Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Inhibitory Effects of Propolis, Bee Pollen, and Beeswax against Staphylococcus aureus in Ground Beef

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Abstract

THIS study assessed the antibacterial efficacy of honeybee-derived products—propolis, bee pollen, beeswax, and their combination—against multidrug-resistant *Staphylococcus aureus (S. aureus)* inoculated in raw ground beef at a concentration of 5 log 10 CFU/g, in response to increasing consumer demand for natural food preservatives. A blend of bee products demonstrated the greatest minimum inhibitory concentration (MIC), followed by propolis, then beeswax, and bee pollen. The antibacterial capabilities, physicochemical characteristics, and sensory quality of the meat were assessed during cold storage at 4°C. The combination of bee products and propolis exhibited the most potent antibacterial efficacy, markedly decreasing *S. aureus* counts by the 3rd and 5th days and entirely suppressing growth by day 7. Treated samples had an increased shelf life, lasting satisfactory until days 9 and 11, but untreated controls deteriorated by day 5. Sensory analysis indicated a postponed decline in sensory attributes, however physicochemical evaluations validated enhanced acceptable quality in treated samples. Electron microscopy demonstrated significant damage to the cell membranes and internal structures of *S. aureus*, especially in samples subjected to propolis and the bee product mixture. The findings indicate that propolis and mixes of bee products may serve as excellent natural preservatives, improving both the microbiological safety and quality of ground beef under refrigeration.

Keywords: *S. aureus*, Ground beef, Propolis, Bee pollen, Beeswax, TBA, TVB-N, Shelf life, Electron Microscope.

Introduction

Meat serves as a significant source of protein and vital vitamins for global populations. It is essential for the growth, repair, and maintenance of bodily cells and facilitates daily physiological functions. Minced meat is especially vulnerable to contamination and can be a major source of food borne pathogens when managed or processed in unsanitary circumstances [1]. The safety and quality of meat products, particularly minced meat, are frequently jeopardized by food borne pathogens like *S. aureus*, a bacterium that produces enterotoxins responsible for food poisoning and significant public health risks. In light of these hazards, there is a rising interest in natural antimicrobial agents as substitutes for synthetic preservatives, propelled by heightened consumer demand for clean-label and sustainable food items [3].

Bee-derived products, including propolis, bee pollen, and beeswax, have garnered interest for their antibacterial properties and capacity to enhance food flavor and quality. These products suppress microbial proliferation and improve sensory attributes, rendering them appealing choices for food preservation without sacrificing quality [4]. Propolis is a resinous material synthesized by honeybees from plant resins and their own glandular excretions. Abundant in flavonoids and phenolic acids, it demonstrates potent antibacterial, antifungal, and antiviral capabilities that safeguard the hive from microbial threats [5]. In culinary applications, propolis has demonstrated a considerable reduction of *S. aureus* populations in minced meat by

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(Received 05 July 2025, accepted 04 September 2025)
DOI: 10.21608/EJVS.2025.400899.2950

compromising bacterial cell walls and obstructing essential cellular functions [6]. Bee pollen, composed of pollen grains combined with honeybee saliva and nectar, is acknowledged for its abundant phytochemical constituents, antioxidant capabilities, and antibacterial effects. Bee pollen extracts exhibit inhibitory actions against Gram-positive foodborne pathogens, such as *Staphylococcus species* [7]. Beeswax, excreted by worker bees to construct honeycomb formations, is esteemed for its hydrophobic and defensive characteristics. In the food industry, it serves as a natural coating or additive (E901) to safeguard products and prolong shelf life. Shelf life is the period during which a food product is safe for eating, maintaining adequate microbiological, chemical, and sensory quality. Transmission Electron Microscopy (TEM) can be utilized to elucidate the mechanisms of antimicrobial action, providing high-resolution images of bacterial cell shape and structural changes [9]. This study aimed to assess the antibacterial activity of propolis, bee pollen, beeswax, and their combination against multidrug-resistant *S. aureus* inoculation in raw ground beef. The study also evaluated the impact of these treatments on physicochemical and sensory characteristics, shelf life, and bacterial cell membrane integrity as observed using TEM.

Material and Method

Preparation of S. aureus bacterial strains

The *S. aureus* strain (ATCC 25923) was supplied by the Reference Laboratory for Food Safety at the Animal Health Research Institute (AHRI) in Dokki, Egypt. The strain was preserved on tryptic soy agar slants with 3% NaCl at 4°C. Prior to the experiment, fresh microbial cultures were calibrated to 0.5 McFarland, approximately corresponding to 5 log 10 CFU/ml.

Preparation of extracts:

Propolis, bee pollen and beeswax were brought as powder from National Research Center in Cairo, Egypt.

Preparation of Propolis, Bee pollen and Beeswax [10]:

One hundred grams of powdered product underwent alcoholic extraction with 400 milliliters of 70% ethanol. The mixture was stirred for 2 hours and subsequently stored overnight at 4ºC. Subsequently, it was filtered using Whatman No. 2 filter paper with a Buchner funnel. The filtrate was concentrated by evaporating the ethanol using a rotary evaporator. The crude ethanolic extract was preserved in dark brown bottles under refrigeration for future use. The experimental working concentration was established according to the minimal inhibitory concentration (MIC), ensuring that it did not affect the sensory attributes of the minced meat.

Antibiotic sensitivity test of S. aureus isolate:

The bacterial strain was evaluated in vitro for its susceptibility to the following antimicrobial discs: Penicillin (P) 10 mcg, Erythromycin (E) 15 mcg, Flucloxacillin (FL) 5 mcg, Clindamycin (DA) 2 mcg, Vancomycin (VA) 30 mcg, Linezolid (LNZ) 30 mcg, and Cefotaxime (CTX) 30 mcg [11]. An inoculum for each isolate was created by emulsifying colonies from an overnight pure culture in sterile normal saline, thereafter adjusting the suspension turbidity to the 0.5 McFarland standard. The bacterial suspension was evenly streaked on the dried surface of Mueller Hinton agar plates using sterile cotton swabs and allowed to rest for 3 minutes before the application of antibiotic disks using sterile forceps. Plates were incubated at 35 °C for 24 hours, and sensitivity was assessed by measuring the widths of the inhibitory zones in millimeters [12].

Determination of Minimum Inhibitory Concentration (MIC) Using Microtiter Plate Method:

The Minimum Inhibitory Concentration (MIC) of propolis, bee pollen, beeswax, and their combination against *S. aureus* was assessed utilizing 96-well microtiter plates. Each well of the microtiter plate was filled with 100 µL of sterile nutritional broth. Subsequently, 100 µL of either extract (100 mg/ml) or the combination was introduced. A two-fold serial dilution was performed across wells 1 to 10 to establish a spectrum of concentrations for MIC assessment. Following dilution, 100 µL of S. aureus suspension (standardized to 5 log 10 CFU/mL) was introduced into the wells. Well 11 served as the positive control, comprising nutritional broth and bacterial culture alone (without extract), whilst Well 12 functioned as the negative control, holding solely sterile distilled water (absent of bacteria or extract). The plates were incubated at 37°C for a duration of 24 hours. Bacterial proliferation was evaluated visually using turbidity assessment. The MIC was established as the minimal concentration of the extract that exhibited no observable bacterial proliferation [13].

