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Scientific Research

Investigation of antimicrobial, antioxidant, physical, and mechanical properties of a nano-composite film (nano-chitosan/ aloe vera) along with hydrolyzed tomato seed protein

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ABSTRACT

This research aimed to investigate the physical, mechanical, antioxidant, and antimicrobial properties of a smart nanocomposite film based on chitosan/ aloe vera containing hydrolyzed tomato seed protein. For this purpose, the hydrolyzed tomato seed protein was first prepared using the Alcalase enzyme under different time conditions (30, 60, 90, 120 minutes). Then, 5 edible films including nano-chitosan, nano-chitosan and aloe vera gel with different concentrations of hydrolyzed protein (0, 0.5, 1 and 1.5%) were prepared, and the film properties were evaluated. Based on the results of the hydrolyzed protein, the hydrolyzed protein had a high protein content and degree of hydrolysis .This protein also had a high content of hydrophobic amino acids (31.78%) and aromatic amino acids (11.74%). The mechanical test results of the films showed that increasing the protein concentration led to a decrease in tensile strength and an increase in elongation at break of the polylactic acid films. According to the physical test results, increasing the protein concentration did not have a significant effect on moisture and solubility, but increased water vapor permeability and turbidity ($p<0.05$). the hydrolyzed tomato seed protein had high DPPH radical scavenging activities and increasing the concentration had a positive effect on these parameters ($p<0.05$). These films also had high antimicrobial activity against pathogenic bacteria, with higher antimicrobial activity against *Staphylococcus aureus* than *Escherichia coli*. The nanocomposite film containing 1.5% hydrolyzed protein had the highest antioxidant and antimicrobial activity ($p<0.05$). This study showed that hydrolyzed protein can improve the physical and mechanical properties of chitosan/aloe vera based films. Specifically, films containing 1.5% hydrolyzed protein had better properties such as higher antioxidant activity and antimicrobial activity.

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1-Introdaction

Edible films and coatings can be defined as a thin layer of edible material that is placed on the surface of food through dipping, spraying and rolling, and it limits microbial contamination and controls the loss of moisture and respiration rate, thereby reducing deterioration. It delays [1]. Today, various types of antimicrobial compounds are used in the design of food packaging and coatings, and nanoparticles that have antimicrobial properties are considered an important group of them. Chitosan is a hydrogenated heteropolymer with a positive electrical charge, which is obtained from the deacetylation of the natural polymer chitin, and due to its antimicrobial properties, biodegradability and non-toxicity, alone or together with other compounds, it is used as a coating to preserve a variety of food products. takes [2, 3]. Nanochitosan is a natural substance with excellent physical and chemical properties that is compatible with nature and bioactive. In addition, the antibacterial and antioxidant activity of chitosan nanoparticles has also been reported [4, 5]. The use of chitosan nanoparticles in food packaging is very promising because of its compatibility with food [5]. Aloe vera gel is a polysaccharide coating, which is extracted from the inner parts of aloe vera plant leaves and has features such as a protective layer, reducing the loss of fruit juice and reducing the amount of gas in relation to the skin of the fruit and reducing the production of ethylene in the raw fruit. [6, 7]. So far, various studies have been conducted on the effect of aloe vera gel in edible coatings to increase the shelf life of fresh fruits and vegetables such as mushrooms, bell peppers, kiwi and strawberries [6, 7, 8, 9].

In recent years, hydrolyzed protein containing bioactive peptides using enzymatic hydrolysis from different plant sources such as clover, wheat germ, sesame flour, rapeseed and watermelon seeds have been used in various studies [10, 11, 12, 13]. Hydrolyzed proteins are produced by enzymatic, chemical or fermentation methods. Meanwhile, the use of enzymes to hydrolyze protein sources is more interesting due to favorable and more suitable production conditions and less side products [14]. Hydrolysis of proteins involves breaking them into smaller peptides and free amino acids. Enzymes used for protein hydrolysis are possible. have plant, animal and microbial origin. In comparison with enzymes of plant and animal origin, microbial enzymes have more advantages, including the variety of proteolytic properties and greater stability in pH and different temperatures [15]. In general, alcalase enzyme because of its function in alkaline pH, A higher degree of hydrolysis and a shorter peptide chain length have attracted the most attention in the production of hydrolyzed protein [16, 17]. In Iran, every year about 8100 tons of wet tomato pomace are created by factories, which are often removed from the consumption chain without considering the practical cases [18]. Tomato seeds contain 33.9-22-2% protein and 20.5-29.6% oil, so it is a very good source for edible oils and vegetable proteins [19, 20]. Tomato seed protein contains amino acids arginine, lysine, histidine, phenylalanine, tryptophan, leucine, isoleucine, methionine, valine, cysteine, alanine, proline, glutamine, serine, asparagine and threonine [21, 22]. Therefore, according to the appropriate amount of protein and high nutritional value, tomato waste can be used to produce hydrolyzed protein with appropriate biological characteristics [20, 21].

According to the investigations, there has been no research on the effect of hydrolyzed tomato seed protein in the nanocomposite film containing nanochitosan-aloeura. Therefore, the purpose of this research is to investigate the physical, mechanical, antioxidant and antimicrobial properties of the nanocomposite film containing nanochitosan-aloeura along with the hydrolyzed protein of tomato seeds.

2- Materials and methods

2-1- Raw materials

The required tomato pomace (waste from the processing of tomato processing industries) was obtained from Rab Ata factory, Iran. Aloe vera leaves were purchased fresh to use its gel. Fresh aloe vera leaves were purchased from a production greenhouse in Amol, Iran. Nanochitosan (Nanovin Polymer Company, Iran), Folin Ciocaltive reagent and free radicals 2 and 2 diphenyl 1-picrylhydrazyl (DPPH) was obtained from Sigma Aldridge, USA. Other chemicals used were of laboratory purity and produced by German Merck and American Sigma Aldridge companies.

