



Effects of *Hibiscus rosa-sinensis* flower powder as a natural colorant and antioxidant on the physicochemical and sensory properties of frozen lamb burgers

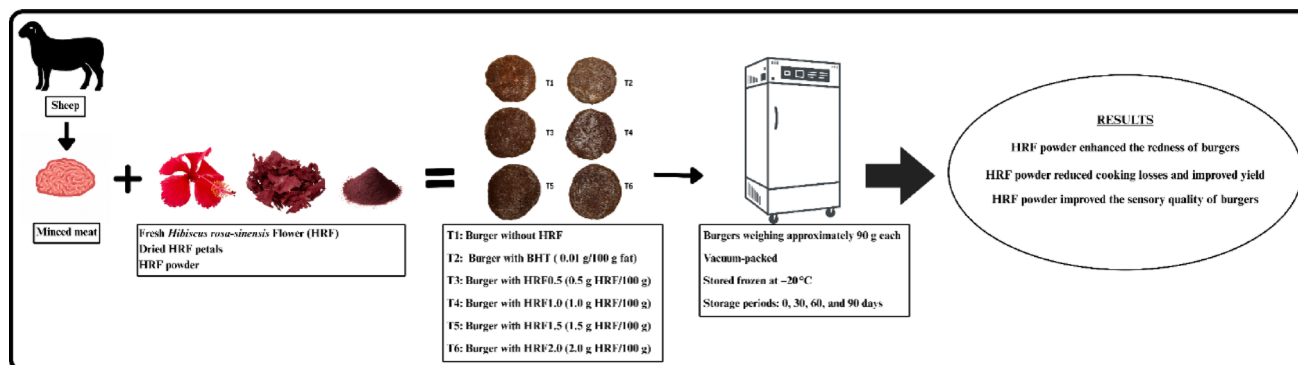
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Abstract

The demand for natural ingredients in meat products has increased due to consumer concerns regarding the safety of synthetic additives. Plant-derived compounds with antioxidant and coloring properties represent promising alternatives for clean-label formulations. This study evaluated the use of *Hibiscus rosa-sinensis* flower (HRF) powder as a natural colorant and antioxidant in frozen lamb burgers. Six treatments were tested: control, BHT, and four HRF levels (0.5, 1.0, 1.5, and 2.0 g/100 g). Burgers were stored at -20°C for up to 90 days and analyzed for physicochemical, textural, and sensory properties. HRF affected instrumental color ($P<0.001$), increasing redness and hue angle. pH was not affected by treatments ($P=0.195$), and although no differences were observed for TBARS ($P=0.784$), the values remained below the threshold for sensory detection in all treatments. Cooking weight loss was reduced in the HRF1.5 and HRF2.0 treatments ($P=0.0006$). HRF0.5 received higher scores for tenderness ($P<0.001$) and juiciness ($P=0.001$) compared to the control, and was preferred in appearance ($P=0.033$) over BHT. These findings indicate that HRF powder can enhance key technological and sensory attributes in lamb burgers and may be used as a clean-label alternative to synthetic additives.

Graphical abstract



Highlights

- *Hibiscus rosa-sinensis* flower (HRF) powder was applied in lamb burger production.
- HRF powder enhanced the red color of lamb burgers.
- HRF powder reduced cooking losses and increased yield.
- HRF powder improved sensory acceptance of lamb burgers.

Keywords Additive-free · Clean label · Cooking losses · Meat derivatives · Lipid oxidation

Extended author information available on the last page of the article

Abbreviations

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Official Method Chemists Society
BHT	Butylated hydroxytoluene
DPPH	2,2-difenil-1-picrilhidrazil
EC50	Concentration inhibiting 50% of the DPPH radical
EtOH	Ethanol extract
MDA	Malonaldehyde
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TPA	Texture profile analysis
UV	Ultraviolet
HRF	Hibiscus rosa-sinensis flower
HS	Hibiscus sabdariffa

Introduction

Oxidative reactions are among the primary factors responsible for the quality deterioration of meat and meat products, leading to reduced shelf life and nutritional value. These reactions promote undesirable changes in color, flavor, aroma, and texture, ultimately diminishing consumer acceptance and limiting marketability (Borella et al. 2019). To counter these effects, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate have been widely incorporated into meat formulations to delay lipid and protein oxidation and preserve sensory attributes. However, growing consumer concerns regarding the potential toxicity and carcinogenicity of synthetic additives have prompted a shift toward safer, naturally derived alternatives (Esazadeh et al. 2024).

In this context, plant-derived antioxidants have garnered significant attention due to their abundance of bioactive compounds, particularly polyphenols, and their proven efficacy in enhancing the oxidative stability of meat products. Among these, *Hibiscus sabdariffa* (HS) has been extensively studied for its antioxidant and antimicrobial properties, as well as for its intense red pigmentation, which contributes to the visual appeal of meat products (Bermúdez et al. 2023; Feridoni and Shurmasti 2020). Although HS extracts have demonstrated potential to extend shelf life and improve food safety, their pronounced acidity may negatively impact sensory acceptability in meat systems (Perez-Báez et al. 2021; Santos et al. 2022). Moreover, incorporating HS flower purée or powder as natural antioxidants in burger formulations has also been associated with reduced pH and increased cooking losses (Bermúdez et al. 2023; Ismail et al. 2022).

An alternative species with similar biofunctional properties is *Hibiscus rosa-sinensis* flower (HRF), a plant traditionally cultivated for medicinal and ornamental purposes (Thakre and Barse 2018). HRF petals are rich in phenolic compounds, particularly flavonoids, with well-documented antioxidant activity and health-promoting effects (Saeed et al. 2025). Furthermore, the intense red hue of its petals highlights its potential as a natural colorant in food systems (Hayat and Jacob 2019). Since color is a critical organoleptic attribute influencing consumer perception and purchase intent, especially in meat products, where it is closely associated with freshness and quality, natural ingredients that combine antioxidant functionality with color-enhancing properties are of great interest to the meat industry (Cömert et al. 2020).

