Meat Science 200 (2023) 109166

Contents lists available at ScienceDirect

Meat Science

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

Applicability of the probiotic *Lactiplantibacillus plantarum* BFL as an adjunct culture in a dry fermented sausage

Noelí Sirini [a], María Stegmayer [a], María J. Ruiz [a], Facundo Cuffia [b], Eugenia Rossler [c], José Otero [d], Lorena Soto [a,d], José M. Lorenzo [e,f], José Ángel Pérez-Álvarez [g], Marcelo Rosmini [a,d,*], Laureano Frizzo [a,d]

[a] *Laboratory of Food Analysis Med. Vet R. Dalla Santina, Institute of Veterinary Science (ICiVet Litoral), National University of the Litoral (UNL)- National Council of Scientific and Technical Research (UNL/CONICET), Esperanza, Province of Santa Fe, Argentina*
[b] *Institute of Food Technology/Sensory Analysis Department, Faculty of Chemical Engineering, National University of the Litoral (UNL), 1° de Mayo 3250, Santa Fe, Santa Fe, Argentina*
[c] *Laboratory for the Analysis of fodder concentrates and agricultural supplies of the Faculty of Agricultural Sciences (FCA), National University of the Litoral (UNL), R.P Kreder 2805- S3080, Argentina*
[d] *Department of Public Health, Faculty of Veterinary Science, National University of the Litoral, (UNL) Esperanza, Province of Santa Fe, Argentina*
[e] *Centro Tecnológico de la Carne de Galicia, Avd. Galicia No. 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain*
[f] *Universidade de Vigo, Área de Tecnoloxía dos Alimentos, Facultade de Ciencias, 32004 Ourense, Spain*
[g] *IPOA Research Group (UMH-1 Grupo REVIV-Generalitat Valenciana), Dept Tecnología Agroalimentaria, Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández, Ctra Beniel, Km 3.2, 03312 Orihuela, Spain*

ARTICLE INFO

Keywords:
Fermented sausages, residual nitrites
Healthy meats
Probiotic
Sensory analysis

ABSTRACT

The addition of probiotic bacteria to a meat batter allows the development of functional fermented sausages. The aim of this work was to study the effect of microencapsulated *Lactiplantibacillus plantarum* BFL (EP) and as free cells (FP) on microbiological, physicochemical, and sensory parameters of fermented sausages during the drying stage and on the product ready for consumption. The microencapsulation of *L. plantarum* BFL did not improve its viability during the drying stage. In addition, sausages inoculated with L. *plantarum* BFL (FP and EP) caused lower residual nitrites values, pH values and *Escherichia coli* counts than the Control (C). However, only the presence of free cells of L. *plantarum* BFL (FP) caused a decrease in the *Enterobacteriaceae* and mannitol salt-positive *Staphylococcus* counts. In the sensory analysis, no significant differences were found in the acceptability of the different sausages. However, the acidity in probiotic sausages (FP and EP) was an attribute that consumers highlighted. The probiotic L. *plantarum* BFL could adapt and survive at high doses in the matrix of an industrial fermented sausage. Therefore, its use could represent a strategy both for biocontrol of pathogens and for the development of functional meat products.

1. Introduction

Since ancient times, man has sought to preserve meat to extend its shelf-life and, therefore, have food supplies. As regards the production of sausages, it did not proliferate until the valuable discovery of salt, when seasoned meat and fish began to be marketed. This is how the manufacture of artisan sausages emerged as a way of preserving the surplus meat that was not consumed fresh. Later, the industrial revolution brought about the mass production of food and industrial meat products. Nowadays, there is advanced machinery technology for the manufacturing of fermented sausages. This technology preserves the traditions of past generations, while offering guarantees on product durability and safety. However, meat products proposed as functional foods are a pending issue of the technological and scientific area (Ojha, Kerry, Duffy, Beresford, & Tiwari, 2015).

Probiotics are defined as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (Swanson et al., 2020). Several beneficial effects have been demonstrated from probiotics bacteria and some studies have shown it use in fermented sausages (Bis-Souza, Barba, Lorenzo, Penna, & Barretto,

* Corresponding author at: Laboratory of Food Analysis Med. Vet R. Dalla Santina, Institute of Veterinary Science (ICiVet Litoral), National University of the Litoral (UNL)- National Council of Scientific and Technical Research (UNL/CONICET), Esperanza, Province of Santa Fe, Argentina
E-mail address: mrosmini@unl.edu.ar (M. Rosmini).

https://doi.org/10.1016/j.meatsci.2023.109166
Received 18 January 2023; Received in revised form 6 March 2023; Accepted 11 March 2023
Available online 16 March 2023
0309-1740/Published by Elsevier Ltd.

2019; Pavli, Argyri, Chorianopoulos, Nychas, & Tassou, 2020; Sirini et al., 2020a; Xia, Liu, Li, Ren, & Liu, 2023). However, viability losses of probiotic strains in dry cured or fermented sausages are unavoidable due to the presence of different factors such as the amount of NaCl and nitrites, low pH, and decreased water activity values during the fermentation and maturation stages. To solve this problem, the microencapsulation of probiotics emerged as an alternative to protect the probiotic strain during food production, preservation, and passage through the gastrointestinal tract (Vivek et al., 2023). This methodology is based on the fact that certain materials (solid, liquid or gaseous) form part of an inert layer or capsule that covers the cell, which is released at a controlled rate under specific conditions. Among the most used encapsulation techniques are spray drying, extrusion and emulsification. With these techniques capsules of highly variable size are obtained, ranging from a few micrometers to a few millimeters. As for spray-drying, it is a useful and economical tool to prolong the viability of probiotic cultures. When stored under suitable conditions, the cells encased in the vitreous powder remain viable and resume their active viable state after reconstitution. Specific formulations are used to improve cell survival during spray-drying and subsequent storage (Shoji et al., 2013). The powders tend to be composed mainly of protective agents such as carbohydrates and, in a minority, proteinaceous agents approved as food additives. (Rokka & Rantamäki, 2010). In this context, the use of maltodextrin (MTX) and whey protein isolate (WPI) has become a promising alternative to be used as wall material for the microcapsules that cover *Lactiplantibacillus plantarum* BFL (Behboudi-Jobbehdar, Soukoulis, Yonekura, & Fisk, 2013; Sharifi, Rezazad-Bari, Alizadeh, Almasi, & Amiri, 2021; Ying, Sun, Sanguansri, Weerakkody, & Augustin, 2012).

The microbiota present in the meat matrix of a fermented meat product is unique to each species, area, or industry. This depends on the inoculant used and the way in which it works. Salamín Criollo is a long-established Argentinian fermented sausage. It does not exceed 15 cm in length or 3.5 in width, and its bacon is coarsely chopped. This traditional fermented sausage can maintain probiotics viability during the drying stage, giving rise to functional meat products. Probiotics viability could be improved with microencapsulation by spray-drying. In this way, industrial meat products with beneficial bacteria could be accessible to the general population (Sirini et al., 2022; Sirini et al., 2022a; Vivek et al., 2023). Therefore, the aim of this work was to study the effect of microencapsulated L. *plantarum* BFL (EP) and as free cells (FP) on microbiological and physicochemical parameters of Salamines Criollos during the drying stage and then to evaluate sensory parameters in the finished product.

2. Materials and methods

2.1. L. plantarum BFL isolation

The isolation of the probiotic strain L. *plantarum* BFL was made from an Argentinian commercial product called Bioflora ™ (BIOSIDUS S.A) (only for research purposes). This is a product certified by the health authorities.

2.2. Detection of L. plantarum BFL antagonisms

The agar diffusion technique was used to evaluate L. *plantarum* BFL ability to inhibit bacterial pathogens. Two pathogenic reference strains (*Escherichia coli* EDL 933 and *Staphylococcus aureus* ATCC 25923), whose species are frequently found in fermented sausages, were studied. The implicit factors were also analyzed against the experimental starter RC20 used in the manufacture of Salamines Criollos. The inhibition test was performed following the puncture inoculation procedure proposed by Ruiz, Colello, Padola, and Etcheverría (2017). The reading was performed by observing translucent zones indicative of growth inhibition of pathogenic strains by the action of Lactic Acid Bacteria (LAB). The presence of inhibition halos >2 mm around the punction was considered a positive test. As a positive control, lactic acid (85%) (Cicarelli, Santa Fe, Argentina) diluted 1/2 was used. As a negative control, the same strain evaluated (*E. coli* EDL 933, *S. aureus* ATCC 25923 and experimental starter RC20, as appropriate) was used.

2.3. Bacterial cultures

The probiotic cultures were obtained as explained by Sirini, Loyeau, et al. (2022a); Sirini et al. (2022b).

A bioreactor (BIOSTAT® A Sartorius Stedim Biotech, Guxhagen, Germany) was used to cultivate 4 L of economic culture medium in continuous agitation (120 rpm) and with constant pH of 6.0 ± 0.2 (18 h/ 37 °C). Its formulation was: 60 g/L Whey permeate (VARIOLAC 850, Arla Foods, Porteña, Argentina); 10 g/L casein peptone (Microquin S.R. L., Santa Fe, Argentina); 8 g/L Yeast extract (Biokar Diagnostics, Beauvais, France); 1 mL/L tween 80; and 0.05 g/L Magnesium sulfate monohydrate (MnSO4) (Merck, Darmstadt, Germany).

After centrifugation (8 °C/10 min/ 6000 xg Thermo Scientific™ Sorvall™ RC 6 Plus Centrifuge) and cell washing steps (phosphate buffered saline, Biopack, Buenos Aires, Argentina) two total inocula of 13. 3 ± 0.1 log UCF were developed: free probiotic inoculum (FP) and encapsulated probiotic inoculum (EP). In case of FP inoculum, the obtained pellet was mixed with a cryoprotective solution (10% whey protein isolate and maltodextrin (WPI-MTX)) and then it was preserved at −80 °C for seven days. For EP inoculum, the pellet was mixed with the microcapsules wall material (WPI-MTX solution). The final WPI-MTX-pellet solution (40% *w*/w) was used to feed a laboratory scale Spray Dryer (Mini Spray Dryer ADL311S, Yamato, Japan) (inlet temperature:160 °C, outlet temperature: 66 °C, feed flow rate: 6.74 mL/ min, air pressure: 0.24 Mpa). Thus, a *L. plantarum* BFL powder (EP inoculum) was obtained.

