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Impact of propolis extract on shelf life of fresh beef burger

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Abstract

Natural preservatives are more increasingly preferred over synthetic than synthetic additives because they tend to have fewer adverse effects on human health. Propolis extract was assessed in the current investigation as a natural preservative to increase the beef burgers' shelf life. The samples were categorized into four groups: a control group and three treatment groups with (0.25%, 0.5%, and 1%) of propolis extract, respectively. Microbiological, chemical, and sensory analyses were conducted every 48 hours during refrigerated storage at 4ºC until signs of spoilage appeared. The findings showed that the control samples started to deteriorate after 4 days, whereas the samples treated with 1% propolis extract stayed acceptable for up to 10 days. Treated samples exhibited significantly lower microbial load and increases in the pH, total volatile bases nitrogen (TVB-N), and thiobarbituric acid (TBA) value were delayed compared to the control group. Moreover, the sensory evaluations confirmed that that incorporated propolis did not adversely affect the overall appearance, aroma, or texture of the beef burgers.

Keywords: *Propolis; beef burger; meat quality; pathogen*

Introduction

Beef burgers are one the most popular protein sources worldwide and offering considerable nutritional benefits. However, due to their moisture and nitrogen content along with optimal pH, Microbial contamination and spoilage are common problems with these products **(Alahakoon *et al.* 2015 and Soares *et al.* 2021).**

Every year, almost one-third of the food intended for human consumption is wasted because of spoiling, which deters the purchasing **(Principato *et al.* 2021)**. Microbial development, lipid oxidation, and enzymatic self-decomposition are the primary causes of meat spoiling and nutritional loss. These processes negatively affected flavours and odours, slime formation, and colour changes, rendering the meat unsuitable for eating **(Pellissery *et al.* 2020)**.

Natural or artificial ingredients are utilized as food additives to prolong the beef products' shelf life while preserving their quality **(Bobko *et al.* 2015)**.

Natural food preservatives are now seen by consumers as superior to synthetic ones in terms of safety and quality since the latter are frequently associated with different carcinogenic and teratogenic characteristics, along with residual toxicity. Propolis has become

widely embraced by individuals from numerous Western and Eastern nations **(El-Bassiony *et al.* 2012)**.

Research into novel approaches in the food industry has been spurred by the rising customer demand for fresh, minimally processed, chemical-free meals, alongside the exploration of natural compounds as substitutes for chemical preservatives **(Tumbarski *et al.* 2022)**. These natural compounds, derived from bacteria, fungi, plants, or animals, are intended to increase the shelf life of food while ensuring its safety **(Barcenilla *et al.* 2022)**. Propolis, a distinctive natural substance obtained from bees, exhibits numerous biological activities and health advantages due to its extensive antimicrobial properties against spoilage microorganisms and foodborne pathogens. This technology is utilized in food production and biopreservation of various products like meat, fish, eggs, poultry, milk, fruits, vegetables, juices, and drinks **(Siheri *et al.* 2017)**.

In accordance with Directive 2002/46/EC, propolis is a dietary supplement that contains concentrated elements with physiological effects **(European Parliament and Council of the European Union 2002).**

The beneficial health effects of propolis are due to its chemical compositions, which includes antimicrobial and antiviral compounds**,** antioxidant properties,anti-allergic**,** anticancer**,** anti-inflammatory and immunostimulants **(das Neves *et al.* 2016; Kismet *et al.* 2017; Azemin *et al.* 2018)**. The important bioactive chemicals are utilized in numerous fields, involving as medicine, pharmaceuticals, and the food industry **(Apriantini and Budiman 2021)**.

The antimicrobial characteristics of propolis are potent against various bacterial types, including gram-positive plus gram-negative strains, and molds and fungi **(Pobiega *et al.* 2019a)**. Propolis extract, with varying amounts, has been used in livestock products like fermented meat sausage, fresh oriental sausage, beef patties, milk, and ice cream for its antibacterial properties **(Pobiega *et al.* 2019b)**.

Initial research has been conducted on propolis extract application for preserving beef products over a 24-hour storage period. The data obtained indicates that higher concentrations lead to improved quality **(Apriantini & Budiman 2021)**.

The application of propolis as a preservative in beef patties successfully prevented the growth of mesophilic and psychrotrophic bacteria **(Vargas-Sánchez *et al.* 2014)**, as well as inhibited molds and yeasts on the surfaces of sausages **(Ozturk 2015**). Propolis is a natural additive and preservative used in food products to enhance their quality and function as a natural enhancement **(Pobiega *et al.* 2019b; Seibert *et al.* 2019).**

Propolis is recognized as an effective decontaminant and antioxidant for fresh sausage since it decreases microbial levels, reduces TBA value and TVB-N content, and enhances sensory quality. Ultimately, it prolonged the shelf-life of the specially formulated Egyptian sausage **(El-Mossalami** and **Abdel-Hakeim 2013).**

Refrigeration is the conventional approach for storing and keeping fresh meat. Fungi and psychrotrophic bacteria can spoil perishable items at refrigeration, reducing shelf life. Propolis extract's efficacy as a naturally occurring preservative in livestock products is correlated with its antimicrobial activity and TBAR's value in various storage conditions **(Kim *et al.* 2013)**.

Mycotic contamination in meat products can arise from the meat, substandard flavoring ingredients, especially spices, as well as inadequate hygiene practices during processing and storage conditions **(Gourama and Bullerman 1995)**.Meat products contaminate with fungal poses a significant public health risk, leading to mycosis, mycotoxicosis, and allergies **(Abuzaid *et al.* 2020)**.

Thereby, to inhibit food spoilage and prolong the storage life of meat products, propolis can be incorporated into the food matrix as an extract, used as a bioactive film or edible coating on the product's surface, or added to the formulation of food bio-packaging materials **(Tumbarski *et al.* 2022)**.

As a consequence, the intent of the present investigation was to assess the consequences of incorporating propolis into beef burgers at three distinct levels (0.25%, 0.5%, and 1%) by analyzing microbial counts (bacterial and fungal counts with identification of isolated fungi), chemical properties, and sensory qualities during storage at 4ºC.

Materials and Methods

1- Preparation of propolis extracts:-

Ten grams of propolis were extracted using twenty milliliters of 95% ethanol to create an ethanolic extract of propolis (EEP). After being diluted to final concentrations of 0.25%, 0.5%, and 1%, the extract was kept in a dark bottle until it was required **(Han and Park 2002).**

2-Preparation of beef burgers:

The beef burgers were prepared according to Egyptian Standard Specifications (ESS 1688/1991) **(Kassem *et al.* 2011**). Fresh beef, delivered to the lab in an ice cooler, was minced with an electric meat grinder (Moulinex, 2000 Watt, France). The formulation consists of 65 g of minced beef, 20 g of fat, 5 g of soybean, 0.3 g of black pepper, 1.8 g of salt, and 10 g of water. The ingredients were mixed thoroughly for five minutes at a medium speed of 80 rpm and then processed through a plate with a smaller opening to ensure uniformity. The prepared mixture was divided into four portions; one as a control with no additives and three groups containing propolis extract at 0.25%, 0.5%, and 1% (w/w). The patties were performed using a commercial shaping tool (10 cm diameter), wrapped in plastic film, and stored on foam plates at 4 ºC. Samples were collected at 2, 4, 6, 8, and 10 days of storage to assess their sensory properties, microbial count, and chemical properties.

3-Physical and Chemical analysis:

A. Determination of pH value acc. to **(EOS 2006c)**
B. Determination of Thiobarbituric acid (TBA) acc. to **(EOS 2006b)**
C. Determination of Total Volatile Nitrogen (TVN) acc. to **(EOS 2006a)**

4-Microbiological examination:

A- Total viable bacterial count:

The standard plate count method **(APHA, 1992)** was applied to ascertain the total number of viable bacteria. The plates underwent two days incubating at 37 °C.

