Italian Journal of Food Safety

https://www.pagepressjournals.org/index.php/ijfs/index

eISSN 2239-7132

Ital J Food Saf 2026 [Online ahead of print]

Please cite this article as:

Vianello S, Ramini M, Buratti L, et al. **Inactivation of *Listeria innocua* and *Salmonella* spp. in traditional Italian *Spianata Piccante*: a comparative study of three formulations**. *Ital J Food Saf* doi: 10.4081/ijfs.2026.15539

Submitted: 16-04-2026
Accepted: 07-05-2026

Inactivation of *Listeria innocua* and *Salmonella* spp. in traditional Italian *Spianata Piccante*: a comparative study of three formulations

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Key words: *Spianata Piccante*, Italian salami, *Salmonella* spp., *Listeria innocua*, high-pressure processing.

Contributions: Silvia Vianello, Lia Bardasi: conceptualization. Silvia Vianello, Mattia Ramini, Lucia Buratti, Maria Francesca Pelliconi, Nica De Prisco: methodology. Giorgio Galletti, Alfonso Rosamilia: data analysis. Silvia Vianello: writing - original draft preparation. Mattia Ramini, Alfonso Rosamilia, Gabriele Gardini, Giuseppe Merialdi, Lia Bardasi: writing - review and editing. Lia Bardasi: project administration, supervision. All the authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare that they have no competing interests.

Ethics approval and consent to participate: not applicable.

Availability of data and materials: not applicable.

Declaration of generative artificial intelligence and artificial intelligence-assisted technologies in the writing process: during the preparation of this manuscript, the authors used OpenAI ChatGPT to improve the readability, language, and clarity of the text. After using this tool, the authors carefully reviewed, revised, and edited the content as necessary and take full responsibility for the content of the published article.

Abstract
A challenge test was conducted to evaluate the efficacy of three *Spianata Piccante* formulations (A: traditional; B: with a bacteriocin-producing starter culture; C: with added dextrose) in reducing experimentally inoculated *Listeria innocua*, used as a surrogate for *Listeria monocytogenes*, and *Salmonella* spp. The aim was to identify the formulation that best ensured pathogen inactivation in compliance with United States (US) export requirements. Traditional Italian salami are considered shelf-stable due to multiple hurdle technologies, including pH reduction, decreased water activity and competitive microflora. However, these hurdles are often insufficient to reduce foodborne pathogens, particularly *L. monocytogenes* and *Salmonella* spp., to the levels required by the Food Safety and Inspection Service (FSIS) regulations (≥3 $\log_{10}$ CFU/g for *L. monocytogenes* and ≥5 $\log_{10}$ CFU/g for *Salmonella* spp.). High-pressure processing (HPP), a non-thermal treatment capable of inactivating vegetative pathogens, can be applied to achieve these reductions. In this study, meat batter was inoculated prior to stuffing (6 $\log_{10}$ CFU/g), and pathogen reductions were evaluated after ripening and HPP treatment. Significant differences in microbial inactivation were observed among formulations ($p<0.05$). Under the worst-case scenario, defined as the lowest $\log_{10}$ reduction among all tested units, only formulation B met the FSIS validation targets, achieving reductions of 3.89 $\log_{10}$ CFU/g for *L. innocua* and 5.77 $\log_{10}$ CFU/g for *Salmonella* spp. These findings suggest that the use of bacteriocin-producing starter cultures, combined with HPP, represents an effective strategy to enhance the microbiological safety of fermented dry-cured sausages intended for the US market.

