Mesquite propolis: An antioxidant and antibacterial preservative for pork meat

Propóleos de mezquite: Un conservador antioxidante y antibacteriano para carne de cerdo

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Scientific Article
Received: February 26, 2024
Accepted: August 29, 2025

ABSTRACT. Honeybee-derived propolis is a promising source of bioactive compounds that enhance meat quality. This study evaluated the antioxidant and antibacterial effects of Mesquite propolis extracts (MPE) on pork meat homogenate. Raw propolis from two apiaries was characterized for pollen origin, physicochemical, and sensory properties. Extracts (MPE1 and MPE2) were evaluated for their polyphenol content, antioxidant properties, and antibacterial activity against foodborne pathogens. Pork meat homogenate was treated with MPE1 and MPE2 (500 mg kg^{-1}), synthetic antioxidant BHT (500 ppm), or left untreated (control). The samples were then thermally processed (65 °C/0-120 min) and analyzed for quality parameters. MPE2 exhibited the highest ($p \leq 0.05$) total polyphenol content and antioxidant values, and both extracts demonstrated effectiveness against Gram-positive bacteria. Incorporation of MPE, especially MPE2, significantly reduced pH variation, color degradation, lipid oxidation, and microbial growth ($p \leq 0.05$). Mesquite propolis shows potential as a natural preservative in meat products.
Keywords: Beekeeping, pollen, extract, bioactivity, meat.

RESUMEN. El propóleo derivado de abejas es una fuente prometedora de compuestos bioactivos para mejorar la calidad de la carne. Este estudio evaluó los efectos antioxidantes y antibacterianos de los extractos de propóleo de mezquite (MPE) en homogeneizado de carne de cerdo. El propóleo crudo de dos apiarios se caracterizó por su origen del polen, propiedades fisicoquímicas y sensoriales. Los extractos (MPE1 y MPE2) se evaluaron por su contenido de polifenoles, propiedades antioxidantes y actividad antibacteriana contra patógenos transmitidos por alimentos. El homogeneizado de carne de cerdo se trató con MPE1 y MPE2 (500 mg kg^{-1}), antioxidante sintético BHT (500 ppm), o se dejó sin tratar (control). A continuación, las muestras se procesaron térmicamente (65 °C/0-120 min) y se analizaron sus parámetros de calidad. MPE2 exhibió el contenido total de polifenoles y valores antioxidantes más altos ($p \leq 0.05$), y ambos extractos demostraron efectividad contra bacterias Gram-positivas. La incorporación de MPE, especialmente MPE2, redujo significativamente la variación del pH, la degradación del color, la oxidación lipídica y el crecimiento microbiano ($p \leq 0.05$). El propóleo de mezquite muestra potencial como conservante natural en productos cárnicos.
Palabras clave: Apicultura, polen, extracto, bioactividad, carne.

How to cite: Vargas-Sánchez RD, Sánchez-Escalante A, Torres-Martínez B del M, Sotelo-Mundo RR, Torrescano-Urrutia GR. (2025) Mesquite propolis: An antioxidant and antibacterial preservative for pork meat. Ecosistemas y Recursos Agropecuarios 12(3): e4020. DOI: 10.19136/era.a12n3.4020.

INTRODUCTION

Pork plays a crucial role in the Mexican diet, with a *per capita* consumption of approximately 22 kg in 2023. In the same year, Mexico reported pork production of around 1.6 million metric tons (Mt), while imports and exports nearly reached 1.3 and 0.3 Mt, respectively (COMECARNE 2024, USDA 2024). Despite its popularity, pork quality remains a challenge due to the oxidation of lipids and proteins, as well as microbial spoilage, which negatively impacts shelf life, safety, and consumer acceptance (Liu *et al*. 2023, Papanagiotou *et al*. 2013).

To reduce these issues, synthetic antioxidants and antibacterial agents are commonly used in the meat industry. However, consumer concerns about the health risks and perceived unnaturalness of these additives have led to an increased demand for natural alternatives. Among natural sources, plant polyphenols have been extensively investigated for their antioxidant and antibacterial properties in meat products (Kane *et al*. 2024, Papuc *et al*. 2012).

