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Fermented salami-type meat sausage with *Lacticaseibacillus paracasei* and plant extract of rosemary (*Rosmarinus officinalis* L.) and green tea (*Camellia sinensis*)

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Abstract

The aim of the study was to develop a salami-type meat sausage enriched with the probiotic bacteria *Lacticaseibacillus paracasei* and a mixed plant extract containing rosemary (*Rosmarinus officinalis* L.) and green tea (*Camellia sinensis*) and to evaluate its physicochemical and microbiological characteristics. In the study, salami-type meat sausages were developed and divided into 11 equal portions, which were added with different concentrations of mixed extract (1–3.4%) and probiotic (1.5–3.5%), totaling 11 formulations according to a Central Composite Rotating Design. Lipid oxidation, instrumental color, pH, aw, and microbiological analyses (count of lactic acid bacteria, thermotolerant coliforms, coagulase-positive staphylococci, and *Salmonella*) were performed to evaluate the developed products. There was a significant effect of varying the mixed extract and probiotic concentration on the lipid oxidation, color parameters, and lactic acid bacteria count responses. Lower lipid oxidation values were observed in the combination of lower concentrations of mixed extract (0.5–1.5%) with intermediate concentrations of the probiotic (2.25–2.75%). All formulations contained lactic acid bacteria above 8 Log_{10} CFU/g throughout the shelf life. It was concluded that the addition of *L. paracasei* to salami-type sausage is viable and, in combination with the mixed extract of rosemary and green tea, can inhibit lipid oxidation.

Keywords: green tea; probiotic lactic acid bacteria; lipid oxidation; natural antioxidant; rosemary.

Practical Application: Salami may be a viable vehicle for probiotic microorganisms.

1 INTRODUCTION

The meat industry has recently prioritized the development of products that go beyond basic nutritional value, focusing on additional health benefits. An emerging approach is the incorporation of natural ingredients and probiotics into the formulation of these products, reducing the need for potentially harmful synthetic additives (Kaveh et al., 2023).

In this context, rosemary (*Rosmarinus officinalis* L.) and green tea (*Camellia sinensis*) emerge as promising natural ingredients, particularly due to their recognized antioxidant effects (Bortolini et al., 2021; Hu et al., 2022). Furthermore, probiotics—which by definition are live microorganisms that, when ingested in adequate amounts, provide health benefits (Hill et al., 2014)—can also be added to potentially functional foods, including meat products. Interestingly, some probiotic bacteria, such as *Lacticaseibacillus paracasei*, exhibit antioxidant properties, expanding their potential application in meat products (Rwubuzizi et al., 2023; Vougiouklaki et al., 2023).

Processed meats are often considered less healthy products due to their high fat and additive content. Therefore, adding probiotics and replacing synthetic additives with natural extracts to these foods could contribute to the supply of foods that meet the needs of a new consumer profile interested in foods that, in addition to basic nutrients, can also offer health benefits (Silva et al., 2016). Among processed meats, salami has the potential for probiotic inclusion because it is not subjected to cooking during production and/or consumption, which does not compromise the viability of probiotic microorganisms until the end consumer. In order to offer consumers new functional food options, the objective of this work was to develop a salami-type meat sausage added with probiotic bacteria *L. paracasei* and mixed plant extract containing rosemary and green tea as a natural antioxidant and evaluate its physicochemical and microbiological characteristics.

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1.1 Relevance of the work

New food products must meet new consumer demands. In this case, a meat product containing plant extracts as antioxidants and probiotics was developed to present a potentially functional product.

2 MATERIAL AND METHODS

2.1 Material

The pork leg and bacon used in the production of the salami-type meat sausage were provided by the Federal Institute of Education, Science, and Technology of the Triângulo Mineiro Campus Uberaba (IFTM Campus Uberaba), and the other ingredients were purchased from local businesses in Uberaba, MG. *L. paracasei* and the mixed plant extract of rosemary and green tea were kindly donated by the companies LEMMA Supply Solutions Ltda (Jardinópolis/SP-Brazil) and Kemin do Brasil, respectively.

2.2 Activation of the probiotic microorganism

An aliquot of lyophilized culture of *L. paracasei* was previously replicated successively three times in MRS (de Man, Rogosa, and Sharpe) broth at 37°C for 18 h. The suspension was then centrifuged twice at 3,700 rpm for 5 min and diluted in 0.85% saline solution until reaching a concentration equivalent to standard 4.0 on the McFarland Scale (approximately 1.2×10^9 cells/mL). This suspension was then used, on the same day, to manufacture the salami-type product.

