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REGULAR ARTICLE

# Antimicrobial Edible Cellulose-Based (CB) Films and Coatings for Enhancing Microbial Safety of White Cheese During Storage

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## ABSTRACT

In this research, the antioxidant and antibacterial characteristics of edible cellulose-based film (CBEF) coating incorporated with β-carotene, Hesperedin and Propolis on the quality of the white cheese at 4°C were investigated. Samples evaluated were: Control, CBE films with 8 different contents containing natural or non-natural antimicrobial agents for 15 days at 5 day intervals. The antimicrobial effects of edible cellulosic films prepared with antimicrobial agents against *Staphylococcus aureus* and *Escherichia coli*, which are important food pathogens, were directly evaluated with artificially contaminated food samples under cold storage conditions. Fresh white cheese samples, which are one of the semi-hard traditional Turkish cheeses, were used for artificial contamination. The antimicrobial activity of the tested antimicrobial substances was measured by the paper disc method. The DPPH free radical scavenging activities, the total phenolic content, and the characteristics (FT-IR, DSC and TGA) of the CBEFs were investigated. All of the samples significantly reduced *E. coli, S. aureus* and total viable counts, as compared with control during the storage time. Specifically, it was determined that hesperidin sprayed films showed an antimicrobial effect on *E.coli* growth and carotene sprayed films showed an antimicrobial effect on *S. aureus* growth. Growth of both bacterial species was highly inhibited (>50%) in the presence of 20% propolis. It was concluded that β-carotene, hesperidin and propolis can be substituted as natural preservatives in white cheese. With this advanced packaging technology, food safety can be ensured, the shelf life of the product can be extended and food losses in this sector can be reduced.

**Keywords:** ß-carotene; Hesperidin; Propolis; Antimicrobial activity; Cellulose-based edible film

## INTRODUCTION

The world population is increasing rapidly, but sufficient food production cannot be provided to meet this demand. Obviously, the preservation of food is as important as its production. Although, various packaging materials can be used for this purpose, some of them may cause environmental pollution. Due to the consumer's demand for microbiologically safe, practical and long shelf-life food, different preservation and packaging techniques are used in the food industry to delay enzymatic and bacterial spoilage and to ensure food safety. Currently, most of the innovative scientific studies have focused on preventing further microbial spoilage of food products and inhibiting microbial growth (Costa et al., 2018). Among the existing food packaging techniques, especially edible antimicrobial films and coatings have received great attention due to the diversity of materials used, application methods and food products. Traditional packaging is insufficient in preventing the development of some undesirable microorganisms in foodstuffs (Quintavalla & Vicini, 2002). Plastics, which are widely used in food packaging, are produced from petroleum-derived materials. Such packaging materials are considered by consumers and environmental activists as an important source of solid waste and environmental pollution (Risch, 2009 and Ramos et al., 2013). With the migration of petroleum-derived substances into foodstuffs, the degradation in foods accelerates and with the continuous consumption of these degraded foods, benzene derivatives cause accumulation of substances in the body (Assis et al., 2020). Since traditional plastic packaging is insufficient to transport food and deliver it to the consumer, alternative packaging methods have been needed (Quintavalla & Vicini, 2002). New and functional active packages continue to be produced. It has become more important to produce edible packaging with the use

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of antioxidant and/or antibacterial agents that can be applied directly into or on the surface of the food (Chhikara & Kumar, 2021). Edible films are organic biopolymer based materials (Galus et al., 2012). Petrochemical packaging materials have a non biodegradable structure and cannot decompose in nature while edible films and coatings produced from organic biopolymers can decompose in nature. Due to this feature, edible films and coatings have recently met in high demand as environmentally friendly packaging materials (Lee & Min, 2014).

Food spoilage can be controlled with antimicrobial substances that can be added to the structure of edible films and coatings. There are generally two reasons for food spoilage; microbial growth and oxidation reactions occurring on the product surface (Yangılar & Oğuzhan Yıldız, 2015). During storage, uncontrolled opportunistic microorganisms can cause off-taste or odor, discoloration, and/or produce toxic secondary metabolites. These can be risky for most consumers and are also associated with a negative impact on product acceptance (Lund et al., 2003). Initial high numbers of coliforms are due to the sub-pasteurisation of cheese milk. Although coliforms are suppressed by the growth of starter bacteria, they can easily re-contaminate cheeses through the environment (Moatsou et al., 2015). There are recorded cases of food poisoning, and the number is high given the amount of cheese consumed worldwide. Spoilage bacteria such as proteolytic and lipolytic bacteria can also grow in milk during storage and alter the quality and shelf life of the milk (O'Connell et al., 2016). Enterococci had the potential to survive pasteurisation and contribute to product spoilage during refrigeration (McAuley et al., 2015). Staphylococcal food poisoning (SFP) is very common in foods such as salads, baked goods, meat and meat products, milk and milk products (Hennekinne et al., 2012). SFP has occurred in the United States, Canada, and Europe with the ingestion of contaminated dairy products (Newkirk et al., 2011). Research over the past few decades has confirmed the ability of several foodborne pathogens to survive longer than 60 days of storage in cheese (Boor et al., 2017). When the 60-d rule was first adopted, it was understood that the ability of pathogens to survive the holding period varied among cheese species due to differences in properties such as pH, salt, moisture, water activity, and temperature (Johnson et al., 1990). Researchers from various institutions collaborated to provide more details on the range of product parameters found in modern raw milk cheeses (Trmčić et al., 2017), but further studies are needed to understand the impact of these parameters and storage conditions. Elimination and control of spoilage organisms and post-pasteurization contamination will also remain an important area in cheese (Boor et al., 2017).