Despite the recognized antioxidant potential of HRF, no studies to date have investigated the use of its powder as a functional additive in meat products. We hypothesized that HRF powder could serve as a dual-purpose ingredient, acting as both a natural antioxidant and colorant in the formulation of lamb burgers, thus replacing synthetic additives while improving consumer acceptability.

Therefore, the objective of this study was to evaluate the effects of HRF petal powder on the physicochemical and sensory properties of frozen lamb burgers.

Materials and methods

Methods

Experiment location

The experiment was conducted at the Agricultural Sciences Campus of the Federal University of the São Francisco Valley (CCA–UNIVASF), Petrolina, Pernambuco, Brazil.

Collection and botanical identification of hibiscus flower

HRF was collected at the CCA–UNIVASF (coordinates: 9°19'30" S, 40°33'39" W; 349 m above sea level). The plant was identified by comparison with specimens from the collection of the Center for Ecology and Environmental Monitoring (NEMA), exsiccate no. 11,548.

Hibiscus flower petal powder production

Fresh HRF petals were manually selected, with any damaged or discolored parts discarded to ensure uniformity and raw material quality. The petals were washed with distilled water to remove surface impurities, and excess moisture was carefully blotted with paper towels. The material was

then spread in a single layer on stainless steel trays and subjected to natural drying at room temperature (approximately 25 ± 2 °C) for 48 h under controlled ventilation and low humidity to prevent microbial growth, compound volatilization, and color degradation.

After drying, the petals were ground using a domestic blender (Philco, All In One, Manaus, AM, Brazil) until a fine and homogeneous powder was obtained. To standardize particle size, the resulting powder was passed through a 60-mesh stainless steel sieve. HRF powder was immediately vacuum-sealed in polyethylene bags and stored at -20 °C, protected from light until use in burger production.

Lamb burger production formulated with HRF powder

To produce the lamb burgers, 25 kg of meat from wether lambs obtained from an officially inspected slaughterhouse was used. The previously frozen meat (-20 °C) was cut into 5×5 cm cubes using a band saw and then ground with an 8 mm cutting disc in an industrial meat grinder.

The meat and all ingredients corresponding to each treatment were weighed individually. Burger production was performed in three independent replicates. The meat mass was manually mixed with the ingredients of the basic formulation (BF) of the burgers (95% lamb meat, 1% oregano, 1.25% onion powder, 1.25% garlic powder, and 1.5% salt). The control treatment consisted only of the BF (without antioxidant). From this BF, five additional treatments were formulated: BHT (BF+0.01 g BHT/100 g fat; Adicel, Belo Horizonte, MG, Brazil), HRF0.5 (BF+0.5 g HRF/100 g), HRF1.0 (BF+1.0 g HRF/100 g), HRF1.5 (BF+1.5 g HRF/100 g), and HRF2.0 (BF+2.0 g HRF/100 g). The BHT concentration followed the maximum limit for additives in frozen meat products established by Brazilian regulations (0.01 g/100 g of fat).

A total of 250 lamb burgers, each weighing approximately 90 g, were produced. All burgers were vacuum-sealed, labeled, and stored frozen at -20 °C for different periods (0, 30, 60, and 90 days).

Analytical methods

Chemical composition of HRF powder

Chemical composition analyses were performed according to the official methods described by the Association of Official Analytical Chemists (AOAC 2016), including the determination of moisture (Method 967.03), ash (Method 942.05), crude protein (Method 981.10), and dietary fiber (Method 985.29). Fat content was determined using

ANKOM XT4 filter bags and an ANKOM XT15 fat extractor (ANKOM Technology, Macedon, NY, USA), which uses petroleum ether as the solvent, following the Am 5–04 protocol established by the American Oil Chemists' Society (AOCS 2017). Carbohydrates were estimated by difference, as described by Sousa et al. (2014).

pH and instrumental color of HRF powder

HRF was placed in a 100×20 mm Petri dish for pH and instrumental color analyses. For pH measurement, the probe was inserted directly into the powder at three different points using a portable digital pH meter (TESTO-205, Testo SE & Co. KGaA, Campinas, São Paulo, Brazil). For colorimetric analysis, a colorimeter (Konica® Minolta, model CR-400, Osaka, Japan) was used. The CIELAB system was applied, considering the parameters L^* , a^* , and b^* , with illuminant C and a 10° standard observer. Readings were taken at three different points.

Phytochemical profile of the crude extract of HRF powder

To obtain the crude extract, 40 g of HRF were diluted in 200 mL of an alcoholic solution (160 mL ethanol and 40 mL Milli-Q water), under light-protected conditions. The mixture was centrifuged at 120 rpm for 120 min, and the supernatant was subjected to ultrasonic homogenization (40 kHz for 60 min) using an ultrasonic bath (Unique, Maxi-Clean 1400 A, Indaiatuba, SP, Brazil). The extract was then filtered under vacuum (Primatec, model 132, Type 2VC, Itu, SP, Brazil), evaporated in a rotary evaporator (50 °C for 120 min; Tecnal, TE 211, Piracicaba, SP, Brazil), and dried in a forced-air oven (50 °C for 12 h).

For qualitative phytochemical analysis, the HRF extract was applied to silica gel plates (5×6 cm) using a glass capillary tube for analytical thin-layer chromatography (TLC; Macherey-Nagel, Düren, Germany). The extract was then eluted and developed in solvent systems specific to each class of secondary metabolites, and the plates were visualized under ultraviolet (UV) light at 254 and 365 nm (Wagner and Bladt 1996).

Antioxidant activity of the crude extract of HRF powder

The antioxidant activity was evaluated based on the scavenging of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma Chemical Co., St. Louis, MO, USA), according to Mensor et al. (2001). Samples of the HRF extract and a control prepared with BHT (1.0 mg/mL) were diluted in ethanol at concentrations of 121, 100, 81, 60, 40, and 20 µg/

mL. A 1 mL aliquot of DPPH ethanolic solution (50 µg/mL) was added to 2.5 mL of each solution, at room temperature and protected from light. After 30 min, absorbance was measured at 518 nm and converted into percentage of antioxidant activity (%AA) using the following formula: %AA = [(absorbance of the control - absorbance of the sample) / absorbance of the control] × 100.

