



Studying the effect of zinc oxide nanoparticles and cellulose nanofiber on the morphological, structural, thermal, mechanical and barrier properties of nanocomposite film based on Barhang (*Plantago major* L) mucilage

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ABSTRACT

The aim of this research was to prepare a nanocomposite film based on Barhang (*Plantago major* L) seed mucilage. Zinc oxide (ZnO) and cellulose nanofiber (CNF) nanoparticles at the concentrations of 4 and 8% w/w were incorporated for improving the morphological, structural, thermal, water vapor permeability and mechanical properties of films. The FT-IR results confirmed the occurring of new interactions between nanoparticles and mucilage polysaccharides. XRD results indicated that the effect of ZnO on semi-crystalline structure of Barhang film is higher than the effect of CNF. The neat film has a smooth surface, but the roughness increased by addition of nanoparticles. According to TGA results, the thermal stability of films was affected by incorporation of nanoparticles, but the effect of CNF on improving the thermal stability of film was more than ZnO. The addition of nanoparticles at the concentration of 4% had no effect on the thickness of films but by increasing to 8%, the thickness increased. Moisture content and moisture absorption of films was also decreased significantly by incorporation of nanoparticles. The water vapor permeability of films was dependent on the concentration of nanoparticles and at 4%, it decreased significantly but at 8% concentration, the permeability increased again due to the aggregation of nanoparticles and their hydrophilic nature. The water contact angle of films' surface increased by addition of ZnO but the CNF caused to decrease this value due to its hydrophilicity. The effect of CNF on improving the mechanical properties of films was better than ZnO. The CNF had the most effect on increasing tensile strength, elastic modulus and elongation to break. The effect of CNF on the improving of the barrier and mechanical properties of Barhang seed mucilage based films was more than ZnO due to its better compatibility. In general, this research indicated that the Barhang seed mucilage based film incorporated with organic and inorganic nanoreinforcements, has desired physicochemical properties and can be introduced as a suitable candidate for food packaging applications.

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1. Introduction

The harmful environmental effects caused by the accumulation of plastic and non-degradable materials is one of the most important concerns in the world today. Due to the fact that a significant amount of this waste is related to food packaging, food industry experts have sought to find alternatives for synthetic polymers. One of the suitable alternatives for these polymers is biodegradable films based on biopolymers. Proteins, polysaccharides, lipids or their combination are used to produce biodegradable films. Among biopolymers, polysaccharides have received more attention due to their abundance, good film-forming ability, and favorable mechanical and gas-inhibiting properties [1].

During the last decade, efforts to produce biodegradable films from mucilage and gum obtained from various plant seeds have increased. Mucilage is a hydrophilic carbohydrate biopolymer that is extracted from different parts of plants as well as some microorganisms, and in the presence of water, it creates a consistency and produces a gel. High purity polysaccharide extracted from mucilage is also called gum [2]. In recent years, various studies have been conducted on the production of biodegradable films from mucilage and gum extracted from different seeds, such as Beh seed [3], Chia seed [4], Balingo seed [5] and Basil seed [6].

A source of plant gum with favorable properties, Barhang (*Plantago major L.*) Is. Barhang is a plant belonging to the family *Plantaginaceae* and grows in temperate regions. This plant produces tiny, monocotyledonous seeds that are surrounded by a coating of various polysaccharides around the seed and produce mucilage by absorbing water. The polysaccharide composition of the gum extracted from the seeds of barberry includes xylose, rhamnose, arabinose, galacturonic acid, glucuronic acid, galactose and glucose [7]. This linear polysaccharide shows good film-making ability [8].

Despite the characteristics of biodegradability and abundant and cheap access to plant polysaccharide sources, the films obtained from plant mucilages have a series of disadvantages that limit their use in food packaging. Among the disadvantages of biodegradable films derived from mucilages and plant gums are the hydrophilic nature, weak barrier properties to

water vapor and moisture, as well as poor mechanical properties. One of the effective ways to strengthen these properties in biopolymer films is the use of nanoreinforcers and the production of nanocomposite films. Nanoreinforcers are organic or inorganic substances on the nanoscale (below 100 nm) that have unique physical and chemical properties due to the reduction of particle size, and adding them to the composition of packaging films can improve their inhibitory, mechanical, and thermal properties [9].

One of the most important and common nanofillers in the production of biodegradable nanocomposite films is zinc oxide (ZnO) nanoparticles. ZnO nanoparticle has various applications in the food industry due to its antimicrobial properties, photocatalytic effect and nutritional value of zinc element. The use of this nanoparticle as a structure enhancer as well as an antimicrobial compound in the formulation of various types of polymer and biopolymer films has been studied and has provided good results [10-12]. Another organic nanomaterial whose use has increased in the production of nanocomposite packaging is cellulose nanofibers (CNF). CNF, which can be produced from plant and microbial sources, is a fiber with a diameter of 10 to 30 nm and a length of several microns. High degree of crystallinity, favorable mechanical strength, biocompatibility, ease of production and availability, and favorable compatibility and mixing with biopolymer films are among the favorable characteristics of CNF, which has increased its use in the production of nanocomposite packaging. Cellulose nanofibers have also been used in the production of different types of biopolymer films [13].

There are few researches in the field of producing biodegradable film from barhang seed mucilage. Niknam et al [8] investigated the effect of different vegetable oils on the hydrophobic properties of this mucilage film. ArdebilchiMarand et al. [14] used nanoclay and fennel essential oil in the composition of borage seed mucilage film and investigated the effect of the film in increasing the shelf life of local butter. AlizadehBehbahani et al. [15] prepared an edible coating based on mucilage of burdock seed and dill essential oil and investigated its effect in increasing the shelf life of beef. So far, no study has been conducted in the field of the production of mucilage film containing

nanoparticles, and the effect of any nanoenhancer on the properties of this film has not been investigated. The aim of this research is to produce a nanocomposite film of the mucilage of barberry seeds¹ (BSM) was ZnO and CNF nanoparticles were used to enhance the properties of castor bean mucilage film and the effect of these two nanoparticles on the morphological, crystallinity, thermal, mechanical and water vapor retarding properties of castor bean mucilage film was investigated.

2- Materials and methods

2-1- Materials

Fresh barhang seeds were obtained from a local perfume shop in Urmia city. ZnO nanoparticles with an average particle size of 30 nm were obtained from Iranian Pishgaman Nanomaterials Company (Mashhad). Cellulose nanofibers obtained from the wood of coniferous trees with an average diameter of 35 nm, an average length of 5 micrometers and a purity of 99% were purchased from Nanovinopolymer Company (Sari). Ethanol was purchased from Kimia Alkal Company (Zanjan). All chemicals, including potassium sulfate, calcium sulfate, and other laboratory-grade salts, were obtained from the representative of Sigma (Germany).

