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Research Article

Investigating the Antioxidant, Antimicrobial and Sensory Effects of Honey Propolis Extract as a Natural Preservative in Beef Burgers

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Abstract
In this study, the antioxidant, antimicrobial and sensory effects of honey propolis extract as a natural preservative in beef burgers were investigated. First, propolis extract was produced at different percentages (0.5, 1.0, and 2.0%), and the control treatment, containing 0% propolis extract, was produced with a beef burger. The pH, antioxidant 2,2-diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), total volatile base nitrogen (TVB-N), and microbial characteristics (total microbial count, *Escherichia coli*, *Staphylococcus aureus*, mold, and yeast) and sensory characteristics (color, smell, texture and overall acceptance) during the storage period were subsequently investigated over a period of 8 days (0, 4, and 8). The results of the evaluation of different properties revealed that adding propolis extract to hamburger samples caused a significant decrease in pH, TBA, TVN, and microbial properties (total microbial count, *E. coli*, *S. aureus*, mold, and yeast) ($p < 0.05$). On the other hand, the amount of antioxidant property and sensory evaluation (color, smell, texture, and overall acceptance) also increased with the addition of propolis extract ($p < 0.05$). Additionally, the duration of storage caused a significant increase in pH, TBA, TVN, and microbial characteristics (total microbial count, *E. coli*, *S. aureus*, and mold and yeast), and a reduction in antioxidant properties and sensory evaluation (color, smell, texture, and overall acceptability) were found in hamburger samples ($p < 0.05$). The use of 2% propolis extract improved the oxidation properties and maintained the sensory properties during 8 days of storage for hamburger. Therefore, the use of 2% propolis extract improves the antioxidant and antimicrobial properties of hamburgers during storage and is a superior treatment.

Keywords: antioxidant, beef burger, propolis extract

1. INTRODUCTION

The importance of animal husbandry and meat production is increasing worldwide, particularly in developing countries, driven by the rising demand for protein fuel by growing populations. However, traditional methods of animal husbandry and meat production must be transformed to adopt a more comprehensive approach. This new approach should address various factors, such as sustainable development, environmental concerns, the use of traditional medicines and drugs, minimizing chemical usage, and adapting to climate change [1]-[4]. One of the most widely consumed meat products is hamburger, which has high nutritional value and is popular because of its deliciousness and ease of consumption [5]. Meat products provide various compounds with high biological value for the physiological and biochemical stabilities of the human body. Burgers are popular among other meat products because of their convenience, availability, nutritional value, affordability, and sensory acceptability, and these products are considered essential components of the human diet because of their high amounts of vitamins, proteins, and minerals [6].

Given that this product is raw until consumption, it may be out of the consumption cycle for two main reasons: microbial growth and chemical spoilage. Bacteria such as lactic acid bacteria, Escherichia, Bacillus, and Staphylococcus are among the most important microorganisms contaminating hamburgers, and the most common chemical spoilage is accelerated lipid oxidation [7]. Although the most common method of preserving meat and its products is the use of cold refrigeration and freezing conditions, food additives (especially synthetic types) are widely used to increase the shelf life of these products and prevent fat oxidation and microbial growth. However, today, consumers are increasingly aware of the side effects of using chemical preservatives and demand healthier foods, especially those that use natural ingredients and

Table 1. Analysis of variance (ANOVA) results for pH levels of hamburger samples.

P-value	F-value	Mean Square	Sum of Squares	Degrees of Freedom	Source of Variation
0.000	166.163	0.114	0.341	3	Experimental treatment
0.000	739.195	0.505	1.010	2	Day
0.000	47.577	0.033	0.195	6	Treatment* Day
		0.001	0.008	12	Experimental error
			869.398	24	Total
			1.554	23	Percent Coefficient of Variation

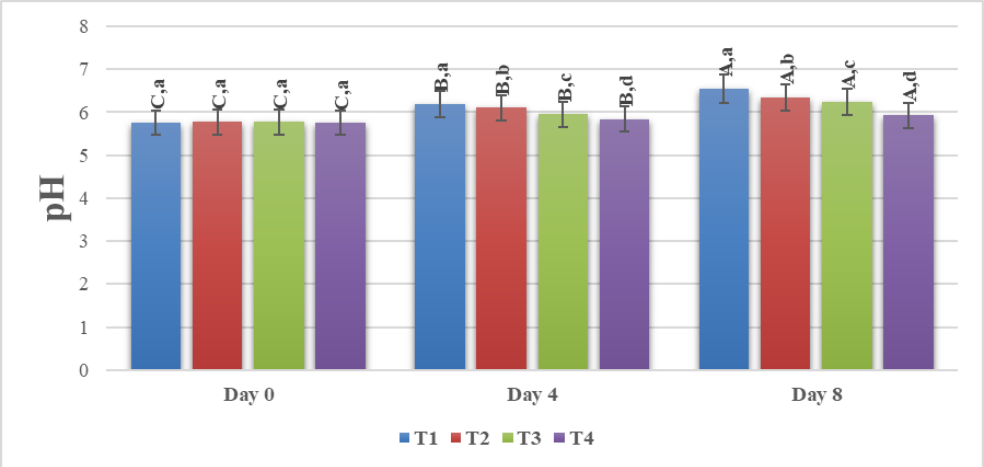

Figure 1. The pH levels of hamburger samples containing propolis extract.
Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$). T1: ControlT2: Treatment with 0.5% propolis extract T3: Treatment with 1% propolis extract T4: Treatment with 2% propolis extract.

additives. Overall, the application of various organic and natural extracts for preservation or as antimicrobial and antibacterial agents is increasing [8][9].

