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Original research

The effect of whey protein-based edible coating containing natamycin and lysozyme-xanthan gum conjugate on the shelf life of ultrafiltrated white cheese

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A B S T R A C T

The effect of whey protein concentrate based edible coating incorporating different concentrations of natamycin and lysozyme-xanthan gum conjugate on ultrafiltrated white cheese shelf life was investigated. *Escherichia coli* O157: H7 (as an indicator for gram negative bacteria), *Staphylococcus aureus* (as an indicator of gram-positive bacteria), and *Penicillium chrysogenum* (as a mold) were inoculated to the surface of experimental ultrafiltrated cheese samples followed by coating trials. The microbial, physicochemical, and organoleptic properties of cheese samples were evaluated during 60 days of ripening. The results showed that coating of cheese had inhibitory effect on growth of *Penicillium chrysogenum*. Natamycin-containing coatings were more effective in reducing the mold population than coatings incorporating lysozyme-xanthan. Coating with 600 ppm lysozyme-xanthan reduced 2.09 log *E. coli* O157:H7 growth compared to that of control (with no coating). Moreover, the populations of *Staphylococcus aureus* were lower in all coated samples containing lysozyme-xanthan than that of control. There was no significant difference ($p > 0.05$) between the pH, acidity, salt, and fat in the dry matter of the coated samples and those of the control sample during 60 days of ripening. Edible coatings reduced moisture loss in cheese (5.03%) during 60 days. Coating improved the textural properties of cheese samples, meanwhile did not have a significant effect ($p > 0.05$) on the taste and overall acceptance of cheese. The results of this study showed that whey protein based edible coating can be used as a carrier of natamycin and lysozyme-xanthan to increase the shelf life of ultrafiltrtated cheese.

Keywords: Edible coating, *Escherichia coli* O157:H7, Lysozyme-xanthan conjugate, *Staphylococcus aureus*, Ultrafiltrated white cheese

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1. Introduction

Ultrafiltrated cheese (UF) is one of the most important types of cheeses produced in industrial scale in Iran. This type of cheese contains at least 34% total solid (w/w), 12% protein, maximum acidity of 1.8% (lactic acid), pH of 4.6 to 5.2, and 5% salt (Jalilzadeh et al., 2018). This product is susceptible to contamination by unfavorable microorganisms, especially molds and yeasts, due to low pH levels. Another problem with the dairy industry is the failure of thermal pasteurization to deactivate certain microorganisms. Although *E.coli* has been reported to be destroyed by pasteurization, there are reports of its ability (including its pathogenic species, O157:H7) to form biofilm in pasteurization equipment, indicating the failure of pasteurization (Dewanti & Wong, 1995). *Staphylococcus aureus* and *Escherichia coli* O157:H7 are among the pathogens that have attracted considerable attention in dairy industry. *Staphylococcus aureus* can survive in white cheese, especially in the presence of yeasts, even at low pH and high salt levels. *Escherichia coli* O157:H7 is a potential risk factor in soft and semi-hard cheeses and several reports have shown that *E. coli* O157:H7 is able to survive and grow in different kind of cheese (Ioanna et al., 2018). It was shown that *Escherichia coli* O157:H7 was completely inhibited in the scalded-brined cheese within 30 days of ripening, however, this pathogen remains active in unscalded cheese even at high salt concentrations (e.g. 17.5% salt) (Robinson, 2014). Various strategies have been proposed to control cheese contamination to increase its shelf life. The application of edible coatings and films incorporating antimicrobial

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agents such as lysozyme, natamycin, herbal extracts, etc., is one of these strategies that have been studied for various cheeses among them, soft, semi-hard, and hard cheeses (Mehyar et al., 2018). Whey protein based films and coatings have been noticed in recent years because, in addition to being biodegradable, they utilize whey as a major by-product of cheese industry (Ramos et al., 2012). Moreover, they have excellent oxygen and water vapor barrier properties, and consequently can be used in food packaging (Schmid et al., 2013).

Lysozyme has been used in cheese production to prevent the growth of lactate-fermenting and gas-forming *Clostridia* spp. (Crapisi et al., 1993). Lysozyme attacks the β-1, 4 glycosidic linkage between N-acetylmuramic acid and N-acetylglucosamine groups found in peptidoglycan layer in bacterial cell walls. Gram-positive bacteria are sensitive to attack by lysozyme. However, Gram-negative bacteria are less susceptible (Duan et al., 2006). It has been reported that conjugating lysozyme enzyme with some polysaccharides can improve the antimicrobial activity spectrum and functional properties such as solubility, emulsion stability, foam stability, thermal stability, water binding capacity, and the antioxidant activity of lysozyme (Aminlari et al., 2005; Dickinson, 2003). Amiri et al. (2008) reported that conjugation of lysozyme-dextran had an excellent antimicrobial effect on *E.coli* compared to that of untreated enzymes, while there is no significant difference between *S. aureus* and non-conjugated lysozyme enzymes ($p > 0.05$). Takahashi et al. (2000) attempted to conjugate lysozyme and glucose-stearicmonoester, which improved antibacterial activity, thermal stability and emulsifier activity of lysozyme

Natamycin is a natural antifungal agent produced by *Streptomyces natalensis* and related strains. Natamycin is active against almost all yeasts and fungi, but has no effect on bacteria, proteases, or viruses (Delves-Broughton et al., 2005). The direct application of natamycin on food surface by spraying or immersion, has limited benefits due to inappropriate adhesion, and its antimicrobials activity generally exhibits a rapid loss due to rapid migration to food bulk, allowing surface fungal growth. While the use of film or coating based on antimicrobial polymers provides more efficiency resulting by maintaining a higher concentration of active material on the surface (Resa et al., 2014). Moreover, incorporation of antimicrobials in edible coating formulations can reduce the compound activity loss by interacting with food.

To the best of our knowledge, there has been no research reporting the effect of whey protein based edible coating incorporating conjugated lysozyme-xanthan and natamycin on UF white cheese shelf life. Therefore, the aim of this study was to investigate the effect of whey protein based edible coating containing lysozyme-xanthan conjugate and natamycin on the physical, chemical, microbial, and sensory properties of UF cheese during 60 days of ripening.

2. Material and Methods

2.1. Materials

The target microbial strains including one gram-negative bacterium (*Escherichia coli* O157:H7), one gram-positive bacterium (*Staphylococcus aureus* PTCC 1189) and one mold (*Penicillium chrysogenum* PTCC 5035) were obtained from the Iranian Research Organization for Science and Technology (Tehran, Iran) in freeze-dried form. Whey protein concentrate (WPC) with 81% protein (on a dry weight basis), 5.5% fat, 10.0% carbohydrate, 5.3% moisture, 3% ash and pH 6.10 (10% solution) was obtained from Agri-Mark (Massachusetts, USA). Commercial Natamycin containing 50% NaCl and 50% w/w Natamycin, and lysozyme (Mr 14600) was provided by DSM (The Netherlands) and Inovatech, Inc. (Abbotsford, BC, Canada), respectively. The mixture of thermophilic *Lactobacillus bulgaricus* and *mesophilic Lactococcus lactis* from the Chr. Hansen Co. (Hørsholm, Denmark) was prepared and used as a starter culture. The analytical grade reagents used were purchased from Merck (Frankfurt, Germany) and Sigma Chemical Co. (St. Louis, Mo, USA).

