https://doi.org/10.17113/ftb.63.03.25.8774 original scientific paper

Effect of Propolis on the Quality Characteristics and Shelf Life of Raw Beef Meatballs during Refrigerated Storage

Running title: Effect of Propolis on the Properties and Shelf Life of Meatballs

Aslihan Cevik Ozkir[1], Ahmet Sukru Demirci[1], Harun Uran[2]*, Recep Gunes[2], Berkay Kopuk[3] and Bayram Cetin[2]

[1]Tekirdağ Namık Kemal University, Faculty of Agriculture, Dept. of Food Engineering, 59030, Tekirdağ, Türkiye
[2]Kırklareli University, Faculty of Engineering, Dept. of Food Engineering, Kayalı Campus, 39000, Kırklareli, Türkiye
[3]Kırklareli University, Food R&D, Innovation and Entrepreneurship Application and Research Center, Kayalı Campus, 39000, Kırklareli, Türkiye

Received: 12 July 2024
Accepted: 23 March 2025

SUMMARY

Research background. Recently, natural ingredients have come to the fore instead of synthetic additives used in various meat and meat products. In this context, the use of extracts prepared with different solvents of propolis, a natural bee product, is becoming quite widespread. From this perspective, this study aimed to determine the contribution of ethanolic extract of propolis on the shelf life of beef meatball samples and to investigate to what extent it maintains the quality properties of the samples during 4 °C storage.

Experimental approach. In the study, an ethanolic extract of propolis was added to meatball samples at different concentrations (0.1 %, 0.3 %, 0.5 %, and 1 %). After production, the samples

*Corresponding author:
Phone: +902882140514
Fax: +902882140516
E-mail: harunuran@klu.edu.tr

were packaged and stored at 4 °C. During storage, the meatball samples were subjected to physicochemical, microbiological, and sensory analysis.

Results and conclusions. The addition of propolis did not significantly affect the water activity values, however, significantly lower pH values were observed at prolonged storage periods, especially in the samples containing higher propolis concentrations. The addition of propolis was also effective at delaying the oxidation and there was a concentration-dependent decrease in the TBARS values. The use of propolis in meatball production did not show a significant effect on the initial CIELab parameters of the samples, but changes in a^* and b^* values were detected compared to the control sample at the end of storage. It was observed that there was a significant increase in the total phenolic content, $DPPH^{\bullet}$ and $ABTS^{+}$ radical scavenging activities of the meatballs depending on the propolis concentration. Considering the microbiological analysis results, it was determined that propolis could increase the microbiological quality of meatballs, but the addition of propolis at a concentration of more than 0.5 % affected the general acceptability of the samples.

Novelty and scientific contribution. As a result, propolis application in certain amounts was found a potential alternative to synthetic counterparts that could be used for the preservation of meatballs stored at refrigeration conditions in terms of delaying oxidation and microbial spoilage without affecting the sensory properties of the samples.

Keywords: meatball; propolis; quality; shelf life

INTRODUCTION

It is well-known that meat is a rich and important source of proteins and other nutrients (*1*). However, the susceptibility of meat products due to their composition, oxidative changes and microbial growth are the two important factors that determine the product's storage stability. In meat products such as minced meat, oxidative stability depends on the interaction between endogenous pro- and antioxidant substances (*2,3*). For instance, lipid and protein oxidations decrease the quality of meat and meat products, which all ultimately influence consumer acceptance. Thus, artificial antioxidant compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tert-butylhydroquinone (TBHQ), and erythorbates are extensively used to reduce or inhibit the oxidative deterioration in these products (*4*,*5*).

Another important factor for the quality and safety of meat products is microbial activity. In general, fresh meat and meat products have a higher water activity (a_w) value than 0.85 and a pH that favors spoilage and pathogenic microorganisms to survive in the product (*6*). For this reason,

synthetic antimicrobials such as chlorides, nitrites, sulfites, and sorbates are widely used to extend refrigerated storage as the most common method for the preservation of meat products (*7*). However, the use of these synthetic antimicrobials and antioxidants in food products has been associated with carcinogenic effects and health risks. To address these concerns, it may be necessary and useful to develop and apply natural ingredients with both antioxidant and antibacterial activity that ensure consumption safety in various meat products (*8-11*).

Moreover, consumer demands are increasing toward more natural and minimally processed products with higher bioactive properties and sensory quality. In this context, some processing strategies including the use of non-thermal processing technologies, the addition of polyphenols from vegetable and fruit by-products, and the incorporation of different herbs and spices, are being studied to improve oxidative and microbial quality of meat products and therefore extend the shelf life of the samples (*11*). In this perspective, a resinous bee product called “propolis”, which has significant antibacterial and antioxidant properties, stands out as a potential natural ingredient for the substitution of synthetic additives in meat products (*5*,*12*,*13*). As is known, propolis is a hive product produced by honey bees (*Apis mellifera*) through collecting resins from plant blossoms and secretions and combining them with salivary secretions and beeswax. The bioactive properties of propolis can be varied depending on factors such as botanical origin, chemical composition, season, bee age, and collection area or time, consisting more than 300 different compounds, mainly plant resins (50 %), bee-wax (30 %), essential oils (10 %), pollen (10 %) and vitamins (B_1, B_2, B_6, C, E), benzoic acid and derivatives, flavonoids and derivatives, amino acids and minerals (*14-17*). The antioxidant (*18*), antibacterial (*19*), antifungal (*20*), anti-inflammatory (*21*), and anti-carcinogenic (*22*) properties of propolis combined with the fact that the substances detected in propolis are commonly present in foods, make it an excellent candidate as an alternative to conventional synthetic preservatives in meat and meat products.

Nevertheless, the use of propolis in food preservation is limited because it can affect the sensory properties of the product due to its intense taste and odor. Thus, it would be very important to determine the propolis concentration that can be applied for preservation purposes without changing the sensorial characteristics of the products. For instance, adding approximately 0.5 % propolis extract to various food products, such as fish sausages and poultry products was reported to provide good sensory results (*17*). However, the literature about the effect of propolis on different quality factors and sensorial characteristics of meat and meat products is very limited. Therefore, in the present study, it was aimed to investigate the effects of different propolis concentrations on the

quality properties of meatballs and to determine the acceptable concentration that capable of providing preservation during the refrigerated storage period (9 days at 4 °C).

MATERIALS AND METHODS

Materials

The beef meat containing 20 % fat, all spices, and other ingredients used in the production of meatball samples were purchased from a local producer in Kırklareli, Türkiye. Ethanolic extract of propolis (30 %, w/w) was supplied by Tekirdağ Namık Kemal University, Faculty of Agriculture, Food Engineering Department. The extract was prepared at 30 % (w/w) using 70 % ethanol (Merck KGaA, Darmstadt, Germany) of a crude propolis sample taken from an apiary in the Mersin region, located in the southern part of Türkiye. Then, the sample was homogenized (IKA T 25, Germany) for 30 min, and kept at room temperature in the dark for 15 days. Finally, the prepared extract was filtered using Whatman No. 4 filter paper (Millipore, USA) and stored at 4 °C until further analysis (*23*). All chemicals used for analysis were supplied by Merck (Darmstadt, Germany) and of analytical grade.

Preparation of meatballs

The beef meat used in the production of meatballs was first cleaned from cartilage and nerves. Then, the lean beef (84 %), bread crumb (10 %), and red onion (3 %) were ground into minced meat in a meat grinder (Ari machine, Türkiye). Followingly, 0.5 % cumin, 0.5 % black pepper powder, and 2 % table salt were added to the dough and kneaded by hand using sterile gloves for 30 min to obtain a homogeneous mixture (*24*). The obtained meat dough was divided into five batches and propolis was added at different concentrations; control (no propolis included), M0.1 (meatball containing 0.1 % propolis), M0.3 (meatball containing 0.3 % propolis), M0.5 (meatball containing 0.5 % propolis), and M1 (meatball containing 1 % propolis). Raw meatball samples (30 g, 5 cm diameter) were shaped by hand, transferred into disposable food grade plastic (PET) containers with lids, and then tightly sealed, labeled and stored aerobically in a refrigerator at 4 °C throughout the study period (9 days).