Introduction
Foodborne pathogens, such as *Salmonella* spp. and *Listeria monocytogenes*, represent a major public health concern due to their widespread occurrence in the food supply and their ability to cause severe illness (Bintsis, 2017). In the United States (US), *Salmonella* spp. is among the leading causes of foodborne infections, with approximately 1.3 million cases reported annually, while *L. monocytogenes*, although less prevalent, is associated with high hospitalization and mortality rates (Scallan Walter *et al*., 2025). Both European Union (EU) and US regulations require the absence of *Salmonella* spp. in ready-to-eat (RTE) foods. However, regulatory approaches differ for *L. monocytogenes*: EU legislation allows levels below 100 colony forming units (CFU)/g under specific conditions, whereas US regulations enforce a zero-tolerance policy in RTE products (Commission of the European Communities, 2005; FSIS, 2023).
For the RTE meat products intended for export to the US, the Food Safety and Inspection Service (FSIS) requires validation studies demonstrating at least a 5 $\log_{10}$ CFU/g reduction of *Salmonella* spp. and a 3 $\log_{10}$ CFU/g reduction of *L. monocytogenes*, along with the application of an effective post-lethality treatment (FSIS, 2023). Traditional fermented and dry-cured meat products, such as Italian salami, are generally considered shelf-stable due to the combined effects of fermentation, pH reduction, decreased water activity (a_w), salt content, and competitive microflora, which act synergistically to inhibit pathogen growth (Barbuti and Parolari, 2002; Dučić and Marcow, 2022). However, these hurdles alone may not be sufficient to consistently achieve the microbial reductions required by US regulations (Bonilauri *et al.*, 2019; Bonilauri *et al.*, 2021). High-pressure processing (HPP) has emerged as an effective non-thermal post-lethality treatment for RTE meat products. It inactivates vegetative pathogenic microorganisms while minimizing thermal damage and preserving sensory and nutritional characteristics. However, its antimicrobial effectiveness may be influenced by product composition, pH, a_w, fat content, and the presence of protective matrices, making product-specific validation essential (San Martín *et al.*, 2010; Bolumar *et al.*, 2021; EFSA Panel on Biological Hazards, 2022). Therefore, challenge testing is often required to substantiate the efficacy of both the manufacturing process and HPP treatment (Merialdi *et al.*, 2015).
Spianata Piccante is a traditional Italian fermented dry-cured sausage characterized by its flattened shape and spicy formulation. Variations in formulation composition, such as the inclusion of bacteriocin-producing starter cultures or the addition of fermentable sugars, may influence

microbial dynamics during fermentation and ripening, potentially affecting pathogen reduction (Barbuti and Parolari, 2002). The aim of this study was to evaluate the effectiveness of the production process and subsequent HPP treatment in achieving the microbial reductions required by FSIS guidelines for *Salmonella* spp. and *Listeria innocua,* used as a surrogate microorganism for *L. monocytogenes* (Hu and Gurtler, 2017), in three different formulations of *Spianata Piccante*. The formulations included a traditional one, one with a bacteriocin-producing starter culture, and one supplemented with dextrose, with the objective of identifying the most suitable formulation for compliance with US export requirements.

Materials and Methods

Bacteria strains and inocula preparation

The *L. innocua* inoculum, used as a *L. monocytogenes* surrogate, was prepared using a cocktail of five strains: IZSLER 111373/1 and IZSLER 111373/2 isolated from environmental swabs collected in a pork meat transformation plant, IZSLER 257259/1 and IZSLER 257259/2 isolated from fresh pork sausages and fresh pork meat, respectively, and the reference strain ATCC 33090. The *Salmonella* spp. inoculum consisted of three strains: monophasic *S.* Typhimurium IZSLER 118174/1 isolated from fresh pork sausage, *S.* Derby IZSLER 106463/1 isolated from fresh pork meat, and the reference strain *S.* Typhimurium ATCC 14028. The inocula were prepared in accordance with ISO 20976-2:2022 (ISO, 2022). Briefly, one cryobead (-80°C) of each strain was transferred into 10 mL of brain-heart infusion (BHI) broth and incubated for 24 h at 30°C. Subsequently, 100 µL of each culture were transferred into 1,000 mL of fresh BHI broth and incubated at 8°C for at least four days for *L. innocua*, and at 20°C for at least two days for *Salmonella* spp. Before use, the five *L. innocua* subcultures were mixed in equal volumes, to obtain a final concentration of approximately 10^9 CFU/mL; the same procedure was applied to the three *Salmonella* spp. subcultures.

Salami contamination and production process

Three different formulations of *Spianata Piccante* salami (designated A, B, and C) were used in the challenge test: (A) the traditional formulation, (B) the traditional formulation with a bacteriocin-producing starter culture (BSC) and (C) the traditional formulation with added dextrose. The detailed composition of each formulation is reported in Table 1.