Meats are susceptible to lipid and protein oxidation during food processing and storage, which leads to a loss of nutritional value, flavor, texture, and shelf-life. A common way to preserve or improve the nutritional quality of meat and derived products is to add synthetic antioxidant foods (including 1BHA, 2BHT, and 3TBHQ). Unfortunately, these traditional chemical antioxidants used in meat and meat products have a negative impact on the health of consumers because of their toxic and carcinogenic effects. In addition, meat and its products, which are considered among the main sources of protein for humans, are subject to spoilage due to contamination with pathogens, which leads to discoloration of the meat and the production of volatile amines [10]. Propolis is a valuable compound made by honeybees after resinous substances are collected from various parts of plants and mixed with wax and enzymes from their saliva. Aqueous and alcoholic extracts of propolis have antibacterial, antifungal and antioxidant properties. The type of solvent used to prepare propolis extract and its phenolic and flavonoid contents have important effects on its antimicrobial and antioxidant properties [11]. This study examined the antioxidant, antimicrobial, and sensory impacts of honey propolis extract, which is used as a natural preservative in beef burgers.

2. MATERIALS AND METHODS

The ethanolic extract of propolis was supplied by the Iranian Herbal Medicine Institute. To prepare 90% beef burger paste, raw materials were prepared according to the methods of the referenced article with slight modifications. Equal amounts of veal

meat from the neck and shoulder parts will be used and ground using a meat grinder with 13 mm holes. Other ingredients, including onion, spices, breadcrumbs, wheat, and propolis extract (0, 0.5, 1.0, and 2.0%), were subsequently weighed via a scale and added to the onion and meat mixture. All the components were mixed together for 1 h to achieve a uniform blend and then ground again with a meat grinder with 2.5 mm holes. The ground burger paste will be shaped into 100-g portions via a manual mold, packaged in polyethylene or polyamide bags, and stored in a refrigerator at 4 °C for 8 days. Microbial, physicochemical, and sensory tests will be conducted on days 0, 4, and 8 [12][13]. Ten grams of 90% burger slice will be mixed with 100 mL distilled water via a homogenizer for 10 s at 13,000 rpm. The pH of the homogenate will be measured via a digital pH meter, which will be calibrated with pH 4 and 7 buffers before use [14].

A 0.2 mL of the prepared emulsion will be added to 4 ml of 60 µM methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution and kept in the dark at room temperature for 1 h. The absorbance was then read at 517 nm via a spectrophotometer [15].

A 2 g of burger sample will be transferred to a centrifuge tube, and 8 mL of 5% trichloroacetic acid (TCA) and 5 mL of 0.8% butylated hydroxytoluene (BHT) in hexane will be added. The mixture will be homogenized at low speed for 1 min and centrifuged at 5000×g for 10 min. The supernatant will be filtered through 0.45 µm cellulose acetate filters into a 10 mL flask. The filtrate was diluted with 5% TCA, and 2.5 mL of this mixture was mixed with 1.5 mL of 0.8% thiobarbituric acid (TBA) in a 10 mL tube. A blank will be prepared similarly. The tubes will be incubated in a water bath at 70 °C for 30 min to enhance the reaction between malondialdehyde (MDA) and TBA, forming a chromophore complex.

Table 2. ANOVA results for DPPH radical scavenging activity of hamburger samples.

P-value	F-value	Mean Square	Sum of Squares	Degrees of Freedom	Source of Variation
000/0	241/16801	025/206	076/618	3	Experimental treatment
000/0	243/12459	781/152	563/305	2	Day
000/0	900/631	749/7	492/46	6	Treatment* Day
		0/012	147/0	12	Experimental error
			573/44680	24	Total
			278/970	23	Percent Coefficient of Variation

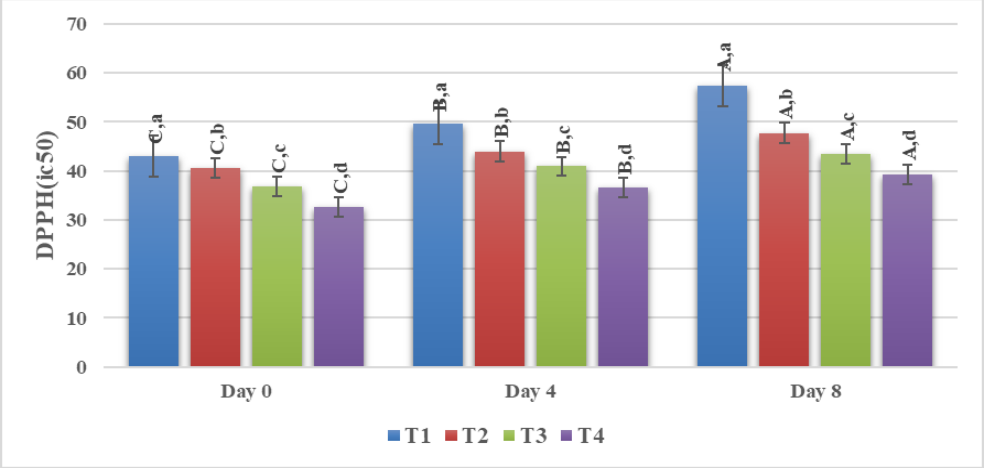

Figure 2. DPPH radical scavenging activity of hamburger samples containing propolis extract. Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$). T1: ControlT2: Treatment with 0.5% propolis extract T3: Treatment with 1% propolis extract T4: Treatment with 2% propolis extract.

Table 3. ANOVA results for TBA values of hamburger samples.

