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Investigation of Nano-Biocomposite for Kashar Cheese and Ground Meat Packaging

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ABSTRACT

Background: Plastic and polymeric materials remain in the soil given the fact that they are derived from petroleum resources. However, such pollution has created a special challenge for human societies. The use of biodegradable packaging has received more attention. The general purpose of this study was to investigate the use of a mixture film of whey protein concentrate and reinforced hydroxypropyl methylcellulose with chitosan nanoparticles to package ground meat and Kashar cheese. Furthermore, this package was compared with ordinary polyethylene coating. **Methods:** Two samples of ground meat and Kashar cheese were packaged using a nano-biocomposite film of whey protein concentrate/ hydroxypropyl methylcellulose (70:30) containing 3% chitosan nanoparticles. The antimicrobial properties of the optimal produced film were examined. The total population of microorganisms and pH for ground meat were tested during 6 days of storage. The total population of microorganisms, weight loss, moisture content, pH, and mold count and yeast for Kashar cheese were examined during 2 months of storage. The results of the tests were analyzed by Duncan one-way analysis of variance with 95% confidence and 5% error by Minitab16 software. **Results:** Samples of ground meat and cheese packaged in whey protein film and hydroxypropyl methylcellulose containing chitosan nanoparticles had less mold count, yeast, and total microorganism population than polyethylene packaging ($P \leq 0.05$) after storage period. **Conclusion:** The use of biodegradable films based on plants and the loading of nanoparticles can lead to the use of this type of packaging for perishable food to prevent environmental hazards in addition to greater safety of perishable food products.

Keywords: *Hydroxypropyl methylcellulose; Chitosan nanoparticles; Nano-biocomposite films; Polyethylene film*

Introduction

The increasing development of petrochemical industries on the one hand and the high need for industrial plastics and their use in packaging industries on the other hand, have led to the increasing use of petroleum polymers such as polypropylene, polyethylene, polystyrene, etc. in the packaging industry, especially in special food packaging. Contamination by synthetic plastics,

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known as white pollution, constitutes a major part of environmental pollution in industrialized as well as developing countries such as Iran, which have a weak plastic recycling system. During recent years, less natural places contaminated with plastic waste have been found in Iran (Ghanbarzadeh, 2009). Given the above-mentioned issues, the necessity of using biodegradable biopolymers, as green polymers, is felt as a suitable alternative to synthetic polymers with petroleum origin. Biodegradability refers to the decomposition of a polymer in the natural environment, which involves changes in chemical structure, loss of structural and mechanical properties, and eventually conversion to other compounds such as water, carbon dioxide, minerals, and intermediate products including biomass and organic matter. Since these biopolymers are of agricultural origin, their production and extraction can increase the added value of agricultural products and preserve the renewable resources for the next generation (Ghanbarzadeh, 2009).

Among the raw materials that can produce edible films, hydroxypropyl methylcellulose, which is a derivative of cellulose and whey protein concentrate, which is a purified form of whey protein can be mentioned. Hydroxypropyl methylcellulose is an ether hydrocolloid of cellulose that is soluble in water and has good film-forming properties. Films produced from Hydroxypropyl methylcellulose have been used successfully to improve properties such as obstructiveness against oxygen and moisture for foodstuffs, while there are defects in their mechanical and thermal properties in comparison to synthetic polymers (Atarés *et al.*, 2011). Moreover, whey protein concentrate, one of the most abundant proteins derived from milk, is one of the by-products of cheese or casein production process. This compound has good film formation properties and excellent gas obstructiveness properties. However, its application in food packaging is limited due to its low tensile strength, inherent stiffness and poor obstructiveness against moisture (Oymaci and Altinkaya, 2016). Accordingly, several solutions have been proposed to eliminate the defects related to biopolymers, including mixing biopolymers with other polymers (natural or synthetic), using plasticizers, coatings and laminates, utilizing of various nanoparticles such as nano-zinc, nano-silver, nano-clay, nanocellulose, nano-chitosan, nano-zein, creating cross-link between polymer chains through chemicals materials, as well as PH changes, heat, enzymes, irradiation, etc. (Chang *et al.*, 2010). In this regard, chitosan nanoparticles have received a lot of attention due to their renewability, optimal mechanical properties, strong antimicrobial properties, and high specific surface. Chitosan nanoparticles, due to their size, have a higher specific surface and therefore more atomic bonds with matrix in comparison to macroscopic enhancers (Martelli *et al.*, 2013).

