Karamanoğlu Mehmetbey Üniversitesi
Mühendislik ve Doğa Bilimleri Dergisi
Karamanoğlu Mehmetbey University
Journal of Engineering and Natural Sciences
e-ISSN: 2687-5071 © KMUJENS

DOI: 10.55213/kmujens.1488048 Research Article (Araştırma Makalesi) 6(2): 9-17 (2024)

The Effects of Various Ethanolic Extracts on Some Microbiological Properties of Chicken Sausages

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Alındı/Received: 22/05/2024; Kabul/Accepted: 20/07/2024; Yayın/Published: 29/11/2024

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Abstract

In this study, the antimicrobial effects of different levels (0%, 5%, 10%) of ethanolic extract of propolis (EEP), sage (EES), lavender (EEL), yarrow (EEY), and St. John's Wort (EEC) on total psychrotroph aerobic bacteria (TPAB), total coliform and fecal coliform, yeast-mold in chicken sausage were investigated. The sausages were divided into seven groups. The first and second groups were the control samples. The surface of second control group of chicken sausage was sprayed with only ethyl alcohol (70%). The third, fourth, fifth, sixth, and seventh groups were sprayed with EEP, EES, EEC, EEY, and EEL at two levels, respectively: 5%, and 10%. Prior to each treatment, sausages were left to be stored at +4ºC for ten days. All extracts significantly ($P<0.05$) reduced the numbers of total psychrotroph aerobic bacteria (TPAB), total coliform and fecal coliform, and yeast mold compared with control samples. 10% EEP reduced the TPAB count to 6.61 log cfu/g compared to the control (8.82 log cfu/g). 5% EEC showed the least antimicrobial effect (33.03 log cfu/g) on coliform bacteria. The antimicrobial effect of 5% EEY (1.21 log cfu/g) and 5% EEP (0.61 log cfu/g) were determined higher than 10% EEY (16.14 log cfu/g) and 10% EEP (3.87 log cfu/g) on coliform bacteria, respectively. Yeast-mold number in chicken sausages reached 10.5 log cfu/g on the 10th day of storage. EEP decreased the population more efficiently than the other extracts and ethanol treatments. The results indicate EEP, EEL, EES, EEY, and EEC as antimicrobial agents might be used to reduce the number of microorganisms in sausages.

Key Words: Propolis, sage, lavender, St. John's Wort, yarrow

Çeşitli Etanol Ekstraktlarının Tavuk Sosislerinin Bazı Mikrobiyolojik Özellikleri Üzerine Etkileri

Öz

Bu çalışmada farklı düzeylerde (%0, %5, %10) propolis (EEP), adaçayı (EES), lavanta (EEL), civanperçemi (EEY) ve sarı kantaron (EEC) etanolik ekstraktlarının antimikrobiyal etkileri araştırılmıştır. Tavuk sosisinde toplam psikrotrof aerobik bakteri (TPAB), toplam koliform ve fekal koliform, maya-küf analizleri gerçekleştirilmiştir. Sosisler 7 gruba ayrılmış olup birinci ve ikinci grup kontrol örnekleri olarak işaretlenmiştir. İkinci kontrol grubu tavuk sosisinin yüzeyine sadece etil alkol (%70) püskürtülmüştür. Üçüncü, dördüncü, beşinci, altıncı ve yedinci gruplara sırasıyla %5, %10 olmak üzere iki düzeyde EEP, EES, EEC, EEY ve EEL püskürtülmüştür. Her uygulamadan önce sosisler +4°C'de 10 gün süreyle depoya bırakılmıştır. Tüm ekstraktlar, kontrol örnekleriyle karşılaştırıldığında toplam psikrotrof aerobik bakteri (TPAB), toplam koliform ve fekal koliformu, maya-küf sayılarını önemli ölçüde azaltmıştır ($P<0.05$). %10 EEP'nin, kontrole (8,82 log kob/g) kıyasla TPAB sayısını 6,61 log kob/g'a düşürdüğü tespit edilmiştir. %5 EEC, koliform bakteriler üzerinde en az antimikrobiyal etkiyi (33,03 log kob/g) göstermiştir. Koliform bakteriler üzerinde %5 EEY (1,21 log kob/g) ve %5 EEP'nin (0,61 log kob/g) antimikrobiyal etkisinin %10 EEY (16,14 log kob/g) ve %10 EEP'den (3,87 log kob/g) daha yüksek olduğu belirlenmiştir. Tavuk sosislerinde maya-küf sayısı depolamanın 10. gününde 10,5 log kob/g'a ulaşmıştır. EEP'nin, populasyonu diğer ekstraktlara ve etanol muamelelerine göre daha etkili şekilde azalttığı tespit edilmiştir. Sonuçlar, antimikrobiyal ajan olarak EEP, EEL, EES, EEY ve EEC'nin sosislerdeki mikroorganizma sayısını azaltmak için kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Propolis, adaçayı, lavanta, sarı kantaron, civanperçemi

