Capítulo 31

Effect of Yerba Mate (Ilex paraguariensis) and Propolis on the Oxidative Stability and Microbiological Quality of Jerked Beef

Efeito da Erva-Mate (Ilex paraguariensis) e da Própolis na Estabilidade Oxidativa e na Qualidade Microbiológica do Jerked Beef

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Abstract: This study demonstrated that the use of natural antioxidants, specifically yerba mate and propolis extracts, is effective in maintaining the oxidative stability and quality of jerked beef during 60 days of storage at 25°C. Both extracts exhibited significant antioxidant capacities in DPPH, ABTS, and FRAP assays, with propolis showing higher values (up to 3,878 µmolTE/g in ABTS) compared to yerba mate (up to 638 µmolTE/g in ABTS). Regarding lipid oxidation, formulations with yerba mate and propolis significantly delayed rancidity compared to the control group, which reached 4.332 mg malonaldehyde/kg by the end of the period; yerba mate proved more efficient, maintaining lower TBARS values (1.843 mg/kg versus 2.121 mg/kg). Physically, an increase in luminosity (L*) and shear force was observed, with the latter rising from an average of 69 N to 74 N, indicating meat hardening due to dehydration and protein oxidation during storage. Microbiologically, the product remained safe and within legal standards, with no detection of Salmonella spp. or thermotolerant coliforms, and Staphylococcus aureus counts remained below 10 CFU/g. It was concluded that these natural agents are viable alternatives to synthetic additives, aligning with the trend toward clean-label products and ensuring the shelf life of meat at room temperature.

Keywords: natural antioxidant; salted meat; oxidative stability; coagulase-positive staphylococcus.

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Resumo: Este estudo demonstrou que o uso de antioxidantes naturais, especificamente extratos de erva-mate e própolis, é eficaz na manutenção da estabilidade oxidativa e da qualidade do jerked beef durante 60 dias de armazenamento a 25 °C. Ambos os extratos apresentaram capacidades antioxidantes significativas nos ensaios DPPH, ABTS e FRAP, com a própolis apresentando valores superiores (até 3.878 µmol TE/g no ensaio ABTS) aos da erva-mate (até 638 µmol TE/g no ensaio ABTS). Quanto à oxidação lipídica, as formulações com erva-mate e própolis retardaram significativamente a rancificação em comparação ao grupo controle, que atingiu 4,332 mg de malonaldeído/kg ao final do período. A erva-mate mostrou-se mais eficiente, mantendo valores inferiores de TBARS (1,843 mg/kg, em comparação a 2,121 mg/kg). Em relação às características físicas, observou-se aumento da luminosidade (L*) e da força de cisalhamento, que passou de uma média de 69 N para 74 N, indicando endurecimento da carne devido à desidratação e à oxidação proteica durante o armazenamento. Do ponto de vista microbiológico, o produto permaneceu seguro e em conformidade com os padrões legais, sem detecção de Salmonella spp. Ou coliformes termotolerantes, e as contagens de Staphylococcus aureus permaneceram abaixo de 10 UFC/g. Concluiu-se que esses agentes naturais são alternativas viáveis aos aditivos sintéticos, alinhando-se à tendência de produtos clean label e assegurando a vida útil da carne em temperatura ambiente.

Palavras-chave: antioxidante natural; carne salgada; estabilidade oxidativa; estafilococos coagulase-positivos.

INTRODUCTION

Salting and dehydration are the oldest forms of meat preservation, predating modern refrigeration systems. This traditional curing process involves a complex combination of physical, biochemical, and bacteriological mechanisms, in which the meat matrix absorbs salt, sugars, and nitrates/nitrites while simultaneously losing water-soluble nutrients, such as proteins, vitamins, and minerals, to the curing medium (Mora; Toldrá, 2023). A significant technological evolution in this category was the emergence of Jerked Beef (JB). Unlike its predecessors, which used only curing salts, JB is classified as an intermediate moisture meat (IMM) product due to the addition of curing salts and vacuum packaging. It maintains its specific characteristics at room temperature due to its stability, with a final water activity ranging from 0.60 to 0.90 (Alves *et al.,* 2022).