Physicochemical analysis

Meatball sample (10 g) were homogenized in 90 mL of distilled water for 1 min and the pH value was determined by immersing the probe of the pH meter (HI 2211, Hanna Instruments, USA). Prior to water activity (a_w) determination, samples were allowed to equilibrate at 25 °C for 30 min and a_w was measured using a benchtop a_w meter (Novasina LabSwift, Switzerland). To investigate lipid

oxidation in raw meatballs, the method proposed by Gokoglu *et al.* (*25*) was used to measure thiobarbituric acid reactive substance content (TBARS). Briefly, 47.5 mL distilled water and 2.5 mL of 4 N HCl were added to 10 g of homogenized sample, and the distillation process was carried out using a distillation apparatus equipped with heating mantle (MS-EAM 9202-03, MTOPs, Korea) at 100 °C for 10 min. Following the transfer of 5 mL of distilled solution into the stoppered test tube, 5 mL thiobarbituric acid (TBA) reagent was added. Then, the solution was homogenized and allowed to react with TBA for 35 min at 110 °C. At the end of time, the measurements were performed at 538 nm against a blank using a UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan), and the results were expressed as mg malondialdehyde (MDA)/kg sample. The CIELab color properties of meatballs were determined using a colorimeter (CR-400 Konica Minolta, Chroma Meter, Tokyo, Japan). Five consecutive measurements were taken from the surface of the samples with the illuminator D65 and an observer angle of 2°. The *L**, *a**, and *b** values were taken as the mean of five readings and were used for the calculation of total color difference (ΔE) values by using the following equation (*26*). All analyses were carried out on days 1, 3, 6, and 9 of the storage period.

$$\Delta E = \sqrt{\Delta L^{*^2} + \Delta a^{*^2} + \Delta b^{*^2}} \qquad /1/$$

Total phenolic content and antioxidant activity analysis

Extraction of phenolics

The extraction of phenolics was performed by mixing the 4 g of raw meatball sample with 16 mL of 80 % methanol, homogenization at 1200 rpm for 1 min, and centrifugation at 9500 rpm for 10 min (15 °C).

Total phenolic content (TPC)

The slightly modified method of Nugboon and Intarapichet (*27*) was used for the measurement of TPC. Briefly, 200 µL of diluted extract, 1 mL of 0.2 N Folin-Ciocalteu reagent (10 %, w/v), and 1 mL of sodium carbonate (75 g/L) solution were mixed in a glass test tube and allowed to rest for 3 min. Then, the solution was completed to 10 mL with distilled water and kept at room temperature in the dark for 90 min. Followingly, the absorbance of the solution was measured at 725 nm using a spectrophotometer (Shimadzu, Tokyo, Japan). The analysis was carried out on days 1, 3, 6, and 9 of the storage period and the results were expressed as mg gallic acid equivalent (GAE)/kg sample.

$DPPH^{\bullet}$ and $ABTS^{+}$ radical scavenging assays

DPPH$^{\bullet}$ radical scavenging activity of the meatballs was measured using the method proposed by Nugboon and Intarapichet (*27*) with slight modifications. For this purpose, 150 µL diluted extract was mixed with 2.850 mL 0.1 mM DPPH$^{\bullet}$ radical solution in pure methanol, which was then kept at 30 min at room temperature in dark conditions. The absorbance of the mixture was measured at 517 nm against pure methanol and the following equation was used to calculate the % inhibition values of the samples. The same procedures were performed on the Trolox standard solution prepared with concentrations ranging from 50 to 1000 µM, and the obtained % inhibition values were plotted against the Trolox concentration to obtain the calibration curve (R^2=0.9991). Then, the results were calculated using this calibration curve and considering all dilution factors, and were expressed as µM Trolox which is equal to µmol Trolox/kg meatball sample. The analysis was carried out on days 1, 3, 6, and 9 of the storage period and each measurement was performed in triplicate.

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \qquad /2/$$

where A_0 is the absorbance of the blank solution and A_1 is the absorbance of the sample solution.

The method of Huang *et al.* (*28*) was used to measure the ABTS^{+} radical scavenging activity of the samples with slight modifications. To prepare ABTS^{+} radical, 7 mM ABTS and 2.45 mM of potassium persulfate solutions were mixed in equal volumes and the reagent was left in the dark at room temperature for 16 h. Then, the radical solution was diluted with pure methanol to an absorbance of 0.700±0.01 at 734 nm before the assay to obtain the working solution. For the spectrophotometric assay, 2 mL of ABTS^{+} radical working solution was added on 20 µL of aliquot and the measurements were taken at 734 nm against pure methanol after 7 min of reaction. The percentage (%) inhibition values of the samples were calculated using the above equation. The same procedures were performed on the Trolox standard solution prepared with concentrations ranging from 50 to 2000 µM, and the obtained % inhibition values were plotted against the Trolox concentration to obtain the calibration curve (R^2=0.9993). Then, the results were calculated using this calibration curve and considering all dilution factors, and were expressed as µM Trolox which is equal to µmol Trolox/kg meatball sample. The analysis was carried out on days 1, 3, 6, and 9 of the storage period and each measurement was performed in triplicate.

Microbiological analysis

Microbiological analysis carried out during the study included the determination of *Enterobacteriaceae*, total yeasts and molds (TYM), *Staphylococcus aureus*, and total aerobic mesophilic bacteria (TAMB) counts. Briefly, 10 g of raw sample was homogenized with 90 mL of 0.1 % peptone water using a Stomacher Lab-Blender 400 (Seward Medical, London, UK). Violet red bile dextrose agar used for the enumeration of *Enterobacteriaceae* performing pour plating method and the incubation was performed at 37 °C for 24 h (*29*). Potato dextrose agar (5 days at (25±1) °C) was used for the total count of TYM (*30*). Baird-Parker agar with egg yolk/tellurite emulsion was used for the determination of *S. aureus* and incubation was performed at 37 °C for 30–48 h (*31*). The verification of *S. aureus* colonies was carried out by coagulase test. Plate count agar was used for the TAMB count and incubation was performed at 30 °C for 24-48 h under aerobic conditions with pour plate method (*32*). All analyses were carried out on days 1, 3, 6, and 9 of the storage period and the results were expressed as log CFU/g.

Sensory evaluation

Before the evaluation, each side of the meatball sample was cooked for 4 min at 180 °C via the preheated grill. After cooking, the samples were coded using letters and randomly presented to the panelists. Sensory parameters (color liking, odor, taste, texture, and general acceptance) of cooked samples were evaluated by 30 untrained panelists. The panelists were chosen especially from people who habitually consume meatballs. All panelists were informed about the study at the beginning of the panel. In the analysis, a 9-point hedonic scale was preferred due to its widespread use. According to the scale, 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, and 9=like extremely for the relevant parameters. Water has been consumed before the assessment of each sample (*33*). The sensory evaluations were carried out under the supervision and coordination of the project administrators. The informed permission was acquired from each individual before their involvement and appropriate protocols to protect the rights and privacy of all participants were utilized during the conduct of the study. The analysis was carried out on days 1, 3, and 6 of the storage period.

Statistical analysis

The results were presented as mean ± standard error. The one-way analysis of variance (ANOVA) test was carried out using IBM's Statistical Software (SPSS version 22, IBM Corp., New York, USA) software (*34*). The Duncan's Multiple Comparison Test was used to identify significant differences between the results ($p<0.05$).

RESULTS AND DISCUSSION

Physicochemical properties results

The physicochemical properties of meatballs produced within the scope of the study are presented in Table 1. As seen in the table, the increase in propolis concentration did not significantly affect the pH values of the meatballs on the 1st and 3rd days ($p>0.05$). However, this result changed on the 6th and 9th days, and it was determined that there was a difference between the samples ($p<0.05$). It was observed that the M1 sample had the lowest pH values (5.89 and 6.00) compared to the control sample on the 6th and 9th days. In addition, considering the entire storage period, the highest pH values were detected on the 9th day ($p<0.05$). According to the literature, the increase in the pH values during storage was also observed for other meat products such as burgers (*35*). It was stated that the increase in the pH values might be due to the production of endogenous or microbial enzymes such as protease and lipase triggered by bacterial growth during prolonged storage (*36*). According to the current results, the use of propolis suppressed the pH increase in the samples compared to the control, and it was concluded that especially 0.5 % and 1 % propolis concentrations had a significant effect on pH control. Similar results were reported by Mehdizadeh and Langroodi (*37*), who investigated the effects of coating chicken breast meat with chitosan including propolis extract and *Zataria multiflora* Boiss oil on the quality parameters and found that the combined treatment was more effective at controlling the pH and microbial activity. Considering the a_w values, it was determined that the addition of liquid propolis extract did not cause a difference between the samples ($p>0.05$). This was considered a positive result in that the addition of propolis did not increase the moisture value of the samples (*38*). Meanwhile, although minor changes were observed in a_w values depending on the storage period, this phenomenon did not create a significant result.

