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***Lacticaseibacillus paracasei* and mixed extract of rosemary and green tea:
a green approach to replace synthetic antioxidants
and confer healthy properties to dry-fermented sausages**

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***Lacticaseibacillus paracasei* e extrato misto de alecrim e chá verde:
uma abordagem sustentável para substituir antioxidantes sintéticos
e conferir propriedades saudáveis a salames fermentados**



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ABSTRACT: The primary objective was to assess whether applying *Lacticaseibacillus paracasei* alone or combined with a mixed extract of rosemary and green tea could give dry-fermented sausages the same oxidative stability as synthetic antioxidants. Moreover, the study investigated whether the combination with the plant extract could enhance the viability of the probiotic culture during the storage of the dry-fermented sausages. The results indicated that the dry-fermented sausages containing *L. paracasei* exhibited lower pH values, providing beneficial implications for the product's microbiological safety. Regarding oxidative stability, the TBARS values of the samples treated with *L. paracasei* and the extract were similar to those with the synthetic antioxidant. Interestingly, the combination of *L. paracasei* with the extract increased the viability of the probiotic culture during storage at 4 °C, even though a gradual reduction was observed over 60 days. The coloration of the dry-fermented sausages was also not adversely affected. In conclusion, the combined approach presents a viable alternative to synthetic antioxidants, making it a promising innovation in dry-fermented sausage production. However, further studies are needed to evaluate other product attributes, such as sensory quality and the profile of bioactive compounds produced.

Key words: *Lacticaseibacillus paracasei*, probiotic cultures, natural antioxidants, lipid oxidation, fermented meat products, clean-label.

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RESUMO: O objetivo principal deste estudo foi avaliar se a aplicação de *Lacticaseibacillus paracasei*, isoladamente ou em combinação com um extrato misto de alecrim e chá verde, poderia conferir aos salames fermentados a mesma estabilidade oxidativa que antioxidantes sintéticos. Para isso, foi realizada uma investigação para verificar se a combinação com o extrato vegetal poderia aumentar a viabilidade da cultura probiótica durante o armazenamento dos salames fermentados. Os resultados indicaram que os salames contendo *L. paracasei* apresentaram menores valores de pH, o que pode contribuir para a segurança microbiológica do produto. Em relação à estabilidade oxidativa, os valores de TBARS das amostras tratadas com *L. paracasei* e com o extrato foram semelhantes aos observados para o antioxidante sintético. Além disso, a combinação de *L. paracasei* com o extrato aumentou a viabilidade da cultura probiótica durante o armazenamento a 4 °C, embora tenha sido observada redução gradual ao longo de 60 dias. A coloração dos salames fermentados não foi negativamente afetada. Conclui-se que a abordagem combinada representa uma alternativa viável aos antioxidantes sintéticos, configurando-se como uma estratégia promissora na produção de salames fermentados com apelo mais saudável. No entanto, estudos adicionais são necessários para avaliar outros atributos do produto, como qualidade sensorial e perfil de compostos bioativos formados.

Palavras-chave: *Lacticaseibacillus paracasei*, culturas probióticas, antioxidantes naturais, oxidação lipídica, produtos cárneos fermentados, clean label.

1 **INTRODUCTION**

2
3 The meat industry has recently prioritized
4 the development of products that go beyond basic

nutritional value, focusing on additional health
benefits. An emerging approach is incorporating
natural ingredients and probiotics into these product
formulations, reducing the need for synthetic additives

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that are increasingly questioned by consumers seeking clean-label foods (BARCENILLA et al., 2022; KAVEH et al., 2023; ZHANG et al., 2022). In dry-fermented sausages, this strategy is particularly relevant because lipid oxidation may occur during ripening and storage due to the combined effects of fat content, salt, dehydration, and prolonged exposure to processing conditions (HU et al., 2020). Although synthetic antioxidants such as sodium erythorbate are effective in delaying oxidation, their use does not meet the increasing consumer demand for products formulated with natural and multifunctional ingredients (MUNEKATA et al., 2020). Therefore, replacing or reducing the dependence on synthetic antioxidants remains a technological challenge, especially when oxidative stability, color preservation, microbial safety, and product functionality must be maintained simultaneously.