Historically, the food industry has relied on synthetic additives as the primary means of delaying lipid oxidation in these products. Although complete oxidation inhibition is unattainable, antioxidants can significantly delay oxidative rancidity by intercepting singlet oxygen and decomposing primary oxidation products into non-radical species (Domínguez *et al.,* 2019). These agents are essential for protecting carotenoids and vitamins, thereby maintaining the product's nutritional and sensory quality over long periods. However, recent toxicological concerns regarding the potential side effects of synthetic compounds have driven a prominent shift in the industry.

Currently, there is a global trend toward incorporating natural substances with high antioxidant potential into meat formulations (Lorenzo *et al.,* 2021). Contemporary

research focuses on evaluating these natural agents as partial or total replacements for synthetic antioxidants and sodium nitrite. These studies aim to satisfy the growing consumer demand for "clean label" products while ensuring the oxidative stability and microbial safety of Jerked Beef in non-refrigerated environments (Santos *et al.,* 2024). Based on this scenario, the present study aims to test natural antioxidant agents in JB, evaluating their effectiveness when associated with or without sodium nitrite.

MATERIAL AND METHODS

For the experiments, brisket was used as the raw material. The samples of yerba mate extracts were obtained by aqueous extraction with the aid of an ultrasound bath Elmasonic P, brand Elma, using distilled water as a solvent, in the ratio of 1:10 (m/m) yerba mate/solvent (Campos *et al.,* 2008; Beal *et al.,* 2011). Then, the extracts were centrifuged (Routine centrifuge 420R, Hettich Zentrifugen brand) at a rotational speed of 8000 rpm for 10 minutes at 20ºC, and the supernatant was filtered. The extracts were dried in a Spray Dryer, LabMaq MSD 1.0, at 150°C and 0.77 L h-1, and stored in dark bottles under refrigeration until analysis. The samples of the alcoholic propolis extract at 30% were supplied by the company Apis Flora.

Before formulating, the antioxidant activity of the products to be used must be determined. The antioxidant capacity was evaluated using the DPPH (Brand-Williams *et al.,* 1995) and ABTS (Re *et al.,* 1999) methodologies, which measure radical scavenging via hydrogen or electron transfer, and the FRAP (Benzie; Strain, 1996) method, which evaluates the reducing potential of iron. These spectrophotometric methodologies enable quantification of the bioactive potential of compounds across different chemical media.

The briskets were packed and transported in isothermal boxes to maintain the storage temperature. Trimming was performed to remove excess skin and surface fat, thereby maintaining the homogeneity of the raw material. For the subsequent step of wet salting, which enables the production of more delicate products with a more homogeneous distribution of salt (Nunes; Pedro, 2011), the parts were randomly divided into 6 groups and subjected to 6 different formulations. Formulation 1: Control sample; Formulation 2: Addition of 200 ppm of sodium nitrite (Standard); Formulation 3: Addition of 300 ppm of Yerba Mate (EM); Formulation 4: Addition of 400 ppm of Propolis Extract 30% (PRO); Formulation 5: Addition of 100 ppm of sodium nitrite and 150 ppm of extracts of yerba mate (EM+NO) and Formulation 6: Addition of 100 ppm of sodium nitrite and 200 ppm of Propolis extract 30% (PRO+NO).

The process of wet salting consisted of immersing the samples in a 20% (p/v) saline solution at a 2:1 (saline solution/meat) ratio for 2 hours under stirring. After the immersion time, the parts were packed into plastic trays, identified, and covered with a 2:1 (p/p) mixture of coarse salt and beef, forming piles intercalated with layers of coarse salt and beef. This process was carried out at room temperature, and after each 24-hour period, the piles were inverted; thus, the top parts moved to the

bottom, and vice versa, until the parts reached a water activity of 0.75, with the salt being changed at each step. After this step, the excess salt was removed, the parts were duly identified, hung with metal staples, and then dried in an oven at 35°C for 24 hours to evaporate surface moisture; they were then vacuum-packed and stored at 25°C until the analytical procedures were implemented.

Contents of moisture, ash, lipids, and proteins were determined according to AOAC (1995). Aw was determined at all stages of processing using an automatic device (Aqualab, Decagon Devices Inc., model CX-2) at 25◦C (±1). For lipid oxidation evaluation, the TBARS (Reactive substances to thiobarbituric acid-2) method was used according to Crackel *et al.* (1988) and the recommendations of Shahidi *et al.* (1985). To evaluate meat texture, Warner-Bratzler Shear Force (WBSF) measures tenderness by calculating the peak force (N) required to shear standardized cores perpendicular to muscle fibers using a V-shaped blade (AMSA, 2015; Bourne, 2002).