Atıf / To cite: Yerlikaya S, Şen Arslan H, Şimşek H, Özkaya N (2024). The effects of various ethanolic extracts on some microbiological properties of chicken sausages. Karamanoğlu Mehmetbey University Journal of Engineering and Natural Sciences, 6(2): 9-17.

1. INTRODUCTION

Nowadays, the number of consumers researching the components of the products they buy is increasing. Conscious consumers have turned to foods that do not contain chemical preservatives. For this purpose, various herbal and livestock (especially beekeeping) products have been used. By using the antimicrobial and antioxidant properties of these products, the shelf life of foods is tried to be increased naturally (Lopez and Belloso 2008; Seçkin et al. 2010).

Pathogenic microorganisms such as *Escherichia coli, Staphylococcus aureus, Salmonella spp, Listeria monocytogenes, Entercoccus spp.* and *Clostridium perfringens* are encountered in the analyzes performed in emulsified meat products due to reasons such as unhygienic production, storage in bad conditions, and non-compliance with the cold chain (Elmalı et al. 2005; Güner et al. 2012). Especially meat products pose a risk due to their nature as with all food products. Food safety rules must be followed at all stages, such as production, processing, packaging, storage, and distribution (Tosun and Demirbaş 2012).

Salvia officinalis L. is a medicinal sage species officially accepted for use in Europe. This species are widely grown in Turkey, too. Its essential oils are known to have an antimicrobial effect (Riahi et al. 2013). According to studies, lavender and sage have high antioxidant and antimicrobial properties due to the phenolic compounds they contain. These plants' oils have antimicrobial properties (Moon et al. 2006).

Yarrow (*Achillea millefolium*) is a plant that is widely distributed in the northern hemisphere and Turkey. Some *Achillea species have been found to have antispasmodic, anti-inflammatory,* and antimicrobial properties. These pharmacological properties are believed to result from flavonoid and phenolcarbonic acid complexes. The antimicrobial effect of yarrow on the microorganisms *Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhimurium, Citrobacter freundii, Candida albicans,* and *Aspergillus fumigatus* was examined by Kazemi (2015). Kazemi (2015) found that fungi were more susceptible than bacteria and that yarrow showed higher antibacterial activity compared to the antibiotics tested (ampicillin and fluconazole). It has been found by many researchers that essential oils obtained from various *Achillea* species have antifungal, antibacterial and antimicrobial effects (Tuberoso et al. 2005; Filippi et al. 2006; Kordalı et al. 2009; Demirci et al. 2011).

Salvia officinalis L. is a type of medicinal sage that is officially accepted in Europe. This species grows widely in Turkey and is used for medicinal purposes. Medicinal sage (*Salvia officinalis L.*), known as the Mediterranean plant, belongs to the Labiatae family and contains essential oil. Since many species of the Salvia genus are medicinal plants used in the treatment of various diseases from ancient times to the present day, they have been studied by various researchers in terms of their chemical components and the substances they carry. According to research data, sage species are important in terms of the flavonoids and essential oils and components they contain. Sage also has a disinfectant effect (Jazo et al. 2023).

Flavonoids, triterpenoids, and 2-hydroxycinnamic acid esters have been identified in the leaves of lavender species. The major flavonoid components in the leaves are flavone glycosides. These are simple flavone glycosides, flavone C-glycosides, 6-hydroxyflavone 7-glycosides, and 8-hydroxyflavone 7-8 glycosides. It has been reported that 0.7% of the weight of the dry leaves of *L. angustifolia* and 1.0-1.9% of the dried leaves of *L. latifolia* are ursolic acid and are found together with 0.5% oleanolic acid. Rosmarinic acid, chlorogenic acid, and caffeic acid were found from 2-Hydroxycinnamic acid esters (El-Feky and Aboulthana 2016)

Lavender flowers contain 1-3% essential oil. 60-65% of lavender essential oil consists of monoterpene alcohols, 20-45% of which is linalool, and 25-46% is linalyl acetate. Other terpenoids include 1,8 cineole, terpinen-4-ol, lavandulyl acetate, α-terpineol, camphor, limonene, geraniol and β-caryophyllene, and the non-terpenoid 3-octanone (Moon et al. 2006).