P-value	F-value	Mean Square	Sum of Squares	Degrees of Freedom	Source of Variation
000/0	944/462	002/0	006/0	3	Experimental treatment
000/0	594/5355	021/0	043/0	2	Day
000/0	247/138	001/0	003/0	6	Treatment* Day
		4/000	800/4	12	Experimental error
			229/1	24	Total
			052/0	23	Percent Coefficient of Variation

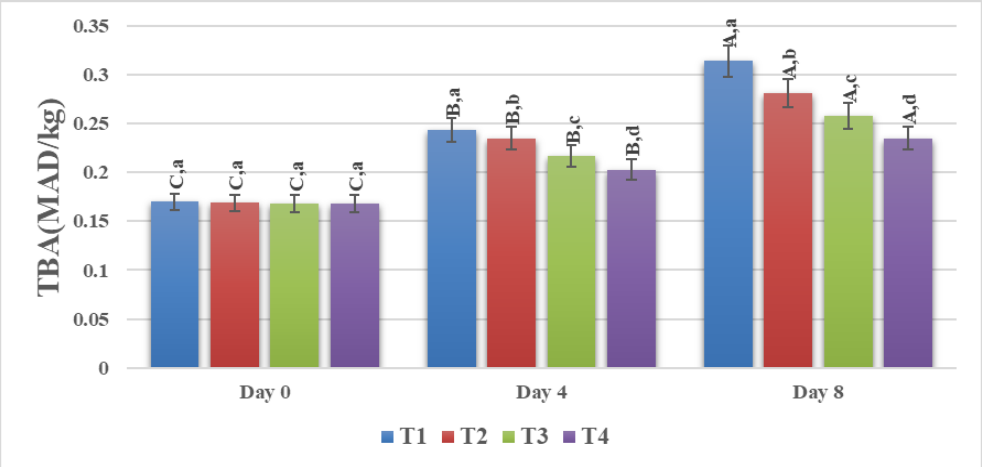

Figure 3. TBA values of hamburger samples containing propolis extract.
Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$). T1: ControlT2: Treatment with 0.5% propolis extract T3: Treatment with 1% propolis extract T4: Treatment with 2% propolis extract.

After cooling, the absorbance was measured at 532 nm with a spectrophotometer (0.1 nm accuracy). TBARS values were calculated from a standard curve (R^2 = 0.99) prepared with 1,1,3,3-tetramethoxypropane and reported as mg MDA per kg of sample [16].

The total volatile base nitrogen (TVB-N) index was measured via the micro-Kjeldahl distillation method to determine proteolytic decomposition. The TVB-N content of each sample was calculated as mg per 100 g of sample.

Using a sterile pipette, decimal dilutions from 10^{-5} to 10^{-10} will be prepared from the initial suspension. Then, 1 mL from dilutions 10^{-3} to 10^{-5} was transferred to sterile plates. The plate count agar mixture will be added via the pour plate method. After incubation at 30±1 °C for 72±3 h, colonies were counted. Using a sterile pipette, 0.1 ml of the initial suspension will be cultured on Baird–Parker agar plates. The samples were spread with a sterile glass rod, left at room temperature for 15 min to absorb, and then incubated at 37 °C for 24±2 h. Suspected colonies will be transferred to brain heart infusion broth and incubated at 37 °C for 24±2 h. Under sterile conditions, 0.1 mL of this suspension will be mixed with 0.3 mL of rabbit plasma and incubated at 37 °C for 4–6 h. A positive coagulase test is indicated by clot formation [17].

E. coli will be counted via lauryl sulfate broth via the most likely number (MPN) method. The first three tubes will contain double-strength medium with 10 mL of diluted sample; the next three tubes will have 1 mL, and the third and fourth series will receive 1 mL from prepared dilutions. The tubes were incubated at 37 °C for 24 h. The contamination levels are determined on the basis of the MPN table. Positive samples were transferred to EC broth and incubated at 44 °C for 24 h. Gas production confirmed the presence of *E. coli*. Positive samples were transferred to peptone water

and incubated at 44 °C for 24 h to assess bacterial growth under these conditions. For the indole test, 3 drops of Kovac's reagent were added; a red color indicates the presence of indole and confirms the presence of *E. coli* [18]. Using a sterile pipette, 0.1 mL of 10^{-2} dilution will be transferred to plates containing Dichloran Rose Bengal chloramphenicol agar. The inoculum will be spread evenly with a sterile spreader. The plates will be incubated aerobically at 25 °C for 5 days, after which the colonies will be counted [17].

Sensory evaluation of the burger samples, including texture, color, taste, odor, and overall acceptance, was conducted after the samples were fried in oil at 148 °C for 7 min via a 5-point hedonic scale (1=unacceptable/very poor, 2=poor, 3=acceptable/medium, 4=good, 5=very good). The samples were coded and provided along with evaluation forms to 8 trained panellists.

3. RESULTS AND DISCUSSION

3.1. Results

According to the results (Table 1, Figure 1), the effects of treatment, storage time and the interaction of variables on the pH of hamburger samples were significant ($p > 0.05$). The pH measurement results revealed that adding propolis extract to hamburgers caused a decrease in pH; thus, the treatment containing 2% propolis extract had the lowest pH, and the control sample had the highest pH during the storage period ($p > 0.05$). Additionally, with increasing storage time, the pH of all the samples increased. The lowest pH of the samples was observed on the first day, and the highest pH of the hamburger treatments was observed on the last day.

The results (Table 2, Figure 2) revealed that the effects of treatment, storage time, and the interaction of variables on the DPPH antioxidant activity of hamburger samples were significant (p >

Table 4. ANOVA results for TVN levels in hamburger samples.