2.3 Evaluation of the phenolic content and antioxidant activity of the extract

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the extract was determined according to the method described by Gao and Wang (2012). The half maximal effective concentration (EC_{50}) was calculated to express the antioxidant capacity. The total phenolic compound (TPC) was quantified using the Folin–Ciocalteu method, as described by Singleton and Rossi (1965), with gallic acid employed as the calibration standard. Results are expressed as milligrams of gallic acid equivalents (mg GAE) per gram of sample.

2.4 Production of salami-type meat products

The salami-type meat sausage was prepared with pork leg (81.56%), pork back fat (14.38%), sodium chloride (2.39%), glucose (0.48%), sucrose (0.48%), garlic powder (0.29%), white pepper (0.19%), nutmeg (0.19%), Bactoferm® T-SPX Starter Culture, Chr. Hansen (0.024%), and curing salt, composed of 90% sodium chloride, 6% sodium nitrate, and 4% sodium nitrite (0.014%). The concentrations of mixed extract (ME) of rosemary and green tea and probiotic culture of *L. paracasei* varied according to the formulations proposed by a central composite rotational design (CCRD) and are presented in Table 1.

Pork leg and pork back fat were ground in an electric grinder (PJ-22 Plus Professional, Jamar Ltda. (Skymsen), Brusque, Santa Catarina, Brazil) with 8 mm discs, and the mixture was transferred to an electric mixer, and the remaining ingredients were added, except for the plant extract and probiotic culture. This mixture was then divided into 11 equal portions, which were homogenized to the respective extract and probiotic concentrations, totaling 11 formulations (Table 1). After homogenization, the mixture was wrapped in cellulose wrap and tied into approximately 6 cm portions and finally immersed in a 20% potassium sorbate solution to inhibit fungal growth.

The pieces were stored at a controlled temperature. The fermentation process started at 25°C and was lowered by 1°C per day until the temperature reached 18°C, which was then maintained until the end of the maturation. The process was completed when the water activity (wa) of the pieces reached a value equal to or less than 0.90, which occurred on the 24th day. Then, the salami-type meat products were vacuum-packed and kept refrigerated (4°C) until analysis.

Table 1. Coded and decoded values of the independent variables and response variables of the salami-type meat sausage formulations containing a mixture of rosemary (*Rosmarinus officinalis* L.) and green tea (*Camellia sinensis*) extracts, and *Lacticaseibacillus paracasei*.

Formulation	Coded values		Decoded values		pH	Water activity	Lipid oxidation (mg MDA/kg)	L*	a*	b*	Lactic acid bacteria (Log_{10}CFU/g)
	X_1	X_2	Mixed extract (X_1) (%)	Probiotic (X_2) (%)							
F1	−1	−1	1	1.5	4.50	0.87	0.63	51.28	11.35	6.20	8.68
F2	−1	1	1	3.5	4.48	0.87	0.65	49.48	11.25	6.41	8.98
F3	1	−1	3.4	1.5	4.50	0.88	0.88	49.45	10.82	7.22	8.91
F4	1	1	3.4	3.5	4.51	0.88	0.81	51.23	11.28	8.05	8.94
F5	−1.4	0	0.5	2.5	4.51	0.89	0.57	51.33	11.83	6.41	8.79
F6	1.4	0	3.9	2.5	4.53	0.88	0.78	49.61	10.77	7.39	8.91
F7	0	−1.4	2.2	1.1	4.51	0.87	0.77	48.26	11.48	7.07	9.15
F8	0	1.4	2.2	3.9	4.51	0.88	0.67	48.38	11.04	7.05	9.22
F9	0	0	2.2	2.5	4.51	0.87	0.64	46.27	10.63	6.35	9.30
F10	0	0	2.2	2.5	4.50	0.87	0.56	47.51	11.04	7.05	9.33
F11	0	0	2.2	2.5	4.51	0.87	0.64	46.90	11.00	6.86	9.27

MDA: malondialdehyde; CFU: colony-forming units.

2.5 Characterization of salami-type sausage meat

The pH was measured directly on the samples using a digital pH meter (T-1000, Tecnal, Piracicaba, Brazil) with a perforating electrode. Water activity (wa) was determined using an electronic wa meter (4TE, Aqua Lab, Decagon Devices, Inc., Pullman, WA, USA) operating at 25°C ± 0.3°C.