B-Psychrophilic bacterial count:

The psychrophilic bacterial count was performed accordance with the conventional procedure of the total plate bacterial count techniques, according to **(APHA 1992)** with the exception of incubation at 4 °C for five days.

5-Mycological examination:

A- Pour plating

In duplicate, A Petri plate was filled with 1 ml of each of the created dilutions (10^2 to 10^6). Subsequently, 10–20 ml of cooled, molten Sabouraud Dextrose Agar (SDA) at 42–45 ºC ought to be added to each petri plate. Swirl the medium and dilutions in both clockwise and anticlockwise directions to combine them, and then let them harden **(Soliman *et al.* 2019).**

B-Isolation and identification of fungi

Petri plates that had been inoculated were given the opportunity to harden at room temperature. After their inversion to avoid condensation, the plates were incubated for three to five days at 25ºC. Using a colony counter, each of the yeast colonies that had regular and irregular forms and looked dull white, creamy, yellow, and pink was counted separately. The yeast count per gm was then computed and documented. The plates for the molds were inverted and incubated for five to seven days at 25ºC. The colonies were enumerated and recorded throughout the incubation period, and the plates were observed every day for development of star-shaped mould **(APHA 1992)**. The methods outlined by **Samson *et al.* (2010)** were followed in the identification of isolated moulds.

6- Sensory assessment:

The burgers were assessed for variations in colour, aroma, texture, and overall acceptability at zero day only using a five-point hedonic scale, where 1 represents a strong dislike and 5 represents a strong like **(Lilic *et al.* 2015)** by a panel of analysts of the Food Hygiene Department at the Animal Health Research Institute in Giza, Cairo, Egypt.

7- Statistical evaluation:

The Statistical Package for the Social Sciences (SPSS) program was applied to analyze the data gathered for this investigation **(Corp, 2013)**. $P < 0.05$ was considered a significant level of significance.

Results and Discussion

Food quality deterioration and shelf life decrease due to factors like bacterial growth, fun-

gal growth, and lipid oxidation. Chemical additives are used to prevent these issues, but consumers are concerned about health risks. They demand minimally processed food without preservatives and clean labels with natural ingredients. To regain trust in food safety, the food industry is exploring natural alternatives with potent antimicrobial and antioxidant qualities **(Ahmad *et al.* 2015)**.

Table (1) and Figure (1), represent the overall aerobic plate count in beef burgers with varying propolis concentrations stored for 10 days at 4°C. On the first day of storage, there was no statistically significant difference ($P < 0.05$) between the control and treatment groups with varying propolis concentrations, However, during storage, the control group and the treatment groups' varying propolis levels differ significantly ($P < 0.05$). The control sample showed the most significant quantity of aerobic plate count 6.31±0.11 (log cfu/g ± SD) by day 4 and it was spoiled after the 4th day of storage. The total aerobic plate count in beef burger samples that were subjected to varying concentrations of propolis, along with the control sample, increase at 4 °C when being stored. Nonetheless, the rise in most of the treated groups occurred at a slower rate in contrast to the control group. The three groups of 0.25%, 0.5% and 1% recorded 6.53±0.07, 6.47±0.8 and 6.54 ±0.03 (log cfu/g±SD) on the sixth, eighth, and tenth days of storage at 4°C correspondingly and they were spoiled after those days.

Shavisi *et al.* (2017); Jonaidi Jafari *et al.* (2018); **Mahdavi-Roshan *et al.* (2022)** found that increasing propolis concentration led to slower growth of bacteria in chicken breast, fillet, ground meat, and fish, confirming previous studies. These findings support previous research on shelf life enhancement in poultry products.

Table (2) and Figure (2), reveal that the total Psychrophilic Count of treated and untreated Beef Burger Samples (Log 10 cfu/g ± SD) during refrigerated storage at 4°C. At zero day, the control group and treated groups with varying propolis concentrations don't differ significantly ($P < 0.05$). However, from the fourth day to the tenth day, there are significant differences ($P < 0.05$) between the various propolis-treated groups. There is gradual increase in total psychrophilic count during storage period till spoiled control group after 4th daywhile the 4th treated group (1% propolis) spoiled at 10th day of refrigerated storage. The antibacterial effectiveness of propolis improves with increased concentrations, as this allows for a greater presence of biologically active compounds **(Yazgan *et al.* 2020).** According to **Gutiérrez (2012),** psychrophilic bacteria declined by about 2 log CFU/g between day 0 (when starting levels were about 4.50 log cfu/g) and day 16 (when they were about 2.50 log cfu/g).

Table (3) and Figure (3), represent the change in beef burger pH value in samples at 4°C refrigerated storage with varying propolis concentrations. There was no significant difference ($P>0.05$) between control groups. The control sample pH value changed from 5.59 ± 0.18 to 6.23±0.06 during storage at 4th day while reach 6.23±0.06 in 2nd treated group (0.25% propolis) at 6th day, 6.27±0.06 in 3rd treated group (0.5% propolis) at 8th day and 6.27±0.06 in 4th treated group (1% propolis) at 10th day of refrigerated storage at 4°C. The study found no significant differences ($P<0.05$) between control and treated groups at zero days. During 2nd and 4th day there are no significant differences between treated groups with 0.25 and 0.5% propolis. At 8th day of storage there are significant differences between 0.5 and 1% treated group with propolis. During storage, the pH levels in both treated and control groups got higher, maybe as a result of an increase in total basic volatile nitrogen compounds spurred on by innate enzymes or proteolytic activities from microbes **(Duman and Ozpolat, 2015)**.

Table (4) and Figure (4), demonstrate the level of TBA (mg malondialdehyde/kg) as a deteriorative chemical criterion for lipid oxidation in samples of beef burgers kept in a refrigerator. Over the course of the storage time, the TBA value gradually increased from 0.36 ±0.05 at zero day in all samples has been observed to reach 1.08±0.20, 1.07±0.12 and 0.99±0.03 mg MDA/kg at 6th, 8th and 10th day of refrigerated storage for 0.25%, 0.5% and 1% of propolis concentrations in beef burgers samples, respectively. These values were not accepted according to the permissible limit of TBA value in beef burger (0.9 mg malondialdehyde/kg) stip-

ulated by (**EOS, 2006b)**. On day 6, There were significant differences (P<0.05) between the different propolis concentrations in the beef burger samples. By day 8, The samples of beef burgers with 0.5% propolis and those with 1% propolis didn't differ significantly (P>0.05). The control sample began to spoil by day 4, whereas the beef burger with 1% propolis extended its shelf life until the 10thday. **Ebadi *et al.* (2019)** found that propolis extract in Nemipterus japonicas fillets reduces lipid oxidation, resulting in lower rancidity in all samples, with TBARS values below 2 mg malondialdehyde/kg at storage end. TBA is a crucial meat test that detects fat oxidation by converting peroxides into ketones and aldehydes, which results in disagreeable smells when refrigerated **(Al-Sabea *et al.* 2022)**.