2.2. Preparation of edible coating solution

Edible coatings were prepared following the protocols established by Perez-Gago and Krochta (2002) with some modifications. Accordingly, 8.0 g of WPC powder was dissolved in 92 mL of distilled water, and heated for 45 min in a 90°C water bath to denature the whey protein. During heating, 5.0 g of glycerol, 5.0 g of beeswax, and 0.15 g of Tween 80 were added to the solution. The mixture was then homogenized for 3-4 min by Ultraurax T25 (IKA, Staufen, Germany) at 15000 rpm. During the heating, the pH of the solution was adjusted to 8 with 5 N sodium hydroxide. After homogenization, the emulsions were placed in an ice bath to prevent further denaturation of whey protein and to crystallize the lipid particles. The emulsions were degassed at room temperature with a vacuum pump and stored at 5°C until application. For cheese coating with different treatments, the coating solution divided in 6 parts and lysozyme-xanthan conjugate at concentrations of 200, 400 and 600 ppm, and natamycin at ratios of 0.01, 0.02 and 0.03% (w/w), were respectively added to the coating formulations.

2.3. Cheese manufacturing and coating

The manufacture of UF white cheese was carried out in East Azarbyajan Pegah Co. (Tabriz, Iran) as introduced by Tetra-pack incorporation (Bylund, 1995) and adapted by Hesari et al. (2006), as shown in Fig. 1. The details of cheesemaking are given in a previous work (Jalilzadeh et al., 2018). After production of cheese samples, a suspension of each targeted microorganisms was prepared and inoculated across the surface of cheese samples using a Drigalski spatula, it was later kept in a sterile cabinet at + 4°C for 15 min for adhesion and absorbtion of the inocula. The cheese samples were then dipped into coating solutions for 10 s and were left to dry in a refrigerated oven for approximately 4-5 h before being packaged. Packed cheese samples were stored at 8°C until the analysis was carried out. The experimental design is given in Table 1.

2.4. Preparation of lysozyme-xanthan gum conjugation

Conjugation of lysozyme with xanthan gum was performed according to procedure of Amiri et al. (2007).

2.5. Microbial analysis

Microbial analysis was performed on the 1st, 3th, 7th, 14th, 21th and 28th days of storage. Tryptic soy broth (TSB; Biolife, Milano, Italy) was used for pre-culture of bacteria, and specific culture

media were used for enumeration of microorganisms. For enumeration of *E. coli* O157:H7 Sorbitol MacConkey Agar containing Cefixime-Tellurite supplement (Conda, Madrid, Spain) was used, and incubated at 37°C for 24 h. *Staphylococcus aureus* was cultured in Baird Parker agar enriched by 5% Egg Yolk Tellurite emulsion (Oxoid) and was later incubated under aerobic conditions at 35-37°C for 24-48 h. *Penicillium chrysogenum* were enumerated after incubation in potato dextrose agar (BD Difco, France) at 25 ± 1°C for 72 h. The starter culture enumeration was done after incubation in M17 agar (HiMedia Laboratories, Mumbai/India) at 37°C for 48 h (US Food and Drug Administration, 2001). Microbial count was calculated in terms of Log_{10} cfu/g of cheese.

Table 1. The experimental design for cheese coating treatments.

Sample	Treatment
Control	without coating
Nat10	coated with WPC containing 0.01% natamycin
Nat20	coated with WPC containing 0.02% natamycin
Nat30	coated with WPC containing 0.03% natamycin
Lyz-Xan200	coated with WPC containing 200 ppm Lysozyme-Xanthan conjugate
Lyz-Xan400	coated with WPC containing 400 ppm Lysozyme-Xanthan conjugate
Lyz-Xan600	coated with WPC containing 600 ppm Lysozyme-Xanthan conjugate

WPC: Whey protein concentrate.

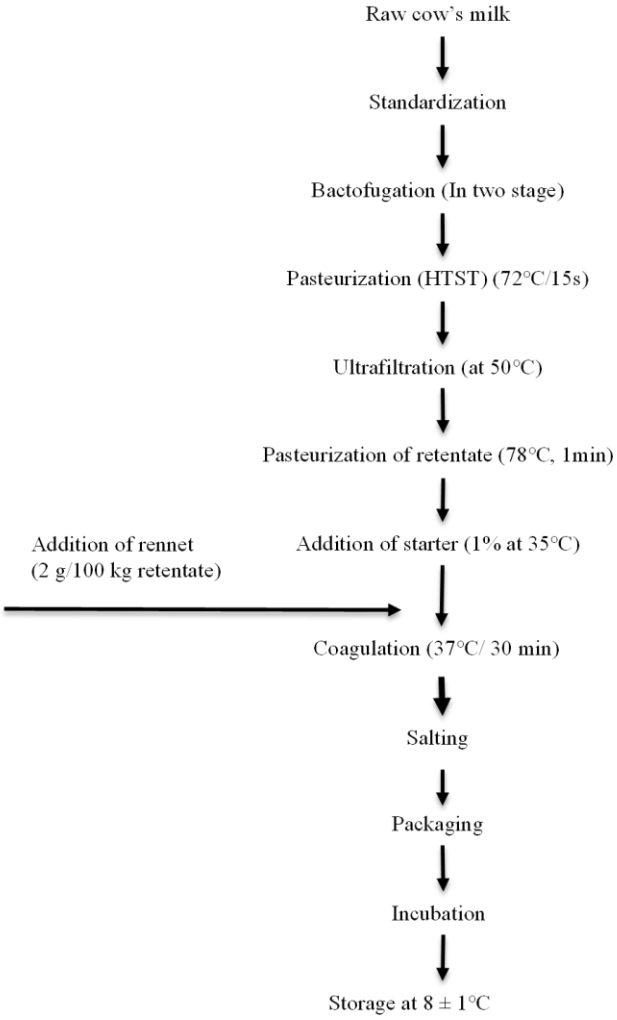

Fig. 1. UF White cheese manufacturers process.

2.6. Physicochemical characteristics

Physicochemical characteristics of cheese samples were studied at 1st, 15th, 30th, 45th and 60th days of ripening. The pH values were examined with a HI 99165 Hanna pH meter (Hanna Instruments, Singapore) by direct insertion of electrode into grated cheese. Titratable acidity was determined (g/100 g of lactic acid) according the method described in AOAC International (1995). Moisture, fat and salt were measured by the methods proposed by IDF (1989), Ardö and Polychroniadou (1999) and Bradley et al. (1992), respectively. Protein content was determined by micro Kjeldahl method (IDF, 1993). Texture profile analysis (TPA) was conducted using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a 36-mm cylinder probe as described by Rashidi et al. (2015). The cheese was carefully cut into 20 × 20 mm cylinders using a stainless steel cylinder knife and was held at room temperature (20°C). The texture analyzer was operated with two compression decompression cycles and pre-test speed of 1.0 mm/s and test speed of 1 mm/s. The recorded TPA parameters were hardness, springiness, gumminess, and cohesiveness.

2.7. Sensory evaluation

To determine if addition of coatings imparted any detectable organoleptic effect on cheese samples, sensory analysis of UF cheese samples were evaluated at 1st , 15th , 30th , and 60th day of ripening according to the procedure of Iranian National Standard No. 4691 (ISIRI, 1998). Samples for sensory analysis were produced separately following the procedure described in section 2.3 but excluding the inoculation with targeted microorganisms. Fifteen panelists at the age 24 to 50 years were randomly selected and trained to evaluate appearance, texture, flavor and overall acceptability on a scale of 0 to 5. The panelists were provided with coded cheese samples (20 g in cubic form) in separate polyester plates and a question sheet to evaluate cheese properties. The panelists used unsalted crackers and water to cleanse their palates between the samples. In order to increase the interrater reliability of the scores and remove the probable effect of raters' scores on each other, evaluators gave their respective scores in separate rooms. Moreover, the analysis was conducted in an odorless, adequately lighted and quiet laboratory (ISIRI, 1998).