To evaluate the culture concentration (before centrifugation step and on the pellet), plates of MRS agar medium were used (Biokar Diagnostics, Beauvais, France). For EP inoculum microbiological counts were also realized on the WPI-MTX-pellet solutions and in a final powdered inoculum.

The corn maltodextrin brand was El Bahiense (Biotechnology y co., China). The WPI (BIPROTM) was given by Davisco Foods International Inc. (Minnesota, USA).

2.4. Salamines Criollos preparation

The Salamines Criollos were manufactured in a local company (Esperanza, Santa Fe, Argentina) as described Sirini, Loyeau, et al. (2022a, 2022b). Briefly, the 100 kg of meat batter already homogenized (lean, fat, condiments, and additives) were divided into 3 equal parts and 3 treatments were prepared (FP, EP and Control without probiotic strain). All treatments had RC20 starter in its formulation. The RC20 is an experimental starter, which is integrated by *Staphylococcus xylosus* and *Pediococcus pentosaceus*. The sausages formulation was as follows: bacon 25.09%, pork trim 25.09%, heart 17.56%, pork shoulder 15.05%, chicken thigh leg 10.04%, spices 3.51%, corn starch 1.51%, soy flour 1.51%, NaCl 2%, BQS 0.50%, sugar 0.15%, and $NaNO_3$ 150 ppm. The FP treatment was carried out by adding 110 mL of FP inoculum to the meat batter. The EP treatment was carried out by adding 330 g of the powdered EP inoculum to the meat batter. The C (control) had no probiotic inoculum in its formulation. To balance the chemical compositions of the three treatments, different amounts of WPI-MTX (without probiotic) were added. To stuff the pastes, naturals bovine casing with a diameter of 30 mm were used. Then, the sausages were kept in the dryer for 5 days (20 ± 1 °C and 72 ± 1% relative humidity (RH)). The probiotic count at d 0 was 8.3 ± 0.2 log CFU/g (EP) and 8.8 ± 0.1 log CFU/g (FP).

2.5. Experimental design

Three complete replicates of the whole study were performed ($n = 3$). Therefore, the design was repeated three times with 100 kg of meat paste each time. Daily, from 0 d to 4 d (drying stage), three sausages from each treatment (FP, EP and Control) were taken to determinate pH changes, A_w, microbiological analysis, residual nitrites, and color measurement. In turn, each of these determinations was performed in triplicate. When the products were ready-to-eat (d8), a consumer-based sensory analysis in single a section was carried out. Fig. 1 summarized the manufacture process and the experimental design.

2.6. pH and A_w analysis

The pH values of sausages were determined at ambient temperature (24 ± 1 °C) by inserting three times the spear-type gel electrode (Oakton ao-35,805–18, USA in different places of samples each time. Altronix TPX-III™ (Altronix, Taipei, Taiwan) pH meterwas used. Water activity (A_w) was measured at 25 °C using the awmeter Aqualab 137 Systems (USA). These determinations were carried out in triplicate.

2.7. Color measurement

Once the Salamines Criollos were obtained from dryer, they were conditioned for color measurements as follow: the samples were cut perpendicular to the longest axis of the sausage into slices >1.0 cm thick, as an infinite solid. Then, they were left in contact with air for a maximum of 3 min to simulate dry-cured color in sliced samples. (Sánchez-Zapata et al., 2011).

The color measurement was based on the CIELAB coordinates: lightness (L^*), red/green co-ordinate (a^*) and yellow/blue co-ordinate (b^*), from which the magnitudes hue (h^*) and chrome (C^*) were calculated (UNE 72–031, 1983). A Minolta CM-2002 (Minolta Camera Co., Osaka, Japan) spectrophotometer with illuminant D65, 10° observer, SCI mode, 11 mm aperture of the instrument for illumination and 8 mm for measurement was used. Between samples and equipment, an optical low-reflectance glass was used.

2.8. Microbiological analysis

All microbiological counts were established according Sirini, Loyeau, et al. (2022a, 2022b). *L. plantarum* BFL counts were made using *Lactobacillus plantarum* selective medium (LPSM) (Bujalance, Jiménez-Valera, Moreno, & Ruiz-Bravo, 2006; Sirini et al., 2020b; Sirini, Loyeau, et al., 2022a) (37 °C/72 h/ anaerobiosis). LAB counts were determined using MRS agar (Biokar, Beauvais, France) (37 °C / 72 h / anaerobiosis). Enterobacteria counts were determined using Violet Red Bile Glucose Agar (Oxoid, Basingstoke, UK) (37 °C/ 24 h/aerobiosis). The *Escherichia coli* counts were determined using Tryptone Bile X-glucuronide Agar (Biokar, Beauvais, France) (44 °C/ 24 h/ aerobiosis) and *Staphylococcus* mannitol salt positives counts (SMSP) were determined in Mannitol salt agar (37 °C /48 h/aerobiosis). The Mannitol salt agar was prepared by components (Sirini, Loyeau, et al., 2022b). All microbiological results were expressed as log CFU/g sausage.

2.9. Residual nitrites

The residual nitrite level was determined according to ISO/DIS 2918 standards (ISO, 1975). A Turner spectrophotometer (model no. 390, Mountain View, CA, USA), setting in 520 nm was used to read the absorbance. The residual nitrites values were expressed as mg $NaNO_2$/kg.

2.10. Consumer-based sensory analysis

2.10.1. Consumer-based sensory characterization

One hundred twenty-four consumers participated in the present study. Each one of those were selected based on their frequency of consumption of fermented sausage and the percentage of women was 50%.

The test was developed in a laboratory that had 10 individual booths for the sensory evaluation (ISO:8589:2007). Samples were given to consumers in a plastic tray, each containing 15 g of the different types of sausages at 25 °C. Each sample were coded with a series of 3 random numbers (Control: C554, FP: P721 & EP: E345).

2.10.2. Overall liking

Consumers were asked to taste the samples and rate them according to their liking, using a 9-point horizontal hedonic scale (Wichchukit & O'Mahony, 2015). One-way ANOVA with fixed factor was carried out to identify differences ($P < 0.05$) in the overall liking of the samples. When differences were found, the means of each of them were contrasted using Tukey's test.

2.10.3. Check-all-that-apply question

Subsequently, consumers re-taste the sausage samples and complete a Check-All-That-Apply (CATA) question with 22 terms related to the sensory, hedonic and not sensory characteristics; usage occasions and concepts of the different type of samples: *nice color, bright, tasty, acid, smoked, spicy, regional product, sweet, dry, soft, for a snack, fatty, smooth, ugly, juicy, unpleasant color, hard, to give away, red color, strange taste, salty, bitter.*

Participants were asked to check all the terms that they considered appropriate to describe each type of sausage. The terms were selected based on published data considering the terms defined by trained personnel and preliminaries works (Dong et al., 2020; Dos Santos et al., 2015).

The frequency with which each of the defined terms was used was

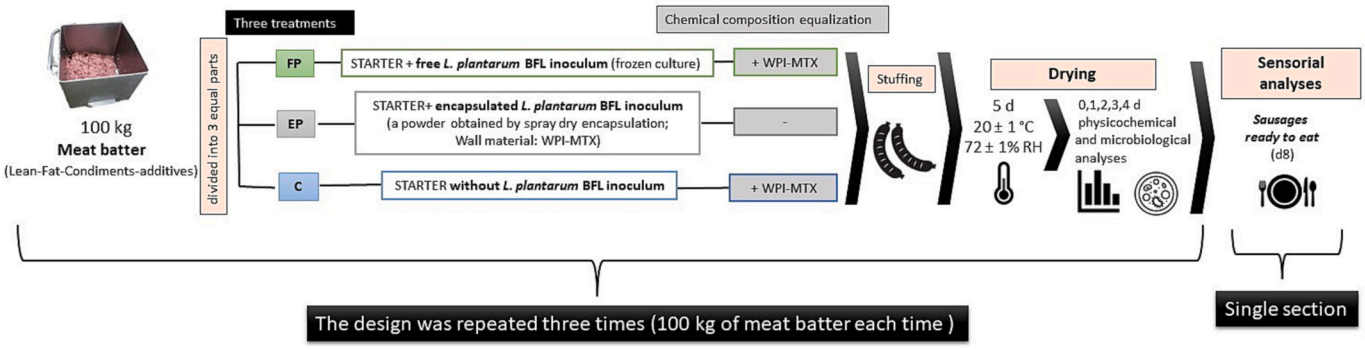

Fig. 1. Graphical summary of Salamín Criollo manufacturing process and experimental design. FP: Free probiotic treatment. EP: Encapsulated probiotic treatment. C: Control.

determined by counting the number of participants who checked it to describe the samples. Cochran's Q test was applied to study differences ($P < 0.05$) between sausages for each CATA term (XLSTAT 2014, Addinsoft). Likewise, a Principal Coordinate Analysis (PcoA) and a Correspondence Analysis (CA) were carried out on the contingency table that contains the frequency of use of each term for each sample.

2.10.4. Penalty analysis

In order to link information from CATA question with that from acceptability, Penalty Analysis (PA) and Correspondence Analysis (CA) were performed. PA was performed by calculating, for each evaluated product, its average acceptability across all consumers that evoked (checked) the attribute in the CATA question and all that did not. The difference between these two values can be considered as an estimation of how much product acceptability changes when the attribute is present or not.

2.11. Statistical analysis

All collected data from microbiological and physicochemical analysis were analyzed by applying generalized lineal mixed model with gamma distribution and logistic linked function with repeated measurements in time and lotes as random variable. There was one factor (treatment) with three levels: Salamines Criollos with L. *plantarum* BFL as a free cell (FP); Salamines Criollos with encapsulated L. *plantarum* BFL (EP) and the Control group (Salamines Criollos without L. *plantarum* BFL). In addition, to know if there were differences between treatments at the end of the processing, a generalized lineal mixed model with gamma distribution and logistic linked function (lotes as random variable) was performed. All determinations (microbiologial and physicochemical) were made in triplicate. The trial plan was a completely randomized design. The InfoStat software (National University of Córdoba) for Windows was used. All the results were expressed as mean values and the S.E of the mean. To define significant differences between treatments level of $P < 0.05$ was used. In sensorial analysis, all the consumers participated in a single section where the 3 treatmenrs were evaluated.

