Received: 26 April 2019 | Revised: 29 July 2019 | Accepted: 28 August 2019

DOI: 10.1002/pts.2483

RESEARCH ARTICLE

WILEY Packaging Technology and Science An International Journal

Evaluation of alginate and zein films as a carrier of natamycin to increase the shelf life of kashar cheese

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The aim of this study was to develop alginate and zein films containing natamycin, a natural antifungal agent, in order to limit/prevent the mould growth on the surface of kashar cheeses. The films were prepared by casting, and characterized in terms of antimicrobial and mechanical properties (tensile strength, elongation-at-break, and elastic modulus), and their morphology was examined by scanning electron microscopy (SEM). Mechanical properties of the zein films were found to be weaker than the alginate films. SEM analysis indicated that alginate films have a more regular structure than zein films, and a more homogenous distribution was observed at lower concentrations of natamycin. The antifungal activities of both films increased as the natamycin concentration (100, 200, 500, 1000, 2000, and 4000 ppm) increased; however, alginate films exhibited relatively high antifungal activity. The effects of films on the shelf life of kashar cheeses inoculated with *Aspergillus niger* and *Penicillium camemberti* were investigated during their storage under refrigerator conditions for 45 days. At high-natamycin concentrations, zein films showed higher antifungal activity against both fungi at the end of the storage period.

KEYWORDS

active packaging, antimicrobial packaging, biofilm, biopolymer, mould

1 | INTRODUCTION

Surface contamination of foods by undesirable microorganisms during processing and packaging significantly disrupt the quality of the food products.[1] Direct application of antimicrobial agents on the food surfaces by dipping, spraying, or coating could be used to inhibit the microorganisms and increase the shelf life of foods.[2] However, incorporation of antimicrobial agents onto food surface with these methods not only lead to inactivation of the active substances but also result to rapid diffusion within in the bulk of foods.[3] Recently, there has been a growing interest in antimicrobial packaging, which is considered to be member of active packaging technology to eliminate the microorganisms found on the surface of the foods.[3,4] In this context, biopolymer-based antimicrobial packaging has received great attention because of the increase in consumer demands for minimally processed foods and sustainable packaging.[5] Several types of biopolymers have been tested as potential antimicrobial packaging materials.[6-9] Among these polymers, alginate can be considered as promising biopolymer since it is non-toxic, biodegradable and has good film-forming properties. Films from alginate are uniform, transparent, and have good oxygen barrier properties at low-humidity conditions.[10] Zein is another important renewable polymeric material since it is the major coproduct of the rapidly growing oil and bioethanol industries. Films prepared from zein possess suitable gas barrier and heat seal properties suitable for packaging applications.[11,12]

A variety of synthetic antimicrobial agents such as sorbates, propionates, and benzoates have been added into different types of films to be used as antimicrobial packaging.[13-15] However, there is a growing interest in packaging materials containing natural antimicrobial agents because of consumer demand for natural food ingredients.[2] Among natural antimicrobial agents, special attention can be given to bacteriocins such as nisin, pediocin, and enterocin.[16] Natamycin (E-235) is another bacteriocin with GRAS (generally recognized as safe) status generated by *Streptomyces natalensis*, and it is very effective against

yeast and molds, even at low concentrations.[17] For these reasons, it is commonly used in dairy products, especially in cheese. Several types of polymers have been used as natamycin carrier such as whey, cellulose, wheat gluten, methylcellulose, and chitosan.[3,18-20] However, to the best of our knowledge, there has been no work on the use of zein and alginate films as a carrier of natamycin to retard mould growth on kashar cheese.

Kashar is the most produced cheese type after white cheese in Turkey, and surface contamination of this cheese is a common issue encountered in dairy companies and households mainly as the results of inappropriate ripening and storage conditions applied following its production. Significant economic losses and serious health problems arise as a result of the consumption of mould contaminated cheese.[21] Thus, the aim of this study was to develop polysaccharide (alginate) and protein (zein)-based films by adding natamycin, in order to eliminate the molds found on surface of the kashar cheese. Initially, antifungal effects of films containing natamycin at different concentrations were determined by the agar disc diffusion method using the selected model microorganisms; *Aspergillus niger* and *Penicillium camemberti*. In the second part of the work, the mechanical properties (tensile strength [TS], elongation at break [EB], and elastic modulus [EM]) of the films were determined, and their suitability for food packaging was evaluated. Also in this section, the cross-sectional structure and surface morphology of the films were examined by SEM. Finally, the effect of prepared films on the shelf life of kashar cheese was tested for 45 days at refrigerator temperature using the tested mould.

2 | MATERIALS AND METHODS

2.1 | Preparation of zein and alginate films

Zein (3.5 g) was homogenized in 20-mL ethanol (80%) (Riedel-de Haen, Germany) using a hot plate magnetic stirrer at 350 rpm. The preliminary studies indicated that excess glycerol leads to a sticky structure while low-glycerol concentrations result in a brittle texture. Therefore, an optimum amount of 500 μL of glycerol (Sigma-Aldrich, Germany) was added slowly to the solution once it starts boiling. Film solutions (10 g ≈ 11-12 mL) was poured into polyester petri dishes and dried at room temperature for 72 hours. Alginate (0.4 g) was homogenized in 20-mL distilled water using a hot plate magnetic stirrer at 350 rpm. Similar to the zein film preparation, once alginate solutions start boiling, 10 g of film solution was poured into polyester petri dishes of 90-mm diameter and dried at room temperature for 72 hours.

2.2 | Preparation of films containing natamycin

After cooling the film solutions to the room temperature, natamycin (Delvecoid®, DSM Food Specialties, Netherlands) was added at appropriate concentrations (100, 200, 500, 1000, 2000, and 4000 ppm), and the mixture was stirred for approximately equal to 15 minutes in a magnetic stirrer and then poured into petri dishes and allowed to dry. Films that do not contain natamycin were used as the control.

2.3 | Preparation of fungal cultures

Penicillium camamberti was isolated from camembert cheese while *A niger* was procured from Ankara University culture collection. Potato dextrose agar (PDA; Merck, Germany) and dichloran rose bengal chloramphenicol agar (DRBC; Merck, Germany) were used for the cultivation and enumeration of both fungi, respectively. *A niger* and *P camemberti* were incubated at 30 °C for 1 week on PDA for stock culture preparation, and fresh subcultures were prepared weekly. *A niger* ve *P camemberti* spores collected in sterile saline. A number of spores were determined on DRBC agar, and the final concentrations of each fungi were adjusted to 1×10^4-spore/mL saline.