2-2- Extraction of the mucilage of barberry seed

The method of Niknam et al [8] was used to extract mucilage. Barhang seeds were carefully cleaned and immersed in distilled water at a ratio of 1:15. Then, ultrasonic treatment with 400 W power for 30 minutes at 35 °C was performed on the dispersion of seeds by an ultrasonic bath (OPTIMA, XL100K, Germany). Then the mixture was kept at 80°C for 1 hour under gentle stirring to absorb water and produce mucilage. The produced mucilage was separated from the seeds using a linen cloth and mixed with 96% ethanol at a ratio of 1:3. BSM was precipitated by the addition of ethanol, and after separation, it was dried at 50 °C for 18 h to be used for film production. The percentage of mucilage extraction was equal to 2.7% of dry seed weight.

2-3- Preparation of nanocomposite films of mucilage of barberry seeds

The method of Niknam et al. [8] with some modifications was also used to produce the films. The amount of 1.2 grams of extracted mucilage was gradually added to 80 ml of distilled water and stirred for 2 hours at 80°C to complete the dissolution of mucilage. To produce nanocomposite films, ZnO and CNF were added to 20 ml of distilled water in amounts of 4 and 8% of mucilage weight, and were subjected to ultrasonic treatment for 20 minutes. Next, the dispersion of nanoparticles was added to the mucilage solution of borage seed and stirring was continued for another hour. Then, the temperature of the solutions was reduced to 30°C and glycerol as a softener was added to the film mixture at the rate of 60% of the biopolymer weight and stirred. 50 ml of the film-forming solutions were poured on polystyrene plates and the plates were dried at 60°C for 18 hours to obtain the final films. A sample of BSM film without nanoparticles was also prepared as a control film. Nanocomposite films containing 4% and 8% ZnO were shown with codes BSM-ZnO4 and BSM-ZnO8, respectively, and nanocomposite films containing 4% and 8% CNF were shown with codes BSM-CNF4 and BSM-CNF8, respectively. Including the control film sample, a total of 5 film samples were prepared.

2-4- Characterization of nanocomposite films

2-4-1- FT-IR spectroscopy test²

FT-IR analysis was used to investigate the structure and interactions between barberry seed mucilage and nanoparticles. For this work, FT-IR spectrometer (SHIMADZU IRPrestige/FTIR-8000) was used. About 2 mg of the film was manually ground and mixed with KBr at a ratio of 1:100 and pressed into a tablet with a thickness of about 1 mm. In the continuation of the FT-IR spectroscopy test of the samples in the range of cm wave number⁻¹ 400-4000 and with a resolution of cm⁻¹ 4 done.

2-4-2- X-ray diffraction test (XRD)³ X

In order to study the microstructure of the produced films and to determine the dispersion of nanoparticles in the film matrix, X-ray diffraction was used. Bruker D8 Advance X-ray

¹, Barhang seed mucilage

². Fourier Transform Infrared Spectroscopy

³. X-ray diffraction

diffractometer (Karlsruhe, Germany) was used to perform X-ray diffraction test. To perform the test, the X-ray generator was set at 40 kV and 40 mA, and the samples were exposed to X-rays with a wavelength of 0.154 nm. Reflected radiation from the sample was collected at ambient temperature and in the range of angle $\theta_2 = 2-80^\circ$ and the graph related to their reflection intensity was drawn. The speed of the analysis was 1/min and the size of the steps was 0.02° .

2-4-3-scanning electron microscope (SEM)⁴

The surface morphology and fracture surface of nanocomposite films were investigated by scanning electron microscope (SEM) (Hitachi 4300S, Japan) at room temperature. The accelerator voltage used was 5 kV. The broken surfaces were covered by a thin layer of gold with a thickness of about a few nanometers. In the cross-sectional study, the samples were observed perpendicular to the broken surfaces.

2-4-4- thermal gravimetric analysis⁵(TGA)

TGA analysis on nanocomposite film samples was performed by PerkinElmer TGA-7 device (PerkinElmer Cetus Instruments, Norwalk, CT, US) in the temperature range of 25-600°C. Heating rate equal to $C.min^{-1}$ It was 10° and nitrogen gas atmosphere was used. DTG curves were also drawn and the maximum thermal degradation temperature (T_{dmax}) and also the amount of remaining weight was calculated from the graphs.

2-4-5- Determining the thickness of films

The thickness of the films was measured using a digital micrometer (Fowler, USA) with an accuracy of 0.01 mm. Measurements were made at five different points on the film surface and averaged.

2-4-6- Measurement of moisture content

The method of determining the difference in weight before and after baking was used to determine the moisture content of the films. Pieces of the film were cut with dimensions of 2 x 2 cm and after weighing, they were placed inside the oven at a temperature of $105^\circ C$ and dried until a constant weight was reached. Then the final weight of the films was also determined and the moisture content of the film

was calculated based on the difference between the two.

2-4-7- Determining the amount of moisture absorption

Ghadiri Alamdari et al.'s method [16] was used to measure the moisture absorption of films. Samples of films with dimensions of 2 x 2 cm were prepared and placed in a desiccator containing calcium sulfate (RH=0%) for 24 hours. After the initial weighing, the samples were transferred to a desiccator containing a saturated solution of calcium nitrate at (RH=47%) and placed at a temperature of 20-25 degrees Celsius. Then the weight of the samples was measured after 72 hours and the amount of moisture absorption was calculated from the following relationship:

$$\frac{W_t - W_o}{W_o} \times 100$$

(Contact 1) $\frac{W_t - W_o}{W_o} =$ moisture absorption (%)

IN_t : sample weight after 72 hours at RH=55%

IN_o : initial weight of the sample

2-4-8- Determination of permeability to water vapor⁶ (WFP)

The method of ArdebilchiMarand et al [17] was used to measure water vapor transfer. For this purpose, special vials with a diameter of 2 cm and a height of 4.5 cm were used. The cap of these vials had a hole with a diameter of 8 mm, where a piece of the test film was cut and placed in this part. 3 grams of calcium sulfate were placed inside the vials. The vials with all their contents were weighed and placed in a desiccator containing potassium sulfate saturated solution (RH=97%). Then, the weight of the vials was measured every few hours for four days. The amount of water vapor transferred from the films was determined by increasing the weight of the vials. The weight increase curve of the vials was drawn with the passage of time and after calculating the linear regression, the slope of the resulting line was calculated. From dividing the slope of the line corresponding to each vial by the total film surface exposed to water vapor transfer, the water vapor transfer rate (WVTR)⁷ Obtained. From dividing WVTR by the vapor pressure difference on both sides of the film, the