3. Results and discussion

3.1. Detection of L. plantarum BFL antagonisms

The antagonistic property of lactic acid bacteria against different pathogens may be the result of lactic acid, hydrogen peroxide and/or carbon dioxide production, pH reduction, nutrient depletion, oxidation-reduction potential decrease and antibiotic-like compounds production. In the present work, the L. *plantarum* BFL strain showed antagonistic capacity against *S. aureus* ATCC 25923 and *E. coli* EDL 933 (Table 1). Some strains of lactobacilli could be utilized as bioprotective cultures by controlling spoilage microorganisms and foodborne pathogens in meat products (Aymerich, Artigas, Garriga, Monfort, & Hugas, 2000; Muthukumarasamy & Holley, 2006). Essid, Medini, and Hassouna (2009) studied the technological properties of 17 strains of L. *plantarum* isolated from a traditional Tunisian meat product, finding that all the L. *plantarum* strains studied presented antagonistic activity against *S. aureus* ATCC 25923 and 94% against *E. coli*. In addition, Luciana, Jamile, Manoela, Eliane, and Eduardo (2015) studied the antimicrobial activity of different strains of L. *plantarum* isolated from sausages in Brazil against pathogens bacteria such as *S. enteritidis* SE86, *S. typhimurium* ATCC 14028, *S. aureus* ATCC 14458, *E. coli* O157:H7 ATCC 43888, *B. cereus* ATCC 33010, and L. *monocytogenes* ATCC 6477, finding that all the strains inhibited all the pathogens studied. On the other hand, no antagonistic effects were observed between the experimental starter RC20 and the L. *plantarum* BFL strain (Table 1). Further studies should be conducted to find the antagonistic mechanism against *S. aureus* ATCC 25923 and *E. coli* EDL 933 by L. *plantarum* BFL.

Table 1
Characterization of L. *plantarum* BFL by determining its antagonistic activity by the puncture method against *E. coli* EDL 933, *S. aureus* ATCC 25923 and experimental RC20 starter.

	Antagonism (+ or -) / Inhibition halo (mm)		
	***E. coli* EDL 933**	***S. aureus* ATCC 25923**	**Experimental RC20 Starter**
L. plantarum BFL	+ 10 ± 1	+ 21 ± 1	–
Positive control*	+ 9 ± 2	+ 25 ± 1	+ 20 ± 2
Negative control**	–	–	–

*Lactic acid (85%); **(same strain).

3.2. Changes in pH and aw

The pH decrease of sausages at early fermentation is a crucial requirement since it participate to the inhibition of undesirable foodborne microorganisms, accelerates the red color development, and ensures an adequate drying process by reducing the water retention capacity of proteins. As shown in Fig. 2, the pH decreased for all treatments during the drying stage ($P < 0.001$). Moreover, *L. plantarum* BFL had a significant effect on the pH decrease in Salamines Criollos ($P < 0.001$). Regardless of the probiotic being encapsulated (EP) or as a free cell (FP), the presence of L. *plantarum* BFL in the sausage formulation caused a greater decrease in pH compared to the Control group (C) (Zhang et al., 2023). This may be related to a greater increase in the lactic acid content in FP and EP due to a greater degradation of the carbohydrates present caused by the L. *plantarum* BFL metabolism. This result could indicate that the spray-drying stress on L. *plantarum* BFL did not affect its acidifying capacity completely. However, a lower acidification for treatment EP compared to treatment FP was observed, mainly at the beginning of drying (Fig. 2). Similar findings were informed by Cavalheiro, Ruiz-Capillas, Herrero, & Pintado (2021), who studied the dry-fermented sausages inoculation with *E. faecium* CECT 410 as free cells or encapsulated in alginate beads, reporting that in both treatments (encapsulated and unencapsulated) the pH values were lower than the control. However, de Oliveira Gomes, de Mesquita Oliveira, de Marins, Gomes, and Feihrmann (2021) investigated the incorporation of encapsulated *B. animalis* ssp. *Lactis* BB12 in salami, finding no significant differences in pH values compared with the control. The authors claim that encapsulated probiotics may not have favoured to the reduction in the sausages pH, which must have been given by the naturals LAB

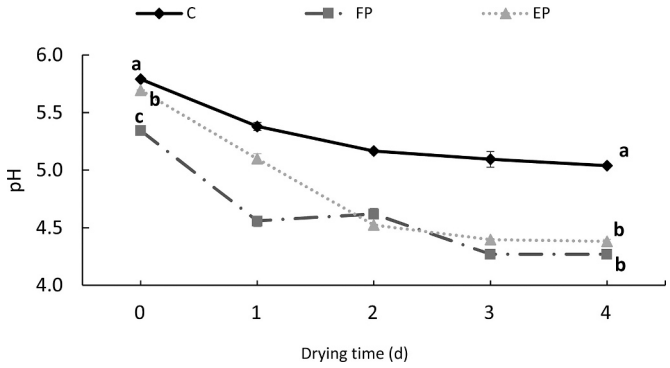

Fig. 2. Evolution of the pH values (Means ± standard error of the mean (SEM)) during the drying stage of Salamines Criollos formulated with L. *plantarum* BFL. The different letters indicate significant differences between treatments on the same day ($P < 0.05$). Random effect (generalized lineal mixed model with repeated measurements in time): $P = 0.292$; Random effect (generalized lineal mixed model): $P = 0.921$.

present in meat. In this present work, a scanning electron microscope to test the efficiency of the microencapsulation before the addition of the capsules to the product was used. This analysis showed that no cells were outside the WPI-MTX microcapsules, so a high encapsulation efficiency could be confirmed before the addition of probiotic powder to the meat batter (Sirini, Loyeau, et al., 2022b). For this reason, in this work the similarity in pH values of FP and EP treatment at final of drying could indicate some erosion in the WPI-MTX microcapsules. This could be due to WPI-MTX wall material is water soluble, and the initial high humidity of the meat paste could have partially disintegrated them. More studies need to be done to confirm this hypothesis. Regarding the A_w values, there was no difference between the Control group and the FP and EP treatments ($P = 0.201$) (Lorenzo, Gómez, & Fonseca, 2014). The A_w values on the last day of drying reached lower values than 0.95, which are considered acceptable for food quality (Ge et al., 2019) (Fig. 3).

3.3. Color

The different FP and EP treatments had significant effects on color parameters. The presence of the probiotic as a free cell (FP) caused higher Lightness (L^*) values and lower redness (a^*) ($P > 0.001$) values than the Control group during the drying stage (Table 2). The increment in L^* could be related with a greater fermentative activity of L. *plantarum* BFL and the lactic acid generation (Fig. 2). The pH values of the FP treatment could be associated with values closer to the isoelectric points of meat proteins. The nearer the pH is to the isoelectric point of proteins, the lower the water retention capacity. In this work, cross sections to measure color were made; free water could have moved to the surface, thus allowing the detection of higher L^* values.

As previously shown, during the drying of the sausages the meat paste becomes acidic. As a result of this, nitrosomyoglobin is partially or totally denatured and a^* values may decrease. In this article, the lowest values of a^* were found in the free Probiotic treatment (FP), which is consistent with the residual nitrites values (Fig. 4). Thus, the a^* value could be lower in the FP treatment because of the presence of the nitrite reductase enzyme, leaving fewer NO_2 groups available to develop color reactions. Regardless of this decrease, the color of sausages with L. *plantarum* BFL (FP and EP) continues to be in the range of pink expected for this type of product and, moreover, consumers defined the color as "nice" with no difference between treatments (Table 4). Reduced values of a^* in the ripening of Longaniza de Pascua have also been informed by Sayas-Barberá, Viuda-Martos, Fernández-López, Pérez-Alvarez, and Sendra (2012), with the incorporation of citrus fiber and L. *casei* CECT

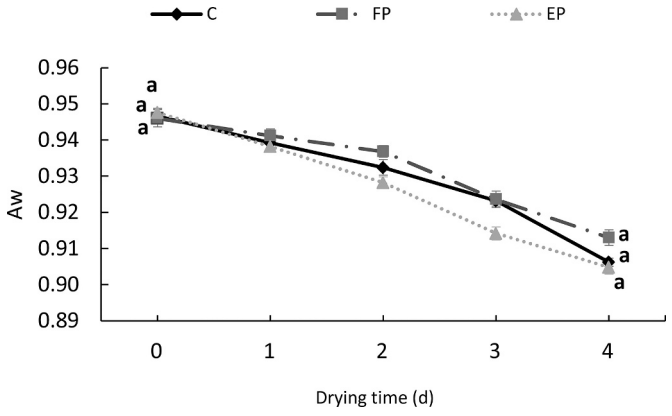

Fig. 3. Evolution of Aw (Means ± SEM) during the drying stage of Salamines Criollos formulated with *L. plantarum* BFL. The different letters show differences between treatments on the same day (P < 0.05). Random effect (generalized lineal mixed model with repeated measurements in time): $P = 0.516$; Random effect (generalized lineal mixed model): $P = 0.481$.

Table 2
Luminosity (L^*), redness (a^*), yellowness (b^*), hue (h*) and Chroma (C*) (mean ± S.E) of Salamines Criollos in C, FP and EP during the drying stage.

Color parameters	Treatments		
	C	**FP**	**EP**
L^*	37.4 ± 0.19 ^{b}	41.87 ± 0.22 ^{a}	39.97 ± 0.86^{ab}
a^*	19.5 ± 0.15 ^{a}	18.44 ± 0.13 ^{b}	19.01 ± 1.56 ^{ab}
b^*	9.35 ± 0.08 ^{a}	9.04 ± 0.11 ^{a}	9.19 ± 0.15 ^{a}
h*	0.44 ± 0.07 ^{a}	0.49 ± 0.13^{a}	0.45 ± 0.09 ^{a}
C*	21.62 ± 0.85 ^{a}	20.91 ± 0.84^{b}	20.5 ± 0.83 ^{b}

a-bThe different letters show differences between treatments. FP: free L. *plantarum* BFL treatment. EP: Encapsulated L. *plantarum* BFL treatment. C: Control group.

