

A portable colorimeter (Konica Minolta, Minolta Chroma Meter 300, USA) was used to measure meat color in the CIELAB space: lightness, L^* ; redness, a^* ; yellowness, b^* . Calibration was performed using a white standard plate ($L^*=97.06$; $a^*=+5.28$; $b^*=-3.49$).

Sensory analysis

A quantitative descriptive analysis was performed. A semi-trained 9-taster panel was used to evaluate sausage samples regarding to eleven sensory parameters: color characteristic, brightness, odor characteristic, strange odor, hardness, flavor characteristic, bitter taste, meat binding, adhesiveness in the mouth, spicy flavor, and acid taste. A scale of intensity of 1 to 13 (1-low intensity; 13-high intensity) was used; the standard sample was previously scoring 7. The testers classified the differences detected from this central point, except for the strange smell parameter, where the reference point of the sample was the value 1.

Statistical analysis

All data were statistically analyzed using an ANOVA procedure (IBM SPSS Statistics 25). Tukey HSD test was used to investigate significant differences in physico-chemical parameters on a significant level of $p<0.05$. A Principal Component (PC) analysis was carried out to reduce the number of variables and select the few that better characterized the product in sensory evaluation. In a first approach, all data was used to calculate the first two PCs. Loadings of each variable to each PC was performed using the 11 original variables after Varimax normalized rotation from two PCs in order to determine which aspects were of greatest importance in “Chouriço Vinha d’Alhos” characterization.

Results

The bacteriocin produced by *Lb. plantarum* ST153Ch was produced at maximum levels (12800 AU/ml against *L. monocytogenes* ScottA and 3200 AU/ml against *L.*

monocytogenes 3701) after 16 h and until 39 h of growth in MRS broth. During the first 39 h of growth, the pH decreased from 6.4 to 3.73 and the counts increased ~4 log (data not shown).

Results of the enumeration of *Lb. plantarum* and *L. monocytogenes* in “Chouriço de Vinha d’Alhos” are presented in Figure 1. In the presence of *Lb. plantarum* ST153Ch-fresh or lyophilized (S2BLAF+LM and S2BLA+LM), *L. monocytogenes* counts were reduced by 2 logs until the end of storage (Figure 1-A). However, in samples only inoculated with cocktail of *L. monocytogenes* (S2BC+LM), the reduction was similar, showing that the addition of *Lb. plantarum* ST153Ch did not produce any additional inhibition.

No influence was observed in *Lb. plantarum* populations with or without *L. monocytogenes* and with or without fresh or lyophilized culture (Figure 1-B). The studied pathogenic organisms, including *Salmonella*, *S. aureus*, *Yersinia*, sulfite-reducing *Clostridium* spores, and *E. coli* were not detected in each sample. Also, counts of Enterobacteriaceae and *B. cereus* were below the detection limit of the enumeration technique. However, in samples without inoculation, the presence of endogenous *L. monocytogenes* was detected until the end of storage.

Levels of moisture, pH, a_w , peroxide index, and acidity index throughout storage period are summarized in Table 1. No significant differences were found in moisture content ($p>0.05$) during processing and storage. Inoculated samples presented lower pH values compared to the control group. Some fluctuations in a_w were noticeable in some sampling points but overall the non-inoculated samples presented lower a_w values than both inoculated samples. Peroxide index values were significantly different ($p<0.05$) at 0, 30, 45, and 75 days. Initially the peroxide value on non-inoculated samples was higher, but no significant differences ($p>0.05$) were found at the end of storage. Acidity index increased during storage and significant differences ($p<0.05$) were found in inoculated samples compared to the control, at the end of storage.

A significant decrease in hardness was found in inoculated samples at the end of storage period ($p<0.05$) compared to the control. With respect to adhesiveness, significant differences were found at the last day of storage; the inoculated samples showing lower values (data not shown). The addition of *Lb. plantarum* ST153Ch had no effect on color parameters a^* (red) and b^* (yellow) but significant differences were found in L^* (brightness) at the end of the storage period; inoculated samples showing lower brightness (data not shown).

The sensory profile of “Chouriço Vinha d’Alhos” during storage at 4 °C in an intensity scale of the main descriptors is shown in Figure 2. No significant differences ($p>0.05$) were found throughout the sampling period in

the following parameters compared to the control: brightness, meat binding, adhesiveness in the mouth, spicy flavor, and acid taste; but significant differences ($p < 0.05$) were detected in color, odor characteristic, strange odor, hardness, flavor characteristic, and bitter taste parameters (data not shown).

