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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effects of LLDPE/Clay Nanocomposite Films Including Bioactive Compounds on The Microbiological Quality of Fresh Kashar Cheese*

LLDPE/Kil Nanokompozit Filmler İçeren Biyoaktif Bileşiklerin Taze Kaşar Peynirinin Mikrobiyolojik Kalitesine Etkileri

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Abstract

Emerging as a significant innovation in the food industry, active packaging extends shelf life, enhances safety, and maintains food quality by incorporating bioactive components like antimicrobial agents directly into packaging materials. In this context, polymer nanocomposites—particularly those reinforced with clay nanoparticles—have recently gained significant interest due to their enhanced functional properties. The cheese industry is one of the sectors with strong potential for applying such materials, as evidenced by recent developments in edible films for cheese**.** This study aimed to investigate the effects of active clay nanocomposite packaging films based on linear low-density polyethylene (LLDPE) containing eugenol (EUG) / thymol (THY) or carvacrol (CRV) loaded layered montmorillonite (MMT) or tubular halloysite (HNT) nanoclays. The study focused on their impact on the natural microbial load of kashar cheese, including total mesophilic aerobic bacteria (TMAB), total coliforms (TC), total lactic acid bacteria (LAB), and total yeast-mold (TYM), as well as on the presence/ growth of *Listeria monocytogenes, Staphylococcus aureus,* and *Aspergillus niger,* along with pH values, after storage at 10 °C for 30 days with analysis conducted every 10 days. The results showed that when prioritizing the packaging materials with the greatest impact on the microbial populations of kashar cheese, the order observed was as follows: THY-HNT for TMAB; EUG-MMT, CRV-HNT and THY-HNT for LAB; and CRV-HNT and THY-HNT for TYM. Moreover, *S. aureus* was not found in any of the kashar cheese samples. Furthermore, CRV-HNT and EUG-MMT films exhibited fungicidal properties throughout storage, leading to a decrease in mold spore counts, whereas THY-HNT films showed fungistatic effects. Additionally, CRV-HNT and THY-MMT packaging samples effectively limited growth of *L. monocytogenes.* As well, the pH values (5.15-5.52) of the cheese samples fluctuated during the storage period except for the control samples. Overall, the study showed that active nanocomposite films (ANFs) have the potential to extend shelf life, maintain safety and improve the overall quality of kashar cheese during storage.

Keywords: Halloysite clay nanotubes, Montmorillonite, Linear low-density polyethylene, Essential oil, Kashar cheese

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Öz

Gıda endüstrisinde önemli bir yenilik olarak ortaya çıkan aktif ambalajlama, antimikrobiyal ajanlar gibi biyoaktif bileşenlerin doğrudan ambalaj malzemelerine entegre edilmesiyle raf ömrünü uzatmakta, gıda güvenliğini artırmakta ve gıda kalitesini korumaktadır. Bu kapsamda, özellikle kil nanopartiküllerle güçlendirilmiş polimer nanokompozitler, üstün fonksiyonel özellikler göstermeleri nedeniyle son zamanlarda büyük ilgi çekmektedir. Yenilebilir peynir filmleri üzerine yapılan son çalışmalar, bu malzemelerin peynir endüstrisinde geniş kullanım potansiyeline sahip olduğunu ortaya koymaktadır. Bu çalışmada, öjenol (EUG) / timol (THY) veya karvakrol (CRV) ile yüklenmiş katmanlı montmorillonit (MMT) veya tübüler halloysit (HNT) nanokillerin lineer düşük yoğunluklu polietilen (LLDPE) esaslı aktif kil nanokompozit ambalaj filmlerinin üretiminde kullanılarak etkilerinin araştırılması amaçlanmıştır. Çalışmada, üretilen bu ambalaj malzemelerinin kaşar peynirinin doğal mikrobiyal yükü (toplam mezofilik aerobik bakteri (TMAB), toplam koliform (TC), toplam laktik asit bakterisi (LAB) ve toplam maya-küf (TYM)), *Listeria monocytogenes, Staphylococcus aureu*s ve *Aspergillus niger* varlığı/ gelişimi ile birlikte pH değerleri üzerine etkileri, 10 °C'de 30 gün boyunca her 10 günde bir yapılan analizlerle değerlendirilmiştir. Elde edilen bulgular, kaşar peynirinin mikrobiyal populasyonları üzerinde en büyük etkiye sahip ambalaj malzemelerinin TMAB için THY-HNT; LAB için EUG-MMT, CRV-HNT ve THY-HNT; TYM için CRV-HNT ve THY-HNT olarak göstermiştir. Ayrıca, hiçbir kaşar peyniri örneğinde *S. aureus* belirlenmemiştir. Öte yandan, CRV-HNT ve EUG-MMT filmleri depolama süresince fungisidal özellikler sergileyerek küf spor sayılarında azalmaya neden olurken THY-HNT filmleri ise fungistatik etkiler göstermiştir. Ek olarak, CRV-HNT ve THY-MMT ambalaj örnekleri *L. monocytogenes*'in büyümesini etkili bir şekilde sınırlamıştır. Ayrıca, peynir örneklerinin pH değerleri (5,15-5,52) kontrol örnekleri dışında depolama süresince dalgalanma göstermiştir. Genel olarak, bu çalışma aktif nanokompozit filmlerin (ANF'lerin) kaşar peynirinin raf ömrünü uzatma, güvenliğini koruma ve depolama sırasında genel kalitesini iyileştirme potansiyeline sahip olduğunu göstermiştir.

Anahtar Kelimeler: Halloysit kil nanotüpleri, Montmorillonit, Lineer düşük yoğunluklu polietilen, Esansiyel yağ, Kaşar peyniri

1. Introduction

Packaging is a crucial application which plays a critical role in extending shelf life and preserving food quality in modern food technology (Atlar et al., 2023; Akman et al., 2023; Celik et al., 2024; Karpuz and Palabıyık, 2024). Nanotechnology has brought to traditional food packaging new insights especially in the field of active and intelligent food packaging. Nanoclays, inorganic mineral silicates in nanoscale, have been widely used as reinforcement agents/nanofillers in the production of polymer nanocomposites. They have many advantages such as being natural origin, cost-effectiveness and biocompatibility, making them ideal for incorporation in biodegradable and high-performance nanocomposite films (Azeredo, 2009; Tornuk et al., 2015). Halloysite nanotubes (HNTs) [$Al_2Si_2O_5(OH)_4$ x nH_2O] are a type of aluminosilicate clay with a two-layered (1:1) structure, measuring 500 to 1000 nm in size with inner diameters between 15 and 100 nm (Lvov et al., 2008). HNTs are incorporated into clay-polymer nanocomposites based on their non-toxic properties, elevated aspect ratio, and impressive mechanical and dispersal attributes (Boonsiriwit et al., 2020). As well, montmorillonite (MMT), with its layered silicate structure of approximately 1 nm thickness and 100-500 nm lateral dimensions, also enhances the composite's permittivity and shielding characteristics. MMT's negatively charged and covered with exchangeable Na^+ and Ca^{2+} ions (Moučka et al., 2011; Tan et al., 2008). Moreover, linear low-density polyethylene (LLDPE), valued for its superior mechanical strength and barrier properties, has garnered significant interest in the development of LLDPE-clay nanocomposite films, although studies on their preparation and characterization remain limited (Manikantan and Varadharaju, 2012).