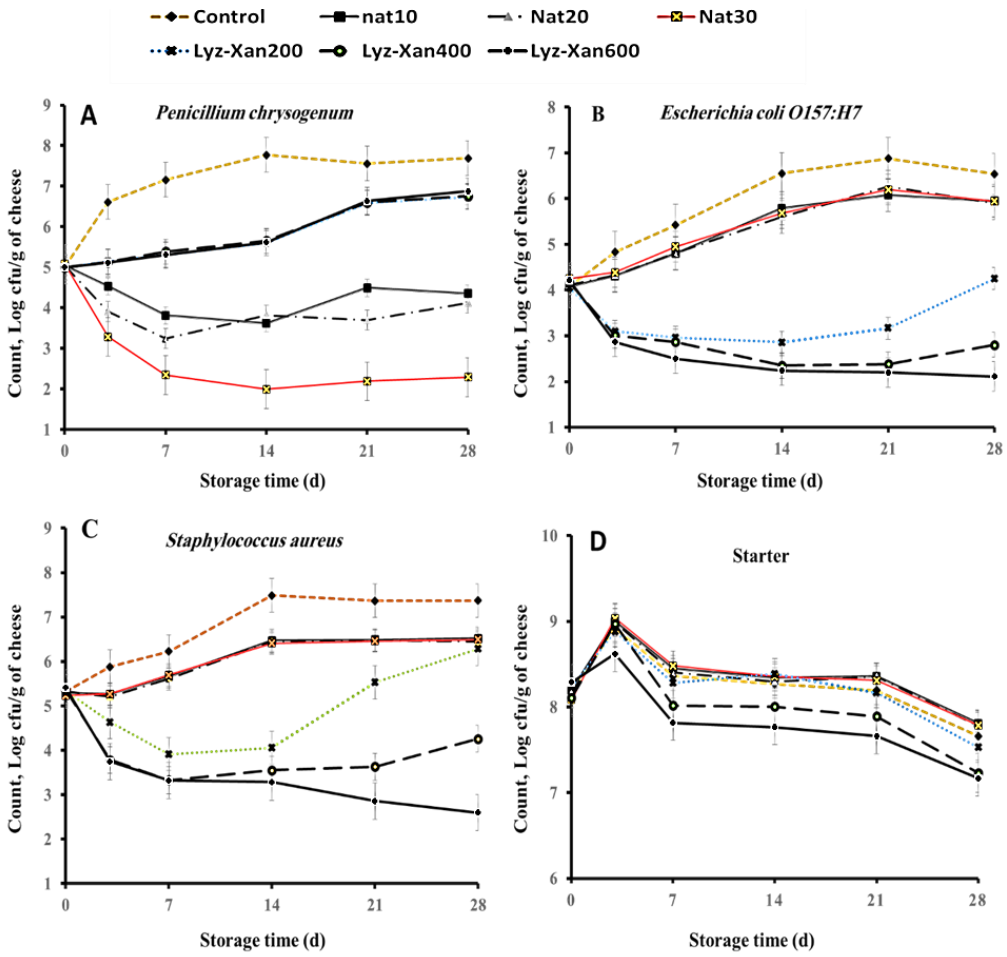

Fig. 2. The Effect of whey protein concentrate- based edible coatings containing natamaycin and lysozyme-xanthanate conjugate on *Penicillium Chrysogenum* (A), *Escherichia coli* O157: H7 (B), *Staphylococcus aureus* (C), and starter activity (D) in UF White cheese during 28 days. Values are mean of at least 3 replicates; error bars indicate standard deviations between means.

Table 2. The Effect of whey protein concentrate - based edible containing natamycin and lysozyme-xanthanate conjugate on pH, acidity and chemical composition in UF cheese during 60 days.