Table (5) and Figure (5), show the variations in TVN levels in beef burger samples containing varying concentrations of propolis during storage at 4°C. The control group and the various propolis concentrations in the beef burger samples at day 0 didn't vary significantly (P<0.05) at the beginning of storage. The TVN of control group was 13.15 ±0.45 mg N/100 g till reach 21.63±0.42 mg/100 g at day 4. While there are significant different (P <0.05) between control and groups with various propolis concentrations in treated beef burger samples during storage period. The TVN were 21.93±0.85 mg/100g at 6th day in 2nd group (0.25% propolis), 21.83±0.64 mg/100g at 8th day in 3rd group (0.5%propolis) and 22.23±0.55 mg/100gm in 4th group (1% propolis).The TVN value is a crucial metric for evaluating meat quality, indicating the presence of amines and ammonia in food, which can indicate potential contamination **(Al-Sabea *et al.* 2022)**. After 28 days of storage, a higher concentration of propolis decreased TVB-N in the treated hot-smoked rainbow trout group in contrast with the control group **(Güngoren *et al.* 2023)**. TVN increased considerably less when the propolis extract concentrations were raised while the marinated chicken breast was being stored (**Mahdavi-Roshan *et al.* 2022)**. According to research by **Shavisi *et al.* (2017)** and **Jonaidi Jafari *et al.* (2018)**, different amounts of propolis extract were applied to ground beef and chicken fillet stored at 5°C in order to extend their shelf life. **El-Mossalami** and **Abdel-Hakeim (2013)** stated that propolis has a strong reputation as an antioxidant and decontaminant. Because it lowers the microbiological burden, lowers the TBA and TVB-N values, and improves the sensory aspect of fresh meat. Lastly, it extended the experimentally created Egyptian sausage's shelf life. In order to replace chemical preservatives in the production of Egyptian sausage, propolis has been proposed as a natural antioxidant and decontaminant. The sensory assessment indicated that incorporating propolis does not adversely affect the overall appearance, aroma, or texture when compared to the control treatment.

Table (6) and Figure (6), represent the total fungal plate count in beef burger stored at 4° C for 10 days with varying propolis concentrations as a natural preservative. On the zero day of the storage period, there was not a significant difference (P < 0.05) between the treatment and control groups at different propolis concentrations; However, significant differences (P < 0.05) between the control group and the treatment groups at various propolis concentrations were noted during the storage period. The greatest count of total fungal plate count was found in control group 4.28±0.12 (log cfu/g±SD) at fourth day. After the fourth day of storage, it became spoiled. The total fungal plate count in beef burger samples**,** which were subjected to various concentrations of propolis along with a control, rise during storage at 4°C. However, the majority of the treated samples revealed a slower rate of increase than the control sample. The three groups of 0.25%, 0.5% and 1% recorded 4.61±0.09, 4.42±0.08, and 4.60±0.01 (log cfu/g±SD) on the 6th,8th and 10th day of storage at 4°c respectively and they were spoiled after 6th, 8th and 10th day of storage. It was discovered that when the propolis content rise, fungal growth decreased.

The findings concurred with those published by **Ali *et al.* (2010)**, and **Vargas-Sánchez *et al.* (2014)**, who evaluated the antimicrobial and lipid oxidation characteristics of non-commercial and commercial propolis extract (PE) in beef patties kept in refrigeration. Raw beef patties were covered with polyvinyl chloride (PVC) and stored at 2°C for

eight days. Numerous factors were assessed, such as pH levels, colour, microbial count, and lipid oxidation. In every metric that was evaluated, non-commercial polyethylene (PE) showed the best results. Furthermore, lipid oxidation was inhibited and bacteria numbers were decreased. Another study **(El-Demery *et al.* 2016)** evaluated the effectiveness of propolis and turmeric powders at varying concentrations (1.5–2.5%) as natural preservatives in packed minced beef. Aqueous extracts of propolis and turmeric both effectively suppressed the development of *Saccharomyces cerevisiae, L. monocytogenes, P. aeroginosa,* and *Fusarium oxysporum.* Additionally, propolis was found to have potent antifungal qualities against three strains of *Penicillium spp.* and one strain of *Zygomycetes spp.* **(Silici *et al.* 2005)**. Propolis successfully prevented *Penicillium expansum* from producing patulin in apple juice. Additionally, it was discovered that propolis inhibited mould and yeast just as well as potassium sorbate and sodium benzoate **(Koc *et al.* 2007; Silici and Karaman, 2014; Yang *et al.* 2017)**. Propolis has the ability to effectively preserve dairy products, such as milk and yoghurt, extending their shelf life. He observed that when the concentrations of propolis water extract increased at 5, 10, and 20%, the overall quantities of bacteria, coliform, molds, and yeasts gradually decreased in comparison to the control group (**El-Deeb, 2017)**.

In study of **Shaban *et al.* (2021),** who investigated the boosting and preservation qualities of propolis extract in both raw and pasteurized milk, discovered similar results. No signs of mould or yeast were discovered until the fourteenth day, demonstrating the effectiveness of propolis in reducing bacterial numbers. **Tumbarski *et al.* (2021)** discovered that the shelf life of Kashkaval cheese may be increased by adding propolis in different amounts to edible films made of 1% carboxymethyl cellulose (CMC). No deterioration was seen after 56 days of storage, and the growth of fungus on the cheese's surface was prevented.

Mahdavi-Roshan *et al. (2022)* explored how the microbiological characteristics of marinated chicken breast were affected by an aqueous extract of propolis 0, 4, 8, and 12% v/w concentrations. *Escherichia coli*, Staphylococcus aureus, yeasts moulds, and total counts were all assessed during a 12 day period at 5 °C. As the propolis extract content in the samples rise, it was discovered that the rate of microbiological growth during the 5°C storage period decreased. In another study, at the storage period end, all propolis extract treated groups demonstrated a significant decline in both psychrophilic and mesophilic bacterial growth, along with lowered counts of molds and yeasts.

Table (7) displays the isolated mold generafrom the examined samples. The most commonly isolated genus from the samples was *Asperigullus* spp. (43%) followed by *Penicillium* spp. (22.7%) then *Mucor*, *Rhizopus*, *Cladosporium*, *Fusarium* spp. *Alternaria*, *Sporotricum*, and *Curvularia* (7.6%, 6.2%, 6.1%, 4.5%, 4.5%, 3.0%, and 1.5%) respectively.

The consequences align to the findings of **Mustafa *et al.* (2019).** With an incidence of 58.23%, they found *Aspergillus* spp. to be the mold species that is most frequently recovered from burger samples, followed by *Penicillium* spp. at 15.18%. *Acremonium*, *Geotrichum*, *Cladosporium, Mucor*, *Emericella nudulans*, *Eurotium, Claveolaria, Fusarium*, and *Scorulopsis* were among the other mould isolates. **Mizakova *et al.* (2002); Abdo *et al.* (2017)** noted that in meat product samples, *Aspergillus* and *Penicillium* species were the most prevalent mould genera (48.4% and 24.2%, respectively), followed by *Mucor* (23.1%), *Cladosporium* (3.3%), and *Alternaria* (1.1%). By breaking down the components of meat products and generating different acids and gasses, these molds can cause them to degrade., resulting in alterations to their smell and taste. Additionally, mold growth on meat products results in financial losses due to discoloration, unattractive appearance, and undesirable flavors. Additionally, some moulds can develop toxic byproducts called mycotoxins, like aflatoxins, which are proven carcinogens **(Pitt and Hoching, 2009).**

From the stand point of public health, *Aspergillus* species have been connected to diseases including otitis, sinusitis, skin infections, pulmonary aspergillosis, and pulmonary allergies

in those who handle meat. According to reports **(Afoakwah *et al* 2024)**, some *Penicillium* species are linked to "yellow rice illness," which has killed several people, as well as lung and urinary tract infections. Lung lesions, gastrointestinal tract infections, skin infections, intraocular infections, external otomycosis, cellulitis, and deep wound infections can all be brought on by *Mucor* and *Rhizopus* species **(Banwart, 1980)**. Species of *Cladosporium* can cause chromoblastomycosis and brain abscesses **(Edris, 1986)**.

Table (8) presents the occurrence of various *Aspergillus* species, as this genus was the most frequently isolated from the examined samples. Additionally, Aspergillus species are the most disease-producing fungi in addition to the production of harmful mycotoxins, including aflatoxins and ochratoxins. *A. niger* was highest isolated species (37.9%) and *A. flavous* (31%) and then *A. terreus*, *A. fumigates*, *A. parasiticus* and, *A. ochraceus*, (13.8%, 6.9%, 6.9%, and 3.5%) respectively.