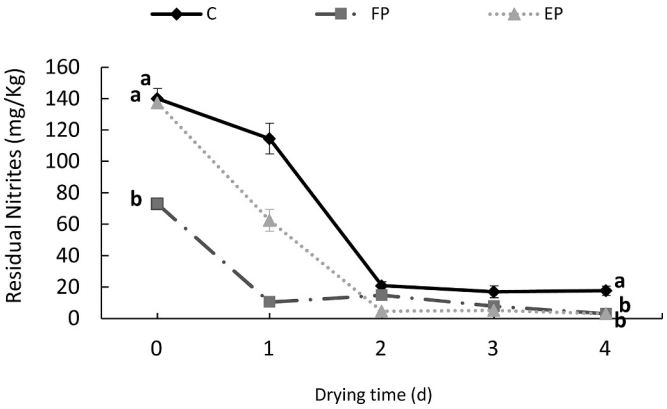

Fig. 4. Evolution of residual nitrites (Means ± SEM) during the drying stage of Salamines Criollos formulated with *L. plantarum* BFL. The different letters show differences between treatments on the same day ($P < 0.05$). Random effect (generalized lineal mixed model with repeated measurements in time): $P = 0.893$; Random effect (generalized lineal mixed model): $P = 0.987$.

475 as a free cells. On the other hand, Chen et al. (2016) reported that L. *plantarum* CMRC6 and L. *sakei* CMRC15 isolated from fermented pork sausages, were able to promote myoglobin nitrosation and produced the common pink color in sausages as a result of their nitrite reductase activity.

In accordance with to Pérez-Alvarez, Sayas-Barberá, Fernández-López, and Aranda-Catalá (1999), changes in b^* values are related to oxygen consumption by microbial cultures inserted in the meat product, both probiotics and starter cultures, during their exponential growth phase. However, the presence of the probiotic had no effect on the b^* values ($P = 0.396$) in this work (Table 2).

Regarding the hue (h*), there were no differences between treatments during the drying stage ($P = 0.373$). However, as regards the Chroma (C*), a lower range of C* values were observed due to the addition of L. *plantarum* BFL ($P < 0.001$), thus inferring that the presence of the probiotic dulls or reduces the vividness of the color. The detriment of saturation or decline in chroma values could be the result of higher values of lactic acid present in FP and EP, as occurred with the Longaniza de Pascua studied by Sirini et al. (2022).

3.4. Microbiological analysis

3.4.1. L. plantarum BFL survival and LAB counts

Table 3 shows the viability of encapsulated L. *plantarum* BFL (EP) and as a free cell (FP) during the drying stage. In both treatments (FP and EP), L. *plantarum* BFL had a survival rate >96% and remained higher than 8 log CUF/g throughout the whole drying period. This dose is very promising since it meets the requirements of probiotic functional foods (Vivek et al., 2023). In addition, this gives an indication of its survival capacity and adaptability as an adjunct culture to the environment,

Table 3
Lactiplantibacillus plantarum BFL (L.p BFL), lactic acid bacteria (LAB), *E. coli*, Enterobacteriaceae, and mannitol salt-positive *Staphylococcus* (SMSP) counts (log CFU/g) (mean ± SEM) of Salamines Criollos in C, FP and EP during drying stage.

Treatments	time (d)	Microbial counts (log CFU/g)				
		L. p BFL	**LAB**	***E. coli***	**Enterobacteria**	**SMSP**
C	0	2.22 ± 0.19[b]	6.57 ± 0.08[b]	3.90 ± 0.04[a]	5.50 ± 0.06[a]	4.75 ± 0.10[a]
	1	2.70 ± 0.14	8.54 ± 0.03	3.91 ± 0.11	4.76 ± 0.16	5.04 ± 0.23
	2	3.34 ± 0.14	8.63 ± 0.11	3.52 ± 0.13	5.38 ± 0.10	6.03 ± 0.48
	3	3.77 ± 0.17	8.80 ± 0.14	3.59 ± 0.19	5.25 ± 0.07	6.16 ± 0.17
	4	3.80 ± 0.20[b]	8.88 ± 0.07[a]	3.37 ± 0.24[a]	4.37 ± 0.52[a]	5.60 ± 0.29[b]
FP	0	8.84 ± 0.07[a]	8.94 ± 0.05[a]	3.72 ± 0.21[a]	5.48 ± 0.12[a]	4.74 ± 0.13[a]
	1	8.70 ± 0.10	8.50 ± 0.02	3.30 ± 0.19	4.61 ± 0.30	3.12 ± 0.26
	2	8.56 ± 0.09	8.72 ± 0.09	2.95 ± 0.12	5.08 ± 0.20	4.46 ± 0.33
	3	8.47 ± 0.10	8.47 ± 0.17	2.44 ± 0.25	2.74 ± 0.38	3.16 ± 0.20
	4	8.78 ± 0.05[a]	8.79 ± 0.08[a]	2.25 ± 0.37[b]	3.13 ± 0.14[b]	4.72 ± 0.34[c]
EP	0	8.20 ± 0.11[a]	8.39 ± 0.24[a]	3.85 ± 0.22[a]	5.78 ± 0.12[a]	4.95 ± 0.10[a]
	1	8.38 ± 0.10	8.71 ± 0.06	3.56 ± 0.15	5.34 ± 0.43	4.97 ± 0.35
	2	8.13 ± 0.14	8.96 ± 0.07	3.69 ± 0.26	5.54 ± 0.08	6.02 ± 0.47
	3	8.05 ± 0.10	8.82 ± 0.23	2.79 ± 0.39	4.36 ± 0.49	5.92 ± 0.45
	4	7.91 ± 0.13[a]	8.90 ± 0.07[a]	2.42 ± 0.16[b]	4.10 ± 0.18[a]	6.97 ± 0.44[a]

The different letters show differences between treatments on the same day ($P < 0.05$).
L.p BFL: Random effect RMT (generalized lineal mixed model with repeated measurements in time): $P = 0.857$; Random effect GLM (generalized lineal mixed model): $P = 0.944$.
LAB: Random effect RTM $P = 0.971$; Random effect GLM: $P = 0.937$.
E. coli: Random effect RTM $P = 0.773$; Random effect GLM: $P = 0.995$.
Enterobacteria: Random effect RTM $P = 0.646$; Random effect GLM: $P = 0.996$.
SMSP: Random effect RTM $P = 0.912$; Random effect GLM: $P = 0.945$.

which in this case is the meat matrix of industrial Salamines Criollos. On the other hand, no differences in L. *plantarum* BFL counts between the FP and EP treatments were observed ($P = 0.068$) during the Salamín Criollo drying stage. The strain L. *plantarum* BFL as a free cell showed it is capable of widely tolerating the conditions of this meat matrix. Therefore, the viability of L. *plantarum* BFL was not improved by spray-drying encapsulation (Table 3). Otherwise, significant differences in L. *plantarum* BFL counts were found between the probiotic treatments (FP and EP) and the Control group (C) ($P < 0.001$).The LAB species present in fermented sausages are usually variable depending on the manufacturing region. In contrast to the findings by Sirini et al. (2020b); Sirini, Lucas-González, et al. (2022) in Longaniza de Pascua, in Salamín Criollo L. *plantarum* sp. was detected as a native bacteria in the Control group. Nevertheless, this did not cause any difficulties in inoculated L. *plantarum* BFL counting, since the native L. *plantarum* was not a dominant strain and was detected in greatly reduced counts compared to the FP and EP treatments (Table 3). As it was expected, a higher LAB count was achieved in the Free Probiotic (FP) and Encapsulated Probiotic (EP) treatments compared to the Control group ($P = 0.014$). This difference is noticeable on d 0, when the starter is beginning to grow, while L. *plantarum* BFL is already inoculated at high doses. All treatments (C, FP and EP) showed LAB counts close to or >7 log CFU/g during the drying stage (Table 3). Fermented meat products require appropriate levels of LAB to control the undesirable microbiota and to enhance the efficiency of the manufacture of this type of meat products. Since the three treatments studied in this work had a starter inoculum (experimental RC20) in their formulation, the results of the LAB counts were expected.

3.4.2. *Enterobacteria, E. coli and SMSP counts*

The addition of L. *plantarum* BFL as free cells (FP) in the Salamines Criollos formulation caused lower Enterobacteria counts ($P = 0.001$) and SMSP counts ($P < 0.001$) in comparison with the Control group (C) (Table 3). However, the encapsulated probiotic (EP) did not show any antagonistic effect on Enterobacteria and SMSP. While L. *plantarum* BFL as a free cell could enhance the biocontrol capacity of the starter, *L. plantarum* BFL microencapsulation by spray-drying could be a disadvantage in the biocontrol capacity. To and Etzel (1997), found that spray-drying delayed lactic acid production in some LAB. The higher the outlet air temperature, the longer the delay time before acid production. As is shown in Fig. 2, in the EP treatment the start of acidification was delayed for the first days of drying compared to the P treatment. That initial period could have been enough for indicators or pathogens to increase their counts. This could be the reason why the antagonistic effect of encapsulated L. *plantarum* BFL (EP) on Enterobacteria and SMPS was not observed. Similar results were found by Muthukumarasamy and Holley (2006) who studied the effect of two microencapsulated co-cultured probiotics (*Lactobacillus reuteri* and *Bifidobacterium longum*) on the survival of *E. coli* O157:H7 in fermented sausages. The authors observed that, although microencapsulation increased the survival of both probiotics, their inhibitory action against *E. coli* O157:H7 was reduced. Therefore, they proposed to inoculate the sausages with probiotics in both forms (encapsulated and unencapsulated). Regarding this work, more studies must be carried out to explain the inhibition mechanism exerted by L. *plantarum* BFL as a free cell (FP) on Enterobacteria and SMSP, and the reason why it is prevented when L. *plantarum* BFL is microencapsulated. In this way, it would be possible to work on microencapsulation systems avowing such inhibitions. These results disagree with those reported by Cavalheiro et al. (2020), who studied different incorporation strategies of *Lactobacillus plantarum* in sausages and found a marked inhibitory effect of L. *plantarum* with respect to Enterobacteria both when the probiotic was added as free cells or encapsulated. As explained by the authors, this efficacy was because L. *plantarum* is a great producer of lactic acid and bacteriocin. In addition, Silva, Carvalho, Teixeira, and Gibbs (2002) showed that the spray-drying process did not affect the antagonistic effect against *Staphylococcus aureus*, *Listeria innocua* and *Listeria monocytogenes*.

