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Different commercial technological ingredients: impact on the shelf life of refrigerated fresh sausages

Diferentes ingredientes tecnológicos comerciais: impacto na vida útil de linguiças frescas refrigeradas

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Abstract: This study aimed to evaluate the effects of incorporating sodium erythorbate (PC) the absence of sodium erythorbate (NC), citric acid (CA), ascorbic acid (AA), sodium lactate (SL), potassium lactate (PL), TARI L 96 Forte (TL96 - a blend of lactic acid, dextrose, citric acid, acetic acid), and rosemary extract (RE) on the pH, TBARS values, and microbial growth of vacuum-packed fresh sausages, throughout days 1, 15, 30, 35, and 40 of storage, further assessed through mathematical modeling. Mathematical modeling revealed a relevant influence of the different technological ingredients on the final bacterial population, evidencing a reduction in growth rates, mainly in the treatment TL96. However, this trend was not corroborated by statistically significant differences in the experimental data ($P > 0.05$). Regarding TBARS values, the use of sodium erythorbate (PC) alone, and together with the organic acid blend (TL96) was effective in delaying lipid oxidation. The treatment CA exhibited the lower constants k_1 and k_2, indicating a slower acidification followed by a gradual pH recovery over time. The treatments with the most favorable results in microbiological and TBARS analyses, as well as superior performance in mathematical modeling (RE, TL96, and PL) were selected for sensory evaluation. The sensory analysis revealed no statistically significant differences among treatments, except for the flavor attribute in the treatment PL. The incorporation of TARI L96 Forte and potassium lactate into the sausage formulations contributed positively to the preservation of quality and microbiological safety of the fresh sausages during refrigerated storage.

Key-words: organic acids; natural extracts; lipid oxidation; microbial growth; meat products.

Resumo: Este estudo teve como objetivo avaliar os efeitos da adição de eritorbato de sódio (CP) e sem adição eritorbato de sódio (CN), ácido cítrico (AC), ácido ascórbico (AA), lactato de sódio (LS), lactato de potássio (LP), TARI L 96 Forte (TL96 – uma blenda contendo de ácido láctico, dextrose, ácido cítrico, ácido acético) e extrato de alecrim (EA), sobre os valores de pH, TBARS e crescimento microbiano de linguiças frescas embaladas a vácuo, ao longo dos dias 1, 15, 30, 35 e 40 de armazenamento avaliados por modelagem matemática. A modelagem matemática demonstrou efeito significativo dos diferentes ingredientes tecnológicos sobre a população bacteriana final, com diminuição da taxa de crescimento, principalmente para o tratamento TL96, entretanto, essa tendência não foi estatisticamente significativa ($p > 0,05$) nos dados experimentais. Em relação ao TBARS, o uso de eritorbato de sódio (CP) sozinho e em conjunto com a mistura de ácidos orgânicos (TL96) foi eficaz em retardar a oxidação lipídica. O tratamento AC apresentou

as menores constantes k_1 e k_2, indicando redução seguida de aumento lento do pH. Os tratamentos que apresentaram os melhores resultados de TBARS e modelagem matemática (EA, TL96 e LP) foram submetidos à avaliação sensorial, que não demonstrou diferença estatística entre os tratamentos, exceto para o atributo sabor do tratamento LP. A adição de TARI L96 Forte e lactato de potássio às formulações de linguiças apresentaram potencial efeito positivo na manutenção da qualidade e segurança das linguiças frescas durante o armazenamento refrigerado.

Palavras-chave: ácidos orgânicos; extratos naturais; oxidação lipídica; crescimento microbiano; produtos cárneos.

1. Introducion

The global pork production reached 121.7 million tons, with an increase forecasted to reach 129 million tons by 2031 [(1)]. Fresh sausage, a widely produced and consumed processed pork product (2), is highly perishable [(3)]. Even when stored under refrigeration temperatures, its high fat and water content, combined with the absence of heat treatment in the industry, makes it susceptible to lipid oxidation and microbial growth [(4)]. Lipid oxidation leads to the development of unacceptable sensory characteristics, whereas microbial growth can lead to product deterioration and foodborne illnesses. Therefore, delaying lipid oxidation and preventing microbial growth are factors that can significantly contribute to extending the shelf life of fresh sausages [(5)].

The meat industry has used various strategies to enhance the stability of meat products [(6)], such as the use of synthetic antioxidants and antimicrobial agents [(7)]. Given the wide range of commercially available additives, studies are needed to validate the efficiency of these additives [(6)] as potential alternatives for application in meat products.

In this context, this study aimed to evaluate the efficacy of various commercial technological ingredients, including organic acids (citric acid, ascorbic acid, lactic acid, acetic acid) and their corresponding salts (sodium erythorbate, sodium lactate, potassium lactate) and a natural rosemary extract, as potential alternatives for the processed meat industry. Particular attention was given for their inhibitory effects against spoilage microorganisms and their effects on the physicochemical properties and storage stability of refrigerated vacuum-packed fresh sausages.

2. Material and methods

2.1 Material

The selection of ingredients and the definition of the treatments were based on a collaboration with a private company in the food additives sector. The raw materials (pork leg, pork fat, garlic, sodium chloride, and sugar) were obtained from local markets in Pinhalzinho/SC. The commercial additives citric acid, ascorbic acid, sodium lactate, potassium lactate, TARI L 96 Forte (lactic acid, dextrose, citric acid, and acetic acid), rosemary extract, sodium erythorbate, cochineal carmine, FIBRISOL 414 phosphate, sodium nitrite, and sodium nitrate were provided by a company specializing in meat additives. All chemicals used in this study were of analytical grade.

2.2 Manufacture of fresh sausages

Eight treatments were performed in duplicate. The formulations were made with the addition of ingredients according to the manufacturer's instructions (Table 1). Except for the negative control (NC), all treatments contained sodium erythorbate plus the target ingredient, aiming to evaluate the potential synergy between them.

Table 1. Types and quantities of additives used in each treatment.

Additives (g/100 g)	Treatments							
	NC	PC	AA	CA	SL	PL	TL96	RE
Sodium erythorbate	-	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Ascorbic Acid	-	-	0.10	-	-	-	-	-
Citric Acid	-	-	-	0.15	-	-	-	-
Sodium Lactate	-	-	-	-	1.00	-	-	-
Potassium Lactate	-	-	-	-	-	1.00	-	-
TARI L 96 Forte	-	-	-	-	-	-	0.15	-
Rosemary Extract	-	-	-	-	-	-	-	0.03

Treatments: NC: Negative Control; PC: Positive Control; AA: Ascorbic Acid; CA: Citric Acid; SL: Sodium Lactate; PL: Potassium Lactate; TL96: Tari L96 Forte; RE: Rosemary Extract.

The base formulation consisted of pork leg (63.52 g/100 g), pork back fat (20 g/100 g), water (14 g/100 g), sodium chloride (1.3 g/100 g), garlic (0.15 g/100 g), sugar (0.5 g/100 g), cochineal carmine (0.02 g/100 g), FIBRISOL 414 phosphate (0.4 g/100 g, blend composed of sodium acid pyrophosphate, tetrasodium pyrophosphate, sodium polyphosphate and sodium tripolyphosphate), and a commercial blend of sodium nitrite and nitrate (0.013 g/100 g). The sausages were produced in batch duplicates.