Lipid oxidation of the salami product was determined using the rapid thiobarbituric acid reactive substances (TBARS) test according to the methodology described by the American Meat Science Association (King et al., 2023). The values were expressed in milligrams of malondialdehyde (MDA) per kilogram of product.

Color parameters were determined directly on the surface of the internal part of the samples using a portable colorimeter (CR 400, Konica Minolta, Japan). The CIELab scale (coordinates L*: luminosity, a*: green/red, and b*: blue/yellow) with illuminant D65 and an observation angle of 10° was used.

2.6 Microbiological profile of salami-type sausage meat

Microbiological analyses were conducted following the procedures described in the Compendium of Methods for the Microbiological Examination of Foods (Salfinger & Tortorello, 2015). To count lactic acid bacteria, the deep plating technique with an overlay on MRS agar medium and incubation at 37°C for 48 h was used.

Thermotolerant coliforms were evaluated by the multiple-tube technique in lauryl sulfate tryptose broth and incubated at 37°C for 24–48 h. The surface plating technique on Baird-Parker agar and incubated at 37°C for 48 h was used to evaluate coagulase-positive Staphylococci. After counting the colonies on the plates, the results were expressed as Log_{10} CFU/g, where CFU is colony-forming units.

The *Salmonella* evaluation assay began with the pre-enrichment of 25 g of sample in 225 mL of buffered peptone water at 37°C for 24 h. After this period, 1 mL was transferred to a tube containing 10 mL of tetrathionate (TT) broth, which was incubated at 37°C for 24 h, and 0.1 mL to a tube containing 10 mL of Rappaport-Vassiliadis (RV) broth, which was incubated in a water bath at 41.5°C for 24 h. After selective enrichment in TT and RV broths, aliquots were placed on three plates containing Hektoen enteric agar, xylose lysine deoxycholate agar, and bismuth sulfite agar, and all were incubated at 37°C for 24 h. The absence of typical colonies in these media was decisive for the analysis of *Salmonella*.

2.7 Statistical analysis

To study the effects of the independent factors, ME (X1) and the probiotic (X2), on the dependent variables (pH value, wa, TBARS value, color parameters, and lactic acid bacteria count), a CCRD was used. The CCRD consisted of a 2^2 full factorial design, including four axial points and three replicates at the central point. For this study, the initial concentrations (lower limits) of the ME (0.5%) and the probiotic culture (1%) were determined based on pretests.

Eleven experimental trials were conducted in random order, with three replicates at the central point and single runs for the remaining CCRD treatments (Table 1). All response variable determinations were performed in triplicate, and quality characteristics were evaluated using a response surface model with two factors: ME and probiotic.

The planning allowed the obtaining of a quadratic model, where the value of the dependent variable is a function of the independent variables, as described in Equation 1. The multiple regression method was used.

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (1)$$

The polynomial coefficients are represented by β_0 (constant term), β_1 and β_2 (first-order effects), β_{11} and β_{22} (second-order effects), and β_{12} (interaction between effects). The significance level was assessed using the p-coefficient at the 5% level ($p \leq .05$).

For the counts of thermotolerant coliforms and coagulase-positive *Staphylococci*, arithmetic means of the triplicates were calculated, and the results for *Salmonella* were expressed only as presence or absence.

3 RESULTS AND DISCUSSION

3.1 Mixed extract evaluation

The ME presented a TPC of 44.06 ± 1.25 mg GAE/mL and antioxidant activity expressed by EC_{50} (concentration required to reduce the initial absorbance of the DPPH• radical by 50%) of 0.11 ± 0.005 µL/mL.

Although the TPC values were lower than those found for rosemary extract (89.45 mg GAE/mL) (Pisheh et al., 2023). The antioxidant capacity of the ME was lower than what had been reported for rosemary extract, grape seed extract, and green tea polyphenol (IC_{50} of 2.5, 0.46, and 0.16 g/mL) (Zhou et al., 2020). This indicates that the ME offers the product greater protection against oxidation by free radicals, probably due to the formation of synergy with the addition of green tea.

Few studies have yet performed the combined application of a mixed rosemary and green tea extract, but even so, the TPC and antioxidant capacity values have been shown to be close to the values reported in the literature for the isolated extracts.