2-2- Preparation of tomato flour

Sedimentation method was used to separate seeds from tomato pomace. For this purpose, the wastes were immersed in water in large plastic containers, while the light crust and heavy seeds settled on the surface. After separation, the seeds were dried in the sun and then turned into flour with a homemade mill and passed through an 80 mesh sieve. The resulting flour was oiled with hexane solvent at a ratio of 1:10 in 4 stages and after remaining in the open air for 1 day, it was transferred in polyethylene bags and stored in a refrigerator [23].

2-3 production of tomato seed protein concentrate

In order to extract tomato seed protein, first the powder obtained from the previous step was mixed with deionized water at a ratio of 1:10 and its pH was adjusted to 11.5 by adding 1 normal sodium bicarbonate and stirred for 30 minutes, then the

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resulting solution was mixed for 20 minutes with It was centrifuged at 2,600 times. In the next step, the pH of the supernatant was adjusted to the isoelectric pH of tomato seeds (7) and the resulting solution was centrifuged at x2600 for 25 minutes to precipitate the proteins. Then, the resulting protein concentrate was dried with a freeze dryer and kept at a temperature of 20°C until the tests were performed [18].

2-4 hydrolyzed hydrolyzed protein concentrate

The protein content of the samples was checked through the Keldal method. For the process, enzymatic hydrolysis of tomato seed protein concentrate at a concentration of 5% (weight by volume) was dissolved in a phosphate buffer with a pH equal to the optimum limit (8.5) for the alkalase enzyme in 100 ml Erlens and the possibility of complete hydration with continuous stirring. It was provided for 30 minutes at room temperature. When the temperature reached the desired temperature, the samples were placed in the temperature, and after the temperature was fixed, the enzyme was added to the solutions in the desired ratio. The reaction was carried out in a certain time range (30, 60, 90 and 120 degrees Celsius). After the desired time, in order to inactivate the Erlens enzyme, it was placed in a 90°C oven for 5 minutes and then it was cooled using a container containing ice until it reached the ambient temperature. After that, the samples were centrifuged at x8000 for 20 minutes and using a sampler, their supernatant was separated and dried by a freeze dryer and kept at -20 degrees Celsius until the tests were performed. [12].

2-5 degrees of hydrolysis

The degree of hydrolysis was calculated based on the amount of α amino acid in the amount of protein in the sample [24].

2-6 amino acid composition

In order to synthesize the amino acid of the hydrolyzed protein powder, the protein sample was first completely hydrolyzed for 24 hours at 110°C using 6 M hydrochloric acid. Then the available amino acids were derivatized by adding phenyl isothiocyanate (PITC). The amount of total amino acids was measured using a Smart line HPLC device (made in Germany) equipped with a C18 column and a fluorescent detector (RF-530). [15].

2-7 Preparation of chitosan nano film-aloe vera gel

The prepared aloe vera leaves were washed with sterile distilled water. The tip and edge of the leaves were cut and then using a hand knife, the middle part of the leaf was cut longitudinally and the skin of the leaves was separated from the gel in the middle of the leaf. After separation, the gel was crushed and mixed with a blender for 5 minutes. The resulting mixture was collected after passing through a cloth strainer with the aim of producing pure gel, and a concentration of 7.5% by weight and volume was prepared by adding sterile distilled water to the pure gel [7]. To prepare the composite gel, chitosan nanoparticles (1%), aloe vera gel and different concentrations of hydrolyzed protein (0.5, 1 and 1.5%) were added to it. For this purpose, different amounts of these compounds were completely dissolved in 200 ml of distilled water at a temperature of 70 degrees Celsius by a stirrer for 45 minutes [5, 7]. From the casting method[1] Films were obtained by pouring 50 ml of each determined solution into a 12 cm Petri dish and drying at room temperature for 48 hours. Then, the films were separated and stored in Stumiker bags

2-8-Measuring the physical properties of films

1-8-2-Measuring the thickness of films

A micrometer with an accuracy of 0.01 mm was used to measure the thickness. The thickness was measured at 5 points of the film and its average value was reported [25]

2-8-2 Measuring the moisture content of films

Film samples with specific weight were placed in glass plates that had already reached a constant weight and were weighed (W1). Then it was dried in the oven at 105 degrees Celsius for 24 hours. The sample with the plate was removed after this period and after cooling in the desiccator, it was weighed again (W2). The moisture content of the films was calculated based on wet weight from equation 1 [26].

Relationship 1

$$100 \times W1 / (W2-W1) = \text{moisture percentage}$$

3-8-2-Evaluation of solubility of films in water

To measure the solubility of the films, 2 cm square pieces of the films were prepared. The dry weight of the samples was obtained by drying them in an oven at 105 degrees Celsius for 24 hours. Then the samples were placed in a 50 ml container of water for 24 hours. Then the film was removed from the water and placed in an oven at 105 degrees Celsius for 24 hours to dry. Finally, the final weight was measured [26].

4-8-2-Permeability against water vapor

In order to measure the permeability of films to water vapor (ASTM E 96-02), first 10 milliliters of distilled water was poured into the permeability measurement cells, and then the glass cells, whose surface was sealed by the film and with the help of grease, were placed in a desiccator containing silica gel. . Water at a temperature of 25 degrees Celsius creates 100% humidity. The difference in humidity on both sides of the film at a temperature of 25 degrees Celsius creates

1- Casting

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a heater pressure difference equal to 2.337.103 pascals. Cell weight changes were measured over time using a digital scale with an accuracy of 0.0001 grams. The water vapor transfer rate in terms of (gram)-meter-second was equivalent to the slope of the resulting lines divided by the cell surface and was obtained from equation 2 [19]. The area of the cells was 0.00287 square meters. From multiplying the water vapor transmission rate (WVTR) by the film thickness (L) and dividing it by the pressure difference on the two sides of the film (AP), the water vapor permeability (WVP) $(10)^{-11}$ $gs^{-1}m^{-1}Pa$) was obtained [26].

Relationship 2

Cell surface (meters) / slope of the line (g/s) = water vapor transfer rate (g^{1-} $seconds^{2-}$ meters)

5-8-2-turbidity

To determine the turbidity, the films prepared as pieces with dimensions of cm^2 1 x 4 was cut. Then these parts were placed in the cell of the spectrophotometer and their absorbance was read at a wavelength of 600 nm. Equation 3 was used to determine the turbidity. [27].

