LWT - Food Science and Technology 200 (2024) 116166

Contents lists available at ScienceDirect

LWT

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

# Evaluation of the performance of a three-strains lactic acid bacteria cocktail for the control of *Listeria monocytogenes* on marinated lean pork

C. Barcenilla [a,*], A. Puente [a], J.F. Cobo-Díaz [a], M. González-Raurich [a,b], M. López [a,b], M. Prieto [a,b], A. Álvarez-Ordóñez [a,b]

[a] Department of Food Hygiene and Technology, Universidad de León, León, Spain
[b] Institute of Food Science and Technology, Universidad de León, León, Spain

ARTICLE INFO

*Keywords:*
*Listeria monocytogenes*
Food cultures
Lactic acid bacteria
meat microbiota
Food safety

ABSTRACT

The aim of this study was to evaluate the addition of a cocktail of three lactic acid bacteria (LAB) (*Lactococcus lactis*, *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum*) as a protective culture into marinated pork. The pork was artificially inoculated with two *Listeria monocytogenes* strains and stored for 12 days. Two packagings were used: vacuum or modified atmosphere packaging (MAP). LAB, *L. monocytogenes*, psychrotrophic bacteria, pH, $a_w$, color, the metataxonomic profile and the sensorial quality of the product were evaluated. The growth of *L. monocytogenes* under vacuum and MAP was reduced with the use of the LAB up to 0.8 and 0.7 $\log_{10}$ CFU/g, respectively. LAB counts gradually increased, which was accompanied by a slight decrease in pH. In LAB samples, psychrotrophic bacteria showed a reduction at day 12 as compared to non-inoculated samples. Some minor differences were also observed among samples for color and sensory parameters. Regardless of the type of packaging, the microbiota of the marinated pork was dominated initially by *Photobacterium* and subsequently during storage by a diversity of LAB. The application of LAB could help to obtain a safe product, although further evaluation would be required to optimize the application of the LAB cocktail in real-scale commercial scenarios.

## 1. Introduction

Meat is a nutrient-rich food which can allow the growth of contaminating spoilage and pathogenic microorganisms. This makes meat especially perishable and a product with a short shelf life. Traditionally, curing (Honikel, 2008), fermentation (Ashaolu, Khalifa, Mesak, Lorenzo, & Farag, 2021), marinating (Latoch, Czarniecka-Skubina, & Moczkowska-Wyrwisz, 2023) or different preservation and packaging techniques (Zhang et al., 2023) have been employed to prolong the shelf life and enhance the microbiological safety of meat (Toldrá, 2022). Still, meat products are frequently involved in outbreaks related to different zoonotic agents (Omer et al., 2018). *Listeria monocytogenes* is one of the major foodborne pathogens linked to outbreaks caused by consumption of contaminated meat products (European Food Safety Authority, 2023). Recently, some important listeriosis outbreaks caused by meat products have been associated with the consumption of chilled roasted pork meat in Spain in 2019 (Fernández-Martínez et al., 2022), blood sausages in Germany in 2018–2019 (Halbedel et al., 2020), while the world's largest listeriosis outbreak, which took place in 2017–2018 in South Africa, was associated with the consumption of a local ready-to-eat (RTE) processed meat product (Tchatchouang et al., 2020). Outbreaks related to *L. monocytogenes* are specially relevant in RTE meats (European Food Safety Authority, 2023), although cross-contamination between raw meat and cooked meat products can pose a risk for the consumer (Li et al., 2018; Y. Zhang et al., 2022). Also, undercooked pork meat can be an important vehicle of foodborne infection, if the intended use according to the producer is not followed (Wang et al., 2015).This risk has encouraged the conduction of studies to appraise different strategies to ensure the safety of pork intended to be cooked (Lopes, da Silva, & Tondo, 2021; Siroli et al., 2020).

The use of lactic acid bacteria (LAB) for food preservation purposes is being widely studied with promising results in a variety of foods, including meat products (Barcenilla, Ducic, López, Prieto, & Álvarez-Ordóñez, 2022; Kaveh et al., 2023, p. 10154; Lahiri et al., 2022; Martín, Rodríguez, Delgado, & Córdoba, 2022, p. 542). The efficacy of some LAB as preservatives in food can be linked to various of their attributes: the synthesis of bacteriocins and/or the acidification of the product and consequent production of organic acids throughout its shelf life due to their fermentative metabolism, among others (Mozuriene

* Corresponding author. Department of Food Hygiene and Technology, Universidad de León, León, Spain.
*E-mail address:* cbarc@unileon.es (C. Barcenilla).

https://doi.org/10.1016/j.lwt.2024.116166
Received 19 December 2023; Received in revised form 4 April 2024; Accepted 4 May 2024
Available online 6 May 2024

**Abbreviations**

| | |
|---|---|
| V-NC | negative control samples packaged under vacuum |
| V-LAB | samples with the cocktail of lactic acid bacteria packed under vacuum |
| V-LAB + LM | samples with the cocktail of lactic acid bacteria and *Listeria monocytogenes* packed under vacuum |
| V-LM | samples with *Listeria monocytogenes* packed under vacuum |
| M-NC | negative control samples packaged under modified atmosphere packaging |
| M-LAB | samples with the cocktail of lactic acid bacteria packed under modified atmosphere packaging |
| M-LAB + LM | samples with the cocktail of lactic acid bacteria and *Listeria monocytogenes* packed under modified atmosphere packaging |
| M-LM | samples with *Listeria monocytogenes* packed under modified atmosphere packaging |

et al., 2016). Considering the versatility of meat products processing, LAB cultures can be applied in such products in different ways. Up to now, the most common way of application is as starter culture during a fermentation process, such as in dry-fermented sausages (Dučić et al., 2023). Also, in sliced products, such as sliced cooked ham or other RTE cooked meat products, the food cultures can be applied superficially by spraying and/or spreading of a liquid suspension with the strain(s) (Barcenilla et al., 2023). Alternatively, the product can be dipped in these liquid solutions containing LAB alone or in combination with other ingredients and/or additives (Castellano et al., 2018). Thus, marinades can be good candidates as solutions for the application of LAB cultures to meat products (Gargi & Sengun, 2021). Other ways of application can be as part of coatings or encapsulated, which would protect the culture bacteria assuring an uniform release (Xie et al., 2018).

In a previous study by our research group (Barcenilla et al., 2023), three LAB strains with good antimicrobial potential were identified, namely *Lactococcus lactis* ULE383, *Lacticaseibacillus paracasei* ULE721 and *Lactiplantibacillus plantarum* ULE1599. None of the three strains carried antimicrobial resistance genes or virulence factors as demonstrated by whole genome sequencing analyses. Moreover, it was shown that *L. lactis* ULE383 was potentially a nisin Z producer. Finally, the aptitude of these strains as food cultures was demonstrated in cooked RTE meat products. In fact, the three-strains cocktail showed remarkable antimicrobial activity against *L. monocytogenes* in cooked ham, limiting the growth potential of the pathogen with no detrimental effects being observed in other quality attributes of the product.

The objective of the present study was to evaluate the performance of this three LAB strain cocktail in a marinated lean pork product, assessing its efficacy to control the growth of *L. monocytogenes*. The experiment was performed independently under vacuum and modified atmosphere packaging (MAP), two common packagings used with this type of products to evaluate the performance of the strains in both techniques. In addition, the effect of the LAB application on the physicochemical parameters, whole microbial community (*16S rRNA* gene amplicon sequencing) and sensorial characteristics of the product was also studied to understand the overall impact on the marinated lean pork.

## 2. Material and methods

### 2.1. Samples preparation

Lean pork cuts (25 ± 1 g per piece) were obtained from a local supermarket and transferred to the laboratory under refrigeration, where they were processed on the same day of arrival. A marinade was prepared as follows: water (80.8%), salt (5.4%), paprika (4.6%), olive oil (2.7%), white wine (2.7%), white pepper (1.2%), sodium citrate (0.6%), cumin (0.5%), cayenne pepper (0.5%), oregano (0.5%) and garlic powder (0.5%). Once prepared, the marinade was sterilized at 121 °C for 15 min. Lean pork cuts were submerged into the marinade, in a proportion of 25 cuts per 400 mL of marinade, and were kept refrigerated (4 °C) for 18 h.

### 2.2. Bacterial strains and inoculation of samples

*L. lactis* ULE383, *L. paracasei* ULE721 and *L. plantarum* ULE1599, all belonging to the research group culture collection and thoroughly characterized by Barcenilla et al. (2023), were individually grown in 9 mL of De Man, Rogosa and Sharpe broth (MRS, Merck, Germany) at 30 °C for 48 h under anaerobic conditions (Anaerocult A, Merck). Afterwards, 3 mL of each strain suspension were mixed and centrifuged at 3000×*g* for 10 min at 20 °C. The supernatants were discarded and the pellets were resuspended in the marinade liquid to reach a concentration of ~8 $\log_{10}$ CFU/mL.