Essential oils are natural bioactive materials with aromatic properties obtained from various plants and spices through water/steam distillation. As the negative effects of synthetic food preservatives on health have become apparent and consumers have become more conscious about food safety and health standards, the use of essential oils in food products has become increasingly widespread, similar to other natural food additives. Essential oils, generally recognized as safe (GRAS), have no reported adverse effects on health. Essential oils have been used by humans for a long time as flavor enhancers and preservatives in foods, as well as being evaluated as therapeutic agents in traditional medicine (Aktepe et al, 2019; Barman et al., 2025; Feyzioglu and Tornuk, 2016; Mahcene et al., 2021; Shaaban, 2020; Weisany et al., 2022). Scientific research has shown that essential oils and their active components have antibacterial effects on various pathogenic bacteria such as *Bacillus cereus, Pseudomonas, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli,* and *Salmonella* as well as antifungal properties (Burt, 2004; Kalemba and Kunicka, 2003; Mahcene et al., 2020). However, presence of high amounts of volatile oils in packaging material can adversely affect the sensory properties of the packaged foods due to their aromatic characteristics. Additionally, the effects of conventional packaging on the shelf life of foods are insufficient because the active substance tends to leave the packaging quickly. Polymer nanocomposite films reinforced with nanoclays impregnated with essential oils or their constituents have been shown to have better antimicrobial and antioxidant activity as well as food protective properties than conventional films containing essential oils, which clearly demonstrates the positive effect of nanoclays on carrying and controlled release of active volatiles (Abdollahi et al., 2012; Camani et al., 2018; Giannakas, 2020; Lee et al., 2018; Saucedo-Zuñiga et al., 2021; Tornuk et al., 2015; Tornuk et al., 2018).

Kashar cheese is one of the most widely consumed cheese types in Türkiye and is known as "kaskaval" in Balkan countries (Hayaloğlu et al., 2002). This semi-hard cheese is produced using raw or pasteurized milk (Andiç et al., 2011). A significant portion of the kashar cheese produced in Türkiye is consumed fresh, while the remaining portion is offered as aged kashar cheese after being matured. In Türkiye, kashar cheese is sold as whole or sliced in vacuum packs (Kamber, 2007).

Although edible film applications for kashar cheese preservation are limited, various research has shown promising results. Studies have explored chitosan/whey protein films for mold control and quality maintenance (Yangılar, 2015), natamycin films enriched with essential oils for safety enhancement (Yangilar, 2017), aloe vera–infused agar-gelatin films (Isık et al., 2023), and whey protein concentrate/hydroxypropyl methylcellulose nano-biocomposite films containing chitosan nanoparticles (Shojaei et al., 2021). Building upon these developments, the present investigation aims to determine the effects of ANFs on the natural microbial load, pH, as well as the presence/development of *L. monocytogenes* and *A. niger*- the most critical factors limiting the shelf life of kashar cheese and potentially causing health issues - in relation to packaging types. To our knowledge, this is the first

study investigating the impact of LLDPE/clay nanocomposite films containing bioactive compounds on the shelf life and microbial safety of fresh kashar cheese.

2. Materials and Methods

2.1. Materials

The full-fat (~3.5%) fresh cow's milk used in the production of kashar cheese was obtained from Erciyes University Agricultural Research and Application Center (ERÜTAM), Türkiye. The milk was promptly subjected to pasteurization and used in kashar cheese production.

Nanoclays, including sodium MMT with a surface area of 250 $m^2 g^{-1}$ and halloysite with a surface area of 64 $m^2 g^{-1}$ and nanotubes ranging from 30 to 70 nm in diameter and 1 to 3 µm in length, were procured from Sigma, Germany. Moreover, 98% pure THY and 98% pure CRV were acquired from Sigma, Germany, with a vapor pressure of 1 mmHg at 64 °C. EUG with a purity of 99% and nonionic Tween 80 with a molecular weight of approximately 1310 were sourced from Merck, Germany. Lotrene, Qatofin Company Ltd., Doha, Qatar, provided LLDPE with the following specifications: its density at 23°C was 0.918 g cm^{-3}, its melt flow index was 2000 g 10 min^{-1}, and its crystal melting point was 121 °C.

2.2. Film production

For the production of ANFs, we followed the method described in detail in our previous publication (Tornuk et al., 2015; Tornuk et al., 2018). Six distinct film samples were processed using a high shear force twin-screw extruder and categorized as follows: control (pure LLDPE film), CRV-HNT (LLDPE film reinforced with CRV-grafted HNT), EUG-MMT (LLDPE film reinforced with EUG-grafted MMT), CRV-MMT (LLDPE film reinforced with CRV-grafted MMT), THY-HNT (LLDPE film reinforced with THY-grafted HNT), and THY-MMT (LLDPE film reinforced with THY-grafted MMT).

2.3. Production and preparation for analysis of kashar cheese and packaging with ANFs

The production of kashar cheese was carried out at the Food Pilot Application Center of Safiye Çıkrıkçıoglu Vocational School, Erciyes University, Kayseri, Türkiye. Fresh, full-fat (~3.5%) raw cow's milk underwent quality controls (antibiotics, pH, SH, Brix, fat, soda, and peroxide). Following that, it was cooled to the fermentation temperature (30 ± 1°C) and pasteurized at 65 °C for 30 min before being placed in the cheese vat. First, $CaCl_2$ dissolved in 0.05% water was added, followed by rennet enzyme. After a 1 h coagulation time, the formed curd was cut, rested for 10 min, and then heated to 40 °C for whey drainage. The drained curd was pressed and subjected to several molding processes to remove excess whey. Once the desired acidity was reached, the curd was heated at 75 °C in water for 5 min. The cooked cheese was filled into cylindrical molds with a diameter of 3 cm and allowed to rest at room temperature for 48 h. After attaining the desired shape, the cylindrical cheese was removed from the molds. The cylindrical cheese was sliced into 8 mm thick slices (~20 g weight) using a slicing machine. To test the effect of ANFs on the natural load of kashar cheese, cheese slices were used without any treatment. For microorganism contamination, the cheese slices were exposed to UV radiation (254 nm) at a distance of 15 cm from both sides for 10 min for surface disinfection. To test the effect of ANFs in sliced kashar cheese, the cheese slices were placed in the ANF-containing packaging, out of contact with each other and vacuum sealed. Control samples were vacuum packaged using pure LLDPE packaging.

