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Monitoring the physicochemical changes of eggs coated with active nanocomposite incorporating garlic extract (*Allium sativum* L.) during storage

Esmaeil Yousefi Zirabi[1], Dariush Khademi Shurmasti*[2]

1 -Master Science, Department of Agriculture, savadkooh Branch, Islamic Azad University, Savadkooh, Iran

2 -Assistant Professor, Department of Agriculture, savadkooh Branch, Islamic Azad University, Savadkooh, Iran

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*Corresponding Author E-

ABSTRACT

Eggs are one of the low-cost sources of protein and are also perishable, so maintaining their internal quality during storage is particularly important. The current research was carried out with the aim of the effect of nanocomposite coating on the physicochemical changes of eggs during storage for 4 weeks. Uncoated eggs (control) and eggs treated with a two-component coating of polyvinyl alcohol/chitosan (PC), a three-component coating of polyvinyl alcohol/chitosan/montmorillonite (PCM)), and PCM coatings incorporating concentrations of 2 and 4% garlic extract were evaluated in a total of 5 treatments and 3 replicates in a completely randomized design. The results showed that the concentration-dependent antimicrobial effect of garlic extract was higher against Staphylococcus aureus than Escherichia coli. The prepared films of active three-component PCM nanocomposite had greater thickness, higher tensile strength, and elasticity, lower solubility, less elongation at break, and lower water vapor permeability ($p<0.05$). At the end of 4 weeks of storage, the eggs coated with active three-component PCM nanocomposites had a thicker shell (0.400 mm) and a stronger shell (0.310 kg), causing less weight loss (About 4 %), Haugh unit (70.00) and yolk index (0.43) were higher and the lowest of total viable count bacteria (4.1 log cfu/ml). Therefore, using the three-component PCM nano-coating containing 4% garlic extract as a biodegradable packaging is possible to maintain the internal quality and eggshell and increase its storage time at ambient temperature.

1- Introduction

Eggs are one of the main sources of animal protein with a low-cost and excellent quality in developed and developing countries, including our country, Iran. Due to gas and mass exchanges between the internal content of the egg and the environment through the shell, the process of continuous reduction of the internal quality and the shell of the egg is greatly accelerated if the storage conditions are inappropriate [1]. Many approaches have been studied to preserve eggs, which can generally be classified into two categories based on the mechanism: inactivating the microorganisms on the egg shell surface and the other sealing the pores of the shell to prevent the exchange of water vapor, gases and microorganisms [2]. In this regard, shell coating is considered as a type of egg packaging and relatively extensive have been studies using different biopolymer coating materials such as cellulose derivatives [3], pectin [4] and chitosan [5].

Poly (vinyl-alcohol) (PVA) is a synthetic polymer and chitosan (CH) is a non-toxic, biodegradable biopolymer. PVA contains a large number of hydroxyl groups and can easily form hydrogen bonds with free water molecules. Chitosan films have gas permeability and favorable mechanical properties. However, the fragility of the material and its high permeability to water vapor limit its use in food packaging [6]. A simple mechanical mixing of polymers to produce a composite coating is an effective approach to overcome their poor mechanical properties. To overcome the poor mechanical properties of PVA, it is usually mixed with chitosan to use the interactions between these two materials to maximize the performance of the composite coating [7]. In a previous study, it was shown that CH/PVA composite coatings containing about 50 to 75% by weight of PVA, as a biodegradable egg packaging, can be used to extent the shelf life of 2 to 3 weeks at ambient temperature [8]. At the same time, to strengthen the tensile and barrier properties of biopolymer based packaging films, the addition of various nanofillers has been suggested. Layered silicate nanoclays are among the most promising materials among different nanofillers. Adding small amounts of nanofillers such as nanoclay to biopolymers creates a new class of packaging films based on bio-nanocomposite [9]. Besides, the results of previous research showed that the use of increased levels of nanoclay improves the barrier properties and increases the efficiency of nanocomposite coatings [10, 11].

The negative effects of using chemical preservatives have led researchers to use natural preservatives such as plant essential oils and extracts. Due to the presence of terpenoids and phenolic compounds in their compounds, these compounds have been widely used as antimicrobial and antioxidant compounds in the composition of active food packaging. Garlic (*Allium sativum* L.) as a medicinal plant has organic sulfur compounds such as thiosulfates, especially allicin, which is an antibacterial component in garlic. Allicin is a natural weapon against infection, which is thought to be a broad-spectrum antimicrobial compound by blocking two groups of enzymes, cysteine proteinases and alcohol dehydrogenases [12].

This study first aims to prepare and investigate the mechanical, physical and antimicrobial properties of the ternary polyvinyl alcohol-chitosan-montmorillonite active composite film containing concentrations of 2 and 4% garlic extract and then with the aim of monitoring qualitative changes. The eggs packed with the mentioned coating material were stored during the 28-day.