Bee products such as propolis have gained attention due to their high content of bioactive compounds. The bioactivity of propolis depends on its botanical and geographical origin, which Influences Its polyphenol profile (Camacho-Bernal *et al*. 2021, Toreti *et al*. 2013). Previous studies have demonstrated that ethanolic propolis extracts can enhance the oxidative and microbial stability of raw beef and pork patties during refrigeration (Vargas-Sánchez *et al*. 2019). In particular, propolis samples collected in northwestern Mexico have been identified as bifloral, primarily composed of Mesquite and Catclaw (Vargas-Sánchez *et al*. 2020).

Although the antioxidant and antibacterial activity of propolis has been reported, there is limited information on specific effects of mesquite-derived propolis in thermally treated meat systems. Therefore, this study aimed to investigate the impact of Mesquite propolis extracts on the oxidative and microbial stability of a thermally treated pork meat homogenate.

MATERIALS AND METHODS

Materials and chemicals

Samples of propolis were acquired from two apiaries from Pueblo de Alamos (29.1476 N, -110.1239 O, 632 m; 29.1887 N, -110.1273 O, 632 m; respectively) and stored at -20 °C, in the dark. All the chemicals used were analytical grade and were purchased from Sigma Chemicals. At the same time, Brain Heart Infusion (BHI) and Plate Count Agar (PCA) were obtained from Merck.

Raw propolis characterization

The acetolysis method was used to determine the floral origin of propolis (Vargas-Sánchez *et al*. 2016), with slight modifications. Propolis was mixed with distilled water (1:10 *w/v* ratio) at 10 000 rpm (25 °C) for 1 min (Ultraturrax-T25, IKA, Germany) and centrifuged at 5 000 × *g* (4 °C) for 15 min (Sorvall ST18R, Thermo Fisher Scientific, USA). The precipitate was dehydrated with 1 mL of CH_3COOH, mixed with 1 mL of H_2SO_4 (9:1), centrifuged, and washed with distilled water (d-water). The sediment was mixed with 0.5 mL of glycerin-water solution (1:1), and 0.1 mL of the obtained suspension was placed on a microscope slide. Pollen grains were observed using an

optical microscope (CX-31, Olympus®, Japan). At least 500 pollen grains were counted and assigned to four classes: minor (< 3%), important minor (3-15%), secondary (15-45%), and predominant (> 45%). Pollen slides, based on the plant species of the local region, were used to identify pollen grains.

The AOAC procedure was followed to measure the pH values (AOAC 2020), with slight modifications. Samples (1:10 ratio) were homogenized at 6 000 rpm (5 °C) for 1 min with d-water before pH measurements (pH211, Hanna Instruments Inc., USA).

Concerning the color values, L* (lightness), a*(redness), b* (yellowness), and RGB (red-green-blue) values were measured in the sample's surface (CM-508d, Konica Minolta Inc., Japan) (Hernández *et al*. 2016).

Regarding the sensory evaluation, a 15-person panel was used to measure sensory attributes of propolis (Habryka *et al*. 2020), with slight modifications. Color (brightness, intensity, and uniformity), aroma (floral, waxy, resinous, and sweet), flavor (acid, bitter, and sweet), and consistency (viscous, sticky, and solid) were the descriptors used, which were subjected to a hedonic scale.

Mesquite propolis extracts (MPE) obtention

Extracts were obtained from raw propolis samples with d-water (1:10 ratio) by maceration-assisted extraction at 150 rpm (25 °C) for 24 h in the dark (MaxQ-5000, Fisher Scientific, Canada). The resultant solution was filtered (Whatman no. 1 filter paper) under vacuum (FE-1500, Felisa, Mexico), and dried (DC401, Yamato, Japan). The obtained Mesquite propolis extracts (MPE) were stored at -20 °C in the dark (SAGARPA 2007).

Polyphenol's content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Matić and Jakobek 2021). MPE (20 µL, 5 mg/mL) was mixed with 160 µL of d-water, 60 µL of sodium carbonate (7% *w/v*), and 40 µL of Folin-Ciocalteu reagent (2 M). The solution was incubated for 60 min (25 °C) in the dark, and the absorbance was read at 750 nm (Multiskan FC UV-Vis, Thermo Scientific, Japan), and the results were expressed as mg of gallic acid equivalents (GAE) g^{-1}.

