Journal of Tekirdag Agricultural Faculty
Tekirdağ Ziraat Fakültesi Dergisi

Mayıs/May 2024, 23(1)
Başvuru/Received: 31/05/23
Kabul/Accepted: 17/03/24
DOI: 10.33462/jotaf.1307835

http://dergipark.gov.tr/jotaf
http://jotaf.nku.edu.tr/

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Which Method Is Better for Preserving Meat and Cheese: Coating Propolis on The Packaging Material or Spraying Directly onto The Food?*

Et ve Peyniri Saklamak İçin Hangi Yöntem Daha İyi: Ambalaj Malzemesine Propolis Kaplamak mı, yoksa Doğrudan Gıdanın üzerine Püskürtmek mi?

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Abstract

This study was carried out to determine whether it is better to spray a preservative coating of propolis on the food packaging material or directly onto meat and cheddar cheese. To test this, the surfaces of a vacuum bag (Polyamide/Polyethylene-PA/PE), some stretch film (Low Density Polyethylene- LDPE) and a ziploc bag (Oriented Polypropylene-OPP) and food samples were coated by spraying them with a propolis-ethyl acetate (PEA) solution. Moreover, a propolis-propylene glycol (PPG) solution was sprayed directly on the food surfaces (cheese and meat) to study the shelf life of these products without packaging. Meat and cheddar cheese placed in packages and covered with a PPG solution were stored at 4°C for 30 and 45 days, respectively. The predominant phenolic compound in the propolis used in the study was caffeic acid phenethyl ester (CAPE) at a level of 27.523,4 µg g^{-1}, and the lowest amount of phenolic acid was epigallocatechin gallate at 287.53 µg g^{-1}. At the end of the storage period, the *Enterobacteriaceae* count of the meat sample stored in propolis sprayed vacuum packaging decreased by 1.01 log CFU g^{-1} ($p<0.05$) compared to the control, and achieved the best result. It was concluded that the PPG solution applied onto the meat did not adhere well to it and the solution could not achieve its antimicrobial effect. At the end of the storage period, while the cheddar cheese sample kept in a propolis-treated vacuum bag had the lowest TMAB count for 21 days ($p<0.05$), PPG directly sprayed on cheddar cheese had the lowest TMAB count at the end of storage with 6.64 log CFU g^{-1} ($p<0.05$). The PPG solution was able to adhere to the surface of the cheddar cheese and propolis was able to show its antimicrobial activity. In addition, the LAB (MRS) value for the cheddar cheese sample stored in propolis-treated vacuum packaging decreased by 0.60 log CFU g^{-1} ($p<0.05$) compared to the control and achieved the best result. Microbiological analysis showed that propolis coating in vacuum packaging improved the microbiological quality of the meat and the cheddar cheese.

Keywords: Propolis, Packaging, Antimicrobial, Shelf life, Spraying

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Atıf: Karpuz, E., Palabıyık, İ. (2024). Et ve peyniri saklamak için hangi yöntem daha iyi: Ambalaj malzemesine propolis kaplamak mı, yoksa doğrudan gıdanın üzerine püskürtmek mi? *Tekirdağ Ziraat Fakültesi Dergisi,* 21(3): 602-618.
Citation: Karpuz, E., Palabıyık, İ. (2024). Which method ıs better for preserving meat and cheese: Coating propolis on the packaging material or spraying directly onto the food? *Journal of Tekirdag Agricultural Faculty,* 21(3): 602-618.

*Bu çalışma Ezgi Karpuz'un Yüksek Lisans tezinden özetlenmiştir.

Öz

Bu çalışma, propolisin koruyucu bir kaplamasının gıda ambalaj malzemesi üzerine mi yoksa doğrudan et ve kaşar peyniri üzerine mi püskürtülmesinin daha iyi olduğunu belirlemek amacıyla gerçekleştirilmiştir. Bunu test etmek için bir vakum torbası (Poliamid/Polietilen-PA/PE), bir miktar streç film (Düşük Yoğunluklu Polietilen-LDPE) ve bir ziploc torbası (Yönlendirilmiş Polipropilen-OPP) ve gıda örneklerinin yüzeyleri propolis-etil asetat (PEA) çözeltisi püskürtülerek kaplanmıştır. Ayrıca, bu ürünlerin ambalajsız raf ömrünü incelemek için gıda yüzeylerine (peynir ve et) doğrudan propolis-propilen glikol (PPG) çözeltisi püskürtülmüştür. Paketlere yerleştirilen ve PPG çözeltisi ile kaplanan et ve kaşar peyniri sırasıyla 30 ve 45 gün boyunca 4°C'de saklanmıştır. Çalışmada kullanılan propoliste baskın fenolik bileşik 27.523,4 µg g^{-1} ile kafeik asit fenetil ester (CAPE), en düşük fenolik asit miktarı ise 287,53 µg g^{-1} ile epigallokateşin gallat olmuştur. Depolama süresinin sonunda, propolis püskürtmeli vakum ambalajda depolanan et numunesinin *Enterobacteriaceae* sayısı kontrole kıyasla 1,01 log CFU g^{-1} ($p<0,05$) azalmış ve en iyi sonucu elde etmiştir. Et üzerine uygulanan PPG solüsyonunun ete iyi yapışmadığı ve solüsyonun antimikrobiyal etkisini gösteremediği sonucuna varılmıştır. Depolama süresinin sonunda, propolisle muamele edilmiş vakum torbasında tutulan kaşar peyniri örneği 21 gün boyunca en düşük TMAB sayısına sahipken ($p<0.05$), kaşar peynirine doğrudan püskürtülen PPG 6.64 log CFU g^{-1} ile depolama sonunda en düşük TMAB sayısına sahip olmuştur ($p<0.05$). PPG çözeltisi kaşar peynirinin yüzeyine yapışabilmiş ve propolis antimikrobiyal aktivitesini gösterebilmiştir. Ayrıca, propolisle muamele edilmiş vakumlu ambalajda saklanan kaşar peyniri numunesi için LAB (MRS) değeri kontrole kıyasla 0,60 log CFU g^{-1} ($p<0,05$) azalmış ve en iyi sonucu elde etmiştir. Mikrobiyolojik analizler, vakum ambalajda propolis kaplamanın etin ve kaşar peynirinin mikrobiyolojik kalitesini iyileştirdiğini göstermiştir.

Anahtar Kelimeler: Propolis, Ambalaj, Antimikrobiyal, Raf Ömrü, Püskürtme

1.Introduction

Propolis is a natural medicine that has been widely used since ancient times (Castaldo and Capasso, 2002). Propolis is a bee product that is collected from the leaves and buds of poplar, birch, pine, oak, eucalyptus and chestnut trees, and is mixed with wax and used for many purposes in the hive. Among more than 300 components identified in the chemical composition of propolis, phenolic compounds are important since they are responsible for the pharmacological and biological activities of propolis (Escriche and Juan-Borrás, 2018; Galeotti et al., 2018). The anti-inflammatory (Memmedov et al., 2017), antioxidant (Zabaiou et al., 2017), antibacterial (Hames-Kocabas et al., 2013), antiviral and antifungal (Güney and Yılmaz, 2013) properties of propolis have been proven in many studies.

Propolis has an antimicrobial effect on some Gram positive and Gram negative bacteria such as *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, *B. subtilis*, *S. epidermidis* and *Streptococcus* (Aliyazcoglu et al. 2013; Fadaly and El-Badrawy, 2001; Obregón Fuentes and Rojas Hernández, 1990). In the studies which investigated propolis as a natural antimicrobial and antioxidant agent for food preservation, it was determined that samples coated with propolis reduced lipid oxidation and successfully inhibited microbial growth (Coban and Coban, 2020; Correa et al., 2019; Güler et al., 2022; Jonaidi Jafari et al., 2018; Safaei and Roosta Azad, 2020; Yazgan et al., 2020). Properties of packaging materials has become more significant today since it plays a crucial role in delaying the deterioration of food products, extending the shelf life, and protecting the quality and safety of foods (López-Rubio et al., 2004).