Transmission Electron Microscopy (TEM) observation

Samples were prepared for transmission electron microscopy (TEM) following the methodology of Glauert and Lewis, [14] with minor modifications. Samples were initially fixed in 2.5% glutaraldehyde formulated in 0.1 M phosphate buffer (pH 7.4) at 4°C for a duration of 2 hours. Subsequent to fixation, the samples were washed thrice with phosphate-buffered saline (PBS) for 10 minutes each. Post-fixation was conducted using 1% osmium tetroxide

in PBS for 30 minutes at ambient temperature. The samples were subsequently rinsed in PBS three times for 10 minutes each and dehydrated using a graded ethanol series (30%, 50%, 70%, 90%, and 100%), with each step lasting 30 minutes. Subsequent to dehydration, the samples underwent treatment with acetone for one hour to facilitate resin infiltration. Embedding was performed utilizing Araldite 502 resin, succeeded by polymerization. Semi-thin slices (0.5–1 µm) were prepared with an LEICA Ultracut UCT ultramicrotome and stained with 1% toluidine blue to identify areas of interest using a light microscope. Ultrathin slices (about 70 nm) were subsequently prepared from designated areas, stained in succession with uranyl acetate and lead citrate, and examined utilizing a JEOL JEM-100SX transmission electron microscope (JEOL Ltd., Tokyo, Japan) [15].

Preparation of meat Samples

Five kilograms of freshly ground beef were procured from a butcher shop in Tanta City, Egypt, and promptly transferred to the Animal Health Research Institute, Tanta laboratory branch, in an icebox under aseptic circumstances. The ground beef was partitioned into ten equal segments of 500 grams each. Each group was inoculated with 10^5 cfu/g (5 log10 CFU/g) of *S. aureus* suspension, excluding the negative control group (G1) and sensory evaluation groups (G7), (G8), (G9), and (G10). Subsequent to inoculation, the meat was meticulously stirred for 3 minutes to guarantee uniform dissemination of the germs. The groups were subsequently incubated at room temperature (25°C) for 30 minutes to facilitate bacterial attachment and adsorption [16]. Subsequently, natural extracts were incorporated into the designated treatment groups, excluding the positive control group (G2). The experimental groups were organized as detailed below: (G1) Negative control: non-inoculated, untreated; (G2) Positive control: inoculated with *S. aureus*, untreated. (G3) Inoculated and treated with propolis extract at a concentration of 50 mg/ml, (G4) inoculated and treated with pollen extract at a concentration of 50 mg/ml, (G5) inoculated and treated with beeswax at a concentration of 50 mg/ml, and (G6) inoculated and treated with a mixture of propolis, pollen, and beeswax extract (1:1:1) for a total of 50 mg/ml, (G7) non-inoculated and treated with propolis extract at a concentration of 50 mg/ml, (G8) non-inoculated and treated with pollen extract at a concentration of 50 mg/ml, (G9) non-inoculated and treated with beeswax at a concentration of 50 mg/ml, and (G10) non-inoculated and treated with a mixture of propolis, pollen , and beeswax extract (1:1:1) for a total of 50 mg/ml. Each extract was meticulously blended into the meat sample for three minutes to guarantee uniformity. Following treatment, the ground beef samples were placed in sterile polyethylene bags, tagged, and refrigerated at 4°C for further analysis. The groups were assessed on days 0, 3, 5, 7, 9, and 11 of storage until spoiling, during which several tests were performed, including bacterial enumeration of S. aureus, physicochemical analyses (pH, TVN, and TBA), and sensory evaluation for (G1), (G7), (G8), (G9), and (G10). Every experiment was conducted in triplicate.

Microbiological examination [17].

Ten grams from each tested group were deposited in a stomacher bag with 90 mL of sterile 0.1% peptone water (Merck, Germany) and homogenized. Serial decimal dilutions of the homogenates were conducted, and 0.1 mL of each sample was inoculated onto the surface of Baird-Parker Agar (BPA; Oxoid). The plates were incubated at 37 °C for 24–48 hours to enumerate *S. aureus* in ground meat, with results represented as log CFU/g.

Physiochemical analysis

Determination of pH [18]

Approximately 10 g of the material were blended with 10 ml of neutralized distilled water in a blender. The homogenate was maintained at ambient temperature for 10 minutes with constant agitation. The pH value was measured using an electrical pH meter (Bye model 6020, USA). The pH meter was calibrated using two buffer solutions with precisely established pH values (alkaline pH 7.01 and acidic pH 4.01). Consequently, the pH electrode was rinsed with neutralized water and thereafter immersed in the homogenate following the adjustment of the temperature correction system.

Determination of Total Volatile Nitrogen (TVN) [19]:

In a clean distillation flask, 10 g of the test samples were added to 300 ml of distilled water and well stirred using a polytron probe. Subsequently, an antifoaming agent and 2 grams of magnesium oxide were included. To a 500 ml receiving flask, 25 ml of 2% boric acid and several drops of indicator were introduced. The receiving flask was positioned so that the receiver tube submerged beneath the boric acid solution. The distillation flask was heated to boiling within 10 minutes, maintained for 25 minutes, and then distilled for an additional 25 minutes. The titration of TVN in boric acid with 0.1 N H_2SO_4 was documented. Consequently, TVA was computed using the subsequent formula: TVN/l00g = (ml H_2SO_4 n 0.1 for sample – ml H_2SO_4 n 0.1 for Blank) × 14

Determination of Thiobarbituric Acid Number (TBA) [20]

The assay relies on the quantification of malonaldehyde (MDA) as a final result of lipid

peroxidation. Oxidative rancidity is often quantified using the TBA number, expressed in milligrams of malonaldehyde equivalents per kilogram of the materials. Oxidative degradation of unsaturated fatty acids in meat results in the production of free malonaldehyde, which subsequently forms a TBA-malonaldehyde complex. Malonaldehyde production can be quantified as a biomarker of lipid oxidation and food quality. Consequently, 10 g of the prepared meat group sample was placed in a distillation flask and combined with 50 ml of distilled water, followed by the addition of 2.5 ml of hydrochloric acid diluted in 47.5 ml of water. Subsequently, tiny quantities of antifoaming chemicals were incorporated. The distillation flask was heated to distill 50 cc within 10 minutes of the onset of boiling. Subsequently, 5 ml of a distilled solution was placed in a covered tube, followed by the addition of 5 ml of prepared thiobarbituric acid (which was formulated by dissolving 0.2883 g of thiobarbituric acid in 90% trichloroacetic acid and diluting to 100 ml). The tube was sealed and placed in a water bath, boiled for 35 minutes, and subsequently cooled in water for 10 minutes. The sample's absorbance was measured with a spectrophotometer (UNICAM969AA Spectronic, USA) at a wavelength of 538 nm. TBA value = absorbance of sample x 7.8 (Malondialdehyde (mg) / Kg)

Sensory evaluation [21]

A sensory evaluation of control and treated ground beef was conducted during refrigerated storage on days 0, 3, 5, 7, 9, and 11 by a trained panel of nine members (five females and four males, aged 30 to 40 years). Panelists participated in 10 structured training sessions, each lasting one hour, to improve their sensory sensitivity and familiarize themselves with the evaluation procedure. Training was deemed complete when individual scores varied by no more than one unit from the mean score. Randomly selected representative samples were presented on porcelain plates. Each sample was assessed in triplicate for color, odor, appearance, and consistency to guarantee statistical reliability, on a five-point scale (5 = very good, 4 = good, 3 = acceptable, 2 = unsatisfactory, 1 = bad).