The flavone and flavanols content were measured by the aluminum chloride method (Matić and Jakobek 2021). MPE (10 µL, 5 mg/mL) was mixed with 130 µL of methanol and 10 µL of aluminum chloride (5%, *w/v*). The solution was incubated for 30 min (25 °C) in the dark, the absorbance was read (412 nm), and the results were expressed as mg of quercetin equivalent (QE) g^{-1}.

The flavanone-dihydroflavonol content (FDC) was measured using the dinitrophenyl method (Isla *et al*. 2014). MPE (40 µL, 5 mg/mL) was mixed with 80 µL of dinitrophenyl solution, incubated for 50 min (50 °C) in the dark, and diluted with 280 µL of potassium hydroxide (10%, *w/v*). The obtained solution (30 µL) was mixed with 250 µL of ethanol, the absorbance was read (490 nm), and the results were expressed as mg of hesperidin equivalents (HE) g^{-1}.

The chlorogenic acid content (CAC) was measured using the sodium nitrite method (Griffiths *et al*. 1992). MPE (100 µL, 5 mg/mL) was mixed with 200 µL of urea (0.7 M), 200 µL of acetic acid (0.1 M),

and 500 µL of d-water. The obtained solution was mixed with 500 µL of sodium nitrite (0.14 M) and 500 µL of sodium hydroxide (0.5 M), and centrifuged at 2 250 × *g* (4 °C) for 10 min. The mixture was incubated for 10 min (25 °C) in the dark, the absorbance was read (510 nm), and the results were expressed as mg of chlorogenic acid equivalents (CGA) g^{-1}.

Antioxidant activity

The free-radical scavenging activity was measured using the DPPH method (Ozgen *et al*. 2006). MPE (20 µL, 100 µg/mL) was mixed with 180 µL of DPPH solution (300 µM). The solution was incubated for 30 min (25 °C) in the dark, the absorbance was read (517 nm), and the results were expressed as inhibition percentage (%).

The radical cation scavenging activity was measured using the ABTS method (Ozgen *et al*. 2006). MPE (20 µL, 100 µg/mL) was mixed with 180 µL of ABTS solution (absorbance 0.8 nm in ethanol). The solution was incubated for 30 min (25 °C) in the dark, the absorbance was read (730 nm), and the results were expressed as inhibition percentage (%).

The reducing power ability (RPA) was measured using the Prussian-blue method (Işıl-Berker *et al*. 2010). MPE (100 µL, 100 µg/mL) was mixed with 300 µL of phosphate buffer (2 M) and 300 µL of potassium ferrocyanide (1%, *w*/*v*). The solution was incubated for 20 min (50 °C) in the dark (Aquabath, Thermo Scientific, USA). Subsequently, samples were mixed with 300 µL of TCA (10%, *w*/*v*) and centrifuged at 4 200 × *g* (4 °C) for 10 min. The supernatant (100 µL) was homogenized with 100 µL of d-water and 250 µL of $FeCl_3$ (0.1%, *w*/*v*), the absorbance was read (700 nm), and the results were expressed as absorbance (abs).

The ferric reducing antioxidant power (FRAP) method was also measured (Işıl-Berker *et al*. 2010). MPE (20 µL, 100 µg/mL) was mixed with 150 µL of FRAP solution. The solution was incubated for 8 min (25 °C) in the dark, the absorbance was read (595 nm), and the results were expressed as mg of iron equivalents (Fe^{2+}) g^{-1}.

Antibacterial activity

The antibacterial activity was measured using the broth-microdilution method (Jorgensen *et al*. 1999). *Staphylococcus aureus* ATCC 29213B, *Listeria monocytogenes* ATCC 33090, *Escherichia coli* ATCC 25922, and *Salmonella typhimurium* ATCC 14028) were initially reactivated in BHI broth for 24 h (37 °C) in the dark (IC403C, Yamato, Japan). MPE (50 µL) was mixed with 50 µL of bacteria suspension (1.5×10^8 CFU mL^{-1}) and incubated for 24 h (37 °C) in the dark. Gentamicin (25 µg mL^{-1}) was used as a positive control, and BHI broth solution as the blank. The absorbance was read (630 nm), and the results were expressed as absorbance (abs).

