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ANTIOXIDANT AND ANTIBACTERIAL EFFECT OF MESQUITE HONEY ON PORK SAUSAGES DURING STORAGE

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ABSTRACT

Lipid oxidation and microbial growth are factors associated with meat product quality loss. Different synthetic additives are used to reduce both processes, but their use has been linked to health risks. Thus, the use of natural ingredients has been suggested as a strategy to prevent these factors. The aim of this study was to investigate the effect of mesquite honey on the oxidative stability and microbial growth of uncooked and cooked pork sausages during refrigerated storage. Pollen origin, moisture, and phenolic compound content of collected honey were evaluated, and the data were only presented as descriptive statistics. The effect of a natural additive (mesquite honey at 5 and 10 %) and two synthetic additives (caffeic acid phenethyl ester at 100 and 200 ppm and butylhydroxytoluene at 0.02 %) on oxidative stability and microbial growth were also measured, and the data were subjected to a two-way analysis of variance. All data were obtained from three independent experiments. Results showed that honey samples had a moisture content of 15.8 %, and *Prosopis juliflora* (Sw.) DC. (mesquite) was the dominant pollen identified (> 45 %). In addition, kaempferol, pinocembrin, caffeic acid, and luteolin were the major phenolic compounds in mesquite honey. The inclusion of mesquite honey in uncooked and cooked pork sausages increased oxidative stability by reducing pH and thiobarbituric acid reactive substances (TBARS) and decreased microbial growth by reducing mesophilic and psychrophilic counts. The addition of mesquite honey to uncooked pork sausages reduced color changes and MMb development. Furthermore, during storage, the concentration of caffeic acid phenethyl ester (CAPE) decreased. This study found that mesquite honey has greater potential as a natural antioxidant and antibacterial ingredient for uncooked and cooked pork sausages than synthetic additives.

Keywords: phenolic compounds, biological activity, meat quality.

INTRODUCTION

The appearance of meat and meat products is one of the most important sensory attributes that influence the consumer's intention to purchase a product (Papuc *et al*., 2017). Lipid oxidation and microbial growth have been considered the primary factors that determine food quality loss during storage and changes in sensory attributes. They also cause the degradation of macronutrients (lipids and proteins) and create consumer health problems (Pateiro *et al*., 2021). Therefore, the reduction of these factors is key to improving the quality of meat products and prolonging their shelf life (Papuc *et al*., 2017; Pateiro *et al*., 2021). Synthetic additives have been used to reduce quality loss, increase consumer acceptance, and reduce human health risks (Papuc *et al*., 2017).

Synthetic antioxidants such as butylhydroxytoluene, butylhydroxyanisole, butylhydroxyquinone, and propyl gallate are widely used to reduce catalytic chain reactions, which initiate and spread lipid peroxidation. However, it has been reported that synthetic antioxidants pose safety concerns when used in doses not permitted by the corresponding legal authority (Bensid *et al*., 2022). Therefore, several investigations regarding the use of non-toxic natural preservatives with antioxidant and antimicrobial activity have increased (Pateiro *et al*., 2021).

Honey (produced by *Apis mellifera* bees) is considered a valuable food due to its nutritional components, including macronutrients and micronutrients, as well as bioactive substances like phenolic compounds (Mărgăoan *et al*., 2021). The presence of phenolic acids and flavonoids has been associated with bioactive attributes such as antiviral, anti-inflammatory, antimutagenic, antioxidant, and antibacterial activity. Thus, honey has been previously proposed as a natural food additive to reduce lipid oxidation in raw and cooked turkey breast meat and frozen chicken patties (Antony *et al*., 2000; Alabdulkarim *et al*., 2012), as well as an antimicrobial ingredient in chicken slices (El-Kalyoubi *et al*., 2014). Nevertheless, it has also been reported that the phenolic composition and bioactivity of honeys depend on their floral origin (Salvador *et al*., 2019; Viteri *et al*., 2021). Based on the above, the use of mesquite honey as a natural additive for the meat industry is still limited.

Therefore, the aim of this study was to evaluate the effect of mesquite honey on the oxidative stability and microbial growth of cooked and uncooked pork sausages during storage.

MATERIALS AND METHODS

Honey collection

Honey was collected during the period of maximum mesquite flowering (April–June 2020) in the Ures municipality of central Sonora, Mexico (29° 27′ N, 110° 22′ W; altitude 413 m). In terms of temperature (20–22 °C) and annual rainfall (400–500 mm), the climate in the region may be defined as semi-arid (SMN, 2018). The honey samples

were collected directly from the apiary in order to avoid any type of processing. Five racks were randomly chosen from the strongest hives (25 000 bees per hive), centrifuged (homemade equipment), and stored in 1 L plastic jars at -20 °C in the dark until use.

Honey characterization

Moisture content

The moisture content was determined by the 969.38 method (AOAC, 2020a), with minor changes. The honey sample was homogenized with distilled water at a 1:10 ratio (Vortexer 2, Bohemi, USA), and 100 µL of the obtained solution were transferred to the prism surface of a refractometer. Subsequently, the ratio of the refractive index and the percentage of humidity were registered.

Pollen analysis

Pollen analysis was determined using the acetolysis method (Sereia *et al*., 2011), with minor changes. The honey sample (40 mL) was homogenized with 60 mL of boiling water and centrifuged at 4000 ×g at 4 °C for 10 min (Allegra® X-12R; Palo Alto, CA, USA). The resultant sediment was dehydrated in glacial acetic acid, mixed with an anhydride/sulphuric acid solution at a 9:1 ratio, and subsequently centrifuged. The obtained sediment was mixed with 0.5 mL of glycerine, and 100 µL of the suspension was transferred onto a microscope slide. An optical microscope (Olympus CX31; Tokyo, Japan) was used to register the pollen types (at least 1000 counts). Results were assigned to four percentage classes: above 45 % (predominant), between 15–45 % (secondary), between 3–15 % (important minor), and under 3 % (minor). Plant pollen slides were used to identify floral origins.