For PC analysis, five variables were selected based on factor loadings modulus higher than 0.70, including brightness, hardness, acid taste, spicy flavor and strange

odor. Figure 3 shows the projection of the selected five variables on the three-dimensional space defined by the two PCs. The first component was defined mainly by the brightness and hardness and by the strange odor on the right. The most important contribution to the second PC (PC2) came from two sensory attributes, acid taste, and spicy flavor, shown in the upper part of the Figure 3. Hardness and brightness decrease, and strange odor increased compared with the control.

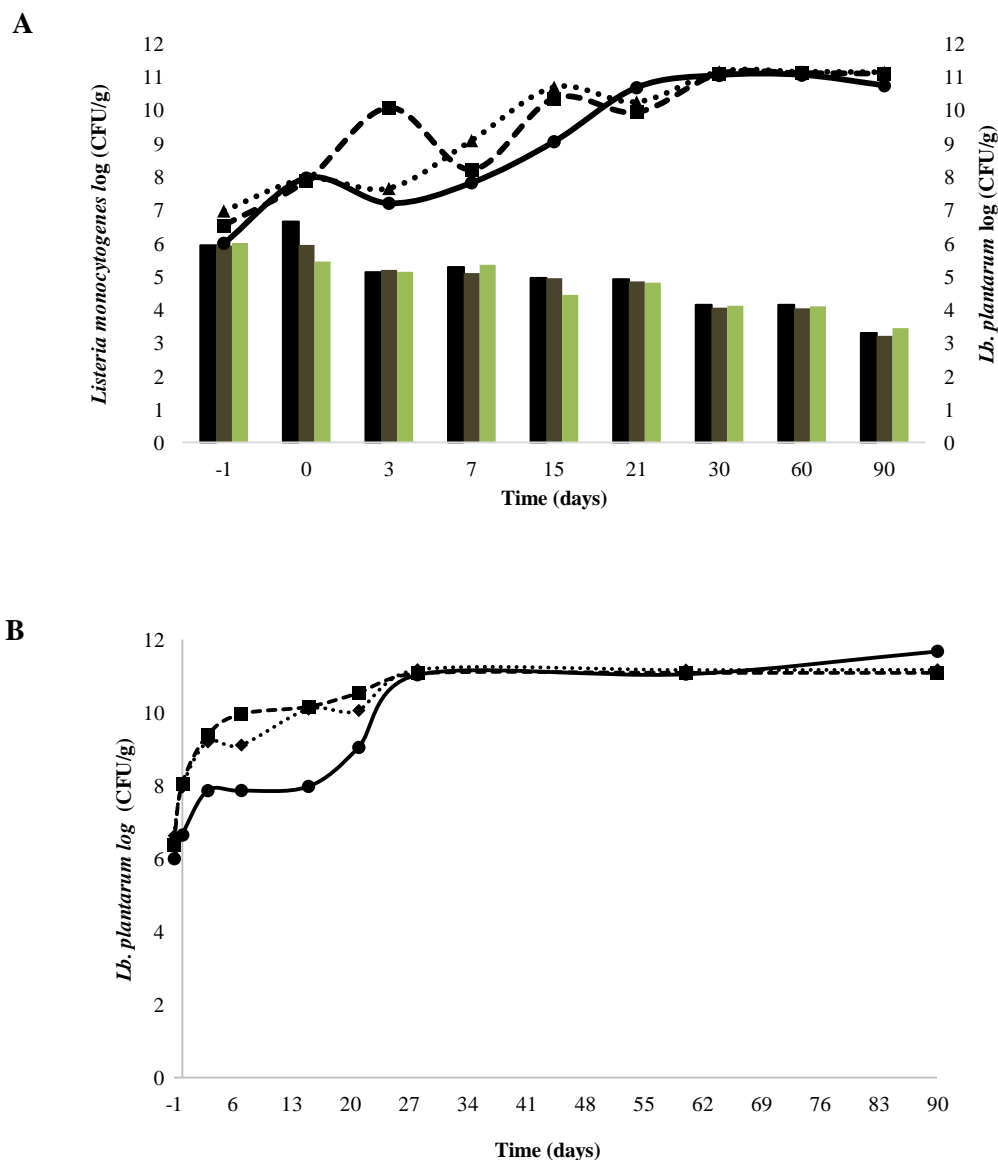
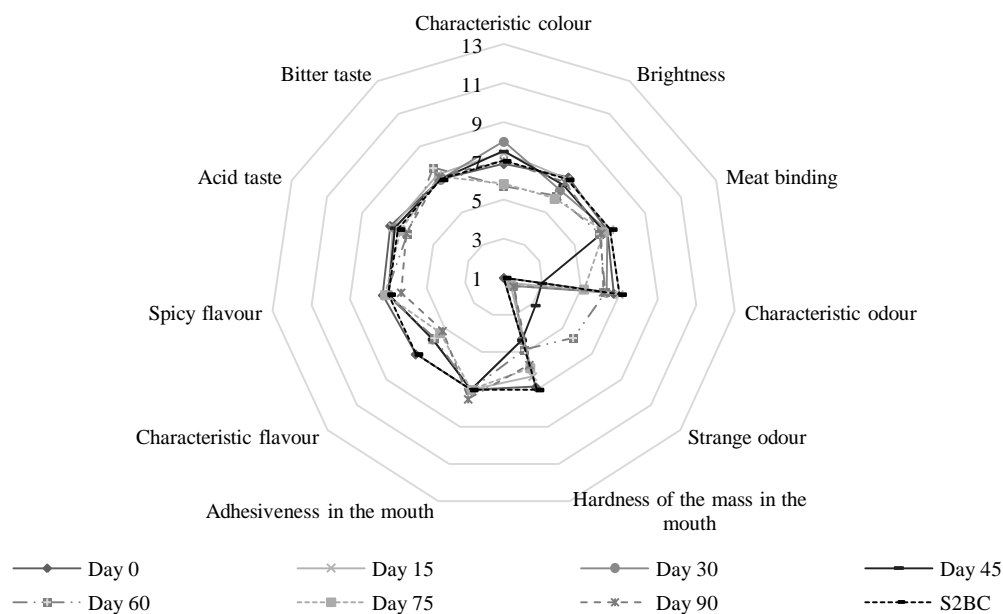