Relationship 3

100x absorbance = turbidity

2-9-Mechanical properties of films

The mechanical tests of the films are based on the modified method of ASTM D0882-02. The films are cut into pieces of 761 cm and conditioned under the conditions of relative humidity of 50% and temperature of 25 degrees Celsius. Their thickness is determined at 5 measurement points and their average thickness. The mechanical properties of the film (extensibility (percentage), tensile strength (MPa) are measured using Instran. In the Instran device, the distance between the two jaws is 50 mm, the movement speed of the upper jaw is 50 mm/min and the lower jaw is fixed. is [26].

2-10- Antioxidant activity of films

The antioxidant activity of the film was measured using the DPPH free radical scavenging method [28]. For this purpose, first, 25 mg of the film was gently mixed in 3 ml of distilled water. Then, 8.2 ml of this solution was added to test tubes containing 0.2 ml of 1 mM DPPH solution in methanol and kept at room temperature for 30 minutes. The optical absorption of the samples and the control sample was measured at a wavelength of 517 nm using a spectrophotometer. The decrease in light absorption compared to the control indicated the ability of the compounds in the film to inhibit the DPPH free radical. Finally, the percentage of inhibitory activity of DPPH free radicals was calculated using equation 3.

relationship 4:

100] × (absorption rate of control / absorption rate of sample - absorption rate of control) - 1 = DPPH free radical scavenging percentage

2-11- Determination of antimicrobial activity of films

Bacterial cultures *Staphylococcus aureus* (PTCC 1189) and *Escherichia coli* (PTCC 2019) was prepared from the microbial collection of Tehran University. Using a sterile loop, an aliquot of each bacterium was removed from sterile ampoules and added to 10 mL of BHI Broth culture medium. The inoculated culture medium was placed in a greenhouse at 37°C for 24 hours. After incubation, bacteria were cultured on nutrient agar plates using a sterile loop. The cultured plates were kept in a greenhouse at 37°C for 24 hours. 3 to 5 isolated and homogenized colonies were transferred to tubes containing 5 ml of physiological serum using a sterile swab. The turbidity (optical absorption) of the bacterial suspensions was measured at a wavelength of 625 nm using a spectrophotometer. Suspensions to a concentration equal to half McFarland, which is equivalent to approx 10^8 Colony per ml, diluted. Using a sterile swab, the bacterial suspensions were uniformly spread on the surface of the culture media. Round discs of film with a diameter of 6 mm were cut using a round knife. The film discs were placed at suitable intervals on the plates impregnated with bacteria and the plates were incubated for 24 hours at 37°C. After incubation, the diameter of the transparent halos around the film discs was measured and reported in millimeters. This method was used to investigate the antimicrobial activity of edible films using the diffusion method in agar [29].

12-2- Statistical evaluation

The experiments were carried out in three repetitions and in the form of a completely randomized design. Data analysis was done using SPSS 18 software. Comparison of means was done with Duncan's test (One way Anova) with 5% error level. Graphs were drawn using Microsoft Excel 2013 software.

3- Results and discussion

3-1- degree of hydrolysis

The degree of hydrolysis is used as a parameter to monitor the amount of protein hydrolysis, this factor is mostly used as an index to compare different hydrolyzed proteins. On the other hand, the degree of hydrolysis is one of the most important factors for investigating the properties of hydrolyzed proteins, which expresses the degree of breaking of peptide bonds and must be controlled [30].

The results related to the degree of hydrolysis in Table 1 show that with the increase in the time of the hydrolysis process, the degree of hydrolysis, which means the breaking of peptide bonds, increases, while the intensity and rate of hydrolysis, which is the same

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as the intensity of the separation of soluble proteins from different types It is insoluble, it decreases [31, 32]. And no significant difference was observed between 90 and 120 minutes. As the hydrolysis time increases, the substrate decreases, which affects the hydrolysis degree values [33]. Similar results were reported by Golpaygani et al. [33] in relation to the degree of hydrolysis of the hydrolyzed protein of rainbow trout eggs. They also stated that the degree of hydrolysis increases with the increase in storage time, but after some time the values of the degree of hydrolysis are almost constant.

2-3-The amount of protein

The primary protein content of tomato seeds in the present study is equal to 0.97±It has been 19.54% and the amount of tomato primary protein in different studies is 5.40 on average±17.71 (between 10.50 and 25.03) percent has been reported [34, 35, 36, 37, 38]. The isolated protein content of tomato seeds is equal to 0.91±It was 36.41 percent. Also, the amounts of protein, hydrolyzed proteins were between 90.66-90.46% (Table 1). These results are consistent with the results of studies related to the amounts of isolated protein and hydrolyzed protein of tomato seeds. They also stated that the amount of protein after hydrolysis is higher than that of isolated tomato seeds. [18]. Also, a significant difference in terms of protein content was observed among the hydrolyzed samples under the influence of different times. So, increasing the time of hydrolysis of protein increased and we did not observe any significant difference between the time of 90 and 120 minutes. As the hydrolysis time increases, the substrate decreases, which probably affects the amount of protein.

Table 1: Degree of hydrolysis and protein content of tomato seed protein hydrolysates at different hydrolysis

Hydrolysis time (min)	Degree of hydrolysis (%)	Protein content (%)
30	14.09±0.87[c]	46.95±1.01[c]
60	21.80±0.48[b]	76.10±0.79[b]
90	24.90±0.30[a]	90.66±1.20[a]
120	24.73±0.54[a]	90.16±0.91[a]

[a] Values represent means ± SE (n = 3).
[b] Values in same columns with different lower letter are significantly different at P < 0.05.