Phenolic compounds extraction

The phenolic compounds were extracted using the column separation method (Yung An *et al*., 2016), with minor changes. The honey sample (100 g) was mixed with 500 mL of acidic water at pH 2. The resultant solution was filtered with cotton to remove solid particles, and phenolic compounds were retained in an Amberlite XAD-2 column (pore size 9 mm and particle size 1.2–3 mm). Phenolic compounds were recovered using ethanol, concentrated at 40 °C at 120 rpm (BÜCHI R-200; Flawil, Switzerland) under high vacuum (10–4 mm Hg). The obtained extract was mixed with 5 mL of distilled water, re-extracted with diethyl ether (1:10 ratio), concentrated, and stored at -20 °C in the dark.

Phenolic compounds identification

The phenolic compounds were identified by the HPLC-DAD method (Yung An *et al*., 2016), with minor changes. An HPLC system (Varian ProStar 320; CA, USA) equipped with a diode array detector (DAD) and an Agilent C18 column (150 × 4.6 mm, 5 µm)

were used. The assignment of peaks was performed by comparing the retention time and by skipping the samples with the respective standard, monitored at 290 and 340 nm. Phenolic compound quantification was performed using calibration curves at 15.6–250 μg mL^{-1} ($r \geq 0.99$). The injection volume was set to 20 μL at 1.5 mL min^{-1}, while formic acid (5 %) and methanol were used as elution solvents (A and B, respectively). The gradient program was as follows: 0 % B (0 min), 30 % (10–15 min), 40 % (15–20 min), 45 % (30–50 min), 60 % (50–52 min), 80 % (52–62 min), 100 % (65–70 min), and 0 % (70–71 min).

Caffeic acid phenethyl ester (CAPE) synthesis

CAPE synthesis was carried out by esterification of caffeic acid catalyzed with *p*-toluenesulfonic acid (Nakanishi *et al*., 1991), with minor changes. The reaction mixture contained caffeic acid and phenethyl alcohol (1:15 molar ratio). The resultant solution and catalyst (*p*-toluenesulfonic acid) were dissolved in anhydrous toluene and kept at reflux for four days. The water formed during the esterification reaction was removed using a Dean-Stark trap. Toluene was removed under reduced pressure (0.1 mm Hg) to obtain concentrated CAPE. In addition, column chromatography was used for CAPE purification. Subsequently, CAPE was crystallized, characterized by ^{1}H, ^{13}C, and IR nuclear magnetic resonance (data not shown).

Pork sausages manufacture

Pork meat (*Semimembranosus* m., 48 h *postmortem*) was acquired from a local processor (Norson®). Visible extra-muscular fat was removed. The pork meat was minced through a 4.8 mm orifice plate (Hobart Dayton 4152; OH, USA) and mixed in separate batches with back fat (40 %) and NaCl (1.5 %). The mass was divided into six different treatments: negative control, sausages without additives (T0); natural additive, sausages with 5 and 10 % mesquite honey (T1 and T2, respectively); synthetic additive #1, sausages with 100 and 200 ppm (fat basis) of CAPE (T3 and T4, respectively); synthetic additive #2, sausages with 0.02 % (fat basis) of butylhydroxytoluene (BHT) (T5). The batches were stuffed (Smith RS-2050; NJ, USA) in a natural lamb casing of 15 mm diameter. This process was maintained at low temperatures (< 5 °C). In addition, some samples were cooked until they reached an internal temperature of 71 °C (George Foreman®, USA). Uncooked and cooked pork sausages were placed on a Styrofoam® tray and wrapped with polyvinyl chloride film (17 400 cm^3 O_2 m^{-2}, at 23 °C for 24 h). Uncooked and cooked pork sausages were stored at 2 °C in the dark and opened for subsequent analysis at each sampling point (days 0, 4, 8, 12, and 16).

Physicochemical analysis of pork sausages

pH measurement

Each sampling day, uncooked and cooked pork sausages were homogenized with distilled water (1:10 ratio) at 4500 rpm at 5 °C for 1 min (T25 IKA®; Wilmington, DE,

USA) and pH was measured with a potentiometer (Ion Analyzer 255; New York, NY, USA) according to method 981.12 (AOAC, 2020b).

Lipid oxidation measurement

Lipid oxidation was determined using the thiobarbituric acid reactive substances method (Pfalzgraf *et al*., 1995). Each sampling day, uncooked and cooked pork sausages (10 g) were homogenized at 4500 rpm at 5 °C for 1 min with 20 mL of trichloroacetic acid solution (10 %). The slurry was centrifuged at 3500 ×g at 4 °C for 20 min, and 2 mL of supernatant were homogenized with 2 mL of 2-thiobarbituric acid (TBA) solution (0.02 M). The obtained mixture was incubated at 97 °C for 20 min, and the absorbance was recorded at 531 nm (Genesys 5; Madison, WI, USA). Results were calculated from a 1,1,3,3,-tetramethoxypropane standard curve ($r \geq 0.99$) and expressed as mg of malondialdehyde (MDA) kg^{-1}.

Color changes and metmyoglobin formation

The color changes and metmyoglobin (MMb) formation of uncooked pork sausages were measured by the spectrophotometric method (AMSA, 2012). Each sampling day, uncooked pork sausages were extracted from their packaging and exposed to atmospheric oxygen at 5 °C for 30 min. After that, lightness (L*), redness (a*), and yellowness (b*) were acquired to measure color changes, while the quotient K/S_{525} nm and K/S_{572} nm parameters were recorded to determine the MMb (CM 508d, Konica Minolta Inc.; Tokyo, Japan).