For the processing of fresh sausages, pork leg and back fat were ground in an industrial grinder/stuffer (7000 Light, MSI-10, Brazil) using an 8 mm disk. Then, the ingredients were mixed in a planetary mixer (PHP500 Turbo, Philco, Brazil), according to each formulation, in the following order: pork leg and FIBRISOL 414 phosphate (mix 1 min), sodium chloride (mix 1 min), pork back fat (mix 1 min), water with dissolved cochineal carmine (1 minute), sodium nitrite and nitrate, garlic, and sugar (mix 1 min), and finally, sodium erythorbate along with the target ingredient (mix 10 min), totaling 15 minutes of mixing to ensure a homogeneous distribution of the ingredients.

The resulting batter was then stuffed into natural pork casings of 30 mm diameter and 10 cm length (approximately 60 g per piece) using the same equipment. The sausages were packed in polyethylene bags, vacuum-sealed (200S, Selovac, Brazil), and stored at 4 °C in an incubation chamber (BOD, SSBODu 342L, Solidsteel, Brazil) for 40 days.

2.3 Proximate composition

The proximate composition of the sausages was determined in triplicate, 1 day after production. The lipid, protein, ash, and moisture contents were determined according to the methodologies 920.39c, 920.152, 940.26 and 925.45b of AOAC [8], respectively.

2.4 Physicochemical characterization

Physicochemical characterization is essential to assess the quality, stability and compliance of meat products with legal and technological standards. These parameters also serve as a basis for interpreting the effects of different formulations and processing conditions on the samples studied. The fresh sausages were sampled on days 1, 15, 30, 35, and 40 of storage, and evaluated in triplicate for pH levels and thiobarbituric acid reactive substances (TBARS). The pH of the samples was measured by direct contact with the probe (LineLab, Akso, Brazil), previously calibrated with standard solutions of pH 4 and 10.

TBARS analyses were carried out using the spectrophotometric method as described by Jo and Ahn [9], with modifications. For that, 5 g of sample was homogenized using a Turrax-type homogenizer (TECNAL TE-139, Brazil) with 30 mL of 7.5% trichloroacetic acid. The mixture was filtered through qualitative filter paper (Qualy 12.5 cm, J. Prolab, Brazil), and a 2 mL aliquot of the filtrate was transferred to a test tube, and 2 mL of 0.02 M thiobarbituric acid (TBA) solution was added. The tubes were heated in a digital thermostatic bath (SSD 5L, Solidsteel, Brazil) at 100 ºC for 20 minutes, and cooled in an iced water bath to room temperature (~25 ºC). Absorbance was measured at 532 nm in a spectrophotometer (80 SA, Femto, Brazil), and the concentration was calculated using a standard curve of 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, Brazil). The results were expressed in mg of MDA per kg of sample.

2.5 Microbiological characterization

Microbiological analysis allows for the detection of microorganisms that indicate contamination, deterioration, or risk to consumer health. This control is particularly important in fresh meat products, due to their high perishability. For that, 10 g of sample were aseptically quantified in a sterile Stomacher bag and mixed with 90 mL of 0.1% (w/v) peptone water, making a 10^{-1} dilution. Then, sequential decimal dilutions were made by transferring 1 mL (from the 10^{-1} dilution) to 9 mL of 0.1% (w/v) peptone water to make a 10^{-2} dilution.

For the enumeration of lactic acid bacteria, 0.1 mL of the diluted sample was transferred to sterile Petri plates containing MRS (Man Rogosa & Sharpe, Millipore, Germany) medium, and incubated at 35±1 °C for 48±3 hours in an oven (SSB 110 L, Solidsteel, Brazil). For the enumeration of mesophilic bacteria and psychrotrophic aerobic bacteria, 0.1 mL of the diluted sample was transferred to sterile Petri plates containing PCA (Plate Count Agar, KASVI, Brazil) medium and incubated at 35±1 °C for 48±3 hours (SSB 110 L, Solidsteel, Brazil), and 7±1 °C for 10 days in an incubation chamber (BOD, SL 200 L, Solab, Brazil), respectively. Before conducting the sensory evaluation, the samples were tested for *Salmonella spp.*, *Escherichia coli*, and coagulase-positive staphylococci according to the methodologies described in Normative Instruction 60 [10].

2.6 Mathematical modeling

The application of mathematical modeling enables the description, prediction, and understanding of variable behavior over time, thereby contributing to formulation optimization and the development of more efficient preservation strategies. To quantify the effects of the different treatments on the quality indicators of fresh sausages (microbiological growth, lipid oxidation, and pH), the Baranyi-Roberts and biphasic mathematical models were applied.

The growth of LAB, mesophilic, and psychrotrophic microorganisms was modeled according to the Baranyi-Roberts model without an adaptation phase, according to Equation 1 [11]:

$$\log_{10}N(t) = \log_{10}N_{max} - \log_{10}\left[1 + \left(10^{\log_{10}N_{max} - \log_{10}N_0} - 1\right)e^{-\mu_{max}t}\right] \quad (1)$$

where $\log_{10}N(t)$ corresponds to the decimal logarithm of the microbial count at time t in log CFU, $\log_{10}N_{max}$ corresponds to the decimal logarithm of the maximum microbial count in log CFU, $\log_{10}N0$ corresponds to the decimal logarithm of the initial microbial count in log CFU, μ_{max} is the maximum growth rate, and t is the time (days).

The increase in lipid oxidation (TBARS) was modeled according to an adapted version of the Baranyi-Roberts model with an adaptation phase, as shown in Equation 2 [11]:

$$(2) \qquad \mathrm{TBARS}(t) = \mathrm{TBARS}_m + \log_{10}\left[\left(\frac{-1+e^{\mu max\lambda}+e^{\mu max t}}{e^{\mu max t}-1+e^{\mu max\lambda}10^{\mathrm{TBARS}_m-\mathrm{TBARS}_0}}\right)\right]$$

where TBARS(t) corresponds to the TBARS values at time t in kg of MDA per kg of sample, $TBARS_m$ corresponds to the maximum TBARS in kg of MDA per kg of sample, $TBARS_0$ corresponds to the initial TBARS in kg of MDA per kg of sample, μ_{max} represents the maximum rate, λ is the duration of the adaptation phase (analogous to the lag phase of the microbial growth curve), and t is the time (days).

Although an initial decrease in pH was observed, followed by a subsequent increase, the pH dynamics were modeled using an adapted version of the biphasic model, as shown in Equations 3(a) and 3(b) [12]:

$$(3a) \qquad \mathrm{pH}(t) = \mathrm{pH}_0 - k_1 t \ \text{ if } t < t^*$$

$$(3b) \qquad \mathrm{pH}(t) = \mathrm{pH}_0 - k_1 t^* + k_2(t - t^*) \ \text{ if } t \geq t^*$$

where pH(t) is the pH at time t, pH_0 is the initial pH, k_1, and k_2 are the rate constants corresponding to the pH decrease and increase, respectively, t is the time (in days), and t* is the time (in days) at which the pH changes occur.