Figure 1: Growth of lactic acid bacteria on MRS agar (lines) and the cocktail of *L. monocytogenes* (bars) in “Chouriço de Vinha d’Alhos”, during storage at 4 °C. **A:** samples inoculated with *Lb. plantarum* ST153Ch and cocktail of *L. monocytogenes*. **B:** samples inoculated only with *Lb. plantarum* ST153CH

Lines: (—●—) S2BC; (—▲—) S2BLAF; (—■—) S2BLA. Bars: (■) S2BC + LM; (■) S2BLAF + LM; (■) S2BLA + LM; Day “-1” after inoculation of *Lb. plantarum* and before smoking

A



B

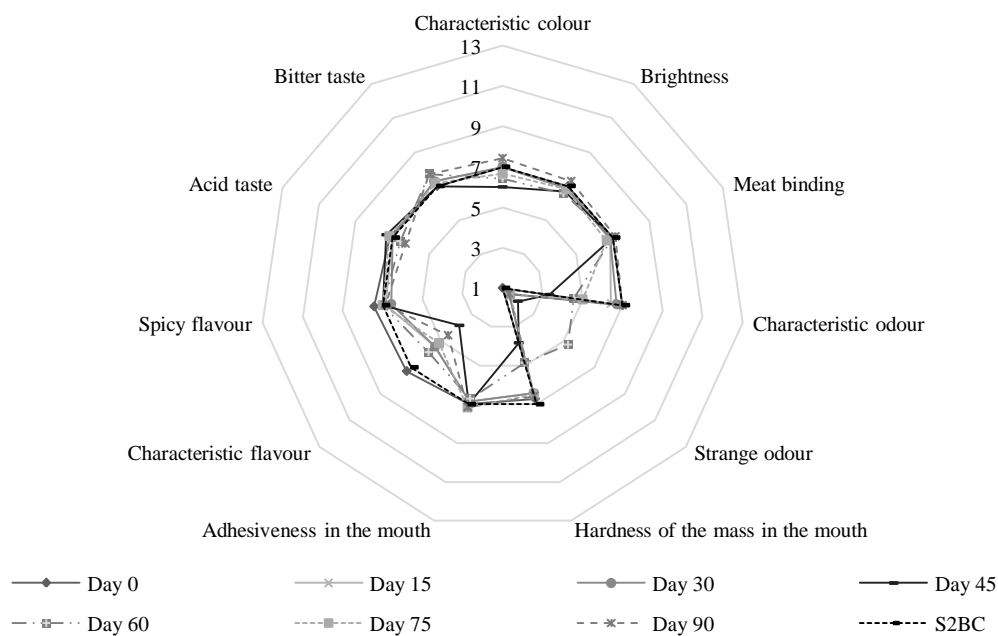


Figure 2: Sensory profile of “Chouriço Vinha d’Alhos” during storage at 4 °C in an intensity scale of the main descriptors considered in a quantitative descriptive analysis for A: S2BLAF with *Lb. plantarum* ST153Ch fresh; B: S2BLA with lyophilized *Lb. plantarum* ST153Ch