P-value	F-value	Mean Square	Sum of Squares	Degrees of Freedom	Source of Variation
000/0	367/2732	407/41	220/124	3	Experimental treatment
000/0	853/26735	160/405	319/813	2	Day
000/0	426/751	387/11	323/68	6	Treatment* Day
		0/015	182/0	12	Experimental error
			617/3343	24	Total
			045/1003	23	Percent Coefficient of Variation

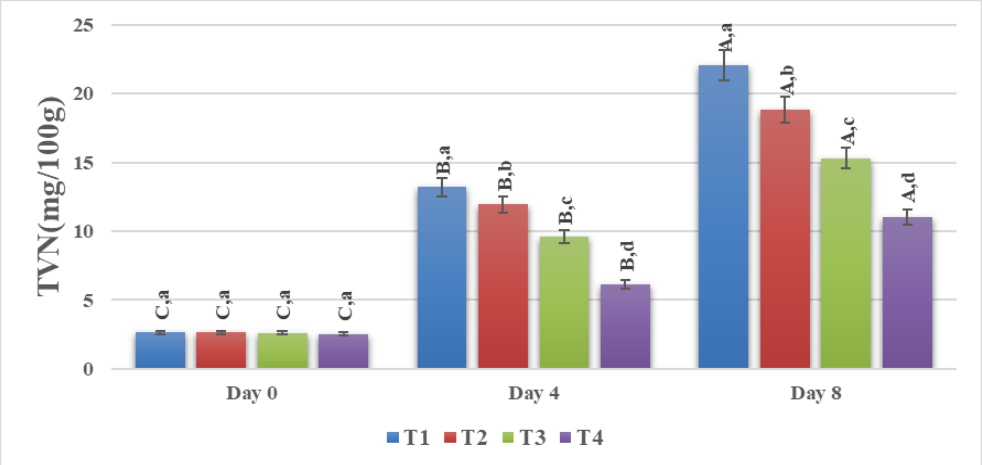

Figure 4. TVN levels of hamburger samples containing propolis extract. Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$). T1: ControlT2: Treatment with 0.5% propolis extract T3: Treatment with 1% propolis extract T4: Treatment with 2% propolis extract. The amount of *Escherichia coli* in all samples and all days of storage was zero.

Table 5. ANOVA results for total microbial count of hamburger samples.

P-value	F-value	Mean Square	Sum of Squares	Degrees of Freedom	Source of Variation
000/0	702/1750	774/1	323/5	3	Experimental treatment
000/0	705/38107	624/38	247/77	2	Day
000/0	578/343	348/0	089/2	6	Treatment* Day
		0/001	012/0	12	Experimental error
			403/964	24	Total
			672/84	23	Percent Coefficient of Variation

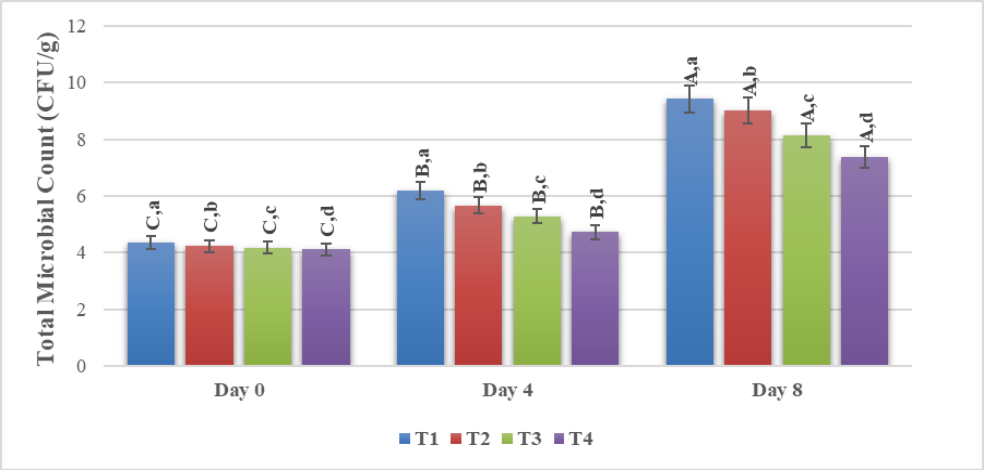

Figure 5. Total microbial count of hamburger samples containing propolis extract. Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$). T1: ControlT2: Treatment with 0.5% propolis extract T3: Treatment with 1% propolis extract T4: Treatment with 2% propolis extract.

Table 6. ANOVA results for *Staphylococcus aureus* count in hamburger samples.

P-value	F-value	Mean Square	Sum of Squares	Degrees of Freedom	Source of Variation
000/0	635/1819	274/1	821/3	3	Experimental treatment
000/0	613/68663	065/48	129/96	2	Day
000/0	058/462	323/0	941/1	6	Treatment* Day
		0/001	008/0	12	Experimental error
			941/288	24	Total
			899/101	23	Percent Coefficient of Variation

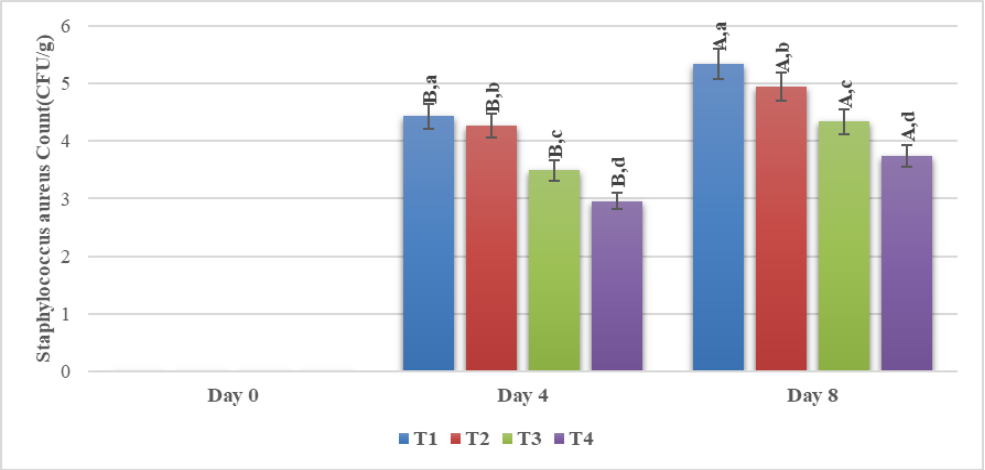

Figure 6. *Staphylococcus aureus* count in hamburger samples containing propolis extract. Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$). T1: ControlT2: Treatment with 0.5% propolis extract T3: Treatment with 1% propolis extract T4: Treatment with 2% propolis extract.