Statistical Analysis [22]

The data underwent analyzed by one-way ANOVA, with results shown as means accompanied by their respective standard deviations (SD). Duncan's multiple range tests were utilized to discern significant differences between treatment groups at a significance level of $P < 0.05$.

Results

Antibiotic sensitivity test

Table 1 displays the outcomes of the antibiotic susceptibility assay, indicating that the strain exhibited resistance to penicillin, flucloxacillin, clindamycin, and cefotaxime.

Minimum Inhibitory Concentration (MIC)

Table (2) presents the results of the Minimum Inhibitory Concentration (MIC). The combination of bee products and propolis exhibited the most significant antibacterial efficacy against *S. aureus*, with inhibition detected at doses as low as 6.25 mg/mL and 12.5 mg/mL, respectively. Pollen and wax demonstrated negligible antibacterial action, with inhibition observed solely at 25 mg/mL. The amalgamation of all three extracts exhibited superior potency relative to pollen and wax alone, indicating possible synergistic effects.

Impact of bee products on S. aureus populations

Table (3) assesses the impact of propolis, pollen, beeswax, and their combination on *S. aureus* levels in intentionally infected minced beef refrigerated at $4 \pm 1°C$ for 11 days. On day 0, all treatment groups (G3–G6) and the positive control (G2) had comparable bacterial counts, whereas the negative control (G1) demonstrated no growth. By day 3, bacterial proliferation markedly escalated in the positive control (5.37 log 10 CFU/g), while both treated groups had decreased counts, with the mixture (G6) demonstrating the most substantial reduction (3.35 log 10 CFU/g). By the fifth day, the positive control was entirely compromised. The combo remained the most efficacious treatment, demonstrating markedly reduced bacterial counts compared to the individual components. By the seventh day, the negative control deteriorated. G6 exhibited total suppression of *S. aureus*, whilst other treatments retained measurable bacterial numbers. By day 9, deterioration was noted in the beeswax group (G5). Only propolis (G3) and the mixture (G6) exhibited no detectable bacterial proliferation. By day 11, G3 and G6 were the sole treatments exhibiting full inhibition of *S. aureus*, hence affirming their superior and sustained antibacterial efficacy.

Physicochemical properties

pH measurements

Table (4) illustrates the effect of several bee products on the pH of *S. aureus*-contaminated minced beef preserved at 4 ± 1 °C for 11 days. Initially, all samples exhibited comparable pH values between 5.62 and 5.67. Spoilage was observed in the positive control (G2) by day 5 and in the negative control (G1) by day 7, as evidenced by pH rises. Conversely, the combination (G6) and propolis (G3) exhibited the lowest pH values during storage, concluding at 5.94 and 6.06, respectively, indicating

superior preservation. Bee pollen (G4) and beeswax (G5) exhibited mild effects. By day 9, G5 (beeswax) surpassed the spoiling pH threshold (>7.0). By day 11, only G3 and G6 sustained pH.

Thiobarbituric Acid (TBA) values

Table (5) illustrates the effect of several bee products on lipid oxidation, quantified by Thiobarbituric Acid (TBA) values, in *S. aureus*-contaminated minced beef during 11 days of refrigeration. All samples commenced with modest TBA values (about 0.07–0.08 mg MDA/kg). Among the treated groups, G3 (propolis) and G6 (combination) exhibited the most potent antioxidant activity, sustaining TBA values beneath the spoilage threshold even on day 11 (0.92 and 0.83 mg MDA/kg, respectively). G4 (pollen) demonstrated moderate efficacy, with values attaining 0.88 mg MDA/kg by day 9 and surpassing the threshold by day 11. G5 (beeswax) exhibited minimal antioxidant protection and indications of deterioration by day 9.

Total Volatile Nitrogen (TVN) values

TVN is considered as an indicative of protein deterioration, are displayed in Table 6, illustrating the impact of bee products over 11 days of refrigerated storage. At the outset, all samples exhibited low total volatile nitrogen (TVN) values (1.87–1.93 mg/100 g), indicative of fresh meat quality. The treated groups (G3 to G6) demonstrated markedly reduced increases in TVN. G6 (mixture) consistently remained beneath the spoiling threshold during the testing, demonstrating significant preservative efficacy. G3 (propolis) also significantly inhibited rotting. G4 (pollen) had moderate efficacy, while G5 (beeswax) showed the least effectiveness in regulating TVN levels.

Evaluation of sensory attributes

Fig 1 assessed the effects of beeswax, pollen, propolis, and their combination on the sensory attributes and shelf life of *S. aureus*-contaminated minced beef under refrigeration. Sensory characteristics such as hue, fragrance, visual aspect, texture, and general appeal were evaluated utilizing a 5-point scale. The control group (G1) deteriorated rapidly, becoming unfit for use by day 5. Beeswax (G9) and pollen (G8) substantially prolonged shelf life to 9 and 11 days, respectively, exhibiting acceptable, albeit diminishing, sensory quality. Propolis (G7) exhibited marginally superior performance, sustaining satisfactory sensory evaluations until day 11. The G10 mixture proved to be the most efficacious treatment, maintaining superior sensory quality throughout the storage duration and postponing deterioration beyond day 11, so more than doubling the shelf life in comparison to the controls.

Transmission Electron Microscopy (TEM)

Fig. (2) depicts the effect of propolis, pollen, beeswax, and their combination on the ultrastructure of *S. aureus* cells as examined via transmission electron microscopy (TEM). The findings demonstrated considerable variability in the extent of bacterial cell wall damage among the treatments. The combination of all three bee products (Fig. E) resulted in the most significant structural damage, encompassing total disintegration of the bacterial cell wall and considerable release of cytoplasmic contents, signifying potent antibacterial activity. Propolis alone (Fig. B) had a marginally less severe yet still significant effect, characterized by observable cell wall breakdown, cellular deformation, and cytoplasmic leakage. Beeswax (Fig. C) and pollen (Fig. D) induced significant, albeit less severe, morphological alterations, including cellular shrinkage and partial depletion of internal components. Conversely, the control group (Fig. A) exhibited intact bacterial cells with well-preserved cytoplasmic and membrane features, signifying the absence of damage.