2 -Materials and methods

2-1 -Materials

Fully hydrolyzed polyvinyl alcohol (MW: 85-146 KD) and nano montmorillonite (MMT, K10) from Sigma Aldrich (Germany), chitosan powder with an MW of 50-190 KD and a deacetylation degree of 85% from Nano Novin Polymer (Iran), glycerol, 99% glacial acetic acid and other chemicals and culture media with analytical purity were purchased from Merck (Germany). Garlic plants were purchased from the local market. Edible white-shelled eggs, without cracks and faecal contamination, with an average weight of 57±5 g, were selected and purchased from a local laying hen farm and transported to the laboratory in compliance with hygiene principles.

2-2 -Preparation of garlic extract

The extract was prepared according to the method proposed by Hassanzadeh et al. [13]. In summary, first, the prepared garlic cloves were soaked in water to separate the skin better, and then the skin was removed and washed with water. After washing, the garlic was completely crushed with a household blender (Moulinex, France) mixed with distilled water at a ratio of 1:1 and kept for 24-48 hours at a temperature below 15^{o}C. At the end of the storage period, the mixture was first passed through a cloth filter to separate coarse garlic particles and then through Whatman No. 1 filter paper. At the end, after centrifugation at 6000 rpm for 15 minutes, garlic extract was prepared.

2-3 -Preparation of film-forming solutions

Chitosan solution (2% w/v) by dissolving a certain amount of chitosan in 1% acetic acid by stirring overnight at room temperature, poly(vinyl alcohol) solution (5% w/v) by dissolving PVA in distillate water was prepared by stirring at 90 C for 2 hours, and nano montmorillonite solution (4% w/w) by dissolving a certain amount of nanoclay in distilled water. Each solution of CH, PVA and MMT was mixed in a mass ratio of 1:1:1 and stirred at room temperature for 1 hour. Two different concentrations (2 and 4%) of garlic extract (Gar) were added to the solutions according to the experimental treatments and stirred for 1 hour at room temperature. To prepare the film, 20 g of the mixtures were poured into a plate and dried in an oven at 40 ^{o}C for 24 hours. The dried films were carefully separated and kept at a temperature of 25^{o}C and a relative humidity of 50% until analysis. The films were prepared using the casting method [14].

2-4 -Measuring the physical, mechanical and antimicrobial properties of films

The thickness of the film was measured using a micrometer with an accuracy of 0.01 mm. Mechanical properties including tensile strength (TS) and elongation at break (EB) were evaluated using a texture analyzer (TA-XT2 model, England) and according to ASTM standard D882-18, directly from the stress-strain curve. Young's modulus (YM) was calculated as the slope of the elastic region of the stress-strain curves. The water vapor permeability (WVP) of the film was measured using the method described by Fu et al. [15] and the water solubility (WS) of the film was measured by immersion in distilled water at a temperature of 25 ^{o}C for 24 hours [16]. The antimicrobial activity of films containing plant extracts was investigated by diffusion disc method. The films were converted into discs with a diameter of 10 mm using a mould. Before placing the discs on the surface of the culture medium, surface culture was performed using 100 microliters of liquid culture medium containing approximately 10^{8} cfu/ml of *Staphylococcus aureus* and *Escherichia coli* bacteria. Discs were placed on mullerhinton agar culture medium in completely sterile conditions and kept in a greenhouse at 37^{o}C for 24 hours. The difference in the diameter of the zone formed around the discs was considered an indicator of the antimicrobial activity of the films [17].

2-5 -Egg coating

Before coating, it was ensured that there were no possible cracks on the shell surface and the eggs were weighed. The eggs of each experimental treatment (20 pieces) were immersed in the coating solutions for 1 minute. After immersing and drying, the treated and control eggs were kept at ambient temperature for 4 weeks and parameters of internal quality and eggshell were measured weekly [18].

2-6 -Measurement of egg parameters

The percentage of weight loss (WL) was measured and calculated by calculating the ratio of the difference between the initial and final weight to the initial weight of the egg in each period. $HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$ was used to evaluate the HU, where H is the albumen height (mm), which was measured using a digital micrometer (INSIZE, Germany), and W is the weight of the egg (g). Yolk index (YI) was determined by measuring yolk height (h) and yolk diameter (d) using the relationship $YI = h/d$ [19]. To determine the pH of egg albumen, about 2 g of each sample was homogenized in 20 ml of deionized water and measured with a digital pH meter (ATAGO, DPH-2, Japan). The strength of the shell was determined using a texture alalyzer and the thickness of the shell was determined using a digital micrometer as the average size of the thickness of 3 points of the shell.

To evaluate the microbial contamination of the eggshell surface, the samples were placed in a special plastic bag containing 25 ml of 0.1% python solution and shaken by hand for 1 minute to separate the bacteria from it. Diluted shell solution samples were cultured on Plate Count Agar (PCA) using the mixed culture method and kept in a greenhouse at 30 C for 72 hours. Then the plates were counted and the results were reported as log cfu/ml [20].