Lipid oxidation is the primary quality failure in meat and meat products that shortens the shelf life of the product. It is a chain reaction of free radicals consisting of three steps, namely initiation, propagation, and termination (*39*). Peroxides, which are primary products of lipid oxidation, can undergo reactions to yield secondary oxidation products. Among them, MDA is one of the most important aldehydes since it gives rancid aromas to meat products even in low amounts. It therefore is used as a marker of lipid oxidation (*4*). In this context, it was observed that the TBARS values of the meatball samples varied between 1.40-2.60 mg MDA/kg sample on the first day, and showed a significant increase up to 2.48-3.88 mg MDA/kg sample on the 9th day. The addition of 0.5 % and 1 % propolis to the meatball formulation provided a significant decrease in TBARS values ($p<0.05$), while lower concentrations were not found to be effective ($p>0.05$). On the other hand, as expected, TBARS values tended to increase during storage and the highest value was found as 3.88 mg MDA/kg

in the control sample on the 9th day of storage ($p<0.05$). For the perception of oxidized taste, although it depends upon the experience and sensitivity of individual, the threshold value of TBARS for a positive sensory perception of beef is reported to be 2 mg MDA/kg (*40*). Since the TBARS values of control and M0.1 samples were higher than the threshold even at the beginning of the storage, it could be concluded that the lipid oxidation occurred during production, which might be due to the prolonged exposure to the oxygen. Although the TBARS values of M0.5 and M1 samples increased above the threshold value after 6 days of storage, the values remained lower compared to other samples. Vargas-Sánchez *et al.* (*12*) found that lipid oxidation in beef patties started from the first day, and continuously increased regardless of low temperature (2 °C) storage. The authors investigated the efficacy of ethanolic extract of propolis as an ingredient to inhibit lipid oxidation and confirmed that the incorporation of 2 % propolis extract into beef patties was effective in retarding lipid oxidation compared to the control sample after 4 and 8 d of storage at 2 °C depending on the polyphenol content of the extract. The results obtained in the current study were also in accordance with Dos Reis *et al.* (*13*), who reported that the burger meats containing microencapsulated propolis co-product extract had lower TBARS values compared to control and sodium erythorbate treated samples during the storage period at -15 °C. As a conclusion, it could be stated that the addition of propolis at concentrations of 0.5 % and 1 % was an effective way to delay lipid oxidation in meatballs stored at refrigerated conditions.

Table 1

Color and color stability are crucial parameters in terms of the quality and freshness of meat, however, the changes in the color during storage affect consumer preference (*41*). In the current study, the incorporation of propolis into the meatball formulation did not significantly change the L^* and a^* values of the samples on the first day ($p>0.05$), while it caused a linear increase in b^* values of propolis-added samples (12.92-14.47) compared to the control sample (12.23) ($p<0.05$). On the other hand, although there was no significant change in the L^* value of the samples depending on the storage period, the a^* values, which varied between 15.21-17.88 on the first day, decreased to the range of 10.68-13.21 on the 9th day. Interestingly, the lowest a^* values on the last day of storage were determined as 11.28±0.58 and 10.68±0.18 in M0.5 and M1 samples, respectively ($p<0.05$). The b^* values of the samples also changed depending on storage time, from 12.23-14.47 on the first day to 11.95-13.02 on the last day, and the highest b^* values were found in propolis-added samples throughout the storage period ($p<0.05$). As is known, long-term storage in an aerobic environment causes the transformation of oxymyoglobin (bright red color) into metmyoglobin (brown color) and this change makes meat and meat products unacceptable (*12*). In this context, considering the current

results, especially changes in the *a** value can be prevented by using vacuum packaging and modified atmosphere applications that prevent the contact of the samples with oxygen in further studies. In a previous study, Vargas-Sánchez *et al.* (*5*) determined that 2 % ethanolic propolis addition to beef and pork patties did not affect the *L** values of the samples regarding the initial and storage period. On the other hand, it was observed that the initial *a** and *b** values of all samples decreased significantly depending on the storage at 2 °C in the dark for 9 days, but contrary to the current results, this decrease was delayed in the propolis-added samples compared to the control sample. In another study, it was determined that increasing the concentration of propolis extract (0.25, 0.50, 1, and 3 %) obtained after 15 days using 70 % ethanol did not affect the *L**, *a**, and *b** values of fresh trout fillets (*42*). According to the study conducted by El Sheikha *et al.* (*43*), while the *L**, *a**, and *b** values of chicken breast meat samples coated with carboxymethyl cellulose (CMC) containing different amounts of ethanolic propolis extract (1, 2, 3, and 4 %) did not change initially, a significant increase was detected in the *L** and *a** values of the sample coated with CMC containing 4 % propolis compared to other samples depending on the storage period of 16 days at 2 °C.

Total phenolic content (TPC) and antioxidant activity results

Reformulation studies using natural ingredients, extracts, or compounds with high antioxidant capacity such as propolis are of great importance for both the meat industry and consumer perception (*44*,*45*). In this regard, the TPC results and antioxidant activity values (DPPH$^{\bullet}$ and ABTS^{+} radical scavenging assays) of raw meatball samples during storage are presented in Table 2. According to the results, the incorporation of propolis as a natural antioxidant compound to the formulation gradually increased the TPC values of meatballs, and this increase became significant at concentrations higher than 0.3 % compared to the control sample. On the other hand, considering the changes in the TPC values of meatballs throughout storage, as expected, a similar decreasing trend was observed in each sample, and the lowest values were obtained on day 9 of storage compared to the initial results. In a different study, beef and pork patties treated with propolis extract showed higher TPC values than those obtained for control sample during all storage time (*5*), which coincide with the current results. Similar to the TPC results, the DPPH$^{\bullet}$ and ABTS^{+} radical scavenging values were also significantly increased with the addition of propolis. In a study performed the addition of 5 % of spray-dried propolis to a optimized fish burger formulation with 10 % of potato flakes and 9 % extra virgin olive oil resulted to about three times greater phenolic content and about four times higher DPPH$^{\bullet}$ radical scavenging activity compared to the control (*46*). In the meantime, considering the storage process, both the DPPH$^{\bullet}$ and ABTS^{+} radical scavenging values showed similar trends in which the

values gradually decreased and became the lowest at the end of storage. The antioxidant activity of propolis can be attributed to its high phenolic content since the phenolic compounds contribute directly to the antioxidant activity by acting as reducing agents, hydrogen donors, and singlet oxygen quenchers (*47*). The propolis was reported to contain mainly flavonoid aglycones that prevent lipid oxidation by breaking the free radical reactions (*48*). Therefore, it was reasonable to relate the decrease in the TBARS values (Table 1) with the increased $DPPH^{\bullet}$ and $ABTS^{+}$ radical scavenging activity of meatballs containing propolis, especially at concentrations of 0.5 % and 1 %.