St. John's wort, known as Hypericum perforatum L., is in the Clusiaceae family. It is naturally distributed in Western Europe, Asia, and North Africa (Walker et al. 2001). It is also found in Australia, New Zealand, South Africa, and other warm regions of the world (Campbell and Delfosse 1984). H. perforatum L. grows naturally in Turkey, at altitudes from sea level to 2500 m and in temperate conditions (Davis 1982).

In many studies, It is stated that Hypericum perforatum L. contains 0.1-0.3% dianthron (hypericin, pseudohypericin, and hypericin-like substances), flavonoids, 3% hyperforin, 0.2-1% essential oil and tannin substances (Wichtl 1986; Berger et al. 1996; Bomme 1997; Wagner 1980).

The whole extract and some identified phytochemicals from some Hypericum species exhibit numerous pharmacological properties, ranging from wound healing and antiseptics to antiviral, anti-inflammatory, antitumoral, and apoptosis-inducing activities. In recent studies, St. John's Wort; antioxidant, antifungal, antimicrobial and cytotoxic activities have been confirmed (Çakır et al. 2005; Hosseinzadeh et al. 2005).

Del Monte et al. (2015) by St. John's Wort Bacillus cereus, an enterotoxigenic strain of E. coli; Its effect on Staphylococcus aureus and P. aeruginosa was examined. It was concluded that the antimicrobial effect of St. John's wort is proportional to the total amount of phenolic substances contained in St. John's wort.

It has been supported by many studies that various active substances found in most Hypericum species have antimicrobial activity (Bombardelli and Morazzoni 1995; Dall'Agnol et al. 2003).

The chemical structure of propolis differs depending on the plant origin from which it was obtained. Propolis contains 45-55% resin, 23-35% waxes and fatty acids, 10% essential oils, 5% pollen and 5% other organic substances and minerals (Scazzocchio et al. 2006). It was reported that wax content of propolis was between 11.2-29.3%, in a study conducted with propolis and honeycomb wax samples. Monoesters formed the largest fractions (62.1–86.6%) in both types of wax. Hydrocarbons (6.9-24.7%) followed monoesters (Polat and Koçan 2006). Various effects, such as antioxidant and antimicrobial, of propolis provide the opportunity to be used in the field of food technology. Propolis' antimicrobial activity is one of the most widely known and most important properties. Antimicrobial effects of propolis on different bacteria, fungi, viruses etc. have been studied in different investigations, in different years (Dığrak et al. 1995; Salomão et al. 2004).

In this study, some antimicrobilogical properties of ethanolic extracts of sage (*Salvia officinalis L.*) (EES), lavender (*Lavandula angustifolia Mill*) (EEL), yarrow (*Achillea millefolium)* (EEY), St. John's Wort (*Hypericum perforatum*) (EEC) and propolis (EEP) sprayed on all of the surface of the chicken sausages were investigated during refrigeration conditions.

2. MATERIAL AND METHOD

2.1. Material

The raw chicken sausage samples were used in this research. Samples were bought from markets in Karaman; sage, lavender, yarrow and St. John's Wort were obtained from Rasayana organic products company located in Konya; propolis was collected from Pertek district of Tunceli. All materials were brought to Karamanoğlu Mehmetbey University, Faculty of Engineering and Food Engineering Department laboratory under aseptic conditions. Sausages were provided on the day of analysis. Plants and propolis extracts were prepared 1 day before analysis and stored at +4°C until analysis.

2.2. Methods

Ethanol was chosen as the organic solvent (due to the high dissolution of propolis in ethanol). In addition, the present study used ethanolic extracts of sage, lavender, yarrow, and St. John's Wort. The insolubility or little dissolution of propolis in water was also effective in the choice of ethanol as the solvent. The analyzes were carried out in two replicates and two parallel.

2.2.1. Preperation of Ethanolic Propolis Extract (EEP)

For 5% EEP, 5 g of propolis was resolved in 95 ml of 70% ethyl alcohol; for the extract of 10% EEP, 10 g of propolis was resolved in 90 ml of 70% ethyl alcohol. The solution was left in a closed container in a light-free environment for one week. The solution was shaken twice a day. Solution was filtered through Whatman no: one filter paper at the end of the period, placed in sterile bottles, and stored at +4°C until used for analysis (maxiumum 3 days before analyses) (Lu et al. 2005).

