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Keywords: functional meat product; lipid oxidation; natural antioxidant



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Article

# Functional Effects of Yerba Mate–Enriched Rabbit Burger: A Dietary Approach to Gastrointestinal Health

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## Abstract

**Background:** Yerba mate (*Ilex paraguariensis*) is a South American plant known for its antioxidant and bioactive properties, which may contribute to gastrointestinal health. This study evaluated the effects of yerba mate (YM) incorporation in rabbit burgers on their physicochemical, technological, and oxidative stability during frozen storage. **Methods:** Burgers were formulated with 0%, 0.5%, 1.0%, 1.5%, and 2.0% of YM and stored at  $-18\text{ }^{\circ}\text{C}$  for 60 days. Quality parameters, including proximate composition, cooking yield, shrinkage, moisture retention, pH, peroxide value, thiobarbituric acid reactive substances (TBARS), and total volatile basic nitrogen (TVB-N), were evaluated. **Results:** No significant changes were observed in dry matter, fat, crude protein, ash content, cooking yield, or shrinkage due to YM addition. Moisture retention decreased at the 2.0% inclusion level. YM incorporation did not inhibit peroxide formation; however, TBARS values were significantly reduced at 1.5% YM. pH remained stable ( $<6.0$ ) in all formulations. TVB-N values decreased in the 1.5% YM formulation after 30 days, suggesting reduced protein degradation. Means were compared using analysis of variance (ANOVA), followed by the *t*-test for significance at  $p < 0.05$ . **Conclusion:** Although yerba mate addition up to 2% had limited antioxidant effects over 60 days of storage, its influence on lipid peroxidation and protein stability indicates potential for developing functional meat products with relevance to gastrointestinal well-being.

**Keywords:** functional meat product; lipid oxidation; natural antioxidant

## 1. Introduction

Rabbit meat is a valuable source of highly digestible protein and essential micronutrients, recommended for athletes, elderly individuals, children, and convalescents. Its low intramuscular fat and favorable fatty acid profile contribute to its classification as a functional food. However, its high susceptibility to lipid oxidation and microbial spoilage limits its shelf life and broader consumer acceptance [1,2].

The incorporation of antioxidants into meat products has been extensively studied to enhance their stability [3]. Although synthetic antioxidants (e.g., BHA, BHT) are effective, concerns about potential toxicity and carcinogenicity have driven consumer preference toward natural alternatives [4,5]. Plant-derived antioxidants are particularly attractive due to their multifunctional properties, including antimicrobial, anti-inflammatory, and gastrointestinal health benefits [6].

Yerba mate (*Ilex paraguariensis*), native to South America, is rich in polyphenols, flavonoids, and other bioactive compounds, exhibiting well-documented antioxidant and antimicrobial activities [7,8]. Prior studies demonstrated its efficacy in reducing lipid oxidation and microbial growth in poultry and fish products [9,10], suggesting its potential application in meat preservation. Moreover,

certain yerba mate polyphenols have been linked to gastrointestinal benefits, including modulation of gut microbiota, reduction of intestinal inflammation, and improved mucosal integrity [11,12].

Therefore, the present study aimed to evaluate the effects of yerba mate addition on the physicochemical, technological, and oxidative characteristics of rabbit burgers during frozen storage. The outcomes may provide insights into the development of functional meat products aligned with current trends in food innovation and dietary interventions for gastrointestinal health.

## 2. Materials and Methods

### 2.1. Sample Preparation

Yerba mate was acquired in Frederico Westphalen (Rio Grande do Sul, Brazil), of native variety, sugar-free, and bearing an organic product certification seal. The samples were ground using a semi-automatic three-blade mill (50 g capacity, 200 W). The resulting material was sieved with a 625-mesh sieve (0.02 mm openings) and stored in plastic bags. This process was carried out 24 hours prior to burger preparation.

### 2.2. Rabbit Burger Production

Rabbit meat (*Oryctolagus cuniculus*) was purchased from the local and used for burger preparation. Five formulations were prepared [13]: control (0% yerba mate [YM]), and formulations with 0.5%, 1.0%, 1.5%, and 2.0% YM. In addition to rabbit meat (83.7%) and YM, the formulation included textured soy protein (4.0%), wheat flour (5.0%), black pepper (0.5%), garlic paste (1.0%), dried spices (4.5%), and salt (1.3%). Burgers were manually molded, packaged in polyethylene, and stored at  $-18^{\circ}\text{C}$  until analysis.