Abdo *et al.* (2017) found that among the strains of *Aspergillus* isolated from meat product samples, *A. niger* had the highest prevalence at 47.7%, followed by *A. flavus* at 43.2%, *A. ochraceus* at 6.8%, *A. glaucus* at 2.3%, and *A. candidus* at 0%. The study's findings on the prevalence of Aspergillus species were similar to their findings. Similarly, *A. flavus*, *A. fumigatus*, *A. niger, A. parasiticus*, and *A. terreus* were isolated from Burger and Kofta by **Brr *et al.* (2004) and Mustafa *et al.* (2019)**. However, from Kofta and Burger, *A. flavus*, *A. niger*, *A. fumigates*, and *A. parasiticus* were isolated **(Morshdy *et al.* 2015)**.

Table (9) illustrated Sensory evaluation which is the assessment that is most useful to determine what actual customers think of a produced or manufactured product. The sensory characteristics of the prepared beef burger, comprising control samples and propolis-added beef burger samples, weren't altered significantly. Color, texture, aroma, and overall acceptability were all assessed, and neither of them altered significantly, confirming that propolis may be incorporated for producing beef burgers. The fact that propolis was added in trace amounts that panelists were unable to perceive and that did not adversely affect the sensory qualities may be the reason for the insignificance in the sensory evaluation assessment.

Conclusion and Recommendations:

Considering the results and discussion of this investigation, it is clear that proplis can control the growth of microorganisms in beef burger during 10 days-storage at 4°C. proplis has antibacterial, antifungal and antioxidant effect as it reduced the microbial load and controlling the increase of PH, TVN and TBA values and it also It prolonged the beef burger's shelf life. Proplis is therefore advised as a natural antioxidant and antibacterial that can substitute chemical preservatives in the manufacturing procedure of beef burgers. Additionally, propolis can stop undesirable alterations in the food's physical, chemical, microbiological, and organoleptic properties, preserving its quality and extending its shelf life in animal-based food products.

Table (1). Mean Value Of Aerobic Plate Count of beef burger Samples (Mean Log $_{10}$Cfu/g ± SD of 3 Trials).

	1st group (Control)	**2nd group (0.25% propolis)**	**3rd group (0.5% propolis)**	**4th group (1% propolis)**
0 day	4.39^{a}± 0.1	4.39^{a}± 0.1	4.33^{a}±0.07	4.26^{a}±0.07
2 day	5.72^{a}±0.05	5.54^{b}±0.07	5.16^{c}±0.16	4.78^{d}±0.04
4 day	6.31^{a}±0.11	6.00^{b}±0.02	5.49^{c}±0.08	5.07^{d}±0.09
6 day	S	6.53^{a}±0.07	5.90^{b}±0.03	5.46^{c}±0.04
8 day		S	6.47^{a}±0.08	5.76^{b}±0.02
10 day			S	6.54±0.03

**Means within a raw followed by different letters showed significant differences (P < 0.05).

Figure (1). Aerobic Plate Count (APC) of beef burger Samples

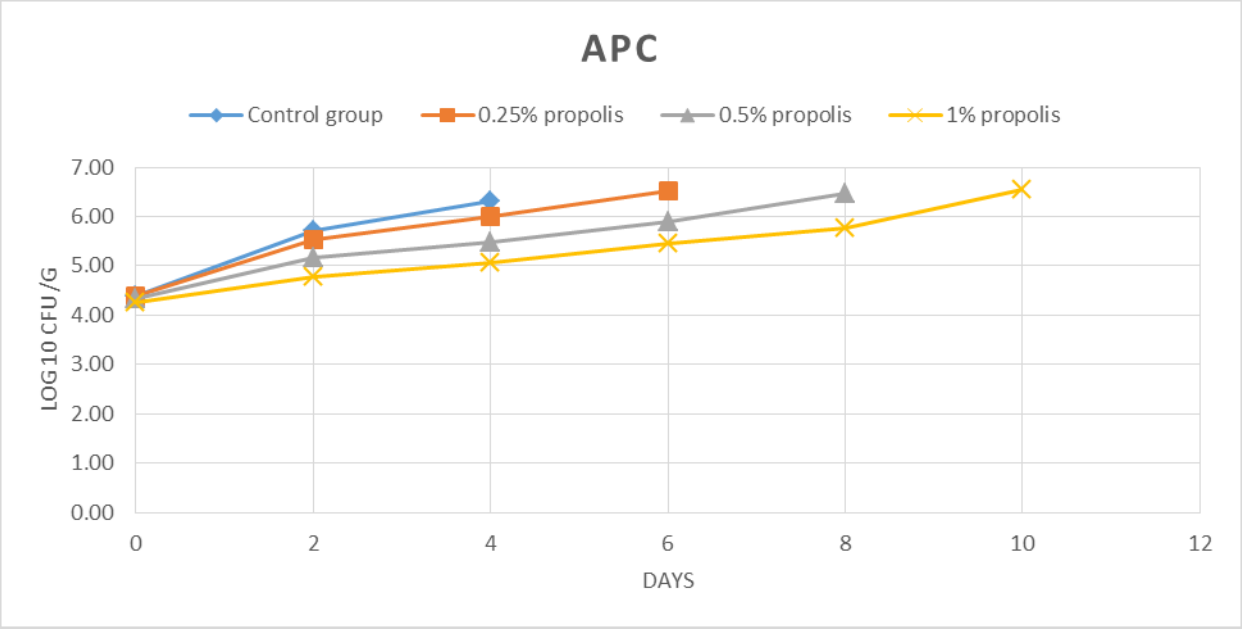

Table (2). Mean Value of Total Psychrophilic Count of Beef Burger Samples (Mean Log $_{10}$Cfu/g ± SD Of 3 Trials).

	1st group (Control)	2nd group (0.25% propolis)	3rd group (0.5% propolis)	4th group (1% propolis)
0 day	3.52±0.18^{a}	3.52±0.18^{a}	3.51±0.18^{a}	3.48±0.19^{a}
2 day	4.55±0.22^{a}	4.51±0.21^{b}	4.38±0.11^{a}	4.15±0.16^{c}
4 day	5.41±0.07^{a}	5.01±0.03^{b}	4.77±0.11^{c}	4.52±0.17^{d}
6 day	**S**	5.32^{a}±0.04	5.01^{b}±0.05	4.83^{c}±0.09
8 day		**S**	5.46^{a}±0.15	5.09^{b}±0.08
10 day			**S**	5.44±0.16