Discussion

Meat products are vulnerable to infection by foodborne microorganisms, including *S. aureus*. This bacterium produces enterotoxins, which cause foodborne diseases and pose a significant public health issue (Pérez-Boto et al. [2]). Our findings align with those of Sharma et al. [23], demonstrating that this strain is multidrug-resistant (MDR) and presents a challenge for treatment with standard antibiotics. antibacterial resistance is regarded as a global concern, necessitating the development of natural antibacterial compounds. The findings suggest that propolis is probably the principal active ingredient accountable for the demonstrated antimicrobial efficacy and highlight its potential as a natural antibacterial agent, while its combination with other bee products may provide formulation advantages. The results surpassed those of Ambi et al. [26], who showed that Russian propolis ethanol extract (RPEE) effectively induced cell lysis and compromised bacterial membranes within mature biofilms at significantly lower doses of about 2–4 µg/mL. Lu et al. [27] similarly observed that Taiwanese propolis demonstrated potent antibacterial activity against S. aureus, with MIC values between less than 3.75 µg/mL and 60 µg/mL. Ikejiofor et al. [28] determined that the MIC values for pollen and wax were elevated, at 125 µg/mL. Additional studies identified comparatively elevated anti-staphylococcal activity in propolis from Turkey, Oman, and Ireland, with minimum inhibitory concentrations (MICs) of 8, 42, and 80 µg/mL, respectively [29-31]. Conversely,

Chilean propolis exhibited significantly diminished efficacy against S. aureus, with MIC values between 200 and 26,900 µg/mL. These comparisons underscore the impact of geographic origins, plant sources, and botanical characteristics on its antibacterial properties [32]. The results unequivocally indicate that propolis and its combination with other apicultural products are highly efficacious in diminishing *S. aureus* populations in contaminated minced beef during refrigeration. The mixture (G6) consistently exhibited the most significant antibacterial effect, presumably due to synergistic interactions among its constituents. The findings corroborate prior research indicating that ethanolic extract of propolis (EEP) significantly diminishes *S. aureus* levels in fish products, especially at doses of 1% and above. Moreover, other researches [34, 35] documented analogous antibacterial properties of EEP. Nevertheless, certain studies [36] saw no significant reduction in bacteria, underscoring heterogeneity presumably because to disparities in propolis composition, concentration, and bacterial strains. EEP exhibits both bacteriostatic and bactericidal characteristics, influenced by various variables. Additionally, Al-Waili [32] and Fratini et al. [33] discovered that beeswax exhibited little effects on *S. aureus*, demonstrating only slight to moderate suppression. Limited studies have shown that beeswax possesses antibacterial properties against some infections, including S. aureus [33]. Subsequent research emphasized the synergistic antibacterial efficacy of propolis and beeswax, revealing that their combined effects surpassed those of each component individually. The combination successfully suppressed the proliferation of *S. aureus* [37]. The pH level of meat is a crucial determinant of its freshness and microbiological stability. Fresh minced beef generally exhibits a pH range of 5.5 to 5.8, with rising pH levels over time indicating deterioration resulting from protein degradation and microbial proliferation [38]. Furthermore, bee-derived products, particularly propolis and its mixes, efficiently regulated the pH of *S. aureus*-contaminated minced meat throughout storage, signifying maintained quality. This impact was ascribed to the antibacterial characteristics of propolis, which suppressed rotting microbes. These results are corroborated by earlier research [39, 40] indicating that propolis-treated beef products exhibited reduced pH levels and prolonged shelf life relative to untreated samples. Lipid oxidation, quantified by TBA, significantly contributes to quality degradation in preserved meat. Propolis (G3) and the mixture (G6) markedly inhibited lipid oxidation, maintaining TBA readings beneath the spoiling threshold (0.9 mg MDA/kg) [41], even on day 11. The antioxidant benefits are ascribed to the elevated levels of flavonoids and phenolic chemicals in propolis, which are recognized for their ability to scavenge free radicals [40, 42]. Pollen (G4) had modest antioxidant activity, presumably attributable to its carotenoids and phenolic compounds, whereas beeswax (G5) shown the least efficacy. These findings corroborate earlier research [43] illustrating the antioxidant capabilities of bee-derived compounds, especially propolis. Total Volatile Nitrogen (TVN) is acknowledged as a dependable indicator for evaluating protein degradation in meat, with increasing values signifying microbiological and enzymatic deterioration [44]. In fresh meat, low total volatile nitrogen (TVN) contents indicate high quality, but elevated levels denote spoilage. As per EOS [41]. The control groups (G1, G2) deteriorated swiftly, whereas propolis (G3) and the mixture (G6) markedly postponed spoiling. G6 consistently kept TVN levels beneath the spoiling threshold during the trial, indicating a synergistic effect when propolis is mixed with bee pollen and beeswax. The results align with previous research [40, 45, 46] that validated the efficacy of bee products, particularly propolis, as natural meat preservatives. The synergistic effect seen in the mixing group further substantiates the application of blended bee products to improve preservation and prolong shelf life during refrigeration. The sensory evaluation results validate the enhanced preservation efficacy of bee-derived compounds, especially when utilized in combination. The mixture (G10) regularly exhibited superior sensory quality and prolonged shelf life beyond day 11, markedly surpassing individual treatments. These findings align with prior studies [47–49], which indicated that bee products enhance the sensory qualities and shelf life of processed meat products. Propolis exhibited significant antibacterial and antioxidant capabilities, enhancing its capacity to maintain meat quality. Pollen and beeswax enhanced sensory characteristics, albeit to a lesser degree, indicating their auxiliary role in preservation when utilized together. Transmission Electron Microscopy (TEM) investigations revealed clear evidence of bacterial cell damage caused by bee products. The combined treatment resulted in the most significant structural disturbance, perhaps owing to the synergistic effects of its bioactive constituents. The detected cytoplasmic leakage and complete cell wall collapse indicate mechanisms include electron transport chain disruption, oxidative phosphorylation interference, and compromised food uptake perhaps attributed to free fatty acid levels [25, 50]. Propolis alone caused considerable cell wall destruction, likely due to the presence of chrysin, a flavonoid recognized for its ability to break bacterial membranes and cell walls [51]. Mirzoeva et al. [52] indicated that propolis compromises the bacterial cytoplasmic membrane, diminishing its functional

integrity and resulting in cell death. This corroborates the present study's finding that propolis alone induced substantial cell wall disruption and cytoplasmic leakage in *S. aureus*. Furthermore, the findings indicate that pollen and wax exert mild to moderate influences on bacterial morphology. aligning with prior studies. Pascoal et al. [53] emphasized that the polyphenolic content of bee pollen is the primary factor responsible for its antibacterial effect. Beeswax has demonstrated antimicrobial properties, presumably attributed to its small bioactive lipid constituents [33]. The untreated control group displayed intact cellular structures, supporting the assertion that bee-derived compounds, particularly in combination, demonstrate potent antibacterial actions at the cellular level. These findings endorse the utilization of bee products as natural antibacterial agents and further validate their contribution to enhancing food safety and shelf life.

Conclusion

This study revealed that honeybee-derived products, especially propolis and its combination with other bee products (pollen and beeswax), have substantial antibacterial efficacy against drug-resistant S. aureus in raw ground beef. Of the treatments evaluated, the mixture demonstrated the most potent inhibitory effect, entirely eradicating bacterial growth by day 7 and prolonging the meat's shelf life for up to 11 days under refrigeration. Propolis had significant bactericidal characteristics, whereas bee pollen and beeswax demonstrated modest effects. These treatments enhanced the physicochemical and sensory attributes of the beef, postponing deterioration signs including odor and discolouration. Electron microscopy validated significant bacterial cell damage in samples treated with propolis and mixtures, corroborating their antibacterial effectiveness.

Ethical considerations

This study was done according to the ethical guidliness of the Animal Health Research Institute.

Conflict of interest

The authors declare they have no conflict of interest.

Authors' contributions

All authors contributed equally to this study.