3-3- Amino acid composition

Amino acids, which are often known as the building blocks of proteins, are compounds that perform many vital roles, including maintaining cell structure, repairing and regenerating muscles and bones, and repairing damaged tissues [39]. In the present study, the amount of 17 amino acids was identified (Table 2). The highest values of amino acids were related to the non-essential amino acid glutamic acid 99.16%, the lowest values related to the non-essential amino acid cysteine, and in relation to essential amino acids, the highest values related to leucine 99.6% and the lowest values related to the essential amino acid methionine. These results are consistent with the results of a study related to tomato amino acid, they also declared the highest values related to the non-essential amino acid glutamic acid and the essential amino acid leucine [37]. Also, other similar results were reported that they also announced that tomato skin contains 14.56% glutamic acid (the most non-essential amino acid) and 5.07% leucine (the most essential amino acid) [36].

The performance of hydrolyzed protein depends on its amino acid profile. Hydrophobic amino acids (phenylalanine, proline, methionine, alanine, leucine, isoleucine, tyrosine, valine) and aromatic amino acids (phenylalanine histidine, tryptophan tyrosine), responsible for most of the functional and biological properties of hydrolyzed proteins, as well as antioxidant, anti-inflammatory, anticancer and Reduction of sugar and blood pressure [40, 41] in the present study, the sum of hydrophobic amino acids and aromatic amino acids were equal to 31.78 and 11.74%, respectively, which due to their high amounts, we can expect their bioactive effects in the host.

Values of valine, isoleucine, leucine, phenylalanine, lysine and tyrosine in tomato seed hydrolyzed protein were higher than the recommendations of FAO/WHO [42] regarding animal proteins. which shows the high nutritional quality of this protein.

Table 2: The amino acid composition tomato seed protein hydrolysates (g 100 g^{-1}) (30 min)

Amino acid(g 100 g^{-1})	Alcalase	FAO/ WHO, 1990
Histidine [a]	2.48	

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Isoleucine [a]	3.44	2.8
Leucine[a]	6.99	6.6
Lysine[a]	3.97	5.8
Methionine[a]	0.74	
Phenyl alanine[a]	5.97	6.3
Tyrosine	3.29	1.1
Threonine[a]	2.59	3.4
Arginine	6.92	
Valine[a]	4.15	3.5
Aspartic acid	8.59	
Glycine	3.95	
Proline	4.11	
Serine	3.47	
Alanine	3.09	
Cysteine	0.37	
Glutamic acid	16.99	
Total amino acid	80.02	
LET[b]	31.78	
AAA[c]	11.74	

a Essential amino acids
b Total hydrophobic amino acids (alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, proline, methionine and cysteine)
c Total amount of aromatic amino acids (phenylalanine, histidine, tryptophan and tyrosine)

3-4- Investigating the physical properties of films

Solubility and moisture content are two important factors of biodegradable films that affect the film's resistance to water, especially in humid environments. According to the results, the highest values of moisture and solubility (Charts 1 and 2) were observed in nanochitosan treatment ($P<0.05$). The addition of aloe vera gel decreased the moisture content and solubility of the film, but the addition of hydrolyzed protein did not have a significant effect on the values of these parameters. In fact, the addition of hydrolyzed protein did not change the moisture content and solubility in the film. Probably, the decrease in solubility in water observed as a result of adding aloe vera gel to chitosan is the increase of hydrophobicity in the resulting composite films. These results are consistent with the results of another study related to the composite film of soybean and sunflower protein containing hydrolyzed plasma protein. They also stated that the addition of hydrolyzed protein had no effect on the moisture content and solubility of the film [43]. Another researcher also stated that the addition of hydrolyzed cottonseed protein to alginate film has no significant effect on the moisture content and solubility of the film. The humidity values in their study were between 12.22-10.75% [44].

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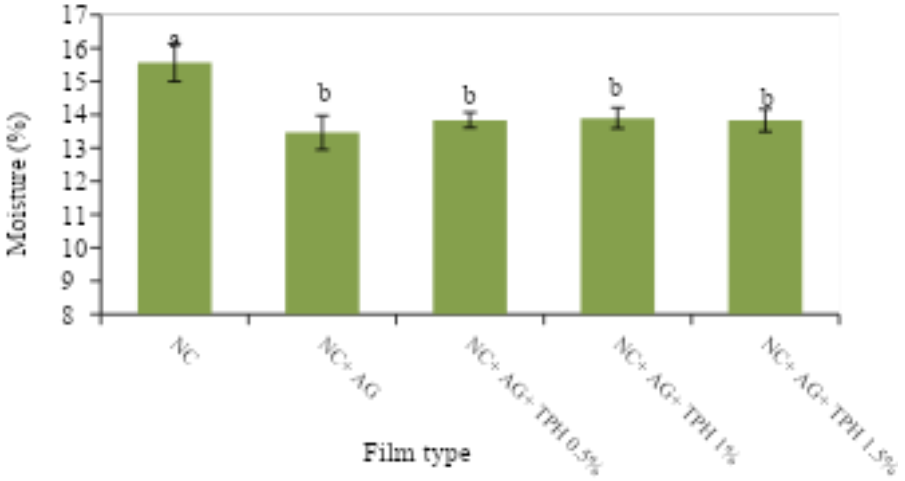

Fig 1. Moisture content of nano-composite film along with hydrolyzed tomato seed protein

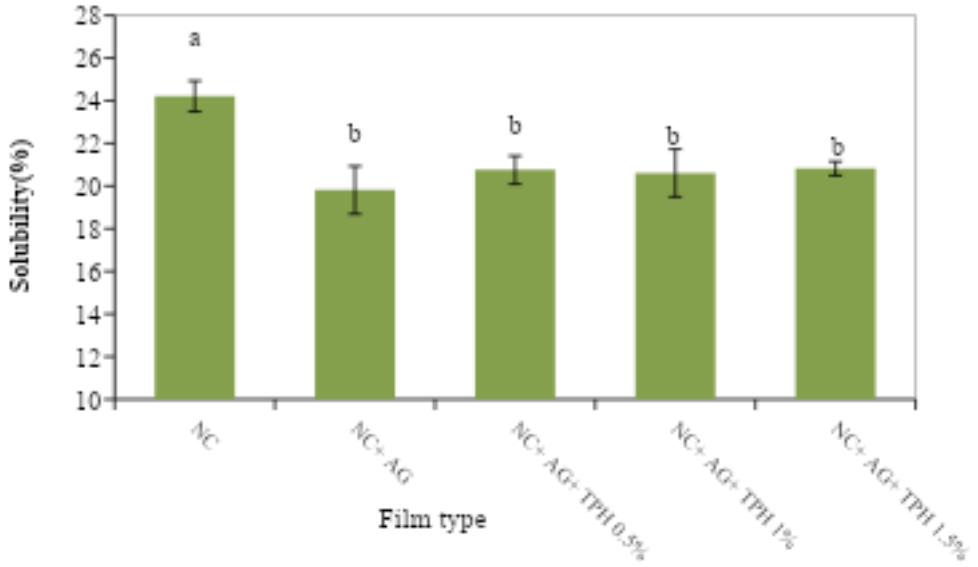

Fig 2. Solubity content of nano-composite film along with hydrolyzed tomato seed protein