A total of 100 kg of meat batter for each formulation was provided by a local meat manufacturer and divided into two batches (Figure 1). Each batch was inoculated with 1% (v/w) of the multi-strain cocktail of *L. innocua* or *Salmonella* spp., to obtain an initial target concentration of approximately 10^6 CFU/g. The mixtures were mechanically blended for 30 seconds and then stuffed into cotton casings to produce salami measuring approximately 300×100×55 mm (L×W×H) and an initial weight of approximately 2.5-3 kg. The products were then pressed using a metal grid to reproduce the characteristic flattened shape of *Spianata Piccante*. The salami were then subjected to fermentation and ripening following the manufacturer's standard processing parameters (Table 1). Weight loss was monitored during production on three samples from each batch and expressed as the percentage of the initial weight. At the end of the ripening stage, salami from each batch of the three formulations were vacuum-packed for HPP treatment, while additional samples served as untreated controls. The HPP treatment was applied at 593 MPa for 290 seconds.

Sampling procedure, physicochemical and microbiological analysis

After inoculation, the samples were collected from each batch of the three *Spianata Piccante* formulations at specific stages of the production process as reported in *Supplementary Table 1*. For each batch, three samples were taken before stuffing (T_0) and at the end of the fermention phase (T_1), while ten samples were collected at the end of ripening (T_end) and after the HPP treatment (T_HPP). At each sampling time, pH and a_w values were measured, and counts of *L. innocua* and *Salmonella* spp. were determined. For physicochemical analysis, the pH was

measured and the a_w was determined in accordance with ISO 18787:2017 (ISO, 2017a). For microbiological analysis, 25 g of contaminated salami were placed in individual plastic one-chamber filter stomacher bags, diluted 1:10 in buffered peptone water (BPW) and homogenized in a Stomacher for 2-3 minutes. Appropriate ten-fold serial dilutions were plated onto Listeria Ottaviani & Agosti agar (ALOA) and incubated at 37°C for 48 h for *L. innocua* enumeration following ISO 11290-2:2017 (ISO, 2017b), or onto Hektoen Enteric Agar (HEA) and incubated at 37 °C for 24 h for *Salmonella* spp. enumeration. After incubation, typical colonies were counted for both *L. innocua* and *Salmonella* spp.. At T_0, T_1, T_end and T_HPP, also mesophilic lactic acid bacteria (LAB) were counted on De Man, Rogosa and Sharpe (MRS) agar, after an incubation period of 72 hours at 30°C, in accordance with ISO 15214:1998 (ISO, 1998).

Data analysis

At the beginning of the challenge test, in accordance with ISO 20976-2:2022, the standard deviation (SD) of the target microorganism concentration in the contaminated batter was required not to exceed 0.3 $\log_{10}$ CFU/g in order to consider the inoculation homogeneous and the study valid.

At each sampling time, mean values and SD of all analyzed parameters (pH, a_w, and counts of *L. innocua*, *Salmonella* spp. and LAB) were calculated. Microbiological results were expressed as CFU/g and subsequently converted to $\log_{10}$ CFU/g. When enumeration of *L. innocua* and *Salmonella* spp. yielded negative results, qualitative analysis (presence/absence) was performed on 25 g of sample, in accordance with ISO 6579-1:2017 for *Salmonella* spp. and ISO 11290-1:2017 for *Listeria* spp. (ISO, 2017c, 2017d). For data processing purposes, positive qualitative results were assigned a value of 5 CFU/g ($\log_{10} = 0.70$), while negative results were assigned a value of 0.04 CFU/g (1 CFU/25g; $\log_{10} = -1.40$).

The $\log_{10}$ reduction (log kill) of the target microorganisms was defined as the difference between the microbial concentration before and after the processing steps. For each batch, the mean $\log_{10}$ concentration at T_0 was calculated and used as the reference value. Individual log kill values were obtained by subtracting each $\log_{10}$ concentration measured at T_1, T_end and T_HPP from the corresponding mean $\log_{10}$ concentration at T_0. The lowest $\log_{10}$ reduction observed among the test units at T_end and at T_HPP was considered the worst-case scenario, to ensure compliance with the FSIS targets for the inactivation of *Salmonella* spp. and *L. monocytogenes*, that requires a minimum reduction of 5 $\log_{10}$ CFU/g and 3 $\log_{10}$ CFU/g, respectively.