Kashar cheese is one of the most delicious, unprocessed, and popular cheeses originating from Turkey and Greece. Fresh Kashar is a smooth, firm, simple, and light cheese usually made from cow's milk. This cheese is very suitable for cutting, melting, grating, or daily consumption. This cheese is often consumed with white cheese for breakfast. It is also widely used in pizzas, sandwiches, and salads. Var et al. (2006) investigated the effect of antimicrobial (Natamycin) and packaging materials (polyvinyl chloride) on the microbiological properties of Kashar cheese during storage (Var *et al.*, 2006). Natamycin and packaging materials had no effect on the total number of aerobic mesophilic bacteria, yeasts, and lipolytic bacteria. However, Natamycin showed the effect of obstructiveness on proteolytic microorganisms. In addition, in samples of Kashar cheese produced using a combination of Natamycin and packaging materials during the five-month ripening period, no significant growth was found in the number of harmful microorganisms (*Enterobacteriaceae*, *Coliform, Salmonella and Staphylococcus aureus)*. Microbiological control of this cheese during ripening and storage is important by active packages containing antimicrobial agents.

Dehnad et al. (2014) evaluated the thermal and antimicrobial properties of packaging films

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produced from chitosan and nanocellulose in extending the shelf life of ground meat (Dehnad *et al.*, 2014). They reported that the film resistance properties to temperature as well as its mechanical resistance were improved by the addition of nanoparticles. The results of microbial analysis showed that the nanocomposites had obstructiveness effects on gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Salmonella enteritidis* and *Escherichia coli*). The use of this nanocomposite reduced the lactic acid bacterial population respectively to 1.3 and 3.1 logarithmic cycles at 3 and 6 days after storage of ground meat samples compared to nylon coating.

Therefore, the general purpose of this study was to: 1) investigate the use of mixed film of whey protein concentrate-hydroxypropyl methylcellulose protein concentrate with chitosan nanoparticles for packaging ground meat and Kashar cheese; 2) evaluate the antimicrobial properties of the optimal film produced for both products and pH, the total population of microorganisms during 6 days of storage for ground meat; 3) study properties of the total population of microorganisms, pH, weight loss, moisture content, mold, and yeast count during 60 days of storage for Kashar cheese.

Materials and Methods

*Chemicals materials used in research***:** Rennet enzyme and thermophilus starter were supplied from CHR HANSEN Company, Denmark. PZ7 phosphate salt was purchased from the JOHA Company, German. In addition, BHI medium was purchased from the Merck Company, German. *Escherichia coli* DH5 alpha and *Staphylococcus aureus* ATCC 25923 were supplied from the Iranian Research Organization for Science and Technology. Other chemicals used in this study were also purchased from Merck Company, Germany.

Using nano-biocomposites to increase the shelf life of ground meat: To this end, freshly ground beef was supplied from the market, enclosed under sterile conditions, and sealed with a heat sealer with nano-biocomposite films obtained from whey protein concentrate-hydroxypropyl methylcellulose (70:30) containing 3% chitosan nanoparticles with physical, mechanical, thermal, and morphological properties optimized previously (Shojaei *et al.*, 2019). Next, the antimicrobial properties of the film, pH, and the total population of microorganisms were compared with the control sample, a polyethylene sample, during 6 days of storage at 4 °C and 25 °C. Given that using nano-biocomposite film, reinforced with 3% chitosan, had no significant effect on the sensory properties (smell, taste, and texture) of ground meat treatments its results were not reported.