The thickness of edible films depends on the concentration of ingredients, the amount of initial film solution per unit area and the speed of pouring on the surface, and this feature has a great impact on important factors in the evaluation of films such as permeability to water vapor and their mechanical behaviors [45]. According to the results, the lowest thickness values (Chart 3) were found in the nanochitosan film. By adding aloe vera gel and hydrolyzed protein to the film, the thickness increased so that the maximum thickness was observed in the film of nano chitosan + aloe vera gel + hydrolyzed protein 1.5%. The reason for the increase in thickness is probably due to the increase in solid content after adding gel and hydrolyzed protein. In general, film thickness depends on the nature and composition of the film. The effect of the nature and composition of the film on the resulting thickness can be seen in the results of other researchers. Similar results were observed in studies related to hydrolyzed protein of cottonseed to alginate film [44] and related to carboxymethyl cellulose film containing hydrolyzed protein of silver carp fish skeleton [46].

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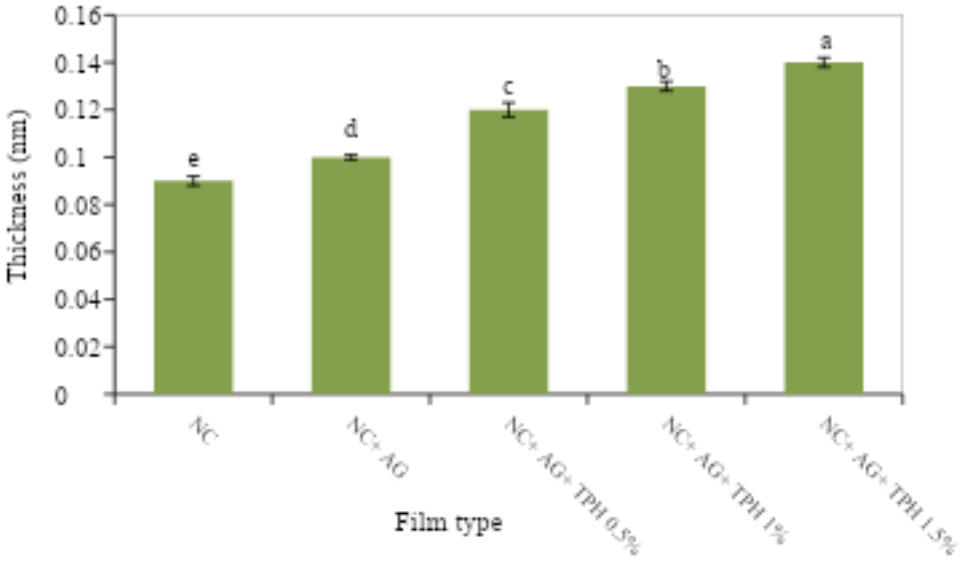

Fig 3. Thickness of nano-composite film along with hydrolyzed tomato seed protein

In general, various factors such as the type of compounds and the degree of interaction between them, thickness, solubility and permeability of water vapor molecules in the film matrix affect the amount of WVP [46]. According to the results, adding aloe vera gel to the nanochitosan film decreased the permeability of the films (Chart 4). In general, the high permeability of the chitosan film to water vapor is related to its hydrophilic nature, which causes water molecules to react with the tissue and leads to an increase in the permeability to water vapor [47]. Therefore, the reduction observed in this study is the result of adding aloe vera gel related to the possible interactions between nanochitosan and various compounds in aloe vera gel, which reduces the permeability to water vapor in the resulting composite films by reducing the ratio of hydrophilic to hydrophobic parts. By adding hydrolyzed protein to the film, the permeability increased so that the highest amount of WVP was observed in the film of nano chitosan + aloe vera gel + 1.5% hydrolyzed protein. This is due to the plasticizing effect of hydrolyzed protein with low molecular weight. The presence of protein leads to an increase in hydrophilic groups in the film structure, and subsequently, the presence of more water molecules in it leads to an increase in the WVP of the film, and the increase in the thickness of the hydrolyzed protein layers also affects the WVP values [44, 46]. Similar results were observed in studies related to hydrolyzed protein of cottonseed to alginate film [44] and related to carboxymethyl cellulose film containing hydrolyzed protein of silver carp fish skeleton [46].