### 2.3. Physicochemical Analysis

Burgers were evaluated for dry matter, ash, and crude protein ( $N \times 6.25$ ) [14] and lipid content [15].

### 2.4. Color Analysis

Color was assessed using a digital colorimeter (FRU® WF-WR10QC) with an 8 mm aperture and D65 illuminant, measuring  $L^*$ ,  $a^*$ , and  $b^*$  parameters (CIELAB scale) at 0 and 60 days. Five readings were taken per sample.  $\Delta E$  was calculated as:

$$\Delta E_{1-2} = \sqrt{(L_2 - L_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad (1)$$

Where  $L_1$ ,  $a^*_1$ ,  $b^*_1$ , and  $L_2$ ,  $a^*_2$ ,  $b^*_2$  refer to the color coordinates of two different experimental groups.

### 2.5. Cooking Characteristics

Cooking yield, moisture retention, and shrinkage were determined [16]. After thawing, samples were weighed and measured before and after cooking ( $250^{\circ}\text{C}$  for 15 min in an electric oven). Calculations were as follows:

$$\text{Cooking yield (\%)} = \frac{\text{cooked weight}}{\text{raw weight}} \times 100.$$

$$\text{Moisture retention (\%)} = \frac{\text{cooked weight} - \text{moisture of cooked burger}}{\text{raw weight} - \text{moisture of raw burger}} \times 100.$$

$$\text{Shrinkage (\%)} = \frac{\text{raw diameter} - \text{cooked diameter}}{\text{raw diameter}} \times 100.$$

### 2.6. Burger Quality Characteristics

Burgers were stored at  $-20^{\circ}\text{C}$  and analyzed at 0, 30, and 60 days. pH was measured using a calibrated portable meat pH meter (Akso Eletronic Products, RS, Brazil). TVB-N was determined using TCA precipitation and titration with standardized sulfuric acid [17].

Lipid oxidation was assessed via peroxide value and TBARS. PV was determined as: lipids were extracted and dissolved (200  $\mu$ L) in benzene: methanol (70:30, v/v), followed by ammonium thiocyanate (10  $\mu$ L) and ferrous chloride (10  $\mu$ L) [18]. Samples were incubated at 50 °C for 2 min, and absorbance was measured at 520 nm. Results were calculated using an iron standard curve (0.7–7.1  $\mu$ mol).

TBARS was evaluated [18]. Samples (1 g) were homogenized in 1.15% KCl, centrifuged (3,000 rpm, 10 min), and 0.75 mL of supernatant was incubated (100 °C, 15 min) with 30% TCA and 0.67% TBA. After cooling, n-butyl alcohol (1.5 mL) was added to extract the colored product. Absorbance was read at 535 nm, and MDA concentration was estimated using a standard curve (0.6–12 nmol).

## 2.7. Statistical Analyses

Physicochemical and cooking data were analyzed by one-way ANOVA. Shelf life parameters (YM concentration  $\times$  storage time) were analyzed by two-way ANOVA. Means were compared by *t*-test for significance at  $p < 0.05$ .

## 3. Results

### 3.1. Physicochemical Composition and Color Characteristics

The proximate composition of rabbit burgers added with yerba mate is shown in Table 1. Crude protein and dry matter content showed variation between rabbit burger formulations. However, this difference is likely due to the fact that the composition was analyzed with reduced and triplicated samples, which reduced data dispersion. Nevertheless, all formulations exhibited low fat content (less than 1%) and high crude protein content (greater than 18%).

It was noticed that before freezing, the color difference between groups is related to the higher concentration of pigments reflecting the green color, indicated by  $a^*$ , which expresses the wavelengths from green ( $-a^*$ ) to red ( $+a^*$ ). However, 90 days after freezing, none of the groups showed a negative  $a^*$  index, meaning there was a reduction of pigments reflecting the green color 90 days after freezing. The higher addition of mate has been associated with a greater perception of the green spectrum in rabbit burgers. The  $b^*$ , which expresses the wavelengths from blue ( $-b^*$ ) to yellow ( $+b^*$ ), was influenced by the incorporation of YM. The higher addition of YM (2%) has been associated with a greater reflection of the positive  $b^*$  index, or yellow. The  $L^*$  (lightness) did not vary between formulations, indicating that there were no perceptible differences in lightness/darkness reflected by the formulations. However, the  $L^*$  index increased in all groups 90 days after freezing.