Packaging is an effective way to restrict the growth of microorganisms and extend shelf life, thereby helping to maintain the quality of the food (Dizaj et al., 2014; Yu et al., 2017). Jonaidi Jafari et al. (2018) removed food fillets after 30 seconds of soaking them in a coating solution containing 0.1% and 2% propolis extract with chitosan to form the coating, and then re-immersed them for 30 seconds after 2 minutes. Correa et al. (2019) immersed cheese slices in 5% and 10% ethanol extract of green propolis solutions for 5 seconds**.** In the study by Coban and Coban (2020), food fillets were dipped in coating solutions (pure chia mucilage (CM), CM with added 0.1% propolis liquid extract, and CM with added 0.3% propolis liquid extract) at 4°C three times at two-minute intervals. After immersion, the fillets were dried in a sterile incubator under an airflow (4°C and 50% RH) for 60 minutes. Safaei and Roosta Azad (2020) developed active polylactic acid films containing 10%, 20% and 40% w w^{-1} propolis extract as active agents in order to extend the shelf life of meat products. The methods of dipping food in propolis solution and the use of propolis as an additive are of limited industrial use on a large scale. However, the spraying method is practical and suitable for industrial-scale use. The spray technique offers potential cost reduction, single-end coating, thickness control and the possibility of very easy application (Andrade et al., 2012; Olivas and Barbosa-Cánovas, 2009; Ustunol, 2009). No study has been found that examines systematically the use of propolis by spraying on the packaging or on foods directly to increase their shelf life at the same time. Meat and cheddar cheese is an excellent source of animal protein with high biological value, containing all essential amino acids and unsaturated fatty acids for human nutrition. These foods are perishable due to their biological composition (Labadie, 1999; Zheng et al., 2021). In this study, the comparison of spraying methods of propolis indirectly on the packaging or directly on meat and cheddar cheese has been investigated. Thus, the more effective of these methods will be determined for the first time in the literature for the perishable foods.

2. Materials and Methods

2.1. Materials

Raw propolis was obtained from beekeepers in Kırklareli province where the oak (*Quercus* spp.) tree is the dominant tree. Materials obtained from the international companies included ethyl alcohol (Merck, Germany), ethyl acetate (Tekkim, Türkiye), propylene glycol (Alfasol, Germany),Whatman A4 filter paper (Merck, Germany), M17 agar acc. to TERZAGHI (Merck, Germany), Man Rogosa and Sharpe (MRS) (Merck, Germany), Violet Red Bile Glucose (VRBG) (Merck, Germany), Plate Count skimmed milk (PCSM) (Merck, Germany), Nutrient Broth (Merck, Germany), Mueller-Hinton Agar (MHA) (Merck, Germany), Nessler reagent (Merck, Germany) and a stomacher bag (PE, 180 × 310 mm, Lp Italiana Spa). Beef meat and cheddar cheese were purchased directly from a local market (Tekirdağ, Türkiye). 80 µm thick vacuum bags (Polyamide/Polyethylene-PA/PE) (Packtech, Türkiye), stretch film (Low Density Polyethylene-LDPE) (Packtech, Türkiye), ziploc bags

(Oriented Polypropylene-OPP) (Packtech, Türkiye) were purchased from the wider market. A spray painting system (TC-SY 600 S, Germany) was used for the spraying application.

2.2. The preparation of propolis solutions

Thirty grams of powdered propolis were mixed with 100 ml of 70% ethanol. Subsequently, the mixture was placed in a shaker incubator (Infors HT Ecotron, Switzerland) at 60°C and 150 rpm for 24 hours. After this, the mixture was filtered through Whatman A4 filter paper, and the ethyl alcohol was evaporated completely in a rotary evaporator (R-100, Buchi, Switzerland). The resulting solvent-free propolis resin was stored in a refrigerator (4±0.5°C) until the experiments were conducted (Bölük et al., 2021).

Proposed weights of solvent-free propolis resin were mixed with liquid ethyl acetate or propylene glycol, and the mixtures were shaken in a shaker incubator (Infors HT Ecotron, Switzerland) at 150 rpm for 5 to 6 hours at room temperature to dissolve propolis resin completely in these solvents. Propolis-ethyl acetate (PEA) solutions were obtained at ratios of 5%, 10%, 20% and 30% (propolis/ethyl acetate, w v^{-1}) to cover the surface of the packages. On the other hand, propolis-propylene glycol (PPG) solutions in ratios of 5%, 10%, 20% and 30% (propolis/propylene glycol, w v^{-1}) were prepared and used as a solution to directly coat the propolis onto meat and cheddar cheese samples.

2.3. Coating packaging and food with propolis solutions

Spray coating was carried out using a compressed air-assisted sprayer (Ronseal Power Sprayer, Sheffield, UK) (Leceta et al., 2015; Meng et al., 2010). Propolis-coated stretch film, ziploc bags, vacuum bags and the PPG solution sprayed onto meat and cheese samples are shown in *Figure 1*. Vacuum packaging, stretch film and ziploc bags are easily accessible and cheap to produce. In addition, these packages are frequently used in the food industry. Considering all these points, vacuum packaging, stretch film and ziploc bags were preferred in this study in order to protect food in a way that is compatible with industry.

A 10% PEA solution was sprayed homogeneously onto the stretch film (30 cm × 50 cm) – opened up to 1 meter in length – for 7 seconds with the help of a compressor. Then, by keeping the stretch film at room temperature for 6 hours, the ethyl acetate was completely evaporated and only propolis remained as a thin yellow layer.

For vacuum and ziploc bags, 5 ml of a 10% PEA solution was taken and poured into the vacuum bags and ziploc bags using a glass pipette and was manually spread all over the packaging. Then, the ethyl acetate was again evaporated off for 6 hours at room temperature. After the packages were covered with propolis solution and dried, the surfaces of the packages became yellow. Propolis is a sticky substance that is less soluble in water and is attached to the packaging (Keskin, 2018).

In addition, a 5% PGG solution was sprayed for 7 seconds with the help of a compressor to cover the entire surface of the meat and cheddar cheese samples. As a result of preliminary experiments, the amount sprayed was chosen by considering the maximum amount of propolis that does not impair the sensory properties of the food, and 0.4 g of propolis was sprayed on each square meter of food surface.

During the preparation of solutions with propolis, propolis was extracted at 50°C and it was not exposed to any higher temperature during the study. Since the coating processes of packaging materials of propolis solutions of different concentrations are created at room temperature, the phenolic compounds in propolis are protected (Yurteri, 2015). For this reason, the method used to treat packages with propolis is also important in terms of protecting the phenolics in it.

Meat and cheddar cheese samples of fixed weights (40 g and 30 g, respectively) were aseptically wrapped in stretch film, put and sealed in vacuum bag and sealed in Ziploc bag without evacuation of the air. Control samples had the packaging materials which were uncoated with propolis. On the other hand, meat and cheddar cheese samples sprayed with PPG solution were aseptically placed in a container without packaging during storage. All the samples were kept in the refrigerator at +4°C; the meats were stored for 30 days and the cheddar cheese for 45 days.

Figure 1. Stretch film with propolis (A), Ziploc with propolis (B), vacuum bag with propolis (C) preparation and coating of cheddar samples and beef meat with PPG solution (D, E)

2.4. Determination of the optimum concentration of propolis

Antimicrobial activity analysis was performed to find the optimum concentrations of propolis solutions to be used for the packaging and for spraying on foods.

Staphylococcus aureus (ATCC6538P) and *Escherichia coli* (ATCC8739) strains taken from the Microbiology Laboratory Culture collection of the Food Engineering Department (Tekirdağ Namık Kemal University) were used to evaluate the antibacterial activity of the solutions. Antibacterial activity was measured using the agar well diffusion method. Bacterial strains cultured in nutrient broth were incubated at 37°C (18 to 24 hours). A standard working culture was formed by setting McFarland at a value of 0.5. A 100 µL amount of bacterial suspension was spread on the surface of Mueller-Hinton Agar (MHA) with the help of a sterile drigalski spatula. Then 10 µl propolis preparations were injected into different petri dishes through two wells opened equidistant from the agar surface. After the solutions were added to the agar, they were incubated at 37°C for 24 hours. At the end of the incubation period, the susceptibility of the test organisms was determined by measuring the diameters of the inhibition zone around the well with digital calipers. The results given are the average of 3 consecutive results.

2.5. The determination of the phenolic compounds in propolis using the HPLC-DAD system

Chromatographic separation was conducted using an Inertsil® ODS-3 (5µm, 4.6 × 150 mm, Tokyo, Japan) column, mobile phase A (0.1% formic acid and deionized water, v v^{-1}) and mobile phase B (acetonitrile) at a flow rate of 1 ml min^{-1}. The column temperature was 30°C. A 5 µl alcoholic extract of propolis injection was done at a wavelength of 270 nm with a gradient system. Stock solutions of corresponding phenolic compounds were prepared with methanol at a concentration of 1 mg ml^{-1}. Spectra were determined by injecting the standards into the HPLC (Agilent, USA) system. Solutions diluted with methanol to concentrations of 3, 6, 12, 24 and 48 µg ml^{-1} were used for the preparation of calibration curves (Pellati et al., 2011).

2.6. Putrefaction analysis

All meat samples (5 g) were taken and placed in a petri dish and Nessler's reagent (Chembio CB2740.0100) was poured over it (15 ml). If the sample putrefied, a color ranging from orange to dark orange-brown was formed (Kirillov et al., 2020).