For each batch, the following were verified: presence of Salmonella spp., count of Staphylococcus aureus, determination of the most probable number of total coliforms and thermotolerant coliforms at time zero, thirty, and sixty days of storage.

The color analyses were performed in triplicate at time 0 and after 60 days of storage using a calibrated Minolta colorimeter, with three readings taken at different points in the inner part of the salted product, where a longitudinal cut was made for all formulations. The values of L*, a*, and b* are expressed in the CIELab color system.

The results were statistically analyzed with analysis of variance and means testing (Tukey test) at a 5% level of significance. For these analyses, the software Statistica® for Windows version 12.0, from STATSOFT (2015), was used.

RESULTS AND DISCUSSION

Propolis and yerba mate possess high antioxidant capacities, albeit at different magnitudes; while propolis extract presents robust values ranging from 1,200 to 4,500 µmolTE/g in the DPPH assay, 1,800 to 6,200 µmolTE/g in ABTS, and 1,500 to 5,800 µmolTE/g in FRAP, yerba mate (Ilex paraguariensis) exhibits ranges of 250 to 650 µmolTE/g (DPPH), 400 to 900 µmolTE/g (ABTS), and 350 to 850 µmolTE/g (FRAP), reflecting the high concentration of phenolic compounds in both matrices (Garcia *et al.,* 2020; Bittencourt *et al.,* 2021; ZangelinI *et al.,* 2022). The data obtained in this study indicate a close correlation with the authors cited above. The data are shown in Table 1.

Table 1 - Antioxidant capacity obtained for Propolis vs. Yerba Mate (averages of triplicates).

	DPPH (µmol TE/g)	ABTS (µmol TE/g)	FRAP (µmol TE/g)
Propolis (Extract)	2.541	3.878	3.102
Erva-Mate (Ilex paragua-riensis)	410	638	614

Table 2 presents the results of the proximal composition, showing that all JB samples were within the standards established by Brazilian legislation (Brasil, 2000) and in accordance with the results reported by Coelho *et al.* (2017), Youssef (2003), and Garcia (2002). Statistically, the formulations differed significantly at the 5% level, and this difference was directly associated with the formulation type. It is noted that current legislation and the available literature recommend values for moisture, ash, and water activity, but this is not the case for lipids and proteins, which may vary and depend directly on the initial characteristics of the raw materials used in processing. Purnomo (2011) analyzed Indonesian spicy dried meat and obtained results comparable to those of this experiment; some factors differ, but the overall profile is similar.

Table 2 - Results of the proximal composition of JB in different formulations regarding the initial (T0) and final time of storage (T60 days).

	Moisture		Ash		Protein		Lipids	
Formulations	T0	T60	T0	T60	T0	T60	T0	T60
Control	55,65^{b} (±1,00)	43,94^{ab} (±0,4)	18,34^{b} (±0,42)	20,61^{b} (±0,55)	30,10^{b} (±1,07)	27,34^{d} (±0,67)	3,86^{b} (±0,33)	5,92^{b} (±1,29)
Standard	57,40^{b} (±0,50)	39,57^{c} (±2,51)	20,19^{a} (±0,27)	19,96^{c} (±0,11)	32,08^{a} (±0,85)	33,89^{a} (±0,48)	4,45^{b} (±1,57)	7,43^{ab} (±0,54)
EM	53,01^{c} (±2,9)	40,37^{c} (±0,50)	17,96^{b} (±0,41)	22,67^{a} (±0,05)	26,71^{c} (±1,42)	29,85^{c} (±0,26)	9,08^{a} (±0,53)	9,07^{a} (±1,06)
PRO	60,10^{a} (±0,33)	42,78^{b} (±0,30)	19,79^{a} (±0,11)	21,12^{b} (±0,27)	30,03^{b} (±0,62)	30,77^{c} (±1,90)	3,63^{b} (±0,91)	2,36^{c} (±0,43)
EM + NO	54,91^{bc} (±0,53)	45,01^{a} (±0,53)	19,66^{a} (±0,17)	19,97^{bc} (±0,50)	30,71^{ab} (±0,12)	32,14^{bc} (±0,35)	2,28^{b} (±0,35)	2,63^{c} (±1,08)
PRO + NO	54,79^{bc} (±0,24)	42,69^{b} (±0,39)	20,16^{a} (±0,17)	19,99^{bc} (±0,17)	30,33^{b} (±0,33)	32,86^{ab} (±0,53)	4,97^{b} (±0,80)	2,09^{c} (±0,25)