Ethanol (1.0 mL)+extract (2.5 mL) solutions were used as blanks. DPPH (1.0 mL)+ethanol (2.5 mL) was used as the negative control. BHT was used as the positive control. All assays were performed in triplicate. EC₅₀ values (the concentration required to inhibit 50% of the DPPH radical) were calculated by linear regression using GraphPad Prism 5.0 software, where the x-axis represented the concentration.

Instrumental color, pH, and lipid oxidation in lamb burgers containing HRF powder

Analyses of pH, instrumental color, and lipid oxidation were conducted on frozen lamb burgers (−20 °C) at 0, 30, 60, and 90 days of storage. Before analysis, the samples were thawed at room temperature for 1 h.

Instrumental color was measured using the same equipment and settings previously described for the HRF powder analysis. The burgers were placed on white acrylic plates and exposed to ambient oxygen for 30 min to allow blooming and stabilize surface color. The L*, a*, and b* parameters were used to calculate total color difference (ΔE), hue angle (H*), chroma (C*), and browning index (BI) according to Sariçoban and Yılmaz (2010), using the following equations:

$$\Delta E = \left[\sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \right]; C^* = \sqrt{a^* + b^*};$$

$$H^* = \tan^{-1} (b^* / a^*); BI = 100 / 0.17^*$$

$$((a^* + 1.75L^*) / (5.645L^* + a^* - 0.012b^*)) - 0.31$$

pH was measured using a portable digital pH meter (TESTO-205, Testo SE & Co. KGaA, Campinas, São Paulo, Brazil), with readings taken at three different points on each burger.

Lipid oxidation was determined using the thiobarbituric acid reactive substances (TBARS) assay, following Tarladgis et al. (1960). Ten grams of sample were homogenized with 50 mL of distilled water for 2 min using an Ultra-Turax digital disperser (T25, Campinas, São Paulo, Brazil). The homogenate was distilled with 2.5 mL of 4 N HCl and 47.5 mL of distilled water until 50 mL of distillate was collected. A 5 mL aliquot of the distillate was mixed with 5 mL of TBA solution, sealed, and incubated in a water bath at 96 °C for 40 min. After cooling, absorbance was measured at 532 nm using a spectrophotometer (Nova Instruments, NI 1248, Piracicaba, São Paulo, Brazil).

Chemical composition of lamb burgers containing HRF powder

The moisture, ash, protein, and fat contents of the lamb burgers were determined using the same methods previously described for the chemical characterization of HRF powder (AOAC 2016; AOCS 2017).

Water holding capacity, cooking losses, and shrinkage in HRF-enriched lamb burgers

Water holding capacity (WHC) was determined by placing 0.5 g of burger sample on filter paper, pressing it between two acrylic plates, and applying a 5 kg weight for 5 min. WHC was calculated based on the weight difference before and after pressing.

Cooking weight loss (CWL) was assessed by weighing the burger before and after cooking on a hot plate (Grill Mondial Redondo Smart Grill G-04, Barueri, São Paulo, Brazil), flipping them every 2 min until an internal temperature of 72 ± 2 °C was reached. Temperature was monitored using a type K thermocouple (HI935005, Hanna, Póvoa de Varzim, Portugal). After cooling to room temperature, samples were reweighed.

Burger diameter was measured before and after cooking using a digital caliper (316119-MTX, São Paulo, Brazil) to determine cooking shrinkage.

Texture profile of HRF-formulated lamb burgers

Texture profile analysis was performed using a texture analyzer (TA-TX2, Stable Micro Systems, version 1.1.12.0, Surrey, UK). Burgers were cooked as previously described, cooled to room temperature, and cut into 2 cm² cubes. Each cube underwent two consecutive compression cycles, reaching 50% of its original height, using a 20 mm diameter cylindrical probe. The test conditions were as follows: pre-test speed of 1 mm/s; test and post-test speed of 2 mm/s; compression distance of 5 mm; 3 s between cycles; and a trigger force of 2 g. The parameters of hardness, elasticity, chewiness, and cohesiveness were calculated from the force–time curves (Bourne 2002).

Microbiological quality of lamb burgers prepared with HRF powder

To ensure the safety of the lamb burgers prior to sensory evaluation, microbiological tests were performed according to Silva et al. (2017). For each analysis, 25 g of burger sample was homogenized with 225 mL of buffered peptone water and serially diluted to 10^{−3}. The samples were inoculated into specific culture media for the detection and

enumeration of microorganisms: *Salmonella* spp. (buffered peptone water, Rappaport broth, XLD agar); total and thermotolerant coliforms and *Escherichia coli* (lactose broth, E.C. broth, EMB agar); total mesophilic aerobic bacteria (PCA agar); and *Staphylococcus* spp. (Baird-Parker agar). All samples were free of *Salmonella* spp. and exhibited bacterial counts within the limits established by Brazilian legislation, confirming their suitability for sensory analysis.

Sensory evaluation of lamb burgers containing HRF powder

This study was approved by the Research Ethics Committee of UNIVASF (Protocol No. 76330820.1.0000.8052). Sensory evaluation was conducted with 62 untrained panelists, all over 18 years of age, who provided informed consent prior to participation. Burgers were cooked on an electric grill to an internal temperature of 72 °C (16 min total; 8 min per side), cut into 2 cm³ cubes (approximately 20 g), and kept in a water bath at 55 °C until serving. Each panelist received coded samples from all treatments, labeled with random three-digit numbers. Appearance, color, aroma, tenderness, flavor, and juiciness were evaluated using a 9-point hedonic scale (1 = “extremely disliked,” 9 = “extremely liked”). Purchase intent was assessed using a 5-point scale (1 = “certainly would not buy,” 5 = “certainly would buy”).