2.7. Microbiological analysis

Microbiological analyses were performed on meat samples at day 0, 7, 15, 21 and 30, and to cheese samples on day 0, 7, 15, 21 and 45. Samples (10 g) were aseptically unpacked and placed in a stomacher bag (PE, 180 × 310 mm, Lp Italiana Spa) and homogenized for 2 minutes in sterile 0.85 g l^{-1} of tryptone salt solution with a blender (Stomacher 400, Germany). Serial decimal dilutions were prepared with peptone at the same dilutions and 1 or 0.1 ml samples of the dilution were spread on agar plates. Plate Count Agar (Merck, Germany) medium was used for a total mesophilic aerobic bacteria count, as well as M17 agar acc. to Terzaghi (Merck, Germany) medium and Man Rogosa and Sharpe (MRS) (Merck, Germany) medium. Violet Red Bile Glucose (VRBG) (Merck, Germany) medium was used for the *Enterobacteriaceae* family, and Plate Count skimmed milk (PCSM) (Merck, Germany) medium was used for *Pseudomonas* bacteria. Incubation conditions were 30°C for 2 days and 30°C for 3 to 4 days for total mesophilic aerobic bacteria and lactic acid bacteria counts, respectively. *Pseudomonas* and *Enterobacteriaceae* were incubated at 37°C for 2 days. The results given are the average of 3 parallel results obtained by observing the characteristics of the colonies (shape, size, pigmentation, etc.) and expressing them as log10 CFU g^{-1} meat and log10 CFU g^{-1} cheese samples (Muhlisin, Utama, Lee, Choi, and Lee, 2016; Talon et al., 2007).

2.8. Statistical analysis

Two-way ANOVA tests and Tukey multiple comparison test were used with the JMP (5.0.1, USA) statistical software package to determine the differences created by TMAB, LAB, *Enterobacteriaceae* and *Pseudomonas* bacteria in meat and cheddar cheese samples by directly spraying on food samples and spraying on the food packaging ($p<0.05$).

3. Results and Discussion

3.1. Profiling the phenolic compounds in propolis

The phenolic compounds found in propolis are shown in *Table 1*. The predominant phenolic compound in propolis was found to be caffeic acid phenethyl ester (CAPE) at a level of 27.523,4 µg g^{-1}, and the lowest amount of phenolic acid was epigallocatechin gallate at 287.53 µg g^{-1}. It has been stated that studies on the bioactivity of propolis should commence with the phenolic profiling of the propolis extracts, since its antibacterial activity is largely due to phenolic compounds (Sforcin and Bankova, 2011). CAPE (Velazquez et al., 2007), ferulic acid (Borges et al., 2013), quercetin (Xu and Lee, 2001), pinocembrin (Rasul et al., 2013; Velazquez et al., 2007), galangin (Cushnie and Lamb, 2005), kaempferol (Xu and Lee, 2001), naringenin and chalcone (Cushnie and Lamb, 2005) are phenolic compounds found in propolis that have antibacterial effects. The antimicrobial activity of propolis is due to the flavonoids, aromatic acids and esters contained in the resin. Galangin, pinocembrin and pinostrobin are the most effective flavonoids against bacteria. Ferulic and caffeic acids also provide an antibacterial effect to propolis (Marcucci, 1995).

Table 1. Phenolic compounds of propolis

Compound	Value (µg g^{-1})
Epigalocatchine gallate	287.53
Caffeic acid	1.304,39
trans-Ferulic acid	556.21
trans-Isoferulic acid	2.529,77
3-4 Dimethoxycinnamic acid	2.203,40
Quercetin	2.062,06
trans-Cinnamic acid	2.105,71
Naringenin	3.823,16
Apigenin	2.335,43
Kaempferol	3.075,68
Chyrisin	6.795,93
Pinocembrin	16.246,77
Galangin	11.809,23
Cafeic acid fenetil ester	27.523,40
trans- Chalcon	11.700,92

3.2. Determination of the optimum concentration of propolis

The antibacterial activity results of PEA and PPG solutions on *Escherichia coli* (ATCC8739) and *Staphylococcus aureus* (ATCC6538P) bacteria are shown in *Table 2*. According to the results in *Table 2*, the antimicrobial effect of 5%, 10%, 20% and 30% PEA solutions on *E. coli* bacteria was not found to be statistically significant ($p>0.05$). The antimicrobial effect of 5% and 10% PEA solutions on *S. aureus* bacteria was found to be statistically significant ($p<0.05$). Again, according to these results, the antimicrobial effect of 5%, 10% and 20% PPG solutions on *E. coli* bacteria was found to be statistically significant ($p<0.05$), and the antimicrobial effect of 5%, 10% and 20% PPG solutions on *S. aureus* bacteria was also found to be significant ($p<0.05$).

It is commercially important to use the minimum amount of propolis to create a solution. For this reason, according to the results in *Table 2*, a 10% PEA solution was preferred for the coating of the packaging and a 5% PPG solution was preferred for the direct coating of the foods due to their statistically highest antimicrobial effect for the least cost. In one study, it was recommended to add 10% water extract of propolis to extend the shelf life of soft cheese (Moawad et al., 2001). Pobiega et al. (2021) investigated the effect of coating with 5% and 10% propolis extract of blueberry to reduce the number of bacteria, mold and reduce the effect of physicochemical properties during storage.

Ertürk (2015) carried out a study and found that the inhibitory effect of 20% ethyl acetate extract of propolis on *E. coli* and *S. aureus* was 17 mm and 13 mm, respectively. In another study, Tosi (1996) found that a 30% propylene glycol extract of propolis had an inhibition zone diameter of 6 to 15 mm against the same bacteria. The results of this study were consistent with some of the results in existing published literature (Apaydın and Gümüş, 2018; Rahman et al., 2010; Vică et al., 2021). According to the antimicrobial activity results in *Table 2*, a 10% PEA solution was preferred for spraying on packaging, and a 5% PPG solution was preferred for direct spraying on meat and cheddar cheese samples.

Table 2. Antibacterial activity on Escherichia coli (ATCC8739) and Staphylococcus aureus (ATCC6538P) bacteria (Diameter of inhibition zone, mm)

***E. coli* (mm)**		***S. aureus* (mm)**	
Solutions		**Solutions**	
E_0	15.68 ± 2.86^{a}	**E_0**	15.68 ± 2.86^{b}
E_1	16.86 ± 3.66^{a}	**E_1**	34.04 ± 7.91^{ab}
E_2	23.06 ± 4.74^{a}	**E_2**	41.44 ± 0.0^{a}
E_3	16.06 ± 2.02^{a}	**E_3**	43.62 ± 5.16^{a}
E_4	15.97 ± 0.63^{a}	**E_4**	35.29 ± 8.65^{ab}
P_0	0 ± 0^{c}	**P_0**	0 ± 0^{b}
P_1	17.13 ± 1.25^{a}	**P_1**	37.97 ± 10.76^{a}
P_2	9.26 ± 0.11^{b}	**P_2**	33.52 ± 6.47^{ab}
P_3	11.74 ± 3.30^{ab}	**P_3**	50.92 ± 14.57^{a}
P_4	11.51 ± 0.00^{ab}	**P_4**	32.74 ± 2.40^{ab}

*The means of replicates ± standard deviations are shown. $^{a-c}$Different lowercase letters within a column indicate significant differences between volumes ($p<0.05$). E_0: ethyl acetate, E_1: 5% propolis-ethyl acetate, E_2: 10% propolis-ethyl acetate, E_3: 20% propolis-ethyl acetate, E_4: 30% propolis-ethyl acetate, P_0: propylene glycol, P_1: 5% propolis-propylene glycol, P_2: 10% propolis-propylene glycol, P_3: 20% propolis-propylene glycol, P_4: 30% propolis-propylene glycol

3.3. Putrefaction analysis

The results of the Nessler analysis after 30 days for meat samples coated with solutions and stored in different packaging are shown in *Figure 2*. Microbial surface contamination was the main cause of spoilage in the raw meat. Putrefaction in meat occurred due to alteration of microbial flora (Nakamura et al., 2021; Ouoba et al., 2003). The total volatile base nitrogen (TVB-N) result is considered to be an important factor when measuring the extent of protein degradation and putrefaction of meat and meat products to amino acids (Han et al., 2001). In many studies it was found that treating meat and meat products with propolis extracts reduced the TVB-N value (Ali et al., 2010; Gutiérrez-Cortés and Suarez Mahecha, 2014; Han et al., 2001; Jonaidi Jafari et al., 2018; Mehdizadeh and Mojaddar Langroodi, 2019). Storage conditions are very important to reduce the putrefaction in foodstuffs (Tucker, 2015). It could be seen in Figure 2 that changing colours to darker tone showed putrefaction took place during storage time. The findings demonstrated that propolis-coated packaging significantly reduced putrefaction at day 15 compared to uncoated packagings as the colours tone were lighter. Yingyuad (2006) stated that the use of a natural antimicrobial coating such as chitosan together with vacuum (PVDC/nylon) packaging promoted the formation of better organoleptic qualities and better microbiological quality of foods compared to conventionally

packaged food products. It was observed that the colour change was the least for meat samples stored by vacuum packaging which meant that the least putrefaction was taken place in vacuum packaging. Compared to the other packaging types used in this research, the vacuum bag ensured a minimum level of oxygen in the environment (Narasimha Rao and Sachindra, 2002) and was more in contact with the meat samples. Ziploc bags and stretch films have less contact with the meat sample and more oxygen permeability compared to vacuum packaging. Therefore, it was observed that the higher the contact area of propolis coated packaging materials with the samples, the stronger the microbial prevention effect of the propolis was.