**Means within a raw followed by different letters showed significant differences (P < 0.05).

Figure (2). Total Psychrophilic Count (TPC) of beef burger Samples

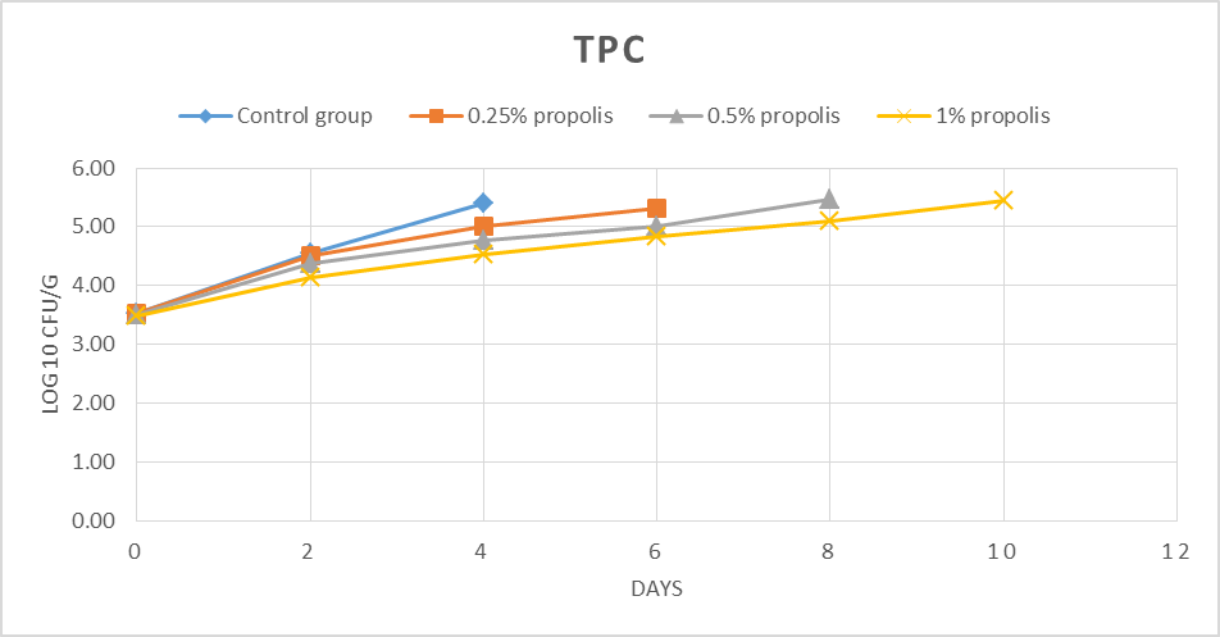

Table (3). Mean value of pH value of beef burger samples (mean of 3 trials ± SD).

	1st group (Control)	2nd group (0.25% propolis)	3rd group (0.5% propolis)	4th group (1% propolis)
0 day	5.59^{a}± 0.18	5.59^{a}±0.18	5.87^{a}±0.18	5.80^{a}±0.21
2 day	5.87^{a}±0.12	5.80^{ab}±0.09	5.69^{ab}±0.12	5.60^{b}±0.15
4 day	6.23±0.06^{a}	5.97±0.11^{b}	5.87±0.07^{bc}	5.73±0.15^{c}
6 day	S	6.23^{a}±0.06	6.03^{b}±0.06	5.88^{b}±0.11
8 day		S	6.27^{a}±0.06	6.03^{b}±0.06
10 day			S	6.27±0.06

**Means within a raw followed by different letters showed significant differences ($P < 0.05$).

Figure (3). pH value of beef burger samples (mean of 3 trials ± SD).

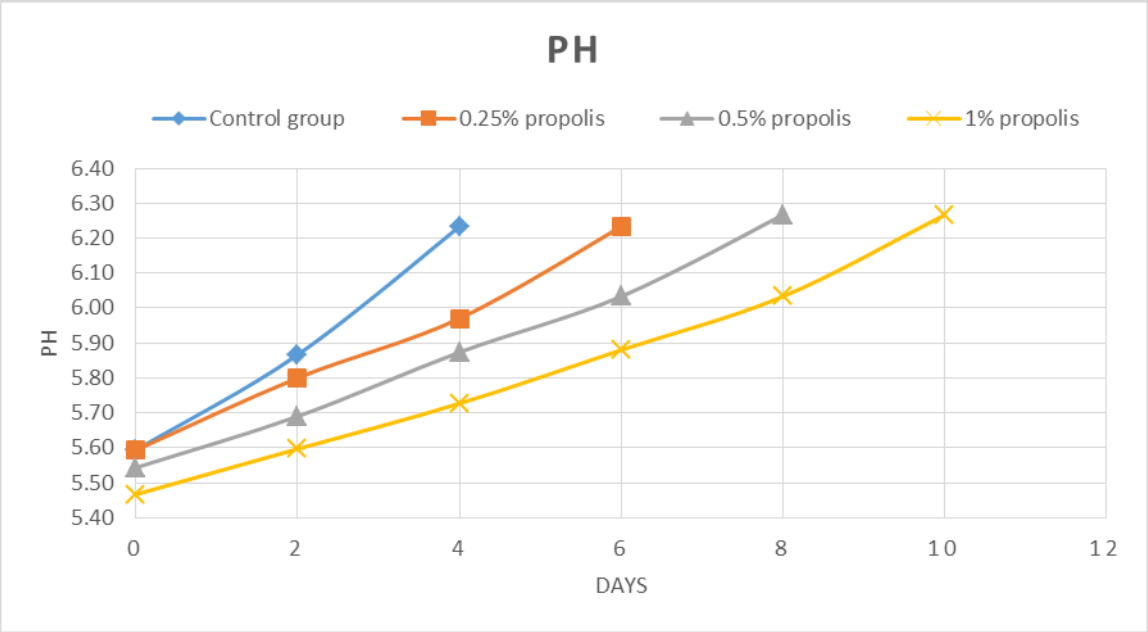

Table (4). Mean value of Thiobarbituric acid (TBA) value of beef burger samples (mean of 3 trials ± SD).

	1st group (Control)	2nd group (0.25% propolis)	3rd group (0.5% propolis)	4th group (1% propolis)
0 day	0.36 [a] ±0.05	0.36 [a] ±0.05	0.36 [a] ±0.05	0.36 [a] ±0.05
2 day	0.69 [a] ±0.09	0.66 [ab] ±0.08	0.61ab ±0.08	0.53 [b] ±0.09
4 day	1.05 [a] ±0.13	0.84 [b] ±0.07	0.72 [cd] ±0.10	0.61 [d] ±0.10
6 day	**S**	1.08[a]±0.20	0.87[b]±0.04	0.72[c]±0.03
8 day		**S**	1.07[a]±0.12	0.83[a]±0.06
10 day			**S**	0.99±0.03

**Means within a raw followed by different letters showed significant differences (P < 0.05).

Figure (4). Thiobarbituric acid (TBA) value of beef burger samples (mean of 3 trials ± SD).

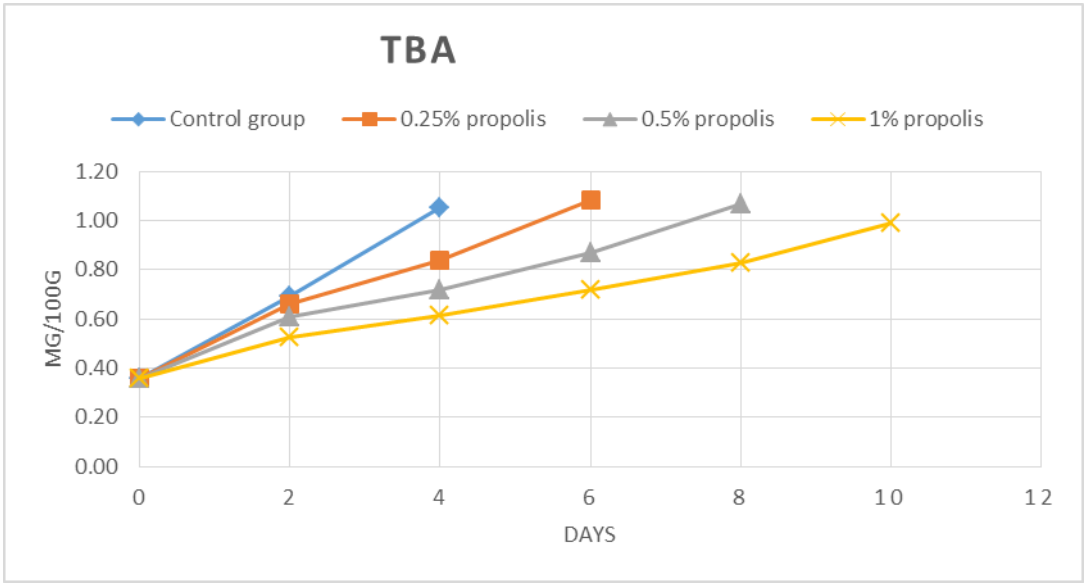

Table (5). Mean value of total volatile bases nitrogen (TVB-N) value of beef burger samples (mean of 3 trials ± SD).