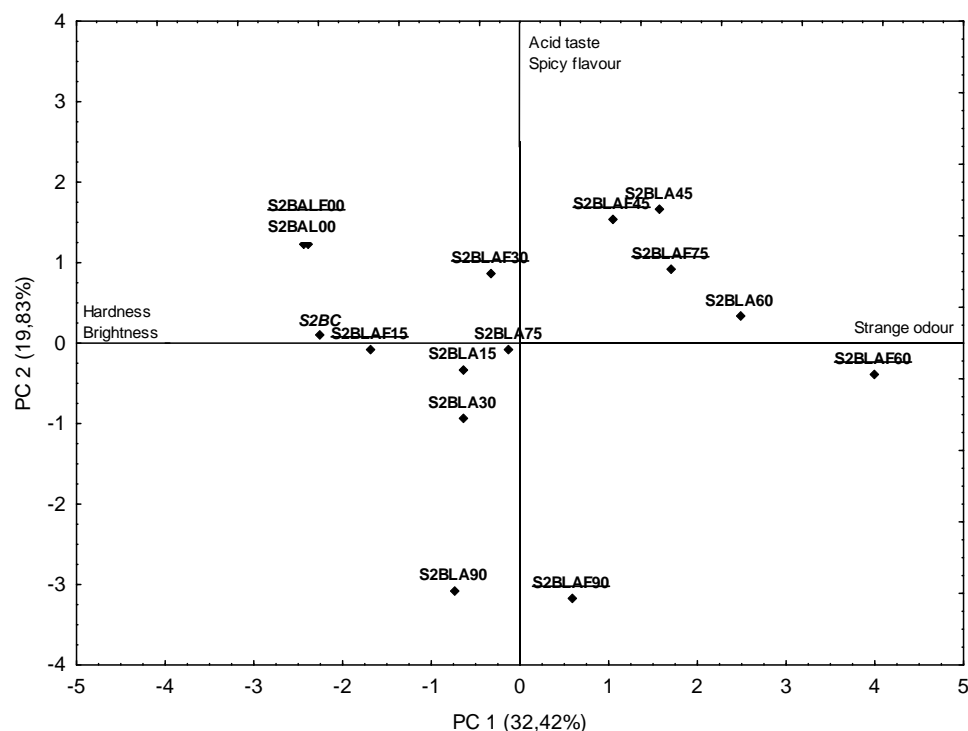


Figure 3: Principal Component (PC) analysis projection applied to the dimensions obtained from the 5 variables selected sensorial analysis data of “Chouriço Vinha d’Alhos” with case projection (storage time). S2BC: control without starter culture; S2BLAF: with *Lb. plantarum* ST153Ch fresh; S2BLA: with *Lb. plantarum* ST153Ch lyophilized

Table 1: Effect of inoculation starter culture on moisture content, pH, water activity, peroxide index, acidity index throughout the storage of “Chouriço Vinha d’Alhos” at 4 °C