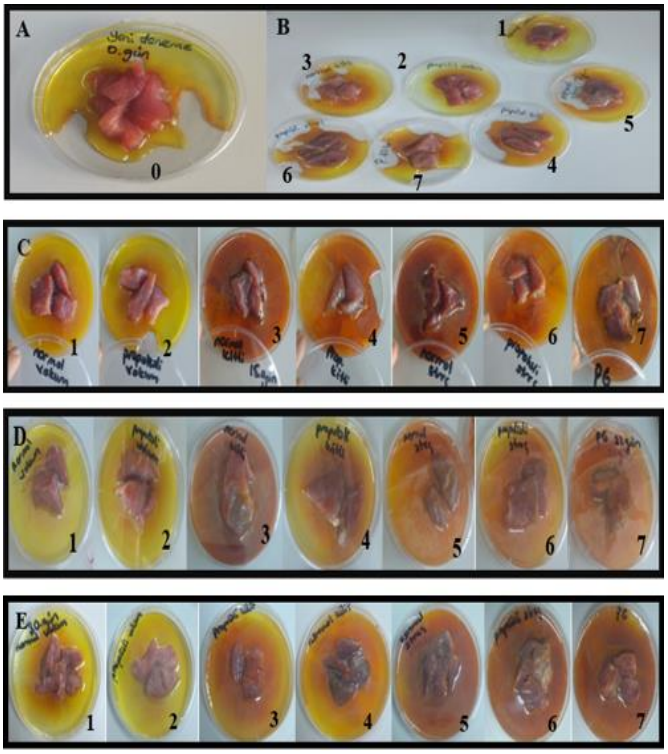

Figure 2. Color change of Nessler solution after dropped on meat samples which were kept by different methods (0) control (1) normal vacuum bag (2) vacuum bag with propolis (3) normal ziploc bag (4) ziploc bag with propolis (5) normal stretch film (6) stretch film with propolis (7) PPG spraying. Storage times (A) day 0 (B) day 7 (C) day 15 (D) day 21 and (E) day 30

3.4. Microbiological analysis

The microbiological analysis results of stored meat samples are given in *Table 3*. According to the findings, the TMAB count increased in the sample stored in a normal vacuum bag, normal ziploc bag, normal stretch film packaging and propylene glycol solution with propolis during storage. In the sample stored in the vacuum bag with propolis, the ziploc bag with propolis and the stretch film with propolis packaging, the TMAB count first increased until day 7, then decreased until day 15 day, and then continued to increase until the end of storage. By the end of day 30, it was determined that the meat samples preserved in propolis-coated vacuum packaging had the lowest ($p<0.05$) TMAB count at 6.57 log CFU g^{-1}. There was a 0.56 log CFU g^{-1} reduction compared to the control vacuum packaging. Vacuum packaging with propolis reduced the TMAB count in the meat sample. Vacuum packaging is more in contact with the meat surface than other packaging due to complete evacuation of the air. Thus, propolis showed more antimicrobial activity by virtue of being more in contact with the meat surface (Siripatrawan and Vitchayakitti, 2016).

Jonaidi Jafari et al. (2018) examined the effect of an edible chitosan (2%) coating – containing a propolis ethanolic extract (1% and 2%) – on the microbiological properties of chicken fillets and observed a 7 log CFU g^{-1} reduction in 12 days. Compared to control samples, Shavisi et al. (2017) found a 4.27 log CFU g^{-1} reduction after 11 days of storage in a film-wrapped meat sample containing 2% essential oil, 2% propolis ethanolic extract and 1% cellulose nanoparticles. In another study, ethanolic extract added to minced carp was found to be effective

against microorganisms that cause spoilage, including psychrotrophic and lactic acid bacteria (Payandan et al., 2017). Duman and Ozpolat (2015) found that the total plate count of fresh shibuta (*Barbus grypus*) fillet samples treated with propolis extracts was lower than the control sample. These results were consistent with our findings.

The effect of *Enterobacteriaceae* on the microflora of meat should be taken into account due to deterioration and potential health hazards during long-term storage (Ercolini et al., 2006). The *Enterobacteriaceae* count increased in the samples stored in normal ziploc bags, ziploc bags with propolis, normal stretch film, stretch film with propolis-treated packaging, and with a propylene glycol solution with propolis during storage. In the sample stored in a normal vacuum bag and vacuum bag with propolis, the *Enterobacteriaceae* count first increased until day 7, then decreased until day 15, and then continued to increase until the end of storage. At the end of day 30, it was determined that the meat sample preserved in propolis-coated vacuum packaging had the lowest ($p<0.05$) *Enterobacteriaceae* count of 4.78 log CFU g^{-1}. There was a reduction of 1.01 log CFU g^{-1} ($p<0.05$) in propolis samples compared to the control vacuum packaging. Vacuum packaging with propolis reduced the *Enterobacteriaceae* count in the meat sample.

Shavisi et al. (2017) reported that on day 11 the *Enterobacteriaceae* population reached 6.24 log CFU g^{-1} in the control sample and 3.66 log CFU g^{-1} in the film-wrapped samples containing 2% essential oil, 2% propolis ethanolic extract and 1% cellulose nanoparticles. Bazargani-Gilani et al. (2021) reported that the *Enterobacteriaceae* count in trout fillets wrapped with a carboxymethyl cellulose coating combined with 2% propolis extract was 6.015 log CFU g^{-1}, whereas counts for the control samples reached 8.69 log CFU g^{-1} in control fillets after 15 days of storage.

Pseudomonas spp. is known as one of the contributors to the bacterial spoilage of meat stored under refrigeration (Labadie, 1999; Molin et al., 1986). The inhibitory effect of propolis on *Pseudomonas* has already been proven (Mascheroni et al., 2014; Petruzzi et al., 2020). The *Pseudomonas* count increased in the sample stored in a normal vacuum bag, vacuum bag with propolis, stretch film with propolis, normal stretch film, normal ziploc bag, ziploc bag with propolis packaging, and the propylene glycol solution with propolis during storage. At the end of day 30, the meat sample preserved in propolis-coated vacuum packaging had the lowest ($p<0.05$) *Pseudomonas* count of 6.44 log CFU g^{-1}. There was a reduction of 0.34 log CFU g^{-1} ($p<0.05$) in the propolis-coated vacuum packaging compared to the control sample. Vacuum packaging with propolis reduced the *Pseudomonas* count in the meat sample.

Mehdizadeh and Langroodi (2019) applied the combination of propolis extract and chitosan enriched with Zataria multiflora essential oil (ZEO) to coat poultry meat, and at the end of the storage period, the samples featuring 1% chitosan with propolis extract and 0.5% ZEO, and 1% chitosan with propolis extract and 1% ZEO had the lowest *Pseudomonas* spp. counts. The number of bacteria in the sample treated with 1% chitosan with propolis extract and 1% ZEO decreased by 2.81 log CFU g^{-1} compared to the control sample. In the study by Shavisi et al. (2017), the beef sample with antimicrobial film containing propolis ethanolic extract had the lowest *Pseudomonas* spp. count compared to the control sample. Rollinia et al. (2017) showed that the potential combination of propolis and chitosan to develop a bio-based active food packaging material increased antibacterial activity against *Pseudomonas putida* ATCC 12633. These results were consistent with our findings.

It was determined that *Enterobacteriaceae* and *Pseudomonas* TMAB numbers were the highest during the storage period where PPG was directly sprayed on meat samples ($p<0.05$). It was concluded that PPG was not suitable for direct use on meat products. Although this result was not consistent with published literature, as it was found that propylene glycol inhibited *Pseudomonas* numbers (Meto et al., 2020; Ramanauskiene et al., 2013), the high water activity in fresh meat might cause this result and decrease the shelf life during storage.

In previous studies, it was stated that the antibacterial activity of propolis was largely due to phenolic compounds (Chaillou and Nazareno, 2009; Sforcin and Bankova, 2011). According to our findings, it was determined that the *Enterobacteriaceae* and *Pseudomonas* TMAB numbers for the meat sample stored in a vacuum bag with propolis were the lowest ($p<0.05$) both during storage and at the end of 30 days. It can be deduced that the reason why the vacuum bag inhibited the growth of the microorganisms more than other packaging was due to the larger contact surface area and lack of oxygen because vacuum packaging completely covered all the surfaces of the samples.