Table 2

Microbiological analysis results

In the present study, the TAMB, *Enterobacteriaceae*, TYM, and *Staphylococcus aureus* counts were determined in order to investigate the effects of different concentrations of propolis on the hygiene indicators in the meatballs. The results are given in Table 3. According to the results, the propolis was effective in decreasing the microbiological counts. Although there was an increase in bacterial counts towards the end of the storage period, it was determined that this increase was suppressed in propolis-added meatballs compared to the control sample. Considering the TAMB counts, the control sample was found to be significantly different from the propolis added samples, except for the 3rd and 9th days of storage ($p<0.05$). On the other hand, an increase in TAMB counts was observed depending on the storage period, but this increase was delayed with the propolis concentration. In particular, when the concentration of propolis added to the meatballs exceeds 0.3 %, significantly lower TAMB counts were determined throughout the storage period ($p<0.05$). In this regard, sample M1 showed significantly lower TAMB count compared to other samples at all storage times ($p<0.05$). Similarly, Kim *et al.* (*49*) reported that the addition of 1 % propolis extract to the meatball formulation clearly inhibits the TAMB growth. In contrast to TAMB counts, the addition of propolis did not affect the *Enterobacteriaceae* counts on day 1 of storage ($p>0.05$). Although there was a slight increase in counts after the 3rd day of storage, *Enterobacteriaceae* counts were determined to be significantly lower, especially in samples containing 0.3, 0.5, and 1% propolis compared to the control sample ($p<0.05$). In a study conducted by Gedikoğlu (*50*), commercial water extract of propolis was added to the raw beef meatballs, and the samples were stored at 4 °C for 7 days. According to the research, the addition of propolis decreased the *Enterobacteriaceae* counts by 2.24 log CFU/g (31.9 %) and TAMB counts by 2.42 log CFU/g (24.9 %). The addition of propolis at concentrations higher than 0.3 % caused a dramatic decrease in the TYM counts at day 1 of storage ($p<0.05$). Although almost similar values were found in days 3 and 6 of storage between the control

and propolis containing meatballs ($p>0.05$), the M0.5 and M1 samples showed significantly lower TYM counts at the end of the storage period, regardless of the total increase ($p<0.05$). Similarly, Ali *et al.* (*51*) stated that the addition of 0.6 % propolis to the sausages improved the shelf life by more than one week by reducing the proteolytic, lipolytic, and TYM counts. With regard to *S. aureus* counts, an insignificant decrease was observed at concentrations up to 0.5 % on the first day of storage ($p>0.05$), while became significant in the M0.5 and M1 samples ($p<0.05$). Moreover, regardless of the total increase, the M0.5 and M1 samples showed dramatically lower *S. aureus* counts compared to the control sample throughout the storage period ($p<0.05$). These results agree with the findings reported in the literature in which the effective concentration of propolis was stated to be in the range of 0.5-2 % (*49*,*51*,*52*). Therefore, based on the microbiological analysis results, it could be concluded that the concentration of propolis added to the meatballs should not be less than 0.5 % under these conditions.

Table 3

Sensory evaluation results

The sensory evaluation results of cooked meatball samples are given in Table 4. Within the scope of the study, the sensory panel was performed on samples stored for 6 days, and the 9th day was not included in the evaluation considering the chemical and microbiological results of the samples. According to results, considering the first parameter, it was determined that the amount of propolis additive and storage time did not create significant difference in the color liking scores of the cooked samples, which varied between 8.15-8.60 throughout the storage period. Similarly, for the odor, taste, and general acceptance parameters, propolis concentration did not create a significant difference in the scores compared to the control sample, however, the highest scores were obtained on the first day in all samples, and then the scores given to these parameters decreased as the shelf life extended in all samples ($p<0.05$). For instance, odor and taste scores varied between 8.13-8.55 and 8.10-8.55, respectively, on the first day, while on the 6th day of storage these scores decreased to 6.76-7.35 and 5.98-6.45, respectively. Given the sensory texture, although propolis concentration slightly increased the scores compared to the control sample, this did not create a significant difference. According to the general acceptance results, all samples received high scores ranging from 7.95 to 8.70 in the first day. Here, it can be stated that only the sample enriched with 1 % propolis affected the general acceptance of the panelists throughout the storage period. This situation can undoubtedly be due to the intense aroma of propolis.

Table 4

Considering the studies applying propolis extract in various meat and meat products, Payandan *et al.* (*53*) investigated the effectiveness of both ethanolic and water extracts of propolis added to minced carp meat at 3 %, 5 %, and 7 % on microbiological and sensory parameters during 9 days of storage at 4 °C and determined that there was no difference in color and texture values of all samples on the first day. Interestingly, samples enriched with the highest amount of propolis obtained with both solvents appeared to have higher initial odor and overall acceptability scores than control sample. On the other hand, there was a decrease in the sensory parameter scores of all samples depending on the storage period, and it was determined that the scores varied between 4.5-6.5 on a 10-point scale on the 6th day, which is consistent with the current results. In addition, all samples had the lowest scores on the 9th day, but this decrease was suppressed especially in the ethanolic propolis-added group compared to the control sample. In another study, Fadhil (*54*) examined the effects of the addition of aqueous propolis extract at different ratios on the shelf life and various quality parameters of chicken meat and reported that the propolis addition (5-15 %) positively affects the odor parameter. In addition, the general acceptability of the samples enriched with 5 % and 10 % aqueous propolis was found to be similar to the control sample, and the author concluded that the aqueous extract of propolis had a potential application as a natural preservative in chicken meat. In this context, in future studies, considering the capacity of ethanol to denaturate proteins by disrupting the non-covalent bonds in the tertiary structures (*55*), the use of ethanol at lower concentrations in the extract solution (<70 %) or the use of different solvents such as water in propolis extraction can be tried in order to delay the formation of off-odors that may arise from this phenomenon. There are also studies in the literature using different "forms" of propolis. For instance, Bernardi *et al.* (*56*) used both free and microencapsulated propolis in the Italian-type salami product, and determined that the propolis-added samples generally had the most acceptable appearance during the 90-day storage period. However, in the same study, it was noted that there were relative differences in aroma and general acceptability criteria in propolis-enriched salami samples compared to the control sample due to the persistent aroma and taste of propolis. In the study conducted by Dos Reis *et al.* (*13*) on the effect of microencapsulated propolis (0.3 g/kg) on the storage stability of burger meat during storage at -15 °C, it was determined that the color, appearance and texture properties of the propolis-added burger meat were at ideal levels, while the aroma and flavor properties were lower compared to the control sample. In the study, burger meat samples containing sodium erythorbate and propolis showed 72.52 % and 63.80 % acceptance rates, respectively. Based on these results, as a different study concept, encapsulated propolis obtained by various techniques

and wall materials such as gelatin, maltodextrin, starch, chitosan may help to alter the perception of its intense aroma on the organoleptic properties of the samples.

CONCLUSIONS

In the present study, different concentrations of propolis (0.1 %, 0.3 %, 0.5 %, and 1 %) were added to the meatballs in order to improve the oxidative and microbiological quality, and therefore prolong the storage period of the raw samples at 4 °C. According to the results, propolis addition did not significantly affect the pH values of meatball samples on the first day, while it suppressed the increase in pH values depending on the storage period. Addition of liquid propolis at all concentrations did not affect the a_w values of the samples. TBARS values increased due to storage time in all samples, but it was observed that the lipid oxidation phenomenon was delayed in the samples with the addition of propolis. The incorporation of propolis into the meatball formulation did not significantly change the *L**, and *a** values, but increased the *b** values of the samples on the first day. During the storage period, no significant change was observed in the *L** values of the samples, while a more significant decrease was observed in the *a** values compared to the *b** parameter of the samples. Moreover, the TPC, $DPPH^{\bullet}$ and $ABTS^{+}$ radical scavenging values increased significantly in parallel with the propolis concentration. It was reasonable to relate the decreased TBARS values with the increased antioxidant activity of propolis containing meatballs. The propolis was effective in delaying all microbiological criteria investigated, but if longer storage time is desired the concentration should not be less than 0.5 %. According to the sensory results, it should be taken into account that increasing concentration may affect the overall acceptability. In conclusion, certain amounts of propolis can be used as a natural antioxidant and antimicrobial ingredient in meatballs stored at 4 °C to improve the oxidative and microbiological properties of the product.

FUNDING

This study was supported by Kırklareli University Scientific Research Projects Coordination Office (Project number; KLUBAP 2020/211). The authors thank to Kırklareli University Scientific Research Projects Coordination Office for their supports. The sensorial analysis of the study was conducted in accordance with the Declaration of Helsinki and ethical approval was granted within the same project.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

A. S. Demirci and H. Uran participated in the conceptualization of this manuscript. H. Uran and A. C. Ozkir were involved in the acquisition of resources for this manuscript. H. Uran and A. C. Ozkir performed the analysis of the physicochemical and oxidation properties of the meatballs. R. Gunes and B. Kopuk analysed the bioactive compounds and participated in the statistical analysis of the results and preparation of the manuscript. A. S. Ozkir, A. S. Demirci, and B. Cetin carried out the microbiological analysis of meatballs and wrote and interpreted these results. H. Uran, R. Gunes, and B. Kopuk contributed to the data curation, drafting, critical revision, and final approval of this manuscript.