TABLE 1. *In vitro* antimicrobial sensitivity testing of *S. aureus* isolates

Antibacterial disc	Code	Concentration	Senstivity
Penicillin	P	10mcg	**R**
Erythromycin	E	15mcg	**S**
Fluxacillin	FL	5mcg	**R**
Clindamycin	DA	2mcg	**R**
Vancomycin	VA	30mcg	**S**
Linezolid	LNZ	30mcg	**S**
Cefotaxime	CTX	30mcg	**R**

N.B: R (Resistance) , S (Sensitive)

TABLE 2. Minimum Inhibitory Concentrations MIC (mg/mL) of bee products on *S.aureus*.

Cocentration / groups	50	25	12.5	6.25	3.125	1.56
Proplies	-	-	-	+	+	+
Wax	-	-	+	+	+	+
Pollen	-	-	+	+	+	+
Mixure	-	-	-	-	+	+

N.B : - No Growth (clear), + Growth (turbid)

TABLE 3. Effect of bee products (propolis, pollen, beeswax and their mixture) on the count of *S. aureus* (log10 CFU/g) experimentally inoculated to ground beef samples.

Storage period \ Groups	G1	G2	G3	G4	G5	G6
0 day	free	4.85 ± 0.1[a]	4.77 ± 0.1[a]	4.82± 0.08[a]	4.82± 0.1[a]	4.75 ± 0.1[a]
3rd day	free	5.37 ± 0.2[a]	4.31 ± 0.1[c]	4.33± 0.1[c]	4.55± 0.05[b]	3.35 ± 0.07[d]
5th day	free	spoiled	3.24± 0.1 [c]	3.52± 0.1[b]	4.32 ± 0.07[a]	2.32 ± 0.09[d]
7th day	spoiled	Spoiled	2.15± 0.1c	3.31± 0.1[b]	3.44± 0.3[a]	No growth
9th day	spoiled	Spoiled	No growth	3.14± 0.09	Spoiled	No growth
11th day	spoiled	Spoiled	No growth	Spoiled	Spoiled	No growth

N.B : Different alphabetical letters within the same raw mean significant using ANOVA at p-value < 0.05.
(G1) negative control, (G2) positive control, (G3) propolis, (G4) pollen, (G5) bee wax and (G6) mixture.

TABLE 4. Effect of bee products (propolis, pollen, beeswax and their mixture) on pH of experimentally inoculated ground beef samples with *S. aureus*.

Storage period \ Groups	G1	G2	G3	G4	G5	G6
0 day	5.67± 0.46[a]	5.67± 0.46[a]	5.63±0.48 [c]	5.63±0.48 [b]	5.64±0.25 [b]	5.62±0.32[b]
3rd day	6.20± 0.22 [a]	6.29±0.32 [a]	5.72±0.29 [c]	5.84±0.15 [b]	5.86±0.11 [b]	5.71±0.26 [d]
5th day	6.46±0.47 [a]	spoiled	5.96± 0.26 [c]	6.12± 0.17[b]	6.18± 0.21 [a]	5.83± ±0.16[d]
7th day	spoiled	Spoiled	6.06± 0.15[c]	6.19± 0.22[b]	6.28± 0.31[a]	5.94± 0.05[d]
9th day	spoiled	Spoiled	6.32± 0.32 [b]	6.52± 0.33 [a]	Spoiled	6.13± 0.16 [c]
11th day	spoiled	Spoiled	6.57±0.33 [a]	Spoiled	Spoiled	6.36± 0.49 [b]

Different alphabetical letters within the same raw mean significant using ANOVA at *p-value* < 0.05.

TABLE 5. Effect of bee products (propolis, pollen, beeswax and their mixture) on TBA (mg MDA/kg) level of experimentally inoculated ground beef samples with *S. aureus*

Storage period \ Groups	G1	G2	G3	G4	G5	G6
0st day	0.08± 0.01[a]	0.08± 0.01[a]	0.07± 0.01[b]	0.08± 0.01[a]	0.08± 0.01[a]	0.07± 0.01[b]
3rd day	0.54± 0.01[a]	0.61± 0.01[a]	0.19± 0.01[c]	0.21 ± 0.01[b]	0.25± 0.01[b]	0.16± 0.02[d]
5th day	0.82± 0.02[a]	spoiled	0.32± 0.02[d]	0.38± 0.01[c]	0.43 ± 0.03[b]	0.22± 0.02[d]
7th day	spoiled	spoiled	0.47± 0.02[c]	0.54± 0.03[b]	0.62 ± 0.02[a]	0.34± 0.04[d]
9th day	spoiled	spoiled	0.77± 0.02[b]	0.88± 0.02 [a]	Spoiled	0.65± 0.07[c]
11th day	spoiled	spoiled	0.92±0.02 [a]	Spoiled	Spoiled	0.83±0.03 [b]

Different alphabetical letters within the same raw mean significant using ANOVA at *p-value* < 0.05

TABLE 6. Effect of bee products (propolis, pollen, beeswax and their mixture) on TVN (mg/100 g) level of experimentally inoculated ground beef samples with *S. aureus*.

Storage period \ Groups	G1	G2	G3	G4	G5	G6
0 day	1.93± 0.33[a]	1.96± 0.37 [a]	1.87± 0.14[b]	1.88± 0.34[b]	1.89± 0.36 [b]	1.87± 0.21[b]
3rd day	12.44 ± 0.25 [a]	12.52±0.52 [a]	4.95± 0.16[b]	5.10± 0.19[c]	5.23± 0.31[b]	4.79± 0.28[e]
5th day	19.32 ± 0.35 [a]	Spoiled	9.34± 0.61[d]	9.59± 0.62 [c]	9.80 ± 0.75[b]	8.94± 0.11[e]
7th day	spoiled	Spoiled	13.22± 0.35[c]	14.60± 0.56[b]	14.84± 0.62 [a]	12.80± 0.21 [d]
9th day	spoiled	Spoiled	17.95±.0.1 [a]	19.22 ± 0.39 [b]	Spoiled	16.78± 0.33 [b]
11th day	spoiled	Spoiled	20.34±0.48 [a]	Spoiled	Spoiled	18.86±0.25 [b]

Different alphabetical letters within the same raw mean significant using ANOVA at *p-value* < 0.05.