In this context, edible antimicrobial films, which are the product of a new technology, are more needed during the storage process. The inclusion of antimicrobials in film formulations can regulate the rate of diffusion into the product, providing a way to maintain high concentrations of the active ingredient at the surface. Fixing the additive to the surface also reduces interaction with food ingredients and/or other additives. Consequently, films or coatings with antimicrobial activity are a suitable form of antimicrobial delivery in relation to food preservation (Costa et al., 2018).

Edible films and coatings have some advantages over normal packaging materials. They can be consumed with food and do not cause pollution in nature. However, there are also disadvantages such as preparing different processes for each product, low number of food materials to be applied and high cost (Oguzhan & Yangilar, 2016). Starch, cellulose, alginate, pectin and chitosan are frequently used in the production of polysaccharide-based edible films (Lai & Wong, 2022). The addition of antimicrobial and antioxidant agents to edible coatings and films, gradual release of the agents, and maintenance of a critical concentration over an extended period of time are important factors during the storage phase of foods (Appendini & Hotchkiss, 2002). The antimicrobial and antioxidant agents used in edible films and coatings can be natural or be synthesized (Silva & Lidon, 2016).

Hesperidin is a flavonoid abundantly found in the peel and membranes of lemon and orange fruit (Erlund et al., 2001). Beta carotene which is also called Provitamin A is a light yellow/orange pigment. Studies have shown that beta carotene has properties such as photoprotection against harmful light during photosynthesis, antioxidant, protection against cancer, increasing immune response, and inhibiting tumor growth. It is also included in the content of some cheese products and margarines (Girard et al., 1994). Propolis is a natural resinous substance that bees collect from parts of plants, plant buds and plant secretions (Ghisalberti, 1979). More than 300 different compounds which have very complex chemical structures have been identified in propolis. It has been determined that propolis can be used both as a nutritional additive and to prevent oxidation in the food industry due to its high antioxidative activity (Mašek et al., 2018). In addition, the functions of propolis such as water activity, pH balancing, color protection and prevention of microbial degradation have been defined in the studies. The advantages of using natural antimicrobial agents are large-scale production, cost-effectiveness, long storage times and long shelf life (Santos et al., 2022).

The production of milk and dairy products in our country is quite high, and most of these products are produced in

small businesses and dairy farms in a less controlled manner (Guven K., et al., 2010). Especially cheeses obtained from raw milk pose great risks to public health. Due to these production conditions, the risk of infection and food poisoning from milk and dairy products increase. They also pose serious risks during the storage process (Pereira et al., 2009). One of the most important problems in the cheese industry is microbial degradation during storage (Garnier et al., 2017). Antimicrobial edible food packaging systems inhibit the growth of pathogenic microorganisms, reduce microbial spoilage and maintain microbial quality. Today, cheeses are usually packaged in plastic packages that are not biodegradable and cause an important environmental problem (Senturk Parreidt et al., 2018)

In this study, the antimicrobial effect of edible cellulosic films prepared by spraying β-carotene, hesperidin, and propolis either separately or as a mixture against *Staphylococus aureus* and *Escherichia coli* was evaluated under cold storage conditions by directly coating artificially contaminated cheese samples.

## MATERIALS AND METHOD

### Chemical materials

Microcrystalline cellulose, potassium sorbate, β-carotene and hesperedin were obtained from Sigma Co (USA). Propolis was obtained from SBS Americas Inc (Turkey). All other chemicals are of analytical grade.

### Microorganisms and cultures

*Escherichia coli* (ES DII) and *Staphylococcus aureus* (F6 III) strains were used as the white cheese contaminants in this study. These strains were obtained from the Refik Saydam National Type Culture Collection (Ankara/Turkey). Bacterial cultures were incubated in brain heart infusion broth (BHIB) medium at 37°C. Before measuring the antimicrobial activity of β-carotene, hesperidin, propolis and cellulosic edible film, 0.1 mL of culture was inoculated into 20 mL of fresh LB broth medium and allowed to incubate for 24 hours.

### Preparation of β-carotene, hesperidin and propolis

β-carotene and hesperidin solutions were prepared in DMSO at final concentrations of 1, 3 and 5% (w/v). Propolis solutions were used in PEG (Polyethylene glycol) at final concentrations of 10% (soluble in water) and 20% (insoluble in water) (v/v).

### Preparation of cellulose-based edible films containing β-carotene, hesperedin and propolis

The edible film mixture was prepared by adding microcrystalline cellulose (500 g/L), NaOH (570 g/L), urea (380 g/L) and distilled water to a final volume of 1L (Yang et al., 2011). The mixture was stirred at 0-5ºC for 15 min on a magnetic stirrer and kept at -8ºC for 6 h. At the end of the incubation period, a clear homogeneous solution was obtained. This mixture was poured into sterile plates to form thin layers. Coagulation was achieved by adding 1 M $CH_3COOH$ dropwise to the mixture. The synthesized films were washed with distilled water after 15 min and left to dry at room temperature (Fig. 1).