**Table 1.** Proximate composition and color parameters of rabbit burgers added yerba mate (YM).

Parameter	Formulations				
	0%YM	0.5%YM	1.0%YM	1.5%YM	2.0%YM
Protein (%)	19.98 $\pm$ 0.66 <sup>a</sup>	18.25 $\pm$ 0.34 <sup>b</sup>	18.50 $\pm$ 0.43 <sup>a,b</sup>	19.18 $\pm$ 0.72 <sup>ab</sup>	19.27 $\pm$ 0.66 <sup>ab</sup>
Dry matter (%)	32.84 $\pm$ 0.23 <sup>ab</sup>	32.26 $\pm$ 0.02 <sup>b</sup>	33.50 $\pm$ 0.23 <sup>a</sup>	32.36 $\pm$ 0.23 <sup>b</sup>	33.57 $\pm$ 0.12 <sup>a</sup>
Ash (%)	2.15 $\pm$ 0.04	2.13 $\pm$ 0.10	2.19 $\pm$ 0.05	2.25 $\pm$ 0.13	2.30 $\pm$ 0.29
Fat (%)	0.85 $\pm$ 0.08	0.79 $\pm$ 0.05	0.89 $\pm$ 0.13	0.73 $\pm$ 0.06	0.80 $\pm$ 0.12
Color at 0 frozen days					
$a^*$	13.42 $\pm$ 7.96	4.83 $\pm$ 5.66	1.12 $\pm$ 10.5	-5.08 $\pm$ 7.45	-9.94 $\pm$ 10.23
$b^*$	66.42 $\pm$ 23.84	55.52 $\pm$ 14.57	58.23 $\pm$ 24.02	47.97 $\pm$ 21.02	43.16 $\pm$ 24.21
$L^*$	29.99 $\pm$ 4.53	31.11 $\pm$ 4.56	27.10 $\pm$ 8.95	27.61 $\pm$ 7.87	23.19 $\pm$ 9.02
X					
		0.5	1.0	1.5	2.0

$\Delta E_{0\%MYM-x}$	-	13.92	15.06	26.24	33.67
$\Delta E_{0.5\%MYM-x}$	-	-	6	12.94	20.83
$\Delta E_{1.0\%MYM-x}$	-	-	-	12	19.10
$\Delta E_{1.5\%MYM-x}$	-	-	-	-	8.14
$\Delta E_{2.0\%MYM-x}$	-	-	-	-	-
Color at 60 frozen days					
a*	12.80±0.82	10.75±0.58	9.64±0.57	8.20±0.44	6.54±0.83
b*	22.12±2.35	21.89±1.61	23.10±1.60	22.00±0.95	21.64±2.12
L*	41.08±1.87	40.09±1.87	38.94±1.21	37.86±0.86	37.11±2.18
X					
		0.5	1.0	1.5	2.0
$\Delta E_{0\%MYM-x}$	-	2.25	3.93	5.61	7.42
$\Delta E_{0.5\%MYM-x}$	-	-	2.04	3.43	5.20
$\Delta E_{1.0\%MYM-x}$	-	-	-	2.10	3.88
$\Delta E_{1.5\%MYM-x}$	-	-	-	-	1.85

Data expressed as mean  $\pm$  standard deviation (n=3). +CP = crude protein. †0%YM = addition of 0% yerba mate; 0.5%YM = addition of 0.5% of yerba mate; 1.0%MYM = addition of 1.0% yerba mate; 1.5%YM = addition of 1.5% of yerba mate; 2.0%YM = addition of 2.0% of yerba mate. Values followed by different letters indicate statistical difference between formulations by *t-test* ( $p < 0.05$ ).

### 3.2. Cooking Characteristics

Cooking yield did not differ among formulations. Shrinkage values varied from 8.51% in the control group to 15.70% in the 0.5% YM group. Although an initial trend toward increased shrinkage was observed with low levels of YM inclusion, no significant differences were detected across treatments.