Two strains of *L. monocytogenes* were used as target microorganisms in the challenge test studies, which were designed considering the "EURL *Lm* Technical Guidance Document for conducting shelf-life studies on *L. monocytogenes* in RTE foods" (European Union Reference Laboratory for Listeria monocytogenes, 2019). *L. monocytogenes* ULE970 is a strain originally isolated from the processing environment of a meat industry (Alvarez-Molina et al., 2021), and *L. monocytogenes* CECT911 was obtained from the Spanish Type Culture Collection (*Colección Española de Cultivos Tipo - CECT*). Each strain was grown individually in 9 mL of Brain Heart Infusion (BHI, Merk) at 37 °C for 18 h. Then, a subculture was prepared by adding 0.1 mL from this bacterial suspension to 9 mL of BHI, followed by storage at 10 °C for 3 days. Four mL of each subculture were then mixed and serial decimal dilutions were prepared before inoculation in the marinade liquid to reach a concentration of ~4 $\log_{10}$ CFU/mL.

Four different batches of marinated lean pork were prepared for each of the packaging conditions tested (Table 1): marinated lean pork without inoculation with LAB or *L. monocytogenes* (NC); marinated lean pork inoculated only with the LAB cocktail (LAB); marinated lean pork inoculated only with the *L. monocytogenes* cocktail (LM); and marinated lean pork inoculated with both the LAB and the *L. monocytogenes* cocktails (LAB + LM).

Lean pork cuts (already marinated for 18h) were submerged in the corresponding marinade and mixed for 14 min, and then the samples (25 ± 1 g) were individually packaged in 30 μm polyamide – 130 μm polyethylene bags, commonly used for meat products (30 $cm^3/(mm^2 \cdot 24$ h bar) permeability to oxygen) (Pargon, Spain). The final concentration of LAB and *L. monocytogenes* on the pork was ~6.5 and ~3 $\log_{10}$ CFU/g, respectively. The lower concentration of the pathogen is closer to what expected in a real scenario, while the higher concentration of the LAB was established based on the results previously obtained in Barcenilla et al. (2023). The two packaging conditions used were vacuum (25 mbar) and MAP (20 % $CO_2$–80 % $N_2$). The packaged samples were stored at 7 °C for 8 days, followed by 12 °C for 4 days, following the recommendations of EURL *Lm* Technical Guidance. Three independent replicates were performed for each type of sample.

### 2.3. Microbiological analyses

Microbiological determinations were performed in triplicate at days 0, 4, 8 and 12 of storage. Each sample (25 ± 1 g) was mixed with 225 mL of 0.1% Buffered Peptone Water (BPW, Merck) and homogenized in a stomacher (IUL Instruments, Spain) at maximum speed for 4 min. Appropriate serial dilutions were prepared in BPW and plated on MRS agar, Agar Listeria Ottaviani and Agosti (ALOA, VWR, US) and Plate Count Agar (PCA, Merck) for the enumeration of LAB, *L. monocytogenes* and total psychrotrophic bacteria, respectively. MRS plates were

**Table 1**
Marinated lean pork samples prepared under different conditions of packaging and inoculation; and types of analysis carried out for each sample type.

| Sample type | Sample preparation | | | | Analyses | |
|---|---|---|---|---|---|---|
| | Packaging | | Inoculation | | | |
| | Vacuum | MAP | LAB cocktail | *L. monocytogenes* | Microbiological | pH, $a_w$, color, sensory analysis, *16S rRNA* sequencing |
| **V-NC** | ● | ○ | ○ | ○ | ● | ● |
| **V-LAB** | ● | ○ | ● | ○ | ● | ● |
| **V-LAB + LM** | ● | ○ | ● | ● | ● | ○ |
| **V-LM** | ● | ○ | ○ | ● | ● | ○ |
| **M-NC** | ○ | ● | ○ | ○ | ● | ● |
| **M-LAB** | ○ | ● | ● | ○ | ● | ● |
| **M-LAB + LM** | ○ | ● | ● | ● | ● | ○ |
| **M-LM** | ○ | ● | ○ | ● | ● | ○ |

incubated at 30 °C for 48 h under anaerobiosis, ALOA plates at 37 °C for 48 h and PCA plates at 10 °C for 7 days. *L. monocytogenes* was never detected in NC samples(data not shown).

### 2.4. pH and water activity

The pH was measured with a pH-meter (VioLab, XS Instruments, Italy) after homogenizing each sample (25 ± 1 g) with 25 mL of distilled water. Water activity was determined using a Decagon CX-2 hygrometer (Decagon Devices Inc., US). Both evaluations were performed in triplicate after 0, 4, 8 and 12 days of storage. Samples were discarded after each analysis.

### 2.5. Color

To simulate a real scenario for consumption, marinated lean pork samples after 1, 6 and 11 days of storage were cooked evenly in an oven until the centre of the piece reached 100 °C prior to color measurement. For color determinations, CIE L*C*h* space color parameters were measured using a CM-5/CR-5 spectrophotometer (Konica Minolta, Japan) with Illuminant D65, 8 mm aperture and 10° standard observer parameters on the surface of cooked marinated lean pork samples. Total color difference (ΔE) was calculated as $\Delta E = [(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{1/2}$. Color analysis was carried out in triplicate.

### 2.6. Sensory analysis

A sensory analysis was conducted to evaluate the organoleptic properties of the cooked marinated lean pork with or without LAB added and packaged under vacuum or MAP after 8 days of storage at 7 °C. The sensory analysis was performed on day 8 of storage due to the logistic organization. The samples were cooked evenly in an oven until the centre of the piece reached 100 °C and immediately served to the panellists. A single session with 50-untrained panellists was performed serving to each individual the four samples randomly with a three-digit identifier. Firstly, a ranking test to evaluate the darkness/lightness (visual), aroma intensity (odor), hardness (texture) and acidity (taste) was conducted. Also, consumers were asked to specify whether the samples were pleasant or unpleasant, based on taste liking.

### 2.7. Metataxonomic analysis through 16S rRNA gene amplicon sequencing

#### 2.7.1. DNA extraction

Total metagenomic DNA was extracted after 1, 6 and 11 days of storage. Firstly, a cell pellet was aseptically obtained after homogenizing 10 g of each sample with 90 mL sterile Phosphate Buffer Saline (PBS, Sigma-Aldrich) in a stomacher for 2 min and centrifuging at 5000×*g* for 15 min. The supernatant was discarded and the pellet resuspended in 5 mL sterile PBS. This washing step was repeated three times. The final pellets were stored at −80 °C until DNA extraction. The DNeasy® PowerSoil® Pro kit (Qiagen, Netherlands) was used to extract total metagenomic DNA from the samples following the manufacturer's instructions. DNA concentration was quantified using the fluorometric assay Qubit ™ dsDNA High Sensitive quantification kit with a Qubit 3.0 fluorometer (Invitrogen, USA).

#### 2.7.2. PCR amplification and high throughput amplicon sequencing

The Illumina Miseq platform was used to sequence *16S rRNA* gene amplicons with 300 bp pair-end sequencing. The V3–V4 hypervariable region was amplified with the primers S-D-Bact-0341-b-S-17 (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3′) (Carrasco et al., 2020). Libraries were elaborated according to the *16S Metagenomic Sequencing Library Preparation* protocol for the Illumina Miseq platform in *Centro de Investigación Biomédica de la Rioja* (CIBIR, Spain).

#### 2.7.3. Taxonomic and biodiversity analyses

Raw reads were processed by *dada2* R-package following the DADA2 v1.8.0 tutorial (Callahan et al., 2016). Primer sequences and ambiguous bases were removed with *cutadapt* and *truncLen* = c(275,250), respectively (Martin, 2011). Chimeras were removed with *removeBimeraDenovo* command in DADA2 pipeline, and the RDP database v18 (Ribosomal Database Project) (Cole et al., 2014) was used with *assignTaxonomy* in DADA2 to create a table with the Amplicon Sequence Variants (ASV) counts for all the samples.

Simpson and Shannon indices and ASV richness values were employed to evaluate the alpha diversity of the microbial community, calculated by using *diversity* and *specnumber* commands from *vegan* R-package. The beta-diversity analysis was done using Bray-Curtis dissimilarity distances with the *cmdscale* command.

### 2.8. Statistical analyses

The experiment was performed in triplicate and results are reported as average values with standard deviations. In microbiological, pH, aw and color analyses, statistically significant differences among different sample groups were determined using Analysis of Variance ANOVA followed by Tukey test in RStudio v4.0.2. In the sensory analyses, data obtained in the ranking test were also analysed with ANOVA and the

Tukey test after data transformation, while the data on the classification of samples as pleasant/unpleasant (frequency, %) were analysed with the statistical tables for estimating significance in paired-preference tests (Roessler, Pangborn, Sidel, & Stone, 1978).