2.4 | Determination of antimicrobial properties of natamycin containing films

The antimicrobial properties of biofilms were determined according to the agar disc-diffusion method. The films were cut in squares (1×1 cm^2) with a scissor and sterilized under UV light (254 nm). The microorganisms were spread-plated on PDA medium, and square-cut films were placed on agar with a sterile forceps. The inhibition zone diameters around the discs were measured using a digital caliper following incubation.

2.5 | Film thickness

A digital electronic calliper was used to measure the approximate thickness of the films. The film thicknesses were expressed as averages of 10 measurements obtained from random points at room temperature.

2.6 | Mechanical characteristics of the films

The mechanical properties of the films were tested according to ASTM Method D882 by the Texture Profile Analyzer (model TA-XT.plus, Stable Micro System, UK). Samples prepared for texture analysis were dried in petri dishes. After drying, the films were maintained at 60% ± 2% relative humidity and room temperature for 48 hours. The relative humidity was obtained with a saturated magnesium sulfate (Tekkim, Turkey) solution in a desiccator. The films were cut into 10 mm width and 50 mm length and clamped to the device by stretching. The test parameters used are as follows: pretest speed: 1 mm/s; test speed: 1 mm/s; posttest speed: 10 mm/s; distance: 15 mm; trigger type: automatic-5 g. TS values, EB, and EM were calculated from stress-strain graphs. The TS value was calculated by dividing the cross-sectional area of the film (thickness × width) to the maximum breaking load of the film while EB value was calculated by dividing the initial length of the specimens to the elongation of the film multiplied by 100. The EM is calculated from the slope of the stress-strain

graph in the first linear region. At least six films have been tested for each film type.

2.7 | Scanning electron microscopy (SEM)

The cross-sectional structure and surface morphology of the films were investigated by scanning electron microscope (SEM) at 15 KV. Samples were fixed with double-sided tape on the sample holder plate, and the surfaces and sections were scanned.

2.8 | Food application of films containing natamycin

2.8.1 | Preparation of specimens and inoculum

Cheeses were purchased from a local supermarket in Ordu, Turkey and brought to the laboratory in isolated boxes with ice packs. Kashar cheese samples were cut to a diameter of 2 cm and a thickness of 1 cm (approximately 20 g). Both sides of the kashar slices were exposed to UV light (254 nm) for 10 minutes, respectively. Then, cheese slices were immersed in mould solution (10^4 spores/mL) for 3 minutes, and each surface was dried for 10 minutes under a laminar hood. Dried kashar slices were sandwiched between two round films (≈4.5-cm diameter), put in locked plastic bags, and stored in a refrigerator. Samples were denoted as follows: (a) no treatment (T1), (b) UV treated (T2), (c) UV treated and inoculated with either *A niger* or *P roquefortii* (T3), (d) UV treated + inoculated + control films (T4), (e) no treatment + control films (T5), (f) UV treated + inoculated + antimicrobial films (T6-T10).[22]

2.8.2 | Microbiological analysis

Cheese samples were stored in a refrigerator, and one sample opened for each analysis period of 0-15-30 and 45th day. Edible films were separated from the cheese slices using a sterile forceps and homogenized (Stomacher/Interscience Bagmixer 400, Paris, France) in 180-mL saline (0.1%). Appropriate dilutions were plated on DRBC agar, and plates were incubated at 30°C for 5 days.

2.9 | Statistical analysis

All analyses were performed in duplicate. The data procured were subjected to ANOVA for variance analysis, and Tukey test was applied to determine the statistical differences ($P \leq .05$) between groups (JMP, USA).

3 | RESULTS AND DISCUSSION

3.1 | Antimicrobial activities of natamycin-containing zein and alginate films

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent to prevent the visible growth of the target microorganism. In the case of zein films, the MIC was found to be 1000 ppm of natamycin (Table 1). Over 1000 ppm, increasing concentrations of natamycin resulted in inhibition zones with greater diameters. Therefore, the maximum inhibition was obtained with the highest dose of 4000-ppm natamycin. Zein films with 4000-ppm natamycin brought about 2.20- and 3.37-cm inhibition zone diameters against *A niger* and *P camemberti*, respectively.

The control films and alginate films containing 100-ppm natamycin showed no antimicrobial activity against the test microorganisms. Therefore, the MIC of alginate films containing natamaycin was determined to be 200 ppm for both fungi tested. The inhibition zone diameters increased as natamycin concentration increased, and maximum inhibition zone diameter was determined in alginate films with 4000 ppm of natamycin. Alginate films containing natamycin had a more effective inhibition against *P camemberti* than *A niger*.

Overall, alginate films showed greater inhibitory activity than zein films against both *A niger* and *P camemberti*. This phenomenon could be attributed to more natamycin liberation to medium in alginate films

TABLE 1 The inhibition zone diameters (cm) of zein and alginate films containing natamycin at different concentrations against *Aspergillus niger* and *Penicillium camemberti*

Natamycin concentration, ppm	Inhibition Zone Diameters, cm			
	Zein films		Alginate films	
	P camemberti	A niger	P camemberti	A niger
0	-	-	-	-
100	-	-	-	-
200	-	-	3.48 ± 1.38[a]	2.63 ± 0.23[a]
500	-	-	4.01 ± 0.88[a]	2.99 ± 0.41[a]
1000	2.30 ± 0.28[a]	1.15 ± 0.21[a]	4.69 ± 0.26[a]	3.38 ± 0.31[a]
2000	2.80 ± 0.26a[b]	1.65 ± 0.20[ab]	4.80 ± 0.22[a]	4.32 ± 1.11[a]
4000	3.37 ± 0.19[b]	2.20 ± 0.14[b]	4.97 ± 0.04[a]	4.40 ± 1.10[4a]

Note. -: no zone.; there is no statistical difference ($P > .05$) between the inhibition zones of different NA concentrations indicated with the same superscripts (a-d). Levels having the same letter are not significantly different.