2.4. Microbiological analyses

Microbiological analyses were performed on the vacuum-packaged cheeses stored at 10 °C for 30 days, with intervals of 10 days, for total mesophilic aerobic bacteria (TMAB), total coliforms (TC), total lactic acid bacteria (LAB), total yeast-mold (TYM), as well as *Staphylococcus aureus*, *Listeria monocytogenes* and *Aspergillus niger* population. For this purpose, 20 g of cheese sample was placed in a stomacher bag under aseptic conditions, to which 180 mL of Maximum Recovery Diluent (MRD, Merck, Germany) was added and then homogenized in a Stomacher device (Stomacher, IUL, Barcelona, Spain) for 120 sec. Subsequently, serial dilutions were prepared, and appropriate dilutions were used for spread plating (Erkmen, 2011). An overview of the analyses performed for this study were given in *Figure 1*.

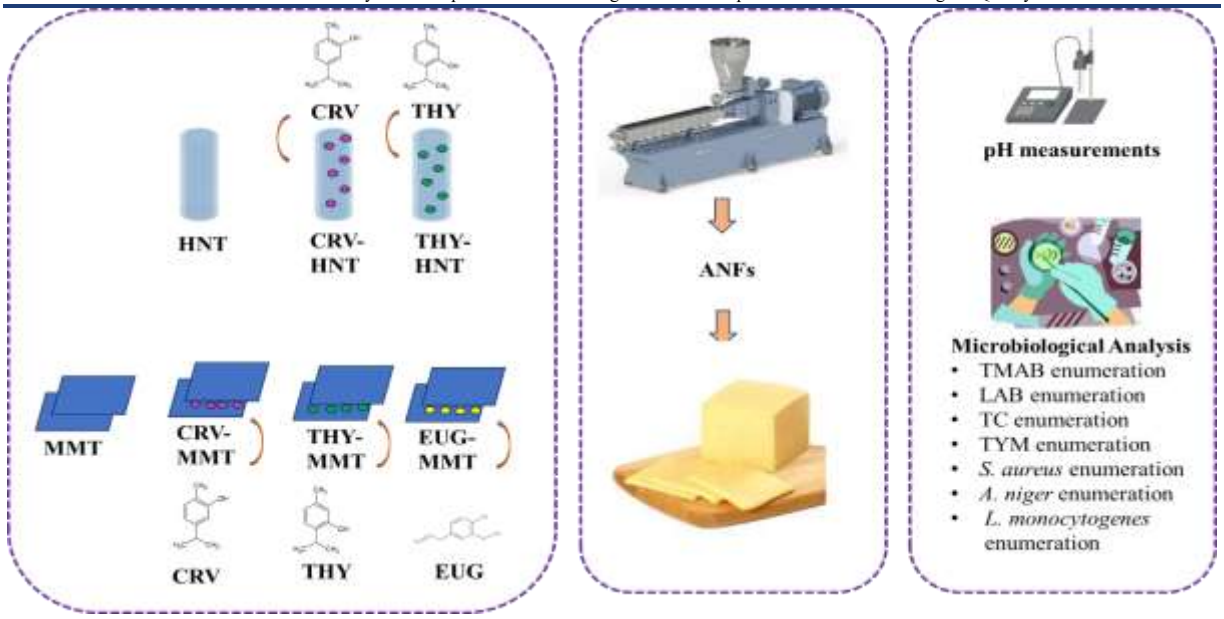

Figure 1. An overview of the analyses performed for this study

2.4.1. TMAB enumeration

0.1 mL of appropriate dilutions corresponding to the estimated microbial count was taken, poured into pre-sterilized sterile petri-dishes, and solidified with Plate Count Agar (PCA, Merck, Germany). Next, the petri dishes were incubated for 24 to 48 h at 30 °C and the developed colonies were counted at the end of incubation (Tornuk et al., 2015).

2.4.2. TC enumeration

0.1 mL of appropriate dilutions corresponding to the estimated microbial count were taken, poured into pre-sterilized sterile petri-dishes, and solidified with Violet Red Bile Agar (VRBA, Merck, Germany). Afterwards, the petri-dishes were incubated at 37 °C for 24 h, and pink-red colonies larger than 1 mm were counted at the end of incubation (Tornuk et al., 2015).

2.4.3. LAB enumeration

0.1 mL of appropriate dilutions corresponding to the estimated microbial count was taken, poured into pre-sterilized sterile petri-dishes, and solidified with De Man, Rogosa and Sharpe (MRS) Agar (Merck, Germany) medium. Subsequently, the colonies were counted after the petri dishes were cultured for 2 to 3 days at 30 °C (Bagdat et al., 2024a; Bagdat et al., 2024b).

2.4.4. TYM enumeration

0.1 mL of appropriate dilutions corresponding to the estimated microbial count was taken, poured into pre-sterilized sterile petri-dishes, and solidified with Dichloran Rose Bengal Chloramphenicol (DRBC) Agar (Merck, Germany). The petri dishes were incubated for 1 to 5 days at 25 °C, and at the conclusion of the incubation period, the colonies were counted (Erol et al., 2024).

2.4.5. S. aureus enumeration

0.1 mL of appropriate dilutions corresponding to the estimated microbial count was taken, poured into pre-sterilized sterile petri-dishes, and solidified with Baird Parker Agar (BPA, Merck, Germany). For 24 h to 48 h, the petri dishes were incubated at 37 °C, and then round, convex, small, shiny zones surrounded by a 2-3 mm black-gray colony were counted as potential *S. aureus* colonies at the end of incubation (Mahcene et al., 2021).