ORCID ID

A. Cevik Ozkir https://orcid.org/0000-0001-7020-1121
A. S. Demirci https://orcid.org/0000-0001-5252-8307
H. Uran https://orcid.org/0000-0002-3161-6698
R. Gunes https://orcid.org/0000-0002-8007-8697
B. Kopuk https://orcid.org/0000-0001-7321-9642
B. Cetin https://orcid.org/0000-0003-1321-7746

REFERENCES

1. Ponnampalam EN, Bekhit AED, Bruce H, Scollan ND, Muchenje V, Silva P, Jacobs JL. Production strategies and processing systems of meat: Current status and future outlooks for ınnovation–a global perspective. In: Galanakis CM, editor. Sustainable meat production and processing. Amsterdam: Elsevier Science Publishers; 2019. pp. 17–44.
https://doi.org/10.1016/C2017-0-02230-9

2. Rather SA, Masoodi FA, Akhter R, Rather JA, Shiekh KA. Advances in use of natural antioxidants as food additives for improving the oxidative stability of meat products. Madridge J Food Technol. 2016;1(1):10–7.
https://doi.org/10.18689/mjft-1000102

3. Shahidi F. Oxidative stability of shelf life of meat and meat products. In: Hu M, Jacobson C, editors. Oxidative stability and shelf life of foods containing oils and fats. Academic Press and AOCS Press; 2016. pp. 373–89.
https://doi.org/10.1016/C2015-0-00077-6

4. Dominguez R, Pateiro M, Gagaoua M, Barba FJ, Zhang W, Lorenzo JM. A comprehensive review on lipid oxidation in meat and meat products. Antioxidants. 2019;8(10):429.
https://doi.org/ 10.3390/antiox8100429

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

6. Dave D, Ghaly AE. Meat spoilage mechanisms and preservation techniques: A critical review. Am J Agric Biol Sci. 2011;6(4):486–510.
https://doi.org/ 10.3844/ajabssp.2011.486.510

7. Jayasena DD, Jo C. Essential oils as potential microbial agents in meat and meat Products: A review. Trends Food Sci Technol. 2013;34(2):96–108.
https://doi.org/10.1016/j.tifs.2013.09.002

8. Fernandez-Lopez J, Zhi N, Aleson-Carbonell L, Perez-Alvarez JA, Kuri V. Antioxidant and antibacterial activities of natural extracts: Application in beef meatballs. Meat Sci. 2005;69(3):371–80.
https://doi.org/10.1016/j.meatsci.2004.08.004

9. Karpinska-Tymoszczyk M. The effect of antioxidants, packaging type and frozen storage time on the quality of cooked turkey meatballs. Food Chem. 2014;148:276–83.
https://doi.org/10.1016/j.foodchem.2013.10.054

10. Aziz M, Karboune S. Natural antimicrobial/antioxidant agents in meat and poultry products as well as fruits and vegetables: A review. Crit Rev Food Sci Nutr. 2018;58(3):486–511.
https://doi.org/10.1080/10408398.2016.1194256

11. Villalobos-Delgado LH, Mateo J, Caro I, Ramos MYL, Mendez NG, Cansino RG, *et al.* Natural antioxidants in fresh and processed meat. In: Galanakis CM, editor. Sustainable meat production and processing. Amsterdam: Elsevier Science Publishers; 2019. pp. 207–36.
https://doi.org/10.1016/C2017-0-02230-9

12. Vargas-Sánchez RD, Torrescano-Urrutia GR, Acedo-Félix E, Carvajal-Millán E, González-Córdova AF, Vallejo-Galland B, *et al.* Antioxidant and antimicrobial activity of commercial propolis extract in beef patties. J Food Sci. 2014;79(8):1499–504.

https://doi.org/10.1111/1750-3841.12533

13. Dos Reis AS, Diedrich C, de Moura C, Pereira D, Almeida JF, da Silva LD, *et al.* Physico-chemical characteristics of microencapsulated propolis co-product extract and its effect on storage stability of burger meat during storage at -15 °C. LWT - Food Sci Technol. 2017;76(B):306–13.
https://doi.org/10.1016/j.lwt.2016.05.033

14. Meneses EA, Durango DL, Garcia CM. Antifungal activity against postharvest fungi by extracts from Colombian propolis. Quim Nova. 2009;32(8):2011–7.
https://doi.org/10.1590/S0100-40422009000800006

15. Yang W, Wu Z, Huang ZY, Miao X. Preservation of orange juice using propolis. J Food Sci. 2017;54(11):3375–83.
https://doi.org/ 10.1007/s13197-017-2754-x

16. Anjum SI, Ullah A, Khan KA, Attaullah M, Khan H, Ali H, *et al.* Composition and functional properties of propolis (bee glue): A review. Saudi J Biol Sci. 2018;26(7):1695–703.
https://doi.org/10.1016/j.sjbs.2018.08.013

17. Pobiega K, Krasniewska K, Gniewosz M. Application of propolis in antimicrobial and antioxidative protection of food quality-A review. Trends Food Sci Technol. 2019;83:53–62.
https://doi.org/10.1016/j.tifs.2018.11.007

18. Gregoris E, Stevanato R. Correlations between polyphenolic composition and antioxidant activity of Venetian propolis. Food Chem Toxicol. 2010;48(1):76–82.
https://doi.org/10.1016/j.fct.2009.09.018

19. Netikova L, Petr B, Petr H. Czech ethanol-free propolis extract displays inhibitory activity against a broad spectrum of bacterial and fungal pathogens. J Food Sci. 2013;78(9):1421–9.
https://doi.org/10.1111/1750-3841.12230

20. Ozturk I. Antifungal activity of propolis, thyme essential oil and hydrosol on natural mycobiota of sucuk, a Turkish fermented sausage: Monitoring of their effects on microbiological, colour and aroma properties. J Food Process Preserv. 2014;39:1148–58.
https://doi.org/10.1111/jfpp.12329

21. McLennan SV, Bonner J, Milne S, Lo L, Charlton A, Kurup S, *et al.* The anti-inflammatory agent propolis improves wound healing in a rodent model of experimental diabetes. Wound Repair Regen. 2008;16(5):706–13.
https://doi.org/10.1111/j.1524-475X.2008.00421.x

22. Ozkul Y, Silici S, Eroglu E. The anticarcinogenic effect of propolis in human lymphocytes culture. Phytomedicine. 2005;12(10):742–7.

https://doi.org/10.1016/j.phymed.2004.06.015

23. Atayoglu AT, Atik DS, Bölük E, Gürbüz B, Ceylan FD, Çapanoğlu E, *et al.* Evaluating bioactivity and bioaccessibility properties of the propolis extract prepared with l-lactic acid: An alternative solvent to ethanol for propolis extraction. Food Biosci. 2023;53:102756.
https://doi.org/10.1016/j.fbio.2023.102756

24. Bingol EB, Akkaya E, Hampikyan H, Cetin O, Colak H. Effect of nisin-EDTA combinations and modified atmosphere packaging on the survival of *Salmonella enteritidis* in Turkish type meatballs. CYTA - J Food. 2018;16(1):1030–6.
https://doi.org/10.1080/19476337.2018.1523810

25. Gokoglu N, Yerlikaya P, Uran H, Topuz OK. The effect of modified atmosphere packaging on the quality and shelf life of Frankfurter type-sausages. J Food Qual. 2010;33:367–80.
https://doi.org/10.1111/j.1745-4557.2010.00317.x

26. Zwolan A, Pietrzak D, Adamczak L, Chmiel M, Kalisz S, Wirkowska-Wojdyla M, *et al.* Effects of *Nigella sativa* L. seed extracts on lipid oxidation and colour of chicken meatballs during refrigerated storage. LWT - Food Sci Technol. 2020;130:109718.
https://doi.org/10.1016/j.lwt.2020.109718

27. Nugboon K, Intarapichet K. Antioxidant and antibacterial activities of Thai culinary herb and spice extracts, and application in pork meatballs. Int Food Res J. 2015;22(5):1788–800.