In metataxonomic analyses, significant differences in alpha-diversity indices were determined using the *t*-test by using the *compare means* command from *ggpubr* R-package. Significant differences in the beta-diversity analyses were analysed through a dissimilarity test (ADONIS), using the *adonis* command from *vegan* R-package. Plots were elaborated in *ggplot2* R-package. All analyses and plots were carried out in RStudio v 4.0.2. Statistical differences were considered significant if associated with a p-value $\leq$0.05.

### 2.9. Data availability

Raw reads from the *16S rRNA* gene amplicon sequencing can be found in the Nacional Centre for Biotechnology Information under the Bioproject ID PRJNA941229.

## 3. Results and discussion

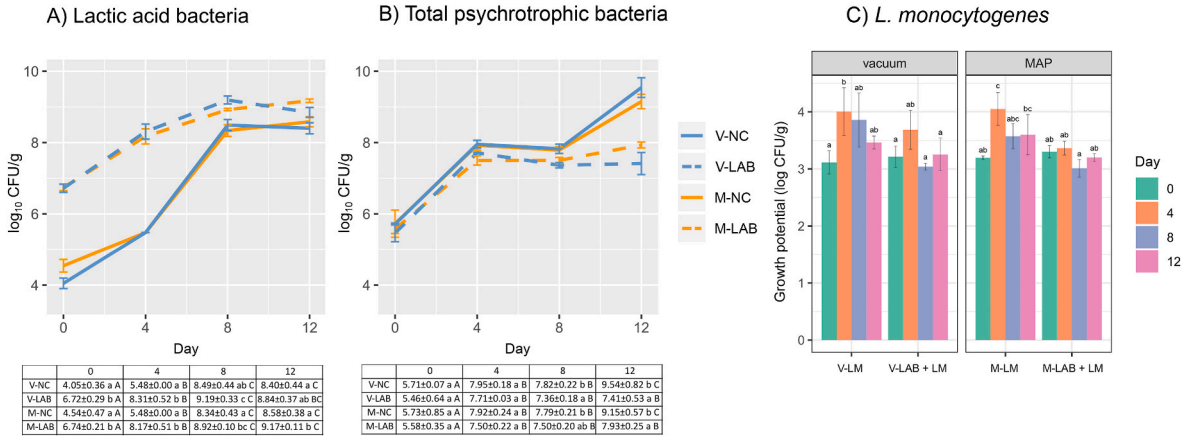
### 3.1. Microbiological analyses

LAB and total psychrotrophic bacterial counts are shown in Fig. 1A and B, respectively. At day 0, LAB counts were 6.7 $\pm$ 0.3 and 6.7 $\pm$ 0.2 $\log_{10}$ CFU/g under vacuum and MAP, respectively. Significantly lower ($p \leq 0.05$) LAB counts, ranging between 4.1 and 4.5 $\log_{10}$ CFU/g, were obtained in non-inoculated samples (NC). In samples inoculated with the LAB cocktail, LAB counts progressively increased throughout storage, except for V-LAB from day 8 to day 12 of storage. The LAB counts reached on the last day of storage for V-LAB and M-LAB were of 8.8 $\pm$ 0.4 and 9.2 $\pm$ 0.1 $\log_{10}$ CFU/g, respectively. LAB counts also showed a progressive increase throughout storage in NC samples, reaching final values of 8.4–8.6 $\log_{10}$ CFU/g at day 12 of storage. M-LAB showed significant higher LAB counts as compared to V-NC samples (Fig. 1A).

LAB are well-known as common members of the microbiota of meat products, including marinated products (Lytou, Panagou, & Nychas, 2016), so a good adaptation of the LAB cocktail to the marinated lean pork was expected. LAB counts showed a very similar pattern under vacuum and MAP, which is in agreement with the findings by other authors (Doulgeraki, Hondrodimou, Iliopoulos, & Panagou, 2012; Nieminen, Nummela, & Björkroth, 2015). LAB are facultative anaerobic microorganisms and thus grow under a variety of environmental conditions and gas composition, either under vacuum or modified atmosphere packaging (Nieminen et al., 2015). This is also evidenced in Barcenilla et al. (2023) where the same LAB cocktail grew similarly in cooked ham (no marinated) packaged under vacuum or MAP (same gas composition). However, the gas composition of the packages could have slightly evolved during the storage period.

An increase in the population of psychrotrophic bacteria (Fig. 1B) was observed from initial values of 5.5 $\pm$ 0.5 $\log_{10}$ CFU/g to 7.6 $\pm$ 0.2 $\log_{10}$ CFU/g at day 4. These counts remained nearly stable until the end of the experiment in the case of LAB inoculated samples, whereas in NC samples increased to 9.4 $\pm$ 0.7 $\log_{10}$ CFU/g at day 12 of storage (Fig. 1B). The significantly lower counts at the last sampling point in LAB inoculated samples, as compared to NC samples, could indicate that the LAB cocktail has limited the growth of some psychrotrophic bacteria on the marinated lean pork, which could have positive effect on the extension of the shelf life of the product.

*L. monocytogenes* counts at day 0, after spiking, were of 3.2 $\pm$ 0.03 and 3.1 $\pm$ 0.2 $\log_{10}$ CFU/g, in MAP and vacuum packaged samples, respectively. The pathogen's growth reached its maximum value of 4.0 $\pm$ 0.5 and 4.1 $\pm$ 0.3 $\log_{10}$ CFU/g at day 4 in NC samples, for vacuum and MAP respectively (Fig. 1C). The growth potential values (calculated as the difference between the counts at day 4, 8 or 12 and the counts at day 0) were 0.9 $\log_{10}$ CFU/g for both packaging conditions. It has been previously established that a growth potential >0.5 $\log_{10}$ CFU/g is generally interpreted as the product supporting the growth of *L. monocytogenes* (Álvarez-Ordóñez, Leong, Hickey, Beaufort, & Jordan, 2015). The growth observed was similar to that observed for other meat products in other similar studies, where the pathogen was able to grow up to reach concentrations in the range 4.8–5.8 CFU/g in chicken wings, 4.8–5.4 CFU/g in chicken drumsticks and 5.2–6.4 CFU/g in chicken breast meat (İncili et al., 2020). *L. monocytogenes* is known to be well adapted to grow at low temperatures under a wide range of pH and $a_w$ (Nyhan et al., 2018). However, different marinades have proved capable of controlling the growth of this pathogen, with some studies reporting up to 6 log CFU/g of difference in growth between different marinated and non-marinated products (Lopes et al., 2021). In the LM samples (only marinated), the pathogen's counts decreased from day 4 to days 8 and 12 of storage. Previous studies demonstrated the antimicrobial effect of different ingredients included in this marinade have, such as paprika, oregano and garlic (Gonzalez-Fandos, de Castro, Martinez-Laorden, & Perez-Arnedo, 2021; Lopes et al., 2021; Martínez, Bastida, Castillo, Ros, & Nieto, 2019). However, the marinade employed in this study was sterilized before submerging the pork cuts in it, hence, the possible antimicrobials present in the ingredients of the marinade itself (oregano, paprika, garlic, etc.) could have been affected by the




| | 0 | 4 | 8 | 12 |
|---|---|---|---|---|
| V-NC | 4.05±0.36 a A | 5.48±0.00 a B | 8.49±0.44 ab C | 8.40±0.44 a C |
| V-LAB | 6.72±0.29 b A | 8.31±0.52 b B | 9.19±0.33 c C | 8.84±0.37 ab BC |
| M-NC | 4.54±0.47 a A | 5.48±0.00 a B | 8.34±0.43 a C | 8.58±0.38 a C |
| M-LAB | 6.74±0.21 b A | 8.17±0.51 b B | 8.92±0.10 bc C | 9.17±0.11 b C |

| | 0 | 4 | 8 | 12 |
|---|---|---|---|---|
| V-NC | 5.71±0.07 a A | 7.95±0.18 a B | 7.82±0.22 b B | 9.54±0.82 b C |
| V-LAB | 5.46±0.64 a A | 7.71±0.03 a B | 7.36±0.18 a B | 7.41±0.53 a B |
| M-NC | 5.73±0.85 a A | 7.92±0.24 a B | 7.79±0.21 b B | 9.15±0.57 b C |
| M-LAB | 5.58±0.35 a A | 7.50±0.22 a B | 7.50±0.20 ab B | 7.93±0.25 a B |