2.2.2. Preperation of Ethanolic Extracts of Sage (EES), Lavander (EEL), Yarrow (EEY) and St. John's Wort (EEC)

Classical method was preferred as extraction method. For 5% ethanolic extract of each material, 10 g of plant in 95 ml of 70% ethyl alcohol; for 10% ethanolic extract, 20 g of plant in 90 ml of 70% ethyl alcohol were kept in shaking water bath by shaking at 65°C for 1 hour. The extracts taken from the water bath were filtered through coarse filter paper and collected into the flask. The remaining part of the filter paper was put back in volumetric bottles and 95 ml of 70% ethanol for 5% and 90 ml of 70% ethyl alcohol for 10% was added and extracted at 65ºC for 1 hour. Each extract was filtered and transferred to the flask. The extracts obtained were stored at -18ºC until used in research, up to 3 days (Şen Arslan and Çam 2022).

2.2.3. Preperation of Sausages for Microbiological Analysis

Raw chicken sausages used in the study were divided into 4 groups. The first and second groups were prepared as control samples (has no any extract). The first control sample was prepared without treatment with any of the ethanolic extracts of sage, lavender, yarrow, St. John's Wort and propolis. The second control group was sprayed with only ethanol (70%). In the third and fourth groups, 2 different levels (5% and 10%) of extracts (sage, lavender, yarrow, St. John's Wort and propolis) were added as spraying (2 ml per sample) to the both surface of the samples. For this purpose, 10 ± 0.5 g sausage sample was weighed into sterile stomacher bags for each group. Then they were stored at +4°C for 10 days. Total psychrotroph aerobic bacteria (TPAB), total coliform and fecal coliform, yeast-mold analysis were performed on 0, 2, 4, 6, 8 and 10 days of storage.

2.2.4. Microbiological Analysis

2.2.4.1. Total Psychrotroph Aerobic Bacteria (TPAB)

Sausage samples were added to 90 mL of sterile peptone water. Then, samples homogenised in a stomacher for 1 min. Decimal dilutions were prepared using sterile peptone water (0.1%, w/v) and the total psychrotrophic viable cells were counted by the spread plate method. Plate count agar (PCA; Merck, Darmstadt, Germany) was used as a medium and incubated aerobically at 4°C for 10 days for the enumeration (AOAC 2000).

2.2.4.2. Total Coliform and Fecal Coliform

The analysis were carried out using the most probable number (MNP-3 tube) method. In MNP method, 3 Lauryl Sulphate Tryptose Broth (LSTB) (Merck, Germany) tubes were used. 1 mL of sample dilutions was added to LSTB. The tubes were left for incubation at 37°C for 24-48 hours. After incubation, gas positive tubes were found out and the number of coliform bacteria was calculated using the MNP table. In order to prove the probability test results,

Brilliant Green Bile Broth (Merck, Germany) medium containing durham tube from all gas positive tubes were inoculated with a loop. After incubating at 37°C for 24-48 hours; the number of proven coliform bacteria in 1 mL of the first dilution was determined using the MNP table. This value was multiplied by the dilution factor of the initial dilution and the number of proven coliform bacteria per 1 gram of sample was calculated. In order to count the fecal coliforms, LSTB tubes, which gave positive results in total coliform analysis, were inoculated with *Escherichia coli* (EC) Broth with a durham tube. Then they were incubated at 45°C for 24-48 hours. The tubes with gas formation were determined, using the MNP table, the possible number of fecal bacteria in 1 mL of the first dilution was determined. This value was multiplied by the first dilution factor and the number of probable fecal coliform bacteria in 1 gram of food was found out (Feng et al. 1998).

2.2.4.3. Yeast- Mold

For the yeast-mold count, the sterilized Patato Dextrose Agar (PDA) medium supplemented with 10% tartaric acid (Merck, Germany) was used. 0.1 mL of the prepared dilutions was transferred to petri dishes. Then the petri dishes were incubated at 28°C for 4-5 days. All colonies that developed at the end of incubation were counted and expressed as yeast-mold (Halkman 2005).

2.2.5. Statistical Analysis

Results were statistically analyzed using SPSS 22 (IBM Corp., Armonk, New York, USA) program. Sample averages were compared by applying one-way and two-way ANOVA. The 95% confidence interval was studied.

3. RESULTS and DISCUSSION

The effect of extracts (Table 1) and storage time (Table 2) were found to be statistically significant (P <0.05) on the TPAB, coliform and yeast-mold. Table 1 shows the effects of ethanolic extracts on mean TPAB, coliform and yeast-mold.