		Storage (days)						
Samples*		0	15	30	45	60	75	90
Moisture content (%)	S2BC	51.05±3.90 ^{ab}	47.76±1.98 ^a	47.76±3.77 ^a	47.37±3.00 ^a	46.83±2.24 ^a	49.32±1.00 ^a	45.82±0.72 ^a
	S2BLAF	48.61±2.94 ^a	48.63±4.11 ^a	49.82±2.66 ^a	49.64±3.78 ^a	47.99±0.76 ^a	50.96±0.92 ^a	50.44±1.96 ^a
	S2BLA	51.54±0.49 ^a	49.17±0.47 ^a	50.31±2.11 ^a	52.07±2.21 ^a	50.17±0.79 ^a	47.17±3.68 ^a	50.15±2.30 ^a
	Sig.***	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
pH	S2BC	5.85±0.11 ^a	5.72±0.06 ^a	5.45±0.02 ^a	5.54±0.02 ^a	5.63±0.01 ^a	5.72±0.06 ^a	5.50±0.08 ^a
	S2BLAF	5.52±0.01 ^b	5.42±0.02 ^b	5.30±0.01 ^b	5.21±0.04 ^b	5.28±0.03 ^b	5.83±0.04 ^b	5.22±0.03 ^b
	S2BLA	5.70±0.16 ^a	5.52±0.07 ^b	5.34±0.02 ^c	5.28±0.03 ^b	5.26±0.03 ^b	5.67±0.02 ^a	5.12±0.04 ^b
	Sig.	S.	S.	S.	S.	S.	S.	S.
a _w	S2BC	0.89±0.02 ^a	0.92±0.01 ^a	0.93±0.01 ^a	0.96±0.01 ^a	0.99±0.01 ^a	0.84±0.03 ^a	0.93±0.01 ^a
	S2BLAF	0.93±0.01 ^b	0.94±0.00 ^b	0.94±0.01 ^a	0.97±0.01 ^b	0.99±0.01 ^a	0.89±0.02 ^a	0.93±0.01 ^a
	S2BLA	0.93±0.01 ^b	0.95±0.01 ^b	0.94±0.01 ^a	0.98±0.00 ^b	0.98±0.00 ^a	0.91±0.00 ^b	0.93±0.01 ^a
	Significance	S.	S.	N.S.	S.	N.S.	S.	N.S.
Peroxide index (mEq/kg of extracted fat)	S2BC	3.71±0.34 ^a	2.37±0.15 ^a	10.92±1.55 ^a	8.22±0.22 ^{ab}	7.20±0.04 ^a	1.42±0.03 ^a	1.92±0.39 ^a
	S2BLAF	2.32±0.62 ^b	2.65±0.18 ^a	8.56±1.03 ^b	10.58±1.95 ^a	8.72±1.66 ^a	1.49±0.18 ^a	2.86±0.64 ^a
	S2BLA	1.67±0.33 ^b	2.75±0.14 ^a	7.53±1.00 ^{ab}	7.18±1.16 ^b	9.32±0.23 ^a	2.89±0.93 ^b	2.94±0.13 ^a
	Sig.	S.	S.	S.	S.	N.S.	S.	N.S.
Acidity index (%)	S2BC	0.80±0.04 ^{ab}	0.74±0.09 ^a	0.82±0.06 ^a	0.73±0.07 ^a	1.12±0.11 ^a	1.46±0.13 ^a	2.21±0.03 ^a
	S2BLAF	0.88±0.04 ^a	0.95±0.04 ^b	0.92±0.02 ^a	0.73±0.13 ^a	1.13±0.11 ^a	1.34±0.09 ^a	1.64±0.15 ^b
	S2BLA	0.75±0.05 ^b	0.80±0.08 ^{ab}	1.00±0.11 ^a	0.64±0.04 ^a	1.04±0.13 ^a	0.86±0.09 ^b	1.53±0.18 ^b
	Sig.	S.	S.	N.S.	N.S.	N.S.	S.	S.

*Samples: S2BC: control without starter culture; S2BLAF: with *Lactobacillus plantarum* ST153Ch fresh; S2BLA: with *Lactobacillus plantarum* ST153Ch lyophilized

**Mean values in the same column (corresponding to the same days of storage) not followed by a common letter differ significantly ($p < 0.05$)

***Statistical significance level (N.S.: not significant; S: significant)

Discussion

Regarding the obtained bacteriocin activity (12800 AU/ml against *L. monocytogenes* ScottA and 3200 AU/ml against *L. monocytogenes* 3701), our results are in agreement with those observed by Delgado et al. (2007) and Albano et al. (2007b). Also, Mataragas et al. (2004) reported that the higher levels of bacteriocin are produced at lower values of pH. In the current study, the addition of *Lb. plantarum* ST153Ch had no further inhibition against *L. monocytogenes*. Several factors could be responsible for this, such as higher hydrophobic matrix of “Chouriço de Vinha d’Alhos” which protects *L. monocytogenes* from bacteriocins and pH effects (Kouakou et al., 2009); the low initial concentration of *Lb. plantarum* ST153Ch that may not be sufficient to have an inhibitory effect. Actually, Vaz Velho et al. (2013) used a higher initial concentration (9 log CFU/g) and, after 7 days, inoculated *Listeria* had decreased 2 log cycles. Alternatively, “Chouriço de Vinha d’Alhos” could have some endogenous bacteriocinogenic lactic acid bacteria, which may act in the same way as inoculated bacteriocinogenic lactic acid bacteria (Franciosa et al., 2018).