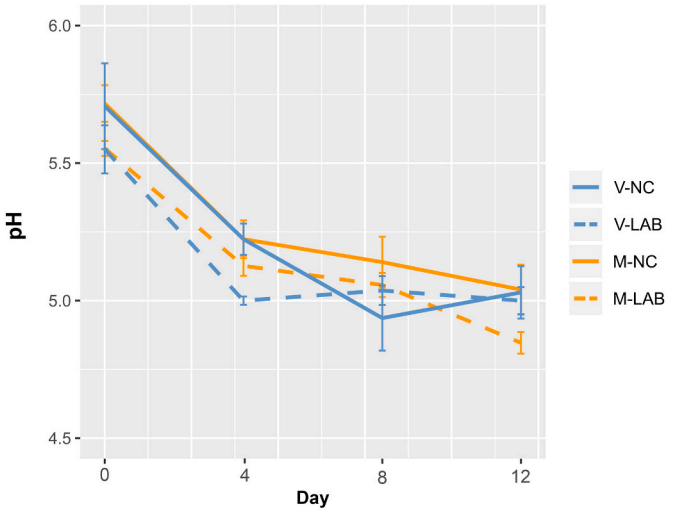
**Fig. 1.** Microbiological evolution during storage of the marinated pork under vacuum (V) (blue) and modified atmosphere packaging (M) (orange), with (dashed) or without (solid) the LAB cocktail added. A) LAB counts, B) total psychrotrophic bacterial counts and C) *L. monocytogenes* counts. In panel A and B, within each row, different capital letters indicate significant differences among days ($p \leq 0.05$); within each column, different lowercase letters show significant differences among sample types ($p \leq 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

thermal treatment. Fig. 1A shows that NC samples also underwent an increase in LAB counts, which could be the reason behind *L. monocytogenes* growth completion. The intentional addition of the LAB cocktail to the marinade resulted in an enhanced reduction of the pathogen's growth compared to the positive control in all samples at days 4, 8 and 12 of storage. Up to a reduction of 0.8 and 0.7 $\log_{10}$ CFU/g were obtained if compared with samples only inoculated with *L. monocytogenes* under vacuum and MAP, respectively. Remarkably, in the LAB inoculated product, the critical limit of a growth potential >0.5 $\log_{10}$ CFU/g was not attained after day 4 in V-LAB. In M-LAB samples, the critical limit of growth potential >0.5 $\log_{10}$ CFU/g, was never exceeded. In another study with cooked ham, where no marination was applied, the growth potential of the same *L. monocytogenes* cocktail reached up to approximately 5 log units (Barcenilla, et al., 2023), which could indicate that these strains are able to grow in meat products without a marination processing and packed under vacuum or MAP (same gas composition as the current study).

### 3.2. pH and $a_w$

The pH of marinated meat can be influenced by several factors, such as the marinade formulation, meat type, duration of the marination process and strains used as biopreservatives (Zavistanaviciute et al., 2023). The pH of the marinated lean pork product oscillated between a maximum of 5.7 at day 0 and a minimum of 4.8 at day 12 of storage (Fig. 2). *L. monocytogenes* is capable to grow within these pH limits, as it has a demonstrated ability to grow at pH values as low as 4.1, according to İncili et al. (2020).

The highest statistically significant difference in the decrease of pH (from 0.43 to 0.55) occurred from day 0 to day 4 of storage. No significant differences ($p > 0.05$) were observed among the pH values presented by the four sample types, except at day 4, where V-LAB samples showed significantly lower pH (Fig. 2). It is widely known that the acidification can be caused by the production of organic acids (de Souza, de Oliveira, & de Oliveira, 2023).




| | 0 | 4 | 8 | 12 |
|---|---|---|---|---|
| V-NC | 5.71±0.27 a B | 5.22±0.10 a AB | 4.94±0.20 a A | 5.03±0.16 a A |
| V-LAB | 5.55±0.15 a B | 5.00±0.03 a A | 5.04±0.09 a A | 5.00±0.09 a A |
| M-NC | 5.72±0.12 b B | 5.22±0.12 b A | 5.14±0.16 a A | 5.04±0.16 a A |
| M-LAB | 5.55±0.05 a C | 5.13±0.06 ab B | 5.06±0.08 a B | 4.85±0.07 a A |

**Fig. 2.** pH evolution in marinated lean pork under modified atmosphere packaging (M) (orange) or vacuum (V) (blue) packaging, with (dashed) or without (solid) the addition of the LAB cocktail to the marinade. Within each row, different capital letters indicate significant differences among days ($p \leq 0.05$); within each column, different lowercase letters show significant differences among sample types ($p \leq 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Other authors (İncili et al., 2020) have found that the pH of marinated chicken meat, on the contrary, increased in 1.0–1.6 units along the 10-days shelf life of the product. A similar increase in pH was also observed in pork treated with a solution containing mytichitin-CB (not marinated), an antibacterial peptide produced by *Pichia pastoris*, during a 8-days storage, which was caused by the degradation of amino acids into volatile alkaline nitrogen-containing molecules (Meng, Sun, Shi, Cheng, & Fan, 2021).

With respect to $a_w$ values, significant differences were not detected among samples along the shelf life, with values ranging from 0.979 to 0.985 (data not shown). This evidences that the addition of the LAB cocktail did not affect the normal evolution of $a_w$ in any type of packaging.

### 3.3. Color

Color parameters, including lightness (L*), chroma (C*) and hue (h*), were recorded at different times of storage of the marinated lean pork product (Fig. 3). Total color difference (ΔE) was also calculated to evaluate the color change during storage, indicating the relative change in color at day 11 compared to day 1. The highest ΔE was found for the samples inoculated with the LAB and packaged under MAP (ΔE = 6.4), followed by NC samples packaged under vacuum (ΔE = 5.9). On the contrary, the ΔE value of M-NC and V-LAB samples was of 3.1. The higher ΔE of the M-LAB and V-NC samples could be due to the significant increase in lightness observed from day 1 to day 11 in both sample types and by the significant decrease in V-NC, or increase in M-LAB, of C* (Fig. 3). According to Gliemmo, Latorre, Gerschenson, and Campos (2009), a ΔE higher than 2 would be detectable by the consumer (Gliemmo et al., 2009). Hence, the color differences here observed could, in principle, be noticeable by the consumer.

Chroma values on day 11 of storage tended to be lower for vacuum-packaged samples than for MAP packaged samples (Supplementary file 1). In fact, the samples with the highest C* values were M-LAB samples. This evidences that color saturation increased with MAP packaging, which corroborates previously reported findings (Stahlke et al., 2019). Both types of packaging excluded the oxygen, so the changes related to the status of myoglobin (Tomasevic, Djekic, Font-i-Furnols, Terjung, & Lorenzo, 2021) are not applicable to this study.

In another study (Mozuriene et al., 2016), pork marinated with a natural potato juice fermented with three LAB strains showed higher L* values than control samples. Meat is observed as lighter when there is less bound water and thus more water available to reflect the light. Also, when the pH declines myoglobin is more easily transformed into met-myoglobin and meat becomes lighter (Mozuriene et al., 2016).

### 3.4. Sensory analysis

The results of the sensory evaluation with 50 panellists are shown in Fig. 4. With respect to color, aroma or acidity, the panellists did not differentiate the samples with the LAB cocktail added from NC samples, neither when packed under vacuum nor under MAP ($p \leq 0.05$). It has been previously reported that the main limitation in the application of LAB as food cultures in meat products is that the significant acidification they may cause in the product could lead to a detectable acid taste (Vermeiren, Devlieghere, Vandekinderen, Rajtak, & Debevere, 2006). On the contrary, in a previous study were a cured-smoked pork product was inoculated with various LAB cultures and packed under MAP, no differences were observed in acidic taste as compared to negative controls (Casquete et al., 2019). However, we found differences among samples in texture, with samples with the LAB cocktail added being ranked as significantly ($p \leq 0.05$) harder (Fig. 4A).

In a previous study by our group (Barcenilla et al., 2023), where the same LAB cocktail was applied in cooked ham, no significant differences