Meat quality measurements

Fresh minced pork meat (*Semimembranosus* muscle) was purchased from a local processor (Norson®, Hermosillo, Mexico). The minced pork meat was mixed with salt (0.5%, *w*/*v*) and pork back fat (10%, *w*/*v*). A 1 g meat sample from the batch was homogenized with 10 mL of d-water at 6 000 rpm (5 °C) for 1 min, and 1 mL of the respective antioxidants: Control, without antioxidant; MPE1 and MPE2, extracts at mg kg^{-1}; BHT, butylated hydroxytoluene at 500 mg kg^{-1}. The obtained

mixture was heated in a water bath for 0, 60, and 120 min (65 °C). After that, meat homogenates were subjected to meat quality assays.

The pH and color of meat homogenates were determined as previously described (AOAC 2020, Hernández *et al*. 2016). Additionally, the TBARS method was employed to measure lipid oxidation (Pfalzgraf *et al*. 1995). Meat homogenates (0.5 mL) were homogenized with 1 mL of TCA (10%, *w*/*v*) at 4 500 rpm (5 °C) for 1 min and centrifuged at 2 500 × *g* (5 °C) for 20 min. Then, 1 mL of the filtered supernatant was mixed with 1 mL of 2-TBA solution (20 mM) and incubated for 20 min (98 °C). After incubating, the absorbance was read (531 nm), and the results were expressed as mg of malondialdehyde (MDA) kg^{-1} of pork meat.

The pour-plate procedure measured the growth of psychrophilic and mesophilic bacteria (SS 1994). Meat product samples were aseptically homogenized with peptone water (0.1%, *w*/*v*) (Seward Stomacher® 400, UK); then, 1 mL of the appropriate dilutions was pour-plated using plate count agar as the standard, incubated during 48 h (37 °C) for mesophilic bacteria), as well during 10 days (5 °C) for psychrophilic bacteria and results expressed as $\log_{10}$ of colony-forming units (CFU) g^{-1}.

Statistical analysis

The study employed a completely randomized design. Results were expressed as mean ± standard deviation (SD) of at least three independent experiments (n = 6). Data from physicochemical, sensory, and polyphenol content were subjected to a Student t-test to compare treatment groups. Data from bioactivity were subjected to a one-way analysis of variance (ANOVA). In contrast, data on oxidative and microbial stability were subjected to a two-way ANOVA, with the treatments and thermal process period as fixed effects. The interaction between these factors was also evaluated. Differences were considered significant at $p \leq 0.05$ using the Tukey-Kramer *post hoc* test (NCSS ver21).

RESULTS

Raw propolis characterization

As shown in Table 1, a total of 14 pollen types from eight botanical families were identified in raw propolis from both apiaries. The Fabaceae family showed the highest frequency of pollen grains ($p \leq 0.05$). *Prosopis velutina* (Mesquite) was the predominant pollen type in both samples ($p \leq 0.05$).

Table 2 presents the physicochemical and sensory properties of Mesquite propolis. The propolis sample from Apiary #1 showed significantly lower pH values compared to the sample from Apiary #2 ($p < 0.05$). Regarding color, Apiary #1 propolis also showed the lowest b* values, while no differences were observed in L* and a* values ($p \geq 0.05$). Based on RGB values and HEX codes, the perceived colors were identified as Black Pepper (Apiary #1) and Dark Lava (Apiary #2). In terms of sensory attributes, Apiary #1 propolis received the highest scores ($p \leq 0.05$) for color (brightness and uniformity), resinous aroma, bitter flavor, and sticky-solid consistency. In contrast, Apiary #2 propolis scored highest only in wax for aroma. Neither sample had a sweet flavor ($p \geq 0.05$).

Table 1. Pollen types identified in propolis samples.