2.7 Sensory evaluation

Sensory analysis serves as a critical tool for assessing consumer perceptions of sensory attributes, complementing physicochemical and microbiological evaluations by providing insights into the potential acceptability of the formulations tested. The treatments exhibiting the most favorable microbiological and physicochemical profiles, combined with superior predictive modeling performance, indicative of improved quality and safety for extended shelf life (days), were selected for sensory evaluation. Sensory analysis was performed three days after sample production, during which time microbiological counts remained within acceptable levels for safe consumption.

The sensory analysis of the fresh sausages was approved by the Research Ethics Committee Involving Human Beings of the Universidade do Estado de Santa Catarina (CAAE: 67501823.1.0000.0118). For that, 60 untrained panelists participated in the test at the sensory analysis laboratory of the Food Engineering and Chemical Engineering Department at Universidade do Estado de Santa Catarina. The evaluations were conducted in individual booths under standardized white lighting, in a temperature-controlled environment (approximately 22 ± 2 °C), free from noise and external odors. The fresh sausages were baked in an electric oven (200 °C) until reaching an internal temperature of 72 °C. The sausage samples were then served warm, sliced into 1 cm thick pieces on white plastic plates, coded with three different random numbers, accompanied by a glass of water and a salt cracker for palate cleansing.

The acceptance test was performed using a 9-point hedonic scale, where 9 = liked extremely and 1 = disliked extremely, to rate the attributes color, appearance, aroma, flavor, texture, and overall impression. The purchase intention test was also performed using a 5 to 1 scale, where 5 = would definitely buy and 1 = would definitely not buy.

2.8 Statistical analysis

The physicochemical, technological, and microbiological parameters of the fresh sausages were evaluated, considering the treatments and time as fixed effects and the repetitions as random effects. For the sensory evaluation, the treatment was considered a fixed effect and the panelists a random effect, using analysis of variance (ANOVA) through STATISTICA 14 Trial (Statsoft). Significant differences were analyzed using Tukey's test at a 5% significance level.

The performance of the Baranyi-Roberts primary model in fitting the experimental data was evaluated using the root mean square error (RMSE) statistical index (Equation 4), calculated in R software (version 4.2.2).

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_{pred,i} - y_{obs,i})^2}{n}} \quad (4)$$

where n is the number of experimental points (n = 5), $y_{pred,i}$ is the value predicted by the models for the experimental observation of i-th treatment, and $y_{obs,i}$ is the experimental observation of i-th treatment.

3. Results and discussion

3.1 Proximate composition

No significant differences (P > 0.05) were observed for protein, lipid, ash, and moisture contents among the treatments (Table 2).

Table 2. Proximate composition (%) of fresh sausages.

Treatment	Protein	Lipid	Ash	Moisture
NC	15.22 ± 0.18^{A}	20.40 ± 0.18^{A}	2.54 ± 0.03^{A}	63.78 ± 0.18^{A}
PC	15.03 ± 0.16^{A}	20.89 ± 0.34^{A}	2.52 ± 0.12^{A}	64.45 ± 0.50^{A}
CA	15.98 ± 0.05^{A}	20.49 ± 0.42^{A}	2.50 ± 0.14^{A}	64.42 ± 0.09^{A}
RE	16.00 ± 0.27^{A}	20.37 ± 0.47^{A}	2.60 ± 0.11^{A}	64.47 ± 0.08^{A}
AA	14.88 ± 0.09^{A}	20.58 ± 0.76^{A}	2.50 ± 0.12^{A}	64.94 ± 0.33^{A}
SL	15.01 ± 0.14^{A}	20.32 ± 0.30^{A}	2.60 ± 0.06^{A}	65.03 ± 0.33^{A}
TL96	15.16 ± 0.36^{A}	20.43 ± 0.21^{A}	2.50 ± 0.11^{A}	65.17 ± 0.28^{A}
PL	14.81 ± 0.03^{A}	20.38 ± 0.15^{A}	2.66 ± 0.03^{A}	64.76 ± 0.26^{A}

Mean ± standard deviation. Averages in the same column followed by the same uppercase letter are not significantly different by the Tukey test (P > 0.05) for the different treatments at the same time. Treatments: NC: Negative Control; PC: Positive Control; CA: Citric Acid; RE: Rosemary Extract; AA: Ascorbic Acid; SL: Sodium Lactate; TL96: TARI L96 Forte; PL: Potassium Lactate.

According to the Technical Regulation of Identity and Quality for Sausages [13], the maximum allowed lipid content is 30%, the minimum protein content is 12%, and the maximum moisture content is 70%. for fresh sausages. All treatments complied with the standards set by the current Brazilian legislation, which has established no requirements for the ash content of fresh sausages.

3.2 Physicochemical characterization

Regarding the pH values (Table 3), no significant differences were observed for the treatments PC, CA, AA, SL, and TL96 (P > 0.05) over the 40 days of storage, indicating a regulatory effect of these ingredients on the pH values of fresh sausages. In turn, a reduction of pH values was observed for the treatments NC, RE, and PL (Table 3) from day 1 to day 15 of storage (P < 0.05), probably due to the activity

of LAB (Table 5), producing lactic acid through carbohydrate metabolism [14], thus reducing the pH values. Although all treatments presented LAB growth (Table 5), a reduction in pH values was observed for NC, RE, and PL, probably due to other chemical reactions occurring in the fresh sausages during storage rather than the development of LAB [15].

An increase in pH values was observed for the treatments RE and PL starting from day 30 and the treatment NC from day 35 ($P < 0.05$). This rise is likely due to decarboxylation and deamination reactions occurring in amino acids, which release ammonia into the environment, thereby increasing its alkalinity, as well as the presence of alkaline compounds from protein decomposition or metabolites resulting from the activity of spoilage microorganisms [16].

Table 3. pH values during refrigerated storage and parameters (± standard errors) obtained from the two-phase model fitted to the pH data.

	Time (days)				
Treatment	**1**	**15**	**30**	**35**	**40**
NC	5.65 ± 0.03^{aAB}	5.32 ± 0.25^{bA}	5.23 ± 0.06^{bC}	5.34 ± 0,03^{bDE}	5.54 ± 0.04^{aBC}
PC	5.62 ± 0.08^{aAB}	5.48 ± 0.24^{aA}	5.39 ± 0.11^{aBC}	5.47 ± 0,14^{aBCDE}	5.65 ± 0.19^{aBC}
CA	5.29 ± 0.12^{aB}	5.38 ± 0.17^{aA}	5.13 ± 0.08^{aC}	5.18 ± 0,08^{aE}	5.40 ± 0.04^{aC}
RE	5.55 ± 0.02^{bAB}	5.21 ± 0.01^{dA}	5.34 ± 0.01^{cBC}	5.37 ± 0,02^{cCDE}	5.61 ± 0.03^{aBC}
AA	5.78 ± 0.26^{aAB}	5.36 ± 0.11^{aA}	5.49 ± 0.19^{aBC}	5.69 ± 0.15^{aABCD}	5.76 ± 0.14^{aABC}
SL	5.85 ± 0.28^{aAB}	5.46 ± 0.11^{aA}	5.64 ± 0.17^{aAB}	5.79 ± 0,14^{aAB}	5.91 ± 0.11^{aAB}
TL96	5.83 ± 0.31^{aAB}	5.39 ± 0.05^{aA}	5.49 ± 0.20^{aBC}	5.64 ± 0.20^{aABCD}	5.77 ± 0.15^{aABC}
PL	6.04 ≤ 0.01^{aA}	5.44 ± 0.03^{cA}	5.90 ± 0.03^{bA}	5.89 ± 0,03^{bA}	6.07 ± 0.06^{aA}