Microbial analysis of sausages

Mesophilic and psychrophilic bacterial growth was measured by the pour plate method (DOF, 1994). Uncooked and cooked pork sausages were homogenized with peptone water (0.1 %) at a 1:9 ratio at 25 °C for 1 min (Seward Stomacher® 400; FL, USA). After that, 1 mL of the resultant dilution was inoculated in a PCA plate and incubated for mesophilic (37 °C for 2 d) and psychrophilic growth (7 °C for 10 d). Results were expressed as $\log_{10}$ colony-forming units (CFU) g^{-1}.

CAPE content of sausages

The CAPE content was determined for uncooked and cooked pork sausages during storage. In this context, 5 g of each sausage was mixed with 100 mL of ethanol, heated in a water bath (60–70 °C for 5 h), and filtered through Whatman filter paper No. 4. The filtrate obtained was concentrated in a rotary evaporator at 64 °C under high vacuum (10–4 mm Hg). The concentrate was homogenized in 10 mL of hexane, and the solvent was evaporated under high vacuum. Subsequently, the concentrate was mixed with 5 mL of methanol, and CAPE was monitored in HPLC equipment under the aforementioned program conditions. The calibration curves were prepared using a mixture of each pork sausage (5 g) with CAPE (200 ppm) for quantification, and linear ranges were determined ($r \geq 0.99$).

Statistical analysis

The data were obtained from at least three independent experimental trials and expressed as the mean ± standard deviation. Pork sausage quality measurements were subjected to a two-way analysis of variance, considering the treatments at six levels (T0–T5) and storage time at five levels (days 0, 4, 8, 12, and 16) as the main effects and a two-way interaction. The Tukey HSD test was carried out for multiple comparisons of means at $p \leq 0.05$ (IBM SPSS Statistics, version 21).

RESULTS AND DISCUSSION

Honey characterization

Moisture content is a quality parameter related to honey maturity, production and harvest method, environmental condition, and nectar source. This parameter is also associated with the granulation and fermentation processes during storage (Rodríguez *et al*., 2012). In this study, the refractive index of the studied honey sample was 1.487, which was used to calculate the humidity percentage (15.8 %). This value is below the limit (20 % of moisture) established by Mexican regulations (DOF, 2018). In addition, the floral origin has also been associated with honey quality (Rodríguez *et al*., 2012; DOF, 2018). Several pollen types were found in the studied honey sample (Table 1). Eight pollen types that belong to three families were identified, mainly from the Fabaceae family (95 % of total pollen grains). While the most common pollen type identified was *Prosopis juliflora* (> 45 %), the honey sample was classified as either monofloral or mesquite honey. In agreement with this study, it has been reported that the mesquite tree (*Prosopis* spp.), which belongs to the Fabaceae family, is one of

Table 1. Pollen type frequencies identified in the honey samples for use in the evaluation of the oxidative stability and microbial growth of pork sausages.

Pollen type	Common name	Family	%
Prosopis juliflora	Mesquite	Fabaceae	67
Olneya tesota	Ironwood	Fabaceae	16
Desmodium spp.	--	Fabaceae	7
Cercidium floridum	Palo Verde	Fabaceae	2
Acacia spp.	--	Fabaceae	2
Brassica spp.	--	Brassicaceae	2
Dalea spp.	--	Fabaceae	1
Citrus spp.	--	Rutaceae	1
Unidentified*	--	--	2
Total	--	--	100

**Ambrosia* spp., Acanthaceae, Malvaceae, and Cactaceae (counts < 1 %).

the primary sources of pollen for honey production in semi-arid regions of Mexico (Medina-Cuéllar *et al*., 2018).

The results showed that mesquite honey is an important source of phenolic acids, esterified phenolic acids, flavones, flavonols, and flavanone compounds. Caffeic acid, kaempferol, pinocembrin, and luteolin were the most abundant in this honey (Table 2). In agreement with this study, it has been reported that there is the presence of caffeic acid, caffeic acid phenethyl ester (CAPE), acacetin, chrysin, galangin, kaempferol, myricetin, pinobanksin, pinocembrin, and luteolin in several mono-floral honeys,

Table 2. Identified and quantified phenolic compounds in mesquite (*Prosopis juliflora)* honey for use in the evaluation of the oxidative stability and microbial growth of pork sausages.

#	Compounds	Retention time (min)	μg 100 g^{-1}
1	Caffeic acid	7.7	157.7 ± 3.12
2	Myricetin	16.2	76.50 ± 1.73
3	Pinobanksin	20.0	(+)
4	Luteolin	23.5	126.25 ± 1.44
5	Kaempferol	25.4	347.70 ± 3.12
6	Apigenin	27.2	33.60 ± 1.62
7	Pinocembrin	30.2	168.10 ± 2.19
8	Chrysin	35.6	11.10 ± 1.27
9	Galangin	36.1	4.75 ± 0.29
10	CAPE	37.0	2.70 ± 0.35
11	Acacetin	38.5	3.35 ± 0.17

(+) compound identified but not quantified. CAPE: caffeic acid phenethyl ester.

although the type of compound and its concentration will depend on the vegetation surrounding the collection area, climate, and geographic region (Mărgăoan *et al*., 2021). Also, it has been demonstrated that honey is an important source of bioactive compounds that exert antioxidant and antimicrobial activity (Salvador *et al*., 2019; Viteri *et al*., 2021). In this regard, the findings obtained highlight that mesquite honey may be employed as a possible antioxidant and antibacterial additive to enhance oxidative stability and reduce the microbial growth of meat products.