Family	Pollen type	Apiary #1		Apiary #2	
		(%)	Classes	(%)	Classes
Agavaceae	*Agave angustifolia*	3	Important minor	3	Important minor
Asteraceae	*Ambrosia*	3	Important minor	3	Important minor
Burseraceae	*Bursera laxiflora*	3	Important minor	3	Important minor
Fabaceae	*Acacia* sp.	10	Secondary	10	Secondary
	Havardia mexicana	3	Important minor	3	Important minor
	Mimosa distachya var. Laxiflora	7	Secondary	7	Secondary
	Olneya tesota	10	Secondary	10	Secondary
	Prosopis velutina	49.8	Predominant	49.6	Predominant
Malvaceae	*Ceiba acuminata*	3	Important minor	3	Important minor
	Herisantia crispa	3	Important minor	3	Important minor
Myrtaceae	*Eucalyptus* sp.	3	Important minor	3	Important minor
Poaceae	*Poaceae* sp.	0.2	Minor	0.2	Minor
Sapindaceae	*Cardiospermum halicacabum*	2	Minor	2	Minor
	Unidentified	0		0.2	Minor
	Total	100		100	

Both apiaries were located in Pueblo de Álamos (t-test; $p \leq 0.05$).

Table 2. Physicochemical and sensory properties of Mesquite propolis.

Item	Apiary #1	Apiary #2	*p*-value
Physicochemical			
pH	4.51 ± 0.01	4.31 ± 0.02	< 0.001
L*	28.43 ± 1.18	31.42 ± 1.49	n.s.
a*	1.82 ± 0.76	2.31 ± 0.47	n.s.
b*	3.16 ± 0.85	5.08 ± 0.66	< 0.001
RGB/HEX code	72, 66, 62/#48423E	81, 72, 66/#514842	
Sensory			
Color - brightness	4.65 ± 0.47	2.75 ± 0.42	< 0.001
Color - uniformity	4.95 ± 0.16	2.90 ± 0.32	< 0.001
Aroma - waxy	2.10 ± 0.32	3.05 ± 0.16	< 0.001
Aroma - resinous	4.85 ± 0.34	3.90 ± 0.32	< 0.001
Flavor - bitter	4.95 ± 0.16	4.05 ± 0.16	< 0.001
Flavor - sweet	-	-	n.s.
Consistency - sticky	4.85 ± 0.34	3.85 ± 0.34	< 0.001
Consistency - solid	4.90 ± 0.21	3.95 ± 0.16	< 0.001

Results expressed as mean ± SD of at least three independent experiments. Apiaries #1 and #2: Sample from Pueblo de Álamos. Lowercase letters indicate statistical differences between treatments (t-test, $p \leq 0.05$).

Polyphenol content and bioactivity of propolis extracts

As shown in Table 3, MPE2 exhibited significantly higher values of TPC, FFC, FDC, and CAC than MPE1 ($p \leq 0.05$). Regarding antioxidant activity, although the synthetic antioxidant BHT showed the highest efficacy, MPE2 showed higher RCSA, RPA, and FRAP values than MPE1 ($p \leq 0.05$); no differences were observed in FRSA values ($p \geq 0.05$). Both extracts demonstrated a higher antibacterial effect against Gram-positive (*S. aureus* and *L. monocytogenes*) than Gram-negative bacteria (*E. coli* and *S. typhimurium*) ($p \leq 0.05$). However, gentamicin remained the most effective.

Table 3. Polyphenol content and bioactivity of Mesquite propolis extracts.

Item	**Assays**			
Polyphenols	TPC (mg GAE g^{-1})	FFC (mg QE g^{-1})	FDC (mg HE g^{-1})	CAC (mg CGA g^{-1})
MPE1	175.04 ± 1.53	29.38 ± 2.94	99.50 ± 2.59	6.83 ± 0.15
MPE2	295.94 ± 8.65	69.44 ± 2.20	149.67 ± 1.86	13.28 ± 0.60
p-value	< 0.001	< 0.001	< 0.001	< 0.001
Antioxidant	FRSA (%)	RCSA (%)	RPA (abs)	FRAP (mg Fe^{2+} g^{-1})
MPE1	89.77 ± 0.25 ^{a}	91.07 ± 0.27 ^{b}	0.30 ± 0.01 ^{a}	1.02 ± 0.03 ^{a}
MPE2	89.47 ± 0.64 ^{a}	90.78 ± 0.23 ^{b}	0.34 ± 0.01 ^{b}	1.38 ± 0.07 ^{b}
BHT	91.20 ± 1.30 ^{a}	64.60 ± 0.55 ^{a}	1.08 ± 0.05 ^{c}	1.40 ± 0.10 ^{b}
p-value	< 0.001	< 0.001	< 0.001	< 0.001
Antibacterial	*S. aureus* (%)	*L. monocytogenes* (%)	*E. coli* (%)	*S. typhimurium* (%)
MPE1	42.50 ± 2.89 ^{a}	62.03 ± 2.84 ^{a}	8.85 ± 2.67 ^{a}	7.39 ± 3.22 ^{a}
MPE2	44.15 ± 3.54 ^{a}	61.17 ± 1.85 ^{a}	6.21 ± 1.19 ^{a}	13.32 ± 3.75 ^{a}
Gentamicin	67.31 ± 3.37 ^{b}	71.43 ± 1.32 ^{b}	67.28 ± 1.53 ^{b}	68.32 ± 2.38 ^{b}
p-value	< 0.001	< 0.001	< 0.001	< 0.001