2.4.6. A. niger inoculation and enumeration

The stock *A. niger* culture was first activated by growing on Malt Extract Agar (MEA, Merck, Germany) for 3 days at 25 °C. Specific mold colonies were then transferred to sterile Malt Extract Broth (MEB, Merck, Germany) and cultured for 3-5 days at the same temperature to form liquid cultures for experiments. The activated *A. niger* culture

was added to sterile MRD solution to create an inoculum with a target microbial count of ~10^3 CFU mL^{-1}. Cheese slices were immersed in this inoculum solution within a biosafety cabinet for 2 min to allow for mold contamination. Each surface was then allowed to dry for 10 min, and the dried slices were placed between two ANFs (4.5 cm in diameter) and then placed inside a sterilized petri dish. The cheese samples in the petri dish were placed in zip-lock plastic bags and stored at 10 °C for 30 days and tests were performed at 10-day intervals (Ture et al., 2011).

For quantifying the *A. niger* load in the cheese, the cheese samples were diluted as described above. Then, appropriate dilutions were spread-plated onto Dichloran Rose Bengal Chloramphenicol (DRBC) Agar, and for one to five days, the plates were incubated at 25 °C to count the development of distinct mold colonies. Results were recorded as logarithmic values (Erkmen, 2011).

2.4.7. L. monocytogenes inoculation and enumeration

To assess the effect of ANF-packed cheese on the viability of *L. monocytogenes* in kashar slices, a bacterial culture with a count of ~10^5 cfu mL^{-1} was prepared using a bacterial culture previously activated for 24 h at 37 °C in Nutrient Broth (Merck, Germany). Cheese slices were immersed in this solution and held for 2 min to contaminate them with bacteria. Subsequently, within a biosafety cabinet, the cheese was allowed to dry, and the bacteria adhere to the cheese surface for 10 min.

Contaminated cheese slices were vacuum packaged using ANFs and stored at 10 °C for 30 days. Neat LLDPE packaging was used for control samples. Samples were taken every 10 days for analysis. The diluted and prepared cheese samples were spread-plated onto Oxford Listeria Selective Agar (Merck, Germany), and after incubating for 24 h at 37 °C, the developed colonies were computed. Results were recorded as logarithmic values (Erkmen, 2011).

2.5. Determination of pH value in kashar cheese

The pH changes in cheese samples vacuum packed with different ANFs and stored at 10 °C for 30 days were measured using a digital pH meter (Kosikowski and Mistry, 1997).

2.6. Statistical evaluation

SAS software package (version 8.2) was used for statistical evaluation of the data obtained from the analyses. Single and two-factor analysis of variance (ANOVA) were applied to the evaluated parameters to determine the effects of factors such as storage duration and packaging type. Duncan's multiple comparison test was used to evaluate group differences at a significance threshold of $\alpha = 0.05$.

3. Results and Discussion

3.1. The influences of ANF-containing packaging on the natural microbial population of kashar cheese

Microbiological spoilage of cheeses occurs primarily due to lipolytic and proteolytic activities. This type of spoilage is more common in cheeses made from raw milk. Lipolytic activity results in rancidity due to the breakdown of triglycerides under the action of the enzyme lipase, while proteolytic activity causes the breakdown of proteins and the formation of undesirable flavor and aroma compounds (Ramos et al., 2022). Important groups of microorganisms responsible for spoilage in cheeses include aerobic psychotropic and mesophilic Gram-negative bacteria, yeasts, molds, heterofermentative LABs, and spore-forming bacteria (Hossain et al., 2024). Fresh unripe and soft cheeses like fresh kashar cheese spoil more rapidly due to their high pH values, water activities, and low salt content (Ritschard and Schuppler, 2024).

Mesophilic bacteria can possess strong lipolytic and proteolytic activities, which can alter the aroma, flavor, and physicochemical features of cheese (Sharafi et al., 2023). *Table 1* shows the variations in TMAB counts of kashar cheeses vacuum-packed with ANFs and kept at 4ºC for 30 days. As can be seen, TMAB counts of the cheeses had a value of 5.25 log CFU g^{-1} at the beginning of the storage period. A significant rise ($p<0.05$) was seen in the TMAB loads of all samples during storage. Only the CRV-MMT film managed to control TMAB growth during the first 10-days of storage, while statistically significant increases were observed in all samples ($p<0.05$). Interestingly, cheese packaged with the control film exhibited a significant decrease in TMAB counts from the 10^{th} to the 20^{th} day, whereas the counts of other samples either remained constant ($p>0.05$) or significantly increased ($p<0.05$). After the 30-day of storage only the cheese packaged with THY-HNT showed a statistically lower TMAB counts compared to the other

cheese samples ($p<0.05$). Previously, the initial TMAB count was 4.58 log cfu g^{-1} (Dıblan and Kaya, 2023), similar to the 4.22 cfu g^{-1} reported in kashar cheese by Ceylan et al. (2021) and increased as storage time progressed.

Table 1. The change in total mesophilic aerobic bacteria (TMAB), lactic acid bacteria (LAB), total coliform (TC), and total yeast and mold (TYM) counts of kashar cheese packaged with different ANFs during storage