Each film was carefully separated from the plate and stored in a polyethylene bag for further analysis. Before each treatment, the films were kept under UV light for 2 min for sterilization. β-carotene, hesperedin and propolis extracts and potassium sorbate (10 mg/g cheese) were sprayed onto the surface of the sterilized films according to the experimental design indicated in Table 1.

### Preparation and artificial contamination of white cheese samples

White cheeses without preservatives were collected from local firms. The samples were transported to the laboratory maintaining cold chain. Thin sections (1-3 mm) with a size of 3×3 cm and a weight of approximately 3 g were taken from three separate points of each cheese mold. Each of the samples was numbered and placed in sterile petri dishes. Artificial contamination was performed by inoculating 0.1 mL of culture containing approximately

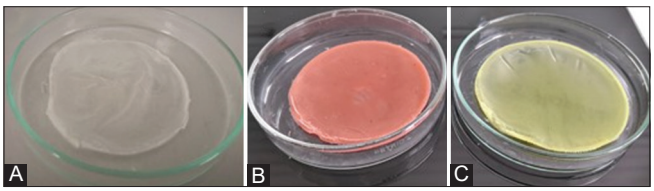


**Fig 1.** Cellulose-based edible film (A), β-carotene (B) and Hesperidin (C) sprayed CBE films.

**Table 1: Experimental design of cellulose-based edible films containing different antimicrobial agents**

| Treatment | Composition |
|---|---|
| CBEF | Microcrystalline cellulose[1], NaOH, urea |
| F1 | CBEF+C[a] |
| F2 | CBEF+C + P[b] |
| F3 | CBEF+C + P[c] |
| F4 | CBEF+H[d] |
| F5 | CBEF+H + P[b] |
| F6 | CBEF+H + P[c] |
| F7 | CBEF+P[b] |
| F8 | CBEF+P[c] |
| F9 | CBEF+PS[d] |

[a]C: 5% (w/v) Carotene
[b]P: 10% (v/v) Propolis
[c]P: 20% (v/v) Propolis
[d]H: 5% (w/v) Hesperedin
[e]PS: Potassium sorbate (10 mg/g cheese)
[1]Measured based on dry cellulose.
CBEF: Cellulose based edible film

$10^4$-$10^5$ CFU/mL bacteria on the cheeses. The contaminated cheeses were kept at 37°C for 15 minutes for better fixation of bacteria on the surface. Cheese samples that were not covered with film used as negative controls. Cellulose-based edible film containing only potassium sorbate (F9) was used as a positive control.

### Coating of cheese

Contaminated cheese samples were covered one by one with the films with different contents (Fig. 2). The coated cheeses were taken into sterile petri dishes and kept at +4°C for 15 days. Uncontaminated cheeses were also prepared as a control group. Viable cell count analyses were performed at 5-day intervals.

### Cheese homogenization and viable cell count

Cheese samples were taken into the sterile stomacher bags. The samples were homogenized with 27 ml of PBS buffer (phosphate buffer, pH; 7.4). The homogenate was exposed to appropriate dilution and were inoculated onto the agar plates with spread plate method.

### Antimicrobial activity of β-carotene, hesperedin and propolis solutions

The antimicrobial activity of β-carotene, hesperedin and propolis was measured with the agar disc diffusion method (Balouiri et al., 2016). Antimicrobial agents were filtered with a 0.45 μm membrane filter to remove possible microorganisms and added into solutions at 1%, 3% and 5% (w/v) concentrations.

The sterilized paper discs (diameter of discs is 12 mm) were placed on agar plates previously seeded with 0.1 mL inoculum containing $10^4$ -$10^5$ CFU/mL of *S. aureus* and *E. coli*. Solutions including antimicrobial agents (0.1 ml) were pipetted onto paper discs. Plates were incubated at 37°C for 24 hours. The diameter of the inhibitory zone surrounding the discs was measured. The experiments were performed in 5 replicates.

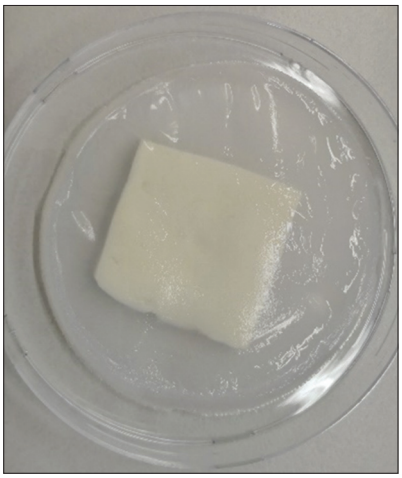

Fig 2. Coating of white cheese with a cellulose-based edible film.

### Antimicrobial activity of CBEFs containing β-carotene, hesperedin and propolis

The antimicrobial activity of cellulose-based edible films was measured with the agar disc diffusion method (Balouiri et al., 2016). Typical cheese bacterial contaminants (*E. coli* and *S. aureus*) were grown in nutrient broth at 37°C. Film discs of 12 mm diameter were cut and placed on nutrient agar plates containing approximately $10^4$-$10^5$ CFU/mL *E. coli* or *S. aureus*. Plates were then incubated at 37°C for 24 hours. The inhibitory zone surrounding the film discs was measured. Triple data are expressed as inhibition zone diameter (mm).

### Antioxidant determination by DPPH radical

DPPH (2,2-diphenyl-1-picrylhydrazil) free radical capture method is used to define antioxidant activity (Torun, 2019). The results were presented as mg TEAC (Trolox Equivalent Antioxidant Capacity)/gr antimicrobial agent.