Statistical analysis

Data were analyzed using the PROC MIXED procedure of the Statistical Analysis System (SAS, version 9.1). Instrumental color, lipid oxidation, and pH were evaluated considering treatments, storage times, and their interaction as fixed effects, with replicates treated as random effects. For EC₅₀, chemical composition, WHC, CWL, shrinkage, and texture profile, only the treatment effect was analyzed. Analysis of variance (ANOVA) was performed, and when significant differences were detected ($P < 0.05$), means were compared using Tukey's test. Sensory analysis data were evaluated using RStudio (version 2021.09.0+351) with the Friedman test ($P \leq 0.05$), followed by the Dunn–Bonferroni post hoc test for multiple comparisons.

Results and discussion

Chemical composition, pH, and instrumental color of HRF powder

HRF powder showed a high carbohydrate content, moderate fiber content, and low fat content. Regarding pH, HRF exhibited low acidity. In terms of instrumental color, HRF

Table 1 Chemical composition, pH and color parameters of Hibiscus rosa-sinensis flower powder

Parameter	Value
<i>Chemical composition (g/100 g)</i>	
Moisture	13.11
Ash	5.55
Protein	6.41
Lipids	2.85
Fiber	14.65
Carbohydrates	72.08
pH	6.06
<i>Color parameters (CIELAB)</i>	
L* (Lightness)	33.25
a* (Redness)	11.85
b* (Yellowness)	5.67
g: gram	

Table 2 Main phytochemical classes identified in the ethanolic (EtOH) extract of hibiscus rosa-sinensis flower powder

Chemical class	St-EtOH
Alkaloids	++
Coumarins	++
Anthracene derivatives	++
Flavonoids	++
Hydrolyzed tannins	++
Condensed tannins	++
Mono-, sesqui-, and diterpenes	+
Naphthoquinones	+
Triterpenes and steroids	+
Saponins	++
Anthraquinones	+
Lignans	+
Anthocyanins	++
Xanthines	–

(–): absence; (+): presence; (++) moderate presence; (+++): high presence of the constituent

powder presented an intense and dark red coloration (Table 1).

Phytochemical profile and antioxidant activity of HRF powder crude extract

The qualitative phytochemical screening of the crude ethanolic extract of HRF powder revealed the presence of thirteen bioactive constituents, including alkaloids, coumarins, anthracene derivatives, flavonoids, hydrolyzable tannins, condensed tannins, saponins, and anthocyanins, with the absence of xanthines (Table 2). These findings are consistent with previous reports indicating that HRF is a rich source of polyphenolic compounds, particularly flavonoids, tannins, and lignans, which are known to exert antioxidant activity through mechanisms such as free radical scavenging and inhibition of oxidative chain reactions (Granato et al. 2020). In the present study, these compound classes

were qualitatively identified, but their quantification was not carried out due to methodological and resource limitations. Nevertheless, the results confirm the presence of key phenolic constituents typically associated with antioxidant activity. Similarly, Mejía et al. (2023) identified and quantified anthocyanins derived from cyanidin, glycosylated flavonols from quercetin and kaempferol, and flavan-3-ols such as catechin and epicatechin in *Hibiscus rosa-sinensis* flowers, which were related to both the color and antioxidant potential of the extracts. Further studies are needed to accurately quantify the phytochemical constituents of HRF and to establish clearer relationships between their concentrations and antioxidant activity.

Corroborating the phytochemical composition, the antioxidant activity of HRF was confirmed via the DPPH radical scavenging assay. HRF exhibited greater antioxidant

capacity than the synthetic antioxidant BHT, as evidenced by its lower half-maximal effective concentration (EC_{50}) value ($P=0.0001$; Fig. 1). This result indicates that a smaller quantity of HRF extract is required to neutralize 1 g of DPPH radicals compared to BHT. According to Kiss et al. (2025), lower EC_{50} values reflect stronger antioxidant potential, underscoring the superior radical-scavenging efficiency of HRF.

The antioxidant performance of HRF can be attributed to its complex matrix of phytochemicals, particularly flavonoids and anthocyanins, which are known to donate hydrogen atoms or electrons to neutralize free radicals and inhibit lipid peroxidation (Silva et al. 2019). This reinforces the functional applicability of HRF as a natural antioxidant additive in meat products, as oxidative stability is critical to

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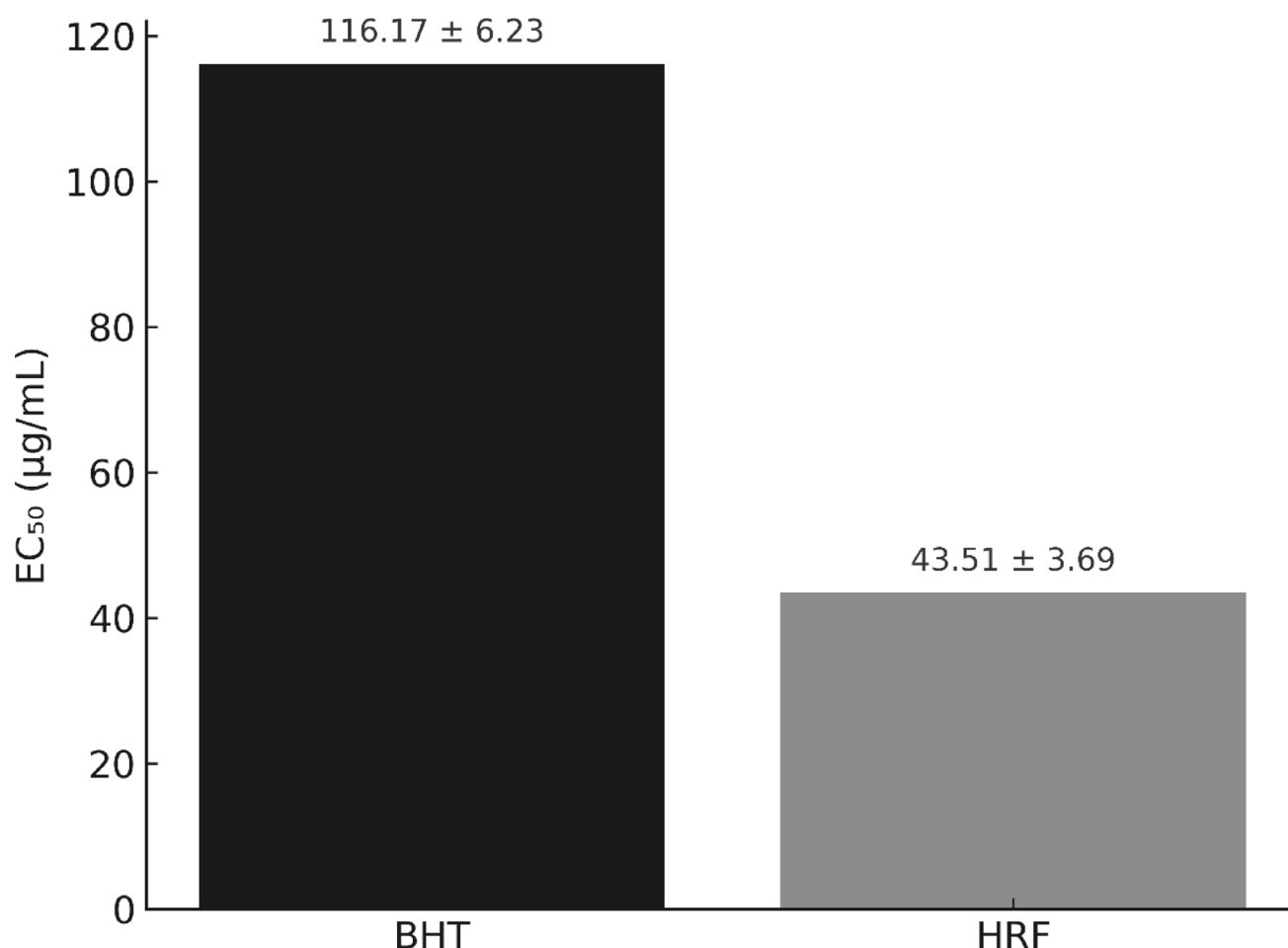