Legend: - Control (salted beef - jerky); Standard (200ppm Nitrite). EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm nitrite); PRO+NO (200ppm Propolis + 100 ppm nitrite); T0 (initial time); T60 (60 days of storage); $^{a-d}$ different letters in the same column statistically differ among themselves ($p<0.05$) by the Tukey test; results are averages of triplicate ± standard deviation.

The addition of propolis results in the greatest changes in proximal composition. Another point to highlight is that moisture values decreased significantly during storage across all tested formulations. This result suggests that, even after drying, the product continued to dehydrate during packing. There are several explanations for this, ranging from the thickness of the packaging to the speed of the air during the product-drying process.

When the moisture content of a food product changes, its entire constitution needs to be reassessed, since water reduction tends to concentrate solids. This phenomenon can be observed in this study, indicating that, with storage, there is an apparent increase in lipids and proteins, as reported by Samejima *et al.* (1992).

Analyzing the results for product color, it should be noted that the Luminosity (L*) values increased over the 60 days of product storage. The lowest L* value at time zero (darker meat) can be explained by the greater capacity for water retention and less loss of liquids in the medium, associated with the membrane integrity (Koohmaraie *et al.* 2002). However, the product in question is already part of low L* values, i.e., low luminosity when compared to in natura meat. Sabadini *et al.* (2001) found that L* values for dried meat increased relative to raw material. These authors mention grayish-colored meat observed in the previous step (wet salting), which was not detected in dry salting, and the samples exhibited a distinctive "glow" characteristic of the product. In Table 3, the evaluated data were recorded for the product color at the initial and final storage times.

Table 3 - Values of L*, a*, and b* were obtained for different formulations of JB between the initial time (T0) and the final days of storage (T60 days).

	L*		a*		b*	
Formulation	T0	T60	T0	T60	T0	T60
Control	27,30^{b} (±1,39)	40,12^{a} (±2,20)	7,20^{b} (±1,32)	9,00^{a} (±0,47)	3,40^{b} (±0,78)	9,67^{a} (±1,04)
Standard	31,61^{b} (±3,67)	34,37^{a} (±4,71)	12,09^{a} (±0,80)	9,40^{b} (±2,64)	5,36^{b} (±1,43)	10,82^{a} (±3,41)
EM	32,04^{b} (±1.40)	41,62^{a} (±4,55)	5,89^{a} (±1,11)	5,03^{b} (±0,09)	5,13b (±0,62)	11,69^{a} (±2,42)
PRO	29,62^{b} (±0,99)	34,20^{a} (±2,63)	9,48^{a} (±0,46)	4,69^{b} (±1,60)	4,38b (±0,66)	6,72^{a} (±0,75)
EM + NO	31,59^{b} (±1,52)	41,58^{a} (4,20±)	9,75^{a} (±0,67)	8,34^{b} (±1,77)	4,38b (±0,50)	8,79^{a} (±1,87)
PRO + NO	29,55^{b} (±2,33)	42,90^{a} (±3,18)	13,23^{a} (±4,09)	4,90^{b} (±3,19)	5,94^{b} (±1,22)	11,50^{a} (±1,51)

Legend: - Control (salted beef - jerky); Standard (200ppm Nitrite). EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm Nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite); (dp) standard deviation of means; a-b different letters in the same column statistically differ among themselves ($p<0.05$) by the Tukey test. Results are averages of triplicate ± standard deviation.

Analyzing the other color parameters, the red intensity (a*) decreased with storage time, which may have resulted from changes in the chemical form of heme pigments (Mancini and Hunt, 2005). Luciano *et al.* (2009) describe that the intensity of red was reduced over the maturation period (1 to 14 days) for in natura beef and went to values around 12 for values below 8, and, concomitantly, these authors observed significant reductions in the amount of heme pigments and an increase in the percentage of metmyoglobin. The increase in the intensity of the yellow (b*) is due to the brownish color of the stored meat, as the product surface is dominated by metmyoglobin (Lawrie 2005).