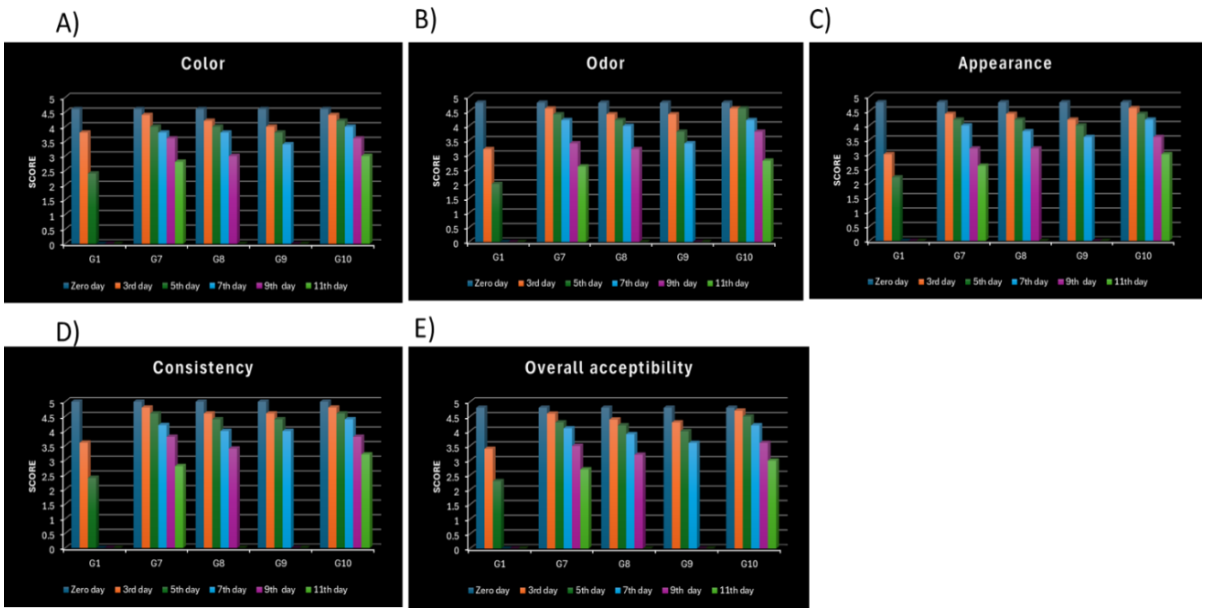

Fig. 1. Sensory evaluation of ground beef samples treated with Propolis, Bee Pollen, Beeswax and their mixture

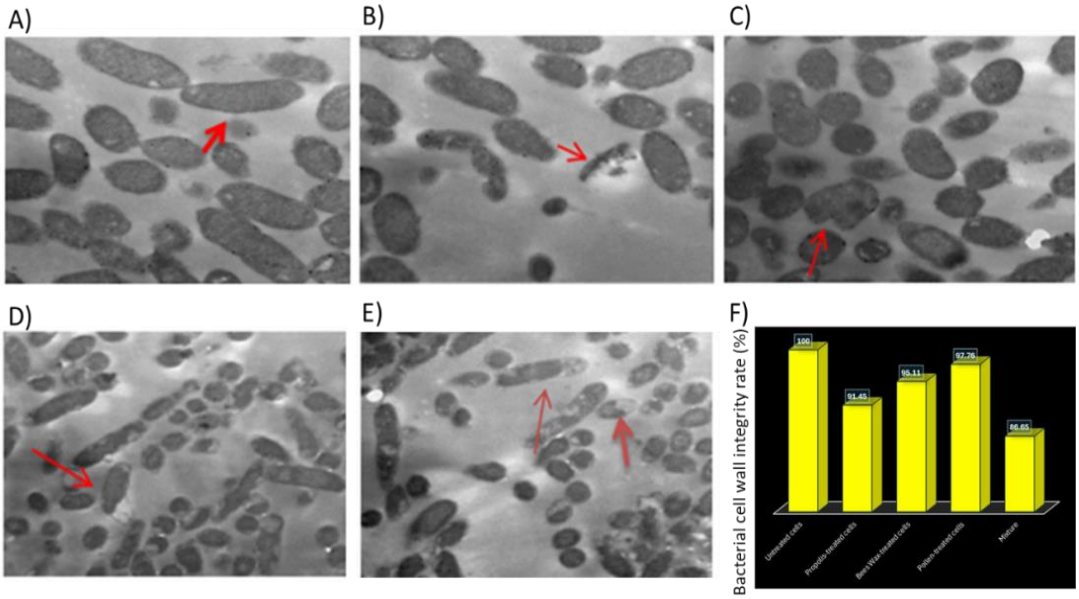

Fig. 2. Transmission electron microscopy (TEM) images of bee products effects on *S. aureus* bacterial cells (A) Untreated control cells, (B) Propolis-treated cells, (C) Beeswax-treated cells, (D) Pollen-treated cells, (E) mixture of bee products, and (F) Bar graph for the effects of the bee products on *S. aureus* bacterial cell wall integrity (%)

References

1. Alexa, E. A., Papadochristopoulos, A., O'Brien, T. and Burgess, C. M. Microbial contamination of food. In *Food Packaging and Preservation* (Chapter 1), 9-13. (2024). https://doi.org/10.1016/B978-0-323-90044-7.00001-X.

2. Pérez-Boto D., D'Arrigo M., García-Lafuente A., Bravo D., Pérez-Baltar A., Gaya P., Medina M. and Arqués JL. *Staphylococcus aureus* in the Processing Environment of Cured Meat Products. *Foods*, **12**(11),2161(2023). doi: 10.3390/foods12112161

3. Mekhloufi, O.A., Chieffi, D., Hammoudi, A., Bensefia, S.A., Fanelli, F. and Fusco, V. Prevalence, Enterotoxigenic Potential and Antimicrobial Resistance of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus (MRSA*) Isolated from Algerian Ready to Eat Foods. *Toxins*, **13**, 835(2021). doi: 10.3390/toxins13120835.

4. Káčanová, M., Vuković, N., Chlebo, R., Haščík, P., Rovná, K., Čubon, J., Džugan, M. and Pasternakiewicz, A. The antimicrobial activity of honey, bee pollen loads and beeswax from Slovakia. *Archives of Biological Sciences*, **64**(3), 927–934. (2012). DOI:10.2298/ABS1203927K

5. Liu, M., Li, X., Chen, H., Pana, F., Zhenga, X., Battinob, M., Tiana, W. and Penga W. Propolis as a promising functional ingredient: A comprehensive review on extraction, bioactive properties, bioavailability, and industrial applications. *Food Science and Human Wellness*,**14**,9250236(2025). https://doi.org/10.26599/FSHW.2024.9250236.

6. Kurek-Górecka, A., Górecki, M., Rzepecka-Stojko, A., Balwierz, R. and Stojko, J. Bee products in dermatology and skin care. *Molecules*, **25**(3),556(2020). https://doi.org/10.3390/molecules25030556

7. Cavallero, A., Vidotto, F., Sbrana, C., Peres Fabbri, L., Petroni, G. and Gabriele, M. Antioxidant-Rich Polyfloral Bee Pollen Exerts Antimicrobial Activity and Anti-Inflammatory Effect in A549 Lung Epithelial Cells by Modulating the NF-κB Pathway. *Foods*, **14**(5),802(2025). https://doi.org/10.3390/foods14050802

8. Fadiji, T., Rashvand, M., Daramola, M.O. and Iwarere, S.A. A Review on Antimicrobial Packaging for Extending the Shelf Life of Food. *Processes*,**11**(2),590(2023). https://doi.org/10.3390/pr11020590.

9. Mokobi, F. Transmission Electron Microscope (TEM)-Definition, Principle, Images. *Microbe Notes*, https://microbnotes.com (2023).

10. Ana, M., Silvia, V., José, L. Bernal., María, J. Nozal. and José Bernal Extraction and determination of bioactive compounds from bee pollen. *Pharm Biomed Anal.*, **147**(5), 110-124.DOI: 10.1016/j.jpba.2017.08.009 (2018).