2-7 -Experiment design and data analysis

A completely randomized design with 5 treatments including PVA-base film-forming solution, PVA/CH two-components, PVA/CH/MMT three-components and active three-components containing concentrations of 2 and 4% of garlic extract in the evaluation of film parameters and with 5 treatments including uncoated eggs (control) and eggs containing two-components, three-components and active three-components coatings according to the formulation of film-forming solutions was carried out in the evaluation of internal and eggshell quality parameters. The data were statistically analyzed using SPSS version 26 statistical software with 5 treatments and 3 replicates using one-way analysis of variance in the case of films and two-way analysis of variance in the case of egg coating as means±standard deviation. Means were compared using Duncan's multiple range test at the 95% probability level.

3- Results and Discussion

3-1 -Mechanical properties of the film

Tensile strength refers to the maximum resistance that a film can withstand. Elongation at break is a measure of a film's ability to stretch. Certain factors, such as the presence of additives, the degree of cross-linking or crystallinity, and the conditions of temperature and humidity during the test, adjust the strength of the films [21]. Determining the mechanical properties of films such as tensile strength, elongation at break and elasticity are important parameters in evaluating the efficiency of food packaging to protect them from external damage. The results obtained in the present study shown in Table 1 indicated that the addition of MMT and garlic extract increased the tensile strength of the films ($p<0.05$). This is due to the interaction bond created by the matrix material and the extract, which strengthens the film [22]. Another important parameter of mechanical properties is strain, which allows the material to resist deformation and fracture under tension. The percentage of strain depends on the flexibility and durability of the material. In other words, films with higher tensile strength have less strain. The decreasing trend in EB and increasing fragility in the active composite film can be explained by the integration of extract masses in the polymer matrix network, which limits the movement of polymer chains [23]. In general, materials with high tensile strength and Young's modulus, but low strain percentage, regardless of material thickness, have higher cross-linking [22]. The results show that basic materials and additives in composite and active films have a significant effect on the mechanical properties of edible films (Table 1).

2-3 -Film thickness and physical properties

The optimal range of film thickness depends on its application goals. As for maintaining the structural integrity of fresh produce, thicker films provide more advantages for coating applications [24]. The results showed that there was a strong direct and positive correlation between the thickness and the tensile strength of the films (0.911). In addition, the thickness of the edible films gradually increased by adding CH and MMT to the base film, while garlic extract did not play a significant role in increasing the film thickness ($p<0.05$). Solubility and WVP of films are important parameters of edible films. Film solubility requirements are different depending on its application. For example, films with lower solubility are preferred for longer-term food storage [22]. Although chitosan is a hydrophilic polymer, the strong hydrophilicity of polyvinyl alcohol compared to chitosan has caused polyvinyl alcohol to absorb more water molecules in competition for absorption ($p<0.05$). Besides, the results of solubility in water were highly correlated with the results obtained in the case of WVP (0.9850). Low water vapor permeability (WVP) is effective in increasing the shelf life of foods; In fact, the efficiency of packaging films is related to their ability to prevent or at least reduce the transfer of moisture between the food and the surrounding environment [25]. As shown in Table 1, the WVP of films containing MMT has decreased compared to other films ($p<0.05$). It has been confirmed that the presence of regularly dispersed nanoparticle layers with relatively large aspect ratios in nanocomposite films leads to a significant reduction in WVP. In this study, the decrease in WVP of films containing MMT can be attributed to the existence of nanoclay layered structures that create a tortuous path for diffusion and thus limit the moisture transfer through the matrix and improve the barrier properties of nanocomposite films. On the other hand, the decrease in permeability to water vapor can be attributed to the strong interaction between clay nanoparticles and bio-polymers [26]. It has been reported that the WVP of a film depends on several variables, especially the thickness [27]. The results showed that WVP has a negative correlation with film thickness (-0.705) and film tensile strength (-0.925). Considering that the addition of composite film components (CH, MMT) to PVA film was related to the increase in thickness and tensile strength of the film, it can be concluded that the triple structure of the composite film has reduced the WVP and increased the efficiency of the film.

Table 1. Thickness (mm), Water Solubility (%), Water Vapor Permeability (gmm/m^2 h Pa) and mechanical properties (Tensile Strength (MPa), Elongation at Break (%), Young's Modulus (MPa)) of films

	WS (%)	Mechanical properties

	Thickness (mm)		WVP (gmm/m^2 h Pa)	TS (MPa)	EB (%)	YM (MPa)
PVA	0.14±0.00^c	65.55±0.21^a	6.85±1.61^b	23.58±1.20^b	75.54±1.16^b	0.11±0.00^b
PVA+CH	0.15±0.02^b	63.32±0.11^b	6.00±1.30^b	21.88±3.15^b	94.52±2.21^a	0.10±0.04^b
PVA+CH+MMT	0.17±0.00^a	58.25±0.12^c	4.70±0.94^a	29.10±5.15^a	64.51±5.12^c	0.13±0.07^a
PVA+CH+MMT+G2%	0.17±0.02^a	57.90±0.10^c	4.35±0.92^a	30.60±3.16^a	56.30±4.17^d	0.14±0.05^a
PVA+CH+MMT+G4%	0.17±0.01^a	58.00±0.11^c	4.10±1.35^a	30.00±2.23^a	61.75±2.14^c	0.14±0.03^a