Fig. 1 Antioxidant activity of *Hibiscus rosa-sinensis* powder extract (HRF) and butylated hydroxytoluene (BHT). Antioxidant activity, determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

scavenging method, is expressed as the inhibitory concentration (EC_{50} , $\mu\text{g/mL}$); * Indicates significant difference ($P<0.05$)

preserving product quality, extending shelf life, and maintaining sensory attributes during storage.

Instrumental color, pH, and lipid oxidation in lamb burgers containing HRF powder

No significant interaction was observed between treatment and storage time for instrumental color parameters (L^* , a^* , b^*), pH, and lipid oxidation ($P > 0.05$; Table 3).

Instrumental color

A significant main effect of treatment was observed for the color coordinates ($P < 0.05$; Table 3). For L^* , the control, BHT, and HRF0.5 treatments exhibited higher values than the formulations containing higher concentrations of HRF ($P < 0.001$). Across storage times, the highest L^* values were recorded at 30 days of frozen storage ($P < 0.001$; Table 3). The reduction in L^* values in burgers with higher HRF levels may be attributed to the naturally darker color of HRF powder compared to meat.

The incorporation of plant-based ingredients rich in pigments can significantly affect the appearance of meat products due to the presence of natural chromophores such as anthocyanins. The anthocyanins in HRF (Table 2), especially in treatments with $\geq$ HRF1.0, are likely responsible for the reduced lightness and enhanced pigmentation observed (Pérez-Báez et al. 2021). Furthermore, the simultaneous reduction in moisture content in these formulations may contribute to product darkening. Despite the decreased brightness, all treatments maintained L^* values above 34, a range considered visually acceptable for burgers according to consumer preference studies (Nascimento et al. 2024).

The red color coordinate a^* was higher in burgers formulated with HRF2.0 compared to the control, BHT, HRF0.5, and HRF1.0, with no significant difference from HRF1.5 ($P < 0.001$). Additionally, the highest a^* values were recorded on day 1 of storage, followed by a gradual decline over time ($P < 0.001$; Table 3). The intensified red hue in HRF-treated burgers is consistent with the anthocyanin content of the powder, which imparts red-to-purple coloration and acts as a natural pigment (Maciel et al. 2018). This finding reinforces the potential of HRF as a natural colorant with functional properties, an aspect of growing interest in the clean-label meat industry. The decline in a^* values during storage is likely associated with oxidative discoloration, particularly the formation of metmyoglobin due to reduced activity of metmyoglobin-reducing enzymes (Ismail et al. 2022).

For the b^* coordinate, the control and BHT treatments showed higher values than the HRF treatments ($P < 0.001$). The highest b^* values were detected on days 1 and 30 of

Table 3 Instrumental color, pH and lipid oxidation of lamb burgers with different levels of *Hibiscus rosa-sinensis* flower powder