Using nano-biocomposite to increase the shelf life of Kashar cheese: Kashar cheese was first produced as follows, enclosed under sterile conditions, and sealed with a heat sealer with nano-biocomposite films obtained from whey protein concentrate-hydroxypropyl methylcellulose (70:30) containing 3% chitosan nanoparticles with physical, mechanical, thermal, and morphological properties optimized previously (Shojaei *et al.*, 2019). The method of producing Kashar cheese was as follows: First, whole cow's milk (with 3.5% fat) was pasteurized at a temperature of 72 degrees Celsius for 15 seconds. Later, its temperature was reduced to 38 degrees Celsius and thermophilic stator was added to the milk along with calcium chloride (amount to 0.02%). Twenty minutes later, when the pH reached (from 6.7) to 6.4, Rennet enzyme and *Sodium Chloride* (0.0015%) were added to milk containing the starter. The enzyme acted 60-70 minutes later and a cheese curd was formed. The curds were then cut in three directions and whey was removed three times at intervals of 20 minutes (where the temperature dropped by 2 degrees and the pH did not change much). Later, the temperature of curd and whey separated by steam reached about 42 degrees Celsius, while the whole cheese was stirred constantly. After heating, the whey was removed from the cheese and the produced crude were poured into a special mold and pressed to extract water as much as possible. In the next stage, the pressed crude (with a pH about 3.5) was cut with a blade and stringed. The

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powdered materials including 1% casein micelle concentrate, 0.85% phosphate salt, 0.8% salt and pH adjusting salts were added to the pressed and cut curds and poured into Stephen machine. The cooking operation was performed at 75 °C for 15 minutes with direct steam by Stephen machine. After ensuring about uniformity of the product, it was poured hot into the molds, its temperature reached 6-8 degrees Celsius immediately, and the molds were returned. Finally, the cheese was transferred to the cold store and was packed under vacuum the next day. The antimicrobial properties of the film, pH, and total population of microorganisms, weight loss, moisture content, mold count, and yeast were compared with the control sample, a polyethylene sample during 60 days of storage at 4 °C and 25 °C. Given that the use of nano-biocomposite film, reinforced with 3% chitosan had no significant effect on the sensory properties (smell, taste and texture) of Kashar cheese treatments, its results were not reported.

Microbial test (agar diffusion method): The antimicrobial properties of biodegradable films of agar diffusion method were performed according to the method applied previously (Dehnad *et al.*, 2014). *Escherichia coli* and *Staphylococcus aureus* lyophilized bacteria were transferred to BHI broth (Brain-Heart Infusion Broth) incubated for 37 hours at 37 °C, and re-cultured for at least 2 consecutive times. Thus, 0.1 ml of bacterial suspension (*Escherichia coli* and *Staphylococcus aureus*) containing 10^8 CFU/ml of microorganisms was spread on BHI agar medium. A spectrophotometer at 600 nm wave was used to calculate and confirm the microbial population. Later, their number was confirmed by culturing on agar. Next, Discs with a diameter of 8 mm were placed on the bacterial medium. The plates were incubated at 37 °C for 24 hours. Finally, the obstructiveness inhibitory zone was calculated using a caliper.

Tests performed on ground meat: All chemical tests of ground meat samples were performed in accordance with the mentioned numbers standard. The pH determination test and total microorganism population count test were respectively performed according to National Iranian Standard with numbers 1028 and 5272. (National standard organization of Iran, 2008)

Tests performed on Kashar cheese: The pH determination test, Moisture content determination test, total microorganism population count test, mold and yeast count test, as well as loss weight test were measured according to National Iranian Standards as 2852, 4451, 2406, 10154, and 2344, respectively. (National standard organization of Iran, 2002)

Investigation of antimicrobial properties of the film: Antibacterial properties of whey protein concentrate (WPC) and hydroxypropyl methylcellulose (HPMC) packaging film, optimized mixture film from HPMC-WPC, and its resulting nanocomposites on key foodborne pathogens are provided in **Table 1**. Optimal compound films have no effect on preventing the growth of *Escherichia coli* and *Staphylococcus aureus*. However, studies on bacteria have shown that addition of 3% chitosan nanoparticles was effective in preventing the growth of *Staphylococcus aureus*. By adding nanoparticles to the compound film substrate, partial preventive effect (+) and somewhat strong preventive effect (++) on *Escherichia coli* was observed. Moreover, addition of nanoparticles to the compound film substrate severely prevented the growth of *Staphylococcus aureus.*