Values are presented as mean±SD, n=3. Means with different superscript indicate significant differences in column (P<0.05), PVA: poly(vinyl alcohol), CH: Chitosan, MMT: Montmorillonite, G:Garlic extract, WS: Water Solubility, WVP: Water Vapor Permeability, TS: Tensile Strength, EB: Elongation at Break, YM: Young's Modulus

3-3 -Antimicrobial properties of the film

The higher antimicrobial efficiency of two-component composite films compared to the base film may be due to the inherent antimicrobial activity of chitosan, which has been reported previously. One of the antimicrobial properties of chitosan against *Staphylococcus aureus* is that chitosan forms a polymer membrane on the cell surface that can prevent nutrients from entering the cell [28]. It was shown that the addition of garlic essential oil had a significant synergistic effect on microbial inhibition in packaging with chitosan films [29]. In the case of both bacteria, the preparation of two-component (PVA+CH) and three-component (PVA+CH+MMT) composite films could increase the antimicrobial efficiency of the films regardless of the presence of the extract. This result may be due to the excessive accumulation of antimicrobial substances caused by the formation of multiple layers, which may prevent the diffusion of the antimicrobial substance [25]. Following the results of this study, it was shown that both clinical and standard isolates of *Staphylococcus aureus* and *Escherichia coli* showed high sensitivity to garlic extract. At the same time, unlike the clinical isolates of *Staphylococcus aureus*, the clinical isolate of Escherichia coli was slightly resistant/non-susceptible, which can be related to the permeability of the membrane of *Escherichia coli* [12]. It was shown in Figure 1 that with the increase in the concentration of garlic extract, its efficiency increased and as a result, the inhibition and growth of the examined bacteria decreased in the form of larger clear inhibitory zones at a higher concentration (4%) (p 0.05). <). Allicin present in garlic has a strong antibacterial role that inhibits the synthesis of DNA, RNA and bacterial protein. The bactericidal effect of garlic can be influenced by the characteristics of the bacteria itself. The antibacterial effect of allicin compound in garlic is more against gram-positive bacteria than gram-negative bacteria. Lipids present in the membrane of Gram-positive bacteria play a role in helping allicin compounds penetrate bacteria [30].

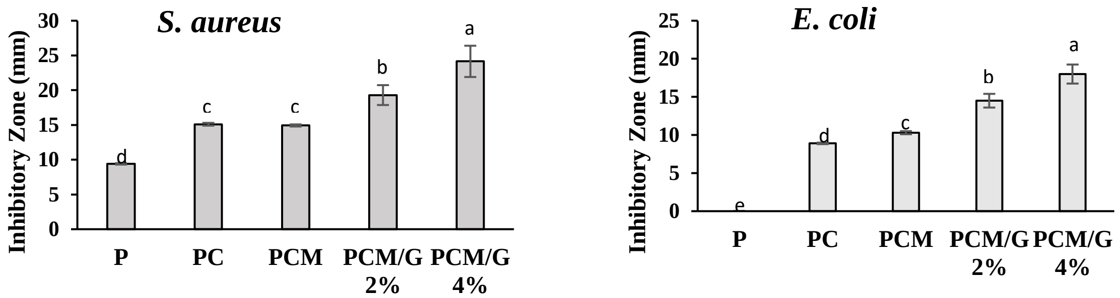

Fig 1. Mean of inhibitory zone (mm) against of *Staphylococcus aureus* and *Escherichia coli* in films; Means with different superscript indicate significant differences in column (P<0.05).

4-3 -Egg weight loss

The type of coating and storage time had a significant effect on egg weight loss (p<0.05). Figure 2 shows that during the storage, the weight loss of the egg increased because with the increase of the storage time, the pores of the eggshell become larger and the gas exchange inside the egg becomes much easier. Enlargement of eggshell pores leads to the loss of carbon dioxide gas and water vapor from inside the egg, the egg white becomes thinner and watery, and this leads to a decrease in the weight of the egg. The loss of carbon dioxide and moisture causes some physicochemical changes in the internal quality of eggs and their destruction, so the weight loss of eggs during the storage period provides important information about the quality and shelf life of fresh eggs [31]. Egg weight loss occurs mainly due to the transfer of moisture from the albumen to the external environment through the pores of the shell. Therefore, the decrease in weight loss in eggs containing three-component coating can be attributed to the presence of layered nanoclay structures, which is related to the reduction of WVP of the produced films and the barrier property of the coating in general. The weight loss of uncoated eggs increased from 1.3±0.5% in the first week to 6.4±1.2% at the end of the storage period. While shell coating reduced the weight loss rate of eggs, at the end of the period, the samples containing the three-component composite coating had the lowest percentage of weight loss ($p<0.05$). Based on these results, it is assumed that the composite coating of three structures closes these pores well and reduces the evaporation process.