Microbiological Analysis	Storage duration (days)	Control	CRV-MMT	CRV-HNT	EUG-MMT	THY-MMT	THY-HNT
TMAB population (log cfu g^{-1})	0	5.25 ± 0.18^{bA}	5.25 ± 0.18^{cA}	5.25 ± 0.18^{cA}	5.25 ± 0.18^{dA}	5.25 ± 0.18^{cA}	5.25 ± 0.18^{cA}
	10	7.54 ± 0.23^{aA}	5.73 ± 0.39^{bcC}	6.40 ± 0.07^{bBC}	6.57 ± 0.04^{cB}	7.00 ± 0.14^{bAB}	6.25 ± 0.06^{bBC}
	20	6.10 ± 0.21^{bC}	6.35 ± 0.26^{bBC}	7.39 ± 0.24^{aA}	7.45 ± 0.21^{bA}	7.18 ± 0.04^{bAB}	6.30 ± 0.00^{bBC}
	30	8.57 ± 0.84^{aA}	7.81 ± 0.73^{aAB}	7.83 ± 0.48^{aAB}	8.45 ± 0.31^{aA}	8.20 ± 0.00^{aAB}	7.13 ± 0.33^{aB}
LAB population (log cfu g^{-1})	0	7.30 ± 0.11^{cA}	7.30 ± 0.11^{cA}	7.30 ± 0.11^{cA}	7.30 ± 0.11^{cA}	7.30 ± 0.11^{dA}	7.30 ± 0.11^{dA}
	10	8.44 ± 0.37^{bA}	8.15 ± 0.25^{bA}	8.32 ± 0.50^{bA}	8.38 ± 0.30^{bA}	8.61 ± 0.03^{cA}	8.18 ± 0.18^{cA}
	20	9.20 ± 0.43^{bA}	8.13 ± 0.77^{bB}	8.91 ± 0.39^{aA}	9.11 ± 0.12^{aA}	9.42 ± 0.10^{bA}	9.13 ± 0.10^{bA}
	30	10.24 ± 0.84^{aA}	9.11 ± 0.89^{aBC}	8.95 ± 0.15^{aC}	8.99 ± 0.26^{aC}	10.03 ± 0.15^{aAB}	8.81 ± 0.28^{aC}
TC population (log cfu g^{-1})	0	2.14 ± 0.16^{dA}	2.14 ± 0.16^{bA}	2.14 ± 0.16^{cA}	2.14 ± 0.16^{aA}	2.14 ± 0.16^{bA}	2.14 ± 0.16^{cA}
	10	2.78 ± 0.10^{cA}	2.56 ± 0.43^{abA}	2.24 ± 0.00^{bcA}	2.85 ± 0.04^{aA}	2.45 ± 0.05^{bA}	2.59 ± 0.03^{bA}
	20	2.82 ± 0.50^{bA}	2.85 ± 0.17^{aAB}	2.73 ± 0.38^{abAB}	2.70 ± 0.36^{aAB}	2.51 ± 0.04^{bB}	3.17 ± 0.12^{aAB}
	30	3.70 ± 0.21^{aA}	2.89 ± 0.25^{aB}	3.00 ± 0.27^{aB}	3.08 ± 0.15^{aB}	3.26 ± 0.21^{aAB}	3.11 ± 0.14^{aB}
TYM population (log cfu g^{-1})	0	2.27 ± 0.15^{cA}	2.27 ± 0.15^{cA}	2.27 ± 0.15^{aA}	2.27 ± 0.15^{bA}	2.27 ± 0.15^{bA}	2.27 ± 0.15^{bA}
	10	3.30 ± 0.22^{bA}	2.93 ± 0.40^{abAB}	2.27 ± 0.15^{aB}	2.29 ± 0.21^{bB}	2.93 ± 0.16^{aAB}	2.65 ± 0.12^{baAB}
	20	3.43 ± 0.23^{bAB}	3.48 ± 0.35^{bA}	2.54 ± 0.40^{aC}	3.04 ± 0.18^{aABC}	2.95 ± 0.06^{aBC}	2.73 ± 0.26^{aC}
	30	4.53 ± 0.17^{aA}	2.88 ± 0.36^{bBC}	2.33 ± 0.37^{aA}	3.21 ± 0.29^{aB}	3.03 ± 0.04^{aB}	2.50 ± 0.08^{baCD}

* THY: Thymol; EUG: Eugenol; CRV: Carvacrol; Control: Neat LLDPE film without nanoclay; CRV-MMT: Carvacrol loaded montmorillonite; CRV-HNT: Carvacrol loaded halloysite; EUG-MMT: Eugenol loaded montmorillonite; THY-MMT: Thymol loaded montmorillonite; THY-HNT: Thymol loaded halloysite; ±: standard deviation; $^{a-c}$: Different lowercase letters within the same column indicate a statistically significant difference ($P<0.05$) between the data; $^{A-C}$: Different uppercase letters within the same row indicate a statistically significant difference ($P<0.05$) between the data.

LAB play a crucial role in enhancing the flavor and aroma of cheese while preserving its quality (Erkaya-Kotan et al., 2023). Hence, assessing LAB counts in the cheese samples was essential in this study to determine whether the ANFs adversely affected these beneficial bacteria. The change in LAB counts during the storage of kashar cheeses packaged with different ANFs are presented in *Table 1*. As observed, LAB counts in the cheeses were determined as 7.30 log cfu g^{-1} on day 0. The LAB counts of the cheeses increased by 2-3 logs during the storage process, and these increases were statistically significant ($p<0.05$). Until the 20th day of storage, there was no statistically significant differences ($p>0.05$) in LAB counts between kashar cheeses packaged with THY-MMT, CRV-HNT, EUG-MMT, THY-HNT, and the control samples. Similarly, at the end of the 30 days of storage, the LAB counts of the control samples reached the highest statistically significant value at 10.24 log cfu g^{-1} ($p<0.05$). In this context, THY-HNT, CRV-HNT, and EUG-MMT films emerged as the most effective ($P<0.05$) packaging materials in delaying LAB growth.

The changes in TC loads of kashar cheeses packaged with different ANFs during the storage process were provided in *Table 1*. As can be seen, the initial TC load of the cheese, which was 2.14 log cfu g^{-1}, showed a statistically significant increase ($p<0.05$) during the 30-day storage. The TC counts after storage in CRV-MMT, CRV-HNT, THY-HNT, and EUG-MMT were statistically lower ($p<0.05$) compared to the other samples. The TC count of the control sample reached 3.70 log after 30 days of storage, which was statistically the highest value ($p<0.05$) among the samples.

For processed cheeses, the presence of deteriorating microorganisms such as molds and yeasts is a particularly significant concern (Erkaya-Kotan et al., 2023). TYM loads in kashar cheese samples stored by vacuum packaging with different produced ANFs are provided in Table 1. As shown, TYM counts of kashar cheese before storage were found to be 2.27 log cfu g^{-1}. CRV-MMT and CRV-HNT significantly reduced ($p<0.05$) the TYM levels of kashar cheese from the 20th to the 30th day of storage. Except for CRV-HNT, all samples exhibited a significant increase ($p<0.05$) in TYM loads during storage. CRV-HNT, however, restrained TYM growth, and there was no statistically significant increase ($p>0.05$) in its TYM load. In this sample, the TYM load was determined to be 2.33 log cfu g^{-1} after 30-days of storage. Previously, Tornuk et al. (2018) reported that HNT nanoclay led to a more gradual release of active compounds compared to MMT over 40 days of storage. This gradual release could contribute to the observed effectiveness of CRV-HNT in inhibiting TYM growth. Moreover, for effective antimicrobial action, a slow diffusion

rate onto the food surface is essential, as a high rate may lead to the loss of the antimicrobial agent into the food matrix, reducing its surface concentration and activity. Rapid diffusion can result in the agent being wasted and may prevent it from inhibiting microbial spoilage, especially if it diffuses faster than the microorganism's lag phase, hindering its effectiveness (Dıblan and Kaya, 2023). Similarly, Saucedo-Zuñiga et al. (2021) observed that MMT's layered structure permits less restricted diffusion of adsorbed oil compared to HNT's hollow structure, which can trap essential oils and impede their diffusion. This observation supports the enhanced control over TYM growth in kashar cheese samples treated with CRV-HNT, which may benefit from the controlled release and diffusion characteristics of the encapsulated forms. Likewise, Erkaya-Kotan et al. (2023) found that an edible coating solution with increasing thyme oil content effectively inhibited yeast and mold growth on Kashar cheese, demonstrating thyme oil's strong fungicidal effect. In cheese samples, antimicrobial films containing various essential oils seem to be more effective against yeast and mold than against mesophilic bacteria. Similarly, Yangılar and Yıldız (2016) demonstrated that protein-based films, like casein with NAT, were more successful in lowering mold counts on kashar cheese after 90 days of storage compared to films made from carbohydrates. According to Sharafi et al. (2023), this increased effectiveness may be due to the direct interaction of essential oils with molds that tend to develop on the cheese surface. Actually, essential oils impact fungal hyphae, leading to compromised membrane integrity and a reduction in ergosterol levels. Moreover, Mani-López et al (2021) noted that essential oils compromise fungal cell walls and membranes, leading to the denaturation of cellular components. They also inhibit ATPases and cytokines, thereby influencing genes associated with cell adhesion, growth, and sporulation.