owing to their more hydrophilic character compared with zein films. Another factor affecting natamycin release from the film is the cross-linking degree. Considering both natamycin and zein have protein structures, there may be a stronger cross-linking between the two leading to higher retention of natamycin inside the zein films.[23] However, the antimicrobial activity is increased against both tested microorganisms as the concentration of natamycin in zein and alginate film increased. Similarly, da Silva et al[24] obtained the largest inhibition zone diameter with the alginate films containing the highest concentration of natamycin (8%) against the model microorganisms of *Debaromyces hansenii*, *Penicillium commune*, and *Penicillium roqueforti*. Türe et al[18] investigated the antimicrobial effects of natamycin against *A niger* and *P roqueforti* microorganisms. As the amount of natamycin added (0.007-0.033 g) into wheat gluten and methylcellulose biofilms increased, the inhibition zone diameters also increased. As a result, they determined that natamycin is more effective against *P roqueforti* than *A niger*, *P roqueforti*, and *P camemberti* are from the same genus *Penicillium*. Similarly, greater inhibition zones formed against *P camemberti* compared with *A niger* in this study, indicating that it is more sensitive to natamycin than *A niger*.

3.2 | Physical and mechanical properties of the films

3.2.1 | Film thickness

The results have shown that the zein films are generally thicker than the alginate films (Table 2). At different natamycin concentrations, it was observed that the thickness of alginate films varied from 0.02 to 0.03 mm while zein films ranged between 0.30 and 0.51 mm. The zein films containing 2000-ppm natamycin were the thickest while those containing 200-ppm natamycin were the thinnest. However, there was no regular pattern found between the thicknesses of the films and the amount of natamycin they contain. It was reported that the film thicknesses increased (47-65 μm) proportionally with increasing natamycin concentrations (0-8 g) in a similar study by da Silva et al[24].

3.2.2 | Mechanical properties

Mechanical properties of alginate and zein films are given in Table 2. No significant differences were observed between the elongation percentages of alginate films with different concentrations of natamycin. As natamycin concentration increased, the TS of alginate films also increased, and it reached to a maximum of 830.9 MPa in films containing 2000 ppm of natamycin. However, it was fairly low (469 MPa) in films containing 4000 ppm natamycin. The EM of alginate films followed a similar trend with the TS but the reduction in EM at 4000 ppm is not that significant.

The elongation percentage of zein films varied from 0.67% to 3.45%. The highest TS was determined in alginate films containing 200 ppm of natamycin. The EM of the zein films changed between 335.6 and 674.2 N/mm^2, and the highest elasticity was determined in the zein films containing 200 ppm of natamycin, while the lowest elasticity was determined in the control (natamycin-free) group. The addition of glycerol as a plasticizer and natamycin as an antimicrobial agent in the formulation of the zein films enhances the elasticity and positively contributes to the development of the film structure (Table 2). Zein films are protein-derived biopolymers with brittle while alginate films have more flexible and strong film structure because of their hydrocolloidal structure. The brittleness and elasticity issues in zein films are outcomes of strong hydrophobic reactions holding zein molecules together; however, glycerol could be used as a plasticizer to compensate these defects and improve its mechanical properties.

3.2.3 | Morphologies of the films by SEM

Surface and cross-sectional structures of alginate and zein films without natamycin and containing 4000 ppm natamycin were investigated

TABLE 2 Thickness values and mechanical properties of alginate and zein films containing natamycin

Film Type	NA, ppm	Thickness, mm	Mechanical Properties		
			TS, MPa	EM, N/mm^2	EB, %
Alginate	0	0.030 ± 0.008^{a}	280.82 ± 159.20^{b}	3333.40 ± 2236.30^{b}	3.447 ± 0.002^{a}
	100	0.030 ± 0.008^{a}	355.07 ± 189.10^{b}	4704.90 ± 1251.30^{ab}	3.446 ± 0.001^{a}
	200	0.023 ± 0.005^{a}	610.04 ± 275.10^{ab}	5926.90 ± 2064.80^{ab}	3.449 ± 0.002^{a}
	500	0.023 ± 0.005^{a}	503.10 ± 161.70^{ab}	6081.00 ± 891.70^{ab}	3.446 ± 0.001^{a}
	1000	0.020 ± 0.002^{a}	629.60 ± 55.40^{ab}	6581.00 ± 20.70^{ab}	3.270 ± 0.248^{a}
	2000	0.020 ± 0.002^{a}	830.90 ± 114.90^{a}	7633.50 ± 646.20^{a}	3.446 ± 0.001^{a}
	4000	0.020 ± 0.010^{a}	469.00 ± 201.30^{ab}	7278.20 ± 554.60^{a}	3.446 ± 0.001^{a}
Zein	0	0.44 ± 0.007^{abc}	1.00 ± 0.17^{b}	335.60 ± 152.72^{d}	3.45 ± 0.001^{a}
	100	0.42 ± 0.024^{ab}	1.30 ± 0.65^{b}	544.20 ± 43.59^{abc}	1.14 ± 0.623^{c}
	200	0.30 ± 0.015^{c}	3.70 ± 1.26^{a}	674.20 ± 9.58^{a}	2.77 ± 0.814^{ab}
	500	0.37 ± 0.051^{bc}	1.60 ± 0.70^{b}	639.96 ± 83.45^{a}	0.96 ± 0.362^{c}
	1000	0.43 ± 0.063^{ab}	1.40 ± 0.76^{b}	565.84 ± 102.95^{ab}	1.09 ± 0.462^{c}
	2000	0.51 ± 0.035^{a}	0.40 ± 0.16^{b}	349.69 ± 53.40^{cd}	0.67 ± 0.155^{c}
	4000	0.44 ± 0.054^{ab}	1.50 ± 0.52^{b}	458.09 ± 50.88^{bcd}	1.76 ± 0.384^{bc}

Note. There is no statistical difference ($P > .05$) between the mechanical properties of different NA concentrations with same superscripts (a-d).