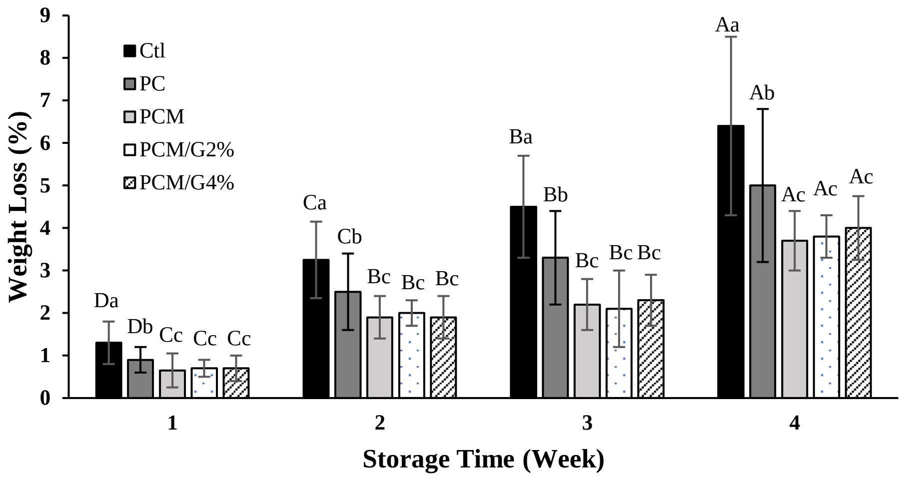

Fig 2. Effect of coatings on eggs weight loss (%) changes during storage; Means with different superscript indicate significant differences in column ($P<0.05$). Crl: control, P: poly(vinyl alcohol), C: Chitosan, M: Montmorillonite, G:Garlic extract

3-5 -Albumen pH

According to the data in Figure 3, pH values increased with increasing storage time. The decomposition of carbonic acid into water and carbon dioxide components increases the pH. The release of water vapor and carbon dioxide from the pores of the eggshell causes a decrease in weight, watery albumen and an increase in pH [32]. Determining albumen pH along with other quality parameters can be used to evaluate egg quality.

Freshly laid eggs contain about 1.44 to 2.05 mg of dissolved CO_2 (in the form of carbonate), which results in a pH between 7.6 and 8.7. Therefore, during the storage duration, CO_2 and water escape through the pores in the eggshell and increase the pH of the albumen from 9.8 to 9.4 [33]. At the end of the storage period, the highest and lowest albumen pH was observed in uncoated and those coated with an active three-component coating containing 4% extract respectively (9.2 vs. 7.9). Considering that albumen pH changes are related to the exchange and transfer of carbon dioxide, therefore, factors such as components of the composite coating, which are related to the integrity and impermeability of the shell, are also effective on this parameter. It can be said that the multi-component nano-coatings used may delay the loss of CO_2 through the pores of the shell and act as a gas exchange barrier.

Following this research, in previous studies, the use of active nanocomposite coating of carboxymethylcellulose, nanoclay and plant extract [11], composite coating of chitosan, polyvinyl alcohol [8], nanocomposite coating of carboxymethylcellulose, oleic acid and nanoclay [34] caused a decrease in weight loss and minimal pH changes during the storage period.

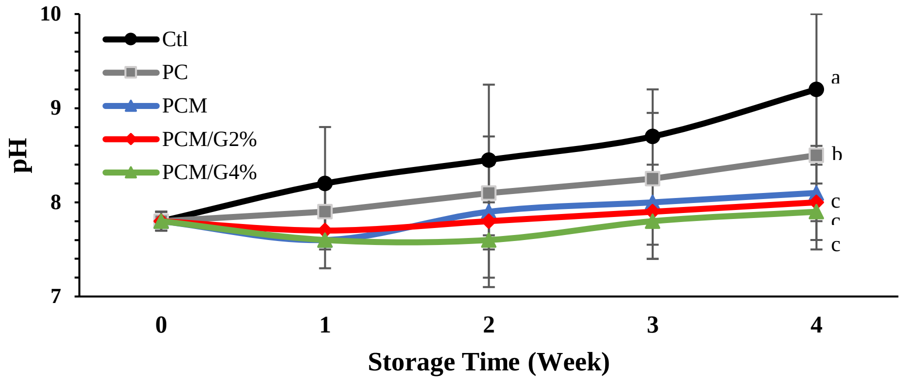

Fig 3. Effect of coatings on eggs pH changes during storage; Means with different superscript indicate significant differences in column (P<0.05). Crl: control, P: poly(vinyl alcohol), C: Chitosan, M: Montmorillonite, G:Garlic extract

6-3 -Eggshell thickness and strength

The cuticle and shell act as the first protective barrier of the egg. The destruction of the cuticle and shell and the presence of any cracks accelerates the process of exiting moisture and carbon dioxide, and on the other hand, the penetration of microorganisms and, as a result, the reduction of egg quality [5]. Therefore, the physical quality of the shell is of great economic importance. Because the thick and of course strong shell, by reducing the percentage of eliminated eggs due to cracking or breaking, increases shelf life and ultimately economic benefits. [35]. It was shown in Figure 4 that the shell thickness of coated eggs was higher compared to uncoated samples ($p<0.05$).