Moisture retention was significantly influenced by YM addition. The 0.5% and 1.0% YM groups demonstrated the highest moisture retention ( $67.36 \pm 1.46\%$  and  $65.29 \pm 1.02\%$ , respectively), whereas the control and 2.0% YM groups exhibited lower values. These findings suggest that moderate concentrations of yerba mate may enhance water-holding capacity during cooking, potentially through interactions between polyphenolic compounds and meat proteins.

**Table 2.** Cooking characteristics of rabbit burgers added with yerba mate (YM).

Parameter	0%YM	0.5%YM	1.0%YM	1.5%YM	2.0%YM
Cooking yield (%)	73.78 $\pm$ 3.00	76.98 $\pm$ 1.67	76.11 $\pm$ 1.19	75.82 $\pm$ 0.87	72.22 $\pm$ 1.76
Shrinkage (%)	8.51 $\pm$ 5.69	15.70 $\pm$ 3.15	12.15 $\pm$ 2.57	9.82 $\pm$ 1.21	10.45 $\pm$ 4.38
Moisture retention (%)	61.38 $\pm$ 2.50 <sup>bc</sup>	67.36 $\pm$ 1.46 <sup>a</sup>	65.29 $\pm$ 1.02 <sup>ab</sup>	62.96 $\pm$ 0.72 <sup>bc</sup>	60.87 $\pm$ 1.49 <sup>c</sup>

Data are expressed as mean  $\pm$  standard deviation (n = 3). Different superscript letters in the same row indicate significant differences between formulations according to *t-test* ( $p < 0.05$ ).

### 3.3. Shelf Life

The shelf life of the rabbit burgers was assessed through physicochemical analyses that evaluated product freshness over frozen storage. Lipid oxidation progression during frozen storage was assessed via peroxide value (Table 3). Peroxide levels increased over time in all formulations.

After 30 days, no differences were detected among treatments, despite a general increase in peroxide levels. At 60 days, the highest peroxide value was observed in the 2.0% YM formulation

( $6.01 \pm 0.38$  mEq/kg fat), which was significantly higher than the control ( $4.07 \pm 0.13$  mEq/kg fat) and other intermediate YM concentrations. Differences were observed both between formulations at each time point and across storage times within the same formulation (Table 3).

**Table 3.** Peroxide values of rabbit burgers added yerba mate (YM) after frozen storage.

Formulations	Frozen days		
	0	30	60
0% YM	$0.92 \pm 0.21^{Cc}$	$3.31 \pm 0.51^{Ba}$	$4.07 \pm 0.13^{Ad}$
0.5% YM	$1.07 \pm 0.45^{Cc}$	$3.46 \pm 0.21^{Ba}$	$4.30 \pm 0.32^{Accd}$
1.0% YM	$2.16 \pm 0.30^{Cab}$	$3.68 \pm 0.49^{Ba}$	$4.69 \pm 0.56^{Abc}$
1.5% YM	$1.84 \pm 0.43^{Cb}$	$3.27 \pm 0.20^{Ba}$	$5.23 \pm 0.17^{Ab}$
2.0% YM	$2.78 \pm 0.47^{Ca}$	$3.58 \pm 0.23^{Ba}$	$6.01 \pm 0.38^{Aa}$

Data expressed as mean  $\pm$  standard deviation (n=3, mEq/Kg fat). 0%YM = addition of 0% YM; 0.5%YM = addition of 0.5% of YM; 1.0%YM = addition of 1.0% YM; 1.5%YM = addition of 1.5% of YM; 2.0%YM = addition of 2.0% of YM. Lower case letters, in the column, indicate statistical difference between formulations within the same storage time by *t-test* ( $p < 0.05$ ). Upper case letters, in the line, indicate statistical difference for each formulation over the storage time by *t-test* ( $p < 0.05$ ).

The secondary products of lipid oxidation, expressed as TBARS (mg MDA/kg), are shown in Table 4. TBARS values progressively increased throughout storage for all formulations. After 30 days, no differences were observed among treatments. At 60 days, the 1.0% YM group exhibited the highest TBARS value ( $5.41 \pm 0.80$  mg MDA/kg), while the 1.5% YM formulation showed the lowest value ( $3.71 \pm 0.27$  mg MDA/kg), suggesting a potential protective effect at this concentration.