Table 7. ANOVA results for mold and yeast counts in hamburger samples.

P-value	F-value	Mean Square	Sum of Squares	Degrees of Freedom	Source of Variation
000/0	334/567	868/0	603/2	3	Experimental treatment
000/0	905/28237	180/43	361/86	2	Day
000/0	988/157	242/0	450/1	6	Treatment* Day
		0/002	018/0	12	Experimental error
			422/245	24	Total
			431/90	23	Percent Coefficient of Variation

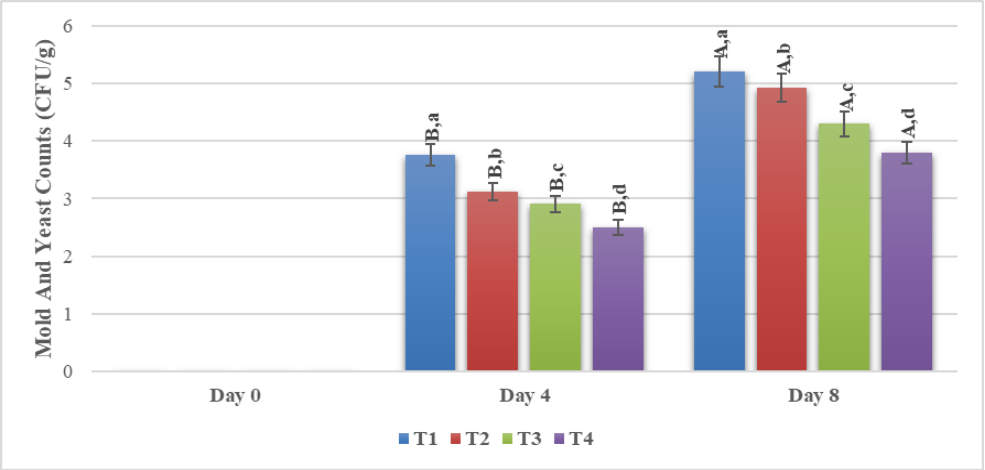

Figure 7. Mold and yeast counts in hamburger samples containing propolis extract. Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$). T1: ControlT2: Treatment with 0.5% propolis extract T3: Treatment with 1% propolis extract T4: Treatment with 2% propolis extract. The *E. coli* count was zero in all the samples throughout the entire storage period.

Table 8. ANOVA results for sensory score of color in hamburger samples.

P-value	F-value	Mean Square	Sum of Squares	Degrees of Freedom	Source of Variation
000/0	819/50	033/2	098/6	3	Experimental treatment
000/0	125/255	205/10	410/20	2	Day
000/0	736/12	509/0	057/3	6	Treatment* Day
		0/040	480/0	12	Experimental error
			060/372	24	Total
			045/30	23	Percent Coefficient of Variation

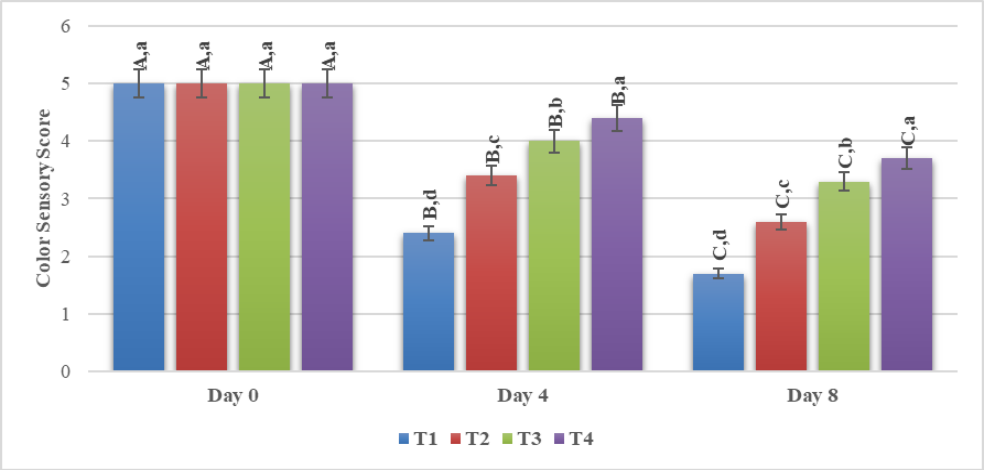

Figure 8. Sensory color scores of hamburger samples containing propolis extract. Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$). T1: ControlT2: Treatment with 0.5% propolis extract T3: Treatment with 1% propolis extract T4: Treatment with 2% propolis extract. Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$).

0.05). The results of the DPPH antioxidant property analysis revealed that adding propolis extract to hamburgers increased the DPPH antioxidant property, such that the treatment containing 2% propolis extract had the highest DPPH antioxidant property and the control sample had the lowest DPPH antioxidant property during storage. ($p > 0.05$). Additionally, with increasing storage time, the level of DPPH antioxidant activity decreased in all the samples; the highest level of DPPH antioxidant activity in the samples was observed on the first day, and the lowest level of antioxidant activity in the hamburger-treated samples was observed on the last day.

The results (Table 3, Figure 3) revealed that the effects of treatment, storage time and the interaction of variables on the TBA level of hamburger samples were significant ($p > 0.05$). The results of the TBA measurement revealed that adding propolis extract to hamburger did not significantly differ between the treatments on the first day ($p < 0.05$). However, during the storage period, the TBA levels of all the samples studied increased significantly. However, the measured values were significantly lower than those of the control sample ($p > 0.05$), so the T4 sample containing 2% propolis extract had the lowest TBA level, and the control sample had the highest TBA level during the storage period ($p > 0.05$). The lowest TBA level of the samples was observed on the first day, and the highest TBA level of the hamburger treatments was observed on the last day.