Abbreviations: EB, percent elongation at break; EM, elastic modulus, TS, tensile strength.

by SEM (Figure 1). Considering the micrographs given, it is proper to say that the film samples with the most homogeneous surface structure are control (natamycin-free) alginate films. Similarly, Bierhalz et al[4] observed crystals and a non-uniform distribution of natamycin in alginate films while control films w/o natamycin exhibited a very homogenous structure. In a study by Türe et al[25], it was observed that the continuity of the methylcellulose and wheat gluten films was disturbed with increasing concentrations of natamycin. No air bubbles or holes were encountered on their surfaces while only the alginate films containing 4000 ppm of natamycin appeared to be rough in some sections (Figure 1D). It was observed that natamycin was in crystallized form when the cross-section of alginate films containing 4000-ppm natamycin was examined. Therefore, this roughness is very likely due to the inadequately dissolved natamycin. Similarly, dos Santos Pires et al[26] observed small antimicrobial crystals in cellulose films containing natamycin using SEM. When SEM micrographs of zein films were examined, air bubbles and holes were determined on the surfaces of the zein films (Figures 1E,G). As the concentration of natamycin increases, the cross-sectional structures of the films become more regular and tight; hence, it is possible to observe the presence of natamycin (Figure 1H). It can be said that increasing concentrations of natamycin and glycerol, which is used as plasticizer to

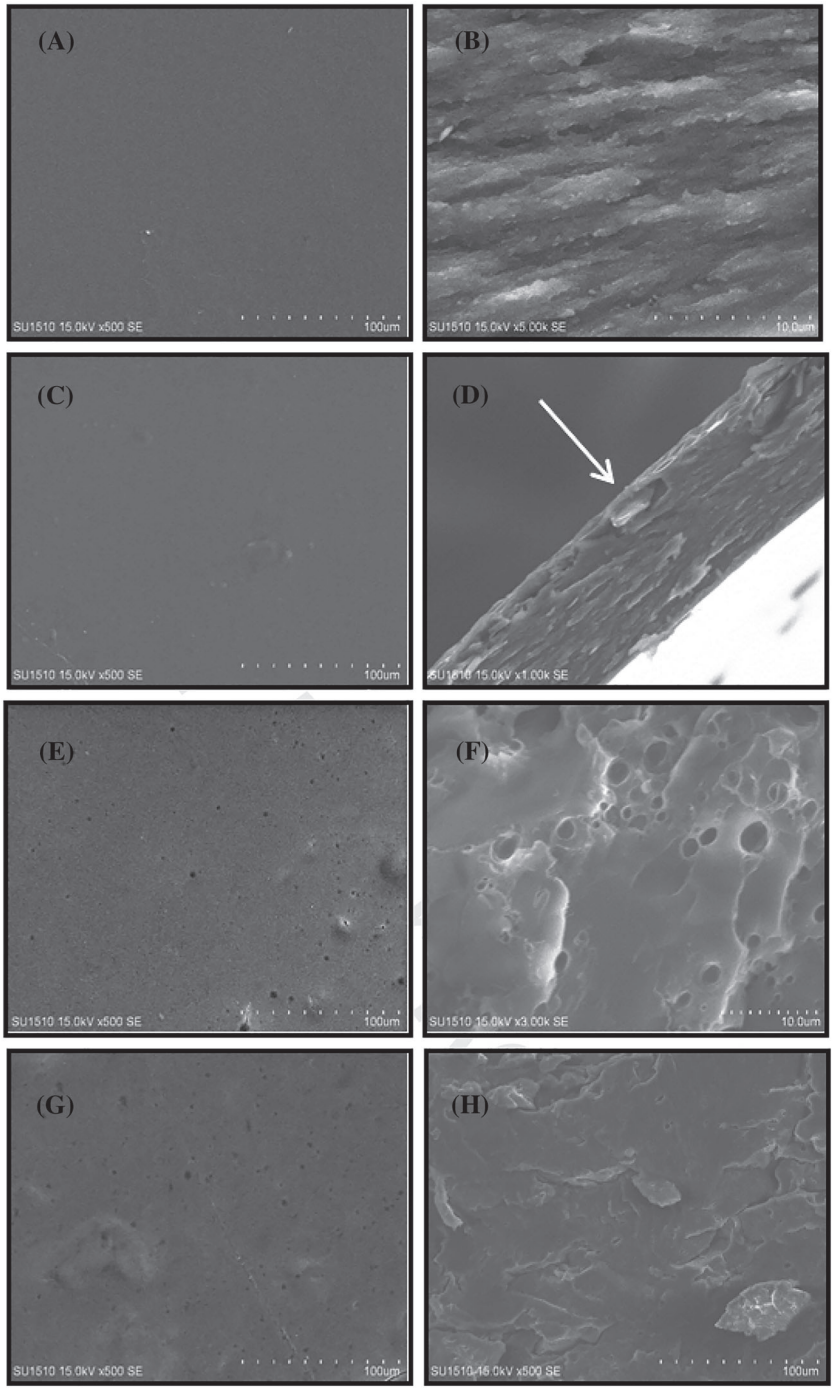

FIGURE 1 Surface and cross-sectional scanning electron microscopy (SEM) images of natamycin containing edible films. A, surface of the control alginate film, B, cross-section of the control alginate film, C, surface of the alginate film with 4000-ppm natamycin, D, cross-section of the alginate film with 4000-ppm natamycin, E, surface of the control zein film, F, cross-section of the control zein film, G, surface of the zein film with 4000-ppm natamycin, H, cross-section of the zein film with 4000-ppm natamycin. The arrow indicates natamycin crystal

improve the structural properties of zein films, are compatible with the zein film structure. In a similar study, the SEM micrographs indicated that the cross-sectional structures of the zein films containing 3.00-mg/cm^2 catechin are more uniform, and their surface structures are more homogenous.[27] At high concentrations, natamycin stratification was more apparent on top while the surface facing down had a smoother texture. Based on the visual evaluation, natamycin at low and high concentrations showed small-on-top and large-on-top stratification behaviours, respectively.[28]

3.3 | Application of the films to kashar cheese

Whey protein isolate and egg white protein based films incorporated with a variety of essential oils including oregano, clove, ginger, lavender, and orange were found successful as antibacterial biofilms in artificially contaminated kashar cheeses with *Listeria monocytogenes*, *Escherichia coli*, and *S aureus*. This study covers inhibition of molds, which is a more common issue in kashar cheeses.[29-32] Both fungi population in the plain cheeses with no treatment (T1), UV-treated samples (T2), and the cheeses packaged with natamycin-free zein and alginate films (T5) increased to the number, which cannot be enumerated on petri dishes at the end of 30 days of storage. Since there was no subsequent inoculation in these samples, this late domination of natural fungi could be explained with their long adaptation phase because of the presence of other competitor microflora.[22] Also, it would be proper to say that UV treatment could not completely eliminate but only suppress the growth of indigenous fungi population until the 30th day of storage. Having said that, the mould counts may not refer to only inoculated fungi species but contain indigenous fungi already present in the kashar cheese.