Native *S. aureus* loads of kashar cheeses, the changes during the storage process, and the effects of ANFs on *S. aureus* were presented in *Table 2*. As indicated, no growth of *S. aureus* was observed in any sample either at the beginning or during the storage. However, Kavas and Kavas (2018) observed that the film containing 2% (v/v) lavender essential oil exhibited a more pronounced antimicrobial effect against *S. aureus* during the storage period, effectively suppressing *S. aureus* growth, in contrast to the control sample, where the levels increased. Additionally, Artiga-Artigas et al. (2017) observed a decrease in the *S. aureus* population from 6.0 to 4.6 log cfu g^{-1} after 15 days of storage in cheese samples coated with 2.0% oregano essential oil. They suggested that carvacrol in the oregano oil might disrupt the fatty acid composition of the bacterial cell membrane, leading to inhibition.

Table 2. The change in S. aureus, A. niger, and L. monocytogenes counts of kashar cheese packaged with different ANFs during the storage

Microbiological Analysis	Storage duration (days)	Control	CRV-MMT	CRV-HNT	EUG-MMT	THY-MMT	THY-HNT
***S. aureus* population (log cfu g^{-1})**	0	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$
	10	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$
	20	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$
	30	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$	$<1.00^{Aa}$
***A. niger* population (log cfu g^{-1})**	0	3.01 ± 0.68^{cA}	3.01 ± 0.68^{bA}	3.01 ± 0.68^{aA}	3.01 ± 0.68^{aA}	3.01 ± 0.68^{cA}	3.01 ± 0.68^{aA}
	10	3.64 ± 0.41^{bcAB}	3.37 ± 0.55^{bABC}	3.10 ± 0.17^{aBC}	2.50 ± 0.58^{aC}	3.85 ± 0.67^{bA}	3.33 ± 0.48^{aABC}
	20	4.53 ± 0.43^{abA}	3.77 ± 0.23^{bABC}	2.77 ± 0.68^{aC}	3.00 ± 0.00^{aBC}	4.00 ± 0.00^{bAB}	3.20 ± 0.17^{aBC}
	30	5.30 ± 0.00^{aA}	5.15 ± 0.21^{aA}	2.17 ± 0.19^{bB}	2.69 ± 0.09^{aB}	5.00 ± 0.00^{aA}	3.00 ± 0.24^{aB}
***L. monocytogenes* population (log cfu g^{-1})**	0	5.19 ± 0.26^{aA}	5.19 ± 0.26^{aA}	5.19 ± 0.26^{aA}	5.19 ± 0.26^{aA}	5.19 ± 0.26^{aA}	5.19 ± 0.26^{aA}
	10	4.72 ± 0.14^{bA}	4.22 ± 0.22^{bcC}	4.36 ± 0.25^{bABC}	4.31 ± 0.26^{bBC}	4.68 ± 0.09^{bAB}	4.10 ± 0.35^{bC}
	20	4.30 ± 0.42^{cA}	3.99 ± 0.24^{cA}	3.85 ± 0.17^{cA}	3.86 ± 0.19^{cA}	3.91 ± 0.21^{cA}	4.09 ± 0.38^{bA}
	30	4.72 ± 0.39^{bA}	4.55 ± 0.20^{bA}	3.62 ± 0.16^{cC}	4.13 ± 0.34^{bcABC}	3.88 ± 0.08^{cBC}	4.36 ± 0.44^{bAB}

* THY: Thymol; EUG: Eugenol; CRV: Carvacrol; Control: Neat LLDPE film without nanoclay; CRV-MMT: Carvacrol loaded montmorillonite; CRV-HNT: Carvacrol loaded halloysite; EUG-MMT: Eugenol loaded montmorillonite; THY-MMT: Thymol loaded montmorillonite; THY-HNT: Thymol loaded halloysite; ±: standard deviation; $^{a-c}$: Different lowercase letters within the same column indicate a statistically significant difference ($P<0.05$) between the data; $^{A-C}$: Different uppercase letters within the same row indicate a statistically significant difference ($P<0.05$) between the data.

At the beginning of storage, a significant microbial population was observed in the kashar cheese. This indicates the presence of starter cultures added after pasteurization of the milk and potential contamination from the environment or equipment during the later stages of production. Additionally, it appears that the subsequent heating process did not sufficiently ensure microbial inactivation. Similar findings were reported in previous studies (Öründü and Tarakçı, 2021). During storage, increases in the natural microbial populations of the cheeses were observed. However, in general,

the ANFs produced in this study exhibited inhibitory/ slowing effects on microbial growth. When ranking the most effective packaging materials to affect the microbial populations of kashar cheese, the following order was observed: THY-HNT for TMAB; EUG-MMT, CRV-HNT, and THY-HNT for LAB; and CRV-HNT and THY-HNT for TYM. Additionally, all ANFs exhibited similar inhibitions for TC, and no *S. aureus* was detected in any of the kashar cheese samples.

Kavas et al. (2015) reported that kashar cheese coated with films containing thyme and clove essential oils (CEO) showed a significant reduction in pathogenic microorganisms such as *E. coli* O157: H7, *L. monocytogenes,* and *S. aureus* during storage. This antimicrobial effect was more pronounced in cheese coated with films containing thyme essential oil compared to those with CEO. Moreover, Isık et al. (2023) showed that aloe vera oil-added gelatin films reduced the bacterial growth to 1.68 log cfu g^{-1} and fungal growth to 2.61 log cfu g^{-1} in kashar cheese as compared to 3.98 log cfu g^{-1} and 5.98 log cfu g^{-1} in controls, respectively.