### Determination of total phenolic substance content

The presence of phenolic substances in antimicrobial agents is important in terms of antioxidant capacity. According to the method proposed by Hatano et al. (1989 the total phenolic content of antimicrobial substances was performed using Folin-Ciocalteu reagent. Results are expressed as mg GAE (Gallic Acid Equivalent)/g antimicrobial agent.

### Instrumental analysis

Fourier-transform infrared (ATI UNICAM system 2000) spectroscopic analyses of all samples were done with a detector at a resolution rate of 4 $cm^{-1}$ in the range of 4000–400 $cm^{-1}$. The FTIR spectra of cellulose film was taken under an attenuated total reflection (ATR) mode (Kumar, 2020).

The thermal stability of the films was performed by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Thermogravimetric analysis was carried out by Shimadzu TGA-50 analyzers. A ten-milligram sample was heated from 30 to 700°C under air atmosphere at a heating rate of 10°C/min. Differential scanning calorimetry was performed using a Shimadzu DSC-60. The scanning rate was 10°C/min in the temperature range of 30-500°C under air atmosphere and the sample weight was 5 mg.

### Statistical analysis

The software package GraphPad Prism 5 (Ver. 5.0; Graph Pad Software Inc, USA) was used for statistical evaluations of the antimicrobial activities. The One Way ANOVA and Tukey tests were used for pairwise comparisons.

## RESULTS AND DISCUSSION

In this study, cellulose-based edible films were synthesized. Structural characterization of the films was obtained with FT-IR. Fig. 3 shows the FTIR spectrum of the cellulose-based edible films. In the FTIR spectra of the cellulose-based edible film, the characteristic peaks at 3600 and 3200 $cm^{-1}$ belong to O-H stretching vibrations. The OH stretching vibration peaks can be asiggned as 3600-3100 $cm^{-1}$. The peak at 3342 $cm^{-1}$ belongs to the symmetric stretching vibrations of -OH groups due to intermolecular hydrogen bonding. The vibration bands between 3000-2800 $cm^{-1}$ and 1500-1250 $cm^{-1}$ were attributed by C-H and $CH_2$ stretching and bending vibrations. The $CH_2$ bending vibration peak can be assigned as at 1430 cm-1. The peak at 1156 $cm^{-1}$ is associated with the C-O-C stretching vibrations. The peak absorption at 1058 and 1018 $cm^{-1}$ attributed to the C-O stretching. O-H bending vibrations bands appeared 894 and 650 $cm^{-1}$.

The TGA and DSC curves of edible cellulose-based films are shown in Fig. 4. The endothermic peak seen in DSC up to 100°C is due to the removal of moisture from the cellulose film structure. Endotherm up to 200°C in DSC and mass loss in TGA at the same temperature indicate removal of solvents in the structure. The exothermic peak observed in DSC at 450-500°C and the absence of weight loss in TGA at this temperature shows that the crystal structure of the cellulose film has changed. The exotherm seen in the DSC curve at 700°C indicates that 33% of the cellulose film remains intact at this temperature, and this proves that the cellulosic film has a polymeric structure.

### Antimicrobial activity of β-carotene, hesperidin and propolis solutions

In microbiological studies of milk and dairy products, *E.coli*, *Salmonella* spp., *Listeria monocytogene*s, *Staphylococcus aureus*, yeast and molds were found to be more effective in the spoilage (Pınar K. 2011). *S. aureus* is one of the most important microorganisms found in raw milk and many studies have been conducted on its pathogenicity on humans and animals. The production of milk and dairy products in Turkey is quite high, and most of these products are produced in small businesses and dairy farms in relatively less controlled manner (Guven et al., 2010). Especially, cheeses made from raw milk pose great risks to public health. Therefore, the risk of infection and food poisoning from milk and dairy products increase during the storage process (Perreira et al., 2009). *E. coli,* as the most important reason of cheese-related poisoning, presents a threat to public health. It is accepted as an indicator microorganism in terms of showing fecal contamination (Pamela et al., 2008).

The inhibition activities of ß-carotene, hesperidin, propolis and potassium sorbate solutions at various concentrations against the tested microorganisms are shown in Table 2.

It was determined that hesperidin and ß-carotene, prepared at 1% and 3% concentrations did not show an antimicrobial effect against either *E. coli* or *S. aureus*. The best antimicrobial activity was obtained with 5% concentration. In contrast, high levels of inhibition were observed in solutions of propolis at both 10% and 20% concentrations. The antimicrobial activity of potassium sorbate was lower. The formation of an inhibitory zone depends on the diffusion of the antimicrobial compound and the growth rate of the microorganisms (Çağrı et al. 2001).