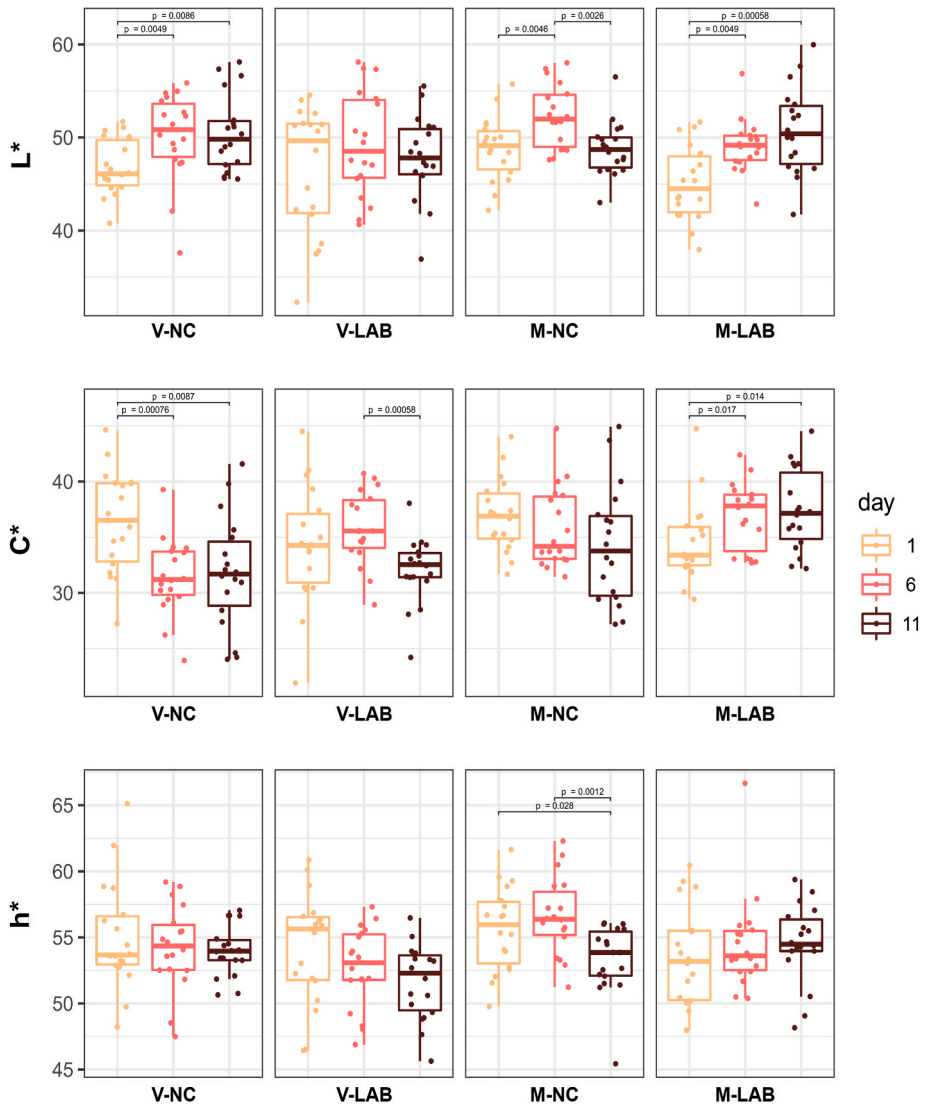



**Fig. 3.** Color parameters of lightness (L*), chroma (C*) and hue (h*) of the marinated lean pork with (LAB) or without (NC) the inoculation with LAB and packaged under vacuum (V) or modified atmosphere packaging (M) during a 11-days storage. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

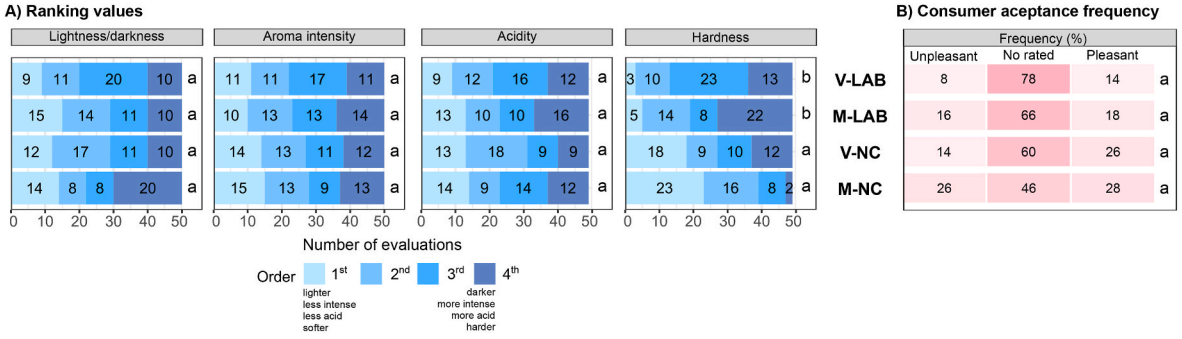



**Fig. 4.** Sensory evaluation of the marinated lean pork with (LAB) or without (NC) the addition of the LAB cocktail and packed under vacuum (V) or modified atmosphere packaging (M). A) Number of times that each of the 50 panellists ranked the samples from position 1 (lighter, less intense aroma, less acid or less hard) to position 4 (darker, more intense aroma, more acid, harder); B) Frequency (in percentage) of panellists that assigned each sample as unpleasant, pleasant or not rated. a–b: statistically significant differences among samples for each parameter evaluated ($p \leq 0.05$).

between LAB inoculated samples and negative controls were found when panellists had to classify the samples as pleasant or unpleasant. The same results were obtained in the current study, although NC samples tended to be more frequently attributed as "pleasant" than LAB inoculated samples, that were more frequently unassigned to any of the two categories ("pleasant" or "unpleasant") by panellists (Fig. 4B).

### 3.5. Metataxonomic analyses

The influence of storage time, type of packaging used and addition of LAB on the composition of the product's microbiota is shown in Fig. 5. Significant differences ($p = 0.001$) in the taxonomic profiles were observed among storage times, with samples being clearly clustered based on this factor in the Principal Coordinates Analysis. The type of packaging method used did not have a significant influence on inter-sample dissimilarity, similar to the results obtained in our previous study with vacuum or MAP packed cooked ham (Barcenilla et al., 2023).

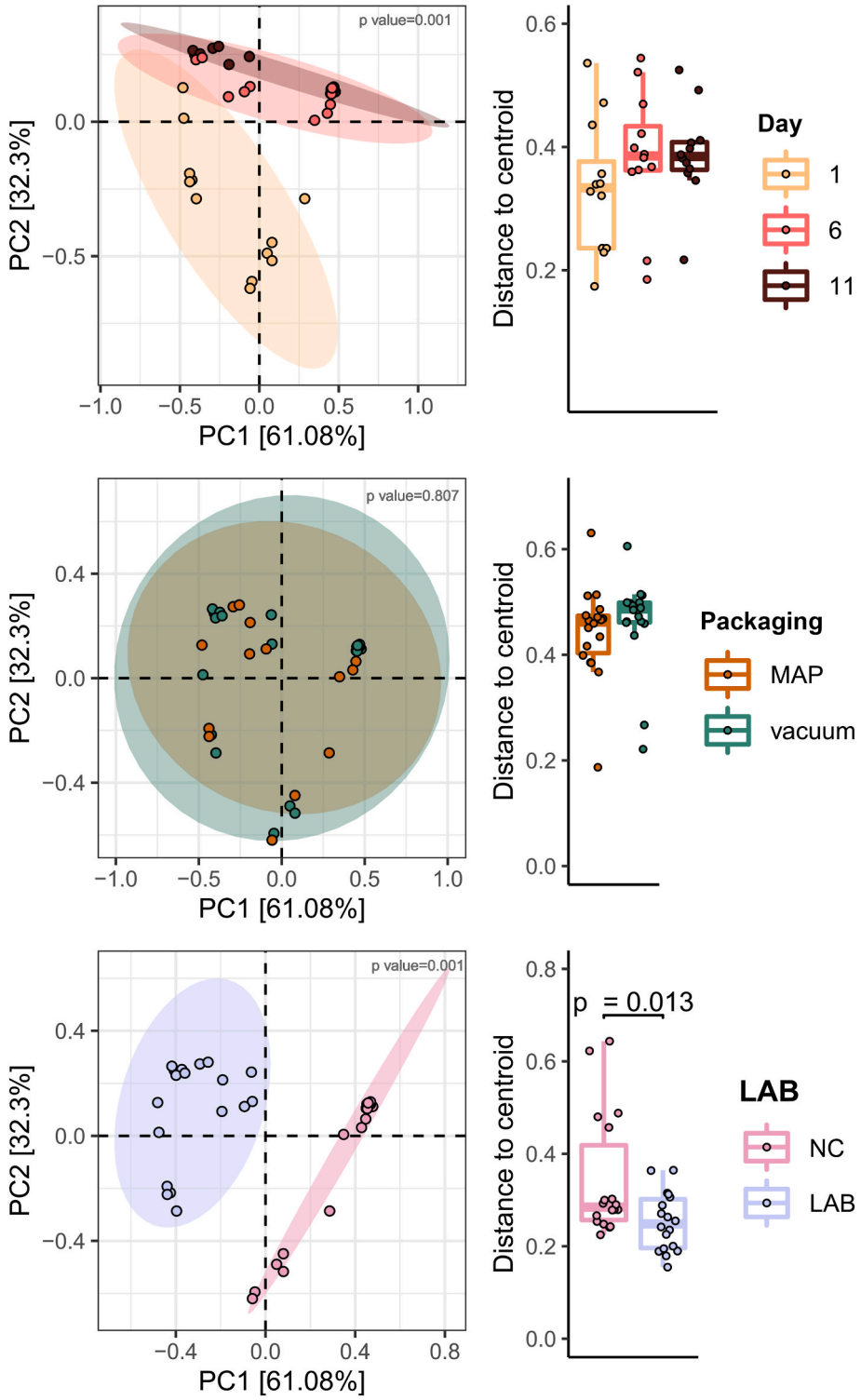



**Fig. 5.** Beta-diversity analyses based on Bray-Curtis dissimilarity of the microbial communities (*16S rRNA* metataxonomic composition) in marinated lean pork without (NC) and with the addition of the LAB cocktail (LAB) packed under vacuum or modified atmosphere packaging (MAP): A) Differences among storage days: 1 (yellow), 6 (red) and 11 (brown); B) Differences between samples packed under vacuum (green) or MAP (orange); and C) Differences between samples inoculated with the LAB cocktail (LAB; purple) and negative control (NC; pink) samples. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

In relation to the effect of the addition of the LAB cocktail, two distinct groups of samples (with or without the LAB strains added) were established with significant differences ($p = 0.001$). Beta-dispersion (assessed as the distance to the centroid) was significantly lower ($p = 0.013$) in LAB inoculated samples than in NC samples, indicating a more homogeneous microbial composition in LAB inoculated samples. The LAB strains used in the cocktail, *L. lactis* ULE383, *L. paracasei* ULE721 and *L. plantarum* ULE1599, were previously studied by our research group in cooked ham and similar effects of their inoculation on beta-dispersion were also observed there.