According to the results, the antibacterial effects of chitosan nanoparticles on gram-positive bacteria (*Staphylococcus aureus*) were higher than gram-negative bacteria (*Escherichia coli*). The exact mechanism of such a phenomenon is still unknown, but several hypotheses have been put forward in this regard. For example, Hosseinnejad and Jafari stated that gram-positive bacteria have a greater ability to form electrostatic bonds between the cell wall of bacteria and functional groups of chitosan nanoparticles compared to gram-negative bacteria, because the cell wall of gram-positive bacteria basically form

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a thick layer of peptidoglycans created by polymers called Teichoic *acids*. Phosphate groups with a negative charge of Teichoic *acids* are able to produce some electrostatic bonds with any of the cationic components such as chitosan and its derivatives (Hosseinnejad and Jafari, 2016). The higher efficiency of chitosan nanoparticles on gram-positive bacteria was confirmed in the literature (Hosseini *et al.*, 2015). It was also hypothesized that chitosan nanoparticles can act as antibacterial components due to their hydrophobic and chelating effects at pHs above pKa or neutral pH (Kong *et al.*, 2010). Moreover, negatively charged chitosan nanoparticles can alter cell membrane integrity and inactivate cell permeability by binding to DNA; thereby, disrupting the process of microorganism proliferation, inhibition of mRNA, protein synthesis, and consequently cause the death of microorganism cells (Divya *et al.*, 2017, Wardani and Sudjarwo, 2018). In this regard, Abdou et al. (2012) reported that a dose of 2-4% chitosan nanoparticles had significant preventive effects on *Staphylococcus* aureus and *coliform* bacteria (Abdou and Sorour, 2014).

Data analysis: The tests were performed in three replications and the results were analyzed by Duncan one-way analysis of variance with 95% confidence and 5% error by Minitab16 software.

Table 1. Antibacterial properties of samples of hydroxypropyl methylcellulose films, whey protein concentrate, optimal mixture film, and its nanocomposites

Treatments	*Staphylococcus* aureus	*E- coli*
Hydroxypropyl methylcellulose film (HPMC)	-	-
Whey protein concentrate film (WPC)	-	-
Optimal mixture film (HW30)	-	-
Optimal mixture film containing 3% chitosan nanoparticles (HW30+3% CNPs)	++	+

-: Lack of growth obstructiveness; +: mild preventive (with obstructiveness area between 6 and 8 mm); ++: moderate preventive (with obstructiveness area between 8 to 10 mm)

Results

The pH of nano-biocomposite samples and polyethylene samples (control sample) in ground meat during storage period are reported in **Table 2**. The results showed that the pH of ground meat decreased in all treatments during the storage period. According to the results, no significant difference was observed between the pH of ground meat packaged in nano-biocomposite film and polyethylene stored at 4 °C at time intervals. No significant difference was found between the pH of ground meat packaged in nano-biocomposite film and polyethylene stored at 25 °C in the first and third days of storage, but this amount was significant on the sixth day.

The pH measurement of the samples confirmed that the edible film could not create change (positive compared to the control sample) in the pH of the meat. A decrease of pH to 21% in the ground meat sample coated with the optimal nanocomposite compared to the control at this temperature confirmed the theory of the effect of acetic acid released from the film containing chitosan nanoparticles on the growth of microorganisms. However, the mechanisms mentioned in the section of antimicrobial properties of the film are effective in creating antimicrobial properties and improving the storage conditions of ground meat with a biopolymer reinforced with chitosan nanoparticles.