28. Huang B, He J, Ban X, Zeng H, Yao X, Wang Y. Antioxidant activity of bovine and porcine meat treated with extracts from edible lotus (*Nelumbo nucifera*) rhizome knot and leaf. Meat Sci. 2011;87(1):46–53.
https://doi.org/10.1016/j.meatsci.2010.09.001

29. ISO 21528-2:2012. Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count method. Geneva, Switzerland: International Organization for Standardization (ISO); 2012.

30. ISO 21527-2:2008. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of yeasts and moulds - Part 2: Colony-count technique. Geneva, Switzerland: International Organization for Standardization (ISO); 2008.

31. ISO 6888-1:2003. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium. Geneva, Switzerland: International Organization for Standardization (ISO); 2003.

32. ISO 4833-2: 2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 2: Colony count at 30 °C by the surface plating technique. Geneva, Switzerland: International Organization for Standardization (ISO); 2013.

33. Ramle NA, Zulkurnain M, Ismail-Fitry MR. Replacing animal fat with edible mushrooms: a strategy to produce high-quality and low-fat buffalo meatballs. Int Food Res J. 2021;28(5):905–15. https://doi.org/10.47836/ifrj.28.5.03

34. IBM SPSS Statistics for Windows, v. 22.0, IBM Corp. Armonk, NY, USA; 2013.

35. Mancini S, Preziuso G, Bosco AD, Roscini V, Szendrő Z, Fratini F, *et al*. Effect of turmeric powder (*Curcuma longa* L.) and ascorbic acid on physical characteristics and oxidative status of fresh and stored rabbit burgers. Meat Sci. 2015;110:93–100. https://doi.org/10.1016/j.meatsci.2015.07.005

36. Bazargani-Gilani B, Aliakbarlu J, Tajik H. Effect of pomegranate juice dipping and chitosan coating enriched with *Zataria multiflora* Boiss essential oil on the shelf-life of chicken meat during refrigerated storage. Innov Food Sci Emerg Technol. 2015;29:280–7. https://doi.org/10.1016/j.ifset.2015.04.007

37. Mehdizadeh T, Langroodi AM. Chitosan coatings incorporated with propolis extract and *Zataria multiflora* Boiss oil for active packaging of chicken breast meat. Int J Biol Macromol. 2019;141:401–9. https://doi.org/10.1016/j.ijbiomac.2019.08.267

38. Lücke FK. Fermented sausages. In Wood BJB, editor. Microbiology of fermented foods. New York, USA: Springer; 1998. pp. 441–83. https://doi.org/10.1007/978-1-4613-0309-1

39. Amaral AB, Da Silva MV, Lannes SCS. Lipid oxidation in meat: mechanisms and protective factors-A review. Food Sci Technol. 2018;38:1–15. https://doi.org/10.1590/fst.32518

40. Campo MM, Nute GR, Hughes SI, Enser M, Wood JD, Richardson RI. Flavour perception of oxidation in beef. Meat Sci. 2006;72(2):303–11. https://doi.org/10.1016/j.meatsci.2005.07.015

41. Wu W, Yu QQ, Fu Y, Tian XJ, Jia F, Li XM, *et al.* Towards muscle-specific meat colour stability of Chinese *Luxi* yellow cattle: A proteomic insight into post-mortem storage. J Proteom. 2016;147:108–18. https://doi.org/10.1016/j.jprot.2015.10.027

42. Güngören A, Patir B, Özpolat E. 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 Sci Technol. 2023;184:115084.
https://doi.org/10.1016/j.lwt.2023.115084

43. El Sheikha AF, Allam AY, ElObeid T, Basiouny EA, Abdelaal AA, Amarowicz R, *et al*. Impact of a carboxymethyl cellulose coating incorporated with an ethanolic propolis extract on the quality criteria of chicken breast meat. Antioxidants. 2022;11(6):1191.
https://doi.org/10.3390/antiox11061191

44. Karre L, Lopez K, Getty KJK. Natural antioxidants in meat and poultry products. Meat Sci. 2013;94(2):220–7.
https://doi.org/10.1016/j.meatsci.2013.01.007

45. Kumar Y, Yadav DN, Ahmad T, Narsaiah K. Recent trends in the use of natural antioxidants for meat and meat products. Compr Rev Food Sci Food Saf. 2015;14(6):796–812.
https://doi.org/10.1111/1541-4337.12156

46. Spinelli S, Conte A, Lecce L, Incoronato AL, Del Nobile MA. Microencapsulated propolis to enhance the antioxidant properties of fresh fish burgers. J Food Process Eng. 2015;38:527–35.
https://doi.org/10.1111/jfpe.12183

47. Negri G, Teixeira EW, Alves MLTMF, Moreti ACCCM, Otsuk IP, Borguini RG, *et al.* Hydroxycinnamic acid amide derivatives, phenolic compounds and antioxidant activities of extracts of pollen samples from southeast Brazil. Journal of Agric Food Chem. 2011;59(10):5516–22.
https://doi.org/ 10.1021/jf200602k

48. Socha R, Gałkowska D, Bugaj M, Juszczak L. Phenolic composition and antioxidant activity of propolis from various regions of Poland. Nat Prod Res. 2014;29(5):416–22.
https://doi.org/10.1080/14786419.2014.949705

49. Kim SK, Han S, Kim S, Bang KW, Choi HM, Moon HJ, *et al*. Improvement of storage stability of meatballs using propolis. J Apic. 2019;34(4):325–8.
https://doi.org/10.17519/apiculture.2019.11.34.4.325

50. Gedikoğlu A. Antimicrobial and antioxidant activities of commercialized Turkish propolis extract and application to beef meatballs. Turk J Food Agric Sci. 2022;10(10):2021–9.
https://doi.org/10.24925/turjaf.v10i10.2021-2029.5340

51. Ali FH, Kassem GM, Atta-Alla OA. Propolis as a natural decontaminant and antioxidant in fresh oriental sausage. Vet Ital. 2010;46(7):167–72.

52. Viera VB, Piovesan N, Moro KIB, Rodrigues AS, Scapin G, Rosa CS, *et al.* Preparation and microbiological analysis of Tuscan sausage with added propolis extract. Food Sci Technol Campinas. 2016;36(Sppl. 1):37–41.
https://doi.org/10.1590/1678-457X.0045

53. Payandan E, Sayyed-Alangi SZ, Shamloofar M, Koohsari H. Study of chemical composition and efficacy of different extracts of Iranian propolis on the microbiological and sensory parameters of minced *Cyprinus carpio* meat at 4 °C storage. J Aquat Food Prod. 2017;26(5):593–603.
https://doi.org/10.1080/10498850.2016.1240281

54. Fadhil YS. Effect of Iraqi propolis on shelf life of poultry meat. Pakistan J Agri Res. 2023;36(2):130-4.
https://doi.org/10.17582/journal.pjar/2023/36.2.130.134

55. Kalathenos P, Russell NJ. Ethanol as a food preservative. In: Russell NJ, Gould G, editors. Food Preservatives. New York, USA: Springer; 2003. pp. 196–217.
https://doi.org/10.1007/978-0-387-30042-9_10

56. Bernardi S, Favaro-Trindade CS, Trindade MA, Balieiro JCC, Cavenaghi AD, Contreras-Castillo CJ. Italian-type salami with propolis as antioxidant. Ital J Food Sci. 2013;25:433–40.
http://www.chiriottieditori.it/pdf/ijfs/IJFS%20254%20ok.pdf