Oxidative stability

In this study, a significant effect on pH and thiobarbituric acid reactive substances (TBARS) values ($p \leq 0.001$) was found by the treatment x storage time interaction. At the beginning of the experiment (day 0), pH values (Figure 1A) of uncooked and

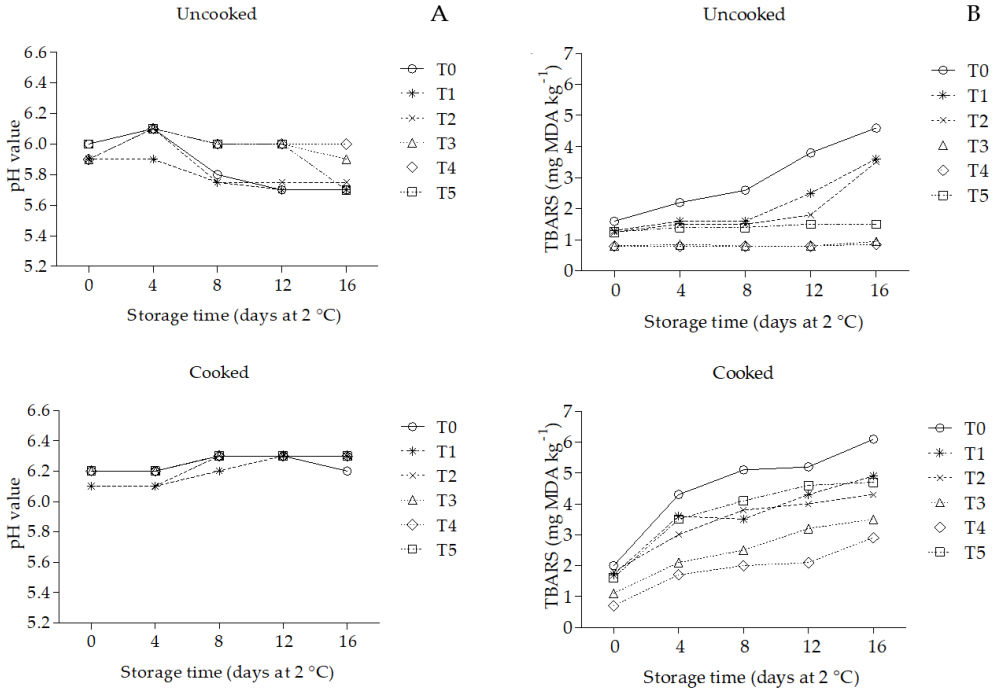

Figure 1. Effect of treatment and storage time on pH and thiobarbituric acid reactive substances (TBARS) values in uncooked and cooked pork sausages. A: effect on pH; B: effect on TBARS. T0: negative control, sausages without additives; T1: natural antioxidant, sausages with 5 % of mesquite honey; T2: natural antioxidant, sausages with 10 % of mesquite honey; T3: synthetic additive #1, sausages with 100 ppm of caffeic acid phenethyl ester (CAPE); T4: synthetic additive #1, sausages with 200 ppm of CAPE; T5: synthetic additive #2, sausages with 0.02 % of butylhydroxytoluene (BHT).

cooked pork sausages were not affected ($p \geq 0.05$) by the inclusion of T1 and T2, as well as T3 and T4 (average pH values of 5.9 and 6.2, respectively). In addition, these values decreased ($p \leq 0.05$) only for uncooked samples during storage. At the end of the experiment (day 16), uncooked and cooked pork sausages treated with T3 and T4 showed the highest ($p \leq 0.05$) pH values compared to T0 (an increase of 3.4 % in this parameter). Additionally, at the beginning of the experiment (day 0), the TBARS values (Figure 1B) of uncooked and cooked pork sausages were reduced ($p \leq 0.05$) by the inclusion of additives (T3 and T4 > T1 and T2 > T5); however, these values increased ($p \leq 0.05$) during storage. At the end of the experiment (day 16), uncooked pork sausages treated with T3 and T4 (80.4 % inhibition), T1 and T2 (22.8 % inhibition), and T5 (67.4 % inhibition) showed lower TBARS values than T0 ($p \leq 0.05$). Cooked pork sausages treated with T3 and T4 (47.5 % inhibition), T1 and T2 (24.6 % inhibition), and T5 (23 % of inhibition) showed lower TBARS values than T0 ($p \leq 0.05$).

Although pH and TBARS data on the effect of the inclusion of mesquite honey in pork sausages during storage is still limited, it has been reported that a natural additive obtained from a honeybee product reduces pH and TBARS changes in an uncooked and cooked meat product during storage, which is associated with the antioxidant effect of the identified phenolic compounds, mainly attributed to the presence of CAPE (Vargas-Sánchez *et al*., 2015, 2019). In disagreement with this study, Yücel *et al*. (2005) reported a not significant effect on pH values of marinated cooked chicken breast incorporated with honey (20 and 30 %; undeclared floral origin) and stored at 4 °C for 7 d; however, a reduction in pH values of chicken meat was observed during storage. Also, a not significant effect was observed on pH values of cooked turkey slices incorporated with 5 and 15 % of dry honey (undeclared floral origin) and stored at 4 °C for 12 weeks under vacuum (Antony *et al*., 2006).

In agreement with this study, a reduction in TBARS values was reported on cooked ground turkey incorporated with 5 % of acacia, clover, soy, and buckwheat honey (53.2, 61.6, 65.1, and 79.4 %, respectively) stored at 4 °C for 3 d (McKibben and Engeseth, 2002). Also, clover and wildflower honey decreased TBARS values of beef patties stored at 4 °C for 12 d and at -18 °C for 45 d (Johnston *et al*., 2005). Sampaio *et al*. (2012) reported a higher reduction in TBARS values for chicken thigh and breast (70 and 82 % inhibition, respectively) incorporated with 5 and 10 % of orange honey than BHT (28.6 and 30 % inhibition, respectively) stored at 4 °C for 4 d. Another study demonstrated that the incorporation of acacia honey (5 and 10 %) reduced TBARS values of fried chicken patties stored at -18 °C for 60 d (Alabdulkarim *et al*., 2012). Similarly, a decrease in TBARS values (26.7 %) was reported in pork sausages incorporated with honey (1.15 %, multifloral origin) stored at 2 °C for 20 d in the dark, which was associated with an increase in phenolic compounds and antiradical activity during storage (Półtorak *et al*., 2018). Recently, a reduction of TBARS values was reported in chicken sausages treated with 2 and 4 % of honey (undeclared floral origin) stored at -20 °C for 30 d (Ali *et al*., 2022).