Microbial populations of nano-biocomposite samples and polyethylene samples (control sample) in ground meat during storage period are reported in **Table 3**. A significant difference was found between the microbial population of the nano-biocomposite sample and the microbial population of the control sample at 4 and 25°C on the third and sixth days. After one week, the mixed

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nanocomposite containing chitosan nanoparticles reduced the lactic acid number of ground meat bacteria in the refrigerator by about 0.9 logarithmic cycles compared to the control sample. The decrease in microbial population of the nano-biocomposite sample (in relation to the control) at refrigerated temperature at the beginning of the period was faster than the end of period. The release of organic acids from the matrix containing chitosan nanoparticles was rapid at the beginning of the period when the ion concentration gradient between the inside of the polymer matrix and the outside environment is high, but the release rate decreased as the reaction progressed. The nanocomposite of the present study reduced the target population compared to the control sample at 25 °C for about one week, which is equal to 3 logarithmic cycles. At high temperatures compared to low storage temperatures, the release of acetic acid, which is obstructiveness of the growth of microorganisms, occurs faster than the polymer bed.

The results of pH changes of packaged Kashar cheese in nano-biocomposite sample and polyethylene sample are reported in **Table 4**. The results showed that the pH of Kashar cheese decreased in all treatments during storage. No significant difference was observed between polyethylene and nano-biocomposite samples. However, the pH changes in the packaged sample with the compound film containing chitosan nanoparticles were smaller than the control sample (1% unit in two months vs. 2% unit). This amount of drop is likely to be due to inhibition of lactic acid bacteria (and prevention of the metabolites production), the main source of dairy products.

The results of weight loss and moisture content of Kashar packaged cheese in nano-biocomposite samples and polyethylene samples are reported in Table 5. In terms of weight loss, a significant difference was found between the nano-biocomposite sample and the polyethylene sample. Weight loss of cheese samples during two months of packing in the packaged cheese sample with polyethylene film was only 1.12%, which was 4.1%in the sample of packaged cheese prepared with nano-biocomposite in this study. No significant difference was observed between the polyethylene sample and the nano-biocomposite sample in terms of moisture content. However, the increasing trend of moisture loss over time in the synthesized nano-biocomposite confirmed that the polyethylene film had a greater ability to retain moisture product due to the optimizations made on it and industrialization, providing a better preventive against the passage of gases and water vapor in Kashar cheese. Although moisture in samples protected by optimal nanocomposite film had little effect on film needs, more research is required in the field of water vapor and gas transmission. Percentage of moisture product is of special importance both qualitatively and quantitatively (due to economic issues). The pattern of moisture loss in packaged cheese samples with optimal nanocomposite film originated from the weight loss of the product, so that the moisture on the first day of packaging was 46.54%, while it reached 44.63% two months after production, which was relatively significant.

The results related to the changes of mold and yeast of Kashar packaged cheese in nano-biocomposite sample and polyethylene sample are reported in **Table 6**. A significant difference was observed between the nano-biocomposite sample and the polyethylene sample. Due to the application of heat process in the production of Kashar cheese, we observed a small and negligible number of molds and yeasts. The compound film containing chitosan nanoparticles was able to control the total number of molds and yeasts (which are among the most important microorganisms in dairy) in two months. At the end of the second month, we observed only 14 unit molds in the product. However, in the packaged samples with polyethylene film, we observed an increase in the number of molds and yeasts over time, which was probably due to the antimicrobial properties of the nanoparticles incorporated in the optimized compound film substrate.

The results of counting the total number of microorganisms are reported in **Table 7**. A

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significant difference was found between polyethylene sample and nano-biocomposite sample. The total count of microorganisms in the sample of Kashar packaged cheese with control film (polyethylene) increased to 1.2 cycle, while it increased only about 0.6 logarithmic cycles in the sample of packaged cheese with nano-biocomposite film after 2 months.

Table 1. Antibacterial properties of samples of hydroxypropyl methylcellulose films, whey protein concentrate, optimal mixture film, and its nanocomposites

Treatments	*Staphylococcus* aureus	*E- coli*
Hydroxypropyl methylcellulose film (HPMC)	-	-
Whey protein concentrate film (WPC)	-	-
Optimal mixture film (HW30)	-	-
Optimal mixture film containing 3% chitosan nanoparticles (HW30+3% CNPs)	++	+

-: Lack of growth obstructiveness; +: mild preventive (with obstructiveness area between 6 and 8 mm); ++: moderate preventive (with obstructiveness area between 8 to 10 mm)