Statistical analysis

Statistical analysis was performed using R Software (v. 4.2.2). Normality of data distribution was assessed using the Shapiro-Wilk test, while homoscedasticity was verified by Levene's test. For normally distributed data, differences between formulations at each sampling time were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test. Significant differences ($p<0.05$) were indicated in tables using superscript letters. For data sets where the normality assumption was violated, the non-parametric Kruskal-Wallis test was applied, followed by Dunn's post-hoc test with Benjamini-Hochberg p-value adjustment. Physicochemical parameters (pH and a_w) were analyzed separately for each pathogen batch (*L. innocua* and *Salmonella* spp.). Statistical significance was set at $p<0.05$. Data visualization, including the generation of box plots to illustrate distribution and outliers, was conducted using the ggplot2 package, while statistical tests were supported by car, dplyr, multcompView, and FSA packages.

Results

The physicochemical characteristics of the three different formulations of *Spianata Piccante* are reported in *Supplementary Table 2*. Overall, a significant consistent decrease ($p<0.05$) in pH and a_w was observed across all formulations during the process. In formulation C (the traditional one with added dextrose), the pH dropped more sharply during the fermentation phase, reaching significantly lower values at T_1 compared to formulation A in both *L. innocua* (4.76±0.01 *vs.*

4.82±0.03; $p<0.05$) and *Salmonella* spp. batches (4.71±0.05 *vs.* 4.82±0.02; $p<0.01$). Regarding the a_w, values decreased significantly from initial levels of 0.94-0.96 (T_0) to 0.92-0.94 at the end of ripening (T_end), with formulation C showing significantly lower values (0.94±0.00; $p<0.05$) at T1 in the *Salmonella* batch compared to the other formulations.
The weight loss increased progressively during the ripening period across all formulations. At T_end, the mean weight loss was 25.19%, 20.93% and 24.36% for formulations A, B and C, respectively. The reduction in weight loss is correlated with the observed decrease in a_w values, which which fell below 0.94 in all samples tested prior to HPP treatment.
In Table 2 are reported the results of the LAB enumeration. In formulation A, at T_1 the LAB count was 8.37±0.45 $\log_{10}$ CFU/g, but the peak was reached later, at T_end (8.81±0.12 $\log_{10}$ CFU/g). In formulations B and C, LAB reached the maximum concentrations immediately after fermentation (T_1), with values of 8.81 ± 0.12 and 9.10 ± 0.25 $\log_{10}$ CFU/g, respectively. The counts of the target microorganisms during the process, from T_0 to T_HPP, are reported in Table 2. The initial inoculation levels were homogeneous across the three formulations (7.11-7.57 $\log_{10}$ CFU/g for *L. innocua* and 6.47-6.94 for *Salmonella* spp.), meeting the ISO 20976-2:2022 validation criteria (SD<0.3 $\log_{10}$ CFU/g). The resulting logarithm reductions for both pathogens are summerized in Table 3; the box plot visualizations of pathogen reductions during the production process and post-HPP treatment are available in *Supplementary Figures 1 and 2*. Statistical analysis confirmed that the formulation significantly influenced biocidal efficacy against both pathogens ($p<0.05$ at all sampling points). Regarding *L. innocua*, formulation B showed the highest efficacy, particularly after the HPP treatment (T_HPP), achieving a reduction of 4.38±0.41 $\log_{10}$ CFU/g, which was significantly higher ($p<0.001$) than both formulation A (3.10±0.51 $\log_{10}$ CFU/g) and formulation C (2.57±0.38 $\log_{10}$ CFU/g). At the end of ripening (T_end), Formulation B also outperformed the others, while no significant difference was observed between A and C. For *Salmonella* spp., formulations B and C exhibited superior performance compared to formulation A starting from the T_end stage ($p<0.001$), with reductions exceeding 5 $\log_{10}$ CFU/g. At T_HPP, formulation B achieved the maximum reduction (7.66±0.66 $\log_{10}$ CFU/g), although it was not statistically different from formulation C (43±1.01 $\log_{10}$ CFU/g, $p>0.05$). In contrast, formulation A showed the lowest inactivation levels throughout the entire process, reaching only 5.51±1.42 $\log_{10}$ CFU/g at T_HPP. For both pathogens, while the ripening process contributed to the reduction of the target microrganisms, the subsequent HPP treatment provided an additional significant inactivation, in particular for formulation B, which was the only one to achieve the FSIS reduction standards under the worst-case scenario both for *L. innocua* (3.89 $\log_{10}$ CFU/g) and for *Salmonella* spp. (5.77 $\log_{10}$ CFU/g), and for formulation C only for *Salmonella* spp. (5.96 $\log_{10}$ CFU/g), as reported in Table 3.