Meat color is a sensory attribute that has a primary influence on consumers' propensity to purchase it, as it changes from red to brown during storage, reducing its acceptability. (Passetti *et al*., 2019). Also, MMb formation has been associated with color changes in meat and meat products due to high MMb values related to the oxidation process. Nevertheless, these parameters can be affected by changes in the pH and TBARS values of meat and meat products during storage (Khan *et al*., 2015; Passetti *et al*., 2019). In our study, a significant effect ($p \leq 0.001$) on color and MMb formation values (Figure 2) was found by the treatment x storage time interaction. No significant effect ($p \geq 0.05$) was shown in L* and b* values during storage (average values of 60.8 and 18.3, respectively) for uncooked pork sausages. However, a* and MMb formation values decreased and increased during storage, respectively ($p \leq 0.05$). At the end of the experiment (day 16), samples incorporated with antioxidants T1 and T2 (29.4 %) > T3, T4, and T5 (18.1 %) increased a* values compared to T0 ($p \leq 0.05$). In addition, T1 and T2 (32.1 %) > T3, T4, and T5 (12.3 %) reduced MMb formation values compared to T0 ($p \leq 0.05$).

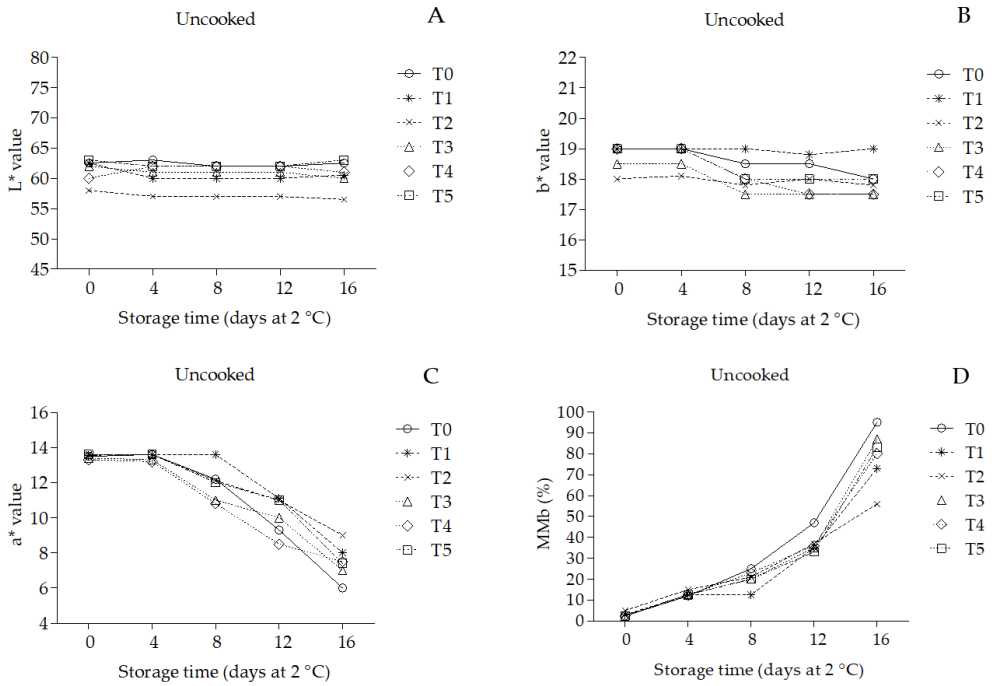

Figure 2. Effect of treatment and storage time on color changes and MMb formation values of uncooked pork sausages. A–D: effect on color changes; D: effect on MMb formation values. T0: negative control, sausages without additives; T1: natural antioxidant, sausages with 5 % of mesquite honey; T2: natural antioxidant, sausages with 10 % of mesquite honey; T3: synthetic additive #1, sausages with 100 ppm of caffeic acid phenethyl ester (CAPE); T4: synthetic additive #1, sausages with 200 ppm of CAPE; T5: synthetic additive #2, sausages with 0.02 % of butylhydroxytoluene (BHT).

Although color and MMb formation data on the effect of the inclusion of mesquite honey in pork sausages during storage is still limited, it has been reported that a natural additive obtained from a honeybee product reduces the color and MMb formation changes of uncooked meat products during storage, which is associated with the antioxidant effect of the identified phenolic compounds (Vargas-Sánchez *et al*., 2015, 2019). In disagreement with this study, Yücel *et al*. (2005) reported that the inclusion of honey (20 and 30 %; undeclared floral origin) in marinated chicken breast reduced color changes (b* value) during storage at 4 °C for 7 d. Although certain phenolic compounds were found in greater concentrations in mesquite honey, a previous study revealed through computational methods (DFT, density functional theory) that CAPE exerted the highest antiradical potential when compared with other phenolic compounds (Vargas-Sánchez *et al*., 2015).

Microbial growth

In this study, a significant effect ($p \leq 0.001$) on mesophilic and psychrophilic counts (Figure 3) was found by the treatment x storage time interaction. At the beginning of the experiment (day 0), no significant effect ($p \geq 0.05$) was found. However, microbial growth increased during storage ($p \leq 0.05$). At the end of the experiment (day 16), samples treated with T1 and T2 reduced mesophilic counts ($p \leq 0.05$) for uncooked and cooked pork sausages (13.3 and 15.2 %, respectively) when compared to T0, while samples treated with T4 also reduced mesophilic growth in uncooked and cooked pork sausages (8.1 and 7.1 %, respectively). Furthermore, samples treated with T1 and T2 showed the lowest ($p \leq 0.05$) psychrophilic counts in uncooked (20.6 and 33.8 %, respectively) and cooked pork sausages (36.4 and 58.2 %, respectively).