3.2. The effect of ANFs on the growth of artificially contaminated A. niger mold in kashar cheese

Molds are microorganisms that grow on the surfaces of cheeses, reducing their shelf life and causing product losses. Another critical concern arising from mold growth in cheeses is that molds produce mycotoxins (Benkerroum, 2016). Some products associated with *A. niger* production are known to be food-grade and safe for consumption. For instance, citric acid derived from *A. niger* is considered a GRAS product by the Food and Drug Administration (FDA) (Mores et al., 2021). *A. niger* is also used in the industrial production of various enzymes such as pectinase, amyl glucosidase, and protease. Although there are reports that *A. niger* can produce mycotoxins, there is no evidence indicating it is an aflatoxigenic mold (Daniel, 2021). However, several *Aspergillus* species, including those containing *A. niger*, have been isolated from semi-hard, semi-soft, salted and unsalted cheeses. These isolates have been associated with spoilage and product losses in cheeses (Özçakmak and Dervisoglu, 2011; Nasser, 2001; Hassanin, 1993; Lund et al., 1995). Therefore, the impact of ANFs on mold, a group of microorganisms that can proliferate and render the cheese inedible, was also investigated in kashar cheese. For this purpose, sliced kashar cheeses were contaminated with *A. niger* at a level of ~3.00 log cfu g^{-1}, and the broad surfaces of the slices were covered with ANFs. The samples were stored for 30 days to observe changes in *A. niger* load. *Table 2* presents the effect of different ANFs on the *A. niger* contaminated in kashar cheese during the 30-day storage period.

The count of *A. niger* inoculated onto kashar cheese slices, each weighing approximately 20 g, was determined to be 3.01 log cfu g^{-1}. Among the samples wrapped in LLDPE film, the control group, as well as those wrapped in CRV-MMT and THY-MMT films, no inhibition was observed on the *A. niger* counts during the storage process; a consistent increase was observed, resulting in an average 2-log increase in fungal load. However, EUG-MMT and THY-HNT films exhibited a measurable fungistatic effect, while CRV-HNT films reduced the *A. niger* count to as low as 2.17 log cfu g^{-1}. The fungal load of the kashar cheese sample wrapped in CRV-HNT film did not show a significant change ($P>0.05$) in the first 20 days of storage but tended to decrease from day 20 until day 30. Films produced with CRV-HNT and EUG-MMT demonstrated fungicidal effects during storage, resulting in a relative reduction in mold spore counts, while THY-HNT films exhibited fungistatic effects. The control, as well as CRV-MMT and THY-MMT films applied on the surfaces of cheese slices, showed an increase in mold counts during storage. Taking into account the effect of nanoclays on inhibitory effect of ANFs on *A. niger* at kashar cheese, incorporation of HNT showed higher antifungal activity than the MMT because lower *A. niger* numbers were observed in kashar cheese samples wrapped with CRV-HNT and THY-HNT films than in the samples with CRV-MMT and THY-MMT at the end of the storage, respectively (*Table 2*).

Various studies have been conducted to prevent mold growth in kashar cheese. In this context, Küçük et al. (2020) examined the use of alginate and zein films with natamycin applied to the surface of kashar cheeses. Their findings indicated that at least 2000 ppm of natamycin in the zein films is necessary to provide sufficient antifungal protection against *A. niger*. Furthermore, Kirci (2020) found that only the films with 3%, 4%, and 5% palmarosa oil showed antifungal activity against *A. niger*. Despite the 5% concentration demonstrating the strongest effect, a 4% concentration was chosen for kashar cheese films to minimize the distinctive odor of palmarosa oil.

3.3. The effect of ANFs on the growth of L. monocytogenes in kashar cheese

Gram-positive, facultative anaerobic *L. monocytogenes* is a species that can thrive in a broad pH range and endure pasteurization temperatures as low as 72 °C (Roberts et al., 2020). In this study, the influences of ANFs on *L.*

monocytogenes, an important foodborne pathogen that can cause illness in humans and is commonly found in various dairy products, especially raw milk and soft cheese, was investigated. For this purpose, kashar cheese slices contaminated with *L. monocytogenes* at a level of ~5.00 log cfu g^{-1} were vacuum packaged with ANPs, and the growth of *L. monocytogenes* during the storage process was monitored. *Table 2* presents the impact of vacuum packaging with different ANFs on the load of *L. monocytogenes* contaminated in kashar cheese.

As seen in *Table 2*, the *L. monocytogenes* count before storage in kashar cheese was 5.29 log cfu g^{-1}. The *L. monocytogenes* counts of all samples, including the control film samples, markedly reduced ($p<0.05$) during the 30-day storage period. The *L. monocytogenes* counts observed after storage in samples packaged with CRV-HNT and THY-MMT were statistically lower ($p<0.05$) than those of the control film samples. A statistically significant difference ($p>0.05$) was not observed in *L. monocytogenes* counts after storage between the control sample and samples packaged with THY-HNT, EUG-MMT, and CRV-MMT. CEO, rich in bioactive compounds like eugenol (76%), effectively targets foodborne pathogens and spoilage bacteria by damaging their cell membranes and walls. This damage allows CEO to penetrate the bacterial cytoplasm, where it disrupts DNA and protein synthesis, ultimately causing cell death (Boro et al, 2022). This reduction in bacterial count aligns with the antimicrobial properties of essential oils. Moreover, eugenol, like carvacrol, is a non-crystallizable phenol. This characteristic influences its bioavailability and how it interacts with bacterial cells, contributing to its ability to disrupt cell membranes and walls without forming crystals. Unlike crystallizable phenols like thymol, non-crystallizable phenols such as eugenol maintain a more fluid molecular structure, which may enhance their antimicrobial efficacy by facilitating better interaction with microbial cell components (Arámbula et al., 2019; Irmak and Erbatur, 2008). Thus, films infused with essential oils such as eugenol, carvacrol, and CEO could be particularly effective in reducing microbial growth during storage.

Although no cases of listeriosis attributed to the consumption of fresh kashar cheese have been reported, considering its pH, water activity, and nutritional composition, it can be regarded as a suitable dairy product for the growth of *L. monocytogenes.* Similarly, Seydim et al. (2020) found that using whey protein isolate films with oregano oil, garlic oil, nisin, and natamycin on kashar cheese slices significantly reduced *L. monocytogenes* counts, with nisin-containing films showing the most pronounced effect. Moreover, Perara et al. (2023) reported that sodium alginate-gelatin films containing nanoclay (NC) and CEO showed complete bacterial inhibition within 24 h, with significant log reductions observed for both gram-positive and gram-negative bacteria, including a 3.71 log reduction for *L. monocytogenes* in the 1% NC-CEO film. Overall, the findings showed that *L. monocytogenes* populations in all vacuum-packaged cheeses decreased during storage. Additionally, it is evident that CRV-HNT and THY-MMT packaging samples effectively limited bacterial growth.