Results expressed as mean ± SD of at least three independent experiments. MPE1 and MPE2: Mesquite propolis extract from Pueblo de Álamos (Apiaries #1 and #2, respectively). TPC, total phenolic content. FFC, flavone, and flavonol content. FDC, flavanone-dihydroflavonol content. CAC, chlorogenic acid content. FRSA, free-radical scavenging activity. RCSA, radical-cation scavenging activity. RPA, reducing power ability. FRAP, ferric-reducing antioxidant power. BHT, butylated hydroxytoluene. %: inhibition percentage. Lowercase letters indicate statistical differences between treatments (t-test; Tukey, $p \leq 0.05$).

Oxidative and microbial stability of meat homogenates

The effect of treatment and thermal processing on pork meat homogenates is summarized in Table 4. A significant interaction was observed for pH, color, TBARS, and microbial count values ($p \leq 0.05$). At 120 min, MPE1 maintained the highest pH values ($p \leq 0.05$). In terms of color, at 120 min, MPE2 and BHT presented the lowest L* values ($p \leq 0.05$), with no differences ($p \geq 0.05$) in a* and b* values. Regarding TBARS, at 120 min, MPE1 showed the lowest TBARS values ($p \leq 0.05$). For microbial stability, at 120 min, MP1 and MPE2 showed the lowest bacterial counts ($p \leq 0.05$).

Table 4. Meat quality measurements of meat homogenates.