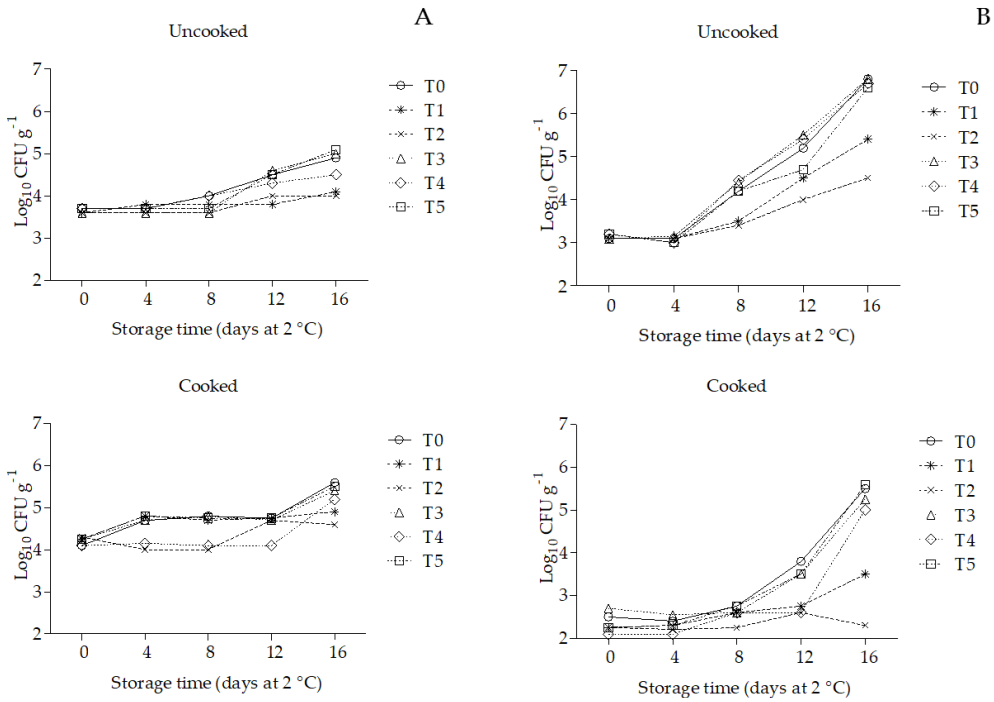

Figure 3. Effect of treatment and storage time on microbial growth values of uncooked and cooked pork sausages. A: mesophilic counts; B: psychrophilic counts. T0: negative control, sausages without additives; T1: natural antioxidant, sausages with 5 % of mesquite honey; T2: natural antioxidant, sausages with 10 % of mesquite honey; T3: synthetic additive #1, sausages with 100 ppm of caffeic acid phenethyl ester (CAPE); T4: synthetic additive #1, sausages with 200 ppm of CAPE; T5: synthetic additive #2, sausages with 0.02 % of butylhydroxytoluene (BHT).

In agreement with this study, a decrease in mesophilic counts (19.1 %) was reported in pork sausages incorporated with 1.15 % of honey (multifloral origin) stored at 2 °C for 20 d in the dark (Półtorak *et al*., 2018). El-Kalyoubi *et al*. (2014) reported that the

incorporation of 5, 10, and 20 % of honey (undeclared floral origin) into chicken slices reduced mesophilic counts (4.0, 3.4, and 5.5 %, respectively) stored at 4 °C for 14 d. Also, McKibben and Engeseth (2002) reported that Chinese milk vetch, acacia, mixed-breed, and buckwheat honey decreased by 82.7, 84.5, 81.8, and 83.6 % the mesophilic counts of raw beef stored at 10 °C for 7 days in the dark. A significant effect was observed in mesophilic counts of marinated cooked chicken breast with honey (20 and 30 %) during storage (Yücel *et al*., 2005). In addition, the literature indicates that the antimicrobial effects of CAPE are probably based on RNA polymerase and cellular protein inhibition (Arasoglu *et al*., 2015).

CAPE content in pork sausages

In this study, a significant effect ($p \leq 0.001$) on CAPE content (Figure 4) was found by the treatment x storage time interaction. At the beginning of the experiment (day 0), uncooked and cooked pork sausages incorporated with T4 showed the highest ($p \leq 0.05$) CAPE values (87 and 130.6 ppm, respectively). However, CAPE content decreased ($p \leq 0.05$) during storage. No detectable CAPE content ($p \geq 0.05$) was shown in the rest of the treatments. At the end of the experiment (day 16), uncooked and cooked pork sausages incorporated with T4 showed the highest ($p \leq 0.05$) CAPE content (62.6 and 89.4 ppm, respectively). In agreement with this study, it was demonstrated that the incorporation of honey and other natural sources rich in phenolic compounds increases phenolic bioavailability in meat products, and storage influenced this content (Półtorak *et al*., 2018; Vargas-Sánchez *et al*., 2019).