In this context, rosemary (*Rosmarinus officinalis* L.) and green tea (*Camellia sinensis*) emerge as promising natural ingredients, notably due to their recognized antioxidant effects (BORTOLINI et al., 2021; HU et al., 2022; SELANI et al., 2022). These effects are mainly associated with phenolic compounds, which can delay lipid oxidation through mechanisms such as free radical scavenging, hydrogen or electron donation, and chelation of pro-oxidant metal ions (ZEB, 2020). Thus, rosemary and green tea extracts may contribute to the oxidative stability of meat products by limiting the propagation of lipid oxidation reactions and reducing the formation of secondary oxidation compounds (TOPCHII et al., 2021). Additionally, probiotics, which provide health benefits when appropriately administered (MA et al., 2023; SIRINI et al., 2023) have been integrated into various foods, including meat products. However, in fermented meat matrices, the use of probiotics cannot be considered only from a nutritional perspective. These microorganisms must survive under adverse conditions such as low water activity, high salt concentration, presence of curing salts, competition with starter cultures, and storage under refrigeration, while also maintaining a potential technological role in fermentation and product stability (AGÜERO et al., 2020; PEREIRA et al., 2024). However, implementing these in the meat industry poses challenges, highlighting the need for additional studies in this field (KARWOWSKA et al., 2021).

Interestingly, some probiotic bacteria, such as *Lacticaseibacillus paracasei*, exhibit antioxidant properties, expanding the potential of their application in meat products (LI et al., 2012; MISHRA et al., 2015; RWUBUZIZI et al., 2023; VOUGIOUKLAKI

et al., 2023). The antioxidant contribution of probiotic cultures may be related to different mechanisms, including the activity of antioxidant enzymes, production of metabolites with reducing capacity, and release of bioactive peptides during protein degradation (ŁEPECKA et al., 2025; SINGH, 2025). In fermented sausages, these mechanisms could be relevant because microbial metabolism occurs in close association with lipid and protein oxidation reactions during ripening and storage. Nevertheless, the combined use of plant extracts and probiotic cultures in dry-fermented sausages remains insufficiently explored, particularly regarding whether phenolic-rich extracts can simultaneously contribute to lipid oxidation control and improve probiotic survival in this complex meat matrix.

Thus, this study investigated the potential of *L. paracasei* and a mixed extract of rosemary and green tea, used separately or in combination, as an alternative strategy to sodium erythorbate for controlling lipid oxidation in dry-fermented sausages. We hypothesized that both the probiotic culture and the phenolic-rich extract would reduce lipid oxidation to levels comparable to those obtained with the synthetic antioxidant, and that the extract could improve the survival of *L. paracasei* during refrigerated storage. Therefore, the effects of these ingredients on oxidative stability, physicochemical properties, instrumental color, and probiotic viability were evaluated during 60 days of storage at 4 °C.

MATERIALS AND METHODS

Natural antioxidant

Kemin of Brazil Ltda (São Paulo/SP, Brazil) provided the natural extract used in this study. The extract consisted of a mix 50:50 of rosemary and green tea (Fortium RGT WS 1200®). The content of total phenolic compounds was evaluated in triplicate using the method described by SINGLETON & ROSSI (1965) using Folin-Ciocalteu reagent (RFC) and gallic acid as the standard for calibration curves, obtaining a value of 44.06 mg of gallic acid equivalents (GAE)/mL.

Probiotic culture preparation

The probiotic culture of *Lacticaseibacillus paracasei* used in this study was obtained through a donation from LEMMA Supply Solutions Ltda (Jardinópolis/SP, Brazil). The lyophilized culture of *L. paracasei* was successively inoculated three times in Man, Rogosa, and Sharpe (MRS) broth (Biolog Inc., Hayward, California, USA) and incubated

at 37 °C for 18 h. The suspension was centrifuged twice at $1530 \times g$ for 5 min and diluted in 0.85% saline solution to reach a concentration equivalent to standard 4.0 on the McFarland scale, corresponding to an estimated concentration of approximately 1.2×10^9 cells mL⁻¹. This turbidity-based procedure was used to standardize the inoculum among experimental batches. The suspension was applied to the dry-fermented sausages on the same day.

Dry-fermented sausages manufacturing

The dry-fermented sausages were made using a proportion of 80% pork meat and 20% pork backfat. Five treatments were created, and each treatment was produced in triplicate. In the treatment named Control (-), the following additives and ingredients were added relative to the meat mass: salt (2.5%), glucose (0.5%), sucrose (0.5%), powdered garlic (0.2%), white pepper (0.2%), nutmeg (0.01%), sodium nitrate (0.015%), sodium nitrite (0.015%), and Bactoferm® T-SPX starter culture (0.15%) (Chr. Hansen, Hørsholm, Capital Region, Denmark) containing the microorganisms *Pediococcus pentosaceus* and *Staphylococcus xylosus*. In the other treatments, in addition to the additives and ingredients added in Control (-), 0.25% sodium erythorbate was also added in the Control (+) treatment; 2% of the *L. paracasei* suspension in the LP treatment; 1.5% mixed extract of rosemary and green tea in the R/GT treatment; and 1.5% mixed extract of rosemary and green tea + 2% of the *L. paracasei* suspension in the R/GT+LP treatment. The commercial starter culture was included in all treatments to reproduce a standard dry-fermented sausage manufacturing process and to ensure adequate fermentation, safety, and technological consistency among batches. Therefore, *L. paracasei* was evaluated as an adjunct culture under conventional processing conditions rather than as the sole fermentative microorganism. Sodium nitrite and sodium nitrate were maintained at the same concentration in all treatments to reproduce a conventional cured dry-fermented sausage formulation and to ensure comparable curing conditions among treatments. Thus, Control (-) should be interpreted as the treatment without sodium erythorbate, probiotic culture, or rosemary/green tea extract, but not as a nitrite/nitrate-free formulation. The inclusion of a nitrite/nitrate-free treatment was not considered within the scope of this study because it would represent a different product system, with direct implications for curing reactions, color development, oxidative stability, and product safety.

The raw material was ground separately in an 8 mm disc grinder. After homogenizing the

raw material with the ingredients and additives, the meat mass was stuffed into collagen casings (20 cm in length and 5 cm in diameter), and the pieces were immersed in a 20% potassium sorbate solution. The immersion in potassium sorbate solution was used as a surface treatment to control undesirable fungal growth on the casing during ripening. This procedure was applied equally to all treatments and was intended to act mainly on the external surface of the casing, while *L. paracasei* was incorporated into the meat batter. The pieces were placed in a fermentation chamber with an initial temperature of 25 °C. The temperature was reduced by 1 °C every 24 h until reaching 18 °C. Relative humidity was maintained between 90 to 95% for the first seven days and 70 to 80% for the remainder of the production period. After reaching a water activity (Aw) of 0.90 on the 24th day post-processing, the sausages' casings were removed, and the products were vacuum packed and stored at 4 °C for 60 days.

pH and Aw analysis

The pH and Aw measurements were carried out in triplicate on storage days 0, 20, 40, and 60. The pH was directly measured in crushed samples using a digital pH meter with a glass electrode (model T-1000, Tecnal Scientific Equipments, Piracicaba/SP, Brazil), previously calibrated with pH 7.0 and 4.0 buffer solutions. Aw was measured at 25 °C $\pm$ 0.3 using the Aqualab 4TE device (Decagon, Pullman, Washington, USA).

Instrumental color analysis

Instrumental color was determined on storage days 0, 20, 40, and 60 on the internal surface of sliced dry-fermented sausages. The analysis used a Minolta CR-400 colorimeter (Minolta Sensing Inc. Konica, Japan) with an aperture size of 8 mm. The D65 illuminant and a 2° observation angle were used. The color variables (L*, a*, and b*) were measured at five points on each side of two samples per treatment.

Thiobarbituric acid reactive substances (TBARS) analysis

Thiobarbituric acid reactive substances (TBARS) were determined in triplicate on storage days 0, 20, 40, and 60. The analysis was conducted following the protocol described by BRUNA et al. (2001), and the results were expressed in milligrams of malonaldehyde per kg of sample.

Probiotic culture count

The presumptive *L. paracasei* count was performed in triplicate on days 0, 20, 40, and 60

of storage following the procedures described by SALFINGER & TORTORELLO (2015). The deep plating technique with an overlay in MRS agar medium was used, and the plates were incubated at 37 °C for 48 h. The count was only performed on treatments with added *L. paracasei* probiotic strain (LP and R/GT+LP). Given that the starter culture added to the sausages contained *Pediococcus pentosaceus*, MRS agar supplemented with tetracycline (10 mg L⁻¹) was used as a selective/differential medium to estimate the viable population of the added *L. paracasei*, according to THARMARAJ & SHAH (2003). However, because tetracycline supplementation is not fully selective at the species level, the results were interpreted as presumptive *L. paracasei* counts rather than definitive species-specific identification. The presumptive *L. paracasei* count was calculated as the difference between the total lactic acid bacteria count in pure MRS agar and the *Pediococcus* count in MRS agar supplemented with tetracycline.

Statistical analysis

The entire experiment was replicated three times (n = 3). The results were evaluated using a general linear model ANOVA, considering treatments and storage period and their interactions as fixed factors and repetitions as random factor. When the fixed factors were significant, the Tukey test was applied for post-hoc comparison of means, with a significance level of 5%.

RESULTS AND DISCUSSION

pH and Aw values were not influenced (P > 0.05) by the interaction between treatments and storage time (Table 1). pH values were significantly influenced (P < 0.05) by the different treatments. Samples with *L. paracasei* showed the lowest pH values. This result suggested that the probiotic strain may have contributed to the acidification of the product, which is relevant for the microbiological stability of dry-fermented sausages (STEGMAYER et al., 2023). However, since lactic acid production or other fermentation metabolites were not quantified, this result should not be interpreted as direct evidence of an enhanced fermentation process. It should also be noted that *L. paracasei* was added in the presence of the commercial starter culture used in all treatments. Thus, the present experimental design does not allow the complete isolation of the probiotic strain's individual contribution from possible interactions with the starter culture. Instead, the results should be interpreted as the effect of adding *L. paracasei* as an adjunct culture in a conventional dry-fermented sausage system. It should be noted that the metabolism of *L. paracasei* is homofermentative, and the amount of lactic acid produced is similar to the *P. pentosaceus* strain (AGÜERO et al., 2020). The pH values increased significantly (P < 0.05) during cold storage. This result can be attributed to the release of alkaline compounds, such as ammonia

Table 1 - Main effects of treatment and storage time on pH and water activity (Aw) values of dry-fermented sausages manufactured with *L. paracasei* and mixed rosemary/green tea extract.

Factor	---Level---	----pH----	---SEM---	--P-value--	----Aw----	---SEM---	--P-value--
Treatment	Control (+)	5.94 ^a	0.04	<0.0001	0.837 ^{ab}	0.004	0.009
	Control (-)	5.90 ^a			0.842 ^{ab}		
	LP	5.56 ^b			0.831 ^b		
	R/GT	5.81 ^a			0.857 ^a		
	R/GT+LP	5.51 ^b			0.856 ^a		
Storage time	Day 0	5.56 ^c	0.03	<0.0001	0.856 ^a	0.005	0.077
	Day 20	5.57 ^c			0.844 ^a		
	Day 40	5.80 ^b			0.840 ^a		
	Day 60	6.05 ^a			0.839 ^a		
Treatment × storage time				0.06			0.87

Different letters within the same factor and column indicate significant differences by Tukey's test (P < 0.05).

Treatments: Control (+), with sodium erythorbate; Control (-), without sodium erythorbate, probiotic culture, or rosemary/green tea extract; LP, with *L. paracasei*; R/GT, with rosemary/green tea extract; R/GT+LP, with rosemary/green tea extract and *L. paracasei*.

SEM: standard error of the mean.

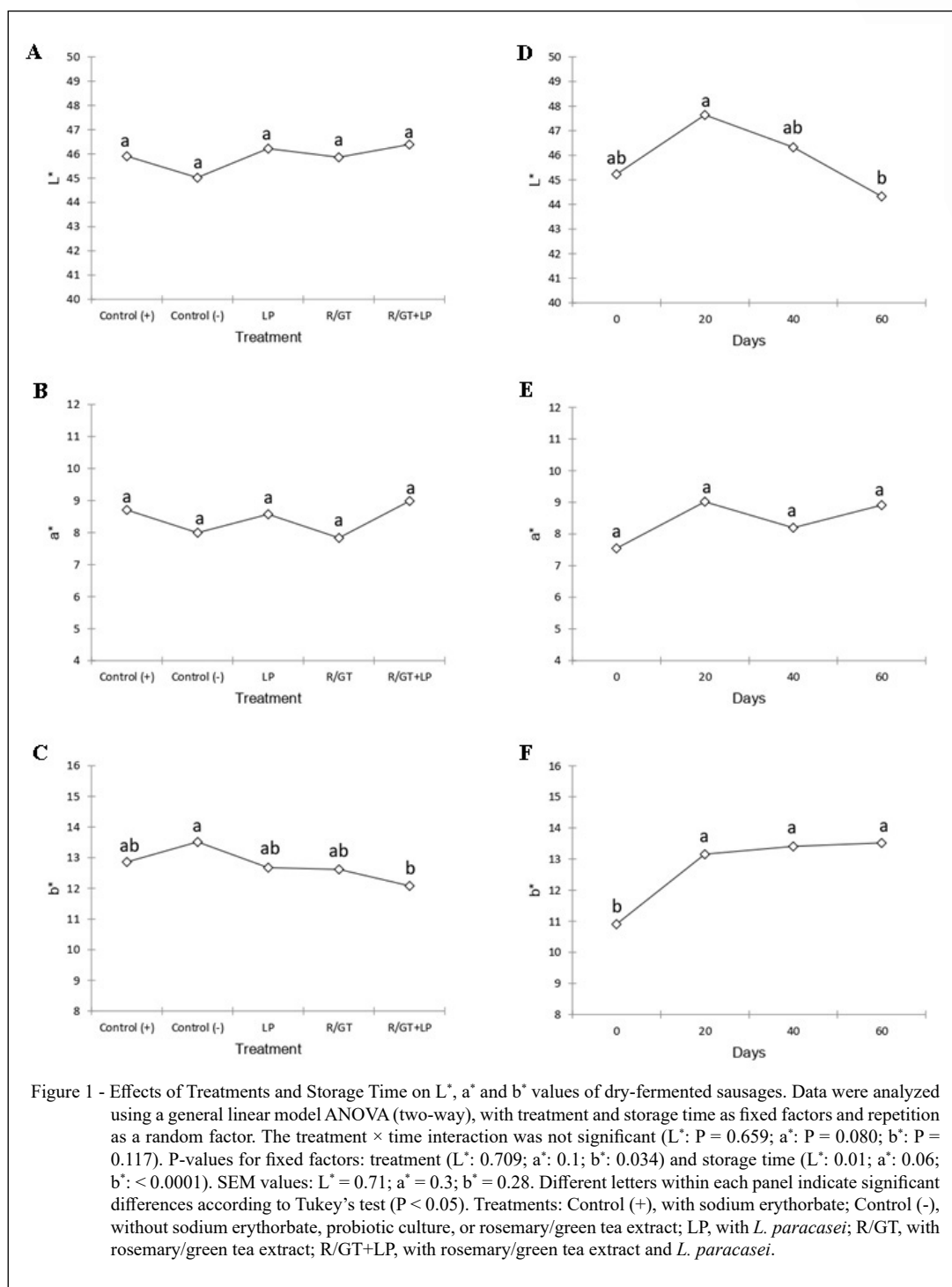
1 and peptides, due to the activity of endogenous and
2 exogenous proteolysis (SERDAROĞLU et al., 2023).
3 Other authors also reported a similar trend when
4 evaluating pH changes during dry-fermented sausage
5 storage (HERRANZ et al., 2004; VAN DER VEKEN
6 et al., 2023).
7 Aw values for all treatments were below the
8 0.90 (Table 1). Samples with the addition of probiotics
9 and natural antioxidants (LP, R/GT, and R/GT+LP)
10 showed Aw values similar ($P > 0.05$) to the control
11 treatments. The LP treatment had a lower Aw value
12 than the R/GT samples, which could be attributed to
13 the water content in the extract. As expected, storage
14 did not affect the Aw values. This indicated that the
15 packaging effectively prevented moisture exchange
16 between the product and the environment.
17 Values of L^* , a^* , and b^* were not influenced
18 ($P > 0.05$) by the interaction between treatments and
19 storage time (Figure. 1). No significant differences
20 were observed in L^* and a^* values between different
21 treatments (Figure 1A and 1B). The b^* values did
22 not differ ($P > 0.05$) between Control (+) and the
23 treatments LP, R/GT, and R/GT+LP (Figure 1C).
24 However, the R/GT+LP treatment had a lower ($P <$
25 0.05) b^* value than the Control (-). Color changes
26 in dry-fermented sausages should be interpreted
27 cautiously because CIELAB parameters are
28 influenced by pigment chemistry, curing reactions,
29 formulation, and oxidative processes. The lower b^*
30 value observed in R/GT+LP compared with Control

(-) may indicate reduced yellowing or discoloration,
which could be associated with lower formation of
lipid-derived oxidation products and lower oxidative
changes in heme pigments (HECK et al., 2021).
However, b^* alone is not a direct marker of oxidative
deterioration. In cured fermented sausages, nitrite and
nitrate contribute to the formation and stabilization
of nitrosylated heme pigments, which may also help
explain why a^* values remain unchanged among
treatments. Therefore, the interpretation based on b^*
values were moderated and should be considered only
as an indirect indication of color stability rather than
direct evidence of antioxidant activity. The values
of L^* and a^* did not differ significantly ($P > 0.05$)
between the beginning and end of storage (Figure 1D
and 1E). An increase in b^* values were observed from
the 20th day of storage (Figure 1F). This expected
outcome is consistent with findings reported in other
studies (GEETA & YADAV, 2017; RUIZ-MOYANO
et al., 2011).
No interaction ($P > 0.05$) between
treatments and storage time was observed for TBARS
values (Table 2). Control (-) showed higher TBARS
values than Control (+). This result was expected
considering the well-known antioxidant action of
sodium erythorbate (WANG et al., 2024; YUAN et
al., 2022). Adding the mixed extract of green tea and
rosemary (R/GT) effectively maintained the same
lipid oxidation level found in Control (+). This result
can be attributed to the high content of phenolic

Table 2 - Main effects of treatment and storage time on TBARS values of dry-fermented sausages manufactured with *L. paracasei* and mixed rosemary/green tea extract.

Factor	-----Level-----	-----TBARS-----	-----SEM-----	-----P-value-----
Treatment	Control (+)	0.92 ^b	0.05	0.002
	Control (-)	1.11 ^a		
	LP	0.90 ^b		
	R/GT	0.83 ^b		
	R/GT+LP	0.82 ^b		
Storage time	Day 0	0.84 ^b	0.04	0.026
	Day 20	0.87 ^{ab}		
	Day 40	0.93 ^{ab}		
	Day 60	1.02 ^a		
Treatment × storage time			0.772	

TBARS values are expressed as mg MDA/kg. Different letters within the same factor indicate significant differences by Tukey's test ($P < 0.05$).
Treatments: Control (+), with sodium erythorbate; Control (-), without sodium erythorbate, probiotic culture, or rosemary/green tea extract; LP, with *L. paracasei*; R/GT, with rosemary/green tea extract; R/GT+LP, with rosemary/green tea extract and *L. paracasei*.
SEM: standard error of the mean.



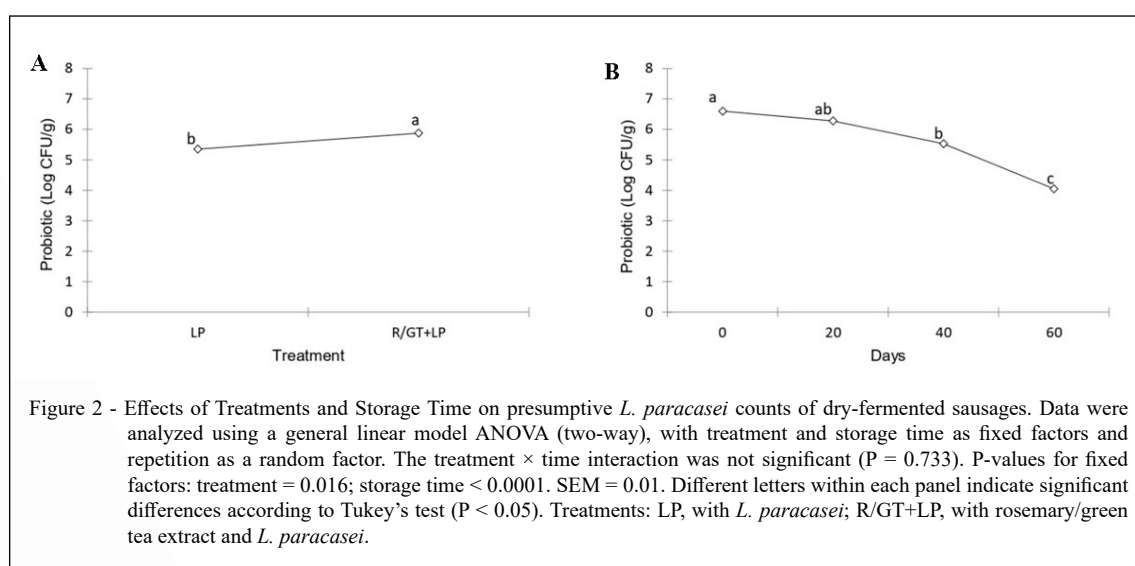
1 compounds present in the extract, which was 44.06
 2 mg GAE/mL, since these compounds are known
 3 for their ability to stabilize free radicals and, thus,
 4 slow down lipid oxidation reactions (ALEXANDRE
 5 et al., 2022; PAIVA et al., 2021). The LP treatment

showed lower TBARS values ($P < 0.05$) than Control
 (-) and similar values ($P > 0.05$) to Control (+) and
 R/GT. These results suggest that the addition of *L.*
paracasei may have contributed to controlling lipid
 oxidation under the conditions evaluated, as indicated

by the lower TBARS values compared with Control (-) and values similar to Control (+) and R/GT. However, the mechanisms involved were not directly investigated in this study. A possible explanation is that *L. paracasei* may contribute to oxidative stability through mechanisms reported for lactic acid bacteria, such as antioxidant enzyme activity, production of metabolites with reducing capacity, or release of bioactive peptides during proteolysis (ALIZADEH BEHBAHANI et al., 2024; HUR et al., 2014). Therefore, this interpretation should be considered a hypothesis that requires confirmation in future studies using specific biochemical or proteomic approaches. In agreement with the findings of this study, other authors recently reported that some strains of lactic acid bacteria, specifically *L. plantarum*, can be very beneficial in increasing the oxidative stability of fermented meat products (LUAN et al., 2021; YU et al., 2020). The LP and R/GT treatments showed similar TBARS values ($P > 0.05$) to the R/GT+LP treatment, demonstrating that the combination of *L. paracasei* and the green tea and rosemary extract did not have a synergistic effect (Table 2). A gradual and significant increase in TBARS values was observed during the 60 days of storage. This result was expected, as having high fat and salt content, lipid oxidation reactions continue to occur in this type of product even when it is vacuum-packed and stored under refrigeration (SIRINI et al., 2020).

The presumptive *L. paracasei* count was not influenced by the interaction ($P > 0.05$) between treatments and storage time (Figure 2). A higher ($P > 0.05$) count was found in the R/GT+LP treatment

(Figure 2A). This result may be partially related to selective interactions between phenolic compounds and the microbial population. Depending on their chemical structure, concentration, and the microbial strain involved, phenolic compounds may exert prebiotic-like effects on some lactic acid bacteria or inhibit susceptible microorganisms, thereby reducing microbial competition (SOUZA et al., 2023; PACHECO-ORDAZ et al., 2018). Thus, in the present study, the rosemary/green tea extract may have favored *L. paracasei* survival either directly, through prebiotic-like interactions, or indirectly, through inhibition of competing microorganisms. However, this mechanism was not specifically investigated and should be confirmed in future studies. A significant reduction in the presumptive *L. paracasei* count was observed during storage (Figure 2B). This result can be attributed to adverse factors that impair its viability over time in this type of product, such as the presence of NaCl, nitrite, and low A_w (SIRINI et al., 2022). Other authors also reported a similar trend when evaluating probiotic strains' viability during the storage of dry-fermented sausages (BIS-SOUZA et al., 2020; SIRINI et al., 2023; STEGMAYER et al., 2023). This decrease is relevant because the functional potential of probiotic foods depends not only on the incorporation of the microorganism but also on its ability to remain viable until consumption. Although there is no universal threshold applicable to all probiotic strains and food matrices, counts around 10^6 - 10^7 CFU/g at the time of consumption are commonly used as a reference for probiotic foods (DODOO et al., 2017). In the present



study, presumptive *L. paracasei* counts remained within or close to this range during the initial and intermediate storage periods, especially in the R/GT+LP treatment. However, the reduction observed at the end of storage indicates that additional strategies may be necessary to maintain probiotic levels compatible with functional claims throughout the entire shelf life of dry-fermented sausages. Therefore, further studies are needed to improve the viability of this microorganism during storage and to confirm the functional relevance of dry-fermented sausages containing *L. paracasei*.

CONCLUSION

The addition of *L. paracasei* as an adjunct culture was associated with lower pH values and did not adversely affect the instrumental color of dry-fermented sausages. Based on TBARS values, *L. paracasei* and the mixed rosemary/green tea extract, used separately or in combination, contributed to lipid oxidation control under the conditions evaluated, reaching values comparable to those obtained with sodium erythorbate. The combination of *L. paracasei* with the phenolic-rich extract improved the viability of the probiotic culture during refrigerated storage, although a reduction in viable counts was observed over time. Further studies are needed to evaluate sensory quality, confirm the functional relevance of the probiotic counts throughout shelf life, and elucidate the mechanisms involved in lipid oxidation control, including the possible formation of antioxidant peptides or other bioactive compounds.

DATA AVAILABILITY STATEMENT

All the original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

We declare that this work did not use any artificial intelligence resources for the conception and writing of the manuscript.

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