We found no significant differences in moisture content of the samples during processing and storage. The study of Lorenzo et al. (2014) on the effect of a *Lb. plantarum* starter culture addition on cured sausages corroborates this finding. The pH reduction may be related to accumulation of some organic acids, such as lactic acids, due to the higher numbers of *Lb. plantarum*, or because of carbohydrate breakdown during the fermentation (Casaburi et al., 2007; Essid and Hassouna, 2013; Lorenzo et al., 2014). Reduction of pH at the start of fermentation is important as it involves to the prevention of undesirable bacteria, accelerates the red color development of fermented sausages, affects taste, and also decreases the water binding capacity of proteins, ensuring the drying (Essid and Hassouna, 2013). Fluctuation of a_w values could be due to heterogeneity of samples; it is expected when dealing with a food matrix, such as “Chouriço Vinha d’Alhos”, because the diversity in its structural organization is noticeable at macroscopic level. Similar behavior was detected in a study with a similar product-“alheira”- inoculated with the same bacterial strains (Barros et al., 2018).

In the present investigation, the peroxide value on non-inoculated “Chouriço Vinha d’Alhos” was initially higher, but at the end of storage no significant differences were found. Peroxide index is useful for monitoring the progress of the initial stages of lipid oxidation. The variation in the level of peroxides occurs in a Gaussian shape; therefore a low level of peroxides at later stages does not guarantee oxidative stability. Also, due to the lipid oxida-

tion process, an increase in the acidity of the fat was expected during storage, as a result of an increasing amount of fatty acids released by lipolytic action (Botsoglou et al., 2003; Fonseca et al., 2013).

One of the main elements of quality and acceptability of food is texture. It is perceived from the sensory sensations of the physical properties of a material (Hussein et al., 2017). Our instrumental and also sensory analysis revealed lower hardness values of inoculated samples which are in agreement with findings of Benito et al. (2007), where starter cultures reduced the hardness of traditional Iberian sausages, probably because of their impact on protein hydrolysis.

Color is one of the most significant quality properties of meat products since it affects overall quality. According to the results of this study, inoculation did not affect color parameters a^* (red) and b^* (yellow); lower brightness was only found at the end of the storage period in accordance to panelists analysis. Panelists also reported a strange odor starting at day 60. Further experiments will be carried out to validate these results, since in previous results these changes did not occur (data not published).

The sensorial properties of the final product are mainly the result of a complex interaction of physicochemical, biochemical, and microbial processes (Simion et al., 2014). The present sensory analysis aimed to compare the effect of the addition of a fresh and a lyophilized *Lb. plantarum* culture, on the sensory properties of “Chouriço de Vinha d’Alhos”, a very heterogeneous food matrix. The present *Lb. plantarum* strain was already applied to “Chouriço Vinha d’Alhos” after processing and slicing (Jácome et al., 2014), but its addition together with other “Chouriço Vinha d’Alhos” ingredients before processing was never previously tested.

Conclusion

Overall, no significant differences were found between application methodologies (fresh or lyophilized *Lb. plantarum*); therefore industry might be able to choose the most suitable method according to their manufacturing process. On the other hand, at 90 days storage of the products, an acid taste and spicy flavor was seen. Additionally, *Lb. plantarum* ST153Ch did not show their antilisterial activity *in situ*, which leads to the conclusion that there may be other factors that influence bacteriocin activity, and it is necessary to understand what they are.

Author contributions

P.T. and M.V.V. designed the study; A.M. and D.B. conducted the experimental work; A.M., D.B., H.A., S.F. and R.P. analyzed the data; A.M., D.B., H.A., M.V.V.,

and P.T. wrote the manuscript. All authors revised and approved the final manuscript.

Conflicts of interest

There was no conflicts of interest.