⁴. Scanning electron microscopy

⁵. Thermogravimetric analysis

⁶. Water vapor permeability

⁷. Water vapor transmittance rate

penetration flux of water vapor (⁸WVPN) was obtained. Due to the presence of calcium sulfate inside the vial, the vapor pressure inside the vial is considered zero. The vapor pressure outside the film was obtained from the product of the relative humidity (RH) inside the desiccator (97%) and the vapor pressure of pure water at a temperature of 25 degrees. Water vapor permeability (WVP) was obtained from the product of WVTR and film thickness.

$$\text{(link 2)} \quad J = WVTR = \frac{\Delta w}{tA}$$

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In this regard, J is the water vapor flux across the film and is also called water vapor transmission rate (WVTR). Δw is the amount of water vapor passing through the film, t is the time of water vapor transfer and A is the surface area of the film.

$$\text{(link 3)} \quad WVP = \frac{WVTR}{P(R_1 - R_2)} \cdot X$$

In relation 3, X is the thickness of the film (m), P is the vapor pressure of pure water at 25 °C (Pa3169), R_1 Relative humidity in the desiccator (97%) and R_2 The relative humidity inside the vial is (0%).

2-4-9- Measuring the contact angle with water

The contact angle test is used to determine the level of hydrophilicity of the film surface. To determine the contact angle with water, a 5 microliter drop of distilled water was placed on the surface of the films and the shape of the drop was photographed using a digital camera (Microsoft, 185 LifeCam, zoom 24x). The contact angle of the droplet with the film surface was calculated using Image.J1.48 software [18].

2-4-10- Measurement of mechanical properties

Tensile strength, elastic modulus and elongation to breaking point of the films were measured using a Zwick/Roell FR010 (Germany) mechanical testing machine and according to the method of de Paiva et al. [18].

First, the samples were qualified for 24 hours in 55% relative humidity (calcium nitrate) and then three samples from each of the films were cut into dumbbells with dimensions of 0.5 x 8 cm and placed between the two jaws of the machine. The initial distance between the two jaws and the upper jaw movement speed were determined as 50 mm and 5 mm/min, respectively, and the data were recorded by a computer.

5-2- Statistical analysis

Except advanced device tests, all tests were performed in three replications in a completely randomized design. Analysis and evaluation (ANOVA) was performed using the linear model (G.L.M) of SPSS 11.5 statistical software at the probability level of 5% ($p < 0.05$) and Duncan's multi-range test to confirm the existence of differences between the means.

3. Results and Discussion

3-1- FT-IR test

In order to investigate the chemical changes in the BSM film structure before and after the addition of nanoparticles, FT-IR test was used. Figure 1 shows the FT-IR spectra of pure and nanocomposite BSM films. According to the report of Niknam et al. [8], hemicellulosic polysaccharides, especially arabinoxylan, is the main component of BSM, and arabinose and xylose are its main monosaccharides. As shown in Figure 1 in the case of pure BSM, a broad peak around $\text{cm}^{-1}3400$ is observed, which is related to the -OH groups of sugars in the polysaccharide structure [8]. Absorption band in $\text{cm}^{-1}2139$ is related to the stretching forces of C-H group of polysaccharide. Peaks appeared at 1870 and $\text{cm}^{-1}1661$ respectively related to the bending forces of C-H and CH groups₂ are [20]. Peak appeared in $\text{cm}^{-1}1462$ is probably related to glycerol [14]. Two other peaks in 1336 and $\text{cm}^{-1}1245$ have appeared, which can be attributed to C-O bonds of saccharide units or phenolic compounds present in BSM mucilage [15]. Small peaks appeared around $\text{cm}^{-1}900-1000$ are related to the glycosidic connections

⁸. Water vapor permeant

of hemicellulose polysaccharides. Also BSM film a sharp peak in $\text{cm}^{-1}708$ showed that it is related to the vibrational forces of the C-O-C bond in the polysaccharide structure. Peak appeared in $\text{cm}^{-1}475$ is also related to the vibrational forces of the C=O bond in the phenolic compounds present in the mucilage [22].

As seen in Figure 1, significant structural changes occurred in the BSM film after the addition of ZnO nanoparticles and CNF. One of the changes is the decrease in intensity or displacement of the peaks in the range of 1462 to cm^{-1} It was 1245. These peaks, which were related to glycerol groups, phenolic compounds

and C-O groups in polysaccharide, changed in intensity and shifted in CNF films. The third group of changes, in peaks between 700 and cm^{-1} It was 950. Especially in the films containing 4% ZnO and 8% CNF, the change in the appearance of the spectrum of this region was greater. In general, the results of FT-IR test showed that both nanoparticles are able to enter into chemical connections with BSM polysaccharide and establish new bonds by making fundamental changes in the spectrum of nanocomposite films. Regarding the addition of CNF to the film of chia seed mucilage [23] and flax seed gum [24], similar results have been reported in the present study.

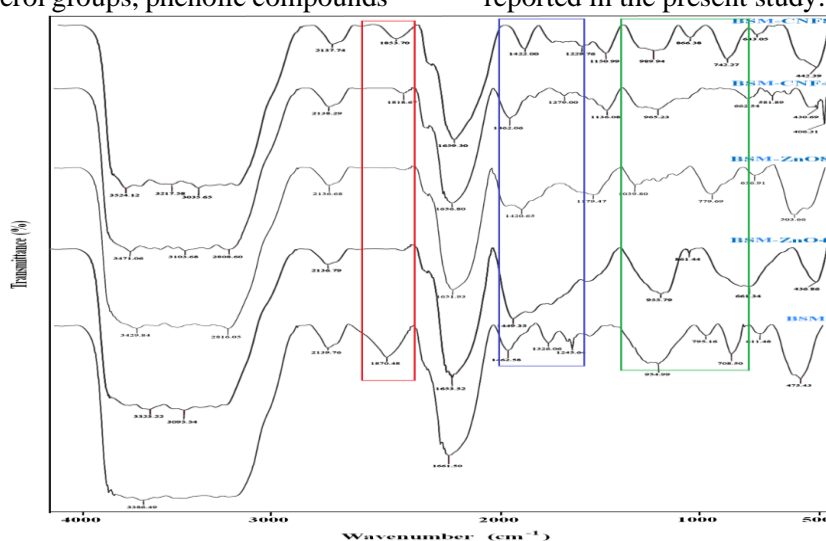


Fig 1 FT-IR spectra of neat BSM film and nanocomposite films containing ZnO and CNF.