A dark color can be observed in products that undergo superficial dehydration, which promotes pigment concentration and, at the same time, changes the optical properties of muscle fibers (Faria *et al.* 2001). This is exactly what is observed in this study: values of a* and b* indicate a darkening of the product, although L* also increased. This increase in L* does not necessarily indicate that the sample was lighter, but rather an increase in its luminosity, which in turn can be associated with dehydration the product undergoes during storage, leading to even more drastic changes in the protein's conformation. It was also verified that a darkening of the samples due to excessive addition of nitrite, which fosters the myoglobin oxidation, leading to the metmyoglobin formation (Walshe and Rose, 1956), can cause the product greening (Judge *et al.,* 1989), a fact that was not observed in this study. On the other hand, the low availability of nitric oxide, resulting from the addition of insufficient quantities of nitrite or reducing agents during the curing, generates a low residual concentration of nitric oxide in the final product, which could result in products with low coloration and little stability (Towsend and Bard, 1971).

The obtained values of water activity (Aw) (Table 4) are in line with those found in the literature. However, additional points should be discussed, as there was a significant difference in Aw between storage times and formulation types. Table 4 shows that for storage times of 0 and 30 days, product Aw shows variation, whereas it stabilizes at 60 days. This is directly associated with the same trend in moisture content, which decreased over time and differed significantly between the initial time and 60 days of storage. Youssef *et al.* (2013) indicated that the water activity in the JB samples at time zero was 0.77. The values of the sun-dried meat Aw ranged from 0.87 to 0.95. Lira and Shimokomaki (1998) reported an Aw of 0.92 for sun-dried meat. Considering that the mean Aw studied was below 0.75, all formulations are close to the recommended value for the product storage without refrigeration.

Table 4 - Results obtained for water activity (Aw) for different formulations of JB stored for 0, 30, and 60 days.

Formulations	Storage Time 25ºC (days)		
	T0	T30	T60
Control	0,724^{a} (± 0,006)	0,722^{b} (±0,014)	0,713^{a} (±0,006)
Standard	0,714^{b} (±0,003)	0,733^{a} (±0,07)	0,712^{a} (±0,012)
EM	0,717^{a} (±0,005)	0,70^{d} (±0,01)	0,714^{a} (±0,002)
PRO	0,722^{a} (±0,002)	0,707^{c} (±0,008)	0,712^{a} (±0,001)
EM + NO	0,724^{a} (±0,001)	0,717^{a,b,c,d} (±0,003)	0,711^{a} (±0,004)
PRO + NO	0,721^{a} (±0,003)	0,718^{a,b,c,d} (±0,003)	0,720^{a} (±0,013)

Legend: - Control (salted beef - jerky); Standard (200ppm Nitrite). EM (300 ppm Yerba mate);

PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm Nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite); (dp) standard deviation of means; a-b different letters in the same column statistically differ among themselves (p<0.05) by the Tukey test. Results are averages of triplicate ± standard deviation.

Aw and the concentration of salt are the main parameters to ensure the JB stability. According to Sabadini *et al.* (2001), in mass transfer studies, it was verified that Aw was directly influenced by salt penetration and water loss, which occurred simultaneously. Whereas Biscontini *et al.* (1995) observed shrinkage of muscle fibers and the formation of intra- and extracellular spaces, leading to the loss of water and other components and resulting in reductions in moisture content and Aw. In another study, Torres *et al.* (1994) found that a value of 0.75 for Aw characterizes the point of balance between the diffusion of sodium chloride into the interior of the meat and the stabilization of the product's moisture content.

Prolonged storage, such as a 60-day period, directly influences Shear Force in intermediate-moisture meats by increasing instrumental hardness due to protein oxidation, which generates cross-linking between fibers, and by limiting proteolysis caused by high salt concentrations that inhibit natural tenderizing enzymes (Table 5). In CH, this hardening is intensified by continuous desiccation and fiber compaction in permeable packaging, whereas in Jerked Beef, the effect is moderated by vacuum packaging protection and higher moisture retention (Domínguez *et al.,* 2019; Alves *et al.,* 2022; Dias, 2022). The Shear Force for CH and JB is higher than that of fresh meat due to the intense salting and desiccation processes; while CH generally presents values above 5.0 kgf (49 N) due to its compact protein structure and low moisture (maximum 45%), JB varies between 4.0 kgf and 5.5 kgf due to its higher moisture content (up to 55%) and vacuum packaging protection, resulting in slightly superior instrumental tenderness compared to CH, although both exceed the 4.6 kgf threshold and are technically classified as tough meats compared to sun-dried beef (Alves *et al.,* 2022; Santos *et al.,* 2024). The treatment was not the determining factor in altering the texture; rather, it was the storage time.