According to the results (Table 4, Figure 4), the effects of treatment, storage time and the interaction of variables on the mold and yeast counts of hamburger samples were significant ($p > 0.05$). The

results of the mold and yeast counts revealed that on the first day, they were zero, but during the storage period, the mold and yeast counts of all the samples studied increased significantly, but the measured values were significantly lower than those of the control sample ($p > 0.05$). The T4 sample containing 2% propolis extract had the lowest mold and yeast count, and the control sample had the highest mold and yeast count during the storage period ($p > 0.05$). The lowest mold and yeast count of the samples were observed on the first day, and the highest mold and yeast counts of the hamburger treatments were observed on the last day.

The statistical results revealed that the effects of treatment, storage time and the interaction of variables on the sensory evaluation (color, odor, texture and overall acceptance) of hamburger samples were significant ($p > 0.05$). The results of the sensory evaluation (color, odor, texture and overall acceptance) revealed that the addition of propolis extract to hamburgers did not significantly differ among the treatments on the first day ($p < 0.05$). During the storage period, the sensory evaluation values color, odor, texture, and overall acceptance) of all the samples increased significantly, but the measured values were significantly lower than those of the control sample (Table 5 - 11 and Figure 5 - 11).

3.2. Discussion

The pH values of different hamburger samples at different storage times (except day zero) were significantly different ($p < 0.05$). Hamburger-treated samples containing propolis extract presented the lowest pH throughout the storage period, whereas the control samples presented the highest pH. Additionally, with increasing storage time, the pH of all the samples increased; however, the treatments with propolis extract tended to decrease the pH. The lowest pH was observed on

Table 9. ANOVA results for sensory score of odor in hamburger samples.

P-value	F-value	Mean Square	Sum of Squares	Degrees of Freedom	Source of Variation
000/0	918/56	300/2	901/6	3	Experimental treatment
000/0	113/280	321/11	643/22	2	Day
000/0	691/12	513/0	078/3	6	Treatment* Day
		0/040	485/0	12	Experimental error
			450/359	24	Total
			106/33	23	Percent Coefficient of Variation

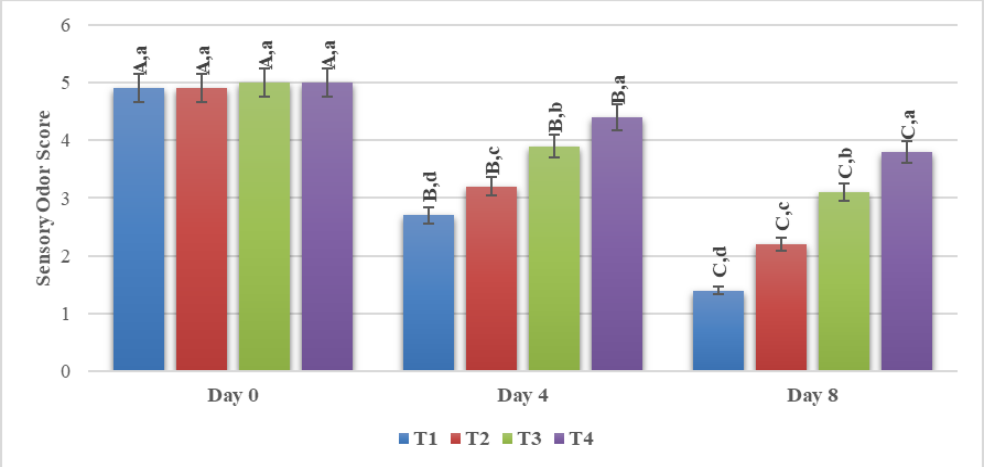

Figure 9. Sensory odor scores of hamburger samples containing propolis extract.
Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$). T1: ControlT2: Treatment with 0.5% propolis extract T3: Treatment with 1% propolis extract T4: Treatment with 2% propolis extract.

Table 10. ANOVA results for sensory score of texture in hamburger samples.

P-value	F-value	Mean Square	Sum of Squares	Degrees of Freedom	Source of Variation
000/0	205/40	955/0	865/2	3	Experimental treatment
000/0	018/464	020/11	041/22	2	Day
000/0	380/10	247/0	479/1	6	Treatment* Day
		0/024	285/0	12	Experimental error
			970/355	24	Total
			670/26	23	Percent Coefficient of Variation

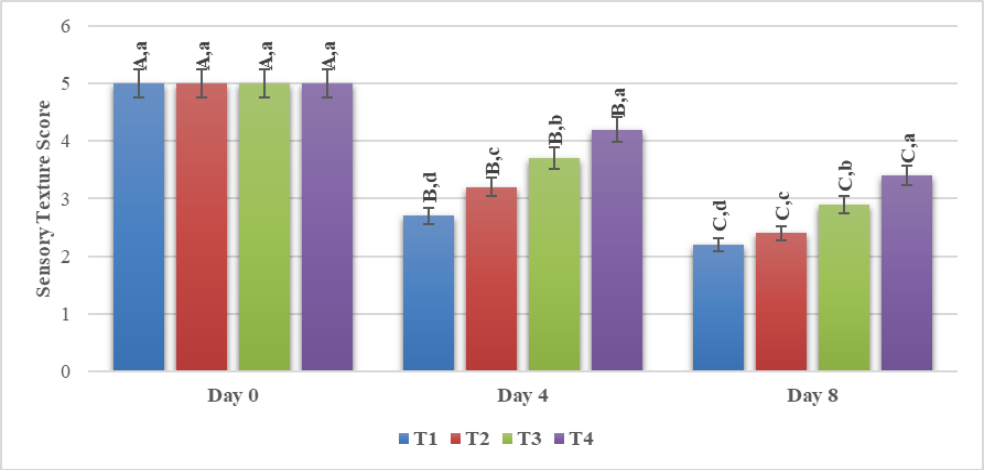

Figure 10. Sensory texture scores of hamburger samples containing propolis extract. Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$). T1: ControlT2: Treatment with 0.5% propolis extract T3: Treatment with 1% propolis extract T4: Treatment with 2% propolis extract.