TABLE 3 Effects of zein films containing different concentrations of natamycin on *Aspergillus niger* population (log cfu/g) in kashar cheese during storage

Kashar cheeses		Day 0	Day 15	Day 30	Day 45
T1	No treatment	0^{b}	0^{c}	tmtc	tmtc
T2	UV treated	0^{b}	0^{c}	tmtc	tmtc
T3	UV treated + *A niger* inoculated	3.24 ± 0.280^{a}	4.73 ± 0.345^{a}	5.59 ± 0.162^{a}	6.46 ± 0.310^{a}
T4	*niger* inoculated + zein control film	3.62 ± 0.090^{a}	2.89 ± 0.669^{ab}	2.49 ± 1.957^{ab}	3.43 ± 0.058^{bc}
T5	No treatment + zein control film	0^{b}	0^{c}	tmtc	tmtc
T6	*A niger* inoculated + zein film (200-ppm NA)	2.98 ± 0.465^{a}	3.12 ± 0.020^{a}	2.04 ± 0.067^{b}	2.67 ± 0.120^{b}
T7	*A niger* inoculated + zein film (500-ppm NA)	2.72 ± 0.315^{a}	2.07 ± 0.148^{ab}	1.41 ± 0.007^{b}	1.89 ± 0.107^{cde}
T8	*A niger* inoculated + zein film (1000-ppm NA)	2.64 ± 0.353^{a}	2.19 ± 0.882^{ab}	1.29 ± 0.412^{b}	2.11 ± 0.212^{bcd}
T9	*A niger* inoculated + zein film (2000-ppm NA)	2.84 ± 0.389^{a}	2.19 ± 0.407^{ab}	1.45 ± 0.171^{b}	1.43 ± 0.070^{de}
T10	*A niger* inoculated + zein film (4000-ppm NA)	2.92 ± 0.243^{a}	2.01 ± 0.253^{b}	1.48 ± 0.013^{b}	1.42 ± 0.007^{e}

Note. There is no statistical difference between the *A niger* populations of different treatments indicated with the same superscripts ($P > .05$).

Abbreviation: tmtc, too many too count.

TABLE 4 Effects of zein films containing different concentrations of natamycin on *Penicillium camemberti* population (log cfu/g) in kashar cheese during storage

Kashar Cheeses		Day 0	Day 15	Day 30	Day 45
T1	No treatment	0^{b}	0^{b}	tmtc	tmtc
T2	UV treated	0^{b}	0^{b}	tmtc	tmtc
T3	UV treated + *P camemberti* inoculated	3.54 ± 0.66^{a}	3.36 ± 0.08^{a}	4.05 ± 0.08^{a}	4.87 ± 0.06^{a}
T4	*P camemberti* inoculated + zein control film	3.72 ± 0.21^{a}	2.96 ± 0.43^{a}	2.06 ± 0.13^{ab}	2.88 ± 0.01^{b}
T5	No treatment + zein control film	0^{b}	0	tmtc	tmtc
T6	*P camemberti* inoculated + zein film (200-ppm NA)	2.95 ± 0.01^{a}	2.08 ± 0.36^{a}	1.66 ± 1.02^{b}	1.89 ± 0.75^{d}
T7	*P camemberti* inoculated + zein film (500-ppm NA)	2.93 ± 0.11^{a}	2.44 ± 0.39^{a}	1.42 ± 0.71^{b}	2.49 ± 0.21^{c}
T8	*P camemberti* inoculated + zein film (1000-ppm NA)	2.88 ± 0.34^{a}	2.60 ± 0.47^{a}	1.30 ± 0.070^{b}	1.19 ± 0.08^{e}
T9	*P camemberti* inoculated + zein film (2000-ppm NA)	3.01 ± 0.23^{a}	2.80 ± 0.27^{a}	1.26 ± 0.02^{b}	1.23 ± 0.01^{e}
T10	*P camemberti* inoculated + zein film (4000-ppm NA)	3.14 ± 0.82^{a}	2.91 ± 0.08^{a}	1.35 ± 0.01^{b}	1.29 ± 0.07^{e}

Note. There is no statistical difference between the *P camemberti* populations of different treatments indicated with the same superscripts ($P > .05$).

Abbreviation: tmtc, too many too count.

3.3.1 | Application of natamycin containing zein films on kashar cheese

In *A niger* inoculated kashar cheese slices (T3), the mould population increased during storage, and the average mould level at the end of 45 days was 6.46 log cfu/g. In T4 samples, the mould population decreased slightly until the 30th day; however, an increase of approximately 1 log was observed at the end of the 45th day. The approximately equal to 3-log difference between T3 and T4 on days 30 and 45 could be attributed to the oxygen barrier role of zein films.[33] However, it is not proper to say that zein film alone has an antimicrobial activity considering there is no mould growth determined in plain kashar cheeses for the first 15 days of storage (Table 3).

A 200-ppm natamycin containing zein films (T6) lead to a fluctuating mould count in cheese samples suggesting that this concentration was just enough to control the fungi population. *A niger* counts in the cheeses packaged with zein films containing 500 ppm (T7) and 1000 ppm (T8) decreased up to 30th day of storage but increased to 1.89 and 2.11 log cfu/g on the 45th day, respectively. The zein films containing 2000- and 4000-ppm natamycin (T9 and T10) provided a constant reduction in *A niger* population with the final counts of 1.43 and 1.42 log cfu/g, respectively. This result indicates that at least 2000 ppm of natamycin is required in the zein films to provide adequate antifungal activity against *A niger*. Effects of zein films on *P camemberti* population are represented in Table 4. The number of *P camemberti* increased during the 45-day storage period and reached 4.87 log cfu/g in the inoculated kashar slices (T3). Regarding T3 and T4 similar to *A niger*, the zein film without natamycin decreased the *P camemberti* population approximately equal to 2 logs on days 30 and 45 probably because of its low oxygen permeability.