In this work, lower *E. coli* counts were achieved ($P < 0.001$) due to the presence of encapsulated (EP) and unencapsulated (FP) *L. plantarum* BFL. This result is in concordance with in vitro L. *plantarum BFL* antagonisms studies (Table 1). With these results, it can be observed that L. *plantarum* BFL could also behave as a biocontroller in the formulation of Salamines Criollos.

3.5. *Residual nitrites*

The evolution of residual nitrites during the drying stage of Salamines Criollos formulated with L. *plantarum* BFL are shown in Fig. 4. The residual nitrites behavior found in this work coincides with that reported by Fernández-López, Sendra, Sayas-Barberá, Navarro, and Pérez-Alvarez (2008) and Mendes et al. (2014), who informed a rapid decrease in residual nitrite values on the first days of fermentation in raw-cured sausages, with these values remaining constant during the following days of maturation. As shows the Fig. 4, the existence of L. *plantarum* BFL (FP and EP) in Salamines Criollos generated lower

residual nitrites values than the Control group (C) during drying ($P < 0.002$) (Sun, Kong, Chen, Han, & Diao, 2017). However, the same rate of residual nitrite reduction was not observed between encapsulated (EP) and unencapsulated (FP) *L. plantarum* BFL. Since nitric oxide reactions are highly dependent on pH reduction (Honikel, 2008), the faster drop in residual nitrite values in the FP treatment could be associated with higher acidification induced by L. *plantarum* BFL (FP) (Fig. 2). The fast interaction between the nitro group and the different meat components during the first days of drying could indicate that the delay in acidification of the EP treatment is responsible for the difference in residual nitrites values. In addition, this greater drop in nitrite levels could be the result of the presence of nitrite reductase enzymes of the L. *plantarum* BFL strain. >30 years ago, Hammes, Bantleon, and Min (1990) had informed that NO_3 could be reduced to NO by the strains L. *sakei* and L. *farciminis*; and this NO could participate in the generation of Mb(Fe^{2+})-NO in fermented meat products which would play a fundamental role in the development of the color of meat products. Years later, Ba et al. (2018) found that L. *plantarum* inoculation in meat products produced a reddish color like that made with commercial starter cultures, suggesting the ability of the probiotic L. *plantarum* to reduce nitrate to nitrite. Since sodium nitrite is the main additive in meat products, the nitrite reductase enzymes of certain microorganisms could become essential allies to reduce the risk of such an additive. Zhu, Guo, and Yang (2020) partially replaced sodium nitrite with *Lactobacillus plantarum*, finding that this substitution could generate a similar amount of Mb (Fe2+)–NO than the control due to nitrite reductase activity of the strain. More studies should be carried out on the probiotic L. *plantarum* BFL used in this current work to determine if it is considered nitrite reductase, and thus be able to confirm that the greater decrease in nitrites is due to the presence of the enzyme.

3.6. Sensory evaluation

3.6.1. Consumer-based sensory characterizations: Overall liking

No significant differences in the overall liking scores of the sausages were found ($F = 1.18$, $P = 0.3093$). Overall liking scores were (mean ± SD): 7.03 ± 1.39 from C554, 6.79 ± 1.29 from E345 and 6.98 ± 1.22 from P721. In addition, the overall liking values of the Salamines Criollos were high according to what was reported by other authors who studied different types of meat products or sausages (Dong et al., 2020; Muthukumarasamy & Holley, 2006; Sayas-Barberá et al., 2012). In agreement with the results obtained in the consumer test, the distributions of the histogram data of the overall liking were very similar for the three samples (Fig. 5).

3.6.2. Consumer-based sensory characterizations: Check-all-that-apply questions

Differences were observed in the frequency with which consumers mentioned 7 of the 22 CATA terms (Table 4). This finding indicates that the CATA question has been able to detect differences in the consumers' perception of the sensory characteristics of the evaluated type of fermented sausages. The terms that presented differences were: "tasty", "red color" and "salty" ($P < 0.05$); "smooth" and "off flavor" ($P < 0.01$); and "bright" and "acid" ($P < 0.001$) (Table 4). The generation of acids in fermented sausages depends on the endogenous microbiota, the type and concentration of sugars present in the meat mixture and the diameter of the sausage (Papamanoli, Tzanetakis, Litopoulou-Tzanetaki, &

Table 4
Number of mentions of the terms of the Check-All-That-Apply (CATA) question used by consumers to describe C554, P721 and E345 sausage samples.

Attributes	*p*-values	Treatments		
		C554	**P721**	**E345**
Nice color ^{ns}	*0.0491*	90 (a)	86 (a)	74 (a)
Bright ***	*0.0000*	64 (b)	51 (b)	25 (a)
Tasty *	*0.0452*	85 (b)	79 (ab)	68 (a)
Acid ***	*0.0000*	11 (a)	38 (b)	52 (b)
Smoky ^{ns}	*0.2116*	14 (a)	13 (a)	22 (a)
Spicy ^{ns}	*0.0390*	14 (a)	26 (a)	28 (a)
Regional Product ^{ns}	*0.7491*	21 (a)	20 (a)	24 (a)
Sweet ^{ns}	*0.4053*	15 (a)	11(a)	9 (a)
Dry ^{ns}	*0.5818*	11 (a)	8 (a)	7 (a)
Soft ^{ns}	*0.0720*	69 (a)	75 (a)	60 (a)
For a Snack ^{ns}	*0.1233*	63 (a)	51(a)	50 (a)
Fatty ^{ns}	*0.4865*	49 (a)	46 (a)	41 (a)
Smooth **	*0.0024*	58 (b)	50 (ab)	32 (a)
Ugly ^{ns}	*0.8669*	3 (a)	2 (a)	2 (a)
Juicy ^{ns}	*0.0626*	26 (a)	30 (a)	17 (a)
Unpleasant color ^{ns}	*0.2636*	3 (a)	1 (a)	5 (a)
Hard ^{ns}	*0.3247*	10 (a)	7 (a)	13 (a)
To Give Away ^{ns}	*0.1383*	27(a)	18 (a)	17 (a)
Red Color*	*0.0305*	37(b)	31(ab)	22 (a)
Off Flavor **	*0.0096*	7(a)	18 (ab)	22 (b)
Salty*	*0.0238*	14 (a)	28 (b)	26 (b)
Bitter ^{ns}	*0.5258*	4 (a)	4 (a)	7 (a)

ns Indicates no significant differences ($P > 0.05$) according to Cochran's Q test. The different letters indicate significant differences between treatments on the same CATA term.
*** Indicates significant differences at $P < 0.001$ according to Cochran's Q test.
** Indicates significant differences at $P < 0.01$ according to Cochran's Q test.
* Indicates significant differences at $P < 0.05$ according to Cochran's Q test.

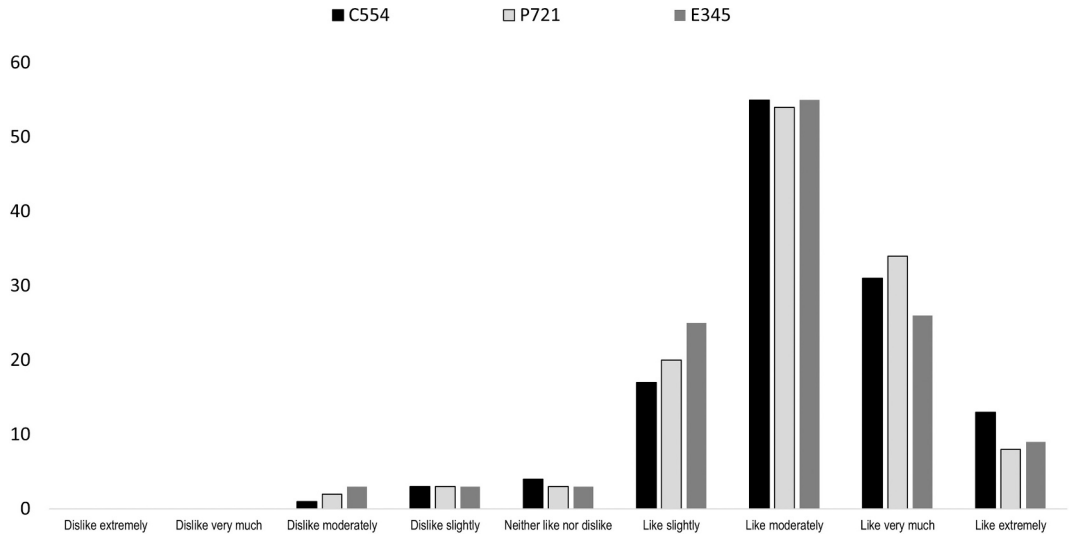

Fig. 5. 9-point hedonic scale histograms corresponding to the C554, P721 and E345 sausage samples.

Kotzekidou, 2003). In this work, the acidification effect that L. *plantarum* BFL had on Salamines Criollos was reflected in the consumer-based sensory analysis. Consumers reported that samples E345 and P721 were more acidic than the Control C554, as it was demonstrated by instrumentally measuring the pH values (Fig. 2). According to Montel, Masson, and Talon (1998), the overall taste of fermented sausages is composed of the acid taste, which is corresponding to the acid content. In this line, the panel expressed that E345 was less tasty and more off flavor than C554. This may be due to such low acidity not being expected in this type of fermented product (Salamines Criollos); therefore, the consumer could not recognize it as a typical or traditional taste. Regarding the activities of the enzymes correlated with carbohydrate catabolism, *N*-acetyl-β-glucosaminidase, β-galactosidase, strong α-galactosidase, and β-glucosidase activities were demonstrated by L. *plantarum* (Papamanoli et al., 2003). Thus, when the L. *plantarum* strains are used in the manufacture of dry fermented sausages, a product with overacidity may be created that is not well accepted by consumers. (Rouhi, Sohrabvandi, & Mortazavian, 2013) claimed that the utilization of probiotics mixed with traditional starter cultures for sausage fermentation has either no considerable effect or improving effects on sensory acceptability of the final product. The consumer also expressed that E345 was less bright than P721 and C554. This result is in concordance with C* values, which showed that the presence of the probiotic reduces the vividness of the color. On the other hand, as was mentioned earlier, the higher acidity could be related to a lower water retention capacity, which could explain the reason why the panel defined E345 and P721 as saltier than C554.