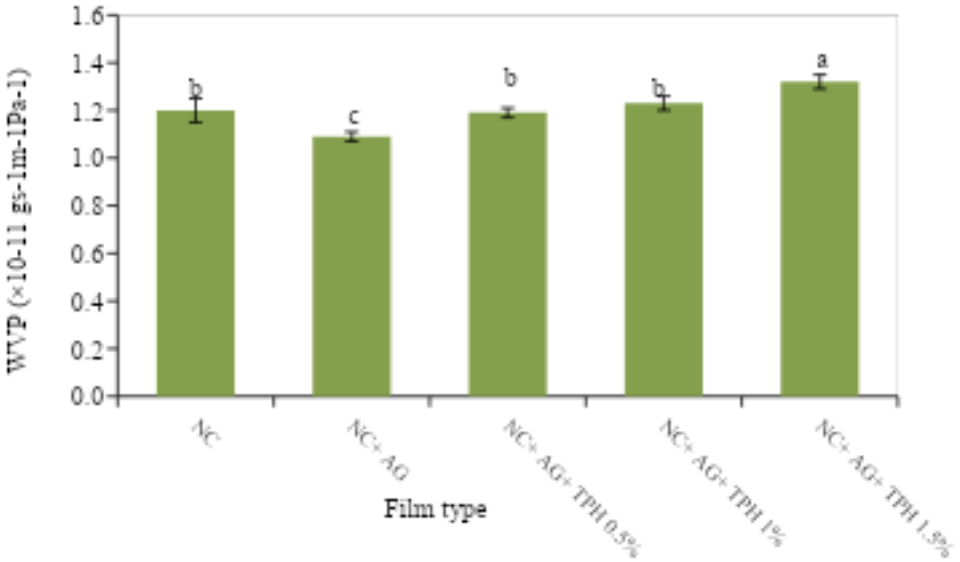

Fig 4. WVP of nano-composite film along with hydrolyzed tomato seed protein

Optical properties of films, such as color, transparency, and light transmission, are important properties that affect their appearance, acceptance, commercialization, marketability, and suitability for

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various applications. According to the results, the lowest turbidity values (Chart 5) were found in chitosan nano film. In general, the addition of aloe vera gel in the film caused a darker appearance, which can be related to the fact that the aloe vera gel extracted in this study can contain anthraquinone compound, which is sensitive to air and light, and it is difficult to remove them from the gel composition. is the result of phenol oxidation [48]. The amount of turbidity also increased with hydrolyzed protein to the film, so that the highest amount of turbidity was observed in the film of nano chitosan + aloe vera gel + 1.5% hydrolyzed protein. Addition of hydrolyzed protein increases the thickness of the film, thus reducing the transparency and increasing the turbidity [46]. Similar results were observed in connection with the hydrolyzed protein of cottonseed to alginate film, in that study it was announced that the increase in light scattering (i.e. decrease in transparency) is related to the increase of protein accumulation in alginate film [44]. Changes in film transparency may reduce consumer demand, but low visible light transmission speed is an advantage for food packaging because the presence of light can cause changes in color and taste, loss of nutrients, and ultimately oxidative spoilage of food.

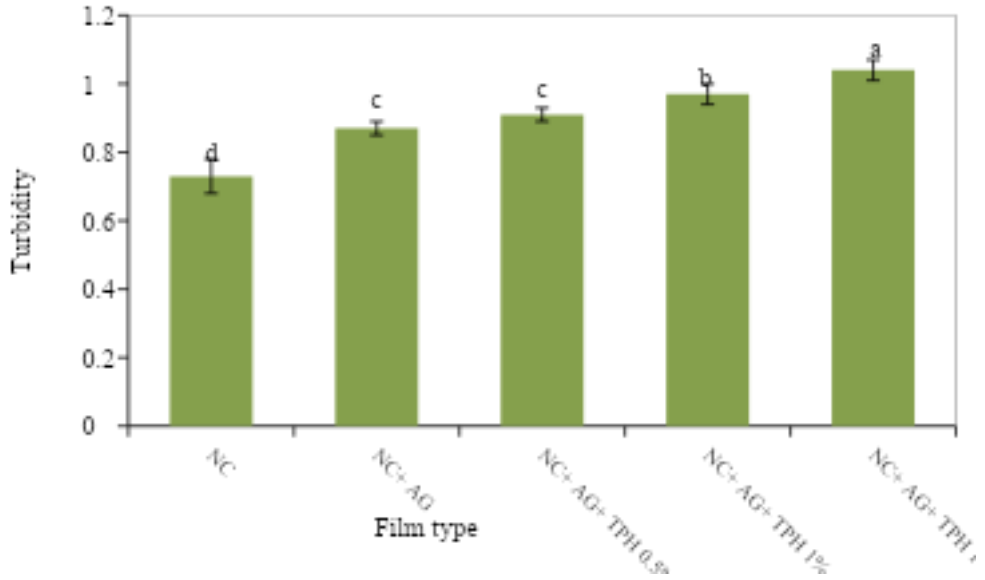

Fig 5. Turbidity of nano-composite film along with hydrolyzed tomato seed protein

3-5- Checking the mechanical properties of the films

The mechanical properties of composite polymers are among the properties that depend on the level of interactions on the common surface of the compounds. In general, the establishment of appropriate interactions between compounds causes a significant improvement in the mechanical properties of films [49]. According to the results, by adding aloe vera gel to the nanochitosan film, the tensile strength increased (Chart 6) and the maximum tension before the breaking point (Chart 7) decreased. This indicated the proper toughness and flexibility of the film after adding aloe vera. In a study, it was also announced that the addition of aloe vera to chitosan film reduced the maximum tension before the chitosan film rupture point. In their study, the reason for this was declared to be the complex and cross-links between chitosan and aloe vera [50]. The addition of hydrolyzed protein decreased the tensile strength and increased the maximum tensile strength before the breaking point. The mechanical properties of films are closely related to the distribution and density of intra- and intermolecular interactions between polymer chains in film networks. Therefore, the addition of hydrolyzed protein to the composite nanofilm can affect the mechanical properties of the films by reducing the intermolecular forces and increasing the mobility of the protein chains [51].

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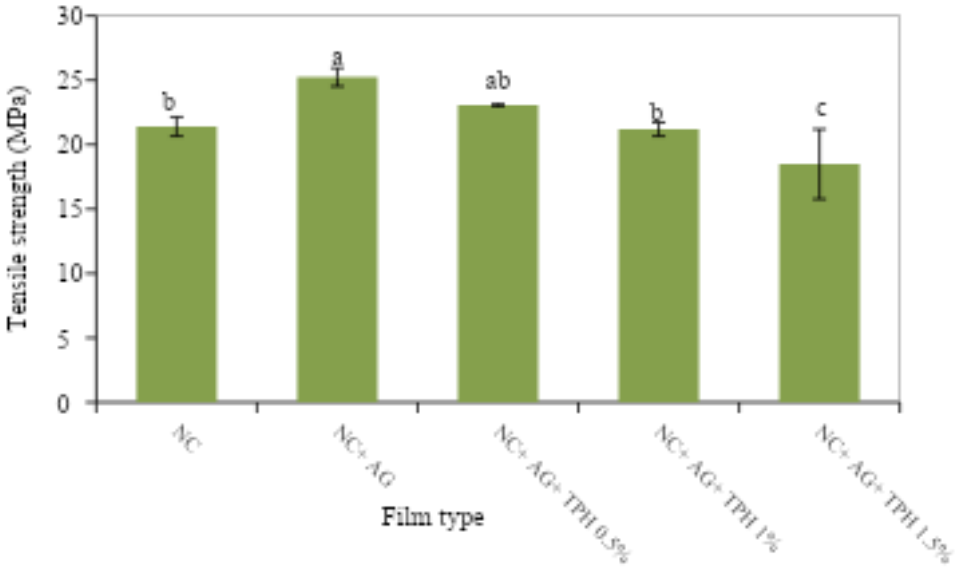