3.1. Total Psychrotroph Aerobic Bacteria (TPAB)

TPAB count of sausages (control) was found as 8.82 log cfu/g, ethanol showed the least antimicrobial effect (8.31 log cfu/g). 10% EEP showed the highest inhibitory effect as 6.61 log cfu/g. If extracts are ranked from the most antimicrobial activity to the lowest, it can be represented as EEP, EES, EEC, EEL and EEY. The inhibitory effect of 5% EES (7.74 log cfu/g) and 5% EEC (7.83 log cfu/g) were determineted higher than 10% EES (7.95 log cfu/g) and 10% EEC (7.90 log cfu/g), respectively. The antimicrobial effect of 10% concentrations of other extracts was found to be higher than 5% concentrations. TPAB was high in all samples (except 10% EEP). 10% EEP in agreement with Turkish Food Codex Microbiological Criteria, *i.e.* <5.10^6 CFU/g (Food Codex 2011).

Table 1. Effects of EES, EEL, EEY, EEC and EEP on TPAB, coliform and yeast-mold count log cfu/g.

Factor	*TPAB*	*Coliform*	*Yeast-Mold*
Sausage	8,82±0,55^{a}	33,73±0,02^{a}	8,55±0,19^{a}
Ethanol	8,31±0,36^{ab}	13,37±0,01^{c}	6,99±0,23^{c}
EES 5%	7,74±0,33^{c}	19,37±0,02^{b}	6,89±0,29^{c}
EES 10%	7,95±0,14^{b}	14,89±0,02^{c}	6,55±0,89^{cd}
EEL 5%	8,05±0,14^{b}	11,04±0,02^{c}	7,65±0,58^{b}
EEL 10%	7,94±0,12^{b}	2,14±0,02^{d}	7,01±0,62^{b}
EEY 5%	8,18±0,26^{ab}	1,21±0,01^{d}	7,53±0,36^{b}
EEY 10%	7,83±0,51^{bc}	16,14±0,21^{bc}	7,67±0,32^{b}
EEC 5%	7,83±0,19^{bc}	33,03±0,23^{a}	7,66±0,57^{b}
EEC 10%	7,90±0,74^{b}	32,83±0,45^{a}	7,32±0,62^{bc}
EEP 5%	7,47±0,32^{d}	0,61±0,01^{e}	6,88±0,45^{c}
EEP 10%	6,61±0,43^{e}	3,87±0,02^{d}	5,60±0,42^{d}

* indicates a significant difference between means (p <0.05), (EES; ethanolic extract of sage, EEL; ethanolic extract of lavander, EEY; ethanolic extract of yarrow, EEC; ethanolic extract of St. John's Wort, EEP; ethanolic extract of propolis)

Table 2 shows the count of TPAB, coliform and yeast-mold of first control group (please check which group is it, I understand first control group, S) of chicken sausage. According to the Table 2the count of TPAB increased during storage. TPAB in the sausage samples was found as 5.98 log cfu/g on 0. day. After 10 days of storage, the level of TPAB in the control sausages reached 11.37 log cfu/g. The treatment of sausage samples with all extracts reduced the levels of TPAB during storage period compared to the control sausages.

Table 2. Effects of only storage days (without samples) on mean TPAB, coliform and yeast-mold log cfu/g.

Storage (day)	*TPAB*	*Coliform*	*Yeast-Mold*
0	5,98±0,03^{c*}	6,50±0,02^{b}	4,83±0,03^{d}
2	6,44±0,03^{c}	6,43±0,01^{b}	6,22±,005^{c}
4	7,60±0,04^{c}	9,59±0,00^{a}	5,65±0,03^{c}
6	8,86±0,31^{b}	4,50±0,01^{d}	7,95±0,03^{b}
8	9,06±0,04^{b}	1,07±0,00^{d}	8,01±0,04^{b}
10	11,37±0,03^{a}	6,04±0,01^{bc}	10,50±0,03^{a}

* indicates a significant difference between means (p <0.05)

Figure 1 shows 10% EEC had the highest antimicrobial effect on TPAB as 4.52 log cfu/g on 4.day. 10% EEP showed the highest antimicrobial effect (7.88 log cfu/g) when the TPAB in the control sample reached 13.42 log cfu/g on the last day of storage. In various studies, it has been stated that the addition of 2% EEP (Viera et al. 2016) and 3-7% EEP (Payandan et al. 2017) additions to sausages have an antimicrobial effect on psychrophilic bacteria. Many studies have reported that EES, EEY, EEL, EEC and EEP has an antimicrobial effect (Ayar et al. 2002; Bakkaloğlu and Arıcı 2019; Çalışkan 2019; Da Silva et al. 2018; Ekiz 2016; Monroy et al. 2017; Yılmaz and Ergün 2012; Yerlikaya 2021). However, there are also studies in which the inhibitory activity of EEP is higher than the others (Candan and Bağdatlı 2018; Yerlikaya and Şen Arslan 2022). It is thought the phenolic compounds it contains cause the high antimicrobial activity of propolis. Vasinauskiene et al. (2006) stated EEY has inhibtory effect against *Pseudomonas spp. and Bacillus spp* as psychrotroph aerobic bacteria.