2-3. XRD test

In order to study the crystalline nature of BSM film and the possible effect of nanoparticles on its structure, XRD test was used. Figure 2 shows the diffraction patterns of pure BSM film and nanocomposite films. Pure BSM film showed a small peak at $\theta=17.8^\circ$, a sharp peak at $\theta=22.4^\circ$ and another peak at $\theta=30.1^\circ$. This XRD spectrum shows the semi-crystalline nature of BSM film. The polysaccharide structure of this gum is able to form a dense and crystalline network, but the presence of glycerol, phenolic compounds, and impurities prevent a significant increase in crystallinity in this film. The results of Niknam et al. [8] about the XRD spectrum of BSM film were also similar to our research. By adding ZnO, the intensity of crystalline peaks decreased significantly. The peak in the 17.8° area disappeared and the intensity of the other two peaks also decreased. But in the case of CNF,

this effect was less and the intensity of the peaks did not decrease. The different effect of nanoparticles on the crystallinity of the BSM film is related to the difference in their chemical nature and their compatibility with the film matrix. CNF is a polysaccharide compound with good solubility in water and due to its structural similarity, it is able to make more connections with BSM strands and thus helps to maintain its crystalline nature. But ZnO has low solubility and miscibility, and its unfavorable distribution in the film substrate causes the destruction of the crystal structure and the reduction of the intensity of the peaks. The distribution of nanoparticles in the BSM matrix will be discussed in the SEM microscopy results. Azizi et al [25] obtained similar results regarding the effect of ZnO nanoparticles and microcrystalline cellulose on the crystallinity properties of chitosan/polyvinyl alcohol composite film.

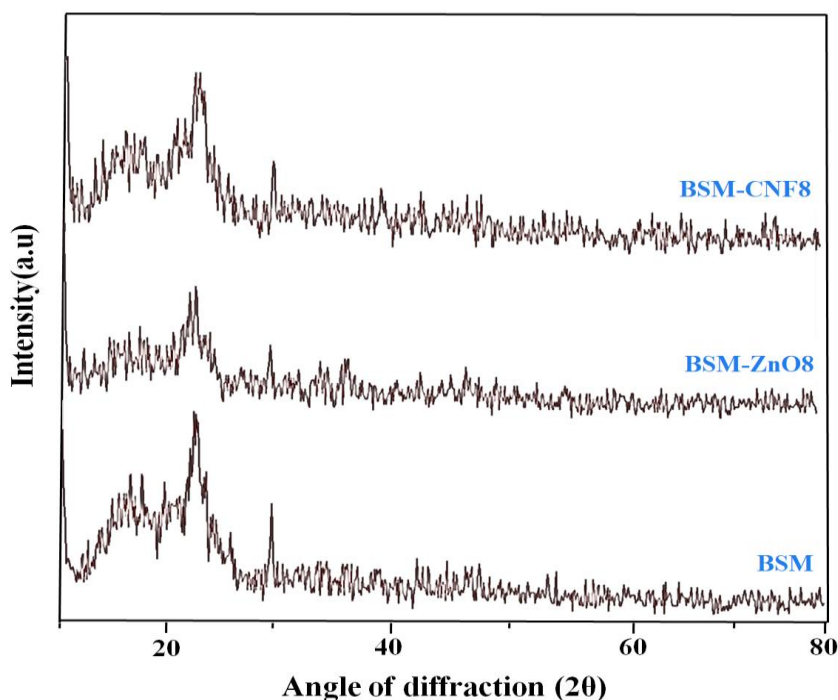


Fig 2 XRD diffractograms of neat BSM film and nanocomposite films containing 8% ZnO and CNF.

3-3- Examining morphology with SEM microscope

Microscopic structure and morphology of BSM films were investigated by SEM microscope. Figure 3 shows the microscopic images of the surface and cross section of the films. As can be seen, the pure BSM film has a smooth surface with few cracks and holes. Also, the cross-section of this film had a high structural density and dense texture. This characteristic shows the good ability of the extracted mucilage to produce a film with desirable characteristics and suitable morphology. After adding nanoparticles, the morphology of BSM film also changed. In the film containing 8% ZnO, the roughness of the film surface increased and ZnO nanoparticle masses were evident as ridges on the film surface. Also, the number and depth of cracks increased in this film. In the cross-section of this film, the masses of ZnO nanoparticles that were spread in the surface and deep layers of this film can be recognized. But when 8% of CNF was used in the composition of the film, the level of heterogeneity and surface roughness was lower than the film containing ZnO. At the same concentration, CNF had a relatively favorable dispersion and the mass of this nanoparticle was not observed on the surface of the film. Also, the amount of cracks and holes in this film was less. In the cross-sectional images, it was also

observed that the BSM-CNF8 sample had a cross-section almost similar to the pure BSM film, and no cracks or nanoparticle masses were observed in the cross-sectional area of the film. The texture density in the cross-section of the film containing CNF was much higher than that of the film containing ZnO. As mentioned in the XRD test results, the compatibility of organic nanoparticles such as CNF with polysaccharide film matrix is more than that of inorganic nanoparticles such as ZnO. The presence of functional groups similar to the film-forming polysaccharide as well as better dispersibility in the film-forming solution and in the aqueous medium is the reason for creating a uniform microstructure in the film containing CNF. Similar results have been reported for the agglomeration of ZnO at high concentrations in the matrix of films such as bacterial cellulose [10], chitosan [26] and starch/kefirin [27]. Also, there are similar findings in the field of favorable distribution of CNF in the substrate of other polysaccharide films such as chia seed mucilage [23] and starch and chitosan [28].