11. Bauer, A., Kirby, W., Sherris, J. and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, **45**(4),493-496(1966). https://doi.org/10.1093/ajcp/45.4_ts.493

12. Clinical Laboratory Standards Institute, (CLSI) M100 Performance Standards for Antimicrobial Susceptibility Testing 30th ed.,Clinical Laboratory Standards Institute,Wayne,Pennsylvania, USA. Available at https://www.nih.org.pk/wpcontent/uploads/2021/02/CLSI **(2020).**

13. Wiegand, I., Hilpert, K and Hancock, R.E.W. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances.*Nature Protocols*, **3**(2), 163–175 (2008). DOI: 10.1038/nprot.2007.521

14. Glauert, A. M. and Lewis, P. R. Biological Specimen Preparation for Transmission Electron Microscopy. *Princeton University Press.* **196**(3), 353-354 (1998).

15. Bozzola, J.J. and Russell, L.D. Electron Microscopy: Principles and Techniques for Biologists. Jones and Bartlett, Boston: 670 (1999).DOI:10.1017/S1431927602029975

16. Dubal, ZB., Paturkar, AM., Waskar, VS., Zende, RJ and Latha C. Effect of food grade organic acids on inoculated *S. aureus, L. monocytogenes, E. coli and S. typhimurium* in sheep/goat meat stored at refrigeration temperature. *Journal of Meat Sci*ence, **66**, 817- 821 (2004). doi: 10.1016/j.meatsci.2003.08.004.

17. ISO 6888-1. Microbiology of the food chain Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) Part 1: Technique using Baird-Parker agar medium (2021).

18. Pearson, D. Chemical Analysis of Foods. 11th Ed, Publishing Co. Churchill Livingstone, Edinburgh, London, United Kingdom (2006).

19. Egyptian Organization for Standardization (EOS**).** Methods of analysis and testing for meat. Part 9: determination of total volatile nitrogen (TVN).ES:63-9 **(**2006).

20. Egyptian Organization for Standardization (EOS). Methods of analysis and testing for meat. Part 10: determination of thiobarbituric acid (TBA). ES: 63-10 (2006).

21. Fik, M. and Fik, A. Microbiological and sensory changes in minced beef treated with potassium lactate and sodium diacetate during refrigerated storage. *Journal International Food Properties*, **10**, 589-598(2007). https://doi.org/10.1080/10942910601048911

22. SPSS 20. "SPSS for Microsoft products screenshot (s) 2011", © Copyright IBM ® SPSS Statistics v20 Corporation. (2011)

23. Sharma, B.S., Rathnapraba, S., Meignanalakshmi, S., Manoharan, S., Saranya, S. and Vijayarani, K. Incidence and Molecular Characterisation of Staphylococcus aureus isolated from meat products. *International Journal of Current Microbiology and Applied Sciences*, **7**(9), 3163-3169(2018). DOI: https://doi.org/10.20546/ijcmas.2018.709.394

24. Abouda, Z., Zerdani, I., Kalaliu, I., Faid, M. and Ahami, M. TThe Antibacterial activity of Moroccan bee bread and bee pollen (fresh and dried against pathogenic bacteria *Research Journal of Microbiol*ogy, **6**,376-384(2011). https://scialert.net/abstract/?doi=jm.2011.376.384

25. Bava,R.,Puteo,C.,Lombardi,R.,Garcea,G.,Lupia,C.,Spano,A.,Liguori,G.,Palma,E.,Britti,D. and Castagna, F. Antimicrobial properities of hive products and their potential applications in human and veterinary medicine. *Antibiotics*, **14**(2), 172(2025). https://doi.org/10.3390/antibiotics14020172

26. Ambi, A., Bryan, J., Borbon, K., Centeno D., Liu, T., Chen, T.P., Cattabiani, T. and Traba C. Are Russian propolis ethanol extracts the future for the prevention of medical and biomedical implant contaminations? *Phytomedicine*, **30**, 50–58(2017). doi: 10.1016/j.phymed.2017.03.006.

27. Lu, L.-C., Chen, Y.W. and Chou C.C. Antibacterial activity of propolis against Staphylococcus aureus. International *Journal of Food Microbiology*, **102**, 213–220(2005). doi: 10.1016/j.ijfoodmicro.2004.12.017.

28. Ikejiofor, O.K.,Ochai, S.,Olabode, P.M. and Olushola, O. Comparative study on antibacterial activities of bee products (Propolis,pollen,bee wax and honey).*International Journal of Pharmacy and Biological Sciences*, **13**(2), 20-27(2018). DOI: 10.9790/3008-1302022027

29. Uzel, A., Sorkun, K., Önçağ, Ö., Çoğulu, D., Gençay, Ö. and Salih, B. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. *Microbiological Research*, **160**, 189–195(2005). doi: 10.1016/j.micres.2005.01.002.

30. Popova, M., Dimitrova, R., Al-Lawati, H.T., Tsvetkova, I., Najdenski, H. and Bankova, V. Omani Propolis: chemical profiling, antibacterial activity and new propolis plant sources. *Chemistry Central Journal*, **7**, 158(2013). doi: 10.1186/1752-153X-7-158.

31. AL-Ani, I., Zimmermann, S., Reichling, J. and Wink, M. Antimicrobial activities of European propolis collected from various geographic origins alone and in combination with antibiotics. *Medicines*, **5**(1), 2(2018). doi: 10.3390/medicines5010002.

32. Bouchelaghem, S. Propolis characterization and antimicrobial activities against Staphylococcus aureus and Candida albicans: A review. *Saudi Journal of Biological Sciences*, **29**(4), 1936-1946(2022). https://doi.org/10.1016/j.sjbs.2021.11.063

33. Al-Waili, N.S. Mixture of honey, Beeswax and Olive Oil Inhibits growth of *Staphylococcus aureus* and *Candida albicans*. *Archives of Medical Re*search, **36**, 10-13(2005). doi: 10.1016/j.arcmed.2004.10.002.

34. Fratini, F., Cilia, G., Turchi, B. and Felicioli, A. Beeswax: A minireview of its antimicrobial activity and its application in medicine. *Asian Pacific Journal of Tropical Med*icine, **9**(9), 839-843(2016). https://doi.org/10.1016/j.apjtm.2016.07.003

35. Suarez, M.H., Jiménez, T.Á. and Díaz, M.C. Determination of microbiological and sensory parameters of fish fillets with propolis preserved under refrigeration. *Reviews MVZ Cordoba*, **19**, 4214–4225(2014). https://doi.org/10.1016/j.apjtm.2016.07.003

36. Saleh, E., Morshdy, A.E. Nassar, A.M.K. and Waly Eldein, M.F. Enhancement of Quality Characteristics and Shelf–Life of Tilapia (Oreochromis niloticus) Fillets by Propolis. *5th International Food Safety Conference Damanhour University* (2018).

37. Palomino García, LR., Martínez Galán, J.P., García Pajón, C.M., Gil González, J.H. and Durango Restrepo, D.L. Caracterización fisicoquímica y actividad antimicrobiana del propóleos en el Municipio de la Unión (Antioquia, Colombia). *Review Faculty of Natural Agricultur*, **63**(1), 5373-5383(2010). http://www.scielo.org.co/scielo.php?script=sci_arttext &pid=S0304-28472010000100013&lng=en&tlng=es.