Table 1. Physicochemical analysis results of raw meatballs stored at 4 °C

Samples	pH	Water activity (a_w)	TBARS (mg MDA/kg)	Color		
				*L**	*a**	*b**
Day 1						
Control	(5.91±0.07)^{aEFGHI}	(0.95±0.00)^{aB}	(2.50±0.07)^{aCD}	(41.52±1.73)^{aA}	(15.21±2.51)^{aABC}	(12.23±0.38)^{bDE}
M0.1	(5.91±0.04)^{aEFGHI}	(0.95±0.00)^{aB}	(2.60±0.11)^{aC}	(43.28±2.64)^{aA}	(15.55±1.95)^{aAB}	(12.92±0.37)^{abBCDE}
M0.3	(5.92±0.04)^{aDEFGHI}	(0.95±0.01)^{aB}	(2.16±0.11)^{aDEF}	(42.94±1.73)^{aA}	(17.88±1.25)^{aA}	(13.16±0.20)^{abABCDE}
M0.5	(5.90±0.05)^{aFGHI}	(0.95±0.00)^{aB}	(1.40±0.03)^{bH}	(44.37±2.81)^{aA}	(15.64±1.90)^{aAB}	(14.02±1.61)^{abAB}
M1	(5.84±0.04)^{aI}	(0.95±0.00)^{aB}	(1.54±0.16)^{bGH}	(43.56±2.22)^{aA}	(15.32±2.48)^{aAB}	(14.47±1.31)^{aA}
Day 3						
Control	(5.98±0.00)^{aDEF}	(0.96±0.00)^{aA}	(3.22±0.08)^{aB}	(41.01±2.03)^{aAB}	(12.87±1.25)^{aBCDE}	(12.24±0.24)^{aDE}
M0.1	(5.94±0.09)^{aDEFGH}	(0.96±0.00)^{aA}	(3.15±0.02)^{aB}	(42.55±0.83)^{aAB}	(12.83±1.46)^{aBCDE}	(12.64±0.45)^{aBCDE}
M0.3	(5.94±0.03)^{aDEFGH}	(0.96±0.00)^{aA}	(2.71±0.23)^{aC}	(44.12±3.02)^{aAB}	(12.41±1.55)^{aBCDE}	(12.96±1.25)^{aABCDE}
M0.5	(5.91±0.07)^{aEFGHI}	(0.95±0.00)^{aB}	(1.87±0.06)^{bEFG}	(43.91±3.93)^{aAB}	(12.24±1.16)^{aBCDE}	(12.93±1.26)^{aBCDE}
M1	(5.87±0.04)^{aHI}	(0.95±0.00)^{aB}	(1.83±0.20)^{bFG}	(43.64±0.67)^{aAB}	(13.02±0.14)^{aBCDE}	(13.68±1.73)^{aABCD}
Day 6						
Control	(5.99±0.03)^{aCDE}	(0.96±0.00)^{aA}	(3.40±0.38)^{aB}	(41.95±3.95)^{aAB}	(11.09±0.62)^{aDE}	(12.08±0.16)^{cE}
M0.1	(5.96±0.07)^{abDEFG}	(0.95±0.00)^{aB}	(3.25±0.12)^{aB}	(43.70±1.68)^{aAB}	(11.48±0.73)^{aDE}	(12.87±0.35)^{bBCDE}
M0.3	(5.95±0.06)^{abDEFGH}	(0.96±0.00)^{aA}	(2.70±0.22)^{abC}	(44.54±2.32)^{aAB}	(14.27±4.77)^{aBCD}	(13.09±0.16)^{bABCDE}
M0.5	(5.95±0.01)^{abDEFGH}	(0.95±0.00)^{aB}	(2.28±0.22)^{bCDE}	(45.66±1.53)^{aA}	(11.80±1.21)^{aCDE}	(13.04±0.25)^{bABCDE}
M1	(5.89±0.02)^{bGHI}	(0.95±0.00)^{aB}	(2.12±0.12)^{bDEF}	(45.28±1.35)^{aAB}	(11.17±1.68)^{aDE}	(13.94±0.76)^{aABC}
Day 9						
Control	(6.17±0.02)^{aA}	(0.95±0.00)^{aB}	(3.88±0.28)^{aA}	(40.75±2.85)^{aB}	(13.21±0.62)^{aBCDE}	(11.95±0.21)^{cE}
M0.1	(6.15±0.02)^{abA}	(0.95±0.00)^{aB}	(3.45±0.24)^{abB}	(43.71±2.50)^{aAB}	(12.44±0.33)^{abBCDE}	(12.16±0.31)^{bcDE}
M0.3	(6.13±0.01)^{bAB}	(0.96±0.01)^{aA}	(3.05±0.10)^{bcB}	(45.13±2.42)^{aAB}	(14.19±2.01)^{aBCD}	(12.45±0.45)^{abcCDE}
M0.5	(6.06±0.00)^{cBC}	(0.95±0.00)^{aB}	(2.60±0.14)^{cC}	(43.42±1.71)^{aAB}	(11.28±0.58)^{bDE}	(12.71±0.09)^{abBCDE}
M1	(6.00±0.01)^{dCD}	(0.95±0.00)^{aB}	(2.48±0.18)^{cCD}	(43.03±1.42)^{aAB}	(10.68±0.18)^{bE}	(13.02±0.49)^{aABCDE}

Data represent means ± standard deviation of three independent sample results

^{a,b} Different superscript lowercase letters within the same column indicate the significant differences between the results of the samples on the same storage day for the same parameter ($p<0.05$)

^{A,B} Different superscript capital letters within the same column indicate the significant differences between the results of the samples during the entire storage period for the same parameter ($p<0.05$)

M: Meatball sample

0.1, 0.3, 0.5, 1: The additive percentage (%) of ethanolic extract of propolis

TBARS: 2-Thiobarbituric acid reactive substance content

Table 2. Total phenolic content and antioxidant activity results of raw meatball samples stored at 4 °C

Samples	Total phenolic content (mg GAE/kg)	DPPH (μM Trolox)	ABTS (μM Trolox)
Day 1			
Control	(630.44±15.17)^{cDE}	(116.33±7.11)^{dHI}	(500.08±17.12)^{eF}
M0.1	(649.62±9.35)^{cD}	(157.22±9.27)^{cG}	(573.29±12.47)^{dD}
M0.3	(680.41±11.02)^{bcC}	(314.77±9.34)^{bD}	(659.59±20.71)^{cC}
M0.5	(691.50±10.83)^{bC}	(506.51±12.28)^{aB}	(728.65±19.14)^{bB}
M1	(789.30±15.18)^{aA}	(532.46±13.08)^{aA}	(850.25±16.68)^{aA}
Day 3			
Control	(614.90±18.30)^{cE}	(114.88±11.17)^{dHI}	(395.19±7.73)^{dI}
M0.1	(627.50±10.60)^{cDE}	(134.18±9.60)^{dHI}	(379.85±11.22)^{dIJ}
M0.3	(636.70±6.11)^{cDE}	(249.75±9.46)^{cE}	(453.13±16.53)^{cGH}
M0.5	(681.90±11.44)^{bC}	(405.41±20.22)^{bC}	(513.76±18.78)^{bEF}
M1	(756.90±12.26)^{aB}	(497.90±15.47)^{aB}	(578.63±13.03)^{aD}
Day 6			
Control	(395.90±5.82)^{dJ}	(91.09±14.05)^{cJ}	(326.97±5.90)^{eK}
M0.1	(455.43±16.44)^{cdHI}	(126.38±14.47)^{bcHI}	(367.44±8.14)^{dJ}
M0.3	(468.74±14.35)^{cdGH}	(131.53±12.05)^{bcC}	(433.99±13.85)^{cH}
M0.5	(537.85±19.75)^{bF}	(159.04±7.23)^{bG}	(459.71±11.81)^{bG}
M1	(683.44±25.99)^{aC}	(236.21±10.15)^{aE}	(529.44±18.26)^{aE}
Day 9			
Control	(387.8±11.43)^{dJ}	(73.92±14.30)^{cJ}	(240.33±5.55)^{eM}
M0.1	(408.41±17.61)^{cdJ}	(111.39±7.15)^{bcI}	(281.42±10.86)^{dL}
M0.3	(435.14±19.75)^{cI}	(115.85±11.17)^{bcHI}	(398.61±12.33)^{cI}
M0.5	(492.28±12.26)^{bG}	(126.38±7.12)^{bHI}	(443.70±14.57)^{bGH}
M1	(639.99±12.46)^{aDE}	(197.19±14.48)^{aF}	(495.13±10.47)^{aF}