### Antimicrobial activity of CBEFs

Antimicrobial agent free CBEF showed no inhibition against both bacteria. F9 film containing potassium sorbate provided limited inhibition on microorganisms. The inhibitions of *E. coli* and *S. aureus* strains were highly

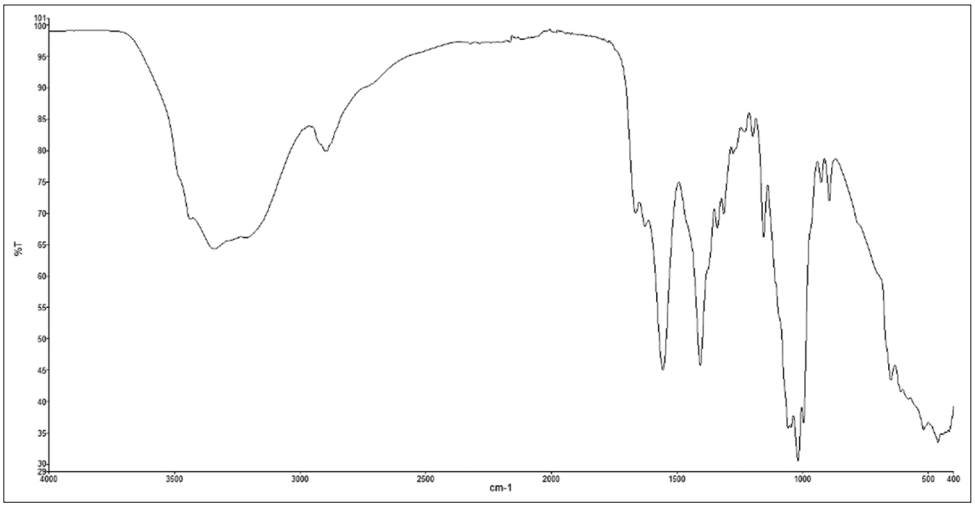


**Fig 3.** Spectrum measurement (FTIR) of cellulose-based edible film.

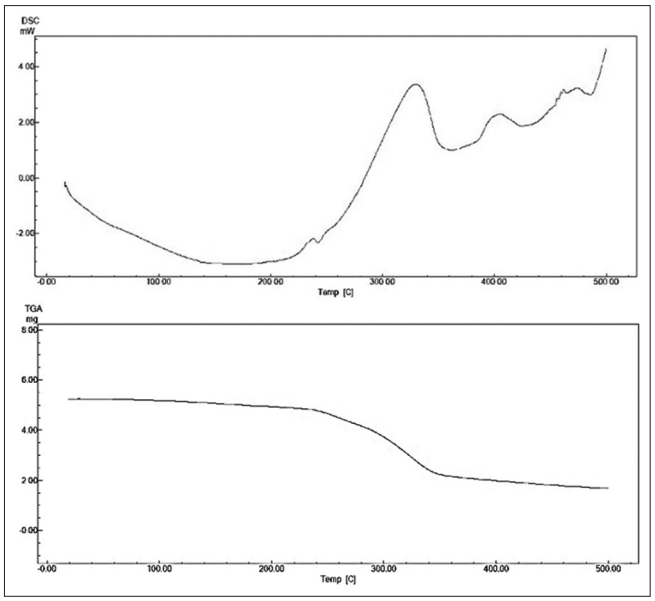


**Fig 4.** Temperature analysis of cellulose-based edible film; Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).

achieved by films containing other antimicrobial agents, especially with F6 and F8 (Table 3).

Antimicrobial activity was obtained with the F8 film containing propolis against *E.coli* and *S.aureus* with a zone diameter of 18.76 and 16.50 mm, respectively. The strong antimicrobial properties of propolis have been reported in most of the previous studies (Yang et al., 2009). Moreover, the antimicrobial effect of propolis was found to be higher against pathogenic microorganisms (Jian-xin et al., 2011). This finding supports previous studies. The differences of growth inhibitions rate may vary depending on the binding of both substances, surface adhesion, and the structure of the compounds (Silva-Carvalho et al., 2015). Morever spray application is the most effective method in coating processes. It offers uniform coating, thickness control and sequential application without contaminating the coating solution (Andrade et al., 2012). In addition, this parameter is also affected by the chemical structure and crosslinking level of the films (Çağrı et al. 2001).

### The effect of antimicrobial films on the levels of *E. coli* and *S. aureus* during the storage period

*E. coli counting*

The level of *E. coli* in the control group was log 6.38 Cfu/g on 5th day of storage (Table 4). With the edible antimicrobial film prepared with hesperidin, a higher bacterial inhibition effect was obtained compared to carotene. However, the highest bacterial inhibition (38%) was achieved with F8 film containing 20% propolis.

At the 10th day of storage, *E.coli* level of the negative control group increased by 13%. Similar bacterial level increase was obtained with the positive control. All films provided better growth inhibition than the positive control.

**Table 2: Inhibitory activities of β-carotene, hesperidin, propolis and potassium sorbate solutions against the tested microorganisms.**

| Antimicrobial agent | Conc. ((w/v) %) | *E. coli* | *S. aureus* |
|---|---|---|---|
| β-carotene | 1 | - | - |
| | 3 | - | - |
| | 5 | ++ | ++ |
| Hesperidin | 1 | - | - |
| | 3 | - | - |
| | 5 | ++ | ++ |
| Propolis | 10% | ++ | ++ |
| Propolis | 20% | ++ | ++ |
| Potassium sorbate | 10 mg/g cheese | + | + |

Antimicrobial activity against different antimicrobial agents was classified by the diameter of the inhibition zone:
-, not detected;
+, diameter of inhibition zone<5 mm;
++, diameter of inhibition zone 5-20 mm.