Table 3. Microbiological quality of meat samples during the storage period *(log CFU g^{-1})

Total Mesophilic Aerobic Bacterial Counts							
Day	V0	V1	K0	K1	S0	S1	PPG
0	4.81±0.06^{a}	4.81±0.06^{a}	4.81±0.06^{a}	4.81±0.06^{a}	4.81±0.06^{a}	4.81±0.06^{a}	4.81±0.06^{a}
7	6.22±0.10^{c}	5.86±0.15^{d}	7.60±0.03^{a}	7.55±0.02^{a}	7.13±0.07^{b}	7.10±0.06^{b}	7.56±0.03^{a}
15	6.28±0.5^{e}	5.83±0.03^{f}	8.80±0.04^{a}	7.16±0.11^{d}	7.96±0.04^{c}	7.01±0.07^{d}	8.21±0.01^{b}
21	7.15±0.02^{e}	6.33±0.01^{f}	8.18±0.03^{ab}	7.92±0.02^{c}	8.32±0.11^{a}	7.60±0.01^{d}	8.03±0.09^{bc}
30	7.13±0.03^{d}	6.57±0.04^{e}	8.39±0.02^{ab}	7.99±0.08^{c}	8.18±0.12^{bc}	8.04±0.09^{c}	8.57±0.1^{a}

***Enterobacteriaceae* Counts**							
Day	V0	V1	K0	K1	S0	S1	PPG
0	0.66±0.57^{a}	0.66±0.57^{a}	0.66±0.57^{a}	0.66±0.57^{a}	0.66±0.57^{a}	0.66±0.57^{a}	0.66±0.57^{a}
7	4.83±0.03^{e}	4.64±0.04^{f}	5.99±0.01^{b}	5.84±0.02^{bc}	5.81±0.03^{c}	5.21±0.04^{d}	6.15±0.11^{a}
15	4.48±0.02^{f}	4.33±0.05^{g}	7.38±0.01^{b}	7.08±0.03^{c}	6.80±0.00^{d}	5.90±0.05^{e}	8.33±0.07^{a}
21	5.50±0.08^{d}	4.47±0.10^{e}	8.33±0.01^{ab}	8.08±0.02^{b}	8.32±0.15^{ab}	7.64±0.07^{c}	8.45±0.11^{a}
30	5.79±0.02^{d}	4.78±0.01^{e}	8.66±0.07^{ab}	8.28±0.03^{b}	7.71±0.36^{c}	7.45±0.27^{c}	8.97±0.02^{a}

***Pseudomonas* Counts**							
Day	V0	V1	K0	K1	S0	S1	PPG
0	4.3±0.07^{a}	4.3±0.07^{a}	4.3±0.07^{a}	4.3±0.07^{a}	4.3±0.07^{a}	4.3±0.07^{a}	4.3±0.07^{a}
7	6.10±0.01^{b}	5.84±0.03^{c}	6.46±0.02^{a}	5.78±0.02^{c}	5.57±0.01^{d}	4.90±0.02^{e}	5.60±0.0^{d}
15	6.30±0.03^{e}	5.89±0.04^{g}	7.48±0.03^{b}	6.48±0.05^{d}	7.25±0.02^{c}	6.13±0.04^{f}	7.84±0.02^{a}
21	6.42±0.03^{d}	6.12±0.06^{e}	7.84±0.00^{b}	7.37±0.03^{c}	7.75±0.03^{b}	7.38±0.05^{c}	8.62±0.10^{a}
30	6.78±0.08^{e}	6.44±0.01^{f}	7.57±0.09^{c}	7.31±0.09^{d}	7.82±0.03^{b}	7.40±0.05^{cd}	8.42±0.04^{a}

*The means of replicates ± standard deviations are shown. $^{a-g}$ Different lowercase letters within a column indicate significant differences ($p < 0.05$). V0: normal (control) vacuum bag, V1: vacuum bag with propolis, PPG: propylene glycol solution with propolis, S0: normal (control) stretch film, S1: stretch film with propolis, K0: normal (control) Ziploc bag, K1: Ziploc bag with propolis.

Microbiological results of cheddar cheese samples during the storage period are shown in *Table 4*. Counts of LAB grown on TMAB, MRS agar and LAB on M17 agar were recorded on days 0, 7, 15, 21 and 45. According to the findings of this study, the TMAB count of the sample stored in a normal vacuum bag, vacuum bag with propolis, normal ziploc bag, ziploc bag with propolis and normal stretch film packaging all increased during storage. The TMAB count of the sample coated with propylene glycol solution with propolis first increased until day 7, then decreased until day 15, then continued to increase until the end of storage. The TMAB count of the sample stored in the stretch film with propolis packaging first decreased until the day 7, then increased until day 15, then continued to increase until the end of storage. At the end of day 45, while the cheddar cheese sample kept in a propolis covered vacuum bag had the lowest ($p < 0.05$). TMAB count at day 21 among the all samples, cheddar cheese with PPG directly sprayed on it had the lowest TMAB count at the end of storage with a value of 6.64 log CFU g^{-1} ($p<0.05$). At the end of the storage, the TMAB value of the cheddar cheese sample coated with PPG was followed by the cheddar cheese sample stored in a vacuum bag treated with propolis with a value of

6.80 log CFU g^{-1}. In previous studies, it was demonstrated that extracts of propolis with different combinations are effective against TMAB (El-Demery et al., 2016; Metwalli, 2011; Nessianpour et al., 2019; Payandan et al., 2017). In another study, the number of TMAB in the control during storage (45 days) was higher than in Tallaga cheese with 5% propolis extract (Saleh et al., 2020). El-Deeb and Omar (2017) suggested that the addition of 6% and 10% water extracts of propolis to karish cheese can create a natural and safe source of antimicrobial agents during storage periods.

Table 4. Microbiological quality of Cheddar cheese during the storage period *(log CFU g^{-1})

Total Mesophilic Aerobic Bacterial Counts							
Day	V0	V1	K0	K1	S0	S1	PPG
0	5.41±0.02^{a}	5.41±0.02^{a}	5.41±0.02^{a}	5.41±0.02^{a}	5.41±0.02^{a}	5.41±0.02^{a}	5.41±0.02^{a}
7	6.08±0.05^{b}	4.79±0.04^{e}	6.62±0.07^{a}	6.16±0.15^{b}	6.04±0.05^{b}	5.28±0.10^{d}	5.70±0.06^{c}
15	6.17±0.01^{c}	4.84±0.06^{e}	6.86±0.01^{a}	6.65±0.04^{b}	6.27±0.01^{c}	6.15±0.04^{c}	5.52±0.13^{d}
21	6.35±0.01^{d}	5.30±0.17^{f}	7.35±0.13^{a}	7.08±0.01^{b}	6.77±0.00^{c}	6.31±0.02^{d}	5.88±0.09^{e}
45	7.13±0.05^{b}	6.80±0.02^{c}	7.24±0.02^{a}	7.18±0.01^{ab}	6.78±0.01^{c}	6.71±0.02^{cd}	6.64±0.07^{d}

Lactic acid Bacterial Counts in the M17 medium							
Day	V0	V1	K0	K1	S0	S1	PPG
0	5.96±0.05^{a}	5.96±0.05^{a}	5.96±0.05^{a}	5.96±0.05^{a}	5.96±0.05^{a}	5.96±0.05^{a}	5.96±0.05^{a}
7	5.51±0.02^{d}	5.03±0.08^{e}	7.10±0.04^{a}	7.06±0.02^{a}	5.86±0.05^{c}	5.17±0.08^{e}	6.21±0.15^{b}
15	6.57±0.02^{d}	6.02±0.12^{e}	7.44±0.02^{a}	7.14±0.03^{b}	7.19±0.01^{b}	6.41±0.05^{d}	6.81±0.08^{c}
21	6.69±0.08^{c}	6.54±0.08^{d}	7.62±0.01^{a}	7.51±0.02^{a}	7.23±0.02^{b}	6.33±0.07^{e}	7.08±0.02^{b}
45	7.38±0.04^{ab}	6.84±0.01^{c}	7.65±0.05^{a}	7.61±0.02^{a}	6.76±0.06^{c}	6.58±0.25^{c}	7.19±0.04^{b}

Lactic acid Bacterial Counts in the MRS medium							
Day	V0	V1	K0	K1	S0	S1	PPG
0	5.88±0.03^{a}	5.88±0.03^{a}	5.88±0.03^{a}	5.88±0.03^{a}	5.88±0.03^{a}	5.88±0.03^{a}	5.88±0.03^{a}
7	5.72±0.02^{c}	5.13±0.11^{d}	7.15±0.06^{a}	6.92±0.04^{a}	6.94±0.04^{a}	4.92±0.17^{d}	6.08±0.07^{b}
15	6.49±0.17^{c}	6.01±0.02^{d}	7.23±0.03^{a}	7.18±0.02^{a}	6.54±0.05^{c}	5.26±0.06^{e}	6.81±0.04^{b}
21	6.79±0.03^{d}	6.43±0.05^{e}	7.37±0.01^{a}	7.19±0.03^{b}	7.00±0.03^{c}	6.36±0.01^{e}	7.11±0.01^{b}
45	7.23±0.09^{b}	6.63±0.07^{d}	7.54±0.07^{a}	7.49±0.08^{a}	6.89±0.11^{c}	6.85±0.09^{cd}	7.20±0.09^{b}

*The means of replicates ± standard deviations are shown. $^{a-g}$ Different lowercase letters within a column indicate significant differences ($p < 0.05$). V0: normal (control) vacuum bag, V1: vacuum bag with propolis, PPG: propylene glycol solution with propolis, S0: normal (control) stretch film, S1: stretch film with propolis, K0: normal (control) Ziploc bag, K1: Ziploc bag with propolis.