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References

- Albano H., Oliveira M., Aroso R., Cubero N., Hogg T., Teixeira P. (2007a). Antilisterial activity of lactic acid bacteria isolated from "Alheiras" (traditional Portuguese fermented sausages): *in situ* assays. *Meat Science*. 76: 796-800.
- Albano H., Todorov S.D., Van Reenen C.A., Hogg T., Dicks L.M.T., Teixeira P. (2007b). Characterization of two bacteriocins produced by *Pediococcus acidilactici* isolated from "Alheira", a fermented sausage traditionally produced in Portugal. *International Journal of Food Microbiology*. 116: 239-247.
- Barbosa J., Borges S., Amorim M., Pereira M.J., Oliveira A., Pintado M.E., Teixeira P. (2015). Comparison of spray drying, freeze drying and convective hot air drying for the production of a probiotic orange powder. *Journal of Functional Foods*. 17: 340-351.
- Barros D.B., Sobrosa C., Pinheiro R., Fonseca S., Vaz-Velho M. (2018). Effect of addition of a starter cultures on physicochemical and sensory properties of "Alheira", a smoked sausage-like product. *Millenium*. 2: 39-48.
- Benito M.J., Martín A., Aranda E., Perez-Nevado F., Ruiz-Moyano S., Cordoba M.G. (2007). Characterization and selection of autochthonous lactic acid bacteria isolated from traditional Iberian dry-fermented salchichón and chorizo sausages. *Journal of Food Science*. 72: 193-201.
- Botsoglou N.A., Grigoropoulou S.H., Botsoglou E., Govaris A., Papageorgiou G. (2003). The effects of dietary oregano essential oil and α -tocopheryl acetate on lipid oxidation in raw and cooked turkey during refrigerated storage. *Meat Science*. 65: 1193-1200.
- Briers Y., Klumpp J., Schuppler M., Loessner M.J. (2011). Genome sequence of *Listeria monocytogenes* Scott A, a clinical isolate from a food-borne listeriosis outbreak. *Journal of Bacteriology*. 193: 4284-4285.
- Casaburi A., Aristoy M.C., Cavella S., Di Monaco R., Ecolini D., Toldra F. (2007). Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (Southern Italy) as affected by the use of starter cultures. *Meat Science*. 76: 295-307.
- Delgado A., López F.N.A., Brito D., Peres C., Fevereiro P., Garrido-Fernández A. (2007). Optimum bacteriocin production by *Lactobacillus plantarum* 17.2b requires absence of NaCl and apparently follows a mixed metabolite kinetics. *Journal of Biotechnology*. 130: 193-201.
- Essid I., Hassouna M. (2013). Effect of inoculation of selected *Staphylococcus xylosum* and *Lactobacillus plantarum* strains on biochemical, microbiological and textural characteristics of a Tunisian dry fermented sausage. *Food Control*. 32: 707-714.
- Ferreira V., Barbosa J., Silva J., Vendeiro S., Mota A., Silva F., Monteiro M.J., Hogg T., Gibbs P., Teixeira P. (2007). Chemical and microbiological characterisation of "Salpicão de Vinhais" and "Chouriça de Vinhais": traditional dry sausages produced in the North of Portugal. *Food Microbiology*. 24: 618-623.
- Fonseca S., Cachaldora A., Gómez M., Franco I., Carballo J. (2013). Effect of different autochthonous starter cultures on the volatile compounds profile and sensory properties of Galician chorizo, a traditional Spanish dry fermented sausage. *Food Control*. 33: 6-14.
- Franciosa I., Alessandria V., Dolci P., Rantsiou K., Coccolin L. (2018). Sausage fermentation and starter cultures in the era of molecular biology methods. *International Journal of Food Microbiology*. 279: 26-32.
- Hussein F.H., Razavi S.H., Emam-Djomeh Z. (2017). Physicochemical properties and sensory evaluation of reduced fat fermented functional beef sausage. *Applied Food Biotechnology*. 4: 93-102.
- International Standards Organization (ISO). (1997). Meat and meat products-Determination of moisture content (reference method). Standard No. 1442.
- International Standards Organization (ISO). (1998). Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of mesophilic lactic acid bacteria-Colony-count technique at 30 degrees. Standard No. 152141.
- International Standards Organization (ISO). (1999). Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species)-Part 1: technique using Baird-Parker agar medium. Standard No. 6888-1.
- International Standards Organization (ISO). (2001). Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*-Part 2: colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. Standard No. 16649-2.
- International Standards Organization (ISO). (2003). Microbiology of the food chain-Horizontal method for the detection and enumeration of *Clostridium* spp.- Part 1: enumeration of sulfite-reducing *Clostridium* spp. by colony-count technique. Standard No. 15213-1.
- International Standards Organization (ISO). (2004). Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of presumptive *Bacillus cereus*-Colony-count technique at 30 degrees C. Standard No. 7932.
- International Standards Organization (ISO). (2009). Animal and vegetable fats and oils-Determination of acid value and acidity. Standard No. 660.
- International Standards Organization (ISO). (2014). Microbiology of food, animal feed and water-Preparation, production, storage and performance testing of culture media. Standard No. 11133.
- International Standards Organization (ISO). (2016). Microbiology of the food chain-Method validation-Part 2: protocol for the validation of alternative (proprietary) methods against a reference method. Standard No. 16149-2.
- International Standards Organization (ISO). (2017a). Microbiology of the food chain- Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp.-Part 1: detection method. Standard No. 11290-1.
- International Standards Organization (ISO). (2017b). Microbiology of the food chain-Horizontal method for the detection, enumeration and serotyping of *Salmonella*-Part 1: detection of *Salmonella* spp. Standard No. 6579-1.
- International Standards Organization (ISO). (2017c). Microbiology of the food chain-Horizontal method for the detection of pathogenic *Yersinia enterocolitica*. Standard No. 10273.