	1st group (Control)	2nd group (0.25% propolis)	3rd group (0.5% propolis)	4th group (1% propolis)
0 day	13.15^{a}±0.45	13.15 ^{a} ±0.45	13.15 ^{a} ±0.45	13.15 ^{a} ±0.45
2 day	17.30 ^{a} ±0.46	16.47 ^{ab} ±0.85	15.87 ^{ab} ±0.93	15.17 ^{b}±0.93
4 day	21.63 ^{a} ±0.42	19.20 ^{b}±0.62	17.60^{c}±0.70	16.97^{cd}±0.71
6 day	**S**	21.93^{a}±0.85	19.53^{b}±0.35	18.40^{c}±0.17
8 day		**S**	21.83^{a}±0.64	19.63^{b}±0.15
10 day			**S**	22.23±0.55

**Means within a raw followed by different letters showed significant differences (P < 0.05).

Figure (5). Mean value of total volatile bases nitrogen (TVB-N) value of beef burger samples

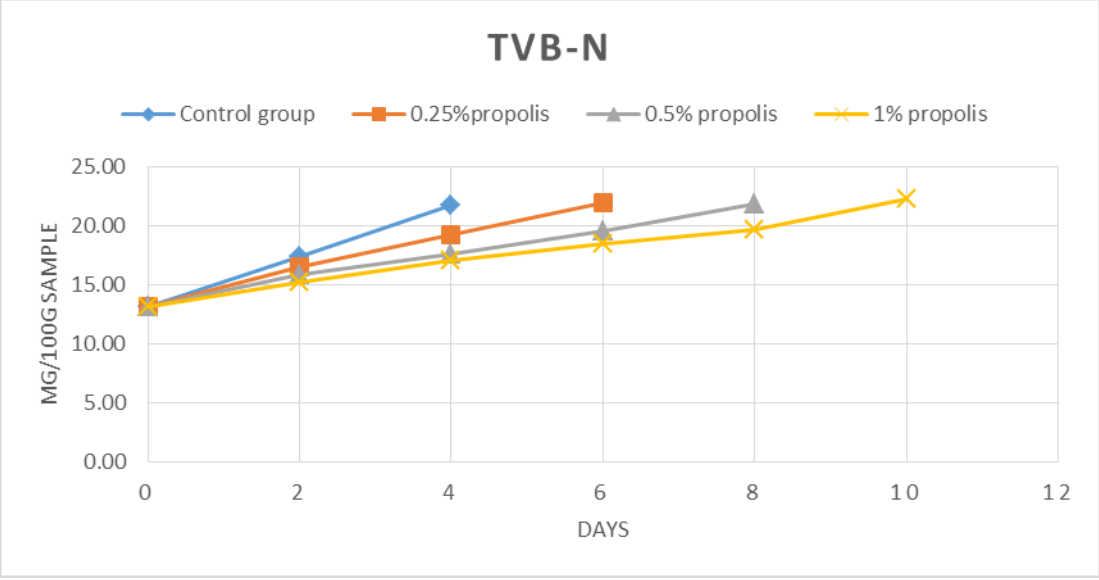

Table (6). Mean Value of Fungal Plate Count beef burger Samples (Mean Log 10 cfu/g ± SD of 3 Trials).

	1st group (Control)	2nd group (0.25% propolis)	3rd group (0.5% propolis)	4th group (1% propolis)
0 day	2.33 ^{a}± 0.09	2.30^{a}± 0.08	2.28^{a}±0.09	2.35^{a}±0.07
2 day	3.45^{a}±0.05	3.39^{b}±0.06	3.25^{c}±0.11	2.42^{d}±0.05
4 day	4.28^{a}±0.12	4.05^{b}±0.03	3.61^{c}±0.09	2.83^{d}±0.08
6 day	S	4.61^{a}±0.09	3.83^{b}±0.04	3.13^{c}±0.04
8 day		S	4.42^{a}±0.08	3.51^{b}±0.03
10 day			S	4.60±0.01

**Means within a raw followed by different letters showed significant differences (P < 0.05).

Figure (6). Fungal Plate Count of beef burger Samples

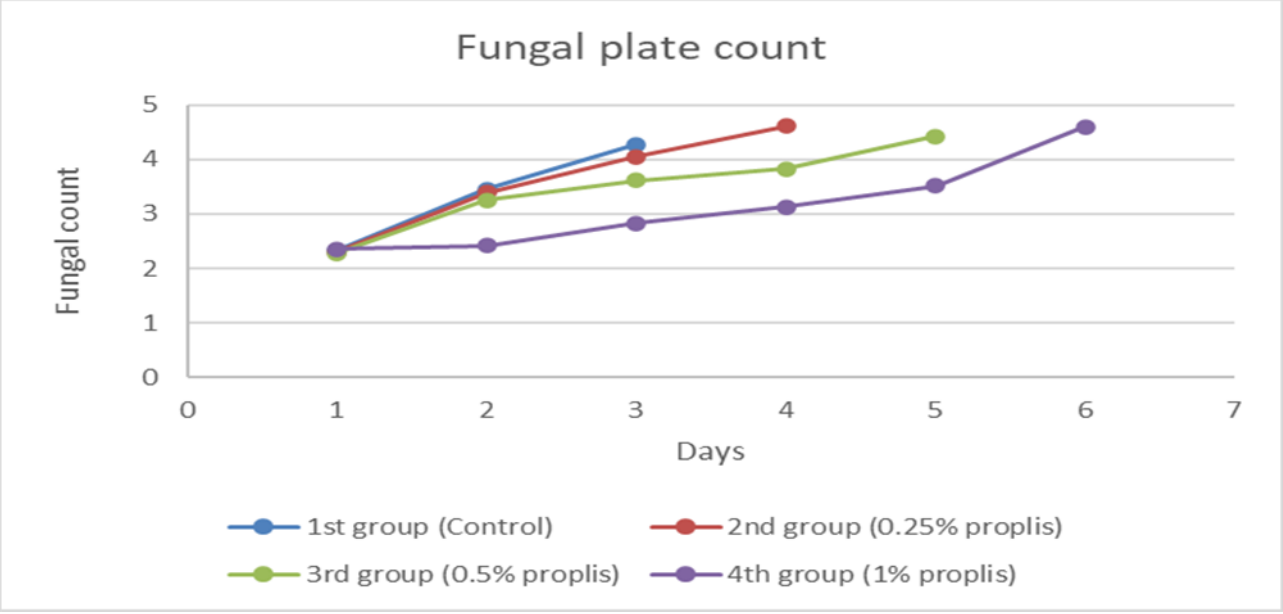

Table (7). Number and percentage of the identified isolates of mould genera from examined samples.

Mould genera	Number of isolates	%
Aspergillus spp.	**29**	**43.9**
Penicillium spp.	**15**	**22.7**
Cladosporium	**4**	**6.1**
Sporotricum	**2**	**3.0**
Fusarium spp.	**3**	**4.5**
Rhizopus	**4**	**6.2**
Mucor	**5**	**7.6**
Alternania	**3**	**4.5**
Curvularia	**1**	**1.5**

Table (8). Percentage of different *Aspergillus* species isolated from examined samples.

Aspergillus species	Number of isolates	%
A. flavus	**9**	**31**
A. niger	**11**	**37.9**
A. fumigatus	**2**	**6.9**
A. parasiticus	**1**	**3.5**
A. ochraceus	**2**	**6.9**
A. terreus	**4**	**13.8**

Table (9). Effect of propolis on sensory evaluation parameters; color, texture, aroma, and over-all acceptability of beef burger.

	Sensory evaluation parameters			
	Color	**Aroma**	**Texture**	**Over All Acceptability**
Control	4.7	4.3	4.0	4.7
Propolis 0.25%	4.5	4.3	4.1	4.5
Propolis 0.5%	4.5	4.1	4.3	4.3
Propolis 1%	4.6	3.9	4.4	4.5

References

Abdo, G.N.; Khafaga, N.I.; El-Hariri, M. and Refai, K.M. (2017). Detection of fungi and total aflatoxins in food additives and some meat products by serological and molecular biological methods. Journal of the Egyptian Veterinary Medical Association;177(2):153-73.