Table 5 - Warner-Bratzler Shear Force (WBSF) measurements of JB in different formulations regarding the initial (T0) and final time of storage (T60 days).

	Shear Force (N)	
Formulations	T0	T60
Control	69.28^{b} (±3,57)	75,94^{a} (±4.48)
Standard	70,40^{b} (±4,0)	74,57^{a} (±5.2)
EM	69,01^{b} (±2,9)	74,37^{a} (±1,5)
PRO	68,93^{b} (±5,33)	73,78^{a} (±3,30)

	Shear Force (N)	
EM + NO	70,91[b] (±5.43)	75,07[a] (±4.82)
PRO + NO	69.05[b] (±4.21)	74,69[a] (±3.9)

Legend: - Control (salted beef - jerky); Standard (200ppm Nitrite). EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm Nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite); (dp) standard deviation of means; a-b different letters in the same line statistically differ among themselves (p<0.05) by the Tukey test. Results are averages of triplicate ± standard deviation.

As a result, the microbiological analyses performed in this study showed that all the tested samples for thermotolerant coliforms were negative. A similar result was observed for Salmonella testing: all tested samples were negative for this microorganism. Tables 4 and 5 present the microbiological results from the study conducted at 0, 30, and 60 days of JB storage. It should be noted that the microbiological quality of the product is in accordance with the recommended procedures for legislation, as stipulated for matured meat products such as raw hams, salami, desiccated sausages, jerky, JB, and the like (Brasil, 2001).

Table 4 - Survey on Total Coliforms (MPN/g) in different JB formulations as a function of the storage period.

Formulation	T0	T30	T60
Standard	<3,0	<3,0	<3,0
EM	<3,0	<3,0	<3,0
PRO	<3,0	<3,0	<3,0
EM + NO	<3,0	<3,0	<3,0
PRO + NO	9,2	<3,0	<3,0

Legend: Standard (200ppm Nitrite); EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite).

Table 5 - Survey of Staphylococcus positive for coagulase (UFC/g) in different JB formulations as a function of the storage period.

Formulation	T0	T30	T60
Standard	<10	<10	<10
EM	<10	<10	<10
PRO	<10	<10	<10
EM + NO	absent	<10	<10
PRO + NO	<10	<10	<10

Legend:(200ppm Nitrite); EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite).

The microorganisms capable of developing into meat products of intermediate water activity are molds, yeasts, and halophilic bacteria (Franco and Landgraf, 1996), the latter belonging to the genera Halobacterium and Halococcus (Buchanan; Gibbons, 1974). These bacteria produce red pigments and a putrefactive alteration, called “redness”. On the other hand, these bacteria grow slowly, even under ideal environmental conditions, and are strictly aerobic (Buchanan and Gibbons, 1974). Vacuum packaging prevents the growth of halophilic bacteria, yeasts, and molds (Geisen *et al.,* 1992).

According to Lara *et al.* (2003), salted meat products may harbor pathogenic microorganisms, a finding corroborated by Santana and Azeredo (2005), who detected Staphylococcus aureus in salted meat. Also, in this issue of microbiological quality, other authors, such as Pinto *et al.* (2002), indicate that coliforms, including Escherichia coli, may be resistant to mild salt concentrations. This author indicates that up to 5% of this microorganism is viable at this salt concentration.

According to Franco and Landgraf (2008), the determination of deteriorative and pathogenic microorganisms in sun-dried meat provides a basis for establishing microbiological standards. Escherichia coli, among coliform bacteria, is the best-known indicator of fecal contamination. Studies conducted by Coelho *et al.* (2017) indicated that sun-dried meat samples, similar to those studied in this work, yielded an average Staphylococcus aureus count exceeding 5.0 UFC/g, a level that poses a considerable risk of enterotoxin production and may lead to food poisoning. These authors also suggest that these organisms are halotolerant, can tolerate up to 15% salt, and are favored because other competing microorganisms are reduced, enabling their easier growth.