The LAB (M17) counts of the samples stored in normal ziploc bags, ziploc bags with propolis packaging and those covered with propylene-glycol propolis solution increased until the end of the storage period. LAB (M17) counts of the samples stored in vacuum bags with propolis, normal vacuum bags, normal stretch film and stretch film with propolis-treated packaging first decreased until day 7 and then continued to increase until the end of storage. It was determined that the cheddar cheese sample, which was kept in a propolis covered vacuum bag had the lowest ($p<0.05$) LAB (M17) number at day 21. At the end of day 45, the cheddar cheese sample covered by propolis stretch film had the lowest LAB (M17) number with a value of 6.58 log CFU g^{-1}. There was a reduction

of 0.18 log CFU g^{-1} ($p<0.05$) compared to the control sample. Stretch film with propolis was able to demonstrate the antimicrobial activity of propolis by wrapping the film around the cheddar cheese. Jonaidi Jafari et al. (2018) revealed that the LAB count at day 12 was 7.7 log CFU g^{-1} in the control sample and 5.5 log CFU g^{-1} in the 2% propolis ethanolic extract sample. Bazargani-Gilani et al. (2021) stated that carboxymethyl cellulose-containing propolis extract was the most powerful application in preventing LAB replication, since it caused a 1.22 log CFU g^{-1} reduction in fillet samples at the end of the storage period. In another study by Duman and Ozpolat (2015), the LAB count was 7.81 log CFU g^{-1} in the control sample and 6.55 log CFU g^{-1} in the 5% propolis water extract sample on day 9. These results were consistent with our findings.

The LAB (MRS) count increased in the sample stored in a normal ziploc bag, ziploc bag with propolis packaging and propylene-glycol propolis solution during storage. The LAB (MRS) count of the sample stored in a vacuum bag with propolis, normal vacuum bag and stretch film with propolis packaging first decreased until day 7 and then continued to increase until the end of storage. The LAB (MRS) count of the sample stored in the normal stretch film package first increased until day 7, then decreased until day 15, and then continued to increase until the end of storage. It was determined that the cheddar cheese sample covered by propolis-treated stretch film had the lowest LAB (MRS) number after 21 days as well. At the end of day 45, it was determined that the cheddar cheese sample covered by a propolis-treated vacuum bag had the lowest ($p<0.05$) LAB (MRS) count of 6.63 log CFU g^{-1}. There was a reduction of 0.60 log CFU g^{-1} ($p<0.05$) compared to the control sample. At the end of storage, cheddar cheese kept in vacuum packaging with propolis was followed by cheddar cheese preserved in propolis-treated stretch film with a value of 6.85 log CFU g^{-1} M17. Vacuum packaging coated with propolis and stretch film coated with propolis showed more antimicrobial activity than the normal ziploc bag.

According to the findings of this study, the reason why the vacuum bag and stretch film showed more antibacterial effect than the ziploc bag was due to the larger contact area with the samples. The reason why the effect of normal ziploc bag packaging on shelf life is low is because of the decrease in the contact between the propolis and the food. Therefore, it is thought that shelf life increase of foods with propolis is not due to propolis' volatile compounds since the results showed that if the contact with propolis was low, the antimicrobial effect of propolis decreased. In addition, the PPG solution sprayed on the surface of the cheddar cheese was able to adhere to the surface and showed antimicrobial activity since the cheddar cheese sample adsorbed the PPG solution well.

4. Conclusions

In the study, the surface of food packaging was coated with propolis by spraying it on ziploc bags, stretch film and vacuum bags. In addition, propolis-PPG solution was formed, and spray coated on the food. According to the antimicrobial activity analysis, a 10% PEA solution was preferred to coat the packages and a 5% PPG solution was preferred to spray directly on the meat and cheddar cheese. The 8.97 log CFU g^{-1} *Enterobacteriaceae*, 8.57 log CFU g^{-1} TMAB and 8.42 log CFU g^{-1} *Pseudomonas* values of the meat samples coated with PPG solution were the highest values at the end of storage. Direct spraying is not suitable for wet and slippery foods such as meat. Because adsorption of propolis solution on this kind of foods could not happen. Nevertheless, the direct spraying process can provide protection for cheddar cheese which are drier than meat, and well adsorption of propolis solution was observed. The 7.24 log CFU g^{-1} TMAB, 7.65 log CFU g^{-1} M17 and 7.54 log CFU g^{-1} MRS values for the cheddar cheese sample stored in a normal ziploc bag were the highest values at the end of storage. As a result of this study, the best results were found in the samples stored in propolis-treated vacuum bags, and the worst results were found in the samples stored in normal ziploc bags. When all these results are evaluated, a new platform can be created where propolis packaging can be produced with a few additional installations in existing established packaging factories.

Acknowledgements

This work supported by the Tekirdag Namık Kemal University Research Project (Project Number: NKUBAP.03.YL.19.225), Türkiye.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Karpuz, E., Palabıyık, İ.; Design: Karpuz, E., Palabıyık, İ.; Data Collection or Processing: Karpuz, E., Palabıyık, İ.; Statistical Analyses: Karpuz, E.; Literature Search: Karpuz, E.; Writing, Review and Editing: Karpuz, E., Palabıyık, İ.

References

Ali, F. H., Kassem, G. M. and Atta-Alla, O. A. (2010). La propoli come decontaminante e antiossidante nella salsiccia orientale fresca. *Veterinaria Italiana*, 46(2), 167–172.

Aliyazcoglu, R., Sahin, H., Erturk, O., Ulusoy, E. and Kolayli, S. (2013). Properties of phenolic composition and biological activity of propolis from Turkey. *International Journal of Food Properties*, 16(2): 277–287. https://doi.org/10.1080/10942912.2010.551312

Andrade, R. D., Skurtys, O. and Osorio, F. A. (2012). Atomizing spray systems for application of edible coatings. *Comprehensive Reviews in Food Science and Food Safety*, 11(3): 323–337. https://doi.org/10.1111/J.1541-4337.2012.00186.X

Apaydın, H. and Gümüş, T. (2018). Inhibitory effect of propolis (Bee Gum) against staphylococcus aureus bacteria ısolated from instant soups. *Journal of Tekirdag Agricultural Faculty*, 15(1): 67–75.

Bazargani-Gilani, B., Pajohi-Alamoti, M., Hassanzadeh, P. and Raeisi, M. (2021). Impacts of Carboxymethyl Cellulose Containing Propolis Extract on the Shelf Life of Trout Fillets. *Archives of Hygiene Sciences*, 10(2): 117–132. https://doi.org/10.52547/archhygsci.10.2.117

Bölük, E., Atik, D. S., Kolaylı, S., Demirci, A. Ş. and Palabiyik, I. (2021). Delivery of phenolics and caffeic acid phenethyl ester by propolis resin: Chewing gum system. *Food Bioscience*, 41:101090. https://doi.org/10.1016/j.fbio.2021.101090

Borges, A., Ferreira, C., Saavedra, M. J. and Simões, M. (2013). Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microbial Drug Resistance*, 19(4): 256–265. https://doi.org/10.1089/mdr.2012.0244

Castaldo, S. and Capasso, F. (2002). Propolis, an old remedy used in modern medicine. *Fitoterapia*, 73(SUPPL. 1):1–6. https://doi.org/10.1016/S0367-326X(02)00185-5

Chaillou, L. L. and Nazareno, M. A. (2009). Bioactivity of propolis from Santiago del Estero, Argentina, related to their chemical composition. *LWT - Food Science and Technology*, 42(8): 1422–1427. https://doi.org/10.1016/j.lwt.2009.03.002