The zein films containing 200 (T6) and 500 ppm (T7) natamycin could suppress the growth of *P camemberti* until the 30th day of storage. The increase in the number of *P camemberti* on the day 45 indicates that natamycin concentrations lower than 1000 ppm was not sufficient to control the growth of this mould. The mould population in kashar samples continuously decreased along 45 days of storage when packaged with 1000-, 2000-, and 4000-ppm natamycin containing zein films. The spore number of *P camemberti* at the end of storage was determined to be 1.19, 1.23, and 1.29 log cfu/g, respectively. The natamycin concentrations greater than or equal to 1000 ppm were adequate in order to inhibit the growth of *P camemberti* for 45 days. Zein films containing lysozyme were found to suppress the growth of *L monocytogenes* in a study by Ünalan et al[34]. Similarly, another protein-based biofilm prepared with casein was found to suppress the growth of fungi in kashar cheeses for a period of 1 month when natamycin was included at a concentration of 0.07%[35] while Yangılar, Oğuzhan Yıldız[36] determined that casein film with natamycin also decreased the ripening index of kashar cheeses.

Assuming that natamycin is completely released from the film, a greater inhibition was achieved against *P camemberti* compared with *A niger* over 45 days of storage. However, further studies are required to determine how much of the natamycin is released from the film into the cheese and make a clear statement.

3.3.2 | Application of natamycin containing alginate films on kashar cheese

The number of *A niger* that was initially 2.54 log cfu/g reached to 5.48 log cfu/g after 45 days in *A niger* inoculated kashar cheese (T3). Although alginate films without natamycin (T4) provided a 15% decrease in *A niger* count at the end of the 15th day, the final count was 5.98 log cfu/g. In the plain kashar slices packed with natamycin-free alginate films (T5), an intensive microbial growth was observed at the end of the storage period. On the basis of these results, it can be said that natamycin-free alginate films alone do not have antimicrobial effect against *A niger*.

As the concentration of natamycin increased in the alginate films, the antimicrobial activity against *A niger* increased (Table 5). Cheese

TABLE 5 Effects of alginate films containing different concentrations of natamycin on *Aspergillus niger* population (log cfu/g) in kashar cheese during storage

Kashar Cheeses		Day 0	Day 15	Day 30	Day 45
T1	No treatment	0^{d}	0^{c}	tmtc	tmtc
T2	UV treated	0^{d}	0^{c}	tmtc	tmtc
T3	UV treated + *A niger* inoculated	2.54 ± 0.06^{a}	3.17 ± 0.08^{a}	4.18 ± 0.26^{a}	5.48 ± 0.05^{a}
T4	*A niger* inoculated + alginate control film	2.88 ± 0.05^{b}	2.42 ± 0.03^{b}	4.65 ± 0.06^{b}	5.98 ± 0.07^{b}
T5	No treatment + alginate control film	0^{d}	0^{c}	tmtc	tmtc
T6	*A niger* inoculated + alginate film (200-ppm NA)	2.42 ± 0.17^{bc}	2.49 ± 0.02^{b}	2.47 ± 0.10^{c}	2.46 ± 0.10^{c}
T7	*A niger* inoculated + alginate film (500-ppm NA)	2.31 ± 0.01^{c}	2.50 ± 0.02^{b}	2.40 ± 0.30^{c}	2.14 ± 0.05^{cd}
T8	*A niger* inoculated + alginate film (1000-ppm NA)	2.82 ± 0.11^{bc}	2.30 ± 0.07^{b}	2.54 ± 0.21^{c}	2.45 ± 0.01^{c}
T9	*A niger* inoculated + alginate film (2000-ppm NA)	2.78 ± 0.05^{bc}	2.40 ± 0.10^{b}	2.20 ± 0.14^{c}	2.13 ± 0.05^{cd}
T10	*A niger* inoculated + alginate film (4000-ppm NA)	2.90 ± 0.04^{b}	2.49 ± 0.02^{b}	2.17 ± 0.10^{c}	1.69 ± 0.03^{d}

Note. There is no statistical difference between the *A niger* populations of different treatments indicated with the same superscripts ($P > .05$).

Abbreviation: tmtc, too many too count.

TABLE 6 Effects of alginate films containing different concentrations of natamycin on *Penicillium camemberti* population (log cfu/g) in kashar cheese during storage

Kashar Cheeses		Day 0	Day 15	Day 30	Day 45
T1	No treatment	0^{c}	0^{c}	tmtc	tmtc
T2	UV treated	0^{c}	0^{c}	tmtc	tmtc
T3	UV treated + *P camemberti* inoculated	2.51 ± 0.66^{a}	3.09 ± 0.08^{a}	6.24 ± 0.08^{a}	6.02 ± 0.06^{a}
T4	*P camemberti* inoculated + alginate control film	2.77 ± 0.03^{b}	2.46 ± 0.02^{b}	6.04 ± 0.29^{a}	6.67 ± 1.08^{ab}
T5	No treatment + alginate control film	0^{c}	0^{c}	tmtc	tmtc
T6	*P camemberti* inoculated + alginate film (200-ppm NA)	2.93 ± 0.03^{b}	2.44 ± 0.23^{b}	5.04 ± 0.08^{b}	5.11 ± 0.22^{bc}
T7	*P camemberti* inoculated + alginate film (500-ppm NA)	2.57 ± 0.12^{b}	2.86 ± 0.02^{b}	4.83 ± 0.14^{b}	4.84 ± 0.22^{c}
T8	*P camemberti* inoculated + alginate film (1000-ppm NA)	2.88 ± 0.10^{b}	2.64 ± 0.01^{b}	4.78 ± 0.17^{b}	4.17 ± 0.10^{c}
T9	*P camemberti* inoculated + alginate film (2000-ppm NA)	2.92 ± 0.05^{b}	2.44 ± 0.15^{b}	4.71 ± 0.22^{b}	4.35 ± 0.01^{c}
T10	*P camemberti* inoculated + alginate film (4000-ppm NA)	3.01 ± 0.14^{b}	2.46 ± 0.02^{b}	4.70 ± 0.10^{b}	4.52 ± 0.07^{c}

Note. There is no statistical difference between the *P camemberti* populations of different treatments indicated with the same superscripts ($P > .05$).
Abbreviation: tmtc, too many too count.

samples covered with alginate film samples containing 200-ppm natamycin (T6) had 2.46 log cfu/g *A niger* at the end of storage period and showed an increase of 1.7%. There were fluctuations in the *A niger* counts of samples packaged with alginate films containing 500- and 1000-ppm natamycin. A permanent reduction in *A niger* load was observed throughout the storage period in kashar cheese slices coated with alginate films supplemented with 2000- and 4000-ppm natamycin. The alginate films containing a minimum of 2000-ppm natamycin showed antifungal activity against *A niger* and delayed microbial deterioration in the cheese slices.