Variables	Treatments		SEM				Storage days				SEM		P-Value		
	CON	BHT	HRF0.5	HRF1.0	HRF1.5	HRF2.0	1	30	60	90		T	D	T × D	
<i>L</i> [*]	37.6 ^a	38.3 ^a	37.8 ^a	35.4 ^b	35.0 ^b	34.8 ^b	0.330	34.7 ^c	38.6 ^a	36.1 ^b	36.5 ^b	0.270	<0.001	<0.001	0.245
<i>a</i> [*]	4.02 ^b	3.81 ^b	3.88 ^b	3.95 ^b	4.26 ^{ab}	4.49 ^a	0.189	4.79 ^a	4.30 ^b	3.64 ^c	3.54 ^c	0.179	<0.001	<0.001	0.225
<i>b</i> [*]	9.88 ^a	9.95 ^a	8.90 ^b	7.75 ^c	7.34 ^{cd}	7.06 ^d	0.181	8.98 ^a	8.72 ^{ab}	7.90 ^c	8.33 ^{bc}	0.162	<0.001	<0.001	0.488
ΔE	2.20 ^{ab}	1.74 ^b	2.92 ^a	2.40 ^{ab}	2.67 ^{ab}	2.78 ^{ab}	0.290	0.00 ^c	4.19 ^a	2.72 ^b	2.90 ^b	0.247	0.030	<0.001	0.712
C [*]	10.67 ^a	10.66 ^a	9.73 ^b	8.72 ^c	8.48 ^c	8.38 ^c	0.143	10.21 ^a	9.76 ^b	8.72 ^c	9.07 ^c	0.117	<0.001	<0.001	0.455
H [*]	21.94 ^c	20.90 ^c	23.49 ^c	26.99 ^b	30.03 ^a	32.25 ^a	1.344	28.53 ^a	26.68 ^{ab}	25.10 ^{bc}	23.43 ^c	1.71	<0.001	<0.001	0.270
BI	7.64 ^c	7.14 ^c	7.37 ^{bc}	8.00 ^{ab}	8.64 ^a	9.15 ^a	0.356	9.81 ^a	7.96 ^b	7.22 ^c	6.97 ^c	0.34	<0.001	<0.001	0.119
pH	6.03	6.04	6.04	6.04	6.05	6.11	0.020	5.97 ^b	5.88 ^c	6.14 ^a	6.21 ^a	0.020	0.195	<0.001	0.190
TBARS	0.364	0.371	0.388	0.356	0.456	0.396	0.015	0.161 ^c	0.145 ^c	0.477 ^b	0.773 ^a	0.149	0.784	<0.001	0.236
CON - basic formulation without antioxidant; BHT - butylated hydroxytoluene (0.01 g BHT/100 g of fat); HRF0.5, HRF1.0, HRF1.5, and HRF2.0 - <i>Hibiscus rosa-sinensis</i> flower powder at 0.5, 1.0, 1.5 and 2.0 g/100 g of the base formulation, respectively; <i>L</i> [*] - lightness; <i>a</i> [*] - redness; <i>b</i> [*] - yellowness; ΔE - total color difference; H [*] - hue angle; C [*] - chroma; BI - browning index; TBARS - thiobarbituric acid reactive substances (mg malondialdehyde/kg); SEM - standard error of the mean; T - treatments; D - storage days; T × D - interaction between treatment and storage day; a,b,c - means within the same row followed by different lowercase letters differ significantly (Tukey's test, <i>P</i> <0.05)															

CON - basic formulation without antioxidant; BHT - butylated hydroxytoluene (0.01 g BHT/100 g of fat); HRF0.5, HRF1.0, HRF1.5, and HRF2.0 - *Hibiscus rosa-sinensis* flower powder at 0.5, 1.0, 1.5 and 2.0 g/100 g of the base formulation, respectively; L^* - lightness; a^* - redness; b^* - yellowness; ΔE - total color difference; H* - hue angle; C* - chroma; BI - browning index; TBARS - thiobarbituric acid reactive substances (mg malondialdehyde/kg); SEM - standard error of the mean; T - treatments; D - storage days; T × D - interaction between treatment and storage day; _{a,b,c} - means within the same row followed by different lowercase letters differ significantly (Tukey's test, $P < 0.05$)

storage ($P < 0.001$; Table 3), with reductions at longer storage times. The inclusion of HRF may have diluted the visual contribution of the fat in the meat, which is naturally yellowish, resulting in lower b^* values in treatments with higher inclusion levels. Similar findings were reported by Nascimento et al. (2024) in beef burgers enriched with HS, reinforcing the influence of botanical powder on yellow intensity. In addition, lipid oxidation during extended storage may have contributed to color degradation, particularly in the yellow spectrum (Ismail et al. 2022).

The values of ΔE differed among treatments ($P = 0.030$), with the highest observed in HRF0.5 and the lowest in BHT. The control and higher HRF treatments showed intermediate values. Throughout storage, ΔE increased from day 1 to day 30, followed by a slight decline on days 60 and 90 ($P < 0.001$; Table 3). This trend suggests that early pigment interactions between HRF and meat proteins lead to a perceptible color change during the initial storage period, whereas prolonged storage may promote pigment stabilization or oxidative changes that homogenize the surface color.

In terms of C^* , which reflects color saturation, the control and BHT treatments exhibited the highest values, while the HRF treatments, particularly HRF1.0, HRF1.5, and HRF2.0, presented lower values, indicating less saturated and more muted colors ($P < 0.001$; Table 3). This reduction may be attributed to the dominant red-purple pigments in HRF, which alter the color profile and reduce vividness. Additionally, C^* values decreased over storage ($P < 0.001$; Table 3), likely due to pigment degradation and oxidative processes.

The parameter H^* increased with higher levels of HRF inclusion ($P < 0.001$; Table 3), reflecting the more reddish-purple hue imparted by anthocyanins (Maciel et al. 2018). However, H^* values gradually decreased during frozen storage ($P < 0.001$; Table 3), indicating progressive pigment degradation and oxidative browning.

Regarding the browning index (BI), the highest values were observed in the HRF1.5 and HRF2.0 treatments compared to the control and BHT ($P < 0.001$; Table 3). This increase is likely associated with the intense pigment contribution of HRF, which, through interaction with meat proteins, results in a darker and slightly brownish tone. Over time, BI values declined from day 0 to day 90 ($P < 0.001$; Table 3), possibly due to structural and oxidative changes affecting anthocyanin stability.

pH

No effect of HRF inclusion was observed on the pH of the burgers ($P = 0.195$; Table 3). However, storage time had a significant impact. Burgers evaluated after 60 and 90 days of frozen storage exhibited significantly higher pH values

than those analyzed on days 1 and 30 ($P < 0.001$; Table 3). All values remained within the acceptable range for meat products (≤ 6.4), as defined by Souza et al. (2022). The progressive increase in pH may be attributed to protein degradation and the generation of basic nitrogenous compounds such as ammonia, resulting from microbial and enzymatic breakdown of amino acids (Zhao et al. 2019).

Lipid oxidation (TBARS)

Lipid oxidation, measured by TBARS, was not significantly affected by the addition of HRF ($P = 0.784$; Table 3). In contrast, storage time had a significant impact, with the highest TBARS values recorded at 90 days ($P < 0.001$; Table 3). Although the EC_{50} value of the HRF extract was significantly lower than that of BHT (Fig. 1), indicating a higher antioxidant activity of the natural extract, no significant differences in TBARS values were observed among treatments.