3.2 Characterization of salami-type sausage meat

Table 1 presents the experimental results obtained for salami-type meat sausage formulations. Table 2 presents the estimated linear and quadratic effects of the ME and probiotic factors, as well as the interactions between them, for the studied responses. Analysis of variance at 95% confidence ($p < .05$) demonstrated the significance of the regressions using the F-test.

No significant regression models were obtained for the pH and wa values obtained for the salami-type meat sausage. On the other hand, significant regression models could be predicted for lipid oxidation, color parameters, and lactic acid bacteria count.

Table 2. *P*-values obtained from the analysis of variance of the results obtained from the salami-type meat sausage formulations containing a mixture of rosemary (*Rosmarinus officinalis* L.) and green tea (*Camellia sinensis*) extracts and *Lacticaseibacillus paracasei*.

Factor/Variable	pH	Wa	L*	a*	b*	Lipid oxidation	Lactic acid bacteria
X_1 extract	.1402	.8087	.3255	.0483*	.0093*	.0030*	.2591
X_1 extract^2	.6628	.0981	.0019*	.1818	.6491	.0857	.0014*
X_2 probiotic	.7758	.3318	.9507	.7535	.3497	.2079	.1853
X_2 probiotic^2	.6078	.8746	.0388*	.2243	.3500	.0219*	.0765
$X_1.X_2$	.2583	.9665	.0800	.3427	.4168	.3805	.2427
R^2	.524	.526	.894	.693	.798	.897	.901

wa: water activity.

3.2.1 pH and water activity values

No significant effect of the concentration of the ME concentration variation and the probiotic culture count on the pH and aw values of the salami-type meat products studied was found. This behavior indicates that the values obtained for these response variables are not dependent on the change in these two variables in the formulation.

The pH values of the salami-type meat products ranged from 4.48 (F2) to 4.53 (F6). The pH values close to 4.5 had already been demonstrated for salami-type processed meat products containing *L. paracasei* (Macedo et al., 2008). These authors demonstrated that the addition of probiotic culture decreased the pH compared to the control treatment, which may indicate fermentation by the probiotic microorganism throughout the product's maturation and shelf life. This decrease in pH occurs because lactic acid-producing microorganisms use carbon to produce energy and, in the process of utilizing this molecule, produce acid that contributes to the decrease in the product's pH (Juturu & Wu, 2016).

pH values lower than 4.5 are considered safer from a microbiological point of view for the development of pathogenic microorganisms and contaminants that can reduce food safety and contribute to the theory of obstacles in the preservation of food products (Stegmayer et al., 2023). Furthermore, values close to 5.0 aim to reach the isoelectric point of the meat's myofibrillar proteins, causing water loss and obtaining texture in the product (Lawrie & Ledward, 2014).

The aging stage of the salami-type meat sausage was carried out with monitoring of wa on different days until the product reached a value < 0.90, which occurred on the 24th day after processing. Thus, the final values of the evaluated formulations ranged from 0.87 (F1, F2, F7, F9, F10, and F11) to 0.89 (F5), corroborating the values that had been reported by Macedo et al. (2008) for the same type of product. Furthermore, all formulations developed in this work comply with Brazilian legislation, which stipulates that ready-to-eat salami products must have a maximum wa of 0.92 (Brasil, 2000).

3.2.2 Lipid oxidation, color parameters, and lactic acid bacteria count

There was a significant effect of varying the concentration of ME and probiotic (P) on the responses of lipid oxidation, color parameters, and lactic acid bacteria count of the salami-type processed meat product (Table 2). Figure 1 demonstrates the response surface and contour plots for each of these response variables.

Regarding lipid oxidation of the developed products, Equation 2 is the modeling of this response variable.

$$Lipid\ oxidation = 0.9530 - 0.0008\ x\ ME + 0{,}065\ x\ P^2 \quad (2)$$

Lower lipid oxidation values were observed in the combination of lower concentrations of ME (0.5–1.5%) in combination with intermediate concentrations of the probiotic (2.25–2.75%) (Figure 1A). Species of the genus *Lacticaseibacillus* (formerly *Lactobacillus*), such as *Lacticaseibacillus rhamnosus*, *Lacticaseibacillus fermentum*, and *Lacticaseibacillus plantarum*, have been reported to exhibit antioxidant activity (Geeta & Yadav, 2017). This may have occurred in the present study, where the probiotic's antioxidant activity may have contributed to reducing lipid oxidation in salami-type meat products.

The salami-type sausage formulations presented lipid oxidation values ranging from 0.56 (F10) to 0.88 (F3), which were close to the values reported by sausage with rosemary extract (~0.7 MDA/kg) (Klančnik et al., 2009).