	Mathematical Modeling			
Treatment	$\mathbf{pH_0}$	$\mathbf{k_1(d^{-1})}$	$\mathbf{k_2(d^{-1})}$	**RMSE**
NC	5.658 (0.027)	0.015 (0.001)	0.032 (0.004)	0.0180
PC	5.621 (0.030)	0.008 (0.001)	0.027 (0.004)	0.0198
CA	5.361 (0.110)	0.007 (0.005)	0.021 (0.016)	0.0738
RE	5.578 (0.066)	0.025 (0.006)	0.025 (0.005)	0.0394
AA	5.808 (0.041)	0.029 (0.003)	0.028 (0.003)	0.0243
SL	5.874 (0.018)	0.027 (0.002)	0.029 (0.003)	0.0108
TL96	5.863 (0.008)	0.032 (0.001)	0.027 (0.001)	0.0050
PL	6.082 (0.073)	0.042 (0.007)	0.024 (0.004)	0.0430

Mean ± standard deviation. Averages in the same column followed by the same uppercase letter are not significantly different by the Tukey test ($P > 0.05$) for the different treatments at the same time. Averages in the same row followed by the same lowercase letter do not differ significantly by Tukey's test ($P > 0.05$) for the same treatment at the different times. Treatments: NC: Negative Control; PC: Positive Control; CA: Citric Acid; RE: Rosemary Extract; AA: Ascorbic Acid; SL: Sodium Lactate; TL96: TARI L96 Forte; PL: Potassium Lactate.

Except for day 15 ($P > 0.05$), the treatment CA showed a significant difference from PL ($P < 0.05$), resulting in lower pH values. This result may be due to the presence of citric acid in this treatment, which originates from three carboxyl groups that can lose a proton in solutions, thus forming a citrate ion and reducing the pH of the medium [17].

The results of the lipid oxidation analysis (Table 4) indicated a gradual increase in TBARS values, except for the treatments PC, RE, SL, and PL ($P > 0.05$), throughout the 40 days of refrigerated storage. The treatments NC, CA, and AA showed an increase in TBARS values from day 30 ($P < 0.05$), with no significant changes until day 40 ($P > 0.05$). The treatment TL96 exhibited an increase in TBARS values from day 35 ($P < 0.05$).

Table 4. TBARS values (mg MDA.kg-1 of sample) of the sausages during refrigerated storage and results and model parameters (± standard errors) obtained from the Baranyi-Roberts model with adaptation phase.

	Time (days)				
Treatment	1	15	30	35	40
NC	0.61 ± 0.10^{bAB}	$1.03 \pm 0.38b^{ABC}$	2.21 ± 0.38^{aA}	2.44 ± 0.04^{aAB}	2.53 ± 0.09^{aAB}
PC	0.54 ± 0.13^{aAB}	0.65 ± 0.02^{aBC}	0.83 ± 0.15^{aB}	0.80 ± 0.28^{aCD}	0.93 ± 0.16^{aCD}
CA	0.53 ± 0.10^{bAB}	0.75 ± 0.01^{bBC}	2.16 ± 0.96^{abA}	2.80 ± 0.84^{aA}	2.83 ± 0.85^{aA}
RE	0.40 ± 0.01^{aB}	0.42 ± 0.05^{aC}	0.51 ± 0.11^{aB}	0.47 ± 0.09^{aD}	0.47 ± 0.06^{aD}
AA	0.68 ± 0.08^{bAB}	$0.86 \pm 0.01b^{ABC}$	1.27 ± 0.04^{aAB}	1.42 ± 0.05^{aBCD}	1.45 ± 0.14^{aBCD}
SL	0.77 ± 0.26^{aAB}	1.36 ± 0.34^{aAB}	1.64 ± 0.62^{aAB}	1.67 ± 0.54^{aABC}	1.73 ± 0.59^{aABC}
TL96	1.09 ± 0.33^{bA}	1.74 ± 0.26^{abA}	2.12 ± 0.39^{abA}	2.30 ± 0.44^{aAB}	2.44 ± 0.64^{aAB}
PL	0.43 ± 0.07^{aAB}	$0.49 \leq 0.01^{aBC}$	0.59 ± 0.16^{aB}	0.70 ± 0.04^{aCD}	0.70 ± 0.24^{aCD}

	Mathematical Modeling				
Treatment	λ (d)	μ_{max} (mg MDA/kg/d)	$TBARS_0$ (mg MDA/kg)	$TBARS_m$ (mg MDA/kg)	RMSE (mg MDA/kg)
NC	20.29 (9.47)	0.12 (0.15)	0.43 (0.05)	0.80 (0.32)	0.0196
PC	22.29 (52.71)	0.03 (0.05)	0.53 (0.07)	8.07 (1.32E7)	0.0290
CA	17.64 (4.26)	0.33 (0.10)	0.56 (0.15)	2.95 (0.21)	0.0685
RE	15.82 (1.21E6)	1.29 (1.89E6)	0.40 (0.03)	0.48 (0.02)	0.0146
AA	17.16 (2.15)	0.15 (0.03)	0.68 (0.03)	1.54 (0.08)	0.0148
SL	-17.52 (25.82)	0.07 (0.04)	0.69 (0.03)	1.84 (0.14)	0.0078
TL96	-22.47 (21.76)	0.05 (0.02)	1.02 (0.07)	10.25 (1.20E7)	0.0228
PL	26.29 (9.47)	0.12 (0.15)	0.43 (0.05)	0.80 (0.32)	0.0196

Mean ± standard deviation. Averages in the same column followed by the same uppercase letter are not significantly different by the Tukey test ($P > 0.05$) for the different treatments at the same time. Averages in the same row followed by the same lowercase letter do not differ significantly by Tukey's test ($P > 0.05$) for the same treatment at the different times. Treatments: NC: Negative Control; PC: Positive Control; CA: Citric Acid; RE: Rosemary Extract; AA: Ascorbic Acid; SL: Sodium Lactate; TL96: Tari L96 Forte; PL: Potassium Lactate.

During refrigerated storage (0 to 4 °C), lipid and protein oxidation occurs due to the presence of unsaturated fatty acids and a high concentration of proteins, and exposure to light [18], which triggers the formation of aldehydes and ketones. Aldehydes are closely associated with the deterioration of meat color and flavor, as well as the loss of protein stability and functionality [19]. The amount of malondialdehyde formed during the process (Table 4) resulted in TBARS values exceeding 2.0 mg MDA per kg for the treatments NC, CA (day 30), and TL96 (day 35), which is recognized as the sensory threshold beyond which lipid oxidation becomes perceptible [14].