Discussion

The results of this study demonstrated that the microbiological safety of *Spianata Piccante* intended for export to the US relies on a multi-hurdle preservation strategy, resulting from the combined effect of product formulation, fermentation-ripening, and HPP treatment (Barbuti and Parolari, 2002; Bonilauri *et al*., 2019; Bonilauri *et al*., 2021). The progressive decrease in pH and a_w observed during fermentation and ripening contributed to pathogen inactivation, while the HPP treatment provided an additional lethality step essential to achieve the microbial reduction targets required for RTE meat products intended for the US market (Bonilauri *et al*., 2019; Bonilauri *et al*., 2021).
The evolution of the physicochemical parameters is consistent with typical Italian fermented dry-cured sausages. During fermentation, the growth of LAB promoted matrix acidification, resulting in a marked pH decrease, particularly in formulation C, which reached a value of 4.71±0.05 $\log_{10}$ CFU/g due to dextrose supplementation. This enhanced carbohydrate fermentation and organic acid production, conditions reported to inhibit foodborne pathogens through intracellular acidification (Sivamaruthi *et al.*, 2025). Accordingly, a pronounced inactivation of *Salmonella* spp. was observed throughout the process. The sensitivity of *Salmonella* spp. to acidic

environments in fermented meat has been widely documented, as organic acid accumulation can impair membrane function and metabolic activity in Gram-negative bacteria (Lücke, 2000; Bonilauri *et al.*, 2019). However, acidification alone was less effective against *L. innocua*, as formulation C showed lower reductions compared with formulation B (2.25 *vs.* 3.89 $\log_{10}$ CFU/g, respectively). This confirms that *Listeria* spp. exhibit greater tolerance to acidic conditions than many Gram-negative pathogens and therefore require additional antimicrobial hurdles for effective control in fermented meat products (Ferreira *et al.*, 2014).

The reduction in a_w during ripening further contributed to microbial inactivation. The progressive moisture loss, reflected in the weight reduction of the products, lowered a_w values below 0.94 in all formulations before HPP treatment, limiting pathogen survival and representing a key hurdle in dry-cured meat products (Hayman *et al.*, 2008).

Among the tested formulations, formulation B, containing a bacteriocin-producing starter culture, showed the highest anti-listerial activity throughout the production process and after HPP treatment, achieving a reduction of 3.89 $\log_{10}$ CFU/g for *L. innocua* and 5.77 $\log_{10}$ CFU/g for *Salmonella* spp. under the worst-case scenario. These results suggest that pathogen inhibition observed during fermentation may result from the synergistic effects of pH reduction, decreased a_w and the metabolic activity of LAB, including the potential production of antimicrobial compounds. Among these compounds, bacteriocins such as pediocin-like peptides have been reported to exhibit inhibitory activity against *Listeria* spp. (Sugrue *et al.*, 2025), although their specific contribution was not directly assessed in this study. Sublethal stress induced during fermentation can increase pathogen susceptibility to HPP, thereby enhancing overall treatment efficacy (Garriga *et al.*, 2002; Campus, 2010). This may explain the higher reductions observed for *L. innocua* in formulation B. Overall, the results indicate that the production process alone is insufficient to consistently achieve the microbial reductions required by the US regulations for RTE meat products.

The lack of evaluation of pathogen re-growth during product storage and shelf-life represents a limitation of the present study. Despite the significant reductions achieved through the applied multi-hurdle approach, the recovery of sublethally injured cells during storage cannot be excluded, particularly under conditions of temperature abuse or changes in packaging atmosphere. Further studies addressing pathogen behavior throughout shelf-life are needed to confirm the long-term microbiological safety of the product.