Table 2. pH of nano biocomposite samples and polyethylene sample (control sample) in the ground meat after applying certain storage conditions

pH	Refrigerator temperature (4°C)			The ambient temperature (25°C)		
	First day	Third day	Sixth day	First day	Third day	Sixth day
Nanocomposite	^{aA} 0.056±5.81	^{aA} 0.127±5.74.	^{aAB} 0.099±6.25	^{aA} 0.014±5.8	^{aAB} 0.028±5.56	^{aB} 0.014±5.39.
Polyethylene	^{aA} 0.028±5.82	^{aA} .084± 5.77	^{aAB} 0.127±5.66.	^{aA} 0.042±5.8	^{aBC} 0.099±5.4	^{bC} 0.042±5.18

Results are shown as mean ±standard deviation; The different lowercase letters indicate a significant difference in each column; The different uppercase letters indicate significant difference in each line

Table 3. Total count of microorganisms (cfu /g) in nano-biocomposite samples and polyethylene samples in ground meat

Population of microorganisms (logcfu/g)	Refrigerator temperature (4°C)			The ambient temperature (25°C)		
	First day	Third day	Sixth day	First day	Third day	Sixth day
Nanocomposite	^{aD} 0.127±2.59	^{aB} 0.155±3.88	^{bA} 0.113±4.45	^{aD} 0.084±2.6	^{bB} 0.056±3.9	^{bC} 0.042±3.06
Polyethylene	^{aD} 0.028±2.59	^{aC} 0.141±4.1	^{aAB} 0.127±5.33	^{aD} 0.07±2.6	^{aB} 0.099±4.99	^{aA} 0.084±5.58

Results are shown as mean ±standard deviation; The different lowercase letters indicate a significant difference in each column; The different uppercase letters indicate significant difference in each line.

Table 4. pH in nano biocomposite sample and polyethylene sample in Kashar cheese packaging

pH	Intervals after packaging				
	First day	First week	Second week	First month	Second month
Nanocomposite sample	^{aA} 0.183±4.83	^{aA} 0.084±4.81	^{aA} 0.396±4.79	^{aA} 0.07±4.75	^{aA} 0.042±4.73
Polyethylene sample	^{aA} 0.155±4.84	^{aA} 0.113±4.79	^{aA} 0.212±4.76	^{aA} 0.042±4.70	^{aA} 0.056±4.61

Results are shown as mean ±standard deviation; The different lowercase letters indicate a significant difference in each column; The different uppercase letters indicate significant difference in each line

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Table 5. Weight loss and moisture content in the shelf life of Kashar cheese by optimal nanocomposite film and polyethylene film sample

Test	Intervals after packaging					
	Package type	First day	First week	Second week	First month	Second month
Weight loss(%)	Nanocomposite sample	^{aE} 0.0±0.0	^{aD} 0.056±1.74	^{aC} 0.07±2.57	^{aB} 0.084±3.28	^{aA} 0.042±4.1
	Polyethylene sample	0.0±0.0^{aD}	^{bC}. 0.028±0.66	^{bBC} 0.042±0.81	^{bB} 0.014 ±0.93	^{bA} 0.084 ±1.12
Moisture(%)	Nanocomposite sample	^{aA} 4.568±46.54	^{aA} 2.135±45.73	^{aA} 2.56±45.34	^{aA} 3.026±45.01	^{aA}. 2.056±44.63
	Polyethylene sample	^{aA} 3.408±46.54	^{aA} 3.182±46.23	^{aA} 2.729±46.16	^{aA} 3.96±46.11	^{aA} 2.404±46.02

Results are shown as mean ±standard deviation; The different lowercase letters indicate a significant difference in each column; The different uppercase letters indicate significant difference in each line.