The absence of sodium erythorbate in NC altered its oxidative stability, resulting in a continuous increase in lipid oxidation throughout storage. This behavior is attributed to the antioxidant role of sodium erythorbate, which acts by quenching singlet oxygen, donating hydrogen atoms, and serving as a reducing agent, thereby delaying lipid oxidation [20]. In turn, the treatment PC containing sodium erythorbate showed lower TBARS values during storage when compared to NC.

Antioxidants are classified based on their mechanism of action and, consequently, exhibit different inhibitory functions depending on the food matrix [20]. Although Fell *et al.* [21] reported positive effects of citric acid in reducing lipid oxidation in sausage formulations, the present results (Table 4) indicate that the combination of citric acid in the treatments CA and TL96, along with sodium erythorbate did not produce a synergistic effect in slowing the lipid oxidation in fresh sausages.

Over the 40 days of storage, the treatment RE exhibited the lowest increase in lipid oxidation (Table 4) when compared to all treatments. Schilling *et al.* [22] and Sun *et al.* [23] also reported promising reductions in TBARS values with the application of rosemary extract in fresh pork and chicken sausages, respectively. In general, the antioxidant potential of plant extracts is largely attributed to their phenolic compound content [24].

3.3 Microbiology characterization

An increase in the lactic acid bacteria (LAB) counts (Table 5) of the fresh sausages was observed for all treatments between days 1 and 15 of storage ($P < 0.05$), followed by stabilization until day 40 ($P > 0.05$), except for PC, which exhibited significant differences throughout the entire storage period ($P < 0.05$). As LAB are part of the natural microflora of fresh sausages, they were already well adapted to the product's environment.

On day 1 after manufacture, a difference was observed in the LAB counts between the treatments CA and SL ($P < 0.05$). Such differences in the initial native bacterial flora are common in meat products [25]. By day 15 of storage, no significant differences were observed between treatments ($P > 0.05$). On day 30, NC exhibited the lowest lactic acid bacteria (LAB) counts, while the treatments RE and AA showed significantly higher values ($P < 0.05$). On day 35, lower counts were detected in PL whereas AA and SL presented the highest counts. By day 40, PC has the lowest and CA has the highest LAB counts ($P < 0.05$). Although the treatment NC did not contain sodium erythorbate, its LAB counts remained stable and comparable to those of the treatment PC. This result is probably due to the presence of nitrite and nitrate in both formulations, which acts as antimicrobial agents by inhibiting or delaying microbial growth [26].

Table 5. Lactic acid bacteria, mesophilic bacteria, and psychrotrophic bacteria counts in fresh sausages during refrigerated storage.

Treatment	Time (days)				
	1	15	30	35	40
	Lactic Acid Bacteria (log CFU.g^{-1})				
NC	3.8 ± 0.2^{cAB}	6.9 ± 0.5^{bA}	7.0 ± 0.1^{abF}	7.7 ± 0.1^{aAB}	7.6 ± 0.1^{aBCD}
PC	3.6 ± 0.6^{cAB}	6.7 ± 0.3^{bA}	7.5 < 0.0^{abCDE}	7.7 < 0.0^{aAB}	7.2 ± 0.1^{abD}
CA	4.1 ± 0.3^{bA}	7.2 ± 0.9^{aA}	7.2 ± 0.1^{aEF}	7.2 ± 0.2^{aAB}	7.8 ± 0.1^{aAB}
RE	3.5 ± 0.4^{bAB}	7.3 < 0.0^{aA}	7.9 ± 0.2^{aABC}	7.3 ± 0.6^{aAB}	7.6 ± 0.1^{aBCD}
AA	3.5 ± 0.1^{bAB}	7.5 ± 0.2^{aA}	7.8 ± 0.2^{aABC}	7.8 ± 0.2^{aA}	7.6 ± 0.4^{aBCD}
SL	2.8 ± 0.8^{bB}	7.3 ± 0.2^{aA}	7.3 ± 0.2^{aDEF}	7.9 ± 0.17^{aA}	7.6 ± 0.2^{aBCD}
TL96	3.7 ± 0.1^{cAB}	6.9 ± 0.2^{bA}	7.7< 0.0^{aBCD}	7.8 ± 0.2^{aAB}	7.6 ± 0.1^{aBC}
PL	4.0 ± 0.1^{bAB}	7.0 ± 0.4^{aA}	7.3 ± 0.1^{aDEF}	6.9 ± 0.7^{aB}	7.3 ± 0.2^{aCD}
	Mesophilic Bacteria (log CFU.g^{-1})				
NC	4.2 ± 0.4^{bA}	7.0 ± 0.2^{aAB}	7.3 ± 0.2^{aBC}	7.5 ± 0.3^{aBC}	7.6 ± 0.2^{aBC}
PC	4.4 ± 0.3^{dA}	6.8 ± 0.1^{cAB}	7.0 ± 0.2^{bcC}	7.5 < 0.0^{abBC}	7.6 ± 0.2^{aBC}
CA	4.0 ± 0.3^{bA}	6.9 ± 0.8^{aAB}	7.4 ± 0.5^{aBC}	6.9 ± 0.3^{aC}	7.7 ± 0.2^{aABC}
RE	4.0 ± 0.2^{cA}	7.2 ± 0.1^{abA}	7.3 ± 0.1^{abBC}	7.1 ± 0.4^{bC}	7.8 < 0.0^{aABC}
AA	4.1 ± 0.2^{cA}	7.3 < 0.0^{bA}	7.7 ± 0.2^{abAB}	8.0 ± 0.2^{aAB}	8.0 ± 0.3^{aAB}
SL	4.0 ± 0.4^{cA}	5.9 ± 0.2^{bBC}	7.,8 ± 0.1^{aAB}	8.1 < 0.0^{aAB}	7.8 ± 0.1^{aABC}
TL96	3.8 ± 0.3^{bA}	5.0 ± 1.0^{bC}	7.8 ± 0.1^{aAB}	7.9 ± 0.3^{aAB}	8.0 ± 0.1^{aAB}
PL	3.9 ± 0.1^{bA}	7.1 ± 0.2^{aAB}	6.9 ± 0.1^{aC}	7.1 ± 0.3^{aC}	7.4 ± 0.2^{aC}
	Psychrotrophic Bacteria (log CFU.g^{-1})				
NC	6.3 < 0.0^{bAB}	7.8 ± 0.3^{aA}	8.0 ± 0.2^{aBC}	8.1 ± 0.1^{aC}	8.1 < 0.0^{aA}
PC	6.4 < 0.0^{cA}	8.1 < 0.0^{aA}	8.2 < 0.0^{aABC}	8.1 ± 0.1^{aC}	8.1 < 0.0^{aA}
CA	6.4 ± 0.5^{bA}	7.8 ± 0.4^{aA}	7.8 ± 0.3^{aC}	8.2 < 0.0^{aC}	8.1 < 0.0^{aA}
RE	6.3 ± 0.1^{cAB}	8.2 < 0.0^{bA}	8.1 ± 0.1^{bABC}	8.1 < 0.0^{bC}	8.4 ± 0.1^{aA}
AA	5.8 ± 0.3^{bABC}	8.4 ± 0.3^{aA}	8.3 < 0.0^{aAB}	8.7 < 0.0^{aA}	8.4 ± 0.2^{aA}
SL	5.6 ± 0.1^{bC}	8.2 ± 0.3^{aA}	8.3 ± 0.1^{aAB}	8.6 ± 0.2^{aAB}	8.6 ± 0.6^{aA}
TL96	5.6 ± 0.2^{bBC}	8.2 ± 0.5^{aA}	8.2 ± 0.1^{aABC}	8.4 ± 0.2^{aABC}	8.1 ± 0.1^{aA}
PL	4.7 ± 0.1^{bD}	8.6 ± 0.2^{aA}	8.4 ± 0.1^{aAB}	8.3 ± 0.2^{aBC}	8.3 ± 0.2^{aA}