Concentrations of 0.5 and 1.0 mg MDA/kg (values reported in this work) are not sensorially described as rancid odor and taste. On the other hand, values between 1 and 2 mg MDA/kg of product are related to the sensory perception of lipid oxidation (Stefanello et al., 2015). This indicates that although there is some lipid oxidation in the developed products, it cannot yet be sensorially perceived.

Equation 3 shows the mathematical modeling of the luminosity response variable (L*). The effects of the ME and probiotic concentrations were positive, indicating that when these concentrations are increased in the formulation, the luminosity response also increases. On the other hand, darker formulations (lower luminosity value) were observed when concentrations of 2.0–3.0% of probiotic and 2.0–2.5% of ME were used (Figure 1B).

$$L* = 64.29 + 1.41\ x\ ME + 0.95\ P^2 \quad (3)$$

The L* values ranged from 46.27 (F9) to 51.33 (F5), which generally represents a darker color in the salami produced from all the formulations studied. A darker color

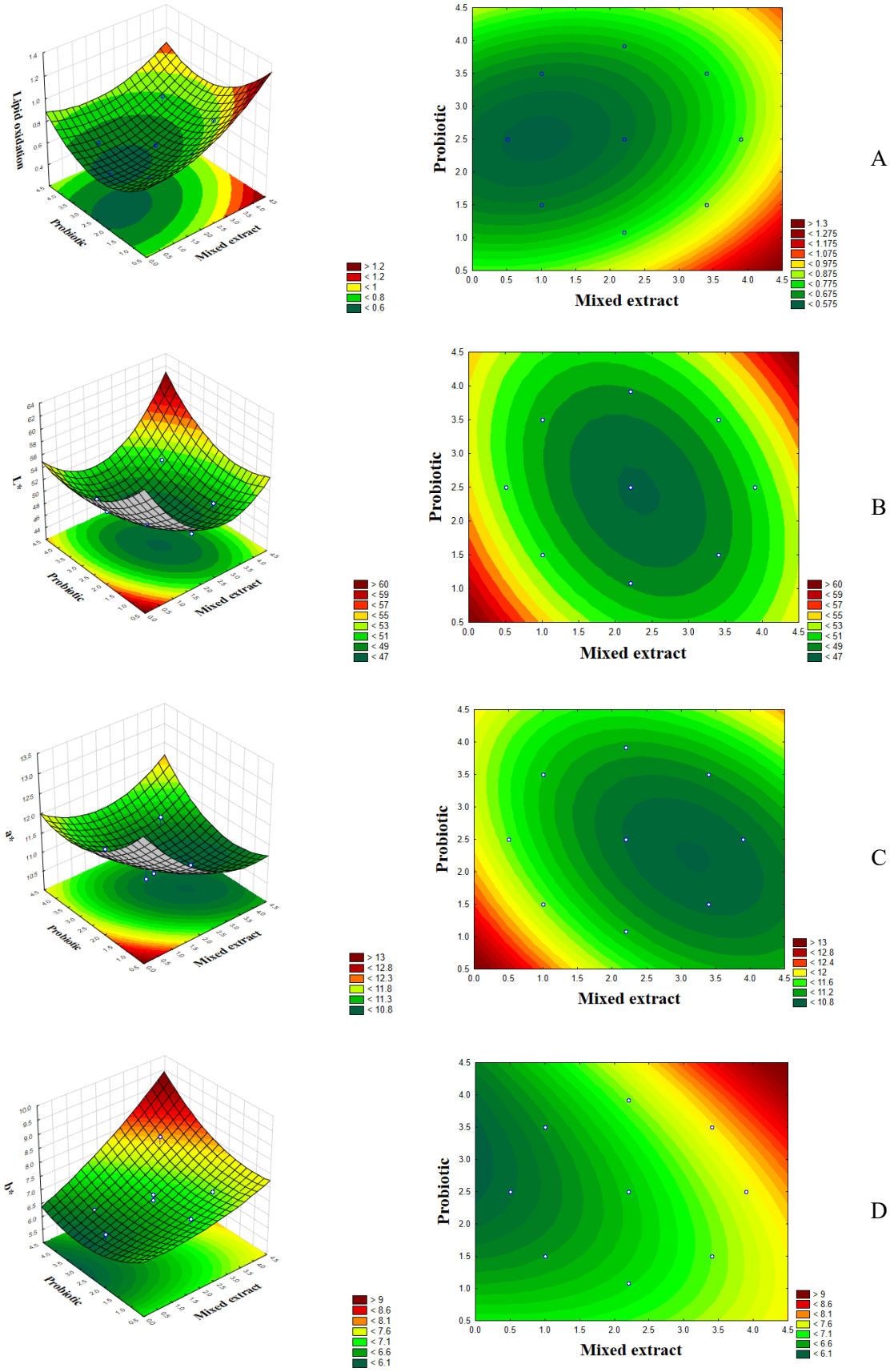

Figure 1. Response surface and contour plot for lipid oxidation (mg MDA/kg) **(A)**, color parameters being lightness (L*) **(B)**, a* **(C)**, and b* **(D)**, and lactic acid bacteria count (Log_{10} UFC/g) (E), respectively, of the salami-type meat sausage formulations containing a mixture of rosemary (*Rosmarinus officinalis* L.) and green tea (*Camellia sinensis*) extracts and *Lacticaseibacillus paracasei*.

is expected in matured meat products, and specifically in salami, which is an effect resulting from the darkening reactions that occur during the product's maturation and may vary according to the components present in its formulation (Bozkurt & Bayram, 2006).

Equation 4 demonstrates the mathematical modeling obtained for the color parameter a*. The ME alone demonstrated a negative effect on this parameter, indicating that the value of a* decreases when the ME concentration is increased (Figure 1C). This may be due to the color of the ME, which, not unlike other plant extracts, contributes to the greenish coloration, reducing the a* value.

$$a^* = 13.66 - 1.04\ x\ ME \quad (4)$$

The a* values ranged from 10.63 (F9) to 11.83 (F5) in the present work. These values were close to those reported by Yoon et al. (2021) for green tea extract and rosemary extract in naturally cured pork sausages with white kimchi powder (8.53) and higher than those found by Barbosa et al. (2019) for fresh beef sausage added with rosemary (*R. officinalis*) (6.20) and green tea (*C. sinensis*) (6.26) extracts.