pH and composition	Treatment	Days 1	15	30	45	60
pH	Control	4.79±0.02^{Da}	4.39±0.05^{Aa}	4.56±0.07^{Ca}	4.49±0.04^{Ba}	4.44±0.05^{ABa}
	10 ppm Nat.	4.69±0.03^{Da}	4.42±0.05^{Aa}	4.55±0.02^{Ca}	4.48±0.02^{Ba}	4.46±0.03^{ABa}
	20 ppm Nat.	4.66±0.07^{Da}	4.39±0.06^{Aa}	4.59±0.06^{Ca}	4.49±0.03^{Ba}	4.48±0.04^{Ba}
	30 ppm Nat.	4.64±0.04^{Da}	4.37±0.04^{Aa}	4.57±0.04^{Ca}	4.51±0.05^{Ba}	4.51±0.04^{Ba}
	200 ppm Lyz-Xan	4.64±0.05^{Da}	4.41±0.06^{Aa}	4.58±0.03^{Ca}	4.51±0.04^{Ba}	4.47±0.05^{ABa}
	400 ppm Lyz-Xan	4.68±0.04^{Da}	4.42±0.07^{Aa}	4.56±0.07^{BCa}	4.54±0.06^{Ba}	4.51±0.05^{ABa}
	600 ppm Lyz-Xan	4.69±0.02^{Da}	4.40±0.08^{Aa}	4.58±0.07^{Ca}	4.51±0.07^{BCa}	4.49±0.04^{BCa}
Acidity	Control	1.63±0.02^{Aa}	1.81±0.03^{Ca}	1.75±0.01^{Ba}	1.77±0.02^{Ba}	1.84±0.04^{Ca}
	10 ppm Nat.	1.65±0.03^{Aa}	1.79±0.04^{Ba}	1.77±0.06^{Ba}	1.77±0.04^{Ba}	1.86±0.03^{Ca}
	20 ppm Nat.	1.62±0.04^{Aa}	1.82±0.04^{Ca}	1.73±0.07^{Ba}	1.79±0.06^{Ba}	1.87±0.07^{Ca}
	30 ppm Nat.	1.63±0.05^{Aa}	1.82±0.06^{Ca}	1.74±0.04^{Ba}	1.78±0.05^{Ba}	1.84±0.04^{Ca}
	200 ppm Lyz-Xan	1.64±0.04^{Aa}	1.80±0.04^{Ca}	1.75±0.06^{Ba}	1.76±0.05^{Ba}	1.85±0.04^{Da}
	400 ppm Lyz-Xan	1.63±0.04^{Aa}	1.81±0.05^{Ca}	1.74±0.04^{Ba}	1.76±0.04^{Ba}	1.84±0.04^{Ca}
	600 ppm Lyz-Xan	1.64±0.01^{Aa}	1.81±0.06^{Ca}	1.74±0.02^{Ba}	1.77±0.03B^{Ca}	1.85±0.03^{Da}
Moisture (%)	Control	66.48±0.62^{Ea}	63.68±0.30^{Da}	62.19±0.40^{Ca}	61.18±0.69^{Ba}	59.65±0.67^{Aa}
	10 ppm Nat.	66.72±0.74^{Ca}	66.29±0.40^{Cb}	66.04±0.29^{Cb}	65.74±0.18^{Bb}	64.65±0.80^{Ab}
	20 ppm Nat.	66.63±0.78^{Da}	66.05±0.30^{Cb}	65.95±0.18^{Cb}	65.34±0.16^{Bb}	64.79±0.24^{Ab}
	30 ppm Nat.	66.35±0.50^{Da}	66.25±0.64^{Db}	65.58±0.54^{CBb}	65.10±0.25^{Bb}	64.22±0.45^{Ab}
	200 ppm Lyz-Xan	67.14±0.79^{Ba}	67.09±0.53^{Bc}	65.99±0.62^{ABb}	65.42±0.60^{Ab}	65.67±0.58^{Ac}
	400 ppm Lyz-Xan	67.06±0.63^{BCa}	66.48±0.89B^{Cbc}	66.31±0.38^{ABb}	65.93±0.70^{Ab}	65.54±0.53^{Ac}
	600 ppm Lyz-Xan	67.01±0.44^{Ca}	67.04±0.07^{Cc}	66.88±0.17^{Bbc}	66.28±0.22^{Bc}	65.67±0.41^{Ac}
Fat in Dry matter (%)	Control	43.25±1.10^{Aa}	43.79±0.43^{Aa}	43.32±1.12^{Aa}	42.61±1.94^{Aa}	42.46±1.40^{Aa}
	10 ppm Nat.	42.26±0.95^{Aa}	43.05±0.52^{Aa}	42.86±1.47^{Aa}	43.61±0.93^{Aa}	43.04±0.50^{Aa}
	20 ppm Nat.	42.29±1.26^{Aa}	43.31±0.97^{Aa}	42.80±0.99^{Aa}	43.28±0.79^{Aa}	43.62±0.63^{Aa}
	30 ppm Nat.	42.32±1.74^{Aa}	43.57±1.41^{Aa}	42.89±0.54^{Aa}	43.13±0.89^{Aa}	44.02±0.63^{Aa}
	200 ppm Lyz-Xan	42.56±1.61^{Aa}	43.18±1.18^{Aa}	43.21±0.70^{Aa}	43.11±1.31^{Aa}	44.55±0.98^{Aa}
	400 ppm Lyz-Xan	43.38±0.75^{Aa}	42.85±0.50^{Aa}	43.05±1.55^{Aa}	43.43±0.64^{Aa}	43.63±0.39^{Aa}
	600 ppm Lyz-Xan	42.54±0.82^{Aa}	42.520.39^{Aa}	42.83±2.45^{Aa}	43.76±1.15^{Aa}	42.71±1.05^{Aa}
Protein (%)	Control	13.89±0.14^{Ca}	13.82±0.12^{Ca}	13.84±0.12^{Ca}	13.52±0.10^{Ba}	13.26±0.14^{Aa}
	10 ppm Nat.	14.09±0.15^{Bb}	13.93±0.10^{Bb}	13.95±0.20^{Bb}	13.82±0.09^{BAb}	13.73±0.28^{Ab}
	20 ppm Nat.	14.12±0.18^{Bb}	13.96±0.07^{Bb}	13.91±0.11^{Bb}	13.86±0.30^{Bb}	13.70±0.22^{Ab}
	30 ppm Nat.	14.10±0.16^{Bb}	13.97±0.27^{Bb}	13.95±0.10^{Bb}	13.81±0.13^{Bb}	13.71±0.08^{Ab}
	200 ppm Lyz-Xan	14.07±0.42^{Bb}	13.99±0.33^{Bb}	13.95±0.18^{Bb}	13.86±0.20^{Bb}	13.69±0.19^{Ab}
	400 ppm Lyz-Xan	14.12±0.21^{Bb}	13.98±0.12^{Bb}	13.89±0.13^{Bb}	13.72±0.07^{Ab}	13.73±0.22^{Ab}
	600 ppm Lyz-Xan	14.14±0.19^{Bb}	13.92±0.1^{Bb}	13.88±0.06^{Bb}	13.57±0.20^{Ab}	13.76±0.21^{Ab}
Salt (%)	Control	2.59±0.06^{Aa}	3.07±0.16^{Ba}	3.02±0.26^{Ba}	2.97±0.15^{Ba}	3.02±0.19^{Ba}
	10 ppm Nat.	2.56±0.13^{Aa}	3.08±0.19 ^{Ba}	3.05±0.27^{Ba}	3.04±0.18^{Ba}	3.00±0.12^{Ba}
	20 ppm Nat.	2.64±0.09^{Aa}	3.06±0.07^{Ba}	3.07±0.16^{Ba}	3.02±0.06^{Ba}	3.07±0.17^{Ba}
	30 ppm Nat.	2.59±0.10^{Aa}	3.08±0.10^{Ba}	3.08±0.23^{Ba}	3.05±0.14^{Ba}	3.07±0.16^{Ba}
	200 ppm Lyz-Xan	2.64±0.05^{Aa}	3.01±0.09^{Ba}	3.07±0.19^{Ba}	3.04±0.06^{Ba}	3.07±0.09^{Ba}
	400 ppm Lyz-Xan	2.62±0.04^{Aa}	3.05±0.21^{Ba}	3.06±0.10^{Ba}	3.10±0.22^{Ba}	2.94±0.45^{Ba}
	600 ppm Lyz-Xan	2.63±0.01^{Aa}	3.06±0.10^{Ba}	3.07±0.12^{Ba}	3.07±0.10^{Ba}	3.00±0.26^{Ba}

$^{A-D}$ Means with different uppercase superscripts differ between ripening times within the same sample ($p < 0.05$). $^{a-c}$ Means with different lowercase superscripts differ between treatments within the same ripening time ($p < 0.05$). Values are expressed as mean ± SD.

2.8. Statistical analyses

All analyses were performed in triplicate, and the results were reported as means ± standard deviations. To examine the influence of coating formulation and ripening period, all the data were analyzed by multifactor ANOVA using the least significant difference (LSD) test ($p < 0.05$). Comparison of the means was

carried out using Duncan's multiple range tests using SPSS statistical software (version 24, IBM Corp., Armonk, NY).

3. Results and Discussion

3.1. Microbial assay

Antimicrobial efficiency of edible coatings against inoculated *Penicillium chrysogenum*, *Escherichia coli* O157:H7 and *Staphylococcus aureus* are given in Fig. 2. All treatments of edible coatings significantly ($p < 0.05$) inhibited the growth of *Pecenicillium chrysogenum*, and the natamycin-containing coatings were more effective than lysozyme-xanthan coatings (Fig. 2A). The coating containing 0.03% of natamycin reduced the growth of *Pecenicillium chrysogenum* from the initial value of 5.8±1.0 to 1.99±0.90 log cfu/g (with 3.81 log cfu/g decrease) at the 15th day. Whereas, the population of *Pecenicillium chrysogenum* in the control sample was 7.69±2.0 log cfu/g (with 2.15 log cfu/g increase) at the same storage time. These results were consistent with the findings of Pintado et al. (2010), Fajardo et al. (2010), Cé and Brandelli (2012) and Kallinteri et al. (2013). In all the coated samples containing lysozyme-xanthan, the growth of *Penicillium chrysogenum* was ascending; however, the growth trend in the coated samples was slower than that of the control. During 28 days of storage, the population of *Penicillium chrysogenum* in the control sample increased significantly ($p < 0.05$) from the initial inoculated amount of 5.01 ± 0.05 to 7.69 ± 0.20 log cfu/g, while in the coated samples, the maximum population was 6.87 ± 0.11 log cfu/g, and there was no significant difference ($p > 0.05$) between different concentrations of lysozyme-xanthan conjugate. Although lysozyme does not have antifungal activity, the decreased rate of mold growth in this sample can be attributed to the reduction of available oxygen in the coated samples. The findings of the present study are consistent with the results of Duan et al. (2007) and Medeiros et al. (2014).

The results also showed that all treatments significantly ($p < 0.05$) decreased the population of *E. coli* O157: H7 in UF white cheeses during 28 days (Fig. 2B). The most decrease was observed for the coating containing 600 ppm lysozyme-xanthan of which the amount of this bacterium decreased from the initial inoculated amount of 4.22 ± 0.17 to 2.11 ± 0.60 log cfu/g on day 28. At the end of storage period, *E. coli* O157: H7 count in samples coated with WPC containing 200 and 400 ppm lysozyme-xanthan were 2.29 and 3.74 log cfu/g lower than those of control, respectively. Although lysozyme has been reported to affect only some of the gram-positive bacteria, the results of Hashemi et al. (2014) showed that conjugating of lysozyme enzymes with polysaccharides can affect the gram-negative bacteria, which is consistent with our findings. In the coated samples containing natamycin, the *E. coli* O157: H7 growth was slower than that of control. This may be accounted for the protective effect of the coating itself, which reduces gas permeability and decreases oxygen transfer rate into the cheese. Therefore, oxygen becomes less available for the growth of aerobic bacteria. These results are in agreement with the data obtained by Ramos et al. (2012), Fajardo et al. (2010), and Resa et al. (2014).