Mean ± standard deviation. Averages in the same column followed by the same uppercase letter are not significantly different by the Tukey test (P > 0.05) for the different treatments at the same time. Averages in the same row followed by the same lowercase letter do not differ significantly by Tukey's test (P > 0.05) for the same treatment at the different times. Treatments: NC: Negative Control; PC: Positive Control; CA: Citric Acid; RE: Rosemary Extract; AA: Ascorbic Acid; SL: Sodium Lactate; TL96: TARI L96 Forte; PL: Potassium Lactate.

Lactic acid bacteria (LAB) make up a substantial part of the natural microbiota of meat products. However, meat products with counts higher than 7 log CFU.g^{-1} are unsuitable for consumption [27], causing sensory changes, such as slime formation and unpleasant flavors. Thus, considering this microbiological threshold for lactic acid bacteria (LAB) counts, all treatments would become unsuitable for consumption starting from day 15 of storage.

Regarding mesophilic bacterial counts (Table 5), all treatments exhibited a significant increase in cell counts over time (P <0.05), followed by a stabilization phase. For treatments NC, CA, and PL, stabilization occurred from day 15 (P >0.05); for AA, SL, and TL96, from day 30 (P >0.05); and for PC, from day 35 (P >0.05). The stabilization of mesophilic counts after day 15 for the treatments NC, CA, and PL, and later in the remaining treatments, may indicate that the microbiota reached a stage where the environment became less favorable for continued growth, possibly due to nutrient depletion, pH reduction, or the accumulation of inhibitory metabolites produced by LAB [28]. This finding is relevant as it suggests that

the risk of exponential proliferation of spoilage microorganisms can be mitigated. The multiplication of mesophilic microorganisms is expected during the storage of meat products [14]. According to the requirements of Normative Instruction 161 [29], the maximum acceptable limit for aerobic mesophilic microorganisms in fresh sausage is 6 log CFU.g^{-1}. Values above this limit are indicative of microbial deterioration and may compromise the safety and quality of the product, with visible deteriorative changes on the sausage surface [30].

On day 1, no differences were observed for the mesophilic bacteria counts between treatments ($P > 0.05$). By day 15, TL96 presented the lowest counts whereas RE and AA exhibited the highest counts ($P < 0.05$). On day 30, lower counts were observed for PC and PL, while AA, SL, and TL96 presented higher counts ($P < 0.05$). On day 35, CA, RE, and PL exhibited lower counts whereas AA, SL, and TL96 maintained significant higher counts ($P < 0.05$). Finally, on day 40 of storage, PL presented the lowest mesophilic bacteria counts, while AA and TL96 had the highest counts ($P < 0.05$). Overall, the treatment PL exhibited lower mesophilic bacteria counts on days 30, 35, and 40 of storage, suggesting a suppressive effect on microbial growth. This antimicrobial action is likely due to the presence of lactic acid salts, particularly the lactate ion, which can penetrate microbial cell membranes and disrupt intracellular pH homeostasis, thereby inhibiting growth or inducing cell death [31]. The beneficial effect of the lactate ion was also reflected in reduced LAB counts on day 1 and 35 in treatments containing sodium lactate (SL) and potassium lactate (PL), respectively, as well as lower psychrotrophic bacteria counts on day 1 in PL.

High mesophilic bacteria counts may indicate the onset of product deterioration. However, in the presence of a significant population of lactic acid bacteria (LAB), such growth should be interpreted with caution. Although LAB are mesophilic, they are not necessarily linked to immediate sensory spoilage and may even exert a bio preservative effect [32]. Thus, high mesophilic counts may reflect LAB proliferation, which reduces the direct association with spoilage risk. The concomitant growth of LAB and other mesophilic microorganisms may compromise product quality, especially if undesirable metabolites are produced or if sensory changes occur - such as acidic or bitter odors and flavors. Moreover, even with high LAB counts, the absence of proper microbial control may allow the simultaneous growth of specific spoilage or pathogenic microorganisms, depending on environmental conditions.

Psychrotrophic bacteria constitute an important part of the microbial population in vacuum-packed meat products, and higher psychrotrophic bacteria counts can cause adverse sensory changes, such as acidic and undesirable aromas, and spoilage in refrigerated foods. All treatments showed an increase in psychrotrophic bacteria counts (Table 5) from days 1 to 15 of storage ($P < 0.05$), followed by stabilization until day 40 ($P > 0.05$), except for the treatment RE, which showed an increase in psychrotrophic bacteria counts on day 40 ($P < 0.05$). Schilling *et al.* [22] evaluated the effect of rosemary and green tea extracts on fresh pork sausage and found psychrotrophic bacteria counts above 7 log CFU.g^{-1} after 14 days of storage, with an increasing trend at the end of the storage period (21 days), with counts exceeding 8 log CFU.g^{-1}.

On the first day of storage, higher psychrotrophic bacteria counts were found in the treatments PC and CA, while PL exhibited the lowest counts ($P < 0.05$). On day 15 of storage, no significant differences were observed between the treatments ($P > 0.05$). By day 30, CA showed the lowest counts while AA, SL, and PL presented the highest counts ($P < 0.05$). By day 35, the treatments NC, PC, CA, and RE presented

the lowest counts while AA maintained the highest counts ($P < 0.05$). Finally, on day 40, no significant differences were observed among treatments ($P > 0.05$), with all samples presenting counts exceeding 8 log CFU.g^{-1}.

The antimicrobial activity of citric acid is attributed not only to its acidifying action since the reduction in pH increases the concentration of undissociated form of the acid, thereby reducing its polarity and enhancing its diffusion across cell membranes and into the cytoplasm [33] but also to its metal-chelating properties, particularly the sequestration of divalent cations such as Ca^{+2} [33].

It is well established that most foods may reach microbial counts above 10^6 CFU.g^{-1} once the microbial biomass becomes sufficient to cause perceptive spoilage for consumers [34], indicating that concerning bacteria growth, vacuum-packed fresh sausages may exhibit detectable sensory changes after 30 days of refrigerated storage.

Regarding the detection of *Salmonella spp.*, *Escherichia coli*, and coagulase-positive staphylococci, all fresh sausages met the standards of Normative Instruction 60 [10] which requires absence of Salmonella spp, counts below 10^{-2} log CFU.g^{-1} for E. coli and below 10 log CFU.g^{-1} for coagulase-positive staphylococci. Therefore, all samples of this study were considered microbiologically safe and suitable for sensory evaluation. It should be noted that the sensory evaluation was carried out in the first three days of sample production, a period in which the counts of mesophilic bacteria, lactic acid bacteria and psychrotrophic microorganisms remained below the limit of 6 log CFU.g^{-1}, ensuring the suitability and safety of the product for sensory tests, without representing a risk to the evaluators or compromising the acceptability of the product.