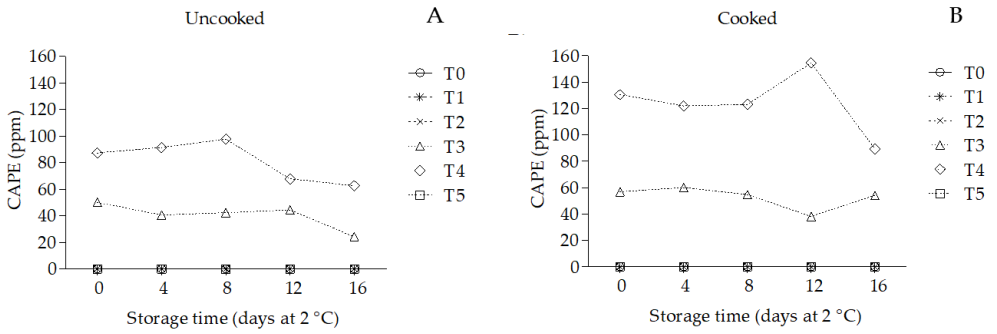

Figure 4. Effect of treatment and storage time on caffeic acid phenethyl ester (CAPE) content of pork sausages. A: cooled sausages; B: uncooked sausages. T0: negative control, sausages without additives; T1: natural antioxidant, sausages with 5 % of mesquite honey; T2: natural antioxidant, sausages with 10 % of mesquite honey; T3: synthetic additive #1, sausages with 100 ppm of CAPE; T4: synthetic additive #1, sausages with 200 ppm of CAPE; T5: synthetic additive #2, sausages with 0.02 % of butylhydroxytoluene (BHT).

CONCLUSIONS

The evaluated honey samples revealed acceptable humidity levels that complied with Mexican regulations. According to the pollen analysis, the floral origin of the sample was mesquite (*Prosopis juliflora*). Mesquite honey showed the presence of phenolic compounds, including phenolic acids and flavonoids. The inclusion of mesquite honey in uncooked and cooked pork sausages increased oxidative stability by reducing pH and thiobarbituric acid reactive substances and decreased microbial growth by reducing mesophilic and psychrophilic counts. A reduction in color changes and metmyoglobin formation was also observed in uncooked pork sausages. In addition, a reduction in caffeic acid phenethyl ester content was observed during storage. In comparison to artificial additives, this study found that mesquite honey has great potential as a natural addition to both cooked and uncooked pork sausages.

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REFERENCES

Alabdulkarim B, Bakeet ZAN, Arzoo S. 2012. Effect of frying oils on quality characteristics of frozen chicken patties incorporated with honey. African Journal of Biotechnology 11 (12): 2985–2992. https://doi.org/10.5897/ajb11.3389

Ali MS, Rahman MM, Habib M, Kabir MH, Hashem MA, Azad MAK. 2022. Quality of spent hen sausages incorporated with bee honey. Meat Research 2 (1). https://doi.org/10.55002/mr.2.1.9

AMSA (American Meat Science Association). 2012. AMSA meat color measurement guidelines. American Meat Science Association: Champaign, IL, USA. 136 p.

Antony S, Rieck JR, Dawson PL. 2000. Effect of dry honey on oxidation in turkey breast meat. Poultry Science 79 (12): 1846–1850. https://doi.org/10.1093/ps/79.12.1846

Antony S, Rieck JR, Acton JC, Han IY, Halpin EL, Dawson PL. 2006. Effect of dry honey on the shelf life of packaged turkey slices. Poultry Science 85 (10): 1811–1820. https://doi.org/10.1093/ps/85.10.1811

AOAC (Association of Official Analytical Chemists). 2020a. AOAC 969.38-1969, Moisture in honey. Gaitherburg, MD, USA. http://www.aoacofficialmethod.org/index.php?main_page=product_info&products_id=2876 (Retrieved: January 2023).

AOAC (Association of Official Analytical Chemists). 2020b. AOAC 981.12-1982, pH in acidified foods. Gaitherburg, MD, USA. http://www.aoacofficialmethod.org/index.php?main_page=product_info&products_id=1159 (Retrieved: January 2023).

Arasoglu T, Derman S, Mansuroglu B. 2015. Comparative evaluation of antibacterial activity of caffeic acid phenethyl ester and PLGA nanoparticle formulation by different methods. Nanotechnology 27 (2): 025103. https://doi.org/10.1088/0957-4484/27/2/025103

Bensid A, El Abed N, Houicher A, Regenstein JM, Özogul F. 2022. Antioxidant and antimicrobial preservatives: Properties, mechanism of action and applications in food–a review. Critical

Reviews in Food Science and Nutrition 62 (11): 2985–3001. https://doi.org/10.1080/10408398.2020.1862046

El-Kalyoubi MH, Khalaf MM, Nadir AS., Wafaa MA, Mansour ME. 2014. Bee honey effect against microbial and oxidation in chicken slices. Middle East Journal of Applied Sciences 4 (4): 884–893.

Johnston JE, Sepe HA, Miano CL, Brannan RG, Alderton AL. 2005. Honey inhibits lipid oxidation in ready-to-eat ground beef patties. Meat Science 70 (4): 627–631. https://doi.org/10.1016/j.meatsci.2005.02.011

Khan A, Allen K, Wang X. 2015. Effect of Type I and Type II antioxidants on oxidative stability, microbial growth, pH, and color in raw poultry meat. Food and Nutrition Sciences 6 (16): 1541. https://doi.org/10.4236/fns.2015.616159

Mărgăoan R, Topal E, Balkanska R, Yücel B, Oravecz T, Cornea-Cipcigan M, Vodnar DC. 2021. Monofloral honeys as a potential source of natural antioxidants, minerals and medicine. Antioxidants 10 (7): 1023. https://doi.org/10.3390/antiox10071023

McKibben J, Engeseth NJ. 2002. Honey as a protective agent against lipid oxidation in ground turkey. Journal of Agricultural and Food Chemistry 50 (3): 592–595. https://doi.org/10.1021/jf010820a

Medina-Cuéllar SE, Tirado-González DN, Portillo-Vázquez M, López-Santiago MA, Franco-Olivares VH. 2018. Environmental implications for the production of honey from mesquite (*Prosopis laevigata*) in semi-arid ecosystems. Journal of Apicultural Research 57 (4): 507–515. https://doi.org/10.1080/00218839.2018.1454377

Nakanishi K, Oltz EM, Grunberger D. 1991. Caffeic acid esters and methods of producing and using same. U.S. Patent No. 5,008,441. Washington, DC, USA. 12 p.