**Table 4.** TBARS values of rabbit burgers added YM after frozen storage.

Formulations	Frozen days		
	0	30	60
0% YM	$0.73 \pm 0.20^{Cbc}$	$4.06 \pm 0.31^{Ba}$	$4.86 \pm 0.34^{Aab}$
0.5%YM	$1.18 \pm 0.37^{Bb}$	$3.72 \pm 0.59^{Aa}$	$4.36 \pm 0.43^{Aab}$
1.0% YM	$0.96 \pm 0.30^{Cbc}$	$3.66 \pm 0.50^{Ba}$	$5.41 \pm 0.80^{Aa}$
1.5% YM	$0.65 \pm 0.20^{Bc}$	$3.41 \pm 0.44^{Aa}$	$3.71 \pm 0.27^{Ab}$
2.0% YM	$3.56 \pm 0.26^{Ba}$	$3.61 \pm 0.51^{Ba}$	$4.38 \pm 0.34^{Aab}$

Data expressed as mean  $\pm$  standard deviation (n=3, mg MDA/Kg sample). 0%MYM = addition of 0% yerba mate; 0.5%MYM = addition of 0.5% of yerba mate; 1.0%MYM = addition of 1.0% yerba mate; 1.5%MYM = addition of 1.5% of yerba mate; 2.0%MYM = addition of 2.0% of yerba mate. Lower case letters, in the column, indicate statistical difference between formulations within the same storage time by *t-test* ( $p < 0.05$ ).

A general increasing trend in pH was observed over the 60-day storage period in all formulations, with significant differences noted both over time and between formulations (Table 5). On day 0, no differences were observed between formulations ( $p > 0.05$ ), with pH values ranging narrowly from  $5.65 \pm 0.01$  (2.0% YM) to  $5.68 \pm 0.01$  (control). However, starting from day 30, the pH values increased in most formulations. The 0.5% YM group reached the highest pH value ( $5.90 \pm 0.02$ ) on day 60, while the 2.0% YM formulation maintained the lowest pH throughout the entire storage period, ending at  $5.79 \pm 0.01$ . These variations in pH may reflect metabolic changes, enzymatic activity, or microbial dynamics during storage.

**Table 5.** pH values of rabbit burgers added YM after frozen storage.

Formulations	Frozen days		
	0	30	60
0% YM	5.68±0.01 <sup>Ba</sup>	5.77±0.01 <sup>Aab</sup>	5.85±0.05 <sup>Aab</sup>
0.5% YM	5.66±0.02 <sup>Ca</sup>	5.80±0.1 <sup>Ba</sup>	5.90±0.02 <sup>Aa</sup>
1.0% YM	5.67±0.01 <sup>Ca</sup>	5.76±0.02 <sup>Bab</sup>	5.83±0.02 <sup>Aab</sup>
1.5% YM	5.67±0.01 <sup>Ca</sup>	5.77±0.02 <sup>Bab</sup>	5.81±0.005 <sup>Ab</sup>
2.0% YM	5.65±0.01 <sup>Ca</sup>	5.74±0.01 <sup>Bb</sup>	5.79±0.01 <sup>Ab</sup>

Data expressed as mean ± standard deviation (n=3). 0%YM = addition of 0% yerba mate; 0.5%YM = addition of 0.5% of yerba mate; 1.0%YM = addition of 1.0% yerba mate; 1.5%YM = addition of 1.5% of yerba mate; 2.0%YM = addition of 2.0% of yerba mate. Lower case letters, in the column, indicate statistical difference between formulations within the same storage time by *t-test* ( $P < 0.05$ ). Upper case letters, in the line, indicate statistical difference for each formulation over the storage time by *t-test* ( $P < 0.05$ ).

No differences were observed among formulations at each storage time or within the same formulation over time in the total volatile basic nitrogen (TVB-N) content (Table 6). At day 0, values ranged from  $6.85 \pm 1.22$  mg N/100 g (2.0% YM) to  $9.95 \pm 1.17$  mg N/100 g (1.5% YM). The control group exhibited the highest value on day 30 ( $16.01 \pm 5.64$  mg N/100 g), followed by a reduction by day 60 ( $11.93 \pm 0.16$  mg N/100 g). Across all formulations, TVB-N remained within acceptable limits throughout frozen storage, indicating no substantial protein degradation during the evaluated period.