3.4 Mathematical modeling

The results of the mathematical modeling (Table 6) showed that lower maximum specific growth rates (μ_{max}) were associated with slower increases in LAB counts, indicating extended shelf life for fresh sausages. Thus, the treatments NC, PC, and TL96 exhibited the lowest growth rates (0.53) while SL, AA, and RE had higher values (0.80, 0.71, and 0.66, respectively). It is noteworthy that concerning the LAB (Table 5), the treatments NC and PC also presented lower counts on days 30 and 35 of storage, respectively.

Concerning the mesophilic microorganisms (Table 6), lower growth rates were obtained for the treatments TL96, SL, and PC (0.32, 0.33, and 0.42, respectively) while PL, RE, and AA (0.65, 0.62, and 0.54, respectively) had the higher mesophilic counts. Finally, lower counts were observed for TL96 on day 15 of storage, when compared to the other treatments.

The mathematical modeling of lactic acid bacteria and mesophilic bacterial growth showed that the treatment TL96 presented the lowest growth rates for both microbial groups. This result is likely due to the combined effect of the organic acids used in the treatment. Acetic acid and lactic acid, which make up the TL96 blend, are known to exert a synergistic antimicrobial effect. This synergy is enhanced by the high pKa value (dissociation constant) of acetic acid [33] as antimicrobial activity tends to increase when the food's pH approaches or fails below the acid's pKa, leading to the accumulation of the acid anion, which plays a key role in cellular inhibition.

Therefore, the efficacy of the treatment TL96 in inhibiting bacterial proliferation may be enhanced by the synergistic action of acetic and lactic acids, especially under low pH conditions that potentiate their antimicrobial effects. Although the experimental data did not show a significant reduction ($P > 0.05$) in microbial growth for the TL96 treatment, especially for mesophilic bacteria (Table 5), its selection for sensory analysis was justified by its favorable performance in mathematical modeling, which indicated a lower growth rate.

Regarding the psychrotrophic bacteria (Table 6), lower growth rates were observed for CA, NC, and RE (0.28, 0.30, and 0.45, respectively) while higher counts were observed for PL, TL96, and AA (0.77, 0.55, and 0.55, respectively). It is noteworthy that the treatment CA achieved the lower psychrotrophic bacteria counts on day 30, along with the treatment NC, on day 35 (Table 5).

Table 6. Counts of lactic acid bacteria, mesophilic bacteria, and psychrotrophic bacteria (± standard errors) in fresh sausages obtained from fitting the Baranyi-Roberts model without an adaptation phase.

Treatment	Mathematical Modeling			
	μ_{max} (log CFU/d)	log N_0 (log CFU)	log N_{max} (log CFU)	RMSE (log CFU)
	Lactic Acid Bacteria			
NC	0.53 (0.11)	3.56 (0.40)	7.44 (0.21)	0.2349
PC	0.53 (0.07)	3.37 (0.28)	7.46 (0.15)	0.1636
CA	0.57 (0.17)	3.84 (0.40)	7.39 (0.21)	0.2338
RE	0.66 (0.10)	3.24 (0.29)	7.61 (0.15)	0.1679
AA	0.71 (0.05)	3.15 (0.14)	7.73 (0.07)	0.0806
SL	0.80 (0.12)	2.48 (0.31)	7.60 (0.16)	0.1789
TL96	0.53 (0.02)	3.45 (0.07)	7.68 (0.04)	0.0384
PL	0.58 (0.15)	3.74 (0.27)	7.17 (0.14)	0.1532
	Mesophilic Bacteria			
NC	0.50 (0.03)	3.97 (0.12)	7.50 (0.06)	0.0698
PC	0.42 (0.09)	4.20 (0.35)	7.37 (0.19)	0.2061
CA	0.49 (0.12)	3.83 (0.42)	7.34 (0.23)	0.2469
RE	0.62 (0.19)	3.72 (0.35)	7.40 (0.18)	0.2017
AA	0.54 (0.05)	3.85 (0.19)	7.91 (0.10)	0.1132
SL	0.33 (0.04)	3.80 (0.20)	8.01 (0.16)	0.1266
TL96	0.32 (0.06)	3.44 (0.47)	8.13 (0.56)	0.3076
PL	0.65 (0.32)	3.65 (0.31)	7.13 (0.15)	0.1636
	Psychrotrophic Bacteria			
NC	0.30 (0.02)	6.16 (0.07)	8.07 (0.04)	0.0384
PC	0.54 (0.31)	6.15 (0.14)	8.14 (0.03)	0.0312
CA	0.28 (0.07)	6.28 (0.19)	8.05 (0.10)	0.1118
RE	0.45 (0.21)	6.12 (0.18)	8.21 (0.09)	0.0936
AA	0.54 (0.20)	5.54 (0.24)	8.45 (0.12)	0.1342
SL	0.50 (0.07)	5.33 (0.17)	8.47 (0.09)	0.0968
TL96	0.55 (0.17)	5.40 (0.17)	8.23 (0.08)	0.0886
PL	0.77 (0.74)	4.41 (0.69)	8.65 (0.33)	0.3583

Treatments: NC: Negative Control; PC: Positive Control; CA: Citric Acid; RE: Rosemary Extract; AA: Ascorbic Acid; SL: Sodium Lactate; TL96: TARI L96 Forte; PL: Potassium Lactate.

In general, although the addition of different ingredients had no significant effect on the final population of LAB, mesophilic, and psychrotrophic bacteria, a reduction in microbial growth rate was observed, mainly in the treatment TL96. The growth rate indicates the speed at which the microbial population increases over time (days), and lower rates are associated with slower development during both the lag and exponential phases. The two phases are of particular interest in food microbiology, as spoilage typically occurs before microorganisms reach the stationary phase [35].

Regarding lipid oxidation (Table 4), higher λ values indicate a longer adaptation phase in TBARS progression, corresponding to the time required for the onset of lipid oxidation, analogous to the *lag* phase in microbial growth. The treatment PL exhibited the highest λ value, suggesting a delayed onset of the oxidation process, and, consequently, a slower rate of lipid oxidation in fresh sausages. In contrast, lower λ values found in TL96 indicated an earlier initiation of lipid oxidation when compared to the other formulations.

As shown in Table 4, lower maximum growth rates (μ_{max}) were associated with a slower increase in TBARS values. The lower rates were obtained for the treatments PC and TL96 (0.03 and 0.05, respectively), suggesting that these ingredients contributed to delaying lipid oxidation, extending the shelf life of fresh sausages. In contrast, higher μ_{max} values were observed for RE and CA (1.29 and 0.33, respectively), indicating a faster progression of lipid oxidation. The use of sodium erythorbate alone (PC) and in combination with organic acids (TL96) proved effective in mitigating oxidative processes. This effect is attributed to the antioxidant capacity of organic acids, which chelate pro-oxidant metal ions such as iron and copper. Moreover, citric acid present in TL96 may have enhanced oxidative stability by acidifying the environment and stabilizing both the primary antioxidant agents and fat globules through synergistic action [36].