Compared with uncoated cheese, the growth of *Staphylococcus aureus* in all coated samples was low (Fig. 2C). The population of this bacterium in the control, increased from 5.32 ± 0.12 on the first day to 7.37 ± 0.07 log cfu/g on the 28th day. In the coated samples containing different concentrations of natamycin, the growth rate of this bacterium was ascending, but it was slower than that of the control. However, there was no significant difference between different concentrations of natamycin in terms of microbial population ($p > 0.05$). At the end of the storage period, *Staphylococcus aureus* counts in coated samples contained 0.01, 0.02, 0.03% natamycin; 200, 400 and 600 ppm lysozyme-xanthan conjugate were 0.85, 0.92, and 0.88; 1.08, 3.11, and 4.77 log cfu/g lower than those of the control. The lowest growth rate of *Staphylococcus aureus* was observed in coated samples containing 600 ppm lysozyme-xanthan on day 28, which was 2.60 ± 0.29 log cfu/g. These results are consistent with the findings of Hashemi et al. (2014).

The data of this study showed that the coating containing natamycin had no inhibitory effect on the starter microflora (Fig. 2D). In spite of a slight decrease over time (due to autolysis), starter population remained at an acceptable level during ripening. In addition, in coating containing natamycin, the starter population was slightly higher than that of uncoated sample, however, there was no significant difference ($p > 0.05$) between the coatings containing different concentrations of natamycin. Hannon et al. (2006) reported that the starter population decreases during the onset of ripening due to autolysis, which is consistent with the results of the present study. The findings of this study were also consistent with those reported by Del Nobile et al. (2009), Yangılar and Yıldız, (2016) and Mehyar et al. (2018).

3.2. Physicochemical profile

Physicochemical analyses were performed by comparing cheeses coated with WPC containing natamycin and lysozyme xanthan conjugate with uncoated cheese samples at 1st , 15th, 30th, 45th, and 60th days of ripening (Table 2). The physicochemical characteristics such as pH, acidity, salt and fat in dry matter content were similarly preserved along the ripening period in all experimental treatments ($p > 0.05$). These results are consistent with the finding of Ramos et al. (2012), Henriques et al. (2013), Kavas et al. (2016), and Cano Embuena et al. (2017), who reported that edible coatings and films have no significant effect on pH changes, acidity, salt, and fat in dry matter content.

In all cases, the moisture loss increased during ripening, with higher rate for the cheeses with no coating. The moisture content of uncoated sample changed from the initial value of 66.48 ± 0.62 to 59.65 ± 0.67% during 60 days of ripening (6.83% moisture loss) while, the maximum moisture loss for coated samples containing natamycin and lysozyme-xanthan were 1.8 and 1.5%, respectively. Moisture loss is related to the water loss of cheese depends on the kinetics of water permeation through the coating. It seems that the presence of xanthan gum in the formulation of coating decreased moisture loss in the cheese. Similarly, Medeiros et al. (2014), Yangılar and Yıldız (2016) and Pena-Serna et al. (2016) reported lower moisture loss for the cheese coated with biodegradable coatings due to their superior water barrier property.

Protein content of uncoated cheese reached from the original value of 13.89 ± 0.14 to 13.26 ± 14.1% (0.63% reduction) during 60 days ripening. For the coated samples, the protein content on the first day was slightly higher (0.22 ± 0.03%) than that of the control sample, which is related to the nature of the protein type of the coating, and during the storage period, protein contents of these treatments were also slightly reduced (0.38% ± 0.01), but the type of treatment did not had significant effect on protein content ($p > 0.05$). A slight decrease in the amount of cheese protein during ripening period has also been reported by Hayaloglu et al. (2005)

and Karami et al. (2009). Further decrease in protein content of control sample in comparison with other types of treatments was probably due to higher water loss, and hence the release of whey proteins.

3.3. *Texture profile analysis (TPA)*

Coated cheese samples with different antimicrobial agents showed similar values of hardness during the ripening period, whereas this parameter was higher in uncoated cheese (Fig. 3A). The hardness of all samples increased over the first 30 days of storage due to possible decrease in moisture content and salt absorption, and then it decreased until the 60th day probably due to a progressive proteolysis. Similar to the previous studies (Zhong et al., 2014; Artiga-Artigas et al., 2017), the obtained results in this study demonstrate that the percentage of high water-content edible coatings maintained softness in the cheese samples.