In NC samples, the richness of the microbiota significantly decreased along the product's shelf life, from 50.7 ± 4.9 ASVs at day 1–34.2 ± 3.3 and 35.3 ± 6.9 ASVs at day 6 and 11, respectively (Fig. 6). On the contrary, in samples with the LAB cocktail added, this parameter was already low at day 1, and no significant differences were observed among different storage times, with richness average values ranging from 31.7 ± 8.8 to 39.3 ± 4.5 ASVs (Fig. 6). This is probably due to the effect on bacterial diversity of the inoculation of samples with high LAB concentrations, which leads to the dominance of these three LAB strains over the rest of members of the product's autochthonous microbiota. Likewise, other alpha-diversity indices, such as the Simpson and Shannon index, were also maximum for NC samples at the start of the experiment and then significantly decreased, as storage time progressed, being lower at the last day of analysis in NC samples than in LAB inoculated samples.

The evolution of the relative abundance of the main genera found in the marinated lean pork samples is shown in Fig. 7. Altogether, no major differences were observed on main genera profile between the two packaging methods applied. The majority of the bacteria found in the marinated pork are facultative anaerobes, and thus can grow either in vacuum or MAP (Pellissery, Vinayamohan, Amalaradjou, & Venkitanarayanan, 2020). This explains the metataxonomic similarities between the samples packaged under vacuum and under MAP. At day 1, samples without the LAB cocktail added showed an important dominance of *Photobacterium*, with >50% relative abundance both in vacuum and MAP packaged samples. Although initially isolated from marine environments, this genus has been previously isolated from meat products and associated to food spoilage (Fuertes-Perez, Hauschild, Hilgarth, & Vogel, 2019). When the LAB cocktail was added, already at day 1, there was a decrease in the relative abundance of *Photobacterium* to ~30%. The addition of the LAB cocktail had a marked influence on the abundance of the main bacterial taxa in the samples. The three LAB strains included in the cocktail belong to the genera *Lactococcus*, *Lacticaseibacillus* and *Lactiplantibacillus* and these were importantly represented in LAB inoculated samples. Among them, *Lacticaseibacillus* was the most dominant genus, with relative abundances from 28 to 57.2%, followed by *Lactiplantibacillus*, with 5.5–28.3%, and *Lactococcus*, with 1.2–12.9%. The dominance of one strain of the LAB cocktail over the other two was also observed in other studies using LAB cocktails, and can be caused by the differences in adaptability to the environmental conditions of the products (Barcenilla et al., 2023; Dučić et al., 2023). Although it is technically not possible to confirm the assignment of ASVs from these three genera (by comparing *16S rRNA* sequences) to the three specific strains used in the cocktail, these three genera were hardly present, with relative abundance between 0 and 0.3%, in the NC samples, which confirm that these ASVs belong to the LAB strains added. Nevertheless, the vast majority of the microbiota members present in the NC samples at day 6 and 11 of storage were also from the LAB group, including *Latilactobacillus* (62.7–72.5%), *Leuconostoc* (11.4–16.8%) and *Liquorilactobacillus* (7.9–17.8%). This high LAB richness can explain the decrease in pH and the limited *L. monocytogenes* growth observed also in those samples without the LAB cultures added. A similar taxonomic profile was previously observed in a study on marinated broiler fillet strips, where members of the former *Lactobacillus* genus, mainly, and *Leuconostoc* dominated in the community (Nieminen, 2012).

As the current study shows, the combination of marinades and biopreservation approaches based on the application of LAB cultures with different types of packaging, as part of a hurdle's technology approach, holds potential for the control of the growth of *L. monocytogenes*, one of the most important foodborne pathogens. The combination of these methods with other novel preservation technologies, such as High Pressure Processing (HPP), has been also explored with success in the past. For example, O'Neill, Cruz-Romero, Duffy, and Kerry (2019) studied the combination of HPP with organic acids in marinated pork and obtained a longer shelf life, of up to 29 days. Other advantages observed were that the pressurization favoured the marinade absorption in the product and that the marinade masked the negative discoloration effect of HPP on the meat. Also, although texture was negatively affected by HPP immediately after treatment, tenderness increased over storage time. Hence, a good combination of hurdles can achieve a safer product while mitigating any potential adverse effects (O'Neill et al., 2019).

## 4. Conclusions

The cocktail of *Lactococcus lactis* ULE383, *Lacticaseibacillus paracasei* ULE721 and *Lactiplantibacillus plantarum* ULE1599 showed potential to be used as a food culture in marinated lean pork. The type of packaging used, i.e. vacuum or MAP, did not influence the results obtained. This fact evidences the good adaptation of the LAB strains employed to both types of packaging, commonly employed in the commercialization of this type of products, although no recommendation for one packaging or the other can be suggested. *L. monocytogenes* was controlled by the use of

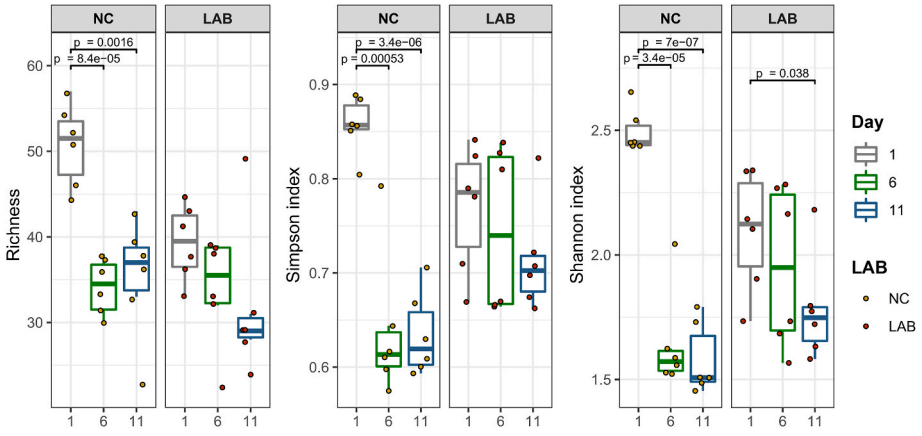



**Fig. 6.** Evolution during storage of alpha-diversity indices (richness, Simpson and Shannon) of the marinated lean pork without (NC) and with the addition of the LAB cocktail (LAB).

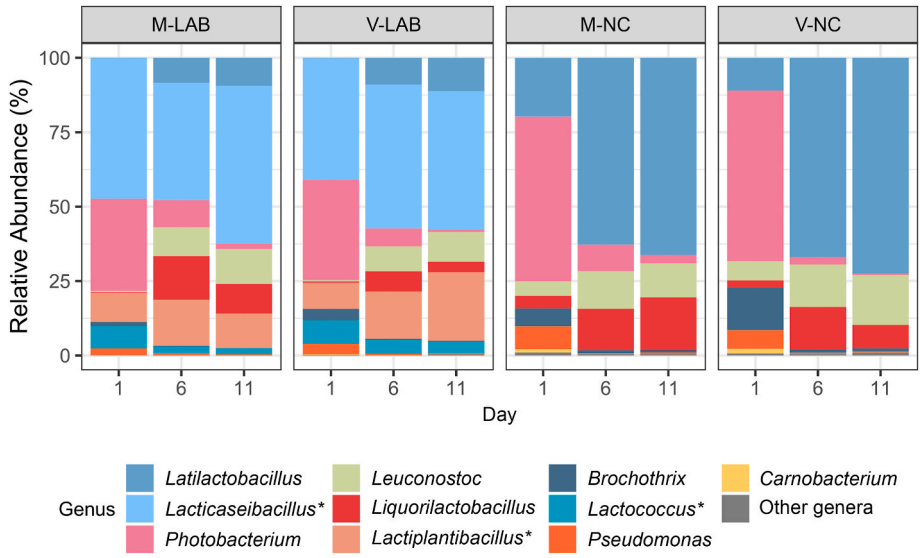



**Fig. 7.** Relative abundance of the main genera present in the marinated pork after 11 days of storage. Samples represented correspond to marinated lean pork with LAB (LAB) or without LAB (NC) added, and packed under vacuum (V) or modified atmosphere packaging (M). *Genera of the three LAB included in the cocktail.

the LAB strains, which made it possible to maintain the pathogen's growth potential below 0.5 log CFU/g for samples stored under MAP throughout the storage period. This study could benefit with future research in various aspects. On the one hand, *L. monocytogenes* was moderately controlled also in the LM samples (positive control), and thus it would be good to include samples of pork cuts without marination to evaluate the effect of the marinade itself. On the other hand, this study could be reproduced in a RTE marinated pork, to assess the potential of controlling the growth of *L. monocytogenes* in this type of products highly related to listeriosis outbreaks. In addition, a wider range of *L. monocytogenes* strains from various serotypes should be evaluated to describe the antilisterial effect of the LAB cocktail in a marinade with wider insights. Also, the individual use of each LAB included in the cocktail could be tried separately. Additionally, a shelf life study would be valuable from a commercial point of view to ensure the safety of the product. Therefore, it is essential to optimize the application of this LAB cocktail before a future application in marinated pork, or any other meat product. This application would need further studies related to the adaptability in a real-scale food chain, where the food cultures are usually added in a lyophilised form and where the amount of LAB to add has to be in ratio to the amount of meat product.