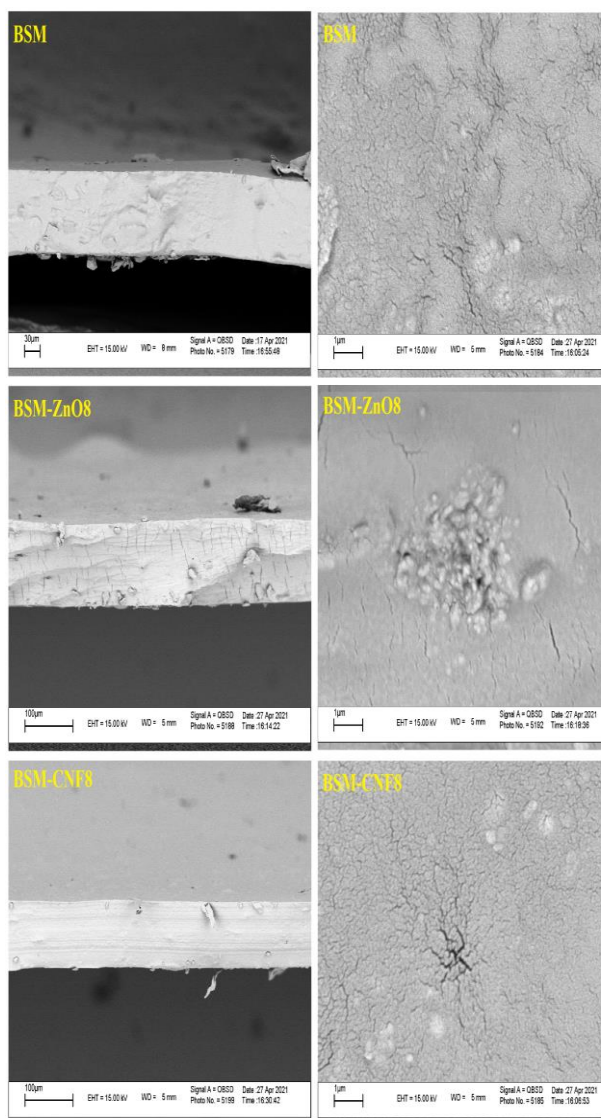


Fig 3 SEM images of surface (right) and cross-section (left) of neat BSM film and nanocomposite films containing 8% ZnO and CNF.

3-4- Determination of thermal properties by TGA test

Thermal properties of biodegradable films are among the important features that determine their usability in various applications. The thermal properties of BSM nanocomposite films were investigated by thermal gravimetric test. Figure 4 shows the GTA and DTG curves of pure BSM film samples and films containing 8% ZnO and CNF. The films showed three distinct degradation zones. The first stage was weight loss around 90 to 100 degrees Celsius, which is related to the release of volatile phenolic compounds in the mucilage composition as well as the evaporation of water in the film. The pure BSM film showed two more stages of thermal degradation, the largest decrease was at 221 °C and the second peak was

observed at 287 °C. As mentioned in the XRD test results, the BSM film had a semi-crystalline nature and crystalline areas were also observed in its structure. Therefore, this two-stage thermal degradation can be attributed to the degradation of amorphous and crystalline regions. Decomposition of amorphous regions, which are less thermally stable, occurs at 221°C. But since the order between the polysaccharide chains and the structural density is greater in the crystalline regions, the thermal degradation of these regions occurs at higher temperatures. Such three-stage thermal degradation has also been observed in the case of films obtained from chia seed gum [4] and sage seed gum [29]. By adding 8% ZnO, the temperature of maximum thermal degradation (T_{dmax}) decreased by 6 degrees and reached 215 degrees Celsius. But the temperature of the destruction of the crystalline region increased by 8 degrees and reached 295 degrees Celsius. This result shows that ZnO is able to increase the density of crystalline regions (and not the amount of crystalline regions), but in amorphous regions, it lowers the thermal resistance of these regions, probably due to agglomeration and reduction of film uniformity. The film containing 8% CNF showed the highest thermal resistance. The amount of T_{dmax} in the BSM-CNF8 film, it reached 229 °C and also the temperature of the degradation of the crystalline regions increased to 295 °C. These changes indicate the good thermal properties of the film due to the addition of CNF. Compatibility and creation of many inter-strand connections and helping to increase texture density, which was also evident in XRD and SEM results, is the reason for strengthening thermal properties due to the addition of this nanoparticle. The effect of adding CNF on the thermal properties of chia seed muslage film [23], chitosan film [30] and acacia gum film (*Acacia nilotica*) [31] was similar to the results of the present study. Another noteworthy point in Figure 4 was the change in the residual weight of the films after thermal degradation. The ash content of pure BSM film was equal to 4%. This residual weight is probably due to the presence of residual compounds and impurities from the extraction stage. In the film containing CNF, this amount increased to 5%, but when ZnO was used in the composition of the BSM film, the amount of residual ash increased to 11.5%. The difference in the nature of the nanoparticles

is the reason for the difference in this final ash. CNF is also degraded like the polysaccharide strands of the film matrix, but ZnO, as a mineral, enters the ash after thermal degradation and increases the residual weight.

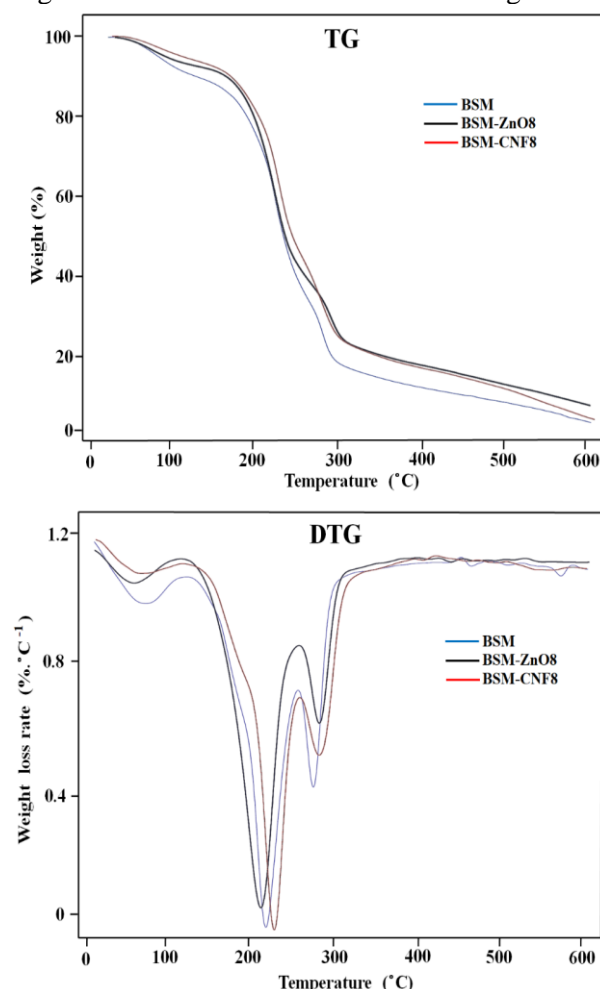


Fig 4 TGA and DTG curves of neat BSM film and nanocomposite films containing 8% ZnO and CNF.