the first day, and the highest pH was observed on the last day in the control, but the pH in the treatment containing 2% propolis extract was lower. pH is an indicator of freshness in meat products because it is closely related to bacterial growth and food spoilage [19]. The decrease in pH is due to the presence of propolis, which affects water absorption and hydrogen ion mobility, causing a decrease in pH and increased acidity. Propolis extract effectively reduces protein decomposition in burger samples by inhibiting microbial growth and preserving quality, and pH reduction itself can reduce microbial flora [20].

The antioxidant properties (DPPH) of different hamburger samples at various storage times were significantly different ($p < 0.05$). Hamburger treatments containing propolis extract had the highest antioxidant activity throughout storage, with the highest activity in the 2% propolis extract treatment. The control sample had the lowest antioxidant activity. With increasing storage time, the antioxidant activity decreased in all the samples, but the decline was slower in those treated with propolis extract. The highest antioxidant activity was detected on the first day, and the lowest activity was detected on the last day in the control. Bioactive compounds (including carotenoids, essential oils, antioxidants, or flavoring agents) are chemicals with health, medicinal, and nutritional properties that are widely used in food products to enhance sensory features or develop nutraceutical properties. One bioactive compound in propolis is phenolics, which are secondary metabolites classified into simple phenols and polyphenols based on the number of phenol units [21]. Phenolic compounds (flavonoids, phenolic acids, anthocyanins) are water-soluble antioxidants that are abundant in plants and are capable of neutralizing free radicals by donating electrons, thus inhibiting lipid oxidation [22]. Hosseini

Khabbazi et al. [11] reported that adding propolis extract increased the antioxidant activity of toast bread, which is consistent with these results. Cottica et al. [23] reported that propolis extract increased antioxidant activity in dairy drinks enriched with conjugated linoleic acid and cake, which is consistent with the findings of this study.

TBA values in different hamburger samples and storage times (except day zero) were significantly different ($p < 0.05$). Hamburger treatments with propolis extract had the lowest TBA values during storage, with the lowest TBA values in the 2% propolis extract treatment; the control had the highest TBA. TBA increased over time in all the samples but rose more slowly in the propolis treatments. The lowest TBA was detected on day one, and the highest was detected on the last day in the control. Food stability and shelf-life extension are critical for new food product development, ensuring the expected nutritional, appearance, texture, aroma, and flavor qualities (Table 5 - 11 and Figure 5 - 11). Food stability includes chemical (oxidative), physical, and microbial stability. Controlling lipid oxidation is crucial for maintaining the oxidative stability and acceptable shelf life of high-oil foods [24]. TBA measures secondary lipid oxidation metabolites (e.g., malondialdehyde, alcohols, ketones, and acids) from monohydroperoxide breakdown, causing rancidity and affecting flavor [23]. Shabani et al. [25] reported that propolis extract reduced TBA in fish burgers, which is consistent with the findings of this study. Vargas-Sánchez et al. [26] reported that propolis extract decreased TBA in fresh meat during refrigerated storage, which is consistent with these results.

The TVN values in different hamburger samples and storage times (except on day zero) were significantly different ($p < 0.05$). Hamburger treatment with propolis extract had the lowest TVN

Table 11. ANOVA results for sensory score of overall acceptability in hamburger samples.

P-value	F-value	Mean Square	Sum of Squares	Degrees of Freedom	Source of Variation
000/0	411/37	354/2	061/7	3	Experimental treatment
000/0	543/135	528/8	056/17	2	Day
005/0	874/5	370/0	218/2	6	Treatment* Day
		0/063	755/0	12	Experimental error
			910/354	24	Total
			090/27	23	Percent Coefficient of Variation

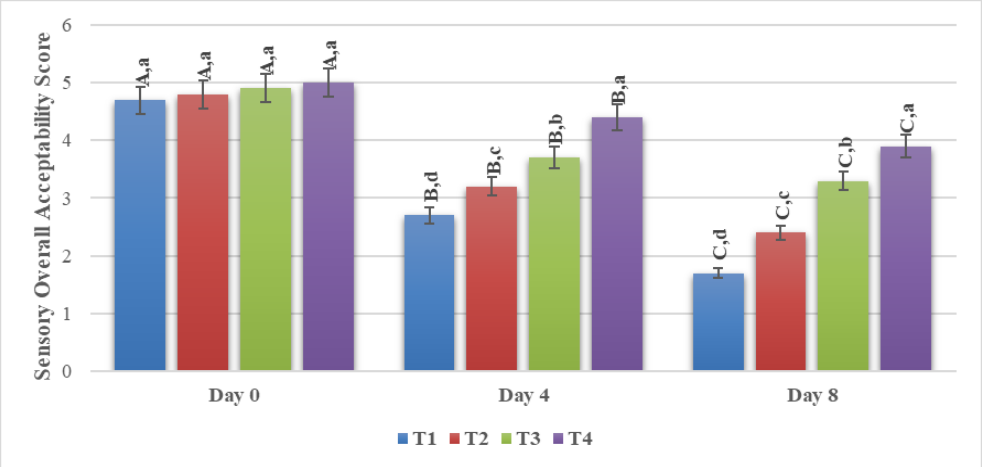

Figure 11. Sensory overall acceptability scores of hamburger samples containing propolis extract. Different uppercase letters within each treatment indicate significant differences between different days ($p < 0.05$). Different lowercase letters within each day indicate significant differences between treatments ($p < 0.05$). T1: ControlT2: Treatment with 0.5% propolis extract T3: Treatment with 1% propolis extract T4: Treatment with 2% propolis extract.