In the Principal Coordinate Analysis (PcoA) graph (Fig. 6), which is a method of representing in a 2- or 3-dimensional graph objects described by a square matrix containing resemblance indices between those objects (GOWER, 1966), we can observe that the consumers' taste was characterized by the terms "to give away", "smooth", "for snacks", "tasty" and "regional product."

Finally, the Correspondence Analysis (AC) (Fig. 7), which consists of seeking the best simultaneous representation of two sets that make up the lines and columns of a contingency table, showed that these two sets played a symmetric role. This is very useful to study the connection between two sets of modalities that make up the lines and columns of a contingency table. The analysis showed that sample C554 was described based on the terms "sweet", "dry" and "to give away"; P721 was described based on the terms "juicy" and "soft"; and E345 for its "smoked" and "regional product" character. As is already known, the texture and flavor of food are defined not only by its composition, but also by the internal structure of the ingredients. Thus, each treatment was described by different terms. Sample C554 could be described as "sweet" since it was significatively less salty than P721 and E345.

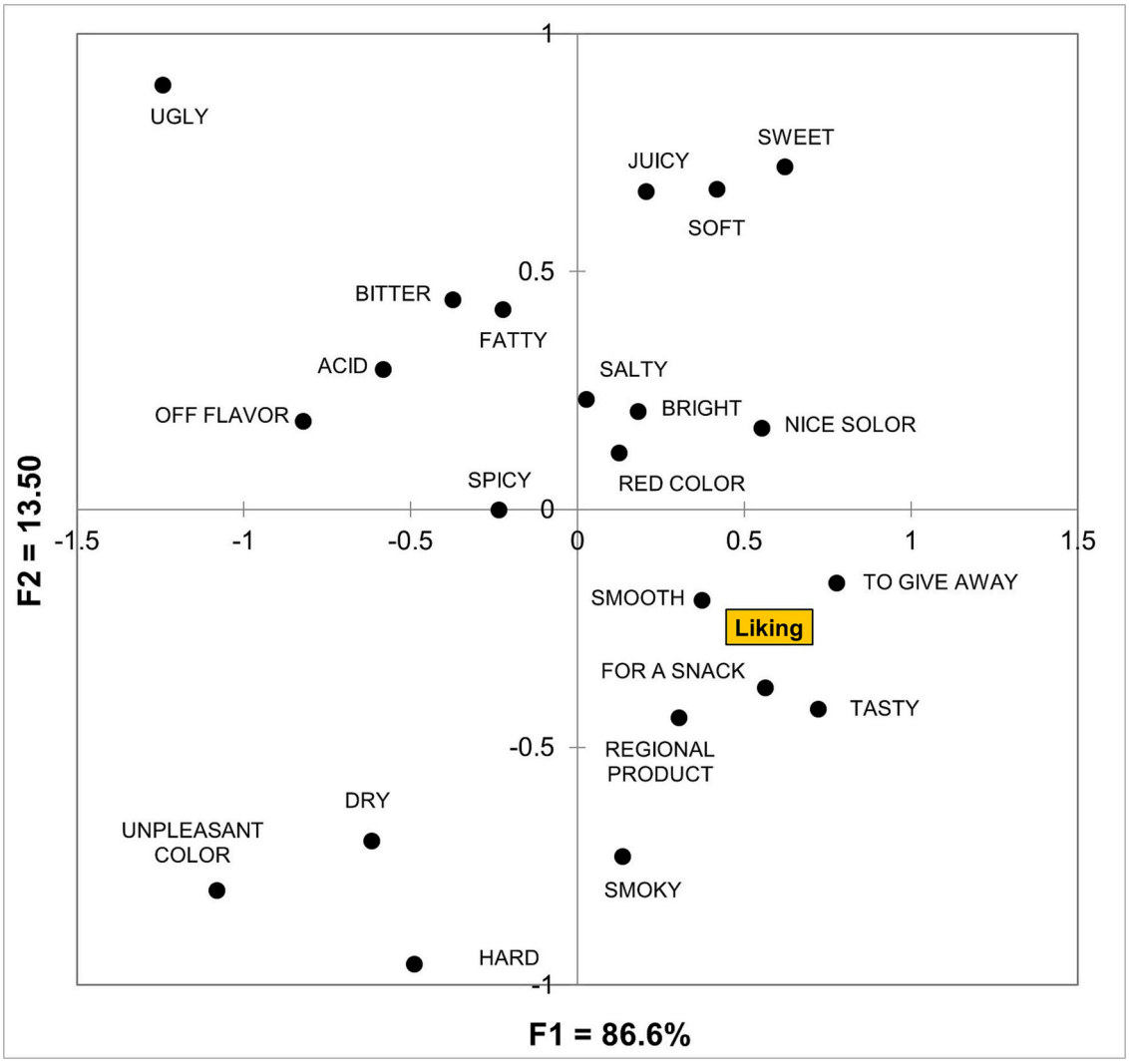

Fig. 6. Representation of the terms and "Liking" of the terms of the C554, P721 and E345 sausage samples in the first and second dimensions of the Principal Component Analysis (PcoA) performed on the frequency table containing the frequency of mention of the terms of the Check-All-That-Apply (CATA) question.

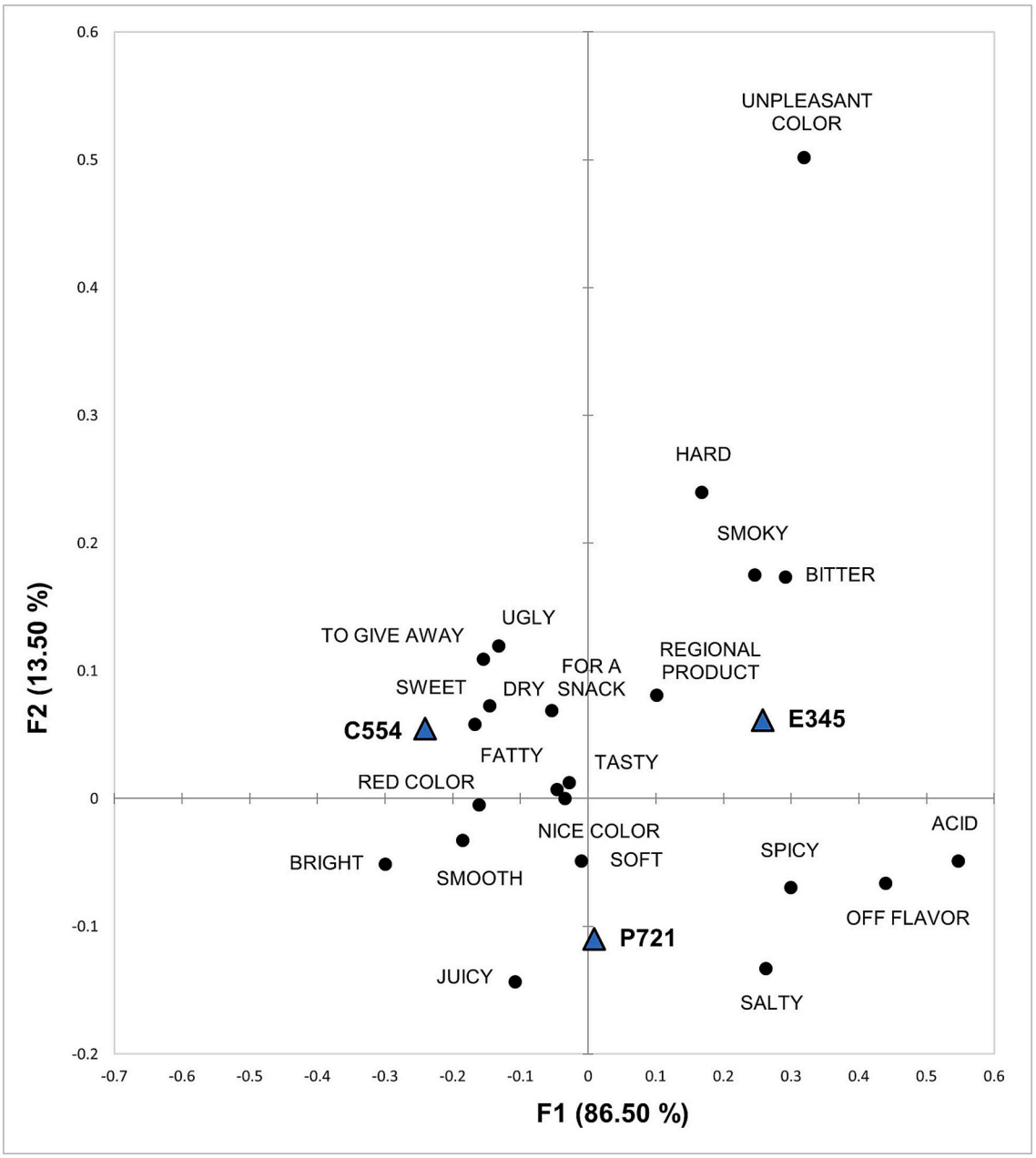

Fig. 7. Representation of the terms of the C554, P721 and E345 sausage samples in the first and second dimensions of the Symmetric Plot of the Correspondence Analysis (CA) performed on the frequency table containing the frequency of mention of the terms of the Check-All-That-Apply (CATA) question.

3.6.3. Consumer-based sensory characterizations: Penalty analysis

The Penalty Analysis indicated that the sausage samples were characterized by presenting a great positive inertia towards the terms that defined them as "nice color", "tasty" and "soft", "for a snack" and "to give away", and as a "regional product". These characteristics were repeated in an equivalent way for each of the Penalty Analyses carried out on each individual sample, indicating that the positive opinions of consumers are consistent among the 3 samples and agree with the global data (Fig. 8).