3-5- Thickness and moisture content

Figure 5 shows the effect of nanoreinforcers on thickness (A) and moisture content (B) of BSM film. The thickness of the reference film was 0.13 mm. The addition of CNF and ZnO at a concentration of 4% had no effect on the film thickness, but when the amount of nanoparticles increased to 8%, the film thickness increased significantly ($p < 0.05$). These results show that at a concentration of 4%, each Two nanofillers are able to be placed in the empty spaces between the resin strands and cause the film network to become denser, and therefore their effect on the thickness will

be insignificant. But when the amount of nanoparticles increases to 8%, its excess amount after filling the holes of the film increases the thickness and leads to the production of a thicker film. Memiş et al. [22] obtained similar results regarding the effect of nanoreinforcers on the thickness of films based on plant resins.

The moisture content of biodegradable films is an important parameter that affects other functional properties of the film for food packaging. Figure B5 shows the moisture content of the nanocomposite films. The moisture content of the control film was 39.28%. This amount is high moisture content for BSM film compared to other biopolymer films such as starch and protein films. The addition of nanoparticles significantly reduced the moisture content of the films ($p < 0.05$). The lowest moisture content was observed in the film containing 4% ZnO (21%). By adding the amount of ZnO to 8%, the moisture content of the film increased, which is probably due to the hygroscopic nature of ZnO nanoparticles and the ability to retain moisture by nanoparticles that are not bound to gum. The addition of CNF also decreased the moisture content of the BSM film. There was no significant difference between the moisture content of the film containing 4 and 8% CNF ($p < 0.05$). But the moisture content of these films was higher than the film containing ZnO. The reason for this could be the more hydrophilic nature of CNF compared to ZnO due to the number of hydroxyl groups in the structure of this nanoparticle. Ranjbaryan et al [32] obtained similar results regarding the effect of CNF on the moisture content of casein film.

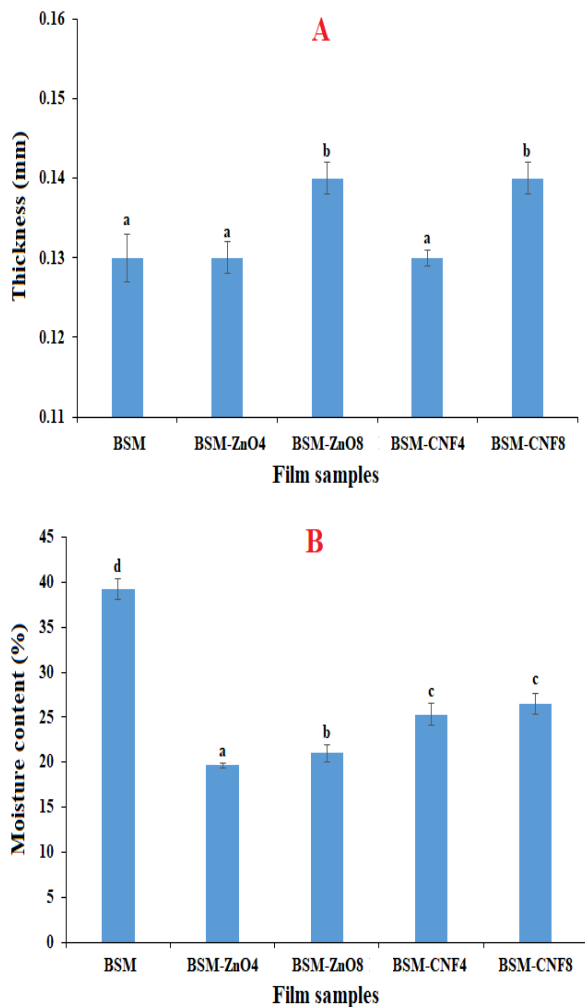


Fig 5 Effect of ZnO and CNF on the thickness (A) and moisture content (B) of nanocomposite films based of Barhang seed mucilage. Different letters show the significant difference in the level of 5%.

6-3-Moisture absorption and permeability to water vapor

The resistance of biodegradable films to moisture absorption affects their usability for different food applications and in different weather conditions. Figure A6 shows the amount of moisture absorption of BSM films after 24 hours exposure to 55% relative humidity. The BSM control film absorbed 37.75% of its weight, which was the highest among all samples. By adding nanoparticles, the amount of moisture absorption decreased significantly ($p < 0.05$). The effect of nanoparticle concentration on moisture absorption content of films was different. ZnO at a concentration of 4% decreased moisture absorption. But when the amount of this nanoparticle increased to 8%, moisture absorption increased again. But the effect of CNF was completely decreasing, and as its

concentration increased, the amount of moisture absorption decreased. These results show that the compatibility and mixing ability of CNF with BSM film is more than that of ZnO nanoparticles. Because CNF in both concentrations was able to establish more connections with mucilage fibers and reduce its active groups that are able to absorb moisture. But in the case of ZnO, when the concentration increases, no noticeable change is observed in the increase in the number of bonds, and the additional nanoparticle not only does not help to improve the structure, but also increases the ability to absorb moisture by the film matrix by disrupting the network of the polysaccharide film. Shahmohammadi and Almasi [10] obtained similar results regarding the effect of ZnO on moisture absorption of bacterial cellulose film. On the other hand, Mujtaba et al. [23] also used CNF to strengthen the chia gum film and obtained similar results regarding its effect on the moisture absorption of the film.