Conclusions

According to the FSIS guidelines, validated processes must ensure at least a 5 $\log_{10}$ CFU/g reduction of *Salmonella* spp. and a 3 $\log_{10}$ CFU/g reduction of *L. monocytogenes* when challenge studies are used for validation purposes. Under the worst-case scenario, only formulation B, containing a bacteriocin-producing starter culture, met both reduction targets after the HPP treatment. These findings highlight the importance of combining formulation strategies, fermentation processes, and post-lethality treatments to ensure microbiological safety and compliance with US regulatory requirements for fermented meat products intended for export markets.

Online supplementary material

Supplementary Table 1. Experimental design with the number of sample units analyzed at each processing step and the corresponding analyses.

Supplementary Table 2. Evolution of pH and water activity in the three formulations of *Spianata Piccante* (A, B and C) throughout the process.

Supplementary Figure 1. Box plot representation of logarithmic reduction (Δ $\log_{10}$ CFU/g) of *L. innocua* for formulations A, B, and C at three processing time points.

Supplementary Figure 2. Box plot representation of logarithmic reduction (Δ $\log_{10}$ CFU/g) of *Salmonella* spp. for formulations A, B, and C across the processing stages.

References

Barbuti S, Parolari G, 2002. Validation of manufacturing process to control pathogenic bacteria in typical dry fermented products. Meat Sci 62:323-9.

Bintsis T, 2017. Foodborne pathogens. AIMS Microbiol 3:529-63.

Bolumar T, Orlien V, Sikes A, Aganovic K, Bak KH, Guyon C, Stübler A, De Lamballerie M, Hertel C, Brüggemann DA, 2021. High-pressure processing of meat: molecular impacts and industrial applications. Compr Rev Food Sci Food Saf 20:332-68.

Bonilauri P, Grisenti MS, Daminelli P, Merialdi G, Ramini M, Bardasi L, Taddei R, Cosciani-Cunico E, Dalzini E, Frustoli MA, Giacometti F, Piva S, Serraino A, 2019. Reduction of Salmonella spp. populations in Italian salami during production process and high pressure processing treatment: validation of processes to export to the U.S. Meat Sci 157:107869.

Bonilauri P, Merialdi G, Ramini M, Bardasi L, Taddei R, Grisenti MS, Daminelli P, Cosciani-Cunico E, Dalzini E, Frustoli MA, Giacometti F, Tomasello F, Piva S, Serraino A, 2021. Modeling the behavior of Listeria innocua in Italian salami during the production and high-pressure validation of processes for exportation to the U.S. Meat Sci 172:108315.

Campus M, 2010. High pressure processing of meat, meat products and seafood. Food Eng Rev 2:256-73.

Commission of the European Communities, 2005. Commission regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuff. In: Official Journal, L 338, 22/12/2005.

Dučić MS, Markov SL, 2022. Overview of treatments for improving the microbial safety of dry fermented sausages. J Food Saf Food Qual 73:33-42.

EFSA Panel on Biological Hazards, 2022. The efficacy and safety of high-pressure processing of food. EFSA J 20:e07128.

Ferreira V, Wiedmann M, Teixeira P, Stasiewicz MJ, 2014. Listeria monocytogenes persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. J Food Prot 77:150-70.

FSIS, 2023. FSIS ready-to-eat fermented, salt-cured, and dried products guideline. Document ID: FSIS-GD-2023-0002. United States Department of Agriculture.

Garriga M, Aymerich MT, Costa S, Monfort JM, Hugas M, 2002. Bactericidal synergism through bacteriocins and high pressure in a meat model system during storage. Food Microbiol 19:509-18.

Hayman MM, Kouassi GK, Anantheswaran RC, Floros JD, Knabel SJ, 2008. Effect of water activity on inactivation of Listeria monocytogenes and lactate dehydrogenase during high pressure processing. Int J Food Microbiol 124:21-6.

Hu M, Gurtler JB, 2017. Selection of surrogate bacteria for use in food safety challenge studies: a review. J Food Prot 80:1506-36.

ISO, 1998. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of lactic acid bacteria – Part 1: Colony-count technique at 30 degrees C. ISO Norm 15214:1998. International Organization for Standardization, Geneva, Switzerland.