Fig 6. Tensile sterngth of nano-composite film along with hydrolyzed tomato seed protein

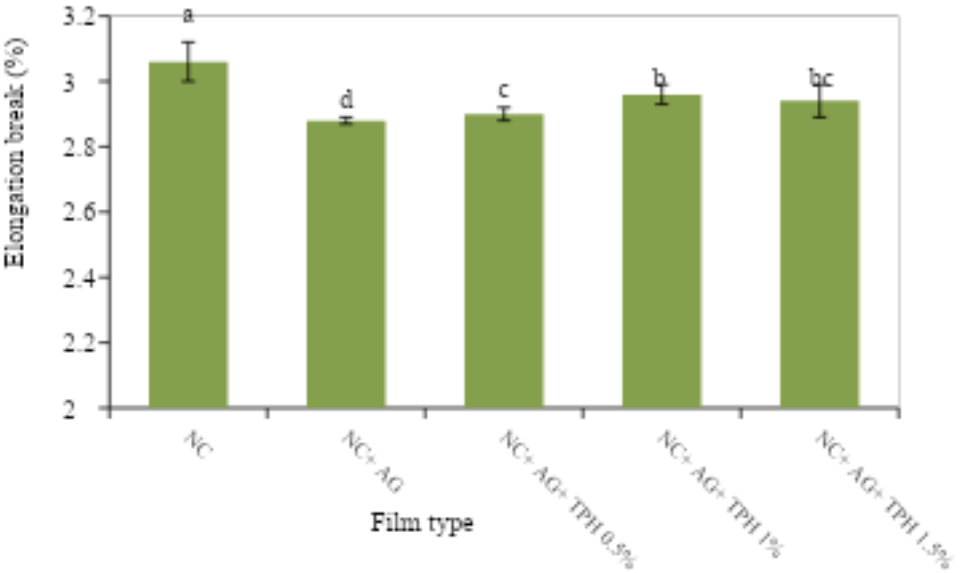

Fig 7. Elongation breaks of nano-composite film along with hydrolyzed tomato seed protein

6-3- Investigating the antioxidant properties of films

One of the common methods to determine the antioxidant activity is the inhibition of DPPH free radicals. DPPH is a stable radical whose methanolic solution has a purple color that shows the highest light absorption at 515-520 nm. The basis of this method is that the DPPH radical acts as an electron acceptor from a donor molecule like an antioxidant, in this case the purple color of the environment turns into yellow. Therefore, the intensity of absorption decreases at 515 nm, by measuring the decrease in intensity of absorption by spectroscopy, one can understand its antioxidant properties [52]. Based on the results (Chart 8), the antioxidant activity of nanochitosan was equal to 26.35%. The antioxidant properties of chitosan have also been reported by other researchers [53, 54]. Also, by adding aloe vera gel, DPPH free radical inhibition increased. Researchers also announced that the antioxidant effect of aloe vera gel is related to the presence of glutathione peroxidase, superoxide dismutase enzyme and phenolic compounds [50, 55].

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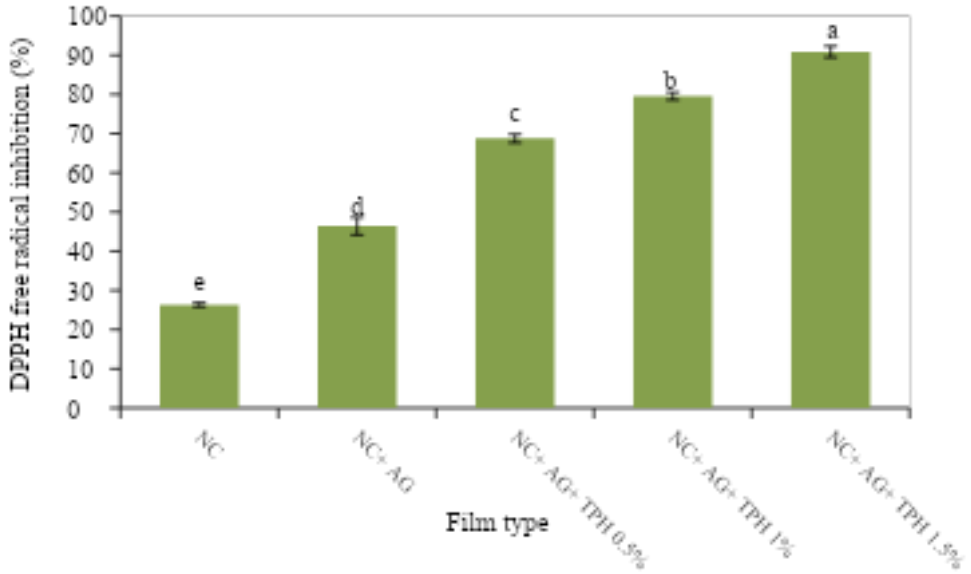

Fig 8. Antioxidant activity of nano-composite film along with hydrolyzed tomato seed protein

Based on the results, adding hydrolyzed protein increased the antioxidant property of the films, and increasing the protein concentration had a positive effect on DPPH free radical inhibition. The antioxidant activity of hydrolyzed protein has been attributed to multiple effects. Some of these features include their ability to remove free radicals, act as metal chelators, oxygen quenchers or hydrogen donors, and the ability to prevent the penetration of fat oxidation initiators by forming a layer around oil droplets [50]. Other researchers also announced that hydrolyzed proteins and plant bioactive peptides have antioxidant properties in laboratory conditions [10, 11, 12, 13, 31]. In a study, it was also announced that nanocellulose film containing hydrolyzed pine seed protein has the ability to inhibit DPPH free radical, and this ability increases with increasing protein concentration [56].