The WVP index shows the moisture permeability from both sides of the packaging films. This parameter plays a significant role in determining the efficiency of the packaging film in preserving food in different humidity conditions. Figure B6 shows the WVP of BSM nanocomposite films. The amount of WVP of the control film is equal to $g/m.h.Pa^{-1}$. It was 3.38×10^{-1} , which is almost close to the values reported for gum films [3], horsetail gum [33], and sage gum [29], which shows the good ability of BSM compared to other film-forming gums. . By adding nanoreinforcers, the WVP of the film decreased significantly ($p < 0.05$). The effect of both additives on the WVP of the films was the same. At 4% concentration, a significant decrease in WVP was observed and there was no significant difference between BSM-ZnO4 and BSM-CNF4 samples. In the samples with 8% nanoparticles, the amount of WVP increased and again no difference was observed between the two nanoparticles. The reduction of WVP with the addition of nanoreinforcers is due to two reasons. First, the binding of nanoparticles to the free hydroxyl groups of the gum strands reduces the absorption and passage of water vapor molecules by the film matrix. Secondly, the presence of nanoparticles in the biopolymer substrate creates zigzag and meandering paths and slows down the movement of water molecules in the space between the biopolymer strands. This phenomenon has also been

reported about the effect of these two nanoparticles on the WVP of other biodegradable films [34,35]. However, at high concentrations of nanoparticles, their aggregation and disruption of the interstrand order in the film structure probably caused this to happen. This is more likely in the case of ZnO, which is less compatible with BSM and has a high probability of agglomeration. In the case of CNF, the hydrophilic nature and its high moisture absorption power at a concentration of 8% probably increase the amount of moisture provided to pass through the cross section of the film and thereby increase the WVP.

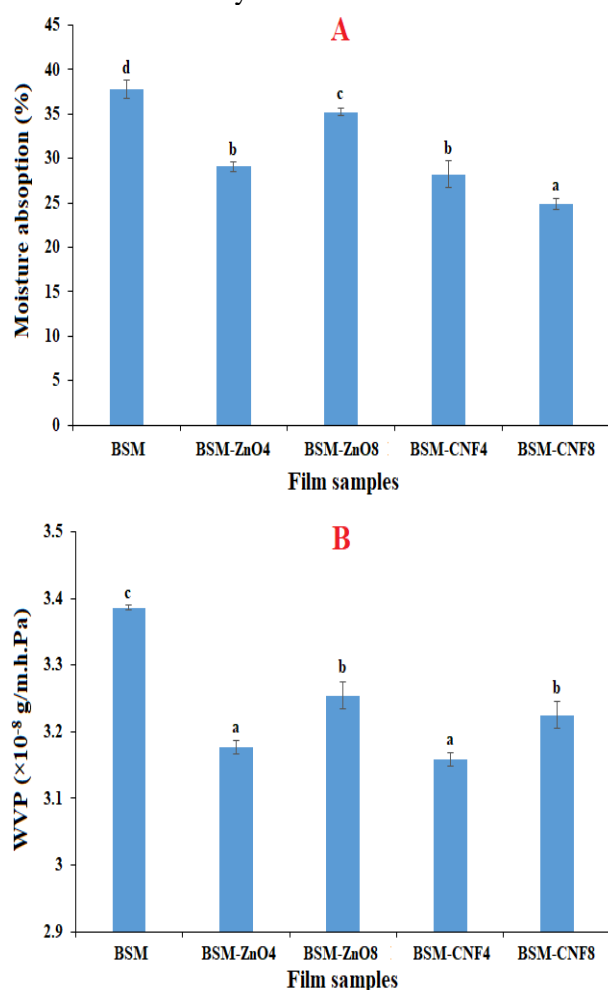


Fig 6 Effect of ZnO and CNF on the moisture absorption (A) and water vapor permeability (B) of nanocomposite films based of Barhang seed mucilage. Different letters show the significant difference in the level of 5%.

3-7-Water contact angle

The contact angle test shows the level of surface hydrophilicity and wettability of packaging films. Figure 7 shows the surface contact angle of BSM films with water. The contact angle of the control film was equal to

107.21 degrees, which indicates the relative hydrophobicity of the film surface based on Barhang gum. By adding 4% ZnO, surface hydrophobicity increased. But at the concentration of 8%, this parameter decreased again. The creation of interstrand connections in the film network at a concentration of 4% and the reduction of free hydroxyl groups is the reason for the increase in the contact angle, and the agglomeration of the nanoparticle at a higher concentration and its water absorption property again causes a decrease in the contact angle at a concentration of 8%. In the case of CNF, the contact angle decreased in both concentrations used. This indicates that the hydrophilic nature of the nanoparticles themselves is more effective on hydrophilicity than their ability to form bonds. Because CNF is able to make more connections, but its inherent hydrophilicity reduces the contact angle of the film surface. Rose Joseph et al [31] reported conflicting results about the effect of CNF on the hydrophilicity of the babul gum film surface. But Mujtaba et al. [36] produced chia mucilage film containing starch nanocrystals and observed that with increasing amount of nanoparticles, the contact angle decreases and the surface of the film becomes more hydrophilic. They also considered the inherent hydrophilicity of starch nanocrystals as the reason for this.

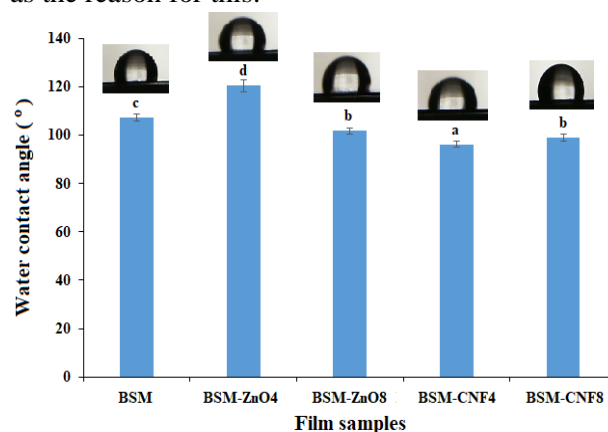


Fig 7 Effect of ZnO and CNF on water contact angle of nanocomposite films based of Barhang seed mucilage. Different letters show the significant difference in the level of 5%.

8-3- Mechanical properties

Mechanical characteristics are one of the most important quality characteristics of packaging materials, which show the film's resistance to force and physical damage to the packaged food. The higher the tensile strength of the packaging material, the greater its resistance to

punctures and tears. Table 1 shows the mechanical properties of BSM films including tensile strength, elastic modulus and elongation to break point. The pure BSM film was an elastic film with poor mechanical properties. So that it showed the lowest tensile strength and elastic modulus and the highest stretchability. The presence of phenolic compounds and other impurities that prevent the formation of strong and large chemical bonds is the reason for the weak mechanical properties of gum and mucilage-based films. By adding nanoparticles, the mechanical properties were significantly improved. ZnO at a concentration of 4% had a favorable effect and helped to increase mechanical strength and reduce elongation. But when the amount of ZnO increased to 8%, the mechanical properties were weakened again. This is probably caused by the accumulation of nanoparticles at a higher concentration. Due to the accumulation of nanoparticles, not only the strengthening effect on the properties of the film is not observed, but also the mechanical

properties are weakened due to the prevention of the formation of a strong interconnected network. This is while CNF had a strengthening effect on mechanical properties in both concentrations used. At the concentration of 8% CNF, not only the tensile strength and elastic modulus, but also the elongation to break point increased. This is the best effect an additive can have on the mechanical properties of the film. The strengthening of mechanical properties due to the addition of CNF is related to the high compatibility of this nanoparticle with the BSM film matrix and the ability to establish high inter-strand connections. Similar results have been reported regarding the effect of CNF on the mechanical properties of the film of various gums and mucilages such as chia seed mucilage [23] and acacia tree gum [31] and even other polysaccharide films such as conjugated mannans [37] and starch [38]. There are similar findings about ZnO agglomeration at high concentrations in the matrix of kefir film [39] and gelatin [34].