The inclusion of nanoclay and extract in the composition of the coating gradually increased the thickness of the shell. The thickness of the eggshell containing active nanocomposite coating was about 11% higher than the shell without coating (0.400 ± 0.05 versus 0.360 ± 0.03 mm). Shell strength followed a similar trend (Figure 4). The composite coating of chitosan/polyvinyl alcohol and also the coating of active three-component nanocomposite caused a gradual increase in eggshell strength ($p<0.05$). The data showed that there was a strong positive correlation (0.929) between shell thickness and strength. Therefore, thicker eggs containing active nanocomposite coating had more strength. It was shown in similar research that the use of nanocomposite coating increased the strength and durability of the eggshell [36 and 37].

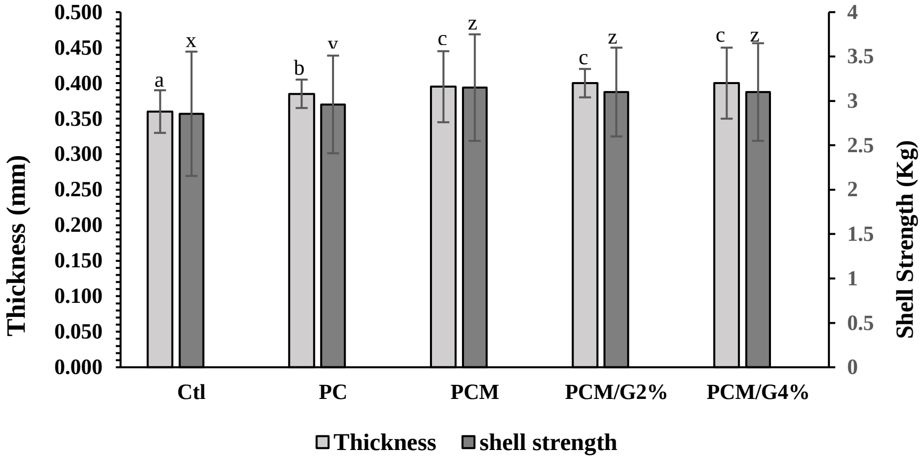

Fig 4. Effect of coatings on eggshell thickness (mm) and shell strength (Kg); Means with different superscript (a-c and x-z) indicate significant differences in column (P<0.05). Crl: control, P: poly(vinyl alcohol), C: Chitosan, M: Montmorillonite, G:Garlic extract

7-3 -Haugh unit and yolk index

Based on the changes in the protein content of the egg, the HU and YI are the main and important parameters in its quality evaluation. The higher of both parameters indicates the better quality of eggs. HU gradually decrease during storage due to the decomposition of the lysozyme-oomucin complex, the decrease in oomucin carbohydrate content, and the increase in pH caused by the release of carbon dioxide [38]. According to the USDA standard, eggs with an index of more than 72 are graded as AA, more than 60 as A, more than 32 as B, and less than 31 as C. In Table 2, it was shown that with the passage of storage time, the values of the HU decreased (p<0.05). At the end of the storage period, the highest index was seen in the eggs containing active nanocomposite coating. Based on the standard, the grade of uncoated eggs gradually changed from AA at the beginning to C at the end of the storage period, while the eggs containing the three-structure nanocomposite coating at the end of the fourth week. They had grade A. In addition, all the eggs coated with nanocomposite until the end of the second week and the eggs coated with active nanocomposite containing 4% extract still had AA grade until the end of the third week.

The index of quality egg YI is about 0.45 and its standard range is (0.45-0.30) [5]. The continuous and progressive penetration of water from the albumen to the yolk through the vitelline membrane due to osmotic pressure causes the yolk to become watery and flattened, and as a result, the YI decreases during the storage period [35]. Therefore, the use of coating reduces the loss of carbon dioxide and moisture and slows down the structural changes of albumen due to the increase in osmotic pressure between egg albumen and yolk, and as a result, improves yolk quality [5]. According to the data in Table 2, the YI of the coated eggs was within the standard range until the end of the storage period, while the YI of the uncoated eggs was outside the standard range at the end of the third week. At the end of the storage period, the highest YI was seen in eggs coated with active nanocomposite (p<0.05).

The structural changes of egg albumen are caused by changes in the osmotic pressure between the egg albumen and the yolk, and it is a function of carbon dioxide and moisture exchanges, therefore, the use of coatings, especially active composite coating, has a positive effect on the properties of the shell, such as permeability, thickness, and strength. Improvement of internal quality characteristics of HU and YI. In accordance with the findings of the present research, it was shown that the use of active composite coating maintained and improved the internal quality of eggs [8, 11, 34, 36 and 37].