ISO, 2017a. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. ISO Norm 18787:2017. International Organization for Standardization, Geneva, Switzerland.

ISO, 2017b. Microbiology of the food chain – Horizontal method for the detection and enumeration of Listeria monocytogenes – Part 2: Enumeration method. ISO Norm 11290-2:2017. International Organization for Standardization, Geneva, Switzerland.

ISO, 2017c. Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella – Part 1: Detection of Salmonella spp. ISO Norm 6579-1:2017. International Organization for Standardization, Geneva, Switzerland.

ISO, 2017d. Microbiology of the food chain – Horizontal method for the detection and enumeration of Listeria monocytogenes – Part 1: Detection method. ISO Norm 11290-1:2017. International Organization for Standardization, Geneva, Switzerland.

ISO, 2022. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Enterobacteriaceae – Part 2: Enumeration method. ISO 20976-2:2022. International Organization for Standardization, Geneva, Switzerland.

Lücke FK, 2000. Utilization of microbes to process and preserve meat. Meat Sci 56:105-15.

Merialdi G, Ramini M, Ravanetti E, Gherri G, Bonilauri P, 2015. Reduction of Listeria innocua contamination in vacuum-packaged dry-cured Italian pork products after high hydrostatic pressure treatment. Ital J Food Saf 4.

San Martín MF, Barbosa-Canovas GV, Swanson BG, 2002. Food processing by high hydrostatic pressure. Crit Rev Food Sci Nutr 42:627-45.

Scallan Walter, EJ, Cui, Z, Tierney, R, Griffin, PM, Hoekstra, RM, Payne, DC, Rose, EB, Devine, C, Namwase, AS, Mirza, SA, Kambhampati, AK, Straily, A, Bruce, BB, 2025. Foodborne illness acquired in the United States—major pathogens, 2019. Emerg Infect Dis 31:669-77.

Sivamaruthi BS, Kesika P, Rafeek SBS, Sivakumar K, Ramasamy SP, Chaiyasut C, Fukngoen P, Alagarsamy K, 2025. Lactic acid bacteria in the meat industry: flavor, function, and food safety. Front Microbiol 16:1703213.

Sugrue I, Ross RP, Hill C, 2024. Bacteriocin diversity, function, discovery and application as antimicrobials. Nat Rev Microbiol 22:556-71.

Table 1. Process parameters and ingredients of the different formulations of *Spianata Piccante* salami used for challenge test.

Formulation	Fermentation		Ripening		Characteristics of different salami					
	Time (days)	Temperature (°C[a])	Time (days)	Temperature (°C[a])	Lipids (%)	Nitrate (ppm)	Nitrite (ppm)	NaCl (%)	Dextrose (%)	BSC (%)
A	7	4-26	21	14-16	29.74	147	n.d.	3.39	0.15	0
B					27.61	65	n.d.	3.38	0	0.20
C					25.90	142	n.d.	3.27	0.40	0

[a] Range of temperature: a variable temperature can be applied during the phase; ppm, part per million; BSC, bacteriocin-producing starter culture; n.d., not detectable.

Table 2. Mean value $\log_{10}$ CFU/g ± standard deviation of *L. innocua*, *Salmonella* spp. and lactic acid bacteria (LAB) enumeration analysis.

Pathogen	Time	Recipe A	Recipe B	Recipe C
L. innocua	T_0	7.11 ± 0.07	7.57 ± 0.12	7.19 ± 0.31
	T_1	5.97 ± 0.18	6.17 ± 0.05	6.5 ± 0.22
	T_end	5.78 ± 0.21	5.61 ± 0.25	5.90 ± 0.16
	T_HPP	4.01 ± 0.51	3.19 ± 0.41	4.62 ± 0.38
Salmonella spp.	T_0	6.94 ± 0.14	6.47 ± 0.11	6.66 ± 0.07
	T_1	5.77 ± 0.06	5.79 ± 0.17	5.35 ± 0.31
	T_end	4.82 ± 0.18	1.41 ± 0.37	1.37 ± 0.91
	T_HPP	1.43 ± 1.42	-1.19 ± 0.66	-0.77 ± 1.01
LAB	T_0	6.94 ± 0.07	7.03 ± 0.05	6.94 ± 0.04
	T_1	8.37 ± 0.45	8.81 ± 0.07	9.10 ± 0.25
	T_end	8.81 ± 0.12	8.50 ± 0.11	8.16 ± 0.08
	T_HPP	5.94 ± 0.39	7.35 ± 0.14	7.75 ± 0.35