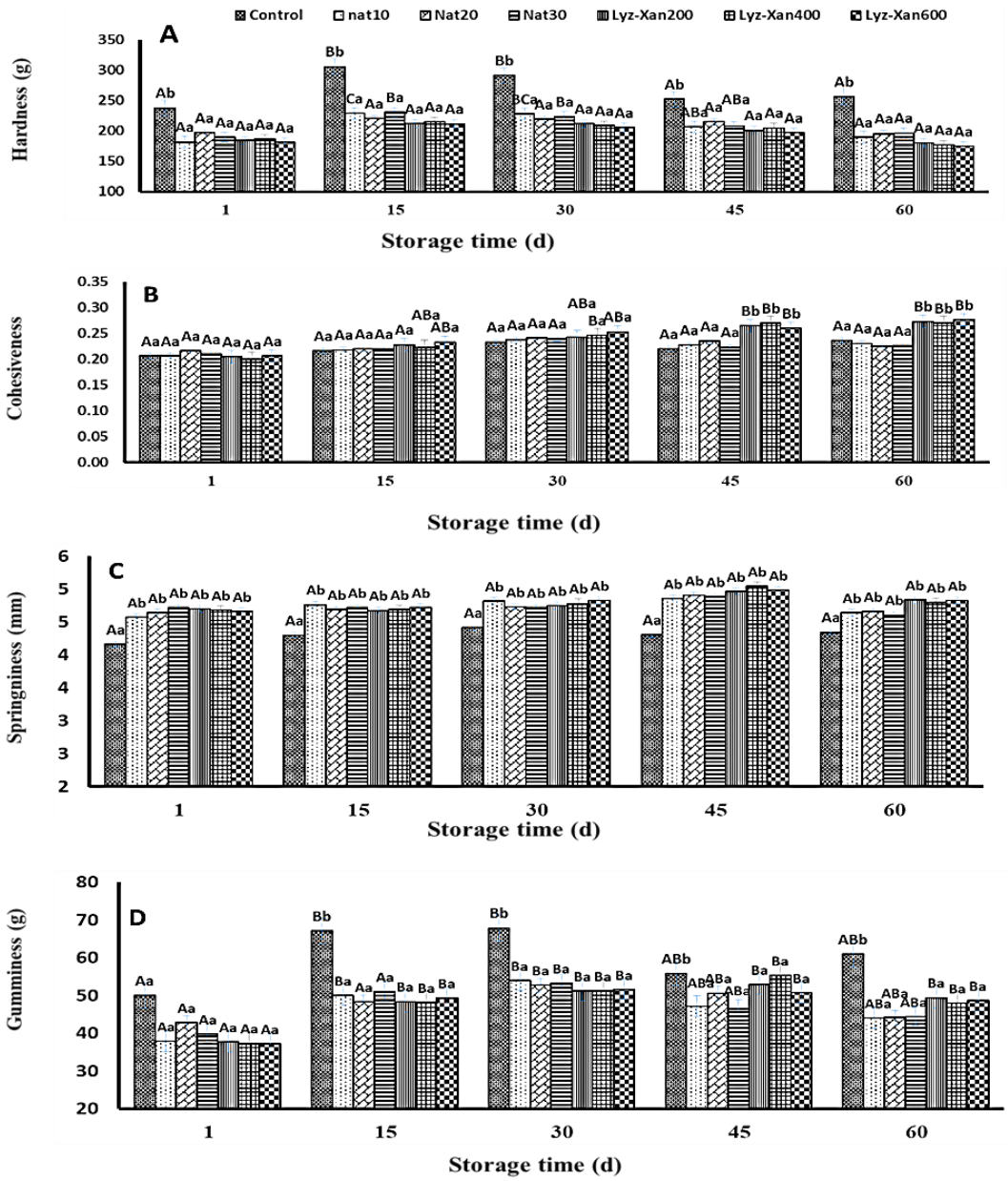

Fig. 3. The Effect of whey protein concentrate -based edible coating containing natamaycin and lysozyme-xanthan gum conjugate on hardness (A), cohesiveness (B), springiness (C), and gumminess (D) of UF white cheese during 60 days of ripening. Values are the mean of at least 3 replicates; error bars indicate standard deviations between means. Different lowercase letters (a–b) show singnificant ($p < 0.05$) differences between treatments within the same ripening time; different uppercase letters (A–B) show singnificant ($p < 0.05$) differences between ripening times within the same sample.

Table 3. The Effect of whey protein concentrate - based edible containing natamycin and lysozyme-xanthanate conjugate on sensory profile of UF cheese during 60 days[1].

		Days			
	Treatment	1	15	30	60
Color and appearance	Control	4.75±0.22^{Ab}	4.77±0.16^{Ab}	4.68±0.23^{Ab}	4.80±0.18^{Ab}
	10 ppm nat.	4.41±0.27^{Aa}	4.30±0.35^{Aa}	4.33±0.17^{Aa}	4.52±0.16^{Aa}
	20 ppm nat.	4.34±0.25^{Aa}	4.43±0.21^{Aa}	4.35±0.19^{Aa}	4.56±0.26^{Aa}
	30 ppm nat.	4.50±0.13^{Aa}	4.53±0.18^{Aa}	4.36±0.24^{Aa}	4.46±0.14^{Aa}
	200 ppm Lyz-Xan	4.30±0.37^{Aa}	4.43±0.22^{Aa}	4.40±0.19^{Aa}	4.37±0.30^{Aa}
	400 ppm Lyz-Xan	4.28±0.34^{Aa}	4.28±0.44^{Aa}	4.38±0.27^{Aa}	4.43±0.25^{Aa}
	600 ppm Lyz-Xan	4.39±0.23^{Aa}	4.41±0.17^{Aa}	4.39±0.16^{Aa}	4.41±0.17^{Aa}
Texture	Control	3.80±0.19^{Aa}	4.41±0.20^{Ca}	4.53±0.15^{Ca}	4.20±0.19^{Ba}
	10 ppm nat.	3.75±0.25^{Aa}	4.88±0.21^{Bb}	4.74±0.13^{BCb}	4.65±0.41^{Bab}
	20 ppm nat.	3.69±0.37^{Aa}	4.37±0.26^{Bb}	4.84±0.16^{Bb}	4.53±0.28^{Bab}
	30 ppm nat.	3.78±0.44^{Aa}	4.700±.55^{Bab}	4.65±0.25^{Bab}	4.60±0.16^{Bb}
	200 ppm Lyz-Xan	3.90±0.34^{Aa}	4.68±0.63^{Bab}	4.77±0.25^{Bab}	4.72±0.15^{Bb}
	400 ppm Lyz-Xan	3.70±0.22^{Aa}	4.66±0.52^{Bab}	4.90±0.40^{Bb}	4.63±0.24^{Bb}
	600 ppm Lyz-Xan	3.83±0.19^{Aa}	4.84±0.33^{Bb}	4.95±0.15^{Bb}	4.70±0.28^{Bb}
Odor	Control	3.56±0.35^{Aa}	4.13±0.20^{Ba}	4.52±0.23^{Ca}	4.53±0.19^{BCa}
	10 ppm nat.	3.54±0.34^{Aa}	3.99±.35^{Ba}	4.55±0.26^{Ca}	4.50±0.32^{Ca}
	20 ppm nat.	3.51±0.41^{Aa}	4.04±0.28^{Aa}	4.57±0.12^{Ba}	4.46±.19^{Ba}
	30 ppm nat.	3.74±0.49^{Aa}	3.97±0.31^{Aa}	4.45±0.16^{Ba}	4.49±0.15^{Ba}
	200 ppm Lyz-Xan	3.64±0.38^{Aa}	4.00±0.19^{Ba}	4.52±0.15^{Ca}	4.33±0.30^{Ca}
	400 ppm Lyz-Xan	3.60±0.36^{Aa}	3.94±0.22^{Aa}	4.50±0.31^{Ba}	4.48±0.22^{Ba}
	600 ppm Lyz-Xan	3.49±0.45^{Aa}	3.90±0.49^{BAa}	4.60±0.32^{Ba}	4.37±0.34^{Ba}
Flavor	Control	4.14±0.41^{Aa}	4.77±0.40^{Ba}	4.88±0.23^{Ba}	3.93±0.31^{Aa}
	10 ppm nat.	4.53±0.28^{Aa}	4.78±0.29^{Ba}	4.81±0.27^{Ba}	4.03±0.50^{Aa}
	20 ppm nat.	3.93±0.40^{Aa}	4.76±0.41^{Ba}	4.83±0.21^{Ba}	3.86±0.43^{Aa}
	30 ppm nat.	3.96±0.22^{Aa}	4.67±0.46^{Ba}	4.60±0.34^{Ba}	3.96±0.56^{Aa}
	200 ppm Lyz-Xan	4.17±0.40^{Aa}	4.60±0.43^{ABa}	4.94±0.16^{Ba}	4.04±0.33^{Aa}
	400 ppm Lyz-Xan	4.11±0.09^{Aa}	4.68±0.41^{Ba}	4.65±0.52^{Ba}	3.82±0.39^{Aa}
	600 ppm Lyz-Xan	3.88±0.33^{Aa}	4.73±0.45^{Ba}	4.89±0.21^{Ba}	3.94±0.31^{Aa}
Overall acceptability	Control	4.06±0.27^{Aa}	4.52±0.06^{Ba}	4.65±0.15^{Ba}	4.35±0.12^{Aa}
	10 ppm nat.	4.01±0.04^{Aa}	4.51±0.24^{BCa}	4.66±0.18^{Ca}	4.42±0.09^{Ba}
	20 ppm nat.	3.84±0.29^{Aa}	4.49±0.07^{Ca}	4.66±0.02^{Da}	4.35±0.15^{Ba}
	30 ppm nat.	4.00±0.19^{Aa}	4.47±0.16^{BCa}	4.52±0.23^{Ca}	4.38±0.13^{Ba}
	200 ppm Lyz-Xan	4.06±0.10^{Aa}	4.43±0.21^{Ba}	4.66±0.06^{Ca}	4.40±0.03^{Ba}
	400 ppm Lyz-Xan	3.92±0.16^{Aa}	4.42±0.24^{BCa}	4.61±0.27^{Ca}	4.34±0.18^{Ba}
	600 ppm Lyz-Xan	3.94±0.17^{Aa}	4.50±4.80^{BCa}	4.73±0.26^{Ca}	4.39±0.19^{Ba}

$^{A-D}$ Means with different uppercase superscripts differ between ripening times within the same sample ($p < 0.05$).
$^{a-b}$ Means with different lowercase superscripts differ between treatments within the same ripening time ($p < 0.05$). Data were analyzed by multifactor ANOVA using the least significant difference test. Values are expressed as mean (of 15 samples) ± SD.