Data represent means ± standard deviation of three independent sample results
^{a,b} Different superscript lowercase letters within the same column indicate the significant differences between the results of the samples on the same storage day for the same parameter ($p<0.05$)
^{A,B} Different superscript capital letters within the same column indicate the significant differences between the results of the samples during the entire storage period for the same parameter ($p<0.05$)
M: Meatball sample
0.1, 0.3, 0.5, 1: The additive percentage (%) of ethanolic extract of propolis

Table 3. Microbiological analysis results of raw meatball samples stored at 4 °C

Samples	TAMBc (log CFU/g)	*Enterobacteriaceae* (log CFU/g)	TYMc (log CFU/g)	*S. aureus* (log CFU/g)
Day 1				
Control	(5.82±0.07)^{aJ}	(4.33±0.09)^{aHI}	(3.52±0.20)^{aG}	(3.80±0.26)^{aIJ}
M0.1	(5.64±0.01)^{bK}	(4.19±0.11)^{aIJ}	(3.21±0.30)^{abHI}	(3.61±0.29)^{abJK}
M0.3	(5.47±0.06)^{cL}	(4.16±0.12)^{aJ}	(2.98±0.10)^{bcIJ}	(3.46±0.30)^{abK}
M0.5	(5.39±0.05)^{cL}	(4.18±0.13)^{aIJ}	(2.92±0.07)^{bcJ}	(3.17±0.25)^{bcL}
M1	(5.13±0.02)^{dM}	(4.16±0.03)^{aJ}	(2.85±0.15)^{cJ}	(2.79±0.09)^{cM}
Day 3				
Control	(6.21±0.07)^{aI}	(4.49±0.01)^{aGH}	(3.89±0.11)^{aF}	(4.44±0.22)^{aG}
M0.1	(6.19±0.11)^{aI}	(4.45±0.02)^{aGH}	(3.86±0.02)^{aF}	(4.19±0.07)^{bGH}
M0.3	(5.88±0.08)^{bJ}	(4.34±0.00)^{bHI}	(3.81±0.04)^{aF}	(4.13±0.05)^{bH}
M0.5	(5.54±0.09)^{cKL}	(4.20±0.01)^{cIJ}	(3.44±0.23)^{bGH}	(4.07±0.07)^{bHI}
M1	(5.22±0.10)^{dM}	(3.82±0.08)^{dK}	(3.26±0.20)^{bGH}	(3.85±0.08)^{cIJ}

Day 6				
Control	(7.24±0.10)^{aC}	(5.30±0.14)^{aD}	(5.38±0.17)^{aE}	(5.50±0.07)^{aDE}
M0.1	(6.95±0.10)^{bDE}	(5.01±0.03)^{bE}	(5.48±0.02)^{aE}	(5.26±0.20)^{bE}
M0.3	(6.65±0.03)^{cGH}	(4.87±0.06)^{bEF}	(5.45±0.03)^{aE}	(4.97±0.03)^{cF}
M0.5	(6.53±0.10)^{cH}	(4.56±0.09)^{cG}	(5.24±0.16)^{aE}	(4.72±0.09)^{dF}
M1	(6.14±0.06)^{dI}	(4.22±0.10)^{dIJ}	(5.30±0.27)^{aE}	(4.41±0.07)^{eG}
Day 9				
Control	(7.81±0.05)^{aA}	(6.66±0.08)^{aA}	(6.80±0.14)^{aA}	(6.18±0.21)^{aA}
M0.1	(7.62±0.18)^{aB}	(6.50±0.15)^{aB}	(6.67±0.17)^{abAB}	(5.97±0.11)^{abAB}
M0.3	(7.09±0.17)^{bCD}	(5.68±0.07)^{bC}	(6.46±0.12)^{bcBC}	(5.82±0.08)^{bBC}
M0.5	(6.86±0.05)^{bcEF}	(5.22±0.08)^{cD}	(6.27±0.07)^{cC}	(5.57±0.08)^{cCD}
M1	(6.77±0.12)^{cFG}	(4.79±0.14)^{dF}	(5.95±0.07)^{dD}	(5.35±0.05)^{dDE}

Data represent means ± standard deviation of two microbiological count results
^{a,b} Different superscript lowercase letters within the same column indicate the significant differences between the results of the samples on the same storage day for the same parameter ($p<0.05$)
^{A,B} Different superscript capital letters within the same column indicate the significant differences between the results of the samples during the entire storage period for the same parameter ($p<0.05$)
M: Meatball sample
0.1, 0.3, 0.5, 1: The additive percentage (%) of ethanolic extract of propolis
TAMBc: Total aerobic mesophilic bacterial count
TYMc: Total yeast and mold count

Table 4. Sensory evaluation results of cooked meatball samples

Samples	Color liking	Odor	Taste	Texture	General acceptance
Day 1					
Control	(8.45±0.19)^{aA}	(8.55±0.20)^{aA}	(8.50±0.27)^{aA}	(8.55±0.45)^{aA}	(8.40±0.34)^{aAB}
M0.1	(8.50±0.38)^{aA}	(8.45±0.26)^{aAB}	(8.40±0.50)^{aA}	(8.50±0.40)^{aA}	(8.80±0.15)^{aA}
M0.3	(8.50±0.41)^{aA}	(8.39±0.47)^{aABC}	(8.55±0.31)^{aA}	(8.50±0.35)^{aA}	(8.70±0.18)^{aA}
M0.5	(8.60±0.32)^{aA}	(8.20±0.25)^{aABC}	(8.48±0.43)^{aA}	(9.00±0.00)^{aA}	(8.50±0.13)^{aAB}
M1	(8.60±0.25)^{aA}	(8.13±0.60)^{aABC}	(8.10±0.35)^{aAB}	(9.00±0.00)^{aA}	(7.95±0.32)^{abBC}
Day 3					
Control	(8.40±0.45)^{aA}	(7.80±0.10)^{aABCD}	(7.62±0.44)^{aBC}	(8.50±0.24)^{aA}	(7.70±0.42)^{bcCD}
M0.1	(8.35±0.25)^{aA}	(7.75±0.35)^{aBCD}	(7.71±0.46)^{aBC}	(8.55±0.35)^{aA}	(7.55±0.63)^{bcCD}
M0.3	(8.48±0.21)^{aA}	(7.85±0.44)^{aABCD}	(7.55±0.23)^{aBC}	(8.50±0.30)^{aA}	(7.30±0.52)^{abCDE}
M0.5	(8.50±0.43)^{aA}	(7.78±0.36)^{aABCD}	(7.43±0.56)^{aBC}	(8.75±0.22)^{aA}	(7.25±0.72)^{aDE}
M1	(8.55±0.35)^{aA}	(7.63±0.21)^{aCDE}	(7.25±0.42)^{aC}	(9.00±0.00)^{aA}	(6.75±0.35)^{cEF}
Day 6					
Control	(8.15±0.58)^{aA}	(6.76±0.52)^{aF}	(6.34±0.25)^{aD}	(8.50±0.40)^{aA}	(6.47±0.15)^{aFG}
M0.1	(8.25±0.33)^{aA}	(6.88±0.41)^{abEF}	(6.38±0.30)^{aD}	(8.50±0.49)^{aA}	(6.35±0.11)^{abFG}
M0.3	(8.40±0.36)^{aA}	(7.16±0.59)^{aDEF}	(6.45±0.48)^{aD}	(8.45±0.30)^{aA}	(6.20±0.27)^{abFG}
M0.5	(8.45±0.28)^{aA}	(7.35±0.33)^{aDEF}	(6.20±0.23)^{aD}	(8.50±0.42)^{aA}	(6.28±0.35)^{abFG}
M1	(8.48±0.19)^{aA}	(6.95±0.59)^{aEF}	(5.98±0.42)^{aD}	(8.65±0.15)^{aA}	(5.90±0.20)^{bG}

Data represent means ± standard deviation of the scores
^{a,b} Different superscript lowercase letters within the same column indicate the significant differences between the results of the samples on the same storage day for the same parameter ($p<0.05$)
^{A,B} Different superscript capital letters within the same column indicate the significant differences between the results of the samples during the entire storage period for the same parameter ($p<0.05$)
M: Meatball sample
0.1, 0.3, 0.5, 1: The additive percentage (%) of ethanolic extract of propolis