Table 1 Effect of ZnO and CNF on the mechanical properties of Barhang seed mucilage based nanocomposite films.

Film sample	Tensile strength (MPa)	Elastic modulus (MPa)	Elongation to break (%)
BSM	3.77±0.56 ^a	13.68±1.26 ^a	102.58±1.11 ^{It is}
BSM-ZnO4	7.23±1.11 ^b	18.28±2.32 ^b	39.58±1.00 ^a
BSM-ZnO8	4.40±0.98 ^a	10.50±1.91 ^a	67.71±0.98 ^d
BSM-CNF4	13.07±1.03 ^c	29.34±0.89 ^c	44.56±1.12 ^b
BSM-CNF8	19.67±1.26 ^d	32.30±1.45 ^d	60.91±0.92 ^c

Different letters in each column show the significant difference at level of 5%.

4 - Conclusion

In this research, a nanocomposite film based on castor bean gum was successfully produced, and zinc oxide nanoparticles and cellulose nanofibers were used to enhance the properties of the film. FTIR test showed that both nanoparticles are able to establish new bonds with polysaccharides of barhang mucilage. The results of XRD test showed the different effect of nanoparticles on the crystallinity of the mucilage film. By adding ZnO, the intensity of crystalline peaks decreased significantly. But in the case of CNF, this effect was less and the intensity of the peaks did not decrease. The SEM test confirmed the smooth surface of the control film, but with the addition of nanoparticles, the roughness increased. The effect of CNF in increasing the film roughness was lower than that of ZnO. Based on the TGA test results, the addition of nanoparticles was also effective on thermal resistance, and the

film containing 8% CNF showed the highest thermal degradation temperature. Examining the inhibitory properties and mechanical properties showed that the addition of both nanofillers can improve the properties of the film. The type of effect of the compounds depended on their compatibility with the film matrix. ZnO had a favorable effect at a low concentration, but as the concentration increased, its effect on the properties of the film decreased due to the accumulation of nanoparticles. CNF was able to improve the mechanical properties better, but due to its hydrophilic nature, it increased the contact angle of the film with water. This research showed that it is possible to produce a film with good mechanical properties and inhibition from castor bean gum, and by choosing the right type of nanoreinforcer, the best film production conditions can be achieved. The use of produced films for packaging real food

products can show their efficiency in food preservation even more.

5- Resources

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مطالعه تأثیر نانوذرات اکسید روی و نانوالیاف سلولز بر روی خصوصیات مورفولوژیکی، ساختاری، حرارتی، مکانیکی و بازدارندگی فیلم نانوکامپوزیت بر پایه موسیلاژ دانه بارهنگ (*Plantago major L.*)

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چکیده

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هدف این پژوهش، تولید فیلم نانوکامپوزیت بر پایه موسیلاژ دانه بارهنگ بود. از نانوذرات اکسید روی (ZnO) و نانوالیاف سلولز (CNF) هرکدام در دو غلظت ۴ و ۸ درصد وزنی، به منظور تقویت خصوصیات مورفولوژیکی و ساختاری، حرارتی، بازدارندگی به بخار آب و خواص مکانیکی فیلمها استفاده شد. براساس نتایج آزمون طیف سنجی مادون قرمز تبدیل فوریه (FTIR)، برقراری پیوندهای شیمیایی جدید بین نانوذرات و پلی ساکارید موسیلاژ تأیید شد. آزمون پراش پرتو ایکس (XRD) نشان داد که ZnO بیشتر از CNF ساختار نیمه بلورین فیلم موسیلاژ بارهنگ را تحت تأثیر قرار می‌دهد. بررسی مورفولوژی فیلمها با آزمون میکروسکوپ الکترونی روبشی (SEM) سطح صاف فیلم شاهد را نشان داد اما با افزودن نانوذرات زبری و ناهمگونی سطحی بیشتر شد. آزمون وزن‌سنجی حرارتی (TGA) نیز تقویت مقاومت حرارتی در اثر افزودن نانوذرات را اثبات نمود اما تأثیر CNF در تقویت خصوصیات حرارتی بیشتر از ZnO بود. افزودن نانوذرات در غلظت ۴ درصد تأثیری بر روی ضخامت فیلمها نداشت اما با افزایش غلظت، ضخامت بیشتر شد. محتوای رطوبت و میزان جذب رطوبت فیلمها با افزودن نانوتقویت کننده‌ها به طور معنی‌داری کاهش یافت. نفوذپذیری به بخار آب فیلم موسیلاژ دانه بارهنگ به غلظت نانومواد وابسته بود و در غلظت ۴ درصد کاهش معنی‌دار داشت اما در غلظت ۸ درصد به دلیل توده شدن و ماهیت آبدوست نانومواد مجدداً نفوذپذیری بیشتر شد. زاویه تماس سطح فیلمها با آب در اثر افزودن ZnO بیشتر شد اما CNF باعث کاهش این زاویه شد. CNF در مقایسه با ZnO عملکرد بهتری در بهبود خصوصیات مکانیکی داشت و بیشترین تأثیر را در افزایش استحکام کششی، مدول الاستیک و درصد ازدیاد طول نشان داد. در مجموع تأثیر CNF در بهبود خصوصیات بازدارندگی و مکانیکی به دلیل سازگاری بیشتر با موسیلاژ بارهنگ، بیشتر از تأثیر ZnO بود. بطور کلی نتایج این پژوهش نشان داد که فیلم نانوکامپوزیت موسیلاژ دانه بارهنگ حاوی نانوتقویت کننده‌های آلی و معدنیاز خواص فیزیکوشیمیایی مطلوبی برخوردار بوده و می‌تواند به عنوان یک گزینه مناسب برای بسته‌بندی مواد غذایی معرفی شود.

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