Coban, M. Z. and Coban, O. E. (2020). Potency and use of chia mucilage coating containing propolis liquid extract for improves shelf-life of sea bass fillets. *Acta Scientiarum Polonorum, Technologia Alimentaria*, 19(3): 255–260. https://doi.org/10.17306/J.AFS.0843

Correa, F. T., de Souza, A. C., de Souza Júnior, E. A., Isidoro, S. R., Piccoli, R. H., Dias, D. R. and de Abreu, L. R. (2019). Effect of Brazilian green propolis on microorganism contaminants of surface of Gorgonzola-type cheese. *Journal of Food Science and Technology*, 56(4): 1978–1987. https://doi.org/10.1007/s13197-019-03664-2

Cushnie, T. P. T. and Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, 26(5): 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

Dizaj, S. M., Lotfipour, F., Barzegar-Jalali, M., Zarrintan, M. H. and Adibkia, K. (2014). Antimicrobial activity of the metals and metal oxide nanoparticles. *Materials Science and Engineering: C*, 44: 278–284. https://doi.org/10.1016/J.MSEC.2014.08.031

Duman, M. and Özpolat, E. (2015). Effects of water extract of propolis on fresh shibuta (*Barbus grypus*) fillets during chilled storage. *Food Chemistry*, 189: 80–85. https://doi.org/10.1016/j.foodchem.2014.08.091

El-Deeb, A. and Omar, S. (2017). Effect of Propolis Extract as a Natural Preservative on the Microbial Content of Kareish Cheese. *Journal of Food and Dairy Sciences*, 8(7): 295–302. https://doi.org/10.21608/jfds.2017.38715

El-Demery, M., Elsebaie, E., Zidan, N. and Essa, R. (2016). Efficiency of Propolis and Turmeric Powders As Natural Preservatives in Minced Beef. *Journal of Food and Dairy Sciences*, 7(1): 45–50. https://doi.org/10.21608/jfds.2016.42805

Ercolini, D., Russo, F., Torrieri, E., Masi, P. and Villani, F. (2006). Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. *Applied and Environmental Microbiology*, 72(7): 4663–4671. https://doi.org/10.1128/AEM.00468-06

Ertürk, Ö., Yavuz, C. and Sıralı, R. (2015). The antimicrobial activity of propolis from Ordu Province of Turkey. *Mellifera*, 15(1): 37–38.

Escriche, I. and Juan-Borrás, M. (2018). Standardizing the analysis of phenolic profile in propolis. *Food Research International*, 106: 834–841. https://doi.org/10.1016/j.foodres.2018.01.055

Fadaly H., E. and El-Badrawy, E. E. Y. (2001). Flavonoids of propolis and their antibacterial activities. *Pakistan Journal of Biological Sciences*, 4(2): 204–207. https://doi.org/10.3923/pjbs.2001.204.207

Galeotti, F., Maccari, F., Fachini, A. and Volpi, N. (2018). Chemical composition and antioxidant activity of propolis prepared in different forms and in different solvents useful for finished products. *Foods*, 7(3): 41. https://doi.org/10.3390/foods7030041

Güler, G., Şen, A., Korkmaz Turgud, F., Tahtabiçen, E., Ağma Okur, A. and ŞAMLI, H. E. (2022). Effects of propolis extract coating on shelf life and quality parameters of eggs obtained from free-range hens. *Journal of Tekirdag Agricultural Faculty*, 19(1): 89–100. https://doi.org/10.33462/JOTAF.893121

Güney, F. and Yılmaz, M. (2013). Propolisin kimyasal içeriği ile antibakteriyel, antiviral, antitümör, antifungal ve antioksidan aktitivitesi. *Arıcılık Araştırma Dergisi*, 5(1): 25–28.

Gutiérrez-Cortés, C. and Suarez Mahecha, H. (2014). Antimicrobial activity of propolis and its effect on the physicochemical and sensoral characteristics in sausages. *Vitae*, 21(2): 90–96.

Hames-Kocabas, E. E., Demirci, B., Uzel, A. and Demirci, F. (2013). Volatile composition of Anatolian propolis by headspace-solid-phase microextraction (HS-SPME), antimicrobial activity against food contaminants and antioxidant activity. *Journal of Medicinal Plants Research*, 7(28): 2140–2149. https://doi.org/10.5897/JMPR2013.4470

Han, S. K., Yamauchi, K. and Park, H. K. (2001). Effect of nitrite and propolis preservative on volatile basic nitrogen changes in meat products. *Microbios*, 105(411): 71–75.

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): 133–136. https://doi.org/10.1111/jfpp.13336

Keskin, M. (2018). Alginat-Propolis mikrokapsüllerin in vitro sindirim sisteminde salınımının ham propolis ile kıyaslanması. *Uludağ Arıcılık Dergisi*, 18(2): 94–100. https://doi.org/10.31467/ULUARICILIK.485031

Kirillov, I., Asrutdinova, R., Yakupova, L., Gilmutdinov, R. and Fayzrakhmanova, G. A. (2020). Veterinary sanitary assessment of chicken meat using squalene. *BIO Web of Conferences*, 17(45): 00185. https://doi.org/10.1051/bioconf/20201700185

Labadie, J. (1999). Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Science*, 52(3): 299–305. https://doi.org/10.1016/S0309-1740(99)00006-6

Leceta, I., Molinaro, S., Guerrero, P., Kerry, J. P. and De la Caba, K. (2015). Quality attributes of map packaged ready-to-eat baby carrots by using chitosan-based coatings. *Postharvest Biology and Technology*, 100: 142–150. https://doi.org/10.1016/J.POSTHARVBIO.2014.09.022

López-Rubio, A., Almenar, E., Hernandez-Muñoz, P., Lagarón, J. M., Catalá, R. and Gavara, R. (2004). Overview of active polymer-based packaging technologies for food applications. *Food Reviews International*, 20(4): 357–387. https://doi.org/10.1081/FRI-200033462

Marcucci, M. C. (1995). Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie*, 26(2): 83–99. https://doi.org/10.1051/APIDO:19950202

Mascheroni, E., Figoli, A., Musatti, A., Limbo, S., Drioli, E., Suevo, R., … Rollini, M. (2014). An alternative encapsulation approach for production of active chitosan-propolis beads. *International Journal of Food Science and Technology*, 49(5): 1401–1407. https://doi.org/10.1111/ijfs.12442

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

Memmedov, H., Aldemir, O. and Aliyev, E. (2017). Propolisin antioksidan ve antiinflamatuvar etkisi. *Arıcılık Araştırma Dergisi*, 9(2): 56–62.

Meng, X. H., Qin, G. Z. and Tian, S. P. (2010). Influences of preharvest spraying Cryptococcus laurentii combined with postharvest chitosan coating on postharvest diseases and quality of table grapes in storage. *LWT - Food Science and Technology*, 43(4): 596–601. https://doi.org/10.1016/J.LWT.2009.10.007

Meto, A., Colombari, B., Meto, A., Boaretto, G., Pinetti, D., Marchetti, L., … Blasi, E. (2020). Propolis affects pseudomonas aeruginosa growth, biofilm formation, eDNA release and phenazine production: Potential involvement of polyphenols. *Microorganisms*, 8(2): 243. https://doi.org/10.3390/microorganisms8020243

Metwalli, S. A. H. (2011). Extended shelf life of kareish cheese by natural preservatives. *Journal of Agricultural Research*, 89(2): 640–648.

Moawad, A., Aly, S. and Galal, E. (2001). Effect of Egyptian honeybee propolis on the growth of Aspergillus ochraceus and ochratoxin A production in Ras cheese. *8th Egyptian Conference for Dairy Science and Technology, Held at the International Agriculture Centre*, Cairo, Egypt.

Molin, G., Ternstrom, A. and Ursing, J. (1986). Pseudomonas lundensis, a new bacterial species isolated from meat. *International Journal of Systematic Bacteriology*, 36(2): 339–342. https://doi.org/10.1099/00207713-36-2-339

Muhlisin, M., Utama, D. T., Lee, J. H., Choi, J. H. and Lee, S. K. (2016). Effects of gaseous ozone exposure on bacterial counts and oxidative properties in chicken and duck breast meat. *Korean Journal for Food Science of Animal Resources*, 36(3): 405–411. https://doi.org/10.5851/kosfa.2016.36.3.405

Nakamura, A., Takahashi, H., Otomo, K., Mizuno, Y., Kuda, T., Kimura, B. … Kobayashi, M. (2021). Dynamics of microbiota in Japanese black beef stored for a long time under chilled conditions. *Food Microbiology*, 100: 103849. https://doi.org/10.1016/j.fm.2021.103849

Narasimha Rao, D. and Sachindra, N. M. (2002). Modified atmosphere and vacuum packaging of meat and poultry products. *Food Reviews International*, 18(4): 263–293. https://doi.org/10.1081/FRI-120016206

Nessianpour, E., Khodanazary, A. and Hosseini, S. M. (2019). Shelf Life of saurida tumbil during storage at refrigeration condition as affected by gelatin-based edible coatings ıncorporated with propolis extract. *International Journal of Food Properties*, 22(1): 1749–1759. https://doi.org/10.1080/10942912.2019.1651738

Obregón Fuentes, A. M. and Rojas Hernández, N. (1990). Acción antimicrobiana de los extractos alcohólicos de propóleo. *Revista Cubana de Farmacia*, 24(1): 34–44.