Item	Treatment	Thermal process at 65 °C		
		0 min	60 min	120 min
pH	Control	5.62 ± 0.02 ^{aA}	5.97 ± 0.01 ^{aB}	6.09 ± 0.01 ^{aB}
	MPE1	5.69 ± 0.01 ^{bA}	6.15 ± 0.01 ^{cB}	6.20 ± 0.02 ^{cC}
	MPE2	5.70 ± 0.01 ^{bA}	6.03 ± 0.01 ^{bB}	6.16 ± 0.01 ^{bC}
	BHT	5.64 ± 0.01 ^{aA}	5.99 ± 0.01 ^{aB}	6.10 ± 0.01 ^{aC}
L*	Control	36.45 ± 1.69 ^{aA}	55.49 ± 2.01 ^{aB}	60.82 ± 1.44 ^{bC}
	MPE1	36.69 ± 0.01 ^{aA}	60.58 ± 0.84 ^{bB}	61.35 ± 0.59 ^{bB}
	MPE2	36.44 ± 1.44 ^{aA}	56.52 ± 0.92 ^{aB}	56.18 ± 0.78 ^{aB}
	BHT	37.28 ± 0.59 ^{aA}	58.75 ± 3.18 ^{abB}	57.80 ± 0.90 ^{aAB}
a*	Control	7.24 ± 0.45 ^{aB}	-2.17 ± 0.24 ^{aA}	-2.14 ± 0.24 ^{aA}
	MPE1	6.88 ± 0.24 ^{aB}	-2.19 ± 0.14 ^{aA}	-2.50 ± 0.11 ^{aA}
	MPE2	6.51 ± 0.37 ^{aB}	-1.87 ± 0.24 ^{aA}	-2.24 ± 0.23 ^{aA}
	BHT	6.79 ± 0.84 ^{aB}	-2.35 ± 0.12 ^{aA}	-2.23 ± 0.25 ^{aA}
b*	Control	9.42 ± 1.09 ^{aB}	4.13 ± 0.74 ^{aA}	5.67 ± 1.26 ^{aA}
	MPE1	9.22 ± 0.75 ^{aB}	5.27 ± 0.48 ^{aA}	5.01 ± 0.37 ^{aA}
	MPE2	9.01 ± 0.58 ^{aB}	4.95 ± 0.67 ^{aA}	3.94 ± 0.89 ^{aA}
	BHT	9.18 ± 0.61 ^{aB}	5.04 ± 1.23 ^{aA}	4.65 ± 0.55 ^{aA}
TBARS	Control	0.299 ± 0.013 ^{cA}	0.537 ± 0.008 ^{dB}	0.675 ± 0.015 ^{dC}
(mg MDA kg^{-1})	MPE1	0.004 ± 0.002 ^{aA}	0.012 ± 0.004 ^{aB}	0.031 ± 0.002 ^{aC}
	MPE2	0.005 ± 0.001 ^{aA}	0.030 ± 0.004 ^{bB}	0.038 ± 0.004 ^{bC}
	BHT	0.223 ± 0.006 ^{bA}	0.439 ± 0.030 ^{cB}	0.517 ± 0.019 ^{cC}
Mesophilic	Control	3.80 ± 0.09 ^{aA}	3.83 ± 0.05 ^{aA}	3.72 ± 0.08 ^{bA}
(Log_{10} CFU g^{-1})	MPE1	3.73 ± 0.08 ^{aB}	3.78 ± 0.08 ^{aB}	3.45 ± 0.08 ^{aA}
	MPE2	3.75 ± 0.05 ^{aB}	3.77 ± 0.08 ^{aB}	3.47 ± 0.05 ^{aA}
	BHT	3.85 ± 0.05 ^{aA}	3.83 ± 0.08 ^{aA}	3.73 ± 0.05 ^{bA}
Psychrophilic	Control	4.48 ± 0.08 ^{aA}	4.45 ± 0.05 ^{aA}	4.35 ± 0.05 ^{bA}
(Log_{10} CFU g^{-1})	MPE1	4.50 ± 0.06 ^{aB}	4.48 ± 0.08 ^{aB}	4.12 ± 0.08 ^{aA}
	MPE2	4.48 ± 0.04 ^{aB}	4.48 ± 0.04 ^{aB}	4.10 ± 0.06 ^{aA}
	BHT	4.52 ± 0.08 ^{aA}	4.53 ± 0.05 ^{aA}	4.33 ± 0.05 ^{bA}

Results expressed as mean ± SD of at least three independent experiments. MPE1 and MPE2: Mesquite propolis extract from Pueblo de Álamos (Apiaries #1 and #2, respectively). BHT, butylated hydroxytoluene. Capital letters indicate statistical differences in each treatment at different thermal process periods; lowercase letters indicate statistical differences between treatments (Tukey, $p \leq 0.05$).

DISCUSSION

Propolis is a resinous material processed by bees from plant resins, whose composition is closely linked to the vegetation surrounding the apiary (SAGARPA 2017). In this context, the Fabaceae

family is frequently identified as a predominant pollen source (Temizer *et al*. 2017), with Mesquite pollen being a representative and particularly abundant component in raw propolis from the Sonoran Desert region (Vargas-Sánchez *et al*. 2016). The botanical origin of propolis is known to influence its physicochemical, sensory, and bioactive properties (Toreti *et al*. 2013, Vargas-Sánchez *et al*. 2020). Although the Mexican NOM-003-SAG/GAN-2017 regulation does not establish standard values for pH or color in propolis, previous research indicates that these parameters vary according to the botanical origin. For Instance, *Quercus* sp. propolis tends to present lower pH values compared to *Populus* sp., *Pinus* sp., and *Castanea sativa* (Dias *et al*. 2012). Botanical source also modifies sensory characteristics, which must align with the standards for color (red, reddish-yellow, dark yellow, brown-green, brown, or black), aroma (resinous), flavor (bitter or sweet), and consistency (solid) (SAGARPA 2017).