The initial mould concentration in *P camemberti*, inoculated (T3) kashar cheese slices increased from 2.51 to 6.02 log cfu/g at the end of storage. Cheese slices packed with alginate films containing no natamycin (T4) showed microbial reduction of 11.2% on day 15, but *P camemberti* level was determined to be 6.67 log cfu/g at the end of storage period. This result showed that alginate films alone did not have antimicrobial activity against the *P camemberti*. The number of *P camemberti* in cheeses coated with alginate films containing 200- and 500-ppm natamycin increased during storage, and the final counts were determined to be 5.11 and 4.84 log cfu/g, respectively (Table 6).

As a result of microbiological analysis on day 15 of cheeses coated with alginate films containing 1000, 2000, and 4000-ppm natamycin, *P camemberti* levels were reduced to 2.64, 2.44, and 2.46 log cfu/g, respectively. However, in the progressive stages of storage, natamycin was ineffective against *P camemberti*, and thus, an increase in its number was observed. Furthermore, it was determined that the in vitro antimicrobial zones (on plates) obtained from *P camemberti* could not be obtained in the in vivo condition, in the kashar cheese.

Ture et al[22] stored kashar cheeses inoculated with *A niger* and *P roqueforti* and packed with natamycin-containing (2-20 mg) wheat gluten (WG) and methylcellulose (MC) films at 10°C for 30 days. While compared with the MC films, WG films containing natamycin provided better antimicrobial activity against *A niger*, they were both not effective against *P roqueforti*. de Oliveira et al[19] found that cellulose films incorporated with natamycin were effective against *P roqueforti* in Gorgonzola cheeses. Chitosan films, another carbohydrate-based biofilm, was found effective in suppression in yeast-mould counts in Saloio cheeses.[3] In a similar study, it was determined that two-layered wax films of chitosan and polyethylene containing natamycin had antimicrobial activity during storage of 20 days against pathogenic strains of *Alternaria alternate* and *Fusarium semitectum* inoculated to Hami melon.[37]

4 | CONCLUSIONS

The alginate and zein films have been observed to have increasing antimicrobial activity against both *A niger* and *P camemberti* when increasing concentrations of natamycin was included in the film formulations. At the same time, it was found that alginate films generated larger inhibition zones than zein films against both mould. The TS, EB, and EM values of the zein films are weaker than alginate films. Micrographs obtained by SEM show that alginate films have a more regular structure than zein films, and lower concentrations of natamycin have a more homogenous distribution in the alginate films. Considering in vitro antifungal effects, physical and mechanical properties, and SEM images, alginate films are more advantageous by far. However, the zein films are more effective in providing antifungal effect in kashar cheese, which is the main aim of this study. Therefore, new studies to improve the hydrophilic properties and to reduce gas permeability of alginate films will be helpful. On the other hand, new methods to improve mechanical properties of zein films are also required. Furthermore, future studies involving physical, chemical, and especially sensory properties of kashar will guide new researchers to the right way in developing the optimum edible films with antimicrobial properties.