Yerlikaya et al. (2021) reported 5% and 10% EEY has antibacterial activity on *Bacillus cereus* as TPAB.

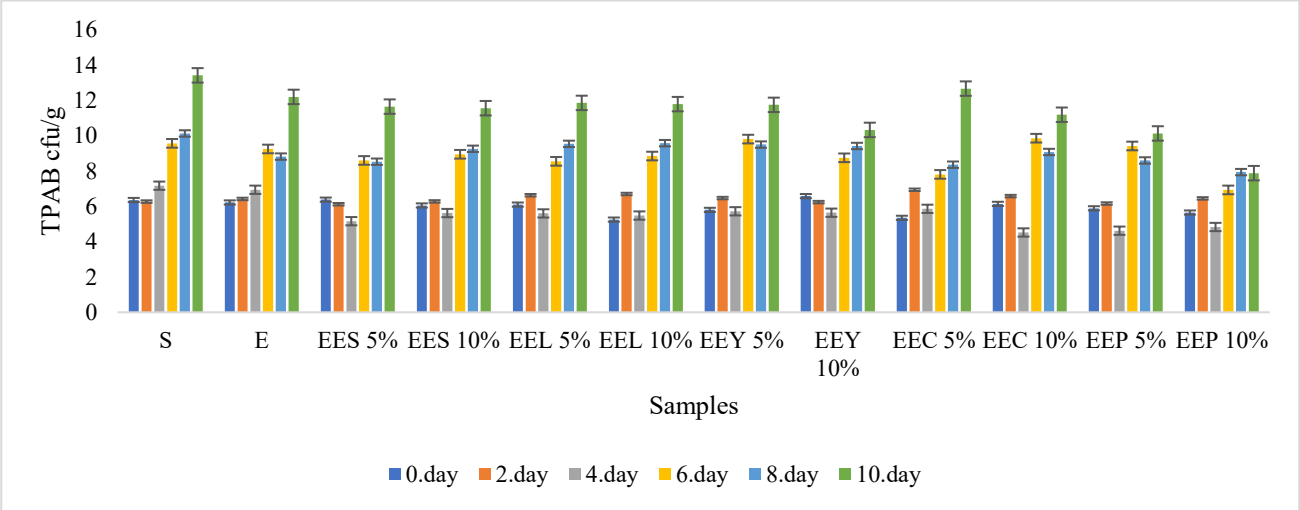

Figure 1. Inhibitory effect of extracts on TPAB depent on storage time.

3.2. Total Coliform and Fecal Coliform

The coliform count of sausages was found as 33.73 log cfu/g, and 5% EEC showed the least antimicrobial effect (33.03 log cfu/g). 5% EEP showed the highest inhibitory effect as 0.61 log cfu/g. If extracts are ranked from the most antimicrobial activity to the lowest, it can be represented as EEP, EEL, EEY, EES and EEC. The inhibitory effect of 5% EEY (1.21 log cfu/g) and 5% EEP (0.61 log cfu/g) were determined higher than 10% EEY (16.14 log cfu/g) and 10% EEP (3.87 log cfu/g), respectively. The antimicrobial effect of 10% concentrations of other extracts was found to be higher than 5% concentrations (Table 1). Table 2 shows that there is a fluctuation in the storage process. Coliform was found as 6.50 log cfu/g on 0.day of storage; this number decreased 1.07 log cfu/g on 8.day of the storage. Containing coliform bacteria, which is accepted as a hygiene indicator, make think insufficient heat and time applications during the cooking of the products, or the possibility of exposure to a secondary contamination of the products. Figure 2 shows 10% EEP had the highest antimicrobial effect on coliform as 0 log cfu/g in all storage days except 0.day. While the number of coliform bacteria in the control samples increased continuously during storage, it was determined that ethanol and ethanolic extract additions decreased this number. EES had the least antimicrobial effect on coliform bacteria. 10% concentration of all extracts showed more effect than 5% concentration.