DOF (Diario Oficial de la Federación). 1994. NORMA Oficial Mexicana NOM-110-SSA1-1994. Preparación y dilución de muestras de alimentos para su análisis microbiológico. Gobierno de México. Secretaría de Salud. Ciudad de México, México. http://www.salud.gob.mx/unidades/cdi/nom/110ssa14.html (Retrieved: May 2020).

DOF (Diario Oficial de la Federación). 2018. NORMA Oficial Mexicana NOM-004-SAG/GAN-2018. Producción de miel y especificaciones. Gobierno de México. Secretaría de Agricultura y Desarrollo Rural. Ciudad de México, México. http://dof.gob.mx/nota_detalle.php?codigo=5592435&fecha=29/04/2020 (Retrieved: July 2021).

Papuc C, Goran GV, Predescu CN, Nicorescu V, Stefan G. 2017. Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products: Classification, structures, sources, and action mechanisms. Comprehensive Reviews in Food Science and Food Safety 16 (6): 1243–1268. https://doi.org/10.1111/1541-4337.12298

Passetti RAC, Resconi VC, Çakmakçı C, Campo MM, Kirinus JK, Passetti LCG, Guerrero A, do Prado IN, Sañudo C. 2019. Number of consumers and days of display necessary for the assessment of meat colour acceptability. Food Research International 121: 387–393. https://doi.org/10.1016/j.foodres.2019.03.036

Pateiro M, Domínguez R, Lorenzo JM. 2021. Recent research advances in meat products. Foods 10 (6): 1303. https://doi.org/10.3390/foods10061303

Pfalzgraf A, Frigg M, Steinhart H. 1995. Alpha-tocopherol contents and lipid oxidation in pork muscle and adipose tissue during storage. Journal of Agricultural and Food Chemistry 43 (5): 1339–1342. https://doi.org/10.1021/jf00053a039

Półtorak A, Marcinkowska-Lesiak M, Lendzion K, Moczkowska M, Onopiuk A, Wojtasik-Kalinowska I, Wierzbicka A. 2018. Evaluation of the antioxidant, anti-inflammatory and

antimicrobial effects of catuaba, galangal, roseroot, maca root, guarana and polyfloral honey in sausages during storage. LWT-Food Science and Technology 96: 464–370. https://doi.org/10.1016/j.lwt.2018.05.035

Rodríguez BA, Mendoza S, Iturriga MH, Castaño-Tostado E. 2012. Quality parameters and antioxidant and antibacterial properties of some Mexican honeys. Journal of Food Science 77 (1): C121–C127. https://doi.org/10.1111/j.1750-3841.2011.02487.x

Salvador L, Guijarro M, Rubio D, Aucatoma B, Guillén T, Vargas-Jentzsch P, Ciobotă V, Stolker L, Ulic S, Vásquez L *et al*. 2019. Exploratory monitoring of the quality and authenticity of commercial honey in Ecuador. Foods 8 (3): 105. https://doi.org/10.3390%2Ffoods8030105

Sampaio GR, Saldanha T, Soares RAM, Torres EAFS. 2012. Effect of natural antioxidant combinations on lipid oxidation in cooked chicken meat during refrigerated storage. Food Chemistry 135 (3): 1383–1390. https://doi.org/10.1016/j.foodchem.2012.05.103

Sereia MJ, Alves EM, Toledo VA, Marchini LC, Serine ES, Faquinello P, de Almeida D, Moreti AC. 2011. Physicochemical characteristics and pollen spectra of organic and non-organic honey samples of *Apis mellifera* L. Anais da Academia Brasileira de Ciências 83 (3): 1077–1090. https://doi.org/10.1590/S0001-37652011000300026

SMN (Servicio Meteorológico Nacional). 2018. Temperature report in Mexico. Gobierno de México. Comisión Nacional del Agua. Servicio Meteorológico Nacional. Ciudad de México, México. https://smn.conagua.gob.mx/es/climatologia/pronostico-climatico/temperatura-form (Retrieved: May 2020).

Vargas-Sánchez RD, Mendoza-Wilson AM, Torrescano-Urrutia GR Sánchez-Escalante A. 2015. Antiradical potential of phenolic compounds fingerprints of propolis extracts: DFT approach. Computational and Theoretical Chemistry 1066: 7–13. https://doi.org/10.1016/j.comptc.2015.05.003

Vargas-Sánchez RD, Torrescano-Urrutia GR, Torres-Martínez BDM, Pateiro M, Lorenzo J M, Sánchez-Escalante A. 2019. Propolis extract as antioxidant to improve oxidative stability of fresh patties during refrigerated storage. Foods 8 (12): 614. https://doi.org/10.3390/foods8120614

Viteri R, Zacconi F, Montenegro G, Giordano A. 2021. Bioactive compounds in *Apis mellifera* monofloral honeys. Journal of Food Science 86 (5): 1552–1582. https://doi.org/10.1111/1750-3841.15706

Yücel B, Önenç A, Bayraktar H, Açikgöz Z, Altan Ö. 2005. Effect of honey treatment on some quality characteristics of broiler breast meat. Journal of Applied Animal Research 28 (1): 53–56. https://doi.org/10.1080/09712119.2005.9706788

Yung An C, Hossain MM, Alam F, Islam MA, Khalil MI, Alam N, Gan SH. 2016. Efficiency of polyphenol extraction from artificial honey using C18 cartridges and Amberlite® XAD-2 resin: A comparative study. Journal of Chemistry: 8356739. https://doi.org/10.1155/2016/8356739