The cohesiveness of cheese samples, defined as the limit point to which the material can deform itself before breaking, did not vary over the time, regardless of the concentration of natamycin (Fig. 3B). Nevertheless, coated samples containing lysozyme-xanthan showed slightly higher cohesiveness values at the end of storage period. It seems that the presence of xanthan gum in the formulation of coating, helped to preserve the diffused water in the cheese matrix maintaining cohesiveness, whereby the disintegration of the product is also less probable. It has been reported that the cohesiveness of cheese is related to its fat content, and any changes in the fat content could affect this parameter, and consequently, the springiness of the cheese (Gunasekaran & Ak, 2002). It is also reported that xanthan gum has been suggested as a polysaccharide based fat replacer to improve the texture of low-fat cheese (McMahon et al., 1996).

Coated cheese samples exhibited higher springiness than that of control during the ripening period (Fig. 3C). The highest springiness value was observed in the sample coated with WPC incorporating 400 ppm of lysozyme-xanhan at day 45 (5.05 ± 0.31 mm), and the lowest value was observed in control at the 1st day (4.16 ± 0.19 mm). Cheese springness is reported to be more dependent on moisture and fat contents (Tunick et al., 1991), and the nature of cheese protein matrix (Bryant et al., 1995), therefore, changes in cheese springiness during the ripening period could be a function of cheese composition. Results showed that the ripening time had not any significant effect on cheese springiness ($p > 0.05$).

Gumminess, understood as the energy required disintegrating a semi-solid food to a state of readiness for swallowing, is dependent on hardness, and it is calculated by multiplying the hardness and cohesiveness values of the sample (Gunasekaran & Ak, 2002). Similar to the hardness, gumminess of all samples increased during the first 30 days followed by a decrease until 60 days. Over the storage time, all coated samples had lower gumminess values than those for uncoated samples which is in agreement with findings of Artiga-Artigas et al. (2017).

3.4. Sensory evaluation

The results of sensory evaluation of cheese samples at 1, 15, 30 and 60 days of storage are shown in Table 3. Coating, significantly ($p < 0.05$) reduced the color and appearance scores. However, sorgate time did not have significant ($p > 0.05$) effect on the color and appearance of cheese samples.

The results also showed that there was no significant difference ($p > 0.05$) between texture scores of coated and uncoated samples on the first day, but on the 15th, 30th, and 60th days, the coated samples gave higher texture scores. The texture scores of all types of treatments decreased slightly from 30th day to 60th day, but the decrease rate was higher for control compared to other treatments. The results also showed that the type of treatment and antimicrobial agents did not affect odor scores of cheese samples, but ripening time improved the cheese odor. The ANOVA results also showed that WPC coating did not negatively affect the taste or overall acceptability of cheese. The highest flavor scores observed on the 30th day and the lowest score was on the 60th day. In the term of overal acceptability, the highest score on the 45th day and the lowest score on the first day was observed but there was not significant difference between treatments ($p > 0.05$). Similarly, Henriques et al. (2013) and Mehyar et al. (2017) reported that edible coatings had no negative effect on the taste or overall acceptability of cheese.

4. Conclusion

Coatings incorporating natamycin and lysozyme-xanthan gum conjugate significantly inhibited the growth of *Penicillium Chrysogenum, Escherichia coli* O157:H7 and *Staphylococcus aureus* compared to uncoated cheese, and consequently extended the shelf life of samples. Edible coating solutions did not inhibit lactic acid bacteria during 60 days of ripening. Based on the ANOVA results, there was no significant difference ($p > 0.05$) between pH, acidity, salt and fat in dry matter content of cheese samples. Edible coatings could reduce the moisture loss of cheese up to 5.33%. The results of TPA showed that the use of WPC-based coatings did not have a negative effect on hardness, cohesivness, springiness, and gumminess of UF white cheese and coated samples had lower hardness, better cohesiveness, and lower gumminess compared to those of uncoated cheese samples. In terms of sensory evaluation, panelists gave the same overall acceptability scores to the tested cheese samples. In conclusion, WPC containing natamycin and lysozyme-xanthan conjugate were found to be effective in extension of shelf life of UF white cheese.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Aminlari, M., Ramezani, R., & Jadidi, F. (2005). Effect of Maillard-based conjugation with dextran on the functional properties of lysozyme and casein. *Journal of the Science of Food and Agriculture*, *85*(15), 2617-2624.

Amiri, S., Ramezani, R., & Aminlari, M. (2008). Antibacterial activity of dextran-conjugated lysozyme against *Escherichia coli* and *Staphylococcus aureus* in cheese curd. *Journal of Food Protection*, *71*(2), 411-415.

AOAC International (1995). Official methods of analysis of AOAC international.

Ardö, Y., & Polychroniadou, A. (1999). *Laboratory manual for chemical analysis of cheese: improvement of the quality of the production of raw milk cheeses*. Publications Office.

Artiga-Artigas, M., Acevedo-Fani, A., & Martín-Belloso, O. (2017). Improving the shelf life of low-fat cut cheese using nanoemulsion-based edible coatings containing oregano essential oil and mandarin fiber. *Food Control*, *76*, 1-12.

Bradley, R. L., Arnold, E., Barbano, D. M., Semerad, R. G., Smith, D.E., Vines, B. K., & Marshall, R. (1992). Standard methods for the examination of dairy products. *Chemical and Physical Methods*, p. 433.

Bryant, A., Ustunol, Z., & Steffe, J. (1995). Texture of Cheddar cheese as influenced by fat reduction. *Journal of Food Science*, *60*(6), 1216-1219.

Bylund, G. (1995). *Dairy processing handbook*. Tetra Pak processing systems AB.

Cano Embuena, A. I., Cháfer Nácher, M., Chiralt Boix, A., Molina Pons, M. P., Borrás Llopis, M., Beltran Martínez, M. C., & González Martínez, C. (2017). Quality of goat′ s milk cheese as affected by coating with edible chitosan-essential oil films. *International Journal of Dairy Technology*, *70*(1), 68-76.

Cé, N., Noreña, C. P., & Brandelli, A. (2012). Antimicrobial activity of chitosan films containing nisin, peptide P34, and natamycin. *CyTA-Journal of Food*, *10*(1), 21-26.

Crapisi, A., Lante, A., Pasini, G., & Spettoli, P. (1993). Enhanced microbial cell lysis by the use of lysozyme immobilized on different carriers. *Process Biochemistry*, *28*(1), 17-21.

Del Nobile, M. A., Gammariello, D., Conte, A., & Attanasio, M. (2009). A combination of chitosan, coating and modified atmosphere packaging for prolonging Fior di latte cheese shelf life. *Carbohydrate Polymers*, *78*(1), 151-156.

Delves-Broughton, J., Thomas, L. V., Doan, C. H., & Davidson, P. M. (2005). Natamycin. Food Science and Technology-New York-Marcel Dekker, *145*, p. 275.

Dewanti, R., & Wong, A. C. (1995). Influence of culture conditions on biofilm formation by *Escherichia coli* O157: H7. *International Journal of Food Microbiology*, *26*(2), 147-164.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, *17*(1), 25-39.

Duan, J., Kim, K., Daeschel, M. A., & Zhao, Y. (2008). Storability of antimicrobial chitosan-lysozyme composite coating and film-forming solutions. *Journal of Food Science*, *73*(6), M321-M329.