Table 3. Logarithmic reduction (log_{10} CFU/g) of *Listeria innocua* and *Salmonella* spp. in three distinct formulations of *Spianata Piccante* (A, B, C) at different processing stages (T_1, T_end, T_HPP).

Pathogen	Time	A	B	C	p-value
L. innocua	T_1	1.14 ± 0.18^{a}	1.40 ± 0.05^{a}	0.69 ± 0.22^{b}	0.0056
	T_end	1.33 ± 0.21^{b}	1.96 ± 0.25^{a}	1.29 ± 0.16^{b}	< 0.001
	T_HPP	3.10 ± 0.51^{a}	4.38 ± 0.41^{b}	2.57 ± 0.38^{a}	< 0.001
WCS	T_end	1.00	1.53	1.11	ne
	T_HPP	2.66	3.89	2.25	ne
Salmonella spp.	T_1	1.17 ± 0.06^{ab}	0.69 ± 0.17^{b}	1.32 ± 0.31^{a}	0.0218
	T_end	2.12 ± 0.18^{a}	5.06 ± 0.37^{b}	5.28 ± 0.91^{b}	< 0.001
	T_HPP	5.51 ± 1.42^{a}	7.66 ± 0.66^{ab}	7.43 ± 1.01^{b}	0.0098
WCS	T_end	1.90	4.32	3.85	ne
	T_HPP	3.99	5.77	5.96	ne

Values are expressed as Mean ± Standard Deviation (n = 3). Different superscript letters (*a, b, c*) within the same row indicate statistically significant differences between recipes ($p < 0.05$). For normally distributed data (Shapiro-Wilk $p > 0.05$), significance was determined using one-way ANOVA followed by Tukey's HSD post-hoc test. For non-normally distributed data (*Listeria* at T_HPP; *Salmonella* at T_end and T_HPP), the non-parametric Kruskal-Wallis test was applied, followed by Dunn's post-hoc test with Benjamini-Hochberg correction. WCS, worst-case scenario; ne, not evaluated for statistical analysis.

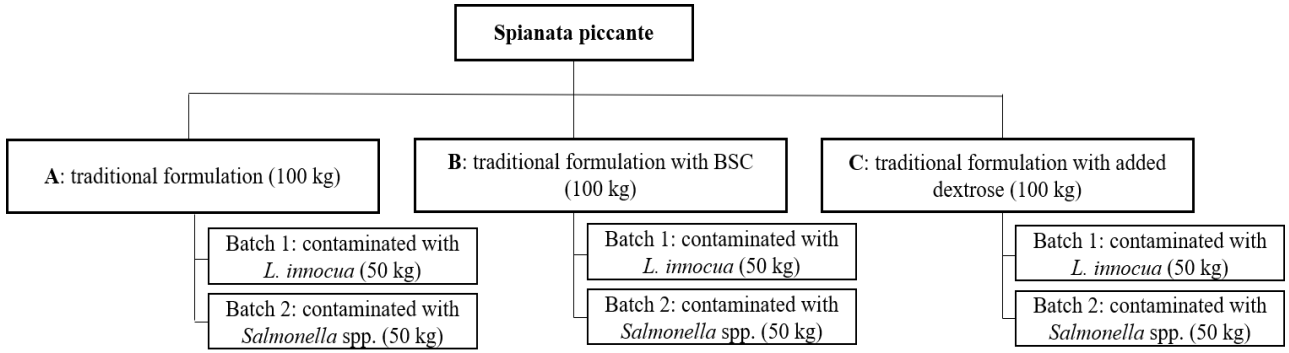

Figure 1. Graphical representation of the experimental design, including the three formulations of *Spianata Piccante* (A, B and C) and their subdivision into batches inoculated with *L. innocua* and *Salmonella* spp. BSC, bacteriocin-producing starter culture.