during storage, with the lowest TVN in the 2% propolis extract treatment; the control had the highest TVN. TVN increased over time in all the samples but rose more slowly in the propolis treatments. The lowest TVN occurred on day one, and the highest TVN occurred on the last day in the control. Total volatile nitrogen refers to nitrogen from protein compounds released by proteolytic enzymes and protein degradation, indicating spoilage onset and progression [25]. It reflects protein breakdown by enzymatic and bacterial activity, producing amines and reducing nutritional value. It increases due to spoilage bacteria and endogenous enzymes. These compounds include methylamine, dimethylamine, trimethylamine, and ammonia, which cause off-flavors in meat products. Amino acid deamination also increases TVN. TVN reduction with higher propolis extract concentrations may be due to antimicrobial effects delaying protein degradation, which are enhanced with higher extract concentrations [14][25][27]. The increase in TVN during storage is attributed to the accumulation of volatile nitrogen from bacterial protein degradation in the early meat spoilage stages. At low temperatures, enzymes and bacteria denature proteins, producing volatile alkaline nitrogen such as ammonia and amines, which can indicate meat freshness and increase pH. This stimulates enzymes such as cathepsins B and L, accelerating protein breakdown and rapid TVN increase [14]. Shabani et al. [25] reported that propolis extract reduced TVN in fish burgers due to its antimicrobial properties, although TVN increased throughout storage, which is consistent with the findings of this study.

Microbial properties (total microbial count, *S. aureus*, mold and yeast) in different hamburger samples and storage times (except day zero for *S. aureus* and mold/yeast) were significantly different ($p < 0.05$). *E. coli* counts were zero in all the treatments until the last storage day. Hamburger treatments with propolis extract had the lowest microbial counts (total, *S. aureus*, mold and yeast) during storage, with the lowest counts in the 2% propolis extract treatment; the control had the highest counts. The microbial counts increased over time in all the samples but rose more slowly in the propolis-treated samples. The lowest counts occurred on day one, and the highest counts occurred on the last day in the control. Essential oils and plant extracts reduce microorganisms by disrupting cell walls, which is likely their main antimicrobial mechanism [28]. Propolis inhibits cell division, damages the cytoplasm and membranes (bacteriolysis), causes leakage of cell components, alters membrane fatty acids and phospholipids, and inhibits DNA and RNA synthesis in microbes [26]. Vargas-Sanchez et al. [26] reported that propolis extract reduced the microbial load in beef.

Sensory evaluation (texture, color, odor, and overall acceptance) of different hamburger samples and storage times (except day zero) revealed statistically significant differences ($p < 0.05$). Hamburger treatments with propolis extract had the highest sensory scores throughout storage, with the highest scores in the 2% propolis extract treatment; the control had the lowest scores. The sensory scores decreased over time in all the samples but declined more slowly in the propolis-treated samples. The highest sensory scores were recorded on day one, and the lowest were recorded on the last day in the control. Propolis has a strong and distinctive odor; adding it to food can cause unpleasant smell and color changes [29], and its strong taste and odor may remain in the product [30]. Proper preparation of propolis extract and the use of appropriate levels of propolis in food formulations are important challenges for the industry. Propolis use should minimize undesirable organoleptic properties while preserving beneficial effects and food preservation [31]. Increasing the propolis extract concentration enhances antimicrobial activity, delays spoilage, prevents bad odors, and improves color and texture at relatively high concentrations. Amadio et al. [32] reported that a chitosan coating enriched with thyme essential oil improved the sensory quality (color, taste, odor, texture, overall acceptance) of refrigerated meat burgers. Hosseini Khabbazi et al. [22] reported that propolis extract increased the sensory score of toast bread, which is consistent with the findings of the present study. The results of this study provide detailed findings on pH, antioxidant activity, lipid oxidation, nitrogenous volatile compounds, microbial counts, and sensory evaluation in hamburger samples treated with propolis extract during storage. Propolis extract generally improved preservation by reducing

spoilage indicators and improving sensory qualities. A holistic approach is essential in every field of science, including agriculture, animal husbandry, and food science. This must multidisciplinary, affecting several aspects such as sustainable development and environmental conservation, reducing the use of chemicals, and adaptation strategies for managing climate change [33]-[35].

4. CONCLUSIONS

In this study, the antioxidant, antimicrobial, and sensory effects of honey propolis extract as a natural preservative in beef burgers were investigated. The results of various characteristic evaluations revealed that adding propolis extract to the burger samples led to a significant reduction in pH, TBA, TVN, and microbial characteristics (total microbial count, *E. coli*, *S. aureus*, mold, and yeast) ($p < 0.05$). On the other hand, the antioxidant property (DPPH) and sensory evaluations (color, odor, texture, and overall acceptance) increased with the addition of propolis extract ($p < 0.05$). Furthermore, the storage period significantly increased the pH, TBA, TVN, and microbial characteristics (total microbial count, *E. coli*, *S. aureus*, mold, and yeast) and decreased the antioxidant properties (DPPH) and sensory evaluations (color, odor, texture, and overall acceptance) of the burger samples ($p < 0.05$). The use of 2% propolis extract improved the oxidative properties and preserved the sensory characteristics of the burgers during 8 days of storage. Therefore, it can be concluded that using a 2% concentration of propolis extract enhances the antioxidant and antimicrobial properties of burgers during storage and can be introduced as a superior treatment.

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Conflicts of Interest

The authors declare no conflict of interest.

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