Equation 5 demonstrates the mathematical modeling obtained for the color parameter b*. The ME alone demonstrated a negative effect on this parameter, indicating that the value of b* decreases when the ME concentration is increased (Figure 1D).

$$b^* = 7.39 - 0.11\ x\ ME \quad (5)$$

The b* values ranged from 6.20 (F1) to 8.05 (F4) in the present work. These values were lower than those reported by Yoon et al. (2021) for cured pork sausages (10.75) and by Barbosa et al. (2019) (14.10 and 13.35) for fresh beef sausage added with rosemary (*R. officinalis*) and green tea (*C. sinensis*) extracts.

All the formulations developed presented lactic acid bacteria counts above 8 Log_{10} CFU/g (Table 1). That is, high counts of these bacteria remained viable after the product maturation stage, which lasted 24 days, during which fermentation is expected to occur.

All developed formulations presented lactic acid bacteria counts above 8 Log_{10} CFU/g after 24 days of maturation (Table 1), indicating that these microorganisms remained viable throughout the fermentation and maturation process. Considering that the initial estimated values of *L. paracasei* added to the formulations ranged from approximately 7.1–7.7 Log_{10} CFU/g, an increase in the lactic acid bacteria population was observed throughout the maturation process. This microbial growth reflects the adaptation and multiplication of probiotic bacteria in the product environment, consistent with what is expected for fermented products, where conditions favor the development of these cultures.

4 CONCLUSIONS

The addition of *L. paracasei* to salami-type sausages in combination with a ME of rosemary (*R. officinalis* L.) and green tea (*C. sinensis*) proved to be a viable strategy for developing healthier meat products.

The results showed that the ME of green tea and rosemary is potential substitute for synthetic antioxidants in salami production and that lower concentrations of the ME combined with intermediate concentrations of probiotics are effective in reducing lipid oxidation without compromising pH and wa parameters. Additionally, all formulations maintained high lactic acid bacteria counts (above 8 Log_{10} CFU/g), indicating not only the technological feasibility of applying *L. paracasei* to this type of meat product but also its stability throughout processing. Thus, this approach represents a promising alternative for reformulating fermented sausages, integrating functional and natural attributes into the formulation, in line with growing consumer demands for healthier foods while maintaining sensory properties and microbiological safety. However, studies monitoring this product over time are needed to evaluate the behavior of the probiotic and extract during storage.

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