Table 2. Effect of coatings on Haugh Unit and Yolk Index of egg during storage (week)

Treatments	Storage Duration (Week)				
	0	1	2	3	4
Haugh Unit					
Ctl	84.75±0.00^{Aa}	61.50±0.55^{Bc}	43.75±0.80^{Cb}	31.45±1.05^{Dd}	27.90±0.85^{Dd}
PC	84.75±0.00^{Aa}	75.70±0.60^{Bb}	70.20±0.75^{Ba}	60.50±0.80^{Cc}	55.00±1.00^{Cc}
PCM	84.75±0.00^{Aa}	80.10±0.50^{Aa}	72.00±0.50^{Ba}	66.50±0.75^{Cb}	61.00±0.90^{Db}
PCM/G2%	84.75±0.00^{Aa}	81.20±0.90^{Aa}	73.10±0.70^{Ba}	71.00±0.55^{Ba}	68.60±0.80^{Ba}
PCM/G4%	84.75±0.00^{Aa}	81.40±0.60^{Aa}	74.85±0.65^{Ba}	72.50±0.60^{Ba}	70.00±0.90^{Ba}

Yolk Index					
Ctl	0.49 ± 0.00^{Aa}	0.40 ± 0.05^{Bb}	0.33 ± 0.08^{Cc}	0.27 ± 0.03^{Dc}	0.25 ± 0.04^{Dd}
PC	0.49 ± 0.00^{Aa}	0.42 ± 0.03^{BCb}	0.40 ± 0.05^{Cb}	0.37 ± 0.05^{Db}	0.36 ± 0.05^{Dc}
PCM	0.49 ± 0.00^{Aa}	0.45 ± 0.05^{BCa}	0.43 ± 0.04^{Cab}	0.42 ± 0.07^{CDa}	0.40 ± 0.06^{Db}
PCM/G2%	0.49 ± 0.00^{Aa}	0.46 ± 0.06^{Ba}	0.44 ± 0.08^{BCa}	0.43 ± 0.03^{BCa}	0.42 ± 0.06^{Ca}
PCM/G4%	0.49 ± 0.00^{Aa}	0.46 ± 0.04^{Ba}	0.45 ± 0.05^{BCa}	0.44 ± 0.05^{BCa}	0.43 ± 0.00^{Ca}

Values are presented as mean±SD, n=3. Means with different superscript (a-d) and (A-D) in columns and rows indicate significant differences respectively (P<0.05), Crl: control, P: poly(vinyl alcohol), C: Chitosan, M: Montmorillonite, G:Garlic extract

8-3 -Total bacteria count

The results of the effect of food coatings on the average total bacterial count (TVC) of the eggshell during the storage period at ambient temperature shown in Figure 5 showed that this parameter was associated with a gradual increasing trend. The total number of egg shell bacteria at the beginning of the experiment was 2.7 ± 0.1 log cfu/ml, indicating the optimal initial hygienic quality of the shell. Environmental factors of storage conditions cause microbial contamination of eggs. Coating significantly had a positive effect on reducing the process of shell pollution. In addition, the type of coating used was also effective on the value of this parameter. The use of chitosan/polyvinyl alcohol composite coating reduced the number of total bacteria compared to uncoated eggs (p<0.05), which could be due to the inherent antimicrobial activity of chitosan, which has been reported previously. At the same time, the inclusion of nano-clay also increased the antimicrobial efficiency of the coating, which could be due to the excessive accumulation of antimicrobial substances caused by the formation of multiple layers, which may prevent the diffusion of the antimicrobial substance. These results show that the antimicrobial effects of edible coatings can be determined based on the type and amount of antimicrobial agents and polymers used, as well as other environmental factors. Also, these results show that the optimal concentration of antimicrobial substances is necessary to be released from the ground substance and to show antimicrobial activity [25]. At the end of the storage period, uncoated eggs had the highest number of bacteria (log cfu/ml 6.9 ± 1.00) and eggs coated with active nanocomposite containing 4% extract had the lowest number of bacteria (log cfu/ml) were 4.1 ± 0.3 (p<0.05).

In this study, it was observed that by increasing the concentration of garlic extract, its efficiency increased and as a result, the microbial contamination of the shell decreased at a higher concentration (4%). The antibacterial effect of garlic extract is influenced by thiosulfinate compounds such as allin, allicin and diallyl sulfonate. Allicin has a strong antibacterial role. Allicin compounds play a role in inhibiting bacterial protein synthesis, which was discussed earlier.