Abuzaid, K.E.A.; Shaltout, F.; Salem, R. and El-Diasty, E.M. (2020). Microbial aspect of some processed meat products with special reference to aflatoxins. Benha Veterinary Medical Journal, 39(2): 24-28. DOI: https://www.doi.org/10.21608/bvmj.2020.44886.1274

Afoakwah, N.A.; Mahunu, G.K. and Apaliya, M.T. (2024). Citrinin Toxin. In Microbial Toxins in Food Systems: Causes, Mechanisms, Complications, and Metabolism (pp. 415-426). Cham: Springer Nature Switzerland.

Ahmad, S.R.; Gokulakrishnan, P.; Giriprasad, R. and Yatoo, M.A. (2015). Fruit-based natural antioxidants in meat and meat products: a review. Critical Reviews in Food Science and Nutrition; 55(11):1503–1513. doi: 10.1080/10408398.2012.701674.

Alahakoon, U.; Jayasena, D.; Ramachandra, S. and Jo, C. (2015). Alternatives to nitrite in processed meat: Up to date. Trends in Food Science and Technology, 45(1): 37-49.

Ali, F.H.; Kassem, G.M. and Atta-Alla, O.A. (2010). Propolis as a natural decontaminant and antioxidant in fresh oriental sausage. VeterinariaItaliana, 46(2):167-172.

Al-Sabea, A.A.; Nasser, A.K. and Al-Rikabi, A.K. (20220. Chemical Parameters of Coated Beef Burger with Edible Oat Film During Refrigerated Preserving. Ann. For. Res. 65(1): 4720-4729.

American Public Health Association (APHA) (1992). Compendium of Methods for The Microbiological Examination of Foods. 1015 fifteenth, NW, Washington, DC 2005.

Apriantini, A. and Budiman, C. (2021). Pengaruh ekstrak propolis sebagai edible coating terhadapkarakteristikkimia dan aktifitasantioksidandagingsapi pada penyimpanansuhuruang. JurnalIlmuProduksi dan Teknologi Hasil Peternakan, 9(2), 72-78.

Azemin, A.; Md-Zin, N.B.; Mohd-Rodi, M.M.; Kim-Chee, A.S.; Zakaria, A.J. and Mohd, K.S. (2018). Application of metabolite profiling and antioxidant activity in assessing the quality of processed and unprocessed stingless bee's propolis. J. Fundam. Appl. Sci. 9: 637-660.

Barcenilla, C.; Ducic, M.; López, M.; Prieto, M. and ÁlvarezOrdóñez, A. (2022). Application of lactic acid bacteria for the biopreservation of meat products: A systematic review. Meat Science, 183, 108661.

Banwart, G.J. (1980). Basic food microbiology. A text book. Ed. Avi. Publishing CO. INK. Weastport, Cooecticut, U.S.A.

Bobko, M.; Kročko, M.; Haščík, P. and Bobková, A. (2015). Oxidative stability of chicken meat after propolis extract application in their diets. Potravinarstvo Slovak Journal of Food Sciences, 9(1): 48-52.

Brr, H.A.; Moustafa, Y.N. and Edris, M.A. (2004). Incidence of moulds and aflatoxiins in some meat products. Benha veterinary medical journal, 15(2): 65-75.

Corp, I.B. (2013). IBM SPSS statistics for windows version 22.0. Armonk, NY: International Business Machines Corp.

das Neves, M.V.; da Silva, T.M.; de Oliveira, Lima E.; da Cunha, E.V. and Oliveira, E.J. (2016). Isoflavone formononetin from red propolis acts as a fungicide against Candida sp. Braz. J. Microbiol. 47:159-166.

Duman, M. and Ozpolat, E. (2015). Effects of water extract of propolis on fresh shibuta (Barbus grypus) fillets during chilled storage. Food Chemistry, 189: 80–85.

Ebadi, Z.; Khodanazary, A.; Hosseini, S.M. and Zanguee, N. (2019). The shelf-life extension of refrigerated Nemipterus japonicus fillets by chitosan coating incorporated with propolis extract. International Journal of Biological Macromolecules, 139, 94–102. https://doi.org/10.1016/j.ijbiomac.2019.07.204

Edris, A.M. (1986). Estimation of mould and yeasts in imported frozen meat and chicken. Ph.D. Fac. Vet. Med. Alex. Edfina University.

El-Bassiony, T.A.; Saad, N.M. and El-Zamakan, M.A. (2012). Study on the antimicrobial activity of Ethanol Extract of Propolis against enterotoxigenic Methicillin – Resistant Staphylococcus aureus in Lab prepared Icecream. Vet. World, 5(3): 155-15.

El-Deeb, A.M. (2017). Utilization of propolis extract as a natural preservative in raw milk. Journal of Food and Dairy Sciences, 8(8): 315–321.

El-Mossalami, H.H. and Abdel-Hakeim, Y.A. (2013). Using of propolis extract as a trial to extend the shelf-life and improving the quality criteria of fresh Egyptian sausage. Assiut. Vet. Med. J. 59: 23-33.

European Parliament and Council of the European Union (2002). Directive 2002/46/EC of the European Parliament and of the council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements (Text with EEA relevance). Off. J. Eur. Communities Legis, 183:51-57.

Egyptian Organization for Standardization (EOS) (2006a). Methods of Analysis and Testing for Meat. Part 9: Determination of Total Volatile Nitrogen (TVN). EOS: 63-9/2006.

Egyptian Organization for Standardization (EOS) (2006b). Methods of Analysis and Testing for Meat. Part 10: Determination of Thiobarbituric acid (TBA). EOS: 63-10/2006.

Egyptian Organization for Standardization (EOS) (2006c). Methods of Analysis and Testing for Meat and meat products. Part 11: measurement of PH . EOS: 63-11/2006.

El-Demery, M.; Elsebaie, E.M.; Zidan, N. and Essa, R. (2016). Efficiency of Propolis and Turmeric Powders as Natural Preservatives in Minced Beef. J. Food and Dairy Sci. Mansoura Univ. 7 (1): 45-50.

Gourama, H. and Bullerman, L.B. (1995). Aspergillus flavus and Aspergillus parasiticus: Aflatoxigenic fungi of concern in foods and seeds: A review. Journal of Food Protection, 58(12): 1495-1404. DOI: https://www.doi.org/10.4315/0362-028x-58.12.1395

Güngoren, A.; Patir, B. and Ozpolat, E. (2023). The effect of propolis application on quality properties of vacuum-packed hot smoked rainbow trout (Oncorhynchus Mykiss, Walbaum 1792) fillets during cold storage. LWT- Food Science and Technology. 148.

Gutiérrez, C. (2012). Evaluación del efecto de propoleoscomobiopreservanteen chorizo (PhD). Universidad Nacional de Colombia, Bogotá.

Han, S.K. and Park, H.K. (2002). Accumulation of thiobarbituric acid – reactive substances in cured pork sausages treated with propolis extracts. J. Sci. Food Agric. 82: 148.

Jonaidi Jafari, N.; Kargozari, M.; Ranjbar, R.; Rostami, H. and Hamedi, H. (2018). The effect of chitosan coating incorporated with ethanolic extract of propolis on the quality of refrigerated chicken fillet. Journal of Food Processing and Preservation, 42 (1), Article e13336.

Kassem, G.M.; Atta-Alla, O.A. and Ali, F.H.M. (2011). Improving the quality of beef burger by adding thyme essential oil and jojoba oil. Arch. Zootec. 60:787–795. doi: 10.4321/S0004-05922011000300066.