Particularly, and in addition to the positive global aspects for all samples, the C554 sausage showed a positive impact/inertia in the "bright" term, the P721 sausage in the "juicy" term, and the E345 sausage in the "smooth" term. On the other hand, the terms with the greatest negative impact/inertia were represented by "fatty" and "acid" for the sample set and for each sausage sample (except for C554), "juicy" for the C554 sausage, and "smooth" for the P721 sausage (Supplementary Figs. S1, S2 and S3). The acid taste in fermented sausages depend on the area in which they were manufactured and the consumption habits. For example, the acid taste is commonly valued in northern Europe, while might be unwanted in southern Europe. Thus, the type of fermented meat product that is projected as a carrier of probiotics is key to pleasing the consumer.

4. Conclusions

The use of L. *plantarum* BFL as an adjunct culture in Salamines Criollos improved its properties. In this regard, the strain L. *plantarum* BFL could be a good candidate to be part of functional fermented sausages since it could survive at high doses as a free cell during the drying stage. Therefore, the encapsulation of the probiotic under the conditions proposed in this work is not recommended or not necessary as a strategy to improve viability. In addition, the antagonisms against pathogens

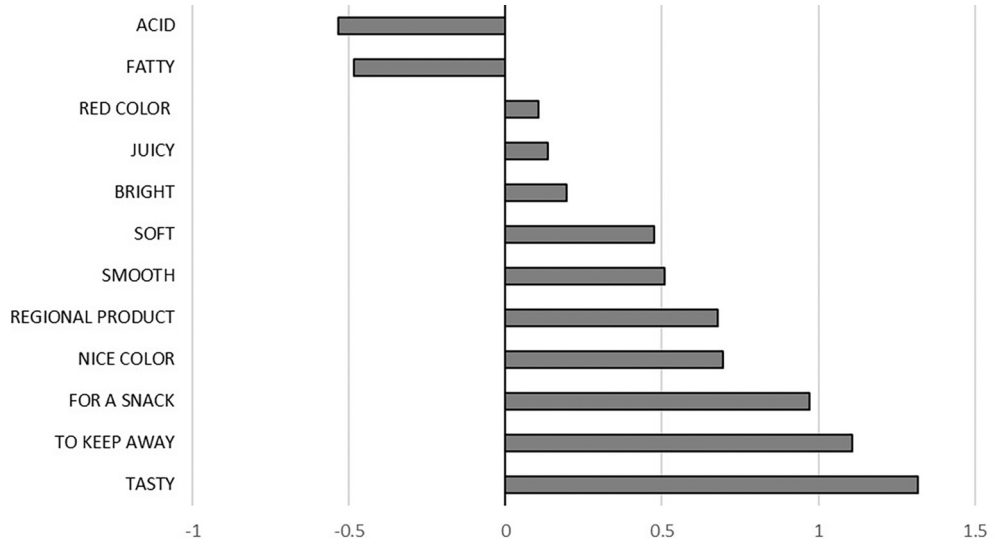

Fig. 8. Penalty Analysis applied to global results of the C554, P721 and E345 sausage samples.

bacteria observed both in vitro and in sausages showed that the L. *plantarum* BFL could be used as a bio controller strain.

Although the general liking values of the samples were high, the notable acidification of Salamines Criollos by L. *plantarum* BFL could be mentioned as a disadvantage since it was a negative attribute for the consumer. The probiotic L. *plantarum* BFL strain could be tested in another type of meat product with a characteristic high acidic taste. In this way, a functional meat product could be developed without changing consumption habits.

Author contributions

Conceptualization, Data curation, Investigation and Formal analysis: M.R, L.F, L.S, J.A.P.A, J.M,L and N·S. Funding acquisition and Project administration: M.R, L.F, L.S, J.A.P.A, J.M.L. Methodology: N·S, M.S., M. J.R, E.R. Supervision; Validation; Visualization; Roles/Writing - original draft and Writing - review & editing: N.S, L.F, J.O and F·C.

Funding

This research was funded by CYTED (ref.119RT0568) and by National Agency for Scientific and Technological Promotion (Proyecto PICT 2016–3495) and Universidad Nacional del Litoral (Proyecto CAI + D 50120150100152LI, CAI + D 50120150100151LI, CAI + D Orientado 2016 2–14, CAI + D 50620190100152LI and CAI + D 50620190100062LI).

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

The authors do not have permission to share data.

Acknowledgments

The authors thank the Universidad Nacional del Litoral (UNL), ITA (FIQ-UNL) and PRODICOM S.R.L., for support. Authors are members of the HealthyMeat network, funded by CYTED (ref. 119RT0568).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2023.109166.

References

Aymerich, T., Artigas, M. G., Garriga, M., Monfort, J. M., & Hugas, M. (2000). Effect of sausage ingredients and additives on the production of enterocin a and B by enterococcus faecium CTC492. Optimization of in vitro production and anti-listerial effect in dry fermented sausages. *Journal of Applied Microbiology, 88*(4), 686–694. https://doi.org/10.1046/j.1365-2672.2000.01012.x

Ba, H. V., Seo, H.-W., Seong, P.-N., Kang, S.-M., Kim, Y.-S., Cho, S.-H., … Kim, J.-H. (2018). Lactobacillus plantarum (KACC 92189) as a potential probiotic starter culture for quality improvement of fermented sausages. *Korean Journal for Food Science of Animal Resources, 38*(1), 189–202. https://doi.org/10.5851/kosfa.2018.38.1.189

Behboudi-Jobbehdar, S., Soukoulis, C., Yonekura, L., & Fisk, I. (2013). Optimization of spray-drying process conditions for the production of maximally viable microencapsulated L. acidophilus NCIMB 701748. *Drying Technology, 31*(11), 1274–1283. https://doi.org/10.1080/07373937.2013.788509

Bis-Souza, C. V., Barba, F. J., Lorenzo, J. M., Penna, A. L. B., & Barretto, A. C. S. (2019). New strategies for the development of innovative fermented meat products: A review regarding the incorporation of probiotics and dietary fibers. *Food Review International, 35*(5), 467–484. https://doi.org/10.1080/87559129.2019.1584816

Bujalance, C., Jiménez-Valera, M., Moreno, E., & Ruiz-Bravo, A. (2006). A selective differential medium for Lactobacillus plantarum. *Journal of Microbiological Methods, 66*(3), 572–575. https://doi.org/10.1016/j.mimet.2006.02.005

Cavalheiro, C. P., Ruiz-Capillas, C., Herrero, A. M., Jiménez-Colmenero, F., Pintado, T., de Menezes, C. R., & Fries, L. L. M. (2020). Effect of encapsulated Lactobacillus plantarum as probiotic on dry-sausages during chilled storage. *International Journal of Food Science and Technology, 55*(12), 3613–3621. https://doi.org/10.1111/ijfs.14695

Cavalheiro, C. P., Ruiz-Capillas, C., Herrero, A. M., & Pintado, T. (2021). *Dry-fermented sausages inoculated with Enterococcus faecium CECT 410 as free cells or in alginate beads*.

Chen, X., Li, J., Zhou, T., Li, J., Yang, J., Chen, W., & Xiong, Y. L. (2016). Two efficient nitrite-reducing Lactobacillus strains isolated from traditional fermented pork (Nanx Wudl) as competitive starter cultures for Chinese fermented dry sausage. *Meat Science, 121*, 302–309. https://doi.org/10.1016/j.meatsci.2016.06.007

Dong, C., Wang, B., Li, F., Zhong, Q., Xia, X., & Kong, B. (2020). Effects of edible chitosan coating on Harbin red sausage storage stability at room temperature. *Meat Science, 159*, Article 107919. https://doi.org/10.1016/j.meatsci.2019.107919

Dos Santos, B. A., Bastianello Campagnol, P. C., da Cruz, A. G., Galvão, M. T. E. L., Monteiro, R. A., Wagner, R., & Pollonio, M. A. R. (2015). Check all that apply and free listing to describe the sensory characteristics of low sodium dry fermented sausages: Comparison with trained panel. *Food Research International, 76*, 725–734. https://doi.org/10.1016/j.foodres.2015.06.035

Essid, I., Medini, M., & Hassouna, M. (2009). Technological and safety properties of Lactobacillus plantarum strains isolated from a Tunisian traditional salted meat. *Meat Science, 81*(1), 203–208. https://doi.org/10.1016/j.meatsci.2008.07.020

Fernández-López, J., Sendra, E., Sayas-Barberá, E., Navarro, C., & Pérez-Alvarez, J. A. (2008). Physico-chemical and microbiological profiles of "salchichón" (Spanish dry-fermented sausage) enriched with orange fiber. *Meat Science, 80*(2), 410–417. https://doi.org/10.1016/j.meatsci.2008.01.010

Ge, Q., Pei, H., Liu, R., Chen, L., Gao, X., Gu, Y., Hou, Q., Yin, Y., Yu, H., Wu, M., Zhang, W., & Zhou, G. (2019). Effects of Lactobacillus plantarum NJAU-01 from Jinhua ham on the quality of dry-cured fermented sausage. *LWT, 101*, 513–518. https://doi.org/10.1016/j.lwt.2018.11.081

Gower, J. C. (1966). Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika, 53*(3–4), 325–338. https://doi.org/10.1093/biomet/53.3-4.325

Hammes, W. P., Bantleon, A., & Min, S. (1990). Lactic acid bacteria in meat fermentation. *FEMS Microbiology Reviews, 7*(1–2), 165–173. https://doi.org/10.1111/j.1574-6968.1990.tb04886.x

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

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. https://doi.org/10.1016/j.foodcont.2014.05.025

Luciana, S., Jamile, Q. P., Manoela, A. V., Eliane, M. Z. M., & Eduardo, C. T. (2015). Isolation and characterization of Lactobacillus plantarum BLS29 as a potential probiotic starter culture for pork sausage production. *African Journal of Microbiology Research, 9*(32), 1887–1895. https://doi.org/10.5897/AJMR2015.7606

Mendes, A. C. G., Rettore, D. M., de Ramos, A. L. S., da Cunha, S. F. V., de Oliveira, L. C., & Ramos, E. M. (2014). Milano type salami elaborated with fibers of red wine byproducts/Salames tipo Milano elaborados com fibras de subprodutos da producao de vinho tinto. *Ciencia Rural, 44*(7), 1291–1297.