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REFERENCES

1. Malhotra B, Keshwani A, Kharkwal H. Antimicrobial food packaging: potential and pitfalls. *Front Microbiol*. 2015;6:611.
2. Irkin R, Esmer OK. Novel food packaging systems with natural antimicrobial agents. *J Food Sci Technol*. 2015;52(10):6095-6111.
3. Fajardo P, Martins J, Fuciños C, Pastrana L, Teixeira J, Vicente A. Evaluation of a chitosan-based edible film as carrier of natamycin to improve the storability of Saloio cheese. *J Food Eng*. 2010;101(4):349-356.
4. Bierhalz AC, da Silva MA, de Sousa HC, Braga ME, Kieckbusch TG. Influence of natamycin loading methods on the physical characteristics of alginate active films. *J Supercrit Fluids*. 2013;76:74-82.
5. Cha DS, Chinnan MS. Biopolymer-based antimicrobial packaging: a review. *Crit Rev Food Sci Nutr*. 2004;44(4):223-237.
6. Azadbakht E, Maghsoudlou Y, Khomiri M, Kashiri M. Development and structural characterization of chitosan films containing Eucalyptus globulus essential oil: Potential as an antimicrobial carrier for packaging of sliced sausage. *Food Packag Shelf Life*. 2018;17:65-72.
7. Nunes MR, Castilho MSM, de Lima Veeck AP, et al. Antioxidant and antimicrobial methylcellulose films containing Lippia alba extract and silver nanoparticles. *Carbohydr Polym*. 2018;192:37-43.
8. Kamari A, Chitosan PE. gelatin and methylcellulose films incorporated with tannic acid for food packaging. *Int J Biol Macromol*. 2018;120:1119-1126.
9. Campos-Requena VH, Rivas BL, Pérez MA, Figueroa CR, Figueroa NE, Sanfuentes EA. Thermoplastic starch/clay nanocomposites loaded with essential oil constituents as packaging for strawberries – In vivo antimicrobial synergy over Botrytis cinerea. *Postharvest Biol Technol*. 2017;129:29-36.
10. Costa MJ, Marques AM, Pastrana LM, Teixeira JA, Sillankorva SM, Cerqueira MA. Physicochemical properties of alginate-based films: effect of ionic crosslinking and mannuronic and guluronic acid ratio. *Food Hydrocoll*. 2018;81:442-448.
11. Kashiri M, Cerisuelo JP, Domínguez I, et al. Zein films and coatings as carriers and release systems of Zataria multiflora Boiss. essential oil for antimicrobial food packaging. *Food Hydrocoll*. 2017;70:260-268.
12. Arcan I, Boyacı D, Yemenicioğlu A. The Use of Zein and Its Edible Films for the Development of Food Packaging Materials. Reference Module in Food Science: Elsevier; 2017.
13. Türe H, Gällstedt M, Hedenqvist MS. Antimicrobial compression-moulded wheat gluten films containing potassium sorbate. *Food Res Int*. 2012;45(1):109-115.
14. Ramos ÓL, Fernandes JC, Silva SI, Pintado ME, Malcata FX. Edible films and coatings from whey proteins: a review on formulation, and on mechanical and bioactive properties. *Crit Rev Food Sci Nutr*. 2012;52(6):533-552.
15. Chen MC, Yeh GHC, Chiang BH. Antimicrobial and physicochemical properties of methylcellulose and chitosan films containing a preservative. *J Food Process Preserv*. 1996;20(5):379-390.
16. Cleveland J, Montville TJ, Nes IF, Chikindas ML. Bacteriocins: safe, natural antimicrobials for food preservation. *Int J Food Microbiol*. 2001;71(1):1-20.
17. Khan M, Pirzadeh M, Förster C, Shityakov S, Shariati M. Role of milk-derived antibacterial peptides in modern food biotechnology: their synthesis, applications and future perspectives. *Biomolecules*. 2018;8(4):110.
18. Türe H, Eroğlu E, Soyer F, Özen B. Antifungal activity of biopolymers containing natamycin and rosemary extract against Aspergillus niger and Penicillium roquefortii. *Int J Food Sci Technol*. 2008;43(11):2026-2032.
19. de Oliveira TM, de Fátima Ferreira Soares N, Pereira RM, de Freitas Fraga K. Development and evaluation of antimicrobial natamycin-incorporated film in gorgonzola cheese conservation. *Packag Technol Sci*. 2007;20(2):147-153.
20. Pintado CM, Ferreira MA, Sousa I. Control of pathogenic and spoilage microorganisms from cheese surface by whey protein films containing malic acid, nisin and natamycin. *Food Control*. 2010;21(3):240-246.
21. Topal Ş. Kaşar peyniri olgunlaşma evresinde gelişen yüzey küfleri ve mikotoksin riskleri. *Gıda Dergisi*. 1987;12(3).
22. Ture H, Eroglu E, Ozen B, Soyer F. Effect of biopolymers containing natamycin against *Aspergillus niger* and *Penicillium roquefortii* on fresh kashar cheese. *Int J Food Sci Technol*. 2011;46(1):154-160.
23. Balaguer MP, Fajardo P, Gartner H, et al. Functional properties and antifungal activity of films based on gliadins containing cinnamaldehyde and natamycin. *Int J Food Microbiol*. 2014;173:62-71.
24. da Silva MA, Iamanaka BT, Taniwaki MH, Kieckbusch TG. Evaluation of the antimicrobial potential of alginate and alginate/chitosan films containing potassium sorbate and natamycin. *Packag Technol Sci*. 2013;26(8):479-492.
25. Türe H, Eroğlu E, Özen B, Soyer F. Physical properties of biopolymers containing natamycin and rosemary extract. *Int J Food Sci Technol*. 2009;44(2):402-408.
26. dos Santos Pires AC, de Fátima Ferreira Soares N, de Andrade NJ, da Silva L H M, Camilloto GP, Bernardes PC. Development and evaluation of active packaging for sliced mozzarella preservation. *Packag Technol Sci* 2008;21(7):375-383.
27. Arcan I, Yemenicioğlu A. Development of flexible zein–wax composite and zein–fatty acid blend films for controlled release of lysozyme. *Food Res Int*. 2013;51(1):208-216.
28. Liu X, Liu W, Carr AJ, et al. Stratification during evaporative assembly of multicomponent nanoparticle films. *J Colloid Interface Sci*. 2018;515:70-77.
29. Kavas G, Kavas N, Saygili D. The effects of thyme and clove essential oil fortified edible films on the physical, chemical and microbiological characteristics of kashar cheese. *J Food Qual*. 2015;38(6):405-412.
30. Kavas N, Kavas G, Saygili D. Use of ginger essential oil-fortified edible coatings in Kashar cheese and its effects on Escherichia coli O157: H7 and Staphylococcus aureus. *CyTA J Food*. 2016;14(2):317-323.
31. Kavas N, Kavas G. Physical-chemical and antimicrobial properties of Egg White Protein Powder films incorporated with orange essential oil on Kashar Cheese. *Food Sci Technol*. 2016;36(4):672-678.
32. Kavas N, Kavas G. Effect of TheLavander Essential Oil Fortified Edible Film on Some Properties of Kashar Cheese During Storage Time. *IOSR J Environ Sci*, Toxicology and Food Technology. 2018;12(11):55-60.
33. Tihminlioglu F, Atik İD, Özen B. Water vapor and oxygen-barrier performance of corn–zein coated polypropylene films. *J Food Eng*. 2010;96(3):342-347.
34. Ünalan İU, Arcan I, Korel F, Yemenicioğlu A. Application of active zein-based films with controlled release properties to control Listeria monocytogenes growth and lipid oxidation in fresh Kashar cheese. *Innov Food Sci Emerg Technol*. 2013;20:208-214.

35. Yildirim M, Güleç F, Bayram M, Yildirim Z. Properties Of Kashar Cheese Coated With Casein As A Carrier Of Natamycin. *Ital J Food Sci*. 2006;18(2).

36. Yangılar F, Oğuzhan Yıldız P. Casein/natamycin edible films efficiency for controlling mould growth and on microbiological, chemical and sensory properties during the ripening of Kashar cheese. *J Sci Food Agric*. 2016;96(7):2328-2336.

37. Cong F, Zhang Y, Dong W. Use of surface coatings with natamycin to improve the storability of Hami melon at ambient temperature. *Postharvest Biol Technol*. 2007;46(1):71-75.

How to cite this article: Küçük GS, Çelik ÖF, Mazi BG, Türe H. Evaluation of alginate and zein films as a carrier of natamycin to increase the shelf life of kashar cheese. *Packag Technol Sci*. 2019;1–10. https://doi.org/10.1002/pts.2483