Fajardo, P., Martins, J. T., Fuciños, C., Pastrana, L., Teixeira, J. A., & Vicente, A. A. (2010). Evaluation of a chitosan-based edible film as carrier of natamycin to improve the storability of Saloio cheese. *Journal of Food Engineering*, *101*(4), 349-356.

Food, U. S. (2001). Bacteriological analytical manual online. http://www. cfsan. fda. gov/~ ebam/bam-toc. html.

Gunasekaran, S., & Ak, M. M. (2002). *Cheese rheology and texture*. CRC press.

Hannon, J. A., Deutsch, S. M., Madec, M. N., Gassi, J. Y., Chapot-Chartier, M. P., & Lortal, S. (2006). Lysis of starters in UF cheeses: behaviour of mesophilic lactococci and thermophilic lactobacilli. *International Dairy Journal*, *16*(4), 324-334.

Hashemi, M. M., Aminlari, M., & Moosavinasab, M. (2014). Preparation of and studies on the functional properties and bactericidal activity of the lysozyme–xanthan gum conjugate. *LWT-Food Science and Technology*, *57*(2), 594-602.

Hayaloglu, A. A., Guven, M., Fox, P. F., & McSweeney, P. L. H. (2005). Influence of starters on chemical, biochemical, and sensory changes in Turkish white-brined cheese during ripening. *Journal of Dairy science*, *88*(10), 3460-3474.

Henriques, M., Santos, G., Rodrigues, A., Gomes, D. M. G. S., Pereira, C., & Gil, M. (2013). Replacement of conventional cheese coatings by natural whey protein edible coatings with antimicrobial activity. *Journal of Hygienic Engineering and Design*, *3*, 34-47.

Hesari, J., Ehsani, M. R., Khosroshahi, A., & McSweeney, P. L. (2006). Contribution of rennet and starter to proteolysis in Iranian UF white cheese. *Le Lait*, *86*(4), 291-302.

IDF (1989). Milk-fat, Products and Butter. Determination of Fat Acidity. Standard 6B Brussel: International Dairy Federation, p.3.

IDF, M. (1993). Determination of the nitrogen (Kjeldahl method) and calculation of the crude protein content, IDF Standard 20B. International Dairy Federation, Brussels, Belgium.

ISIRI, Institute of Standards and Industrial Research of Iran, 1998. Milk and dairy products—Cheese sensory evaluation test method. National Standard No. 4691.

Jalilzadeh, A., Hesari, J., Peighambardoust, S. H., & Javidipour, I. (2018). The effect of ultrasound treatment on microbial and physicochemical properties of Iranian ultrafiltered feta-type cheese. *Journal of Dairy Science*, *101*(7), 5809-5820.

Kallinteri, L. D., Kostoula, O. K., & Savvaidis, I. N. (2013). Efficacy of nisin and/or natamycin to improve the shelf-life of Galotyri cheese. *Food Microbiology*, *36*(2), 176-181.

Karami, M., Ehsani, M. R., Mousavi, S. M., Rezaei, K., & Safari, M. (2009). Changes in the rheological properties of Iranian UF-Feta cheese during ripening. *Food Chemistry*, *112*(3), 539-544.

Kavas, N., Kavas, G., & Saygili, D. (2016). Use of ginger essential oil-fortified edible coatings in Kashar cheese and its effects on *Escherichia coli* O157: H7 and *Staphylococcus aureus*. *CyTA-Journal of Food*, *14*(2), 317-323.

McMahon, D. J., Alleyne, M. C., Fife, R. L., & Oberg, C. J. (1996). Use of fat replacers in low fat Mozzarella cheese. *Journal of Dairy Science*, *79*(11), 1911-1921.

Medeiros, B. G. D. S., Souza, M. P., Pinheiro, A. C., Bourbon, A. I., Cerqueira, M. A., Vicente, A. A., & Carneiro-da-Cunha, M. G. (2014). Physical characterisation of an alginate/lysozyme nano-laminate coating and its evaluation on 'Coalho'cheese shelf life. *Food and Bioprocess Technology*, *7*(4), 1088-1098.

Mehyar, G. F., Al Nabulsi, A. A., Saleh, M., Olaimat, A. N., & Holley, R. A. (2018). Effects of chitosan coating containing lysozyme or natamycin on shelf-life, microbial quality, and sensory properties of Halloumi cheese brined in normal and reduced salt solutions. *Journal of Food Processing and Preservation*, *42*(1), e13324.

Pena-Serna, C., Penna, A. L. B., & Lopes Filho, J. F. (2016). Zein-based blend coatings: Impact on the quality of a model cheese of short ripening period. *Journal of Food Engineering*, *171*, 208-213.

Pérez-Gago, M. B., & Krochta, J. M. (2002). Formation and properties of whey protein films and coatings. *Protein-Based Films and Coatings*, 159-180.

Pintado, C. M., Ferreira, M. A., & Sousa, I. (2010). Control of pathogenic and spoilage microorganisms from cheese surface by whey protein films containing malic acid, nisin and natamycin. *Food Control*, *21*(3), 240-246.

Ramos, Ó. L., Santos, A. C., Leão, M. V., Pereira, J. O., Silva, S. I., Fernandes, J. C., Franco, M. I., Pintado, M. E., & Malcata, F. X. (2012). Antimicrobial activity of edible coatings prepared from whey protein isolate and formulated with various antimicrobial agents. *International Dairy Journal*, *25*(2), 132-141.

Rashidi, H., Razavi, S. M. A., Mazaheri-Tehrani, M., & Ghods-Rohany, M. (2015). Improving textural and sensory characteristics of low-fat uf feta cheese made with fat replacers. *Journal of Agricultural Science and Technology*, *17*, 121-132.

Resa, C. P. O., Gerschenson, L. N., & Jagus, R. J. (2014). Natamycin and nisin supported on starch edible films for controlling mixed culture growth on model systems and Port Salut cheese. *Food Control*, *44*, 146-151.

Robinson, R. K. (2014). *Encyclopedia of food microbiology*. Academic press.

Schmid, M., Hinz, L. V., Wild, F., & Noller, K. (2013). Effects of hydrolysed whey proteins on the techno-functional characteristics of whey protein-based films. *Materials*, *6*(3), 927-940.

Ioanna, F., Quaglia, N. C., Storelli, M. M., Castiglia, D., Goffredo, E., Storelli, A., De Rosa, M., Normanno, G., Jambrenghi, A. C., & Dambrosio, A. (2018). Survival of *Escherichia coli* O157: H7 during the manufacture and ripening of Cacioricotta goat cheese. *Food Microbiology*, *70*, 200-205.

Takahashi, K., Lou, X. F., Ishii, Y., & Hattori, M. (2000). Lysozyme−glucose stearic acid monoester conjugate formed through the maillard reaction as an antibacterial emulsifier. *Journal of Agricultural and Food Chemistry*, *48*(6), 2044-2049.

Tunick, M. H., Mackey, K. L., Smith, P. W., & Holsinger, V. H. (1991). Effects of composition and storage on the texture of Mozzarella cheese. *Nederlands melk en Zuiveltijdschrift*, *45*(2), 117-125.

Yangılar, F., & Oğuzhan Yıldız, P. (2016). Casein/natamycin edible films efficiency for controlling mould growth and on microbiological, chemical and sensory properties during the ripening of Kashar cheese. *Journal of the Science of Food and Agriculture*, *96*(7), 2328-2336.

Zhong, Y., Cavender, G., & Zhao, Y. (2014). Investigation of different coating application methods on the performance of edible coatings on Mozzarella cheese. *LWT-Food Science and Technology*, *56*(1), 1-8.