**Table 3: Antimicrobial activity * of cellulose-based edible films (CBEF) with different contents against the growth of microorganisms.**

| | *E. coli* | *S. aureus* |
|---|---|---|
| CBEF only | - | - |
| F1 | 5.41±0.57 | 5.22±0.70 |
| F2 | 12.67±0.20 | 14.40±0.10 |
| F3 | 13.33±0.23 | 19.12±0.18 |
| F4 | 8.72±0.42 | 6.76±0.30 |
| F5 | 11.33±0.47 | 12.01±0.41 |
| F6 | 21.00±0.15 | 22.09±0.29 |
| F7 | 14.13±0.11 | 12.66±0.32 |
| F8 | 18.76±0.97 | 16.50±0.55 |
| F9 | 3.43±0.29 | 3.01±0.12 |

*, Diameter of the inhibition zone formed around the film discs; (mm)
-, not detected.

**Table 4: The amount of *E. coli* (log CFU/g) in white cheese covered with CBE films containing antimicrobial substances during storage at 4º C.**

| | Storage (day) | | |
|---|---|---|---|
| | 5 | 10 | 15 |
| Control | 6.38±0.41 | 7.24±0.38 | 7.34±0.29 |
| F1 | 5.00±0.23[a] | 4.20±0.23[a] | 3.78±0.34[a] |
| F2 | 5.45±0.40[a,b] | 4.47±0.46[a] | 4.22±0.20[a,b] |
| F3 | 5.34±0.62[a,b] | 4.03±0.38[a,c] | 3.90±0.45[a,b,c] |
| F4 | 4.58±0.88[a,b,c,d] | 3.79±0.22[a,b,c,d] | 3.13±0.19[a,b] |
| F5 | 5.30±0.31[a,b,e] | 4.97±0.27[a,b,c,e] | 3.72±0.26[a,c,e] |
| F6 | 5.34±0.34[a,e] | 4.20±0.49[a,e,f] | 4.04±0.33[a,e,f] |
| F7 | 5.39±0.43[a,b,e] | 4.26±0.66[a,e,f] | 3.75±0.29[a,c,e] |
| F8 | 3.94±0.29[a,b,c,d,e,f,g,h] | 3.11±0.54[a,b,c,d,e,f,g,h] | 3.02±0.34[a,b,d,g,h] |
| F9 | 5.26±0.18[a,b,e,i] | 5.32±0.22 [a,b,c,d,e,g,h,i] | 5.36±0.43[a,b,e,h,i] |

[a],Significantly different from control (P<0.05)
[b],Significantly different from F1 (P<0.05)
[c],Significantly different from F2 (P<0.05)
[d],Significantly different from F3 (P<0.05)
[e],Significantly different from F4 (P<0.05)
[f],Significantly different from F5 (P<0.05)
[g],Significantly different from F6 (P<0.05)
[h],Significantly different from F7 (P<0.05)
[i],Significantly different from F8 (P<0.05)

This result showed that antimicrobial agents are more effective in the long term when compared to potassium sorbate, a synthetic preservative. This can be explained by the fact that antimicrobial substances diffuse slowly from the surface on which they are sprayed and show the capacity to inhibit bacterial growth. The longer-term effect of antimicrobial substances is an important result in terms of their application to products to provide a longer shelf life. The hydroxyl groups in phenolic compounds show an inhibitory effect on bacteria (Shan et al., 2004). These groups interact with the cell membrane of bacteria, disrupting the membrane structure of the cells and causing cellular components to leak out of the cell (Xue et al., 2013). Hydroxyl groups in phenolic compounds cause cell death by helping to delocalize electrons acting as a proton exchanger and reducing the tendency of bacterial cells on the cytoplasmic membrane (Gyawali & Ibrahim, 2014). It has been reported that hydroxyl groups can easily bind the active part of enzymes by changing the cell metabolism of microorganisms. This situation reveals the importance of hydroxyl groups in antimicrobial activity (Farag et al., 1989). The hydroxyl groups in phenolic compounds also show antioxidant effects. Antioxidants provide scavenging of free radicals, prevent the formation of reactive oxygen species, and thus reduce the redox potential of the growth medium (Cueva et al., 2010). However, it was determined that F3, F6, F7, F8 films containing propolis were more effective in the later days of storage.

It was observed that all coated films (F1-F8) continued to inhibit bacterial growth on the 15th day of storage. Bacterial levels in the negative control groups increased depending on the days (Table 4). The highest bacterial inhibition percentages were 59% and 57% in film coatings containing propolis (20%) and hesperidin (5%), respectively. Application of CBF-H/P to samples resulted in 2-4 Logs reduction in the count of *E. coli* during storage period. However, the expected synergistic effect could not be obtained in the applications prepared with Hesperidin-propolis and carotene-propolis mixtures. This can be explained by the alteration of the hydroxyl groups that serve in the antimicrobial activity when each antimicrobial substance becomes a mixture (Gyawali and İbrahim, 2014). Asdagh and Pirsa (2020) reported that the addition of *Carum copticum* essential oil into the pectin/nanoclay films containing β-carotene enchanced the antimicrobial activity of *E. coli*. In our study, when β-carotene and propolis were applied separately to the edible cellulose based films, they showed a more effective antimicrobial effect for both bacteria. These results are not consistent with literature. This is probably due to various factors influencing the antimicrobial activity of films, such as bacterial type, film matrix properties, and film production conditions (Alboofetileh et al., 2014). Non-packaging of cheeses during storage causes them to be affected by environmental contamination. This not only reduces the shelf life of the cheese, but also affects the quality of the cheese (Costa et al., 2018). In this study, as a result of macroscopic observations, contaminations were observed in cheese samples that were not covered with film.