The TBARS results obtained over 60 days of storage are shown in Table 6. In the TBARS test, malondialdehyde is quantified, one of the main products of the decomposition of hydroperoxides of polyunsaturated fatty acids formed during the oxidative process.

Table 6 - Values of TBARS in JB (mg of malondialdehyde/kg of sample) for treatments in different periods of storage.

Formulation	Periods of storage at 25ºC (days)								
	T0	T7	T14	T21	T30	T37	T45	T52	T60
Control	1,298^{a} (±0,022)	1,760^{a} (±0,029)	2,096^{a} (±0,020)	2,646^{a} (±0,031)	3,448^{a} (±0,027)	3,512^{a} (±0,019)	3,639^{a} (±0,065)	4,266^{a} (±0,069)	4,332^{a} (±0,175)
Standard (NO)	0,739^{b} (±0,021)	0,853^{b} (±0,017)	0,880^{e} (±0,017)	1,113^{c} (±0,019)	1,340^{b} (±0,032)	1,295^{e} (±0,013)	1,383^{e} (±0,023)	1,580^{e} (±0,038)	1,619^{b} (±0,029)
EM	0,895^{c} (±0,018)	1,020^{c} (±0,021)	1,019^{c} (±0,026)	1,090^{b} (±0,014)	1,272^{e} (±0,017)	1,483^{b} (±0,017)	1,552^{b} (±0,030)	1,785^{b} (±0,023)	1,843^{b} (±0,051)
PRO	1,055^{d} (±0,015)	1,183^{d} (±0,020)	1,216^{b} (±0,021)	1,671^{d} (±0,019)	1,855^{c} (±0,037)	1,895^{c} (±0,018)	1,976^{c} (±0,043)	2,026^{c} (±0,034)	2,121^{c} (±0,031)
EM + NO	0,767^{b} (±0,020)	0,887^{b} (±0,031)	0,821^{f} (±0,024)	1,054^{b,c} (±0,018)	1,344^{b} (±0,028)	1,460^{b} (±0,020)	1,506^{b} (±0,041)	1,694^{d} (±0,027)	1,758^{b} (±0,038)
PRO + NO	0,860^{c} (±0,029)	0,880^{b} (±0,014)	0,975^{d} (±0,020)	1,178^{d} (±0,031)	1,595^{d} (±0,036)	1,663^{d} (±0,021)	1,718^{d} (±0,030)	1,800^{b} (±0,022)	2,010^{c} (±0,031)

Legend: - Control (salted beef - jerky); Standard (200ppm Nitrite). EM (300 ppm Yerba mate);

PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm Nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite); (dp) standard deviation of means; a-b different letters in the same column statistically differ among themselves ($p<0.05$) by the Tukey test. Results are averages of triplicate ± standard deviation.

As shown in Table 6, the starting point for comparison among the formulations is the presence of sodium nitrite (NO); i.e., the sample set serves as the standard. The formulations were processed with 200 ppm sodium nitrite, as required by legislation limiting the residual level of this salt in cured products to 150 ppm, in this case, JB. The maximum allowed nitrite level in cured Chinese ham at the end of processing is 20 ppm, according to Zhu (1998), and, by Brazilian legislation, the maximum is 150 ppm (ABIA, 1998).

It should be noted that after 60 days of storage, TBARS values increased. A formulation control was performed in order to be able to show clearly the action of these different types of antioxidants in salted meat product. The values obtained in this experiment are, for JB, within the range reported in the literature, keeping in mind that the control formulation showed values much higher than the others, since it does not contain any antioxidant and thus cannot be directly compared to the others. However, it indicates how a salted meat product without curing compares to a salted and cured meat product in terms of oxidation rate.

Pearson *et al.* (1977) suggested that the antioxidant effect of sodium nitrite in cured meat products is interesting. However, salt use should be minimized because excessive consumption is associated with toxic effects (Lijinsky, 1999). The use of sodium nitrate in cured products serves as a reserve, replenishing sodium nitrite levels by reducing bacterial activity and prolonging the salt's antioxidant action (Cassens, 1997). Authors such as Nassu *et al.* (2003), Trindade *et al.* (2008), and Bertolin *et al.* (2010) studied the action of synthetic and natural antioxidants, indicating that they can minimize lipid oxidation in meat products.