7-3- Investigating the antimicrobial properties of films

Based on the results, nanochitosan film had antimicrobial activity against both bacteria. There are two theories regarding the antimicrobial mechanism of chitosan. First of all, chitosan has the ability to chelate metals and necessary elements and remove them from the reach of bacteria by using its application properties. Second, chitosan destroys the bacterial cell wall by forming a bond with the anions of the cell wall [57, 58]. By adding gel Aloe vera to the film, the antimicrobial properties of the films increased. Anthraquinone[2] and divahydroxyanthraquine[3] The active ingredients in aloe vera gel are antimicrobial [59]. In another study, the antimicrobial activity of aloe vera gel against a number of gram-positive and gram-negative bacteria has been shown by several different methods [60]. Also, the addition of hydrolyzed protein increased the antimicrobial activity of the films. So that the highest level of antimicrobial property of the films against both bacteria was observed in nano chitosan film + aloe vera gel + 1.5% hydrolyzed protein. Antimicrobial peptides with membrane permeability function can enter the membrane and disrupt it as a result. These changes cause an imbalance in the cellular contents that disrupts the processes of replication, transcription and translation of the DNA sequence by binding to specific intracellular targets [44, 57, 61].

Tabble 3: Influence of hydrolyzed tomato seed protein on the antimicrobial activity of nano-composite film

Film / Microorganism	*Staphylococcus aureus* (mm)	*Escherichia coli* (mm)
NC	10.90± 0.11^{and}	9.53± 0.40^{and}
NC+ AG	15.54± 0.43^{d}	14.00± 0.03^{d}
NC+ AG+ TPH 0.5%.	19.32± 0.58^{c}	17.32± 0.29^{c}
NC+ AG+ TPH 1%.	21.43± 1.02^{b}	19.27± 0.28^{b}

2 -Anthraquinone

3- Dihydroxyanthraquinone-1,4

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NC+ AG+ TPH 1.5%.	23.80± 0.28[a]	21.04± 0.98[a]

Data are given as mean± standard divition (n=3)
Different small letters in the same column indicate significant differences (P <0.05)

4- Conclusion

This research showed that the addition of hydrolyzed tomato seed protein to chitosan/aloeura nano films can improve the properties of the film. By increasing the hydrolyzed protein concentration to 1.5%, the films had higher antioxidant and antimicrobial properties. Also, the addition of hydrolyzed protein had a positive effect on the mechanical properties of the films, including reducing the tensile strength and increasing the tensile strength up to the breaking point. In general, this study shows that the hydrolyzed tomato seed protein at a concentration of 1.5% can be used as a suitable additive in nanochitosan/Aloehora films.

5- Resources

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چکیده

این تحقیق با هدف بررسی ویژگی‌های فیزیکی، مکانیکی، آنتی‌اکسیدانی و ضدمیکروبی فیلم هوشمند نانوکیتوزان/آلوئه‌ورا حاوی پروتئین هیدرولیز شده دانه گوجه فرنگی انجام شده است. بدین منظور ابتدا پروتئین هیدورلیز شده دانه گوجه فرنگی توسط آنزیم آلکالاز تحت تاثیر زمان های مختلف (۳۰، ۶۰، ۹۰، ۱۲۰ دقیقه) تهیه شد. سپس ۵ فیلم خوراکی شامل، نانو کیتوزان، نانوکیتوزان و ژل آلوئه‌ورا به همراه غلظت‌های مختلف پروتئین هیدرولیز شده (۰، ۰/۵، ۱ و ۱/۵٪) تهیه و ویژگی‌های فیلم‌ها بررسی گردید. بر اساس نتایج مربوط به پروتئین هیدرولیز شده، که پروتئین هیدرولیز شده از میزان پروتئین و درجه هیدرولیز بالایی برخوردار بود. همچنین این پروتئین دارای اسیدهای آمینه هیدروفوب (۳۱/۷۸٪) و اسیدهای آمینه آروماتیک (۱۱/۷۴) بالایی بود. نتایج آزمون مکانیکی فیلم نشان داد افزایش غلظت پروتئین سبب کاهش مقاومت کششی، افزایش کشش تا قبل از نقطه پارگی نانو فیلم شد و همچنین بر اساس نتایج مربوط به آزمون‌های فیزیکی، افزایش غلظت پروتئین بر روی رطوبت و حلالیت تاثیر معنی‌داری نداشت، اما سبب افزایش نفوذ پذیری بخار آب و افزایش کدورت شد ($p<0/05$). پروتئین هیدرولیز شده دانه گوجه فرنگی توانایی بالایی در فعالیت مهار رادیکال آزاد DPPH و افزایش غلظت تاثیر مثبتی بر این پارامتر داشت ($p<0/05$)، همچنین این فیلم‌ها خاصیت ضد میکروبی بالایی علیه باکتری‌های پاتوژن داشتند و خاصیت ضد میکروبی علیه باکتری *استافیلوکوکوس اروئوس* بالاتر از *اشیرشیاکلی* بود. فیلم نانوکامپوزیت حاوی ۱/۵٪ پروتئین هیدرولیز شده دارای بالاترین فعالیت آنتی‌اکسیدانی و ضدمیکروبی را دارا بود ($p<0/05$). این مطالعه نشان داد که پروتئین هیدرولیز شده می‌تواند خواص فیزیکی و مکانیکی فیلم‌های بر پایه نانوکیتوزان/آلوئه‌ورا را بهبود دهد. به طور خاص، فیلم‌های حاوی ۱/۵٪ پروتئین هیدرولیز شده دانه گوجه فرنگی دارای خواص بهتری مانند قدرت آنتی‌اکسیدانی و خاصیت ضد-میکروبی بودند.

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