The results obtained in this study, encourage further investigations in the development of novel strategies for the preservation of meat products using LAB as food cultures in combination with other treatments (e.g. other marinades, HPP, natural compounds with antimicrobial activity, etc.) to mitigate the growth of important pathogens for the meat industry, such as *L. monocytogenes, Campylobacter*, or *Salmonella*.

## CRediT authorship contribution statement

**C. Barcenilla:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **A. Puente:** Investigation. **J.F. Cobo-Díaz:** Formal analysis, Data curation. **M. González-Raurich:** Methodology. **M. López:** Writing – review & editing. **M. Prieto:** Writing – review & editing, Methodology. **A. Álvarez-Ordóñez:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgements

This work was supported by the European Union's Horizon 2020 Research and Innovation Program under grant agreement No. 818368 (MASTER). Coral Barcenilla is grateful to *Junta de Castilla y León* and the European Social Fund for awarding her a pre-doctoral grant (BOCYL-D-07072020-6). We wish to acknowledge the help provided by Sergio Sampedro Pardal.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116166.

## References

Álvarez-Ordóñez, A., Leong, D., Hickey, B., Beaufort, A., & Jordan, K. (2015). The challenge of challenge testing to monitor Listeria monocytogenes growth on ready-to-eat foods in Europe by following the European Commission (2014) Technical Guidance document. https://doi.org/10.1016/j.foodres.2015.06.004.

Alvarez-Molina, A., Cobo-Díaz, J. F., López, M., Prieto, M., de Toro, M., & Alvarez-Ordóñez, A. (2021). Unraveling the emergence and population diversity of <i> Listeria monocytogenes <i> in a newly built meat facility through whole genome sequencing. *International Journal of Food Microbiology, 340*, Article 109043. https://doi.org/10.1016/j.ijfoodmicro.2021.109043

Ashaolu, T. J., Khalifa, I., Mesak, M. A., Lorenzo, J. M., & Farag, M. A. (2021). A comprehensive review of the role of microorganisms on texture change, flavor and biogenic amines formation in fermented meat with their action mechanisms and safety. *Critical Reviews in Food Science and Nutrition, 63*(19), 3538–3555. https://doi.org/10.1080/10408398.2021.1929059

Barcenilla, C., Ducic, M., López, M., Prieto, M., & Álvarez-Ordóñez, A. (2022). Application of lactic acid bacteria for the biopreservation of meat products: A systematic review. *Meat Science, 183*. https://doi.org/10.1016/j.meatsci.2021.108661

Barcenilla, C., Puente, A., Cobo-Díaz, J. F., Alexa, E. A., Garcia-Gutierrez, E., O'Connor, P. M., et al. (2023). Selection of lactic acid bacteria as biopreservation agents and optimization of their mode of application for the control of Listeria monocytogenes in ready-to-eat cooked meat products. *International Journal of Food Microbiology, 403*(April). https://doi.org/10.1016/j.ijfoodmicro.2023.110341

Casquete, R., Fonseca, S. C., Pinto, R., Castro, S. M., Todorov, S., Teixeira, P., et al. (2019). Evaluation of the microbiological safety and sensory quality of a sliced cured-smoked pork product with protective cultures addition and modified

atmosphere packaging. *Food Science and Technology International, 25*(4), 327–336. https://doi.org/10.1177/1082013219825771

Castellano, P., Peña, N., Ibarreche, M. P., Carduza, F., Soteras, T., & Vignolo, G. (2018). Antilisterial efficacy of Lactobacillus bacteriocins and organic acids on frankfurters. Impact on sensory characteristics. *Journal of Food Science and Technology, 55*(2), 689–697. https://doi.org/10.1007/s13197-017-2979-8

Cole, J. R., Wang, Q., Fish, J. A., Chai, B., McGarrell, D. M., Sun, Y., et al. (2014). Ribosomal database Project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Research, 42*(D1), D633–D642. https://doi.org/10.1093/NAR/GKT1244

de Souza, E. L., de Oliveira, K.Á., & de Oliveira, M. E. (2023). Influence of lactic acid bacteria metabolites on physical and chemical food properties. *Current Opinion in Food Science, 49*, Article 100981. https://doi.org/10.1016/J.COFS.2022.100981

Doulgeraki, A. I., Hondrodimou, O., Iliopoulos, V., & Panagou, E. Z. (2012). Lactic acid bacteria and yeast heterogeneity during aerobic and modified atmosphere packaging storage of natural black Conservolea olives in polyethylene pouches. *Food Control, 26*(1), 49–57. https://doi.org/10.1016/J.FOODCONT.2012.01.006

Dučić, M., Barcenilla, C., Cobo-Díaz, J. F., López, M., Álvarez-Ordóñez, A., & Prieto, M. (2023). High pressure processing at the early stages of ripening enhances the safety and quality of dry fermented sausages elaborated with or without starter culture. *Food Research International, 163*(July 2022). https://doi.org/10.1016/j.foodres.2022.112162

European Food Safety Authority (EFSA). (2023). The European union one Health 2022 Zoonoses Report. *EFSA Journal, 21*(12). https://doi.org/10.2903/J.EFSA.2023.8442

European Union Reference Laboratory for Listeria monocytogenes. (2019). *EURL Lm TECHNICAL GUIDANCE DOCUMENT for conducting shelf-life studies on Listeria monocytogenes in ready-to-eat foods*. 1–47. Retrieved from https://sitesv2.anses.fr/en/system/files/private/EURLLm_TechnicalGuidanceDocumentLmshelf-lifestudies_V3_2014-06-06.pdf.

Fernández-Martínez, N. F., Ruiz-Montero, R., Briones, E., Baños, E., Rodríguez-Alarcón, L. G. S. M., Chaves, J. A., et al. (2022). Listeriosis outbreak caused by contaminated stuffed pork, Andalusia, Spain, July to October 2019. *Euro Surveillance, 27*(43), Article 2200279. https://doi.org/10.2807/1560-7917.ES.2022.27.43.2200279/CITE/REFWORKS

Fuertes-Perez, S., Hauschild, P., Hilgarth, M., & Vogel, R. F. (2019). Biodiversity of Photobacterium spp. isolated from meats. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/FMICB.2019.02399/FULL

Gargi, A., & Sengun, I. Y. (2021). Marination liquids enriched with probiotics and their inactivation effects against food-borne pathogens inoculated on meat. *Meat Science, 182*, Article 108624. https://doi.org/10.1016/J.MEATSCI.2021.108624

Gliemmo, M. F., Latorre, M. E., Gerschenson, L. N., & Campos, C. A. (2009). Color stability of pumpkin (Cucurbita moschata, Duchesne ex Poiret) puree during storage at room temperature: Effect of pH, potassium sorbate, ascorbic acid and packaging material. *LWT - Food Science and Technology, 42*(1), 196–201. https://doi.org/10.1016/J.LWT.2008.05.011

Gonzalez-Fandos, E., de Castro, M. V., Martinez-Laorden, A., & Perez-Arnedo, I. (2021). Behavior of Listeria monocytogenes and other microorganisms in sliced Riojano Chorizo (Spanish dry-cured sausage) during storage under modified atmospheres. *Microorganisms, 9*(7). https://doi.org/10.3390/MICROORGANISMS9071384

Halbedel, S., Wilking, H., Holzer, A., Kleta, S., Fischer, M. A., Lüth, S., et al. (2020). Large Nationwide outbreak of Invasive listeriosis associated with blood sausage, Germany, 2018–2019. *Emerging Infectious Diseases, 26*(7), 1456. https://doi.org/10.3201/EID2607.200225

Honikel, K. O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science, 78*(1–2), 68–76. https://doi.org/10.1016/J.MEATSCI.2007.05.030

İncili, G. K., Akgöl, M., Aydemir, M. E., Alan, S., Mutlu, M., İlhak, O.İ., et al. (2020). Fate of Listeria monocytogenes and Salmonella Typhimurium in homemade marinade and on marinated chicken drumsticks, wings and breast meat. *LWT, 134*, Article 110231. https://doi.org/10.1016/j.lwt.2020.110231

Kaveh, S., Mohammad, S., Hashemi, B., Abedi, E., Amiri, M. J., & Conte, F. L. (2023). Bio-preservation of meat and fermented meat products by lactic acid bacteria strains and their antibacterial metabolites. *Sustainability, 15*(13), Article 10154. https://doi.org/10.3390/SU151310154, 2023, Vol. 15, Page 10154.