### *S. aureus counting*

In terms of food microbiology, the most important pathogen species in the *Staphylococcus* genus is *S. aureus*. Approximately 50-70% of *S. aureus* strains could synthesize enterotoxins. It is known that superantigenic *S. aureus* enterotoxins are one of the main causes of food poisoning in developed countries (Erol, 2007). It was determined that the level of *S. aureus* was lower in all film coatings containing antimicrobial substances than the control groups (Table 5).

On the 5th day of storage, the *S. aureus* level in the control group was calculated as log 6.30 Cfu/g. As a result of coating with F3 film, it was observed that bacterial growth was inhibited by 32%. Also, F3 film was able to inhibit *S. aureus* 2 times more than *E. coli*. Antimicrobial packaging materials prolong the "lag period" of bacteria, reduce microorganism growth, extend shelf life and ensure food safety (Weng and Hotchkiss, 1992).

Antimicrobial agent mixtures were more effective against *S. aureus*. In addition, contrary to *E. coli* applications, the mixture of antimicrobial agents managed to reduce the level of *S. aureus* in a short time. The decrease in redox potential limits the growth of many microorganisms, especially aerobics ones. It is claimed that inhibition

**Table 5: The amount of *S. aureus* (log CFU/g) in white cheese covered with CBE films containing antimicrobial substances during storage at 4°C.**

| | Storage (day) | | |
|---|---|---|---|
| | 5 | 10 | 15 |
| Control | 6.30±0.64 | 7.27±0.54 | 7.25±0.16 |
| F1 | 5.25±0.68[a] | 3.98±0.47[a,b] | 3.03±0.48[a] |
| F2 | 5.36±0.45[a] | 4.76±0.58[a] | 4.48±0.33[a,b] |
| F3 | 4.27±0.22[a,b,c] | 4.21±0.29[a,c] | 4.07±0.51[a,b] |
| F4 | 4.74±0.43[a,b,c,d] | 3.64±0.49[a,c,d] | 3.28±0.28[a,c,d] |
| F5 | 4.70±0.48[a,b,c,d] | 4.20±0.37[a,c,e] | 3.75±0.34[a,b,c,d,e] |
| F6 | 5.01±0.56[a,d,f] | 4.64±0.25[a,e] | 4.46±0.2[a,b,d,e,f] |
| F7 | 5.39±0.42[a,d,e,f,g] | 3.91±0.33[a,c,g] | 3.46±0.26[a,c,d,g] |
| F8 | 5.36±0.51[a,d,e,f,g] | 3.86±0.45[a,c,d,f,g] | 3.04±0.40[a,c,d,f,g] |
| F9 | 5.48±0.11[a,d,e,f,g] | 6.08±0.28[a,b,c,d,e,f,g,h,i] | 6.05±0.33[a,b,c,d,e,f,g,h,i] |

[a], Significantly different from control (P<0.05)
[b], Significantly different from F1 (P<0.05)
[c], Significantly different from F2 (P<0.05)
[d], Significantly different from F3 (P<0.05)
[e], Significantly different from F4 (P<0.05)
[f], Significantly different from F5 (P<0.05)
[g], Significantly different from F6 (P<0.05)
[h], Significantly different from F7 (P<0.05)
[i], Significantly different from F8 (P<0.05)

of bacteria may be caused by disruption of membrane integrity, loss of cell content (molecules and ions) due to damage to the selective permeable structure of the membrane, and phenolic compounds causing damage to the cell membrane (Şengün and Öztürk, 2018). In both controls, the bacteria level remains high in the second stage of storage. In contrast, a 38% inhibition percentage was achieved with the F8 film. The film containing potassium sorbate minimally reduced the *S.aureus* level but the level of bacteria in the negative control continued to increase over the 15-day period. A total of 58% reduction in *S. aureus* level was obtained with the F1 film compared to the control. The rapid inhibition of bacterial growth obtained in the first stage of the storage process with the F3 film could not be observed in the later stages of the storage process. It can be explained by the alteration of the hydroxyl groups of the propolis solution, which contains a high percentage of phenolic substances (Gyawali İbrahim, 2014).

It has been found that β-carotene, hesperedin and propolis have stronger antimicrobial effects on Gram-positive bacteria. This can be explained with the cell wall structure of Gram-positive bacteria. The main molecule of the cell wall in gram positive bacteria is peptidoglycan and protein. The cell wall of gram negative bacteria has a more complex structure with lipid-based peptidoglycans and lipopolysaccharides. The lipopolysaccharide layer does not allow hydrophilic compounds to pass through the cell membrane in Gram-negative bacteria. Due to the absence of an extra outer membrane in Gram-positive bacteria, antimicrobial agents can easily penetrate (Siripatrawan & Harte, 2010). Since fresh cheeses may be exposed to adverse conditions such as high humidity and air, light and temperature during the storage process, microbial contamination may occur in cheeses. These results show that β-carotene/hesperidin/propolis penetrates the cheese sample during storage and inhibits the microbial loading of cheese due to their antimicrobial activities. This inhibition was accomplished by film coating.

Total bacterial count indicates that propolis-enhanced CBE films cause the best microbial reduction. The growth of both bacterial species was highly inhibited (>50%) in the presence of 20% propolis. Propolis is added directly to food or applied superficially with a coating. Thus, it reduces the number of pathogens or completely eliminates pathogens in meat, fish, juices and milk. Propolis can be used as an antioxidant, especially in meat and fish products, and can preserve the antioxidant properties of fruit and fruit juices during storage. It also ensures that the quality of them. With a non-ethanolic solvent complex formed on the basis of polyethylene glycol (PEG), a more efficient extraction of active substances from propolis can be achieved compared to extractions containing only water (Kubiliene et al. 2015). In this study, propolis dissolved in PEG was preferred and the results were found in accordance with the literature (Mascheroni et al., 2010). It can be stated that these applications may lead to the formation of "green label" functional foods.