**Table 6.** Total volatile basic nitrogen values of rabbit burgers added yerba mate before and after frozen storage.

Formulations <sup>†</sup>	Frozen days		
	0	30	60
0% YM	8.60±2.45 <sup>Aa</sup>	16.01±5.64 <sup>Aa</sup>	11.93±0.16 <sup>Aa</sup>
0.5% YM	9.40±0.83 <sup>Aa</sup>	12.01±1.20 <sup>Aa</sup>	11.61±0.16 <sup>Aa</sup>
1.0% YM	8.72±0.24 <sup>Ba</sup>	11.44±0.02 <sup>Aa</sup>	10.86±0.67 <sup>Aa</sup>
1.5% YM	9.95±1.17 <sup>Aa</sup>	9.88±0.58 <sup>Aa</sup>	10.32±0.80 <sup>Aa</sup>
2.0% MM	6.85±1.22 <sup>Aa</sup>	10.87±1.66 <sup>Aa</sup>	10.18±0.24 <sup>Aa</sup>

Data expressed as mean ± standard deviation (n=3). 0%YM = addition of 0% yerba mate; 0.5%YM = addition of 0.5% of yerba mate; 1.0%YM = addition of 1.0% yerba mate; 1.5%YM = addition of 1.5% of yerba mate; 2.0%YM = addition of 2.0% of yerba mate. Lower case letters, in the column, indicate statistical difference between formulations within the same storage time by *t-test* ( $P < 0.05$ ). Upper case letters, in the line, indicate statistical difference for each formulation over the storage time by *t-test* ( $P < 0.05$ ).

#### 4. Discussion

The minor variations observed in crude protein and dry matter content among formulations may be attributed to the limited replication ( $n = 3$ ), as previously noted. Nevertheless, even with the addition of up to 2% yerba mate (YM), the formulations maintained a low fat content (<1%) and high protein content (>18%), both desirable characteristics for functional meat products. These findings are consistent with previous studies that evaluated the use of YM as a natural additive in meat products [19,20].

When compared to similar YM formulations applied to panga fish burgers, those products exhibited higher fat content, lower protein levels, and comparable dry matter and ash values [20]. Rabbit meat contains approximately 20.70–21.70% crude protein and 1.05–5.55% lipids, which is consistent with the values obtained in this study [21,22]. The lower lipid content observed compared

to earlier studies may be attributed to advancements in genetic selection and a reduction in slaughter age, both aimed at minimizing intramuscular fat deposition.

The influence of YM on color (negative  $a^*$  values prior to freezing and increased  $L^*$  values over time) aligns with previous reports, as plant extracts rich in chlorophyll can alter the chromatic profile of meat products. Similar behavior was observed in fish burgers enriched with YM, where  $a^*$  values decreased and  $L^*$  values increased following frozen storage [20].

The  $\Delta E$  value of 2.3 units represents the smallest color difference perceptible to the human eye [22]. In the present study, all formulations exhibited perceptible color differences prior to freezing, with the greatest difference observed between the control and the 2% YM group ( $\Delta E = 33.67$  units). After 90 days of frozen storage, perceptible color differences between treatments decreased, with visible differences remaining only between groups with YM concentration differences greater than 0.5% ( $\Delta E > 2.3$  units) (Table 1).

The higher moisture retention observed at 0.5% and 1.0% YM levels suggests possible interactions between YM polyphenols and muscle proteins, enhancing water-holding capacity during cooking. Similar effects have been reported in chicken burgers enriched with plant extracts, where bioactive compounds interact with proteins, improving both texture and moisture retention [23]. This phenomenon occurs as hydroxyl groups in polyphenols form hydrogen bonds with proteins, modifying their structure and increasing hydrophilicity, thereby promoting water retention. Additionally, non-covalent interactions between polyphenols and proteins strengthen the protein network, reducing water loss during thermal processing.

Regarding shrinkage, rabbit burgers formulated with YM exhibited relatively higher shrinkage values compared to other products, such as panga fish burgers enriched with yerba mate (1.01% to 6.59%) [20], beef burgers (10.5% to 18.7%) [24], and chicken burgers (8.3% to 13.6%) [25].

Peroxide formation was not inhibited by YM addition, as peroxide levels were similar to or even higher than those observed in the control formulation. The biphasic behavior observed—no effect on primary oxidation (peroxide formation), but reduction in secondary oxidation (TBARS)—may be explained by reaction kinetics. YM polyphenols may act preferentially during the secondary oxidation phase by neutralizing free radicals and inhibiting the formation of reactive compounds.