3.4. The effect of ANFs on the pH of kashar cheese

One of the physicochemical properties that can be changed by microbial growth in food items like kashar cheese, affecting their shelf life, is the pH level. The changes in pH values of kashar cheeses packaged with different produced ANFs during storage were presented in *Table 3*. During the 30-day storage period, pH values were measured in the following ranges: 5.24-5.52 for control, 5.18-5.52 for CRV-MMT, 5.17-5.52 for CRV-HNT, 5.23-5.52 for EUG-MMT, 5.15-5.52 for THY-MMT and 5.29-5.52 for THY-HNT. According to Yangılar (2015), optimal pH range (5.5–5.8) and the high moisture content (45%) of kashar cheese create favorable conditions for the growth of various microorganisms.

The pH values of all samples exhibited a significant decrease ($p<0.05$) during the storage. Cheeses packaged with neat LLDPE (control group) films and films produced with THY-MMT showed a decrease in pH until the 10^{th} day, followed by a slight increase after the 10^{th} day. The initial decrease in pH might be due to the production of organic acids resulting from bacterial activity during the early stages of storage. The subsequent increase after the 10^{th} day could be attributed to the formation of basic compounds from the activities of proteolytic microorganisms. The ongoing pH drop during storage may suggest that acid generation was prominent over proteolytic action. These results were generally in accordance with the literature findings. For instance, Dıblan and Kaya (2023) stated that the pH values of kashar cheese produced with raw milk and starter culture-enriched pasteurized milk were in the range of 4.91-5.40. Moreover, Kavas et al. (2015) reported that pH values of kashar cheese samples coated with whey protein isolate-based films containing thyme and CEO 1.5% (v/v) ranged from 4.07 to 4.48 during 60 days of storage. In addition, Dıblan and Kaya (2023) incorporated potassium sorbate, nisin, chitosan, or silver-substituted zeolite into low-density

polyethylene (LDPE)/polyamide/LDPE films with 2% antimicrobials and observed no significant pH variation (5.40–5.48). Lim et al. (2020) reported that pH values of soft cheese samples decreased gradually from >6.0 to 5.7-5.9 during the storage for 30 days irrespective from the packaging application type (LDPE, polybutylene adipate-co-terephthalate (PBAT), LDPE with grape seed extract or PBAT with grape seed extract). Additionally, Andiç et al. (2011) investigated the pH changes during for kashar cheese wrapped with either vacuum-sealed or non-vacuum-sealed plastic packaging a 180-day storage period. The study reported an increase in pH values from the initial levels of 5.24 and 5.25 in both cheese samples during storage.

Table 3. The change in the pH values of kashar cheese packaged with different ANFs during the storage

Storage duration (days)	pH					
	Control	**CRV-MMT**	**CRV-HNT**	**EUG-MMT**	**THY-MMT**	**THY-HNT**
0	5.52 ± 0.07^{aA}	5.52 ± 0.07^{aA}	5.52 ± 0.07^{aA}	5.52 ± 0.07^{aA}	5.52 ± 0.07^{aA}	5.52 ± 0.07^{aA}
10	5.24 ± 0.02^{bC}	5.39 ± 0.03^{bAB}	5.31 ± 0.07^{bBC}	5.39 ± 0.01^{bAB}	$5.36\pm0.06b^{AB}$	5.41 ± 0.03^{abA}
20	5.27 ± 0.04^{bB}	5.25 ± 0.02^{cBC}	5.18 ± 0.02^{bcBC}	5.25 ± 0.01^{cBC}	5.15 ± 0.05^{cC}	5.43 ± 0.07^{aA}
30	5.28 ± 0.03^{bAB}	5.18 ± 0.03^{Cc}	5.17 ± 0.05^{cC}	5.23 ± 0.03^{cBC}	5.34 ± 0.02^{bA}	5.29 ± 0.01^{bAB}

* THY: Thymol; EUG: Eugenol; CRV: Carvacrol; Control: Neat LLDPE film without nanoclay; CRV-MMT: Carvacrol loaded montmorillonite; CRV-HNT: Carvacrol loaded halloysite; EUG-MMT: Eugenol loaded montmorillonite; THY-MMT: Thymol loaded montmorillonite; THY-HNT: Thymol loaded halloysite; ±: standard deviation; $^{a-c}$: Different lowercase letters within the same column indicate a statistically significant difference ($P<0.05$) between the data; $^{A-C}$: Different uppercase letters within the same row indicate a statistically significant difference ($P<0.05$) between the data.

4. Conclusions

In this study, the active ANFs prepared from linear low-density polyethylene (LLDPE), which employed layered montmorillonite (MMT) or tubular halloysite (HNT) nanoclays loaded with thymol (THY)/eugenol (EUG), or carvacrol (CRV) for reinforcing polymer nanocomposites were successfully used on kashar cheese and assessed their natural microbial load (including total lactic acid bacteria (LAB), total mesophilic aerobic bacteria (TMAB), total coliforms (TC), total yeast-mold (TYM)), the presence/development of *L. monocytogenes, S. aureus, A. niger* as well as pH values. The results showed that when ranking the packaging materials based on their influence on the microbial populations of kashar cheese, the observed sequence was: THY-HNT showed the most significant impact on TMAB; EUG-MMT, CRV-HNT, and THY-HNT were effective for LAB; and CRV-HNT and THY-HNT were prominent for TYM. Additionally, there was no detectable growth of *S. aureus* observed in any sample, either at the initial stage or throughout the storage period. ANFs have suppressed the natural microbial load of kashar cheese as well as the growth of *A. niger* and *L. monocytogenes* that contaminated the cheese. Foremost, the pH levels of the cheese samples varied during the storage period, with the exception of the control samples. The results suggested that the utilization of active nanocomposite packaging could potentially enhance the shelf life of fresh kashar by decreasing the growth of *A. niger* and *L. monocytogenes.* In conclusion, future research should focus on long-term storage experiments to further evaluate the sustained effectiveness of ANFs on shelf life of different kinds of cheeses and other perishable products, which will confirm their potential benefits and optimize their application in food preservation.

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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: Tornuk, F., Sagdıc, O., Hancer, M., Yetim, H.; Design: Tornuk, F., Yetim, H.; Data Collection or Processing: Fatih, T.; Statistical Analyses: Tornuk, F.; Literature Search: Tornuk, F., Kutlu, G.; Writing, Review and Editing: Tornuk, F., Kutlu, G.

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