Several studies have shown that extracts rich in phenolic compounds often display strong radical-scavenging capacity in vitro (DPPH, ABTS, or FRAP), although this activity does not always translate into reduced lipid peroxidation in complex food matrices. For instance, grape seed extract exhibited high antioxidant activity in DPPH and FRAP assays but increased TBARS values in fresh sausages, acting as a pro-oxidant under specific conditions (Bottoli et al. 2023). As highlighted by Echegaray et al. (2021), the DPPH method, while simple and sensitive, operates in organic solvent systems and mainly measures the ability of antioxidants to donate electrons or hydrogen atoms to a stable radical, which differs from the peroxy radicals generated in lipid oxidation of meat. Additionally, the test is unsuitable for emulsions or protein-rich matrices, such as burgers, where interactions with proteins and salts can alter antioxidant behavior. Shahidi and Samarasinghe (2025) also emphasize that in vitro assays like DPPH, although useful for preliminary screening, cannot fully reproduce the physicochemical environment of real food systems. Therefore, a strong DPPH response may not correspond to lower TBARS values in meat products, since antioxidant efficacy in the matrix depends on compound solubility, phase partitioning, metal interactions, and stability during processing (Bottoli et al. 2023; Echegaray et al. 2021; Shahidi and Samarasinghe 2025).

This discrepancy may also be partially explained by the neutralization of antioxidant compounds in the HRF powder when they come into direct contact with the basic burger matrix, resulting in an immediate and uncontrolled release that could rapidly deplete their antioxidant capacity due to saturation. In addition, possible interactions between HRF powder and other ingredients, such as salt and seasonings, may neutralize the antioxidant activity of the compounds

present in the HRF extract. Therefore, encapsulating HRF powder prior to its inclusion in meat product formulations could be a strategy to enhance its antioxidant activity. Feridoni and Shurmasti (2020) reported higher antioxidant activity of microencapsulated HS extract compared to the non-encapsulated form in chicken nuggets.

It is also important to note that antioxidant activity (DPPH assay) was evaluated using the ethanolic extract of HRF, which may have concentrated the antioxidant phytochemicals. In contrast, in the crude powder, these compounds are diluted by other, more abundant constituents. Therefore, HRF extracts could be more effective in preventing lipid oxidation in lamb burgers.

Extending the storage period beyond 90 days or evaluating the product under refrigerated conditions may provide further insight into the long-term oxidative stability provided by HRF. Notably, throughout the 90-day frozen storage period, TBARS values remained below the threshold for rancidity perception by consumers (<2 mg MDA/kg meat) (Campo et al. 2006).

Chemical composition of lamb burgers formulated with HRF powder

The inclusion of HRF powder at levels above 1.0% resulted in a significant reduction in the moisture content of lamb burgers compared to the control group ($P=0.0052$). In contrast, no significant differences were observed among treatments for crude protein, ash, or fat content ($P>0.05$; Table 4). The observed decrease in moisture content may be attributed to the inherently lower water content of HRF compared to raw meat, leading to a dilution effect upon its incorporation into the burger matrix. A similar result was reported by Bermúdez et al. (2023), who evaluated the

incorporation of HS powder in beef rissoles. The authors found that treatments containing HS powder exhibited significantly lower moisture levels than the control group, an effect attributed to the low moisture and high fiber content of the added powder. These findings support the idea that fiber-rich botanical powder, such as HRF, can modulate water retention and distribution in meat systems, influencing the final composition and technological performance of the product.

Water retention, shrinkage, and cooking loss in lamb burgers enriched with HRF powder

No significant differences were observed among treatments for WHC and dimensional shrinkage during cooking ($P>0.05$; Table 4). However, the HRF1.5 and HRF2.0 treatments resulted in lower CWL compared to the control, and the HRF2.0 treatment showed lower CWL than the BHT, HRF0.5, and HRF1.0 treatments ($P=0.0006$; Table 4). Similar results were reported by Dos Santos et al. (2023), who observed reduced cooking losses in burgers formulated with *Opuntia ficus-indica* powder and attributed this effect to the presence of plant compounds with high WHC. HRF powder is rich in carbohydrates and has a moderate dietary fiber content (Table 1), which may contribute to reinforcing the structural integrity of the meat matrix and reducing fluid exudation during thermal processing. In fact, HRF petals are known to be rich in pectin, a polysaccharide with high WHC and recognized gelling, thickening, and stabilizing properties, which also contributes to the production of high-quality jellies using this ingredient (Thakre and Barse 2018).

In contrast to the findings of our study, previous research using purée and powder from HS flowers in burger formulations reported an increase in CWL, which was partially

Table 4 Chemical composition, water holding capacity, cooking weight loss, shrinkage and texture profile of lamb burgers with different levels of *Hibiscus rosa-sinensis* flower powder

Variables	Treatments						SEM	P-Value
	CON	BHT	HRF0.5	HRF1.0	HRF1.5	HRF2.0		
Moisture, %	70.8 ^a	69.7 ^{ab}	69.6 ^{ab}	69.2 ^b	68.7 ^b	68.7 ^b	0.32	0.0052
Protein, %	18.8	18.9	18.2	18.4	18.8	18.6	0.16	0.0747
Ash, %	2.43	2.62	2.55	2.69	2.72	2.57	0.06	0.0636
Fat, %	5.93	5.45	5.42	5.53	5.54	5.66	0.27	0.7918
WHC, %	71.2	74.3	71.8	67.1	73.9	73.3	2.11	0.2334
CWL, %	16.3 ^a	12.9 ^{ab}	13.7 ^a	13.1 ^{ab}	8.9 ^{bc}	8.3 ^c	0.97	0.0006
SHR, %	11.9	11.3	11.4	11.8	10.3	11.5	0.48	0.2770
Hardness, N	37.6 ^{ab}	34.9 ^b	48.3 ^a	43.0 ^{ab}	36.1 ^{ab}	37.7 ^{ab}	2.31	0.0120
Elasticity, mm	0.84	0.86	0.84	0.84	0.82	0.86	0.011	0.2633
Chewiness, N/m	32.4	30.7	35.2	33.7	30.5	30.3	1.92	0.4261
Cohesiveness	0.75	0.76	0.73	0.74	0.73	0.73	0.015	0.6557