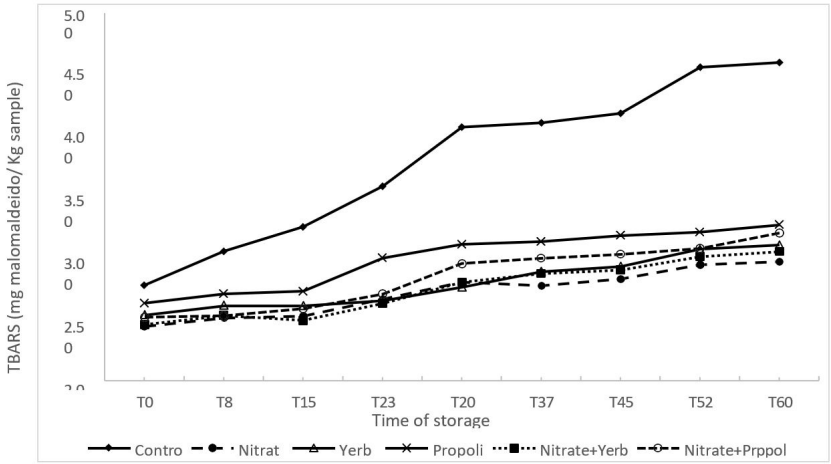
The research by Torres and Okani (1997) presents TBARS values by food group, with the meat group ranging from 0.20 to 1.25mg malondialdehyde/kg sample. TBARS values of 1,240mg/kg of malondialdehyde in samples were obtained by Pinto (1998) at the end of the processing of JB. Youssef *et al.* (2003) reported mean TBARS values in JB samples ranging from 0.23 to 1.38 mg.

In this study, JB with the addition of EM to its formulation showed lower TBARS values than the PRO-added formulation (Han and Park, 2002). Govaris *et al.* (2004) reported that researchers using natural products derived from oregano also obtained positive results in reducing lipid oxidation rates.

It is observed that when a blend of EM with sodium nitrite (NO) was formulated, even though there was a reduction of 50% of the amount of NO used (200 ppm to 100 ppm of NO), the values of TBARS became close to the values obtained only for NO. These data suggest that there may be a reduction when curing salts are combined with another antioxidant, in this case, EM. This hypothesis comes hand in hand with what is currently sought in the industry of cured products, i.e., a reduction in the addition of curing salts to make these products more attractive to the general

public, which is increasingly concerned with the production of potentially disease-causing products, such as nitrosamines and other products originating from the meat curing process. The comparative evolution of lipid oxidation during storage is shown in Figure 1.

Figure 1- Evolution of lipid oxidation by the number of TBARS as a function of the storage time in JB. Legend: Control (salted meat-jerky); Standard (200ppm Nitrite);EM (300 ppm Yerba mate); PRO (400ppm Propolis); EM+NO (150ppm Yerba mate + 100ppm nitrite); PRO+NO (200ppm Propolis + 100ppm Nitrite).



Among the different formulations with tested natural antioxidants, one may realize that the least satisfactory response was observed with the addition of PRO, which can once more be compared to the work of Hans and Park (2002), who, upon examining the antioxidant capacity of PRO, conclude that between this and the first showed the worst performance.

FINALES CONSIDERATION

The present study demonstrates that natural antioxidants, specifically yerba mate (Ilex paraguariensis) and propolis extracts, are effective agents in maintaining the oxidative stability and quality of Jerked Beef. Both extracts exhibited significant antioxidant capacities across DPPH, ABTS, and FRAP assays, with propolis showing higher overall values. Throughout the 60-day storage period, all formulations remained within the legal standards for proximal composition, although significant dehydration was observed even under vacuum packaging. Microbiologically, the use of these natural agents, combined with traditional salting and vacuum packaging, ensured the product's safety, with no detection of Salmonella spp. or thermotolerant coliforms, and Staphylococcus aureus counts remaining within safe limits. Regarding lipid oxidation, formulations containing yerba mate and propolis significantly delayed rancidity compared to the control group. Notably, yerba mate extracts were more

efficient than propolis in keeping lower TBARS values over time. These results suggest that natural antioxidants can serve as viable alternatives or complements to synthetic additives and sodium nitrite, meeting the “clean label” consumer trend while ensuring the shelf-life and safety of intermediate moisture meat products at room temperature.

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