Kim, Y.H.; Kim, A.S. and Chung, H.J. (2013). Synergistic effect of propolis and heat treatment leading to increased injury to Escherichia coli O157:H7 in ground pork. Journal of Food Safety. 34(1):1-8.

Kismet, K.; Ozcan, C.; Kuru, S.; Gencay, Celemli O.; Celepli, P.; Senes, M.; Guclu, T.; Sorkun, K.; Hucumenoglu, S. and Besler, T. (2017). Does propolis have any effect on non-alcoholic fatty liver disease? Biomed Pharmacother. 90: 863-871.

Koc, A.N.; Silici, S.; Mutlu-Sariguzel, F. and Sagdic, O. (2007). Antifungal activity of propolis in four different fruit juices. Food Technology and Biotechnology, 45(1):57-61.

Lilica, S.; Ivana Brankovica, I.; Koricanaca, V.; Vranica, D.; Spalevicb, L.; Pavlovicc, M. and Lakicevic, B. (2015). Reducing sodium chloride content in meat burgers by adding potassium chloride and onion.Procedia Food Science. 5: 164 – 167.

Mahdavi-Roshan, M.; Gheibi, S. and Pourfarzad, A. (2022). Effect of propolis extract as a natural preservative on quality and shelf life of marinated chicken breast (chicken Kebab). LWT, 155, 112942. Microbiology, Ridgeview Publication Co. California.

Mizakova, A.; Pipova, M. and Turek, P. (2002). The occurrence of moulds in fermented raw meat products. Czech Food Sci.20, (3):89 -94.

Morshdy, M.A.; Hussien, A.M.; ElAbbasy, T.M. and Elzwahery, M.R. (2015). Aflatoxins residues in some meat products. 2nd Vet. Med. Suez Canal. Conference of Food Safety, I: 90-95.

Mustafa, Y.; Abd El-Tawab, A.; El-Diasty, M. and Khater, F. (2019). Mycobiota of some meat products and spices. Benha Veterinary Medical Journal, 36(2):325-34.

Ozturk, I. (2015). Antifungal activity of propolis, thyme essential oil and hydrosol on natural mycobiota of sucuk, a Turkish fermented sausage: Monitoring of their effects on microbiological, color and aroma properties. J. Food Process Preserv. 39:1148-1158.

Pellissery, A.J.; Vinayamohan, P.G.; Amalaradjou, M.A. and Venkitanarayanan, K. (2020). Spoilage bacteria and meat quality. In Meat quality analysis (pp. 307– 334). Elsevier.

Pitt, J.I. and Hoching, A.D. (2009). Fungi and Food spoilage. 3rd Ed. Published by Springer Dordrecht Heidelberg London New York.

Pobiega, K.; Kraśniewska, K. and Gniewosz, M. (2019a). Application of propolis in antimicrobial and antioxidative protection of food quality: A review. Trends Food Sci. Technol. 83: 53-62.

Pobiega, K.; Kraśniewska, K.; Przybył, J.L.; Bączek, K.; Żubernik, J.; Witrowa-Rajchert, D. and Gniewosz, M. (2019b). Growth biocontrol of foodborne pathogens and spoilage microorganisms of food by Polish propolis extracts. Molecules 24: 2965.

Principato, L.; Mattia, G.; di Leo, A. and Pratesi, C.A. (2021). The household wasteful behaviour framework: A systematic review of consumer food waste. Industrial Marketing Management, 93: 641– 649.

Samson, R.A.; Houbraken, J.; Thrane, U.; Frisvad, J.C. and Andersen, B. (2010). Food and indoor fungi. CBS Laboratory Manual Series, pp. 19-21. Available at: https://www.worldcat.org/title/food-and-indoor-fungi/oclc/703787403

Seibert, J.B.; Bautista-Silva, J.P.; Amparo, T.R.; Petit, A.; Pervier, P.; dos Santos Almeida, J.C.; Azevedo, M.C.; Silveira, B.M.; Brandão, G.C.; de Souza, G.H.; de

Medeiros Teixeira, L.F. and dos Santos, O.D. (2019). Development of propolis nanoemulsion with antioxidant and antimicrobial activity for use as a potential natural preservative. Food Chem. 287: 61-67.

Shaban, M.M.; Abdel-Aleem, W.M. and Gala, S.M. (2021). Organic propolis extract as a natural fortifier and preservative for milk. Journal of Food and Dairy Sciences. 12 (12):325-9.

Shavisi, N.; Khanjari, A.; Basti, A.A.; Misaghi, A. and Shahbazi, Y. (2017). Effect of PLA films containing propolis ethanolic extract, cellulose nanoparticle and Ziziphoraclinopodioides essential oil on chemical, microbial and sensory properties of minced beef. Meat Science, 124, 95-104.

Siheri, W.; Alenezi, S.; Tusiimire, J. and Watson, D.G. (2017). The chemical and biological properties of propolis. In Bee products-chemical and biological properties (pp. 137-178). Springer, Cham

Silici, S. and Karaman, K. (2014). Inhibitory effect of propolis on patulin production of Penicillium expansum in apple juice. Journal of food processing and preservation, 38(3), pp.1129-1134.

Silici, S.; Koc, N.A.; Sariguzel, F.M. and-Sagdic, O. (2005). Mould inhibition in different fruit juices by propolis. Archivfuer Lebensmittelhygiene, 56:87-90.

Soares, K.S.; Souza, M.P.; Silva-Filho, E.C.; Barud, H.S.; Ribeiro, C.A.; Santos, D.D.; Rocha, K.N.S.; de Moura, J.F.; Oliveira, R.L. and Bezerra, L.R. (2021). Effect of edible onion (Allium cepa L.) film on quality, sensory properties and shelf life of beef burger patties. Molecules. 26: 7202 https://doi.org/10.3390/molecules26237202

Soliman, M.M.; Ata, N.S.; El-Shafei, H.M. and Kandil, M.M.A. (2019). Prevalence of toxigenic Aspergillus flavus in meat and meat products. Bioscience Research, 16(1): 822-829. Available at: https://www.isisn.org/BR_16_1_2019.htm

Tumbarski, Y.D.; Todorova, M.M.; Topuzova, M.G.; Georgieva, P.I.; Ganeva, Z.A.; Mihov, R.B. and Yanakieva, V.B. (2021). Antifungal Activity of Carboxymethyl Cellulose Edible Films Enriched with Propolis Extracts and Their Role in Improvement of the Storage Life of Kashkaval Cheese. Current Research in Nutrition and Food Science, 9(2): 487.

Tumbarski, Y.; Topuzova, M. and Todorova, M. (20220. FOOD INDUSTRY APPLICATIONS OF PROPOLIS: A REVIEW. Journal of Hygienic Engineering & Design. 40.

Vargas-Sánchez, R.D.; Mendoza-Wilson, A.M.; Torrescano-Urrutia, G.R. and Sánchez-Escalante, A. (2015). Antiradical potential of phenolic compounds fingerprints of propolis extracts: DFT approach. Computational and Theoretical Chemistry, 1066: 7-13.

Vargas-Sánchez, R.D.; Torrescano-Urrutia, G.R.; Acedo- Félix, E.; Carvajal-Millán, E.; González-Córdova, A.F.; Vallejo-Galland, B.; Torres-Llanez, M.J. and Sánchez-Escalante, A. (2014). Antioxidant and antimicrobial activity of commercial propolis extract in beef patties. Journal of Food Science, 79 (8): 1499-1504.

Yang, W.; Wu, Z.; Huang, Z.Y. and Miao, X. (2017). Preservation of orange juice using propolis. Journal of Food Science and Technology, 54:3375-83.

Yazgan, H.; Burgut, A.; Durmus, M. and Kosker, A.R. (2020). The impacts of water and ethanolic extracts of propolis on vacuum packaged sardine fillets inoculated with Morganellapsychrotolerans during chilly storage. Journal of Food Safety, 40(2): 1–9.