Olivas, G. I. I., and Barbosa-Cánovas, G. (2009). Edible Films and Coatings For Fruits And Vegetables. In: Edible Films and Coatings for Food Applications, Eds: Huber, K. C. and Embuscado, M. E., Springer Nature. https://doi.org/10.1007/978-0-387-92824-1_7

Ouoba, L. I. I., Rechinger, K. B., Barkholt, V., Diawara, B., Traore, A. S. and Jakobsen, M. (2003). Degradation of proteins during the fermentation of African locust bean (*Parkia biglobosa*) by strains of Bacillus subtilis and Bacillus pumilus for production of Soumbala. *Journal of Applied Microbiology*, 94(3): 396–402. https://doi.org/10.1046/j.1365-2672.2003.01845.x

Payandan, E., Sayyed-Alangi, S. Z., Shamloofar, M., and Koohsari, H. (2017). Study of chemical composition and efficacy of different extracts of ıranian propolis on the microbiological and sensory parameters of minced cyprinus carpio meat at 4°C storage. *Journal of Aquatic Food Product Technology*, 26(5): 593–603. https://doi.org/10.1080/10498850.2016.1240281

Pellati, F., Orlandini, G., Pinetti, D. and Benvenuti, S. (2011). HPLC-DAD and HPLC-ESI-MS/MS methods for metabolite profiling of propolis extracts. *Journal of Pharmaceutical and Biomedical Analysis*, 55(5): 934–948. https://doi.org/10.1016/j.jpba.2011.03.024

Petruzzi, L., Corbo, M. R., Campaniello, D., Speranza, B., Sinigaglia, M. and Bevilacqua, A. (2020). Antifungal and antibacterial effect of propolis: A comparative hit for food-borne pseudomonas, enterobacteriaceae and fungi. *Foods*, 9(5): 559. https://doi.org/10.3390/foods9050559

Pobiega, K., Igielska, M., Włodarczyk, P. and Gniewosz, M. (2021). The use of pullulan coatings with propolis extract to extend the shelf life of blueberry (*Vaccinium corymbosum*) fruit. *International Journal of Food Science & Technology*, 56(2): 1013–1020. https://doi.org/10.1111/IJFS.14753

Rahman, M. M., Richardson, A. and Sofian-Azirun, M. (2010). Antibacterial Activity of Propolis and Honey Against Staphylococcus aureus and Escherichia coli. *African Journal of Microbiology Research*, 4(18): 1872–1878.

Ramanauskiene, K., Inkeniene, A. M., Petrikaite, V. and Briedis, V. (2013). Total phenolic content and antimicrobial activity of different lithuanian propolis solutions. *Evidence-Based Complementary and Alternative Medicine*, 2013: 842985. https://doi.org/10.1155/2013/842985

Rasul, A., Millimouno, F. M., Ali Eltayb, W., Ali, M., Li, J. and Li, X. (2013). Pinocembrin: A novel natural compound with versatile pharmacological and biological activities. *BioMed Research International*, 2013: 379850. https://doi.org/10.1155/2013/379850

Rollini, M., Mascheroni, E., Capretti, G., Coma, V., Musatti, A. and Piergiovanni, L. (2017). Propolis and chitosan as antimicrobial and polyphenols retainer for the development of paper based active packaging materials. *Food Packaging and Shelf Life*, 14: 75–82. https://doi.org/10.1016/j.fpsl.2017.08.011

Safaei, M. and Roosta Azad, R. (2020). Preparation and characterization of poly-lactic acid based films containing propolis ethanolic extract to be used in dry meat sausage packaging. *Journal of Food Science and Technology*, 57(4): 1242–1250. https://doi.org/10.1007/s13197-019-04156-z

Saleh, A., Abd El-Malek, F. and Moussa, M. (2020). Extended shelf life of tallaga cheese by natural preservatives. *Journal of Productivity and Development*, 25(1): 25–37. https://doi.org/10.21608/jpd.2020.81013

Sforcin, J. M. and Bankova, V. (2011). Propolis: Is there a potential for the development of new drugs? *Journal of Ethnopharmacology*, 133(2): 253–260. https://doi.org/10.1016/j.jep.2010.10.032

Shavisi, N., Khanjari, A., Basti, A. A., Misaghi, A. and Shahbazi, Y. (2017). Effect of PLA films containing propolis ethanolic extract, cellulose nanoparticle and Ziziphora clinopodioides essential oil on chemical, microbial and sensory properties of minced beef. *Meat Science*, 124: 95–104. https://doi.org/10.1016/j.meatsci.2016.10.015

Siripatrawan, U. and Vitchayakitti, W. (2016). Improving functional properties of chitosan films as active food packaging by incorporating with propolis. *Food Hydrocolloids*, 61: 695–702. https://doi.org/10.1016/J.FOODHYD.2016.06.001

Talon, R., Lebert, I., Lebert, A., Leroy, S., Garriga, M., Aymerich, T., … Lauková, A. (2007). Traditional dry fermented sausages produced in small-scale processing units in Mediterranean countries and Slovakia. 1: Microbial ecosystems of processing environments. *Meat Science*, 77(4): 570–579. https://doi.org/10.1016/j.meatsci.2007.05.006

Tosi, B., Donini, A., Romagnoli, C. and Bruni, A. (1996). Antimicrobial activity of some commercial extracts of propolis prepared with different solvents. *Phytotherapy Research*, 10(4): 335–336.

Tucker, G. S. (2015). Food Preservation and Biodeterioration. In Food Preservation and Biodeterioration. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118904657

Ustunol, Z. (2009). Edible Films and Coatings for Meat and Poultry. Edible Films and Coatings for Food Applications, Eds: Huber, K. C. and Embuscado, M. E., Springer Nature. https://doi.org/10.1007/978-0-387-92824-1_8

Velazquez, C., Navarro, M., Acosta, A., Angulo, A., Dominguez, Z., Robles, R., … Hernandez, J. (2007). Antibacterial and free-radical scavenging activities of Sonoran propolis. *Journal of Applied Microbiology*, 103(5): 1747–1756. https://doi.org/10.1111/j.1365-2672.2007.03409.x

Vică, M. L., Glevitzky, M., Tit, D. M., Behl, T., Heghedűş-Mîndru, R. C., Zaha, D. C., … Bungău, S. (2021). The antimicrobial activity of honey and propolis extracts from the central region of Romania. *Food Bioscience*, 41: 101014. https://doi.org/10.1016/J.FBIO.2021.101014

Xu, H. X. and Lee, S. F. (2001). Activity of plant flavonoids against antibiotic-resistant bacteria. *Phytotherapy Research*, 15(1): 39–43.

Yingyuad, S., Ruamsin, S., Reekprkhon, D., Douglas, S., Pongamphai, S. and Siripatrawan, U. (2006). Effect of chitosan coating and vacuum packaging on the quality of refrigerated grilled pork. *Packaging Technology and Science*, 19(3): 149–157. https://doi.org/10.1002/pts.717

Yu, D., Jiang, Q., Xu, Y., and Xia, W. (2017). The shelf life extension of refrigerated grass carp (Ctenopharyngodon idellus) fillets by chitosan coating combined with glycerol monolaurate. *International Journal of Biological Macromolecules*, 101: 448–454. https://doi.org/10.1016/J.IJBIOMAC.2017.03.038

Yurteri, Ü. (2015). *Investigation of bioactivity and chemical content of Turkish propolis from Ankara province.* (MSc. Thesis) Middle East Technical University, Institute of Natural and Applied Sciences, Biotechnology Department, Ankara, Türkiye.

Zabaiou, N., Fouache, A., Trousson, A., Baron, S., Zellagui, A., Lahouel, M. and Lobaccaro, J. M. A. (2017). Biological properties of propolis extracts: Something new from an ancient product. *Chemistry and Physics of Lipids*, 207: 214–222. https://doi.org/10.1016/j.chemphyslip.2017.04.005

Zheng, X., Shi, X., and Wang, B. (2021). A review on the general cheese processing technology, flavor biochemical pathways and the ınfluence of yeasts in cheese. *Frontiers in Microbiology*, 12: 703284. https://doi.org/10.3389/FMICB.2021.703284

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 Morganella psychrotolerans during chilly storage. *Journal of Food Safety*, 40(2): 12767. https://doi.org/10.1111/jfs.12767