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# Investigating the Functional, Mechanical and Structural Characteristics of Soy Protein Isolate Biodegradable Film Containing Nanoclay (Montmorillonite) and *Salvia officinalis* Essential oil

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**ABSTRACT**

In this research, production films based on soy protein were produced with two variables of nanoclay at three levels (0, 0.5 and 1%) and Salvia officinalis Essential oil at three different levels (0, 250 and 500 ppm) and the effect of this Two variables were investigated on the functional, mechanical and structural characteristics of the produced biodegradable films. The results showed that by adding different percentages of nanoclay on the soy protein film substrate, the moisture content, solubility, permeability to water vapor and transparency of the samples decreased significantly compared to the control sample. This decrease in indicators was more evident with increasing concentration of Salvia officinalis Essential oil. Among the production samples, the film containing 1% nanoclay and 500 ppm of Salvia officinalis Essential oil had a water vapor permeability of $32.02\times10^{-11}$ g/m.s.pa. Also, the addition of nanoclay and Salvia officinalis essential oil caused a significant increase in the antioxidant content of the samples, and the sample containing 1% nanoclay and 500 ppm of Salvia officinalis essential oil had the highest antioxidant content with 32.88%. Examining the results of spectroscopy and microstructure of the obtained films also shows the proper interaction between nanoparticle and essential oil with soy protein substrate and creating strong and new bonds. The obtained results showed that the addition of nanoclay and Salvia officinalis essential oil can have positive effects on the physical and structural properties of soy protein isolate film.

## 1. Introduction

Packaging is one of the most important methods to maintain the quality of food products for the purpose of preservation, storage, transportation and final use, and in addition, it prevents the loss of food quality and the process of distribution and Marketing makes it easy. In addition to the main function of packaging, which is to preserve food, a good packaging can not only maintain the quality of food, but also has a significant direct relationship with commercial profitability [1]. Proteins and polysaccharides are the main biopolymers used in making edible films, among which, protein films and coatings are more interesting due to their functional and nutritional characteristics. 2]. Soybean protein isolate is an edible product that has the property of replacing animal protein and is used in various biscuit, confectionery and meat industries. Soy isolate is the rest of soybean flakes without fat and is the only soybean product that is commercially produced with the highest protein percentage. Soy protein isolate in dry form contains about 90% protein and actually has the highest amount of protein in all soy products. This substance is the highest food source for providing amino acids. Protein from defatted soybean meal, including soybean isolate and soybean concentrate, can be used for coating films. This protein creates a uniform, transparent and flexible texture that is very resistant to the penetration of oxygen and fat. But due to the hydrophilic properties of soy protein, the film made from it has little resistance to moisture. Soy protein coating in fried products reduces oil absorption and prevents moisture exchange. Various attempts have been made to improve the water resistance of soy protein isolate film by physical and chemical improvers, including improvement by alkalization, alkylation with sodium alginate or propylene glycol, cross-linking using aldehyde or other methods. Enzyme, heat and combination with some hydrophobic additives and polymers have been done [3]. The use of biopolymer films is associated with limitations due to problems related to their performance, inherent sensitivity to water and low resistance, especially in humid environments. In general, due to the weak mechanical and barrier properties of biopolymer films, their use as packaging has limitations. Adding reinforcing compounds and forming biopolymer composites improve their performance. Nano technology in these polymers can provide a new method not only to improve their properties, but also reduce their costs and improve food packaging and improve its capabilities. Nanoclays are the most important and widely used materials used in the production of biopolymer nanocomposites. One of the main advantages of nanoclays, which play an important role in the production of nanocomposites, is their ability to spread in the form of separate layers and the ability to change the surface properties of these materials and create compatibility. with all kinds of polymers and biopolymers [4]. Other reasons that have increased attention to the use of nanoclay in the production of biopolymer nanocomposites are simpler production, as a result of lower prices, easier access and greater compatibility with biopolymers [5]. One of the most important advantages of edible films and coatings compared to synthetic polymers is that these types of coatings can be used as a carrier for various additives and compounds such as antimicrobial substances, antioxidants, etc. act In this case, they are called active packaging. This type of packaging, in addition to having the inhibitory properties of usual packaging, such as preventing gases and water vapor and mechanical stresses, by changing the packaging conditions, safety, shelf life or the sensory characteristics of the food improves and at the same time the quality of food is maintained [6]. The inclusion of essential oils in the formulation of films aims to improve their antimicrobial and antioxidant properties. Due to the health concerns of people, essential oils extracted from plants have been used as a source of polyphenolic compounds instead of synthetic antimicrobial compounds [7]. Sage belongs to the *Lamiaceace* family and the leaves of this plant contain essential oil and tannin. Its essential oil, which is usually prepared from the wild type of the plant, is a yellow or greenish-yellow liquid with a special smell. In addition, this essential oil contains pinene, cineol and borneol right and left [8]. Considering the importance of the health and safety of packaged foods and replacing synthetic polymers with biopolymers in the manufacturing composition of packaging and improving the packaging characteristics, the aim of this research is to produce a biodegradable film based on isolated protein. Soybean and investigating the effect of adding nanoclay and essential oil of *Salvia officinalis* plant as a strengthening agent and improving its physical, mechanical and microstructural characteristics.

## 2- materials and methods

In this research, soy protein isolate (Milad Khorasan milk powder company), montmorillonite, 2 and 2-diphenyl-1-picrylhydrazyl powder to determine the antioxidant activity of the samples from Sigma-America, glycerol, sodium hydroxide and calcium chloride (Merck, Germany) and *Salvia officinalis* essential oil (Zardband Yasouj) were used.

### 2-1- film production

A solution of 50 grams/liter of soy protein isolate was obtained by dissolving 5 grams of soy protein isolate powder in 100 ml of distilled water. Then glycerol softener was added to the prepared film in a ratio of 1:2 (soy protein isolate: glycerol) and it was thoