International Journal of Food Studies | IJFS 2025 | Volume 14 | pages 100–119

Antioxidant and Antimicrobial Activity of Yerba Mate Extract (*Ilex paraguariensis*) in Vienna Sausage

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Received: 26 December 2024; Published online: 31 January 2026

Abstract

This study aimed to evaluate the effectiveness of yerba mate extract, both in its free and microencapsulated forms, in Vienna sausages, focusing on preserving their physical properties, antioxidant capacity, and antimicrobial activity during storage at 5°C and 12°C. The results demonstrated that microencapsulating yerba mate extract significantly reduced weight loss during sausage cooking, maintained antioxidant activity, and inhibited lipid oxidation more effectively than the free extract. Furthermore, yerba mate extract exhibited notable antimicrobial properties against pathogenic microorganisms, enhancing the microbiological safety of meat products. The analysis revealed that storage temperature significantly influenced the characteristics of sausages treated with yerba mate extract. Sausages stored at 5°C retained higher antioxidant activity, exhibited lower levels of oxidative compounds (TBARs), and showed more effective inhibition of microbial growth compared to those stored at 12°C. Regarding sensory acceptability, sausages containing free yerba mate extract were more similar to the control sample than those with the microencapsulated extract. These findings highlight the promising potential of yerba mate extract, particularly in its microencapsulated form, as a functional ingredient in sausages, contributing to physical stability, antioxidant protection, and antimicrobial properties during storage.

Keywords: Natural antioxidants; Encapsulation; Meat products; Food preservation and safety; Shelf life

1 Introduction

The growing global interest in food quality has led consumers to increasingly favor natural and organic products (Aschemann-Witzel et al., 2019). This shift has encouraged the food industry to develop ingredients with functional properties, particularly plant-based compounds rich in bioactive molecules that promote health and help reduce the risk of chronic diseases (Kawski et al., 2017; Torres-Martínez et al., 2022).

In Brazil, the processed meat sector, especially sausage production, has expanded significantly in response to the rising demand for ready-to-eat foods. However, the widespread use of synthetic additives such as antioxidants, stabilizers and preservatives has raised public health concerns. Frequent consumption of these substances has been linked to adverse effects, including allergies, digestive disorders, cancer risk, and reduced bioavailability of essential vitamins (Gonçalves et al., 2020; Ng et al., 2019; Ordoñez, 2005; Soares,

 10.7455/ijfs/14.01.2025.a7

2023), potentially compromising the nutritional quality of processed products.

Among the major causes of deterioration in meat products are lipid and protein oxidation, and microbial contamination. Lipid oxidation of polyunsaturated fatty acids during storage and cooking promotes the formation of harmful compounds, and negatively affects the flavor, color, texture and nutritional value of meat products (de Farias Marques et al., 2022; Torres-Martínez et al., 2022). To mitigate these effects and ensure product stability, the meat industry has increasingly adopted antioxidant strategies, traditionally involving synthetic compounds such as sodium erythorbate, a derivative of ascorbic acid commonly used to prevent lipid oxidation (Delgado-Pando et al., 2021). However, the demand for cleaner-label products has driven interest in plant-based alternatives with both antioxidant and antimicrobial effects (Angiolillo et al., 2014; Vialta & Amaral, 2014).

In addition to their preservative functions, non-meat ingredients may also contribute to cost reduction and offer health benefits, depending on their composition and technological functionality (Owusu-Ansah et al., 2022). Among these, yerba mate (Ilex paraguariensis), a plant native to Latin America, stands out for its high content of phenolic compounds and other bioactives with antioxidant and antibacterial properties (Chaicouski & Lazzarotto, 2021).

To enhance the stability and functionality of these compounds in food matrices, microencapsulation has been proposed as an effective strategy. Although sodium erythorbate shares structural similarities with natural antioxidants, its synthetic origin makes it less appealing to consumers seeking clean-label formulations (Delgado-Pando et al., 2021). Given this scenario, for the first time, this study investigates the application of yerba mate extract, both in its free and microencapsulated forms, as a natural additive to improve the oxidative and microbiological stability of Vienna sausages, thus demonstrating its potential to enhance functionality and stability. This approach meets the growing demand for healthier meat products by applying plant-based bioactives in a cleaner and more sustainable way to preserve meat products while maintaining technological quality and sensory attributes.

2 Material and methods

2.1 Material

The Vienna sausages were produced at the pilot plant of the Meat Technology Center (CTC) of the Institute of Food Technology (ITAL), located in Campinas, São Paulo, Brazil, using its facilities and equipment. The ingredients used for sausage production, including pork trimmings (75/25), water, sodium lactate, cassava starch, isolated soy protein, sodium chloride, curing salt (a mixture of sodium chloride and sodium nitrite), stabilizers (sodium tripolyphosphate, sodium acid pyrophosphate, and sodium hexametaphosphate), seasonings, sugar, flavor enhancers, and sodium erythorbate, were kindly provided by ITAL.

The yerba mate extract was supplied by Heide Extratos Vegetais (Pinhais, PR, Brazil). Canola oil (Liza® brand) was purchased from Cargill (Mairinque, SP, Brazil), and PGPR (polyglycerol polyricinoleate) was obtained from Concepta Ingredients (São Paulo, SP, Brazil). BTM pectin was supplied by CP Kelco (Limeira, SP, Brazil), and calcium chloride was acquired from Dinâmica (Diadema, SP, Brazil).

Emulsions were prepared using a rotor-stator homogenizer (Ultra-Turrax®, model TE-102, Tecnal, Piracicaba, SP, Brazil), and the encapsulation process was conducted with a Büchi Encapsulator B-390 (Flawil, Switzerland). Color measurements were performed with a CR-400 colorimeter (Konica Minolta, Osaka, Japan), and spectrophotometric analyses were conducted using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, USA).

2.2 Methods

Microencapsulation of yerba mate extract

Yerba mate extraxt was microencapsulated by ionic gelation from as double emulsion system (W/O/W), based on a previously described method (Budin et al., 2023) with modifications.

Initially, a simple emulsion (aqueous phase in oil) was prepared by mixing 35g of hydrophilic yerba mate extract, 61g of canola oil, and 4g of PGPR (polyglycerol polyricinoleate) using a rotor-stator homogenizer (Ultra-Turrax®, model TE-102, Tecnal, Brazil) at 11,000 rpm, with a flow rate of 0.7 mL/min in a jacketed beaker maintained at 25 ^{o}C

This emulsion was incorporated into a continuous phase containing 2% (w/w) BTM pectin at a 20:80 ratio, forming a double emulsion through homogenization at 14,000 rpm for 5 minutes. The resulting W/O/W emulsion was dripped into a 4% (w/v) calcium chloride crosslinking solution using a vibrational encapsulator (Encapsulator B-390, Buchi, Switzerland) equipped with a 450 μm nozzle, at a vibration frequency of 1100 Hz, voltage of 2000 V, and pressure between 300–400 mbar. The beads were cured in the crosslinking solution for 15 minutes and then stored in an acidified solution (pH 3.0, adjusted with citric acid) under refrigeration at 5 ^{o}C

Drying was carried out in a fluidized bed dryer (FBD 1.0, Instituto Mauá de Tecnologia, Brazil). After preliminary drying for 3 hours on absorbent paper trays, the microparticles (initial moisture ∼72%) were processed to yield 124 g of dry material with a final moisture content of 15.85 ± 0.51%. The final concentration of encapsulated extract was approximately 7%. The microparticles had a mean diameter of 6.51 ± 0.61 μm.

Sausage manufacturing

The meats and ingredients were weighed and processed in a Kramer Grebe cutter model 65. Salt, curing salt, stabilizer, spices, flavor enhancer, chilled water, sodium lactate, sugar, antioxidant (sodium erythorbate), free or microencapsulated yerba mate extract, and cassava starch were gradually incorporated until the emulsion reached 10 ^{o}C. The emulsified mass was then stuffed into 22 mm cellulose casings using a Handtmann VF610 Plus stuffer and cooked in a Schroter HR1 chamber, reaching a final core temperature of 72 ^{o}C, over 110 minutes. After cooking, the sausages were cooled using water showers, manually peeled, vacuum-sealed in 200 g polyethylene packaging, coded, and stored in a cold chamber.

The samples were prepared as follows: VC (control sausage) without yerba mate extract; VE, containing 0.1% free yerba mate extract; and VP, containing 1.0% microencapsulated yerba mate extract. These levels were calculated based on the phenolic content of the extract to ensure functional equivalency. The formulations were standardized across treatments and included pork trimmings (75/25) (82.46–81.46%), water (10%), sodium lactate (2.0%), cassava starch (2.0%), salt (1.6%), curing mix (0.25%), stabilizers (0.3%), seasonings (0.8%), sugar (0.3%), flavor enhancer (0.2%), and sodium erythorbate (0.09%). The only variable among formulations was the presence and form of yerba mate extract. The encapsulated particles contained approximately 7% yerba mate extract. The total phenolic content in the dry microcapsules was 495.31 ± 1.60 mg GAE/100 g, while the free extract presented 3850.92 ± 19.72 mg GAE/100 g.

Sausage analyses

On the first day of shelf life, centesimal composition analyses were performed, including moisture, fat, protein, and ash content. Stability analyses were conducted every seven days for 65 days on samples stored at 5^{o}C and 12^{o}C. The methodologies used for sausage analysis were:

Centesimal composition

Moisture content was determined by drying the samples in an oven at 105^{o}C until reaching a constant weight ("Chap. 39, Method 981.10," 2012). Lipids were extracted using petroleum ether in Soxhlet units, and their percentage was determined by gravimetry ("Chap. 39, Method 991.36," 2012).

Total protein content was measured using the Kjeldahl method, which quantifies total nitrogen. The nitrogen content was then converted into protein percentage using a conversion factor of 6.25 ("Chap. 39, Method 950.46b," 2012). Ash content was determined by calcining the samples in a muffle furnace at 550^{o}C, followed by drying

in an oven at 105^{o}C until reaching a constant weight (Brasil. Ministério da Agricultura, 2019).

Color

Colorimetric evaluation was performed using a CR-400 portable colorimeter (Konica Minolta Sensing Inc., Osaka, Japan) with the CIE Lab system, which measures the value parameters (L*, a* and b*), chroma and hue (Konica Minolta, 2007). The readings were taken directly on the sample without prior treatment.
Each sausage was vertically divided into two halves, and the results for value parameters, chroma and hue were calculated as the average of nine measurement points.

Substances reactive to thiobarbituric acid (TBARS)

Substances reactive to 2-thiobarbituric acid (TBA), formed during lipid oxidation, were extracted from the samples by distillation in a hydrochloric acid-acidified medium. The reaction between 2-thiobarbituric acid and acetic acid developed the color used to determine the TBA value, expressed as malonaldehyde concentration. Absorbance was measured at 540 nm using a Shimadzu UV-1800 spectrophotometer (Koniecko, 1979).

Actives Extraction

The antioxidant activity was determined by the DPPH and ABTS methods according to Jiménez-Zamora et al. (2016). Fourteen grams of the sausage sample were weighed into a 50 mL beaker and finely chopped. The same mass of Celite 545 P.A Marca: Synth, (Diadema, SP Brazil) and 30 mL of 70% ethanol were added, and the mixture was placed in an ultrasound bath for four minutes. Homogenization was performed using an IKA T 25 digital ULTRA-TURRAX ® disperser for four minutes, followed by vacuum filtration through a Büchner funnel attached to a Kitassato flask.
The filtrate was transferred to a 100 mL volumetric flask, while the residue on the filter paper was collected and subjected to a second extraction. This process was repeated once more, totaling three extractions. Finally, the volumetric flask containing the extract was filled to 100 mL with 70% ethanol.

Antioxidant activity

Antioxidant activity was assessed using the DPPH and ABTS methods, following the methodology of Jiménez-Zamora et al. (2016). Analyses were conducted in triplicate using a UV/Visible spectrophotometer (Agilent Technologies, Cary 60 MY13110012, USA) at wavelengths of 515 nm (DPPH) and 734 nm (ABTS). The results were expressed in μmol TE/g fresh weight (wet basis).

Total phenolic compounds

Total phenolic content was determined using the Folin-Ciocalteu spectrophotometric method, following the procedure of Erkan-Koç et al. (2015). Analyses were conducted in triplicate using a UV/Visible spectrophotometer (Agilent Technologies, Cary 60 MY13110012, USA) at a wavelength of 750 nm. The results were expressed in mg GAE/100 g fresh weight (wet basis).

Microbiological analyses on sausages

The presence of *Salmonella* (ISO 6579, 2007), *Listeria monocytogenes* (ISO 11290-2, 1998), *Clostridium perfringens* (Labbe, 2013) coagulase-positive staphylococci (Bennett et al., 2015), *Escherichia coli* (ISO 725, 2005), and mesophilic aerobic bacteria (Salfinger & Tortorello, 2015) was analyzed. The results were expressed as log CFU/g (log colony-forming units per gram of sample).

Nitrates and nitrites

Nitrate and nitrite levels were quantified following the methodology of Brasil. Ministério da Agricultura (2019). Nitrate was reduced to nitrite after passing through a cadmium/copper column and subsequently measured as nitrite. Detection was performed using a Shimadzu UV-1800 spectrophotometer at a wavelength of 538 nm.

Kinetic study of degradation

The degradation of quality results, including total phenolic compounds, antioxidant activity (DPPH and ABTS), instrumental color (ΔE) and aerobic mesophilic counts, was modeled using kinetic approaches based on Moura et al. (2018). Adjustments to zero- and first-order reaction models were analyzed.

The coefficient of determination (r^2) was used as the criterion for selecting the best model fit to the experimental data. From this, the reaction constant (k), activation energy (E), Q_{10}, and half-life time ($t_{1/2}$) were determined using equations 1 to 4, respectively.

$$\ln\left(\frac{C}{C_0}\right) = -kt \tag{1}$$

$$t_{1/2} = \frac{\ln 2}{k} \tag{2}$$

$$Q_{10} = \frac{k_T}{k_{T-10}} \tag{3}$$

$$E = 0.46 \times T^2 \times \log(Q_{10}) \tag{4}$$

Where: T (Kelvin), Q_{10} (ΔT^oC), E (cal. $g.mol^{-1}$), k (day^{-1})

Equation 5 was used to calculate the total color difference (ΔE).

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \tag{5}$$

Sensory acceptability

The sensory study was evaluated by the Research Ethics Committee of Hospital Municipal Dr. Mário Gatti (HMDMG) in Campinas, Brazil, and approved under CAAE number 38438520.8.0000.5453. The acceptability test was conducted following ABNT NBR ISO 11136:2016 (Associação Brasileira de Normas Técnicas, 2016) guidance to assess overall acceptance and identify potential sensory changes over time. Samples (VC, VE and VP) were evaluated at two time points: immediately after production (freshly manufactured) and after approximately 60 days of refrigerated storage. The evaluation involved 80 untrained sausage consumers.

Prior to the test, samples were removed from refrigeration, allowed to reach room temperature, and reheated in a water bath at 70 ^oC for 10 minutes to simulate typical consumption conditions. Approximately 20 g of each sample was served in disposable containers coded with random three-digit numbers. The samples were presented in a sequential monadic manner, following a balanced complete block design. Palate cleansing was performed with mineral water between samples. Data collection and analysis were conducted using the Compusense Cloud computerized system.

Consumers evaluated overall acceptability and specific attributes such as appearance, odor, and flavor using a nine-point hedonic scale (9 = liked it very much, 5 = neither liked nor disliked it, and 1 = disliked it very much). Additionally, the intensity of saltiness, seasonings/condiments, and firmness was assessed using a five-point ideal scale: 5 = much saltier/more seasoned/much firmer than preferred, 3 = ideal, and 1 = significantly less salty/less seasoned/much softer than preferred. Beyond acceptability analysis, the CATA (Check All That Apply) descriptive method was used to identify the attributes that characterized each sample. Data from the hedonic scale evaluations were subjected to analysis of variance (ANOVA) and Tukey's test for mean comparison.

To evaluate the product's shelf life, the study assessed whether the sample's acceptability significantly declined over time, indicating the end of its shelf life. This was determined using the cutoff point methodology, which defines the minimum tolerable acceptability of samples based on Equation 6. In general, the commercial shelf life of vacuum-packed Vienna sausages stored under refrigeration is approximately 45 days, which served as a practical reference for the evaluation of sensory decline.

$$S = F - Z_{\alpha/2} \cdot \mathrm{MSE}_n \tag{6}$$

where:

S: minimum tolerable acceptability of the stored sample;

F: acceptability of the newly produced sample;

Zα: one-tailed area under the standard normal curve for significance level α;

MSE: mean square error derived from analysis of

variance of consumer data.
n: number of consumers.

Statistical analysis

The study results were statistically analyzed using Analysis of Variance (ANOVA) and Tukey's Test at a 5% significance level, with the aid of STATISTICA® version 7.

3 Results and Discussion

3.1 Centesimal Composition

The centesimal composition of Vienna sausages was analyzed, and the results indicated that all formulations comply with the Technical Regulation of Identity and Quality (IN No. 4, March 31, 2000) (Brasil. Ministério da Agricultura e do Abastecimento, 2000). This regulation establishes maximum limits for moisture (65%) and fat (30%), and a minimum protein content of 12%. Significant differences ($p < 0.05$) were observed in fat, moisture, and ash contents among treatments. The formulation containing microencapsulated yerba mate extract (VP) showed the highest fat content (16.29 ± 0.01 g/100g), while the control (VC) and the formulation with free extract (VE) presented lower values, 14.19 ± 0.01 and 14.94 ± 0.14 g/100g, respectively. Regarding moisture, VP also differed significantly, presenting the lowest value (62.41 ± 0.05 g/100g), in contrast to VC and VE, which had slightly higher contents. In terms of ash content, the differences were less pronounced.
VE showed the lowest mean (3.54 ± 0.03 g/100g), VC the highest (3.62 ± 0.02 g/100g), and the mean for VP (3.57 ± 0.01 g/100g) remained statistically equivalent to both. No significant variation was found for protein content, which ranged from 13.96 ± 0.22 g/100g (VP) to 14.40 ± 0.00 g/100g (VC). Taken together, these results suggest that the addition of yerba mate extract, particularly in encapsulated form, led to subtle modifications in the fat and moisture profiles of the sausages, likely influenced by the characteristics of the encapsulating matrix, without causing substantial changes in the overall nutritional composition.

Nitrate and nitrite levels in Vienna sausage samples (VC, VE, VP) complied with the maximum limit of 150 mg/kg for the sum of sodium nitrate and nitrite, as established by the Brazilian technical standard (MAPA IN 51/2006).
Microbiological characterization was conducted immediately after sausage packaging. The absence of *Listeria monocytogenes* and *Salmonella* spp. in all treatments confirmed the microbiological safety of the products. Additionally, bacterial counts remained below the acceptable limits for *Clostridium perfringens* (max. 10^2 CFU/g), *Escherichia coli* (max. 10 CFU/g), and coagulase-positive Staphylococci, indicating good hygiene practices during processing.

3.2 Stability Analyses

Color

Instrumental color analysis was conducted to evaluate the behavior of Vienna sausage samples during storage at 5 ^{o}C and 12 ^{o}C (Figure 1). Measurements of L*, a* and b* parameters were used alongside the calculation of total color difference (ΔE) in relation to day 0. Overall, L* values ranged from 64 to 66, indicating light-colored formulations. On day 45, the VP sample showed significantly higher L* (65.52 ± 0.76) compared to VC (64.11 ± 0.36) and VE (64.32 ± 0.54), reflecting greater brightness in the sample containing microencapsulated extract ($p < 0.05$). The a* values, associated with redness, did not differ significantly among treatments throughout the storage period. In contrast, b* values, related to yellowness, increased in all samples, with significant differences observed at specific time points. ΔE values, used to quantify overall color variation over time, remained below 2.0 in most cases, suggesting that visual changes were subtle and acceptable for product stability. The VP sample presented slightly higher ΔE values at certain time points (e.g., 1.24 on day 42 at 5 ^{o}C and 1.14 on day 63), indicating a slightly greater degree of variation. However, the trend lines generated from ΔE data yielded correlation coefficients (r^2) below 0.6, indicating no consistent linear pattern and supporting the conclusion that color remained relatively stable throughout storage.

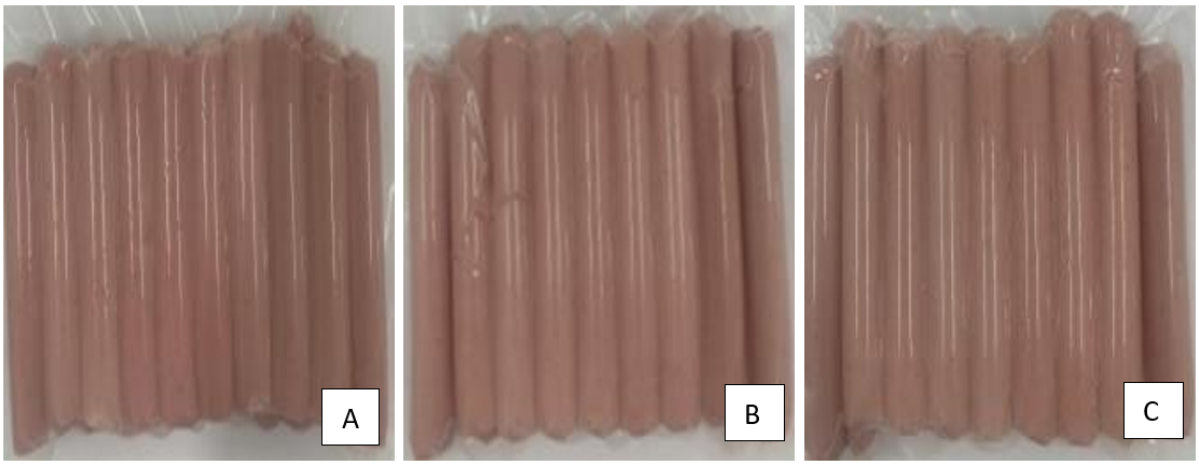

Figure 1: Samples on day zero: (A) Vienna Sausage Control (VC), (B) Vienna Sausage with Free Yerba Mate Extract (VE), and (C) Vienna Sausage with Microencapsulated Yerba Mate Extract (VP).

TBARS

To assess oxidative stability, the TBARS method was used. Secondary lipid oxidation products, which are responsible for sensory changes in food, can be quantified by measuring hydrocarbons such as ethane and pentane, free fatty acids, volatile oxidation compounds (mainly aldehydes, ketones, and alcohols), and malondialdehyde (through the analysis of substances reactive to thiobarbituric acid – TBARS) (Shahidi et al., 1998). Malondialdehyde, one of the primary products formed during the oxidation of polyunsaturated fatty acids, is the most commonly used marker for oxidative stability in meats and meat products (Ganhão et al., 2011).

In meat products, Adam et al. (2017) proposed a maximum acceptable limit of 2.0 mg MDA/kg for malondialdehyde. Trindade et al. (2008) further reported that rancid odors may become perceptible when TBARS values range between 0.5 and 1.0 mg MDA/kg in raw meats and between 0.6 and 2.0 mg MDA/kg in processed meat products. Several studies have demonstrated the effectiveness of natural plant-derived antioxidants in controlling lipid oxidation in meat products. Maqsood et al. (2012) evaluated an ethanolic extract of kiam tree peels (a species common in Thailand) applied to fish sausages. After 20 days of refrigerated storage, sausages containing 0.08% of the extract showed reduced peroxide and TBARS values compared to the control group. Similarly, Özvural and Vural (2011) investigated grape seed powder, a byproduct of wine production, in sausage formulations. The addition of this antioxidant ingredient effectively suppressed lipid oxidation, and its protective effect was dependent on concentration. More recently, Salem et al. (2023) reported that treating chicken fillets with 200 ppm of T. articulata essential oil significantly ($p < 0.05$) reduced lipid oxidation over 12 days of refrigerated storage. Together, these findings highlight the potential of various natural extracts in improving the oxidative stability of different types of meat products. According to Table 1, no statistically significant difference ($P < 0.05$) was observed over time in the control sample or in the sample with microencapsulated yerba mate extract during the storage period. However, the sample containing free yerba mate extract showed a significant difference ($P < 0.05$) on day 63 compared to the other samples. A calibration curve was used for TBARS quantification, with a determination coefficient of $R^2 = 0.9972$.

During storage, malondialdehyde can be oxidized and converted into other organic acids or alcohols that do not react with thiobarbituric acid, leading to a decrease in TBARS values (Liu et

Table 1: Thiobarbituric Acid Reactive Substances (TBARS) in sausages during refrigerated shelf life at 5^{o}C.

Days	VC (mgMDA/kg)	VE (mgMDA/kg)	VP (mg MDA/kg)
0	$0{,}140^{Ba}$	$0{,}133^{Bc}$	$0{,}203^{Aa}$
14	$0{,}143^{Ba}$	$0{,}176^{ABb}$	$0{,}186^{Aa}$
28	$0{,}163^{Aa}$	$0{,}173^{Ab}$	$0{,}183^{Aa}$
42	$0{,}176^{Aa}$	$0{,}180^{Ab}$	$0{,}160^{Aa}$
56	$0{,}140^{Ba}$	$0{,}190^{ABb}$	$0{,}173^{Aa}$
63	$0{,}176^{Ba}$	$0{,}243^{Aa}$	$0{,}166^{Ba}$

MDA (malondialdehyde) is a secondary compound formed during lipid oxidation and is used as an indicator of oxidative degradation in meat products. Means followed by different uppercase letters in the same column and lowercase letters in the same row differ significantly according to Tukey's test ($p < 0.05$).VC: Control Vienna sausage; VE: Vienna sausage with free yerba mate extract; VP: Vienna sausage with microencapsulated yerba mate extract.

al., 2009). The increase in TBARS levels results from the decomposition of hydroperoxides into secondary oxidation products (Yarnpakdee et al., 2012). In the final stages of storage, lipid oxidation may accelerate due to the depletion of antioxidant compounds, which are consumed during the oxidation process (Sbardelotto, 2022). Additionally, MDA is a volatile compound and may evaporate during storage, especially under vacuum packaging, which could explain the decrease in TBARS values observed in the VP sample over time. It is also known that MDA can form complexes with proteins and amino acids, rendering it undetectable by the TBARS method.

These factors may help explain why the microencapsulated extract formulation (VP) presented a higher initial MDA value than the control (VC), but lower values at the end of storage. Moreover, natural antioxidants such as tocopherols, present in the sausage matrix or potentially released from the encapsulated extract, may have contributed to the inhibition of secondary oxidation reactions, particularly in the VP formulation. In contrast, the free extract (VE), although rich in phenolic compounds, may have been less effective due to faster degradation or interaction with other components, resulting in a progressive increase in TBARS values.

Montowska et al. (2025) investigated the use of cold-pressed hempseed oil as a partial pork fat substitute in cooked, vacuum-packed meatballs. Over 12 days of storage, formulations containing 0.8% to 7.5% of the oil showed a significant reduction ($p < 0.05$) in both protein and lipid oxidation, with a 34.9% decrease in carbonyl compounds and a 17.5% reduction in TBARS values. In the present study, TBARS values significantly increased ($p < 0.05$) in sausages containing free yerba mate extract (VE), while no significant difference was observed between the control sample (VC) and the formulation with microencapsulated extract (VP) during storage at 5 ^{o}C. Importantly, malondialdehyde (MDA) levels in all sausage formulations remained below 1 mg MDA/kg. According to Jaberi et al. (2020), MDA values below this threshold indicate that no relevant lipid oxidation has occurred, supporting the oxidative stability of the products throughout the evaluated period.

Scavenging Activity (DPPH)

The control Vienna sausage sample (VC) had an initial DPPH scavenging value of 3.94 ± 0.09 μmol/g fresh weight (fw). The sample containing free yerba mate extract (VE) showed an initial value of 4.64 ± 0.05 μmol/g fw, while the sample with microencapsulated yerba mate extract (VP) had a slightly higher value of 4.94 ± 0.03 μmol/g fw. These results indicate significant differences among treatments at the be-

ginning of storage, with the VP sample exhibiting the highest initial scavenging activity (Figure 2). During storage at 5 ^{o}C, the VP formulation retained greater DPPH scavenging activity than the VE sample, although both yerba mate-containing treatments consistently outperformed the control (VC) throughout the evaluated period. Interestingly, while the VP sample had the highest initial scavenging capacity, the VE sample showed slightly better stability over time. This behavior suggests that microencapsulation enhances initial reactivity, whereas the free extract may offer more sustained scavenging potential.

Fitriana et al. (2016) support these findings, noting that compounds with radical scavenging activity can reduce DPPH radicals by donating hydrogen atoms. In contrast, a marked decline in scavenging activity was observed in all samples stored at 12 ^{o}C, as depicted in Figure 2. This reduction is likely related to the thermal degradation of heat-sensitive bioactive compounds such as flavonoids, anthocyanins, and ascorbic acid, as also reported by Morais et al. (2022).

This trend reinforces the importance of storage temperature in preserving radical scavenging properties. Elevated temperatures accelerate the breakdown of antioxidant compounds, compromising the functional potential of the product.

In summary, the results indicate that yerba mate extract is effective in scavenging DPPH radicals, especially in its microencapsulated form, which demonstrated higher initial antioxidant capacity. However, the free extract exhibited slightly greater stability during storage. Therefore, the selection between free and microencapsulated forms should consider whether the application requires a rapid antioxidant effect upon formulation or enhanced preservation of activity over time under refrigerated conditions.

Antioxidant Activity (ABTS)

The control Vienna sausage sample (VC) had an initial ABTS value of 4.05 $\pm$ 0.26 mmol/g b.u.. The sample containing free yerba mate extract (VE) showed an initial value of 4.48 $\pm$ 0.46 mmol/g b.u., while the sample with microencapsulated yerba mate extract (VP) had 5.07 $\pm$ 0.17 mmol/g b.u..

This increase in initial values suggests that the addition of yerba mate extract, particularly in its microencapsulated form, enhanced antioxidant capacity, as previously observed with the DPPH method. This effect is likely due to the antioxidant properties of yerba mate compounds, which effectively neutralize free radicals.

In samples stored at 5^{o}C, the Vienna sausage containing microencapsulated yerba mate extract showed better antioxidant activity than the sample with free yerba mate extract. All yerba mate-treated samples exhibited superior results compared to the control. Over the weeks, all samples showed a decreasing trend, with the VC sample experiencing the highest rate of decline, followed by VP and, lastly, VE (Figure 2).

In samples stored at 12^{o}C, antioxidant activity measured by the ABTS method declined significantly (Figure 2), consistent with the trend observed using the DPPH method. According to Oliveira de Moura (2023), temperature plays a crucial role in the stability of bioactive compounds, as increased temperatures accelerate their decomposition, likely causing the sharp reduction in antioxidant activity observed in the samples.

At both temperatures, the VC samples exhibited the highest rate of decline. The VP sample showed a slower decline than VE, particularly at 5^{o}C. The degradation of antioxidant compounds and chemical interactions between sausage components and antioxidants may be influenced by temperature. Higher temperatures can accelerate degradation reactions or chemical interactions.

The main difference between the ABTS and DPPH methods for assessing antioxidant activity can be attributed to the greater affinity of the ABTS radical for both hydrophilic and lipophilic compounds, whereas DPPH primarily interacts with hydrophilic compounds (Sridhar & Charles, 2019). Table 2 presents the kinetic parameters for the degradation of antioxidant activity measured by the ABTS method. The Q_{10} value indicates the sensitivity of the reaction rate to temperature changes, with higher values reflecting greater temperature sensitivity. Among the samples, VE had the highest Q_{10}, suggesting that the antioxidant activity of the free extract is more affected by temperature variations than VC and

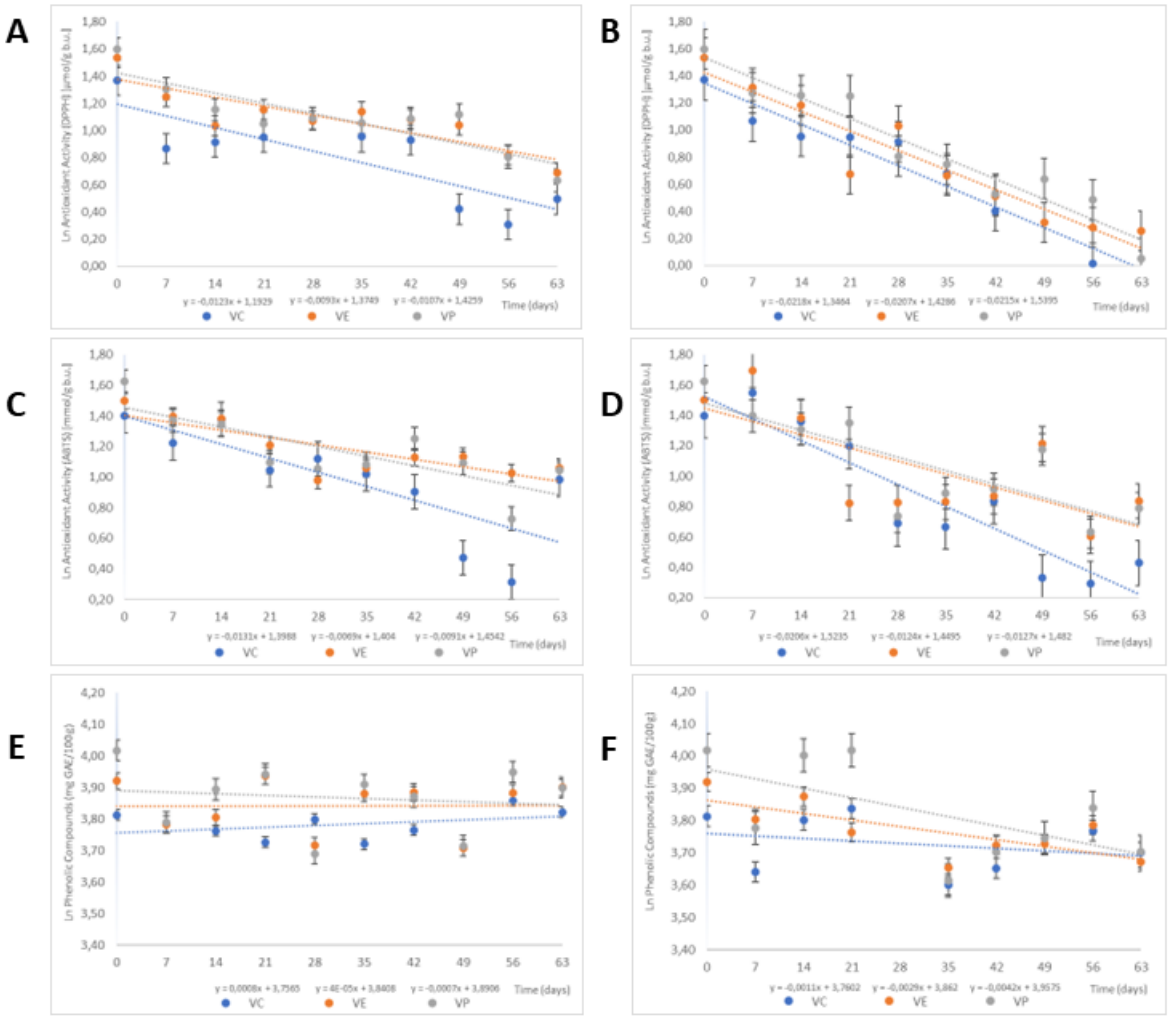

Figure 2: Antioxidant activity and total phenolic content of Vienna sausage samples stored at 5 ^{o}C and 12 ^{o}C for 63 days. (A, B) DPPH radical scavenging activity; (C, D) ABTS radical scavenging activity; (E, F) total phenolic content (expressed in mg GAE/100 g). Samples: VC (control), VE (with free yerba mate extract), and VP (with microencapsulated yerba mate extract). Storage at 5 ^{o}C is represented in panels A, C, and E; storage at 12 ^{o}C in panels B, D, and F. Error bars indicate standard deviation (n = 3).

Table 2: Kinetic parameters of degradation at different storage temperatures.

Sample	Order	T (^oC)	Q_{10}	$t_{1/2}$ (days)	Ea (kcal.g mol^{-1})
DPPH					
VC	1^a	5	2,27	56	12,5
		12		32	
VE	1^a	5	3,14	75	17,48
		12		33	
VP	1^a	5	2,71	65	15,24
		12		32	
ABTS					
VC	1^a	5	1,91	53	9,88
		12		34	
VE	1^a	5	2,31	100	12,8
		12		56	
VP	1^a	5	1,61	76	7,28
		12		55	
Phenolic compounds					
VC	1^a	5	1,58	866	6,95
		12		630	
VE	1^a	5	455	17325	93,56
		12		239	
VP	1^a	5	12,9	990	39,14
		12		165	

VC: Vienna Sausage Control; VE: Vienna Sausage with Free Yerba Mate Extract; VP: Vienna VC: Vienna Sausage Control; VE: Vienna Sausage with Free Yerba Mate Extract; VP: Vienna Sausage with Microencapsulated Yerba Mate Extract

VP.

The free extract was more sensitive to temperature increases than the control, possibly because its antioxidant compounds are more easily activated or degraded at higher temperatures. In contrast, the microencapsulated extract showed lower sensitivity to temperature changes, suggesting that microencapsulation helps protect antioxidant compounds from thermal degradation.

Regarding activation energy, the value found in the VP sample suggests that antioxidant compounds in the microencapsulated extract can be released or activated more easily, consistent with the protective role conferred by microencapsulation.

The free extract had a longer half-life at both temperatures, indicating greater stability than the control. The microencapsulated extract showed an intermediate half-life, suggesting good stability and potential benefits from microencapsulation. Overall, microencapsulation positively influenced the stability of antioxidant activity compared to the free extract.

Like the ABTS method, the DPPH results (Table 2) indicate that the free extract is more sensitive to temperature, suggesting that its antioxidant activity is more affected by temperature variations than the control. Although microencapsulation provides some protection against thermal sensitivity, it is less effective than in the free extract.

The high activation energy suggests that releasing or activating antioxidant compounds in the free extract is more complex and requires more energy. Microencapsulation helps reduce

activation energy compared to the free extract. The free extract also had a longer half-life at both temperatures, indicating greater stability than the control. The microencapsulated extract showed an intermediate half-life, suggesting good stability with potential benefits from microencapsulation.
The DPPH and ABTS results follow a similar trend, where the free extract is more sensitive to temperature than the control. In contrast, the microencapsulated extract helps mitigate this effect.
Table 2 presents the kinetic parameters for the degradation of antioxidant activity measured by the ABTS method. The Q_{10} value indicates the sensitivity of the reaction rate to temperature changes, with higher values reflecting greater temperature sensitivity. Among the samples, VE had the highest Q_{10}, suggesting that the antioxidant activity of the free extract is more affected by temperature variations than VC and VP.
The free extract was more sensitive to temperature increases than the control, possibly because its antioxidant compounds are more easily activated or degraded at higher temperatures. In contrast, the microencapsulated extract showed lower sensitivity to temperature changes, suggesting that microencapsulation helps protect antioxidant compounds from thermal degradation.
Regarding activation energy, the value found in the VP sample suggests that antioxidant compounds in the microencapsulated extract can be released or activated more easily, consistent with the protective role conferred by microencapsulation.
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Total Phenolic Content

In his study, Kharrat et al. (2018) applied 2.5% fig extract to salami, resulting in an initial total phenolic content of 37.0 ± 0.12 mg GAE/100g. The phenolic compounds contributed to the antioxidant activity without altering the sensory characteristics. Similarly, Casarotto (2013) added brewery waste extract to sausages; however, due to its low phenolic concentration, a large volume of extract was required to reach the target antioxidant dosage of 60 mg GAE/kg, ensuring the desired antioxidant effect in the final product. The initial total phenolic content in Vienna sausages was as follows: VC (control): 45.30 ± 0.25 mg GAE/100g, VE (free yerba mate extract): 50.42 ± 0.23 mg GAE/100g, and VP (microencapsulated yerba mate extract): 55.58 ± 0.10 mg GAE/100g. The addition of free yerba mate extract (VE) increased the total phenolic content by 10% compared to the control (VC), while the microencapsulated yerba mate extract (VP) resulted in an 18.5% increase.
According to Mateos et al. (2018) the average concentration of polyphenols in commercial yerba mate tea is approximately 80 mg/g dry mass, emphasizing its relevance as a natural source of these bioactive compounds.
The total phenolic content was monitored weekly for 63 days in Vienna sausages stored at 5^{o}C and 12^{o}C. In Vienna sausages stored at 5^{o}C (Figure

2), the phenolic compound content in the VE sample remained stable throughout the analysis period. In contrast, the VC sample showed a slight increase, while the VP sample exhibited a decline. The antioxidant activity observed is attributed to the polyphenols in yerba mate tea, which are the plant's main bioactive compounds (Sgarioni, 2023). However, this activity declined when the samples were stored at 12^{o}C (Figure 2).

Total phenolic content was quantified using the Folin-Ciocalteu method. According to Erkan-Koç et al. (2015), certain components, such as citric acid and reducing sugars, can interfere with this analysis. Therefore, the method is considered reliable only after these interfering compounds are removed through sample purification. In pectin-based candies, degradation products from sugar hydrolysis and Maillard reactions were retained, which may have contributed to an increase in total polyphenol content. Encapsulation protects substances susceptible to degradation by light or temperature variations, enhancing their stability. By controlling their release, it is possible to extend the duration of their activity in the product (Santos et al., 2017). However, this protection can be influenced by temperature, with higher temperatures accelerating the degradation process.

As shown in Table 2, the degradation of phenolic compounds varies among samples. VE exhibits high temperature sensitivity, as indicated by its high Q_{10} and activation energy values. VP shows moderate sensitivity, with stability between VC and VE. Regarding half-life, VE has an extended shelf life, suggesting greater stability compared to VC. VP also demonstrates increased shelf life, albeit to a lesser extent than VE, but with higher values than VC.

VE's high temperature sensitivity indicates a rapid response to temperature changes. VP exhibits moderate sensitivity, suggesting that microencapsulation enhances stability, although not to the same extent as in the free form. Therefore, for phenolic compounds, the free form may be preferable in applications where stability under varying conditions is crucial. The microencapsulated form, on the other hand, may be advantageous for controlled release, prioritizing this factor over an extended shelf life.

Aerobic mesophiles

Prado Martin et al. (2013) investigated the antimicrobial potential of yerba mate tea extract against foodborne pathogens, including *Listeria monocytogenes, Salmonella* spp. and *Escherichia coli*. The findings demonstrated that yerba mate tea has antimicrobial properties, making it a promising natural preservative for food applications.

Costa et al. (2017) evaluated the *in vitro* antimicrobial activity of different yerba mate tea concentrations against microorganisms isolated from chicken breast samples. The results indicated that yerba mate tea effectively inhibited two strains, *Escherichia coli* and *Proteus mirabilis*, under *in vitro* conditions.

Salem et al. (2023) reported a significant ($p < 0.05$) reduction in microbial flora in chicken fillets treated with essential oil, with a 50.31% decrease at 200 ppm by the 6th day of refrigerated storage. According to Savoia (2012), alkaloids, flavonoids and polyphenols are the primary compounds responsible for the antimicrobial activity of plant species. Additionally, flavonoids exhibit bactericidal properties by forming complexes with extracellular proteins, soluble proteins, and bacterial cell membranes, leading to cellular instability (Fowler et al., 2011).

Mesophilic aerobes require oxygen for metabolism, thriving in environments where oxygen is available. Their optimal growth temperature ranges from 20^{o}C to 40^{o}C, and they are commonly found in environments rich in organic matter. These microorganisms are often monitored as indicators of food quality and hygiene since their presence can contribute to food spoilage. The trendlines in Figure 3 illustrate distinct growth and decline patterns over time under different temperature conditions. Higher temperature (12^{o}C) promoted a greater increase in mesophilic aerobe populations compared to lower temperatures (5^{o}C). The difference in final microorganism counts between VC samples (Vienna Sausage Control) stored at 12^{o}C and 5^{o}C is significant and can be explained by factors related to temperature and the specific characteristics of the samples, as they do not have the addition of yerba mate extract.

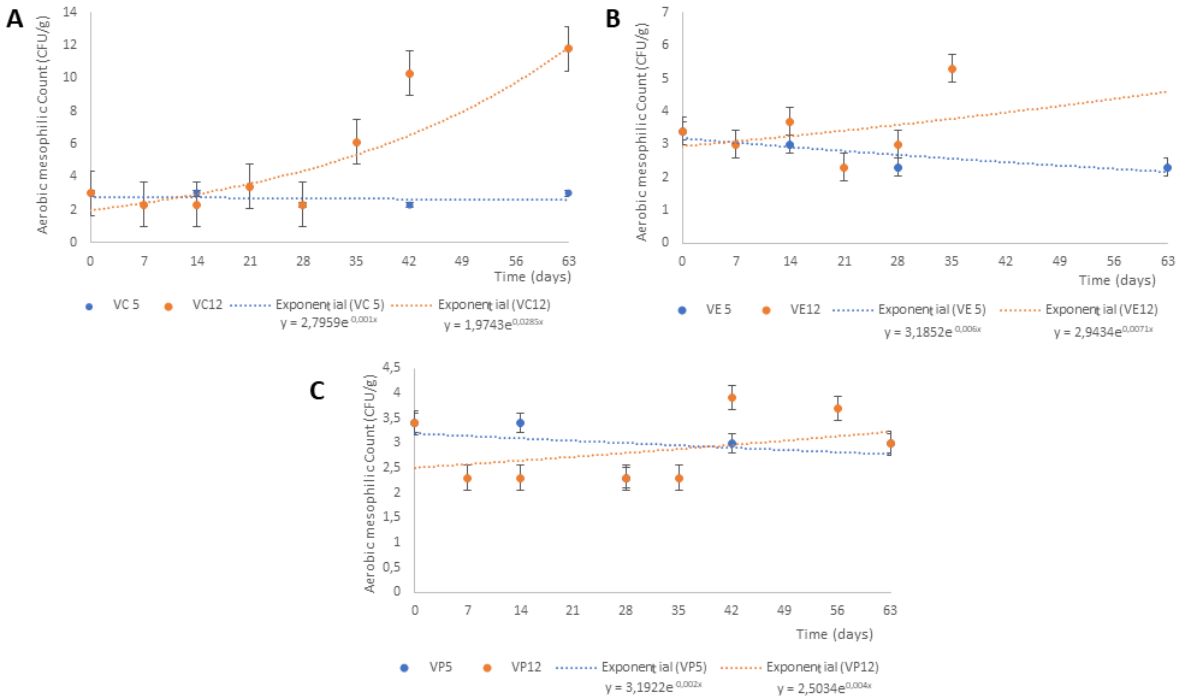

Figure 3: Graph of aerobic mesophile growth in: (A) Vienna Sausage Control (VC), (B) Vienna Sausage with Free Yerba Mate Extract (VE), and (C) Vienna Sausage with Microencapsulated Yerba Mate Extract (VP) stored for 63 days at 5^{o}C and 12^{o}C.

Table 3: . Results from the acceptability test of sausage samples at the initial (i) and final (f) time points, evaluating appearance, odor, flavor, and overall perception.

Acceptability1	**VC (i)**	**VC (f)**	**S2**	**VE (i)**	**VE (f)**	**S2**	**VP (i)**	**VP (f)**	**S2**
Appearance	6,4 (1,7)	7,3 (1,1)*	6	6,4 (1,7)	7,0 (1,3)*	6	5,7 (1,9)	5,9 (1,7)	5,2
Odor	7,0 (1,4)	7,4 (1,3)*	6,6	6,8 (1,5)	7,1 (1,2)	6,4	6,6 (1,5)	6,7 (1,5)	6,2
Flavor	7,0 (1,6)	7,4 (1,1)	6,6	7,0 (1,5)	7,1 (1,4)	6,6	6,1 (2,0)	5,8 (2,0)	5,6
Overall	6,9 (1,6)	7,3 (1,1)	6,5	6,9 (1,5)	7,1 (1,4)	6,5	6,1 (1,9)	5,8 (1,9)	5,6

VC: Vienna Sausage Control; VE: Vienna Sausage with Free Yerba Mate Extract; VP: Vienna Sausage with Microencapsulated Yerba Mate Extract
1- Results expressed as mean (standard deviation) of 80 evaluations per sample.
2- Minimum tolerable sample acceptability at the final time point with a 5% error level.
*- Asterisks indicate a significant difference between the initial and final time points ($p \leq 0.05$; Tukey's test).

The free yerba mate extract in sample VE contributed to the inhibition of microbial growth compared to sample VC. This effect may be attributed to the extract's antimicrobial and antioxidant properties, as well as potential interactions between the extract and sausage components. On day 35, the VE sample stored at 12 ^{o}C presented a higher-than-expected mesophilic count compared to other time points. This isolated increase may reflect natural microbiological variability or localized interactions between the free extract and microbial populations under elevated temperature conditions. Despite this, the overall trend for VE samples stored at 12 ^{o}C remained relatively stable across the storage period.

The influence of free and microencapsulated yerba mate extracts on microbial growth varies, as indicated by the different slopes in the trend equations. The sample containing microencapsulated extract (VP) exhibited lower microbial growth than the other Vienna sausage samples over the 63 days of storage. This difference may be due to specific characteristics of the microencapsulated yerba mate extract and its interaction with the microorganisms present.

Controlled release prolongs the exposure of microorganisms to antimicrobial compounds, effectively inhibiting their growth over time. Microencapsulation further protects the active compounds by isolating them from external factors such as oxygen, light, and moisture, preserving their antimicrobial effectiveness. Microencapsulated compounds can retain their antimicrobial efficacy for longer, contributing to a sustained reduction in microbial growth. Additionally, microencapsulation enables controlled dosing of antimicrobial compounds, preventing excessive release that may occur with free extracts and ensuring more balanced and efficient microbial protection.

Sensory acceptability

The VE sample was similar in description to the VC control sample, indicating that both achieved an acceptance rate of 75% or higher for appearance, odor, taste, and overall liking in both evaluation periods. For salt intensity and firmness, consumers rated the VE sausage as closer to the ideal than the other samples. In the preference ranking, VC and VE samples were preferred by more consumers and did not significantly differ from each other. However, both differed significantly ($p < 0.05$) from the VP sausage, which was the least preferred.

In the CATA descriptive analysis, VC and VE sausages were characterized as having a distinctive, flavorful taste, no off-odor or off-flavor, and a smooth texture, making them the preferred choices among consumers. At the final evaluation, the VP sample was perceived as significantly more sandy/grainy than the VC and VE samples.

All samples remained microbiologically acceptable after 60 days of storage, with aerobic mesophilic counts remaining below the regulatory limit of 6 log CFU/g established for cooked meat products. This result confirms that product quality and safety were maintained throughout the storage period. Table 3 presents the scores for each sausage sample at the initial (i) and final (f) evaluation periods. VC and VE samples had similar scores across almost all evaluated parameters, while VP received lower scores but still met the minimum acceptability threshold.

Price et al. (2013) compared the impact of grape seed and green tea extracts with the effect of sodium ascorbate on the sensory quality of cooked pork meatballs during refrigerated storage. The meatballs were stored at 4^{o}C in aerobic packaging for 0, 4, 8, 12 and 16 days under retail display conditions. Refrigerated storage did not affect the color of the meatballs, however, the addition of extracts introduced brown tones. Antioxidant supplementation did not alter sensory attributes except for color.

Montowska et al. (2025) evaluated the quality characteristics of cooked, vacuum-packed meatballs reformulated with cold-pressed hemp oil as a partial pork substitute (0.8%–7.5%) during 12 days of storage. Sensory analysis revealed no significant changes in texture, odor, or flavor throughout the storage period.

4 Conclusion

The application of yerba mate extract, in both free and microencapsulated forms, demon-

strated promising antioxidant potential in Vienna sausages. In particular, the microencapsulated form contributed to higher initial antioxidant activity and better preservation of phenolic compounds, oxidative stability, and instrumental color during refrigerated storage. These effects are likely associated with the controlled release and protection of bioactive compounds enabled by microencapsulation.

Kinetic modeling parameters, including Q_{10}, half-life ($t_{1/2}$), and activation energy (E_a), confirmed the temperature sensitivity of antioxidant degradation processes, reinforcing the importance of storage conditions in maintaining product quality. In terms of sensory acceptability, sausages containing the free extract (VE) showed greater similarity to the control sample and were better accepted by consumers. Meanwhile, samples with microencapsulated extract (VP) received slightly lower scores, particularly in texture-related attributes.

Although a reduction in microbial counts was observed in VP samples compared to the control, the results do not clearly confirm a significant antimicrobial effect attributable to the extract. Therefore, further studies with targeted microbiological testing are recommended to validate this potential application.

In summary, microencapsulated yerba mate extract stands out as a promising natural antioxidant strategy for meat product preservation, particularly due to its contribution to oxidative stability and sensory quality during storage. These findings support ongoing efforts to develop cleaner-label and more sustainable food preservation alternatives. The novelty of this study lies in demonstrating, for the first time, the incorporation of microencapsulated yerba mate extract into Vienna sausages, highlighting its potential to increase the functionality and stability of meat products. This innovative approach offers new perspectives on the use of microencapsulation as a tool to expand the application of plant-based bioactive compounds in the development of cleaner and more sustainable food preservation alternatives.

Acknowledgements

The authors sincerely thank Heide Extratos Vegetais (Pinhais, PR, Brazil) for providing the yerba mate extract.

The authors declare that they have no conflict of interest.

This research received funding from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil (postgraduate scholarships), and the São Paulo Research Foundation (FAPESP), Brazil (Grant 2019/19647-0).

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