The presence and concentration of polyphenols, the primary contributors to propolis bioactivity, also depend on their biological origin (Kumazawa *et al*. 2012, Papuc *et al*. 2017, SAGARPA 2017). These compounds act as antioxidants by donating hydrogen atoms or electrons to stabilize free radicals and by chelating metal ions involved in oxidative reactions. They also exert antibacterial effects, possibly by altering membrane permeability or inhibiting nucleic acid synthesis (Papuc *et al*. 2017). In propolis from sources such as *Cannabis sativa*, Pine, Quercus spp., *Helianthus annuus*, the phenolic profile includes *p*-coumaric, ferulic, gallic, and chlorogenic acids, as well as flavonoids like quercetin, apigenin, and pinocembrin (Kekecoglu *et al*. 2021, Kolayli *et al*. 2023, Özkök *et al*. 2023).

Functionally, antioxidant activity is essential for determining the efficacy of propolis in preserving meat products. Regulatory standards require that propolis demonstrate free radical scavenging activity, although specific quantitative thresholds are not mandated (SAGARPA 2017). Previous studies have reported variable antioxidant activity, depending on the botanical source and extraction method. Notably, extracts from *C. sativa* and Eucalyptus sp., display high antiradical and reducing power activity, correlating with their phenolic content (Kumazawa *et al*. 2012, Castro-Falcón *et al*. 2016).

In terms of antibacterial properties, multiple studies have confirmed the Inhibitory effects of propolis against foodborne pathogens, particularly *S. aureus* and *E. coli* (SAGARPA 2017, Özkök *et al*. 2023). Interestingly, propolis also shows greater efficacy against Gram-positive bacteria, likely due to structural differences in bacterial cell walls that influence compound penetration (Kekecoglu *et al*. 2021).

The Incorporation of propolis extracts into meat systems has been increasingly studied as a natural alternative to synthetic antioxidants. Lipid oxidation and microbial growth are primary factors contributing to meat spoilage, and both are influenced by pH, thermal treatment, and packaging conditions (Papuc *et al*. 2017, Anton *et al*. 2019). Lipid oxidation is a radical-mediated chain reaction that generates primary products, such as hydroperoxides (ROOH), and secondary products, including alcohols and aldehydes. Phenolic compounds inhibit this process by donating hydrogen, which helps preserve both lipid Integrity ($ROO^{\bullet} + ArOH \rightarrow ROOH + ArO^{\bullet}$) and meat color ($MetMb^{3+} + ArOH \rightarrow Mb^{2+} + ArO^{\bullet}$) (Bai *et al*. 2025, Li *et al*. 2025, Pfalzgraf *et al*. 1995, Vargas-Sánchez *et al*. 2019).

In this study, propolis extract improves the oxidative stability of pork meat homogenates, consistent with findings from Kročko *et al*. (2014), who reported lower MDA values in cooked ham treated with ethanol extracts. Similarly, propolis extracts enhance oxidative stability, reducing pH, color, and lipid oxidation changes, as well as microbial stability, reducing microbial loads in sausages, ground beef, patties, and marinated chicken under various storage conditions (El-Demery *et al*. 2016, Vargas-Sánchez *et al*. 2019, López-Patiño *et al.* 2021, Fadhil 2023).

These results support the potential of propolis as a functional ingredient in meat preservation, aligning with consumer demand for naturally preserved products. However, variability in propolis composition due to geographic and botanical differences remains a limitation. Furthermore, while antioxidant and antibacterial effects were demonstrated in this study, further research is required to address sensory acceptability and scalability

CONCLUSIONS

The results demonstrated that raw Mesquite propolis meets the physicochemical and sensory standards required by Mexican regulations. Mesquite aqueous propolis extract exerts antioxidant and antibacterial activity, mainly associated with its polyphenol composition. Furthermore, the incorporation of this extract into pork meat homogenates reduced pH variation, lipid oxidation, color changes, and microbial growth after thermal processing. These results highlight that Mesquite propolis has great potential as a preservative for meat products. Its application may help extend shelf life by reducing synthetic additives. Future studies should evaluate its acceptability and scalability.

ACKNOWLEDGMENTS

Rey David Vargas-Sánchez gratefully acknowledged the fellowship received from SECIHTI through the "Investigadoras e Investigadores por México" program. Authors also thank CIAD for support through "Proyecto Semilla 10735".

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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