- International Standards Organization (ISO). (2017d). Animal and vegetable fats and oils-Determination of peroxide - Iodometric (visual endpoint determination). Standard No. 3960.
- Jácome S.L., Fonseca S., Pinheiro R., Todorov S.D., Noronha L., Silva J., Gomes A., Pintado M., Morais A.M.M.B., Teixeira P., Vaz-Velho M. (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.
- Kouakou P., Ghalfi H., Destain J., Dubois-Dauphin R., Evrard P., Thonart P. (2009). Effects of curing sodium nitrite additive and natural meat fat on growth control of *Listeria monocytogenes* by the bacteriocin-producing *Lactobacillus curvatus* strain CWBI-B28. *Food Microbiology*. 26: 623-628.
- Kouakou P., Sidibe D., Biego H.G. (2016). Combinations of lyophilized cell-adsorbed bacteriocin of *Lactobacillus curvatus* CWBI-B28 with organic acids or salts to control *Listeria monocytogenes* on smoked salmon stored at 4 °C in vacuum packages. *International Journal of Agricultural and Food Research*. 5: 13-20.
- Lorenzo J.M., Gómez M., Fonseca S. (2014). Effect of commercial starter cultures on physicochemical characteristics, microbial counts and free fatty acid composition of dry-cured foal sausage. *Food Control*. 46: 382-389.
- Mainar M.S., Khaferi R., Samapundo S., Devlieghere F., Leroy F. (2016). Opportunities and limitations for the production of safe fermented meats without nitrate and nitrite using an antibacterial *Staphylococcus sciuri* starter culture. *Food Control*. 69: 267-274.
- Mataragas M., Drosinos E.H., Tsakalidou E., Metaxopoulos J. (2004). Influence of nutrients on growth and bacteriocin production by *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442. *Antonie van Leeuwenhoek*. 85: 191-198.
- Oliveira M., Ferreira V., Magalhães R., Teixeira P. (2018). Biocontrol strategies for Mediterranean-style fermented sausages. *Food Research International*. 103: 438-449.
- Orihuel A., Bonacina J., Vildoza M.J., Bru E., Vignolo G., Saavedra L., Fadda S. (2018). Biocontrol of *Listeria monocytogenes* in a meat model using a combination of a bacteriocinogenic strain with curing additives. *Food Research International*. 107: 289-296.
- Perez R.H., Zendo T., Sonomoto K. (2014). Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. *Microbial Cell Factories*. 13: S3.
- Simion A.M.C., Vizireanu C., Alexe P., Franco I., Carballo J. (2014). Effect of the use of selected starter cultures on some quality, safety and sensorial properties of Dacia sausage, a traditional Romanian dry-sausage variety. *Food Control*. 35: 123-131.
- Singh S., Shalini R. (2016). Effect of hurdle technology in food preservation: a review. *Critical Reviews in Food Science and Nutrition*. 56: 641-649.
- Todorov S.D., Franco B.D.G.M., Wiid I.J. (2014). *In vitro* study of beneficial properties and safety of lactic acid bacteria isolated from Portuguese fermented meat products. *Beneficial Microbes*. 5: 351-366.
- Todorov S.D., Vaz-Velho M., de Melo Franco B.D.G., Holzapfel W.H. (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.
- Van Reenen C.A., Dicks L.M., Chikindas M.L. (1998). Isolation, purification and partial characterization of plantaricin 423, a bacteriocin produced by *Lactobacillus plantarum*. *Journal of Applied Microbiology*. 84: 1131-1137.
- Vaz-Velho M., Jácome S., Noronha L., Todorov S., Fonseca S., Pinheiro R., Morais A., Silva J., Teixeira P. (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.
- Verluyten J., Leroy F., De Vuyst L. (2004). Influence of complex nutrient source on growth of and curvacin A production by sausage isolate *Lactobacillus curvatus* LTH 1174. *Applied and Environmental Microbiology*. 70: 5081-5088.