38. Castaldo, S. and Capasso, F. Propolis, an old remedy used in modern medicine. *Fitoterapia*, **73** (1), 1-6 (2002). DOI: 10.1016/s0367-326x(02)00185-5

39. Yassin, M.N. Effect of storage conditions on the quality parameters of differently treated fish (Ph.D. thesis, Faculty of Agriculture, Ain Shams University, Cairo, Egypt) (2003).

40. El-Demery, M. E., Hoda, A.I.A. and El-Kholy, W.F.A. Efficiency of propolis and turmeric powders as natural preservatives in minced beef. *Journal of Food and Dairy Sciences, Mansoura University*, **7**(1), 45–50(2016). DOI: 10.21608/JFDS.2016.42805

41. Gedikoğlu, A. Antimicrobial and Antioxidant Activities of Commercialized Turkish Propolis Extract, and Application to Beef Meatballs *Turkish Journal of Agriculture - Food Science and Technology*, **10**(10), 2021–2029(2022). DOI: https://doi.org/10.24925/turjaf.v10i10.2021-2029.5340

42. Egyptian standard specification (EOS 1694 / 2005). Egyptian standards specification for minced meat. Egyptian Organization for Standardization and Quality Control (2005).

43. Al-Azee, M. T. A., Sinan, A. and Al-Rubeii, A**.** Effect of adding propolis to improve quality of frozen ground beef. *Indian Journal of Ecology*, **49**(20), 439–445 (2022).

44. Anjos, O., Fernandes, R., Cardoso, S. M., Carpes, S., Delgado, T., Farinha, N., Paola,V., Estevinho, L.M. and Carpes, S.T. Bee pollen as a natural antioxidant source to prevent lipid oxidation in black pudding. *LWT - Food Science and Technology*, **111**, 869–875(2019). https://doi.org/10.1016/j.lwt.2019.05.105

45. Bekhit, A. E. D. A., Hopkins, D. L., Geesink, G. H., Bekhit, A. A. and Franks, P. Total volatile basic nitrogen (TVB-N) and its role in meat spoilage: A review. *Trends in Food Science & Technology*, **109**, 280–302 (2021). DOI:10.1016/j.tifs.2021.01.006

46. Fatma, H. A., Kassem, G. M. and Atta-Alla, O. A. Propolis as a natural decontaminant and antioxidant in fresh oriental sausage. *Veterinaria Italiana*, **46**(2), 167–172 (2010).

47. Turhan, S., Yazici, F., Saricaoglu, F. T., Mortas, M. and Genccelep, H. Evaluation of the nutritional and storage quality of meatballs formulated with bee pollen. *Korean Journal for Food Science of Animal Resources*, **34**(4), 423–433(2014). doi: 10.5851/kosfa.2014.34.4.423.

48. Mehdizadeh, T. and Mojaddar Langroodi, A**.** Chitosan coatings incorporated with propolis extract and *Zataria multiflora* Boiss oil for active packaging of chicken breast meat. *International Journal of Biological Macromolecules*, **141**, 401–409 doi: 10.1016/j.ijbiomac.2019.08.267. **(2019).**

49. Mashhadi, Z., Davati, N., Emamifar, A. and Karami, M. The effect of nano/microparticles of bee pollen on the shelf life of high-fat cooked sausage during refrigerated storage. *Food Science & Nutrition*, **12**(6), 4269–4283(2024). https://doi.org/10.1002/fsn3.4086

50. Taha, I. M., Ismail, K. A., Sami, R., Aljumayi, H., Alshehry, G., Algarni, E., Aljumayi, A., Alshahrani, M. Y., Asseri, T. A. Y., Alsharari, Z. D., Baakdah, F. and Taha, E.K. A**.** Bee pollen: An antioxidant and antimicrobial agent for beef sausage. *Journal of Biobased Materials and Bioenergy*, **18**(1), 31–38(2024). DOI:10.1166/jbmb.2024.2339

51. Desbois A.P. and Smith V.J. Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential .*Applied Microbiology and Biotechnol*ogy, **85**, 1629-1642(2010). doi: 10.1007/s00253-009-2355-3.

52. Mani, R. and Natesan V. Chrysin Sources, beneficial pharmacological activities , and molecular mechanism of action. *Phytochemistry*, **145**, 187-196(2018). doi: 10.1016/j.phytochem.2017.09.016.

53. Mirzoeva, O. K., Grishanin, R. N. and Calder, P. C. Antimicrobial action of propolis and some of its components: The effects on growth, membrane potential and motility of bacteria. *Microbiological Research*, **152**(3), 239–246(1997). doi: 10.1016/S0944-5013(97)80034-1.

54. Pascoal, A., Rodrigues, S., Teixeira, A., Feás, X. and Estevinho, L. M. Biological activities of commercial bee pollens: Antimicrobial, antimutagenic, antioxidant and anti-inflammatory. *Food and Chemical Toxicology*, **63**, 233–239(2014). doi: 10.1016/j.fct.2013.11.010.

النشاط الحيوي للبروبوليس وحبوب اللقاح وشمع النحل ضد المكورات العنقودية الذهبية في لحم البقر المفروم

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الملخص

استجابةً للطلب المتزايد من المستهلكين على المواد الحافظة الطبيعية للأغذية، قيّمت هذه الدراسة فعالية المنتجات المشتقة من نحل العسل، مثل البروبوليس وحبوب لقاح النحل وشمع العسل، وخليطها، ضد ميكروب الأستاف أوريس (S. aureus)، المحقون تجريبيا في لحم بقري مفروم بتركيز 10^5 وحدة ميكروبيه مستعمرة/جم .حيث أوضحت الدراسة التأثيرات المضادة للميكروبات، والخصائص الفيزيائية والكيميائية، والجودة الحسية للحم أثناء التخزين البارد عند درجة حرارة 4 درجات مئوية. من بين المعالجات، أظهر خليط منتجات النحل والبروبوليس أقوى نشاط مضاد للبكتيريا، حيث قلل بشكل ملحوظ من أعداد ميكروب الأستاف أوريس في اليومين الثالث والخامس، وثبط نموها تمامًا في اليوم السابع. كما أظهرت العينات المعالجة مدة صلاحية أطول، حيث بقيت مقبولة حتى اليومين التاسع والحادي عشر، على عكس العينات غير المعالجة التي فسدت في اليوم الخامس. كما أظهر الفحص الظاهري تأخر تدهور الرائحة واللون، بينما أكدت التقييمات الفيزيائية والكيميائية تحسن الجودة في العينات المعالجة. هذا بالإضافة إلي المجهر الإلكتروني الذي أظهر تلف كبير في أغشية خلايا ميكروب الأستاف أوريس وبنيتها الداخلية، وخاصةً في العينات المعالجة بالبروبوليس وخليط منتجات النحل. تدعم هذه النتائج إمكانية استخدام خليط البروبوليس ومنتجات النحل كمواد حافظة طبيعية فعالة تُعزز السلامة الميكروبيولوجية وجودة اللحم المفروم أثناء التخزين المبرد.

الكلمات الدالة: *S. aureus*، لحم البقر المفروم، البروبوليس، حبوب لقاح النحل، شمع العسل، ، TVB-N، مدة الصلاحية، المجهر الإلكتروني.