Montel, M. C., Masson, F., & Talon, R. (1998). Bacterial role in flavour development. *Meat Science, 49*, S111–S123. https://doi.org/10.1016/S0309-1740(98)90042-0

Muthukumarasamy, P., & Holley, R. A. (2006). Microbiological and sensory quality of dry fermented sausages containing alginate-microencapsulated Lactobacillus reuteri. *International Journal of Food Microbiology, 111*(2), 164–169. https://doi.org/10.1016/j.ijfoodmicro.2006.04.036

Ojha, K. S., Kerry, J. P., Duffy, G., Beresford, T., & Tiwari, B. K. (2015). Technological advances for enhancing quality and safety of fermented meat products. *Trends in Food Science and Technology, 44*(1), 105–116. https://doi.org/10.1016/j.tifs.2015.03.010

de Oliveira Gomes, B., de Mesquita Oliveira, C., de Marins, A. R., Gomes, R. G., & Feihrmann, A. C. (2021). Application of microencapsulated probiotic Bifidobacterium animalis ssp. Lactis BB-12 in Italian salami. *Journal of Food Processing & Preservation, 45*(10), Article e15841. https://doi.org/10.1111/jfpp.15841

Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E., & Kotzekidou, P. (2003). Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Science, 65*(2), 859–867. https://doi.org/10.1016/S0309-1740(02)00292-9

Pavli, F. G., Argyri, A. A., Chorianopoulos, N. G., Nychas, G.-J. E., & Tassou, C. C. (2020). Effect of Lactobacillus plantarum L125 strain with probiotic potential on physicochemical, microbiological and sensorial characteristics of dry-fermented sausages. *LWT, 118*, Article 108810. https://doi.org/10.1016/j.lwt.2019.108810

Pérez-Alvarez, J. A., Sayas-Barberá, M. E., Fernández-López, J., & Aranda-Catalá, V. (1999). Physicochemical characteristics of Spanish-type dry-cured sausage. *Food Research International, 32*(9), 599–607. https://doi.org/10.1016/S0963-9969(99)00104-0

Rokka, S., & Rantamäki, P. (2010). Protecting probiotic bacteria by microencapsulation: Challenges for industrial applications. *European Food Research and Technology, 231*(1), 1–12. https://doi.org/10.1007/s00217-010-1246-2

Rouhi, M., Sohrabvandi, S., & Mortazavian, A. M. (2013). Probiotic fermented sausage: Viability of probiotic microorganisms and sensory characteristics. *Critical Reviews in Food Science and Nutrition, 53*(4), 331–348. https://doi.org/10.1080/10408398.2010.531407

Ruiz, M. J., Colello, R., Padola, N. L., & Etcheverría, A. I. (2017). Efecto inhibitorio de Lactobacillus spp. Sobre bacterias implicadas en enfermedades transmitidas por alimentos. *Revista Argentina de Microbiología, 49*(2), 174–177. https://doi.org/10.1016/j.ram.2016.10.005

Sánchez-Zapata, E., Fuentes-Zaragoza, E., Navarro-Rodríguez de Vera, C., Sayas, E., Sendra, E., Fernández-López, J., & Pérez-Alvarez, J. A. (2011). Effects of tuna pâté thickness and background on CIEL*a*b* color parameters and reflectance spectra. *Food Control, 22*(8), 1226–1232. https://doi.org/10.1016/j.foodcont.2011.01.022

Sayas-Barberá, E., Viuda-Martos, M., Fernández-López, F., Pérez-Alvarez, J. A., & Sendra, E. (2012). Combined use of a probiotic culture and citrus fiber in a traditional sausage Longaniza de Pascua. *Food Control, 27*(2), 343–350. https://doi.org/10.1016/j.foodcont.2012.04.009

Sharifi, S., Rezazad-Bari, M., Alizadeh, M., Almasi, H., & Amiri, S. (2021). Use of whey protein isolate and gum Arabic for the co-encapsulation of probiotic Lactobacillus plantarum and phytosterols by complex coacervation: Enhanced viability of probiotic in Iranian white cheese. *Food Hydrocolloids, 113*, Article 106496. https://doi.org/10.1016/j.foodhyd.2020.106496

Shoji, A. S., Oliveira, A. C., Balieiro, J. C. C., Freitas, O., Thomazini, M., Heinemann, R. J. B., … Favaro-Trindade, C. S. (2013). Viability of L. acidophilus microcapsules and their application to buffalo milk yoghurt. *Food and Bioproducts Processing, 91*(2), 83–88. https://doi.org/10.1016/j.fbp.2012.08.009

Silva, J., Carvalho, A., Teixeira, P., & Gibbs, P. (2002). Bacteriocin production by spray-dried lactic acid bacteria. *Letters in Applied Microbiology, 34*(2), 77–81. https://doi.org/10.1046/j.1472-765x.2002.01055.x

Sirini, N., Loyeau, P., Ruiz, M., Stegmayer, M., Soto, L., Werning, M., … Rosmini, M. (2022a). Development of probiotic fermented sausages and viability monitoring of supplemented Lactiplantibacillus plantarum BFL strain. *Fermentation, 8*(10). https://doi.org/10.3390/fermentation8100526. Article 10.

Sirini, N., Loyeau, P., Ruiz, M., Stegmayer, M., Soto, L., Werning, M., … Rosmini, M. (2022b). Development of probiotic fermented sausages and viability monitoring of supplemented Lactiplantibacillus plantarum BFL strain. *Fermentation, 8*(10). https://doi.org/10.3390/fermentation8100526. Article 10.

Sirini, N., Lucas-González, R., Fernández-López, J., Viuda-Martos, M., Pérez-Álvarez, J. A., Frizzo, L. S., … Rosmini, M. R. (2022). Effect of probiotic Lactiplantibacillus plantarum and chestnut flour (Castanea sativa mill) on microbiological and physicochemical characteristics of dry-cured sausages during storage. *Meat Science, 184*, Article 108691. https://doi.org/10.1016/j.meatsci.2021.108691

Sirini, N., Munekata, P. E. S., Lorenzo, J. M., Stegmayer, M.Á., Pateiro, M., Pérez-Álvarez, J.Á., … Rosmini, M. (2022). Development of healthier and functional dry fermented sausages: present and future. *Foods (Basel, Switzerland), 11*(8), 1128. https://doi.org/10.3390/foods11081128

Sirini, N., Roldán, A., Lucas-González, R., Fernández-López, J., Viuda-Martos, M., Pérez-Álvarez, J. A., … Rosmini, M. R. (2020a). Effect of chestnut flour and probiotic microorganism on the functionality of dry-cured meat sausages. *LWT, 134*, Article 110197. https://doi.org/10.1016/j.lwt.2020.110197

Sirini, N., Roldán, A., Lucas-González, R., Fernández-López, J., Viuda-Martos, M., Pérez-Álvarez, J. A., … Rosmini, M. R. (2020b). Effect of chestnut flour and probiotic microorganism on the functionality of dry-cured meat sausages. *LWT, 134*, Article 110197. https://doi.org/10.1016/j.lwt.2020.110197

Sun, F., Kong, B., Chen, Q., Han, Q., & Diao, X. (2017). N-nitrosoamine inhibition and quality preservation of Harbin dry sausages by inoculated with Lactobacillus pentosus, Lactobacillus curvatus and Lactobacillus sake. *Food Control, 73*, 1514–1521. https://doi.org/10.1016/j.foodcont.2016.11.018

Swanson, K. S., Gibson, G. R., Hutkins, R., Reimer, R. A., Reid, G., Verbeke, K., … Sanders, M. E. (2020). The international scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nature reviews. Gastroenterología y Hepatología, 17*(11). https://doi.org/10.1038/s41575-020-0344-2. Article 11.

To, B. C. S, & Etzel, M. R. (1997). Spray drying, freeze drying, or freezing of three different lactic acid bacteria species. *Journal of Food Science, 62*(3), 576–578. https://doi.org/10.1111/j.1365-2621.1997.tb04434.x

Vivek, K., Mishra, S., Pradhan, R. C., Nagarajan, M., Kumar, P. K., Singh, S. S., … Gowda, N. N. (2023). A comprehensive review on microencapsulation of probiotics: Technology, carriers and current trends. *Applied Food Research, 3*(1), Article 100248. https://doi.org/10.1016/j.afres.2022.100248

Wichchukit, S., & O'Mahony, M. (2015). The 9-point hedonic scale and hedonic ranking in food science: some reappraisals and alternatives. *Journal of the Science of Food and Agriculture, 95*(11), 2167–2178.

Xia, B., Liu, X., Li, Z., Ren, J., & Liu, X. (2023). The effects of microbiota-targeted approaches in inflammatory bowel disease: Probiotics, probiotic foods, and prebiotics. *Current Opinion in Food Science, 49*, Article 100956. https://doi.org/10.1016/j.cofs.2022.100956

Ying, D., Sun, J., Sanguansri, L., Weerakkody, R., & Augustin, M. A. (2012). Enhanced survival of spray-dried microencapsulated Lactobacillus rhamnosus GG in the presence of glucose. *Journal of Food Engineering, 109*(3), 597–602. https://doi.org/10.1016/j.jfoodeng.2011.10.017

Zhang, Y., Hou, Y., Zhang, S., Jing, N., Zhang, H., Xie, Y., … Jin, J. (2023). Bifidobacterium animalis A12, a probiotic strain that promotes glucose and lipid metabolism, improved the texture and aroma of the fermented sausage. *Foods, 12*(2). https://doi.org/10.3390/foods12020336. Article 2.

Zhu, Y., Guo, L., & Yang, Q. (2020). Partial replacement of nitrite with a novel probiotic Lactobacillus plantarum on nitrate, color, biogenic amines and gel properties of Chinese fermented sausages. *Food Research International, 137*, Article 109351. https://doi.org/10.1016/j.foodres.2020.109351