Table 6. Mold count and yeast in nano-biocomposite sample and polyethylene sample in Kashar packaged cheese

Mold and yeast count (cfu/g)	Intervals after packaging				
	First day	First week	Second week	First month	Second month
Nanocomposite sample	^{bE} 0.438±3.0	^{bD} 0.636±5.0	^{bC} 0.537±8.0	^{bB} 0.156±10.0	^{bA} 0.311±14.0
Control sample	^{aE} 0.353 ±5.0	^{aD} 0.396±8.0	^{aC} 0.17±13.0	^{aB} 0.141 ±22.0	^{aA} 0.212±39.0

Results are shown as mean ±standard deviation; The different lowercase letters indicate a significant difference in each column; The different uppercase letters indicate significant difference in each line

Table 7. Total count of microorganisms (cfu/g) in nano-biocomposite sample and polyethylene sample in Kashar packaged penny

Total count of microorganisms (cfu/g)	Intervals after packaging				
	First day	First week	Second week	First month	Second month
Nano- biocomposite sample	^{aB}. 0.155±2.33	^{bB} 0.113±2.36	^{bB} 0.396±2.48	^{bAB} 0.099±2.6	0.028^{bA} ±2.95
Polyethylene sample	^{aE} 0.084±2.33	^{aD} 0.042±2.85	^{aC} 0.396±3.11	^{aB} 0.014 ±3.79	^{aA} 0.07±4.46

Results are shown as mean ±standard deviation; The different lowercase letters indicate a significant difference in each column; The different uppercase letters indicate significant difference in each line

Discussion

The findings showed that during storage the total count of microbes in ground meat and cheese samples packed in nano-biocomposite film was reinforced with 3% chitosan nanoparticles, which was significantly less than the samples packed with ordinary polyethylene coating. This can be due to the ability of chitosan to destroy the outer membrane of bacteria. In confirmation of these results, ouattara and Dehand reported that using chitosan and its derivatives in bio-packaging films respectively reduced 0.6 and 1 unit in lactic acid bacteria the population cycle compared to the control sample at refrigerator temperature (Dehnad *et al.*, 2014, Ouattara *et al.*, 2000). Similarly ture et al. reported that packaged film based on wheat gluten and methylcellulose containing was well able to prevent the growth of molds such as *Aspergillus* niger and *Penicillium roqueforti* in Kashar cheese for 30 days at 10 degrees Celsius (Ture *et al.*, 2011). Moreira et al. showed that packaging of Cheddar cheese with two biofilms derived from chitosan and chitosan-sodium caseinate mixture showed different results of antimicrobial properties, so that the film obtained from chitosan-sodium caseinate compound could reduce the microorganisms about 4.5 cycles (as opposed to a 2-cycle

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reduction of chitosan-based biofilm) (Moreira *et al.*, 2011). They attributed this event to ionic interactions between the two macromolecules, resulting in an increase in antimicrobial specificity due to this interaction. Of course, the chitosan nanoparticles used in the preparation of the optimal compound film were also obtained by the process of ionic gelation. These antimicrobial properties can be attributed to the activation of intermolecular interaction. Moreover, Park et al. incorporated 1–8% chitosan into LDPE films; they observed no significant differences in log values of total viable count between control and chitosan-incorporated LDPE films. No antimicrobial activity was also observed for chitosan incorporated films during the storage time (Park *et al.*, 2010).

Conclusions

Based on the findings, ground meat samples packaged in nano-biocomposite containing 3% nanoparticles after 6 days of storage showed less microbial population than the control sample. Furtehrmore, samples of Kashar packaged cheese in nano-biocomposite containing 3% nanoparticles showed less mold count and yeast and microbial population after 60 days of storage than the control sample. The findings of this research indicated that nano-biocomposite packaging, in addition to the biodegradability advantage, could provide the safety of perishable food products such as ground meat and Kashar cheese to a greater extent compared to packaging of polyethylene origin. These findings also show that the use of nanotechnology in the packaging industry can have dramatic effects on the performance characteristics of biodegradable films. Therefore, more extensive efforts are needed to further improve the functional properties of these polymers to use them for commercial purposes.

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Conflict of interest

There was no conflict of interests in this study.

Authors' Contribution

Nateghi L designed the study and guided the writing and analyses; Eshaghi M conducted the experimental research and data analysis. Shojaei M prepared the manuscript's first draft. All authors reviewed the paper and confirmed it.

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