The statistical indices obtained after model fitting revealed high standard deviations, indicating a poor fit for certain datasets, such as the rosemary extract treatment, which showed deviations of 1.21 x 10^6 (λ = 15.82) and 1.89 x 10^6 (μ_{max} = 1.29). Despite the limited fir of the mathematical model to the experimental data, the TBARS values in the treatment RE (Table 4) showed that the combination of rosemary extracts and sodium erythorbate led to a smaller increase in lipid oxidation in fresh sausages.

The rate constants k_1 and k_2 (Table 3) represent the rates of pH decrease and increase, respectively, over the storage period (in days), with a pH reduction during storage with a subsequent increase (see Section 3.2). Lower values of these constants correspond to slower pH changes. Regarding the different treatments (Table 3), the treatment CA had the lowest k_1 and k_2 values, indicating a slower acidification followed by a more gradual pH recovery over time.

Monteiro *et al.* [37] reported that regular pH values for meat products and sausages should not be lower than 5.5 to prevent undesirable flavors, discoloration, gas production, package swelling, and greenish coloring. In turn, high pH values reflect the degree of sample deterioration, due to the increase in the microbial population that produce ammonia, which raises the pH of the environment. However, most authors consider the population of lactic acid bacteria as a more reliable indicator of the shelf life of fresh sausages rather than pH variations.

The results of the mathematical modeling of microbial growth, TBARS, and pH indicated that the treatment TL96 was the most promising in extending the shelf life of fresh sausages.

3.5 Sensory evaluation of the fresh sausages

The results of the physicochemical characterization, microbiological analyses, and mathematical modeling allowed the selection of the treatments with the best performances for the sensory analysis. The treatments RE and PL demonstrated a promising effect in reducing TBARS values throughout the storage period. The microbiological analysis did not reveal significant differences between treatments, limiting the ability to identify superior microbiological performance based solely on empirical data. Therefore, mathematical modeling was employed as a complementary tool to support treatment selection. The model indicated that TL96 presented lower microbial growth rates over time, suggesting a more favorable inhibitory effect. Based on these findings, the treatments RE, PL and TL96 were selected for sensory evaluation in comparison to the control treatment (PC).

Although no significant differences were observed for the attributes color, appearance, aroma, and texture of the samples (Table 7) ($P > 0.05$), lower scores (< 7.00) were obtained for color and appearance in all treatments. These scores correspond to panelist perceptions such as " lacking characteristic color" and "appearance could be improved". This result was probably due to the sample preparation procedure, as the use of aluminum foil packaging may have caused a cooked-like appearance, resulting in diminished color intensity and visual appearance (instrumental color parameters data not shown).

Tabela 7. Sensory attributes and purchase intention of fresh sausages.

	Treatments			
Attributes	PC	RE	TL96	PL
Color	5.92 ± 1.52^{A}	6.13 ± 1.67^{A}	6.52 ± 1.60^{A}	6.45 ± 1.47^{A}
Appearance	6.40 ± 1.52^{A}	6.45 ± 1.62^{A}	6.87 ± 1.44^{A}	6.75 ± 1.26^{A}
Aroma	6.45 ± 1.80^{A}	6.72 ± 1.84^{A}	6.68 ± 1.65^{A}	7.02 ± 1.51^{A}
Flavor	6.97 ± 1.81^{B}	6.98 ± 1.83^{B}	7.15 ± 1.66^{AB}	7.77 ± 0.93^{A}
Texture	7.23 ± 1.42^{A}	7.02 ± 1.73^{A}	7.30 ± 1.49^{A}	7.28 ± 1.34^{A}
Overall impression	6.85 ± 1.63^{A}	6.77 ± 1.79^{A}	6.85 ± 1.74^{A}	7.13 ± 1.47^{A}
Purchase intention	3.37 ± 1.63^{A}	3.50 ± 1.26^{A}	3.67 ± 1.16^{A}	3.85 ± 0.90^{A}

Mean ± standard deviation. Averages in the same row followed by the same uppercase letter are not significantly different by the Tukey test ($P > 0.05$) for the different treatments. Treatments: PC: Positive Control; RE: Rosemary Extract; TL96: TARI L96 Forte; PL: Potassium Lactate.

In addition, no significant differences were observed in overall impression and purchase intention between treatments ($P > 0.05$) (Table 7). Therefore, the addition of rosemary extract (RE), the blend of organic acids (TL96), and potassium lactate (PL) did not alter the sensory characteristics of fresh sausages when compared to the control treatment made with sodium erythorbate (PC).

4. Conclusion

The treatments sodium erythorbate and TARI L 96 Forte showed the slowest increase in thiobarbituric acid reactive substances values, as indicated by the mathematical modeling, suggesting that the use of both potassium lactate and the blend of organic acids as technological ingredients has a positive effect on inhibiting and/or reducing lipid oxidation in fresh sausages. Regarding microbial growth, none of the treatments were effective in reducing the lactic acid bacteria, mesophilic bacteria, and psychrotrophic bacteria counts, indicating that the technological ingredients used in this study, sat the concentrations tested, did not inhibit bacterial growth. In the sensory evaluation, the treatments with sodium erythorbate, rosemary extract, TARI L 96 Forte, and potassium lactate showed no significant differences, except for the attribute flavor with the highest scores for the treatment with potassium

lactate. Attributes such as color, appearance, aroma, texture, overall impression and purchase intention did not differ significantly among treatments. Despite their limited effect on microbial inhibition, the use of the organic acid blend (TL96) and potassium lactate (PL) can be a promising approach for maintaining the safety and quality of fresh sausages, as they delayed lipid oxidation and stabilized pH without negatively impacting the sensory attributes, thus they can be used as technological ingredients in the meat industry. For future studies, it is recommended to reduce the storage period and perform physicochemical and microbiological evaluations at shorter intervals to better access the behavior of these ingredients during the early stages of storage of fresh sausages.

Conflicts of interest statement

The authors declare no conflict of interest.

Data availability statement

The data will be provided upon request.

Author contributions

Conceptualization: A. M. P Amaral, G. A. R. Sehn and D. Cavalheiro. Investigation: A. M. P Amaral, E. M. Amorim, E. G. S. Carmo, J. V. P. Santos, M. T. Canova and P. A. Dalan. Methodology: A. M. P Amaral, L. S. Moroni, G. A. R. Sehn, D. Cavalheiro. Formal analysis: A. M. P Amaral, E. M. Amorim, E. G. S. Carmo, J. V. P. Santos, M. T. Canova and P. A. Dalan. Data curation: A. M. P Amaral and W. S. Robazza. Validation: A. M. P Amaral, E. M. Amorim; E. G. S. Carmo; J. V. P. Santos; M. T. Canova and P. A. Dalan. Supervision: G. A. R. Sehn and D. Cavalheiro. Writing – original draft: A. M. P Amaral, G. A. R. Sehn and D. Cavalheiro. Writing – review & editing: A. M. P Amaral, L. Bettanin, G. A. R. Sehn and D. Cavalheiro.

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