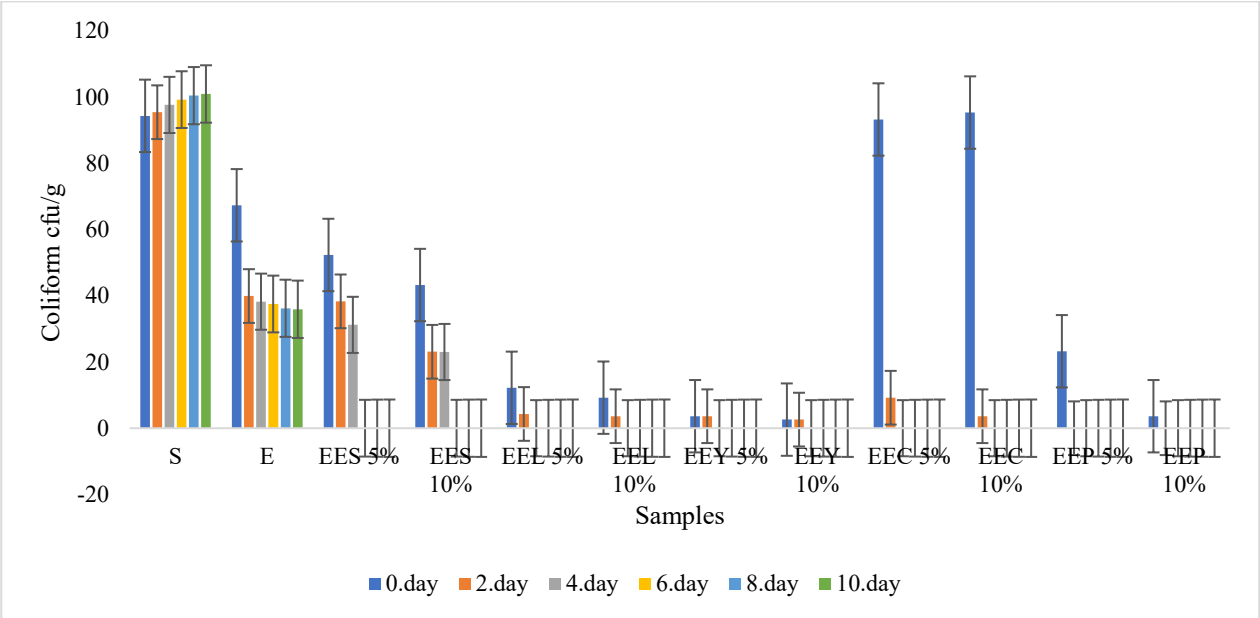

Figure 2. Inhibitory effect of extracts on coliform depend on storage time.

Viere et al. (2016) reported that 2% EEP has no inhibitory effect on total coliform bacteria in sausages. In the current study, 5% EEP showed a full antimicrobial effect on the 2. day of storage. Yerlikaya and Şen Arslan (2022) stated

EEP has more antibacterial effect on *E.coli* (a member of coliform bacteria) than EES and EEL. They also reported as *E.coli* concentration increased, antimicrobial effect of EEL is higher than that of EES. Sariçoban and Yerlikaya (2015) determined different concentration of propolis has antibacterial effect on *E.coli*. İlkimen and Gülbandilar (2018) reported EEL has higher antibacterial effect than EES on *E.coli* (as a coliform), as in this study. Serpi et al. (2012) indicated EEL has inhibitory activity on some pathogen microorganisms. Haşimi et al. (2015) and Nostro et al. (2000) determined different plants' ethanolic extracts have antimicrobial activity *E.coli*, as coliform bacteria.

3.3. Yeast-Mold

While the yeast-mold count of sausages was found as 8.55 log cfu/g, 10% EEY showed the least antifungal effect (7.67 log cfu/g). 10% EEP showed the highest inhibitory effect as 5.60 log cfu/g. If extracts are ranked from the most antifungal activity to the lowest, it can be represented as EEP, EES, EEL, EEC and EEY. The inhibitory effect of 5% EEY (7.53 log was determineted higher than 10% EEY (7.67 log cfu/g). The antifungal effect of 10% concentrations of other extracts was found to be higher than 5% concentrations (Table 1).

Table 2 shows, the count of yeast-mold count increased during storage (except for 4.day). 0.day of storage, the count of yeast-mold was found as 4.83 log cfu/g; this number reached 10.50 log cfu/g on 10.day of the storage. It was determined that 10% EEP showed the most antifungal activity on all storage days (Figure 3). 10% EEP reduced yeast-mold count to 8.2 log cfu/g when S (control) count was 12.49 log cfu/g at the end of storage. The yeast-mold concentration increased on the 10th day of storage in all treatments. On 0th day of storage, there was 5.92 log cfu/g yeast-mold in the S sample; 10% EEP (4.12 log cfu/g) showed the highest antifungal effect.