Based on the results, yerba mate at 1.5% to 2.0% did not prevent primary lipid oxidation. However, after 30 days of storage, a protective effect became evident, confirmed by reduced secondary oxidation as demonstrated by the progressive decrease in TBARS values (Table 4). This antioxidant effect may be attributed to the presence of chlorogenic acids in yerba mate, which have been extensively studied for their radical-scavenging and metal-chelating capacities [7,20].

Previous studies evaluating yerba mate as an antioxidant in panga fish burgers reported TBARS reduction at inclusion levels of 1% and 1.5%, although such reductions were only observed after 120 days of storage [20]. In contrast, the present study demonstrated a faster onset of lipid stabilization in rabbit meat formulations. On day 0, the control group exhibited the lowest lipid oxidation levels; however, after 60 days of frozen storage, the 1.5% YM group showed the lowest TBARS values, indicating superior lipid oxidative stability.

The presence of phenolic compounds, flavonoids, and other molecules such as caffeine, rutin, and chlorogenic acid in yerba mate contributes to its well-documented antioxidant activity [8]. Additionally, YM extracts exhibit greater antioxidant capacity than oregano, thyme, and rosemary extracts when tested in ethanol/water (20:80, v/v) solutions [10].

Regarding freshness indicators, pH and total volatile basic nitrogen (TVB-N), including ammonia, trimethylamine, and dimethylamine, are key parameters used in meat quality assessments [26]. In the present study, pH values ranged from 5.65 to 5.90 (Table 5), without significant variation. However, the 2.0% YM formulation consistently exhibited the lowest pH values across all storage periods (days 0, 30, and 60). In rabbit meat products, acceptable pH values typically range from 5.2 to 6.8 [27].

The lower pH values observed in YM-enriched formulations may be associated with the antimicrobial properties of phenolic compounds, which can suppress the formation of alkaline

byproducts, such as ammonia and other nitrogenous bases, thereby limiting pH increases during storage [28].

A favorable response was observed after 30 days of frozen storage in the burgers containing 1.5% YM, as indicated by the TVB-N values presented in Table 6. Previous studies have reported a correlation between increased pH and elevated TVB-N, as both are influenced by similar spoilage mechanisms [29,30]. TVB-N is widely used as an indicator of meat freshness, based on the quantification of volatile amines (trimethylamine, dimethylamine, and ammonia), which are formed as a result of protein degradation during microbial spoilage [31].

In poultry meat, TVB-N levels typically increase progressively during storage, reflecting ongoing degradation of glycogen, protein, and lipids [32]. Initially, microbial growth is supported by the availability of these substrates; as degradation progresses, volatile bases, hypoxanthine, organic acids, and biogenic amines are released, contributing to the loss of meat quality.

In panga fish burgers enriched with micronized yerba mate, a positive effect of the 2% YM treatment on TVB-N was observed after 30 days of frozen storage, where an increase in values was recorded [20]. In the present study, although no significant statistical differences were found between treatments, an increase in TVB-N from day 0 to day 30 was noted in the 0.5%, 1.0%, and 2.0% formulations, whereas the 1.5% YM group showed a slight decrease, suggesting a potential stabilizing effect.

## 5. Conclusions

The incorporation of yerba mate (YM) into rabbit burgers demonstrated selective functional effects on product stability during frozen storage. Although the addition of YM up to 2.0% did not prevent primary lipid oxidation, its inclusion—particularly at 1.5%—significantly reduced secondary lipid oxidation and contributed to protein stabilization, as evidenced by lower TBARS and TVB-N values. Furthermore, moderate concentrations of YM (0.5–1.0%) improved moisture retention during cooking, without compromising color parameters or overall physicochemical quality.

These findings support the potential of yerba mate as a natural antioxidant for application in functional meat products, contributing to shelf life extension and improved nutritional quality. The observed effects may be attributed to the presence of polyphenols and bioactive compounds with antioxidant and antimicrobial properties. Future studies should explore the specific mechanisms of action and assess the impact of YM on sensory attributes and gastrointestinal health outcomes, reinforcing its role in dietary interventions aimed at promoting functional and clean-label meat products.

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## Abbreviations

The following abbreviations are used in this manuscript:

YM Yerba mate (*Ilex paraguariensis*)

TBARS Thiobarbituric acid reactive substances  
 TVB-N Total volatile basic nitrogen

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