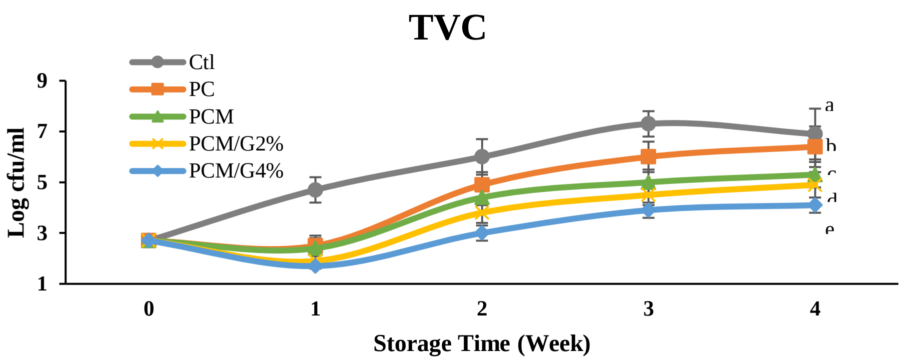

Fig 5. Effect of coatings on eggshell Total Viable Count (log cfu/ml) during storage; Means with different superscript indicate significant differences(P<0.05). Crl: control, P: poly(vinyl alcohol), C: Chitosan, M: Montmorillonite, G:Garlic extract

4 -Conclusion

The findings of the present study, while confirming the antimicrobial effect of garlic extract against gram-positive and gram-negative bacteria, showed that the antimicrobial efficacy of garlic extract against gram-positive bacteria is more than gram-negative and concentration-dependent. At the same time, active tri-structural films (polyvinyl alcohol/chitosan/montmorillonite) having some physical and mechanical properties, especially lower WVP and higher tensile strength, can easily replace films become plastic like polyethylene, which is widely used in the market. Egg coating with an active three-component solution by creating a thicker and stronger shell reduces weight loss, reduces pH changes, and increases yolk index during the storage period and ultimately leads to an increase in egg shelf life. The chicken was kept for at least 2-3 weeks. Therefore, it is recommended to use an active tri-structural nanocomposite coating containing 4% garlic extract as a biodegradable packaging to replace synthetic types to maintain the internal quality and eggshell during the storage period at ambient temperature.

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مقاله علمی_پژوهشی

پایش تغییرات فیزیکوشیمیایی تخم‌مرغ با پوشش نانوچندسازه فعال حاوی عصاره سیر (*Allium sativum L.*) طی دوره انبارمانی

اسماعیل یوسفی زیرابی[۱]، داریوش خادمی شورمستی[*۲]

۱- کارشناس ارشد، گروه کشاورزی، واحد سوادکوه، دانشگاه آزاد اسلامی، سوادکوه، ایران

۲- استادیار، گروه کشاورزی، واحد سوادکوه، دانشگاه آزاد اسلامی، سوادکوه، ایران

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چکیده

تخم‌مرغ یکی از منابع ارزان قیمت پروتئینی و در عین حال فسادپذیر است، لذا حفظ کیفیت داخلی آن طی دوره ذخیره‌سازی اهمیت ویژه‌ای دارد. تحقیق حاضر با هدف تأثیر پوشش نانو چندسازه‌ای بر تغییرات فیزیکوشیمیایی تخم‌مرغ طی دوره نگهداری به‌مدت ۴ هفته اجرا شد. تخم‌مرغ‌های فاقد پوشش (شاهد) و تخم‌مرغ‌های تیمار شده با پوشش دو سازه‌ای پلی وینیل الکل/کیتوزان (PC)، پوشش سه سازه‌ای پلی وینیل‌الکل/کیتوزان/مونت–موریلونیت (PCM)، و پوشش‌های PCM حاوی غلظت‌های ۲ و ۴ درصد عصاره سیر در مجموع ۵ تیمار و ۳ تکرار در قالب طرح کاملاً تصادفی مورد ارزیابی قرار گرفت. نتایج نشان داد اثر ضدمیکروبی وابسته به غلظت عصاره سیر در برابر استافیلوکوکوس اورئوس بیشتر از اشرشیا کلی بود. فیلم‌های تهیه شده نانو سه سازه‌ای PCM فعال دارای ضخامت، استحکام کششی و مدول الاستیسیته بیشتر و حلالیت، کرنش و نفوذپذیری کمتر بودند ($p<0.05$). در پایان ۴ هفته نگهداری، تخم‌مرغ‌های دارای پوشش نانو سه سازه‌ای PCM فعال، پوسته ضخیم‌تر (۰/۴۰۰ میلی‌متر) و مستحکم‌تر (۰/۳۱۰ کیلوگرم) داشته، موجب افت وزنی کمتر (حدود ۴ درصد)، واحد هاو (۷۰/۰۰) و اندیس زرده (۰/۴۳) بالاتر و کمترین شمار باکتری کل (۴/۱ log cfu/ml) بودند. بنابراین می‌توان از پوشش نانو سه سازه‌ای PCM حاوی ۴ درصد عصاره سیر به‌عنوان بسته‌بندی زیست تخریب‌پذیر جهت حفظ کیفیت داخلی و پوسته تخم‌مرغ و افزایش زمان نگهداری آن در دمای محیط استفاده کرد.