EEP decreased the population more efficient than the other extracts and ethanol treatments. Inhibitory activity by phenolic and other substances of propolis can be thought to be the cause of this situation. The higher inhibitory activity of propolis than the other extracts may be related to the flavanols, flavones, flavanones and isoflavones in its composition (Hema´ndez and Bemal 1990; Sforcin et al. 2000). Compared to ethanol (negative control), it is thought the inhibitory activity of propolis is due to other substances, not just ethanol in propolis.

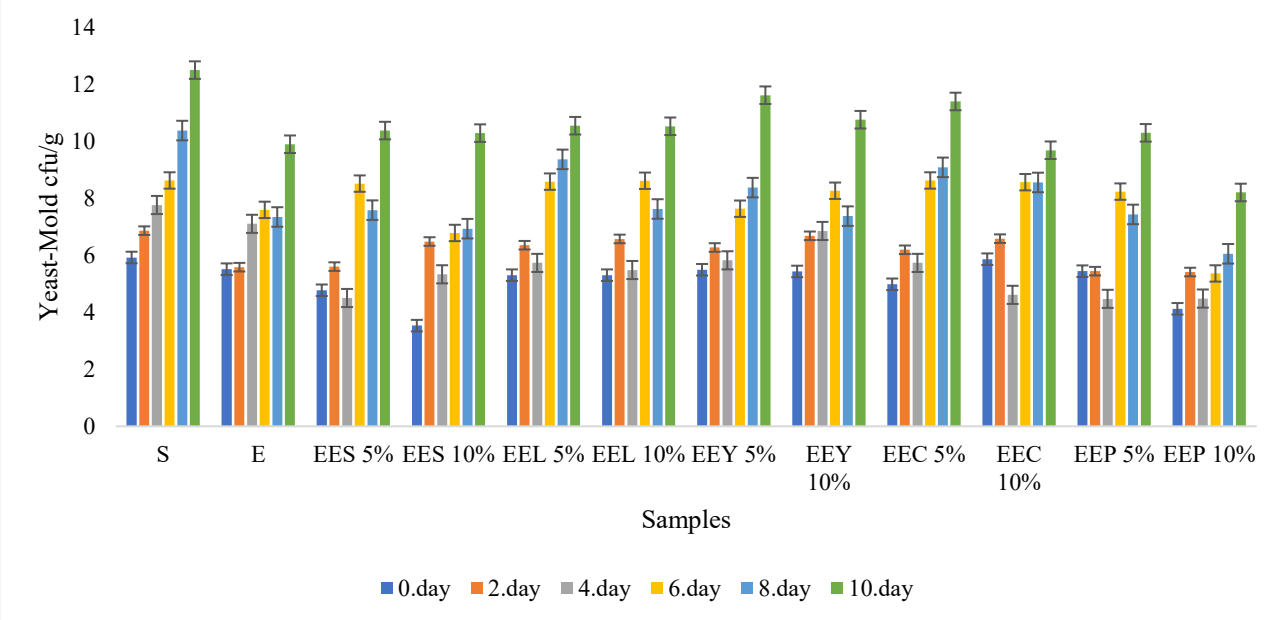

Figure 3. Inhibitory effect of extracts on yeast-mold depend on storage time.

Kujumgiev et al. (1999) stated that propolis collected from different geographical regions has antifungal activity. Bruni et al. (2003) stated that the antimicrobial effect of sage on yeast-mold is due to the phenols, aldehydes and ketones it contains.

4. CONCLUSION

Problems such as the inadequacy of the sausage production methods with modern technologies, the incorrect or insufficient application of heat treatment during production, and the poor microbiological quality of the raw materials used can cause the microbiological quality of the sausages to threaten public health. For this reason, serious microbiological problems may be encountered during storage in sausages. Various techniques are used to avoid these problems. One of them is to add natural additives without using chemical preservatives. In this study, various plant and propolis extracts were used as a natural method. It has been determined that all the extracts (EES, EEY, EEL, EEC and EEP) can be used in the storage of sausages for the inhibition of TPAB, coliform bacteria and yeast-mold. In particular, the antimicrobial effect of propolis was found to be higher than the others.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

THANKS

Financial support (scientific research project) was provided by Karamanoğlu Mehmetbey University (Project no: 08-M-19), Turkey

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