Lahiri, D., Nag, M., Sarkar, T., Ray, R. R., Shariati, M. A., Rebezov, M., et al. (2022). Lactic acid bacteria (LAB): Autochthonous and Probiotic Microbes for meat preservation and Fortification. *Foods, 11*(18), 2792. https://doi.org/10.3390/FOODS11182792, 2022, Vol. 11, Page 2792.

Latoch, A., Czarniecka-Skubina, E., & Moczkowska-Wyrwisz, M. (2023). Marinades based on natural ingredients as a way to Improve the quality and shelf life of meat: A review. *Foods, 12*. https://doi.org/10.3390/foods12193638

Li, H., Wang, P., Lan, R., Luo, L., Cao, X., Wang, Y., et al. (2018). Risk factors and level of Listeria monocytogenes contamination of raw pork in retail markets in China. *Frontiers in Microbiology, 9*, Article 347852. https://doi.org/10.3389/FMICB.2018.01090/BIBTEX

Lopes, S. M., da Silva, D. C., & Tondo, E. C. (2021). Bactericidal effect of marinades on meats against different pathogens: A review. *Critical Reviews in Food Science and Nutrition, 62*(27), 7650–7658. https://doi.org/10.1080/10408398.2021.1916734

Lytou, A., Panagou, E. Z., & Nychas, G. J. E. (2016). Development of a predictive model for the growth kinetics of aerobic microbial population on pomegranate marinated chicken breast fillets under isothermal and dynamic temperature conditions. *Food Microbiology, 55*, 25–31. https://doi.org/10.1016/J.FM.2015.11.009

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal, 17*(1), 10–12. https://doi.org/10.14806/EJ.17.1.200

Martín, I., Rodríguez, A., Delgado, J., & Córdoba, J. J. (2022). Strategies for Biocontrol of Listeria monocytogenes using lactic acid bacteria and their metabolites in ready-to-eat meat- and Dairy-Ripened products. *Foods, 11*(4), 542. https://doi.org/10.3390/FOODS11040542, 2022, Vol. 11, Page 542.

Martínez, L., Bastida, P., Castillo, J., Ros, G., & Nieto, G. (2019). Green Alternatives to Synthetic Antioxidants, antimicrobials, Nitrates, and Nitrites in clean Label Spanish Chorizo. *Antioxidants, 8*(6), 184. https://doi.org/10.3390/ANTIOX8060184, 2019, Vol. 8, Page 184.

Meng, D. M., Sun, S. N., Shi, L. Y., Cheng, L., & Fan, Z. C. (2021). Application of antimicrobial peptide mytichitin-CB in pork preservation during cold storage. *Food Control, 125*, Article 108041. https://doi.org/10.1016/J.FOODCONT.2021.108041

Mozuriene, E., Bartkiene, E., Krungleviciute, V., Zadeike, D., Juodeikiene, G., Damasius, J., et al. (2016). Effect of natural marinade based on lactic acid bacteria on pork meat quality parameters and biogenic amine contents. *LWT - Food Science and Technology, 69*, 319–326. https://doi.org/10.1016/j.lwt.2016.01.061

Nieminen, T. (2012). The effect of marination on lactic acid bacteria communities in raw broiler fillet strips. *Frontiers in Microbiology, 3*(OCT), 376. https://doi.org/10.3389/fmicb.2012.00376

Nieminen, T. T., Nummela, M., & Björkroth, J. (2015). Packaging gas selects lactic acid bacterial communities on raw pork. *Journal of Applied Microbiology, 119*(5), 1310–1316. https://doi.org/10.1111/JAM.12890

Nyhan, L., Begley, M., Mutel, A., Qu, Y., Johnson, N., & Callanan, M. (2018). Predicting the combinatorial effects of water activity, pH and organic acids on Listeria growth in media and complex food matrices. *Food Microbiology, 74*, 75–85. https://doi.org/10.1016/J.FM.2018.03.002

Omer, M. K., Álvarez-Ordoñez, A., Prieto, M., Skjerve, E., Asehun, T., & Alvseike, O. A. (2018). A systematic review of bacterial foodborne outbreaks related to red meat and meat products. *Foodborne Pathogens and Disease, 15*(10), 598–611. https://doi.org/10.1089/fpd.2017.2393

O'Neill, C. M., Cruz-Romero, M. C., Duffy, G., & Kerry, J. P. (2019). Improving marinade absorption and shelf life of vacuum packed marinated pork chops through the application of high pressure processing as a hurdle. *Food Packaging and Shelf Life, 21*, Article 100350. https://doi.org/10.1016/J.FPSL.2019.100350

Pellissery, A. J., Vinayamohan, P. G., Amalaradjou, M. A. R., & Venkitanarayanan, K. (2020). Spoilage bacteria and meat quality. In *Meat quality analysis: Advanced evaluation methods, techniques, and technologies* (pp. 307–334). https://doi.org/10.1016/B978-0-12-819233-7.00017-3

Roessler, E. B., Pangborn, R. M., Sidel, J. L., & Stone, H. (1978). Expanded statistical tables for estimating significance in paired-preference, paired-difference, Duo-trio and Triangle tests. *Journal of Food Science, 43*, 940–947.

Siroli, L., Baldi, G., Soglia, F., Bukvicki, D., Patrignani, F., Petracci, M., et al. (2020). Use of essential oils to increase the safety and the quality of marinated pork Loin. *Foods, 9*(987). https://doi.org/10.3390/foods9080987

Stahlke, E. von R., Rossa, L. S., Silva, G. M., Sotomaior, C. S., Pereira, A. J., Luciano, F. B., et al. (2019). Effects of modified atmosphere packaging (MAP) and slaughter age on the shelf life of lamb meat. *Food Science and Technology (Brazil), 39*(2), 328–335. https://doi.org/10.1590/FST.29617

Tchatchouang, C. D. K., Fri, J., De Santi, M., Brandi, G., Schiavano, G. F., Amagliani, G., et al. (2020). Listeriosis outbreak in South Africa: A Comparative analysis with previously reported cases worldwide. *Microorganisms, 8*(1). https://doi.org/10.3390/MICROORGANISMS8010135

Toldrá, F. (2022). The storage and preservation of meat. III—meat processing. *Lawrie's Meat Science*, 281–314. https://doi.org/10.1016/B978-0-323-85408-5.00002-9

Tomasevic, I., Djekic, I., Font-i-Furnols, M., Terjung, N., & Lorenzo, J. M. (2021). Recent advances in meat color research. *Current Opinion in Food Science, 41*, 81–87. https://doi.org/10.1016/J.COFS.2021.02.012

Vermeiren, L., Devlieghere, F., Vandekinderen, I., Rajtak, U., & Debevere, 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*(3), 532–545. https://doi.org/10.1016/j.meatsci.2006.05.003

Wang, X., Lahou, E., De Boeck, E., Devlieghere, F., Geeraerd, A., & Uyttendaele, M. (2015). Growth and inactivation of Salmonella enterica and Listeria monocytogenes in broth and validation in ground pork meat during simulated home storage abusive temperature and home pan-frying. *Frontiers in Microbiology, 6*(OCT), Article 164760. https://doi.org/10.3389/FMICB.2015.01161/BIBTEX

Xie, Y., Zhang, M., Gao, X., Shao, Y., Liu, H., Jin, J., et al. (2018). Development and antimicrobial application of plantaricin BM-1 incorporating a PVDC film on fresh pork meat during cold storage. *Journal of Applied Microbiology, 125*(4), 1108–1116. https://doi.org/10.1111/jam.13912

Zavistanaviciute, P., Klementaviciute, J., Klupsaite, D., Zokaityte, E., Ruzauskas, M., Buckiuniene, V., et al. (2023). Effects of marinades prepared from food industry by-products on quality and Biosafety parameters of lamb meat. https://doi.org/10.3390/foods12071391.

Zhang, J., Zhang, J., Huang, X., Shi, J., Muhammad, A., Zhai, X., et al. (2023). Study on cinnamon essential oil release performance based on pH-triggered dynamic mechanism of active packaging for meat preservation. *Food Chemistry, 400*, Article 134030. https://doi.org/10.1016/j.foodchem.2022.134030

Zhang, Y., Zhou, C., Bassey, A., Bai, L., Wang, Y., & Ye, K. (2022). Quantitative exposure assessment of Listeria monocytogenes cross-contamination from raw to ready-to-eat meat under different food-handling scenarios. *Food Control, 137*, Article 108972. https://doi.org/10.1016/j.foodcont.2022.108972