CON - Basic formulation without antioxidant; BHT - Butylated hydroxytoluene (0.01 g BHT/100 g of fat); HRF0.5, HRF1.0, HRF1.5 and HRF2.0 - *Hibiscus rosa-sinensis* flower powder at 0.5; 1.0; 1.5 and 2.0 g/100 g of the basic formulation, respectively; WHC - water holding capacity; CWL - cooking weight loss; SHR - shrinkage; SEM - standard error of the mean; ^{a, b, c} means within the same row followed by different lowercase letters differ significantly (Tukey's test, $P<0.05$)

Table 5 Sensory analysis of lamb burgers with the inclusion of different levels of *Hibiscus rosa-sinensis* flower powder

Variables	Treatments						SEM	P-Value
	CON	BHT	HRF0.5	HRF1.0	HRF1.5	HRF2.0		
Appearance	7.13 ^{ab}	6.60 ^b	7.60 ^a	7.11 ^{ab}	7.23 ^{ab}	6.84 ^{ab}	0.089	0.033
Color	7.08	6.50	7.42	7.02	7.24	6.90	0.094	0.101
Aroma	6.98	6.60	7.40	6.94	6.98	6.74	0.080	0.085
Flavor	7.06	6.95	7.19	7.32	7.34	6.65	0.086	0.173
Tenderness	6.34 ^c	6.82 ^{bc}	7.69 ^a	7.66 ^a	7.60 ^{ab}	7.63 ^a	0.081	<0.001
Juiciness	6.50 ^b	6.60 ^{ab}	7.45 ^a	7.37 ^{ab}	7.39 ^{ab}	7.19 ^{ab}	0.088	0.001
Purchase intent	3.32	3.37	3.90	3.66	3.71	3.34	0.061	0.056

CON - Basic formulation without antioxidant; BHT - Butylated hydroxytoluene (0.01 g BHT/100 g of fat); HRF0.5, HRF1.0, HRF1.5 and HRF2.0 - *Hibiscus rosa-sinensis* flower powder at 0.5, 1.0, 1.5 and 2.0 g/100 g of the base formulation, respectively; SEM - standard error of the mean; ^{a, b, c} means within the same row followed by different lowercase letters differ significantly (Friedman's multiple comparison test and Dunn-Bonferroni correction, $P < 0.05$)

attributed to the reduction in burger pH caused by the addition of HS flower purée and powder (Bermúdez et al. 2023; Ismail et al. 2022). pH influences the stability of the meat protein matrix, affecting WHC and, consequently, cooking losses. Values close to the isoelectric point of muscle proteins reduce WHC, compromise the integrity of the protein network, and promote the exudation of water and fat during heating (Glorieux et al. 2017). As previously reported, the inclusion of HRF powder did not affect the pH of the burgers, which remained well above the isoelectric point of myofibrillar proteins (> 6 ; Table 3).

Instrumental texture profile of lamb burgers with HRF powder

Only hardness was affected by the treatments ($P = 0.0120$; Table 4). A reduction in hardness was observed with increasing levels of HRF, particularly in burgers containing HRF1.5 and HRF2.0. The highest value was recorded in HRF0.5. The softer texture in formulations with higher HRF levels may be partly explained by the lower CWL observed in these treatments, which likely resulted in greater moisture retention and a less rigid structure. In addition, the fibrous and mucilaginous components of HRF may have interacted with the meat matrix, altering the protein-gel network and contributing to a more tender product (Dos Santos et al. 2023; Thakre and Barse 2018). These textural changes are relevant from a technological standpoint, as consumer acceptance of burgers is closely related to perceived tenderness and juiciness.

Sensory analysis of lamb burgers enriched with HRF powder

No differences were observed among treatments for color, aroma, flavor, or purchase intent ($P > 0.05$; Table 5). However, appearance scores differed between the HRF0.5

and BHT treatments, with HRF0.5 receiving higher ratings ($P = 0.033$; Table 5). Tenderness also improved in the HRF0.5, HRF1.0, and HRF2.0 treatments compared to the control and BHT groups. HRF1.5 showed higher tenderness scores than the control but did not differ from the other treatments ($P < 0.001$; Table 5). Regarding juiciness, HRF0.5 was rated higher than the control ($P = 0.001$), but did not differ from the other treatments containing HRF powder ($P = 0.001$; Table 5). These improvements in tenderness and juiciness are likely associated with the hydrophilic properties of HRF carbohydrates and fiber, which may help retain moisture during cooking (Dos Santos et al. 2023; Thakre and Barse 2018), and are consistent with the lower CWL and reduced instrumental hardness observed with HRF powder inclusion.

Therefore, the addition of HRF powder up to 2.0% did not compromise the sensory acceptability of lamb burgers. This highlights the potential of HRF powder as a natural and functional ingredient capable of enhancing both the sensory quality and technological performance of lamb burgers. In contrast to the results observed in the present study, the use of higher concentrations of HS extract in the formulation of Frankfurter-type sausages led to reduced sensory acceptance, an outcome attributed to the high acidity of the extract, which contributed to a decrease in sausage pH (< 6 ; Perez-Báez et al. 2021). As previously presented, the inclusion of HRF powder had no effect on the pH of the burgers (> 6 ; Table 3), which may be related to the naturally low acidity of HRF powder itself (> 6 ; Table 1).

Conclusion

The inclusion of HRF powder in lamb burgers highlights its potential as a natural colorant, as well as its ability to improve key technological and sensory attributes. Although the extract obtained from HRF powder showed higher antioxidant activity than BHT, the direct use of HRF powder in

the formulation of frozen lamb burgers stored for 90 days did not result in lower lipid oxidation compared to the synthetic antioxidant. Nevertheless, it was effective in maintaining oxidation levels below the threshold of sensory perception for oxidized meat products. Further studies using concentrated extracts of HRF powder and/or encapsulation strategies are recommended to enhance its antioxidant potential when incorporated into meat product formulations.

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