### Total phenolic substance amount results

The total phenolic substance amounts of antimicrobial substances used as components in the films were calculated by gallic acid calibration (Fig. 5). When the ß-carotene, hesperidin and propolis substances are analyzed separately; it was determined that 10% propolis solution contains the highest amount of phenolic substance. However, ß-carotene, which contains less phenolic substance alone, was found to have the highest phenolic content when mixed with 10% propolis. The mixing of two substances, their dissolution in each other, their degree of surface adhesion affect the release of phenolic substances (Brglez Mojzer et al., 2016).

The phenol compounds containing a hydroxyl group and have important antifungal, antibacterial and antioxidant properties. These phenolic compounds destroy the phospholipid layer in the cell membrane and increase the permeability of this layer, thus helping the substances in the cell to leak out of the cell more easily or the enzyme systems of the bacteria to deteriorate. In this case, inhibition of microorganisms occurs, and thus, it shows an antimicrobial effect (Moreira et al., 2008).

### Antioxidant determination by DPPH radicals

Among the tested materials, 20% propolis had the highest antioxidant properties. Mixtures containing propolis also showed more antioxidant properties (Fig. 6). It has been reported in previous studies that propolis has a high antioxidant capacity (Russo et al., 2004). The measurement results were similar to previous studies. Hesperidin and ß-carotene also have a very high free radical scavenging activity. However, they have been found to have less scavenging effects than propolis.

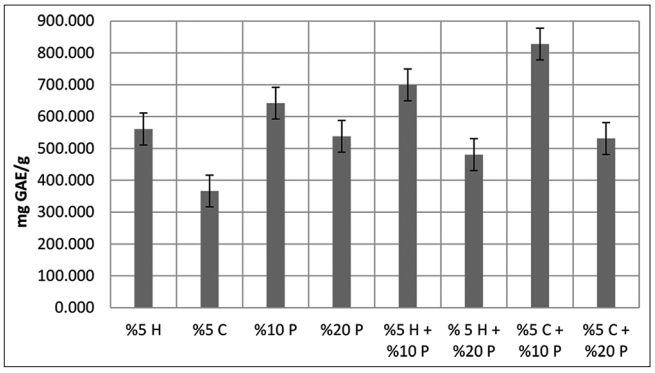


**Fig 5.** Total phenolic content of applied substances.

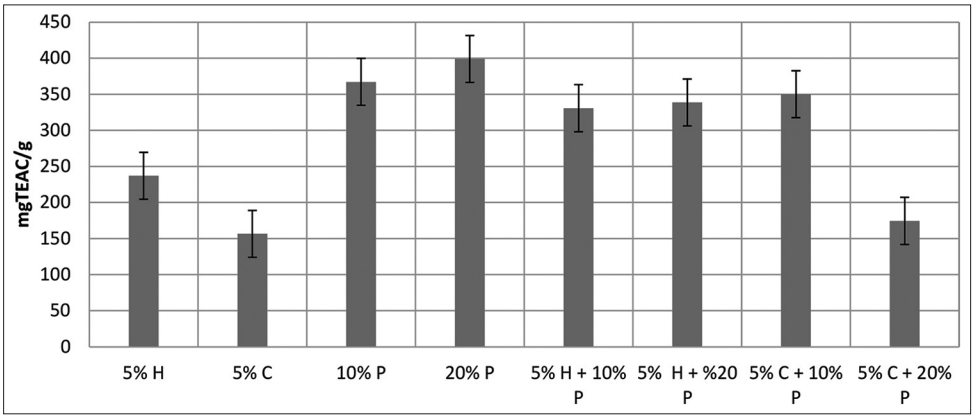


**Fig 6.** The antioxidant activity levels of the applied subtances

## CONCLUSIONS

Edible polymers are an environmentally safe alternative to fossil-based plastics in food packaging. Natural extracts and essential oils can improve the properties of edible polymers. With the application of cellulose-based edible films, the problems caused by *E. coli* and *S. aureus* contamination in white cheese can be prevented by adding ß-carotene, hesperidin, propolis and mixtures of these substances to the film. Hesperidin, ß-carotene and propolis reduced *E.coli* contamination in cheese by 57, 48 and 59%, respectively. Similar results were observed with *S. aureus*; it was observed that hesperidin, ß-carotene and propolis reduced the contamination in cheese by 54, 58, 58%, respectively. With this advanced packaging technology, food safety can be ensured, the shelf life of the product can be extended and food losses in this sector can be reduced. In addition, since the thickness of the plastic packaging material on the outer surface will be reduced, the amount of packaging waste will be reduced and environmental pollution will be prevented to some extent. Edible active packages can ensure the safe storage of foods without contamination. Hesperidin, carotene and propolis samples tested in this study were proven to be highly efficient to be used in cellulose-based film coatings as natural ingredients. As well as efficiency, their biocompatible and eco-friendly nature can pave the way for future food safety approaches including a wide range of foods.

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Writing original manuscript: Seval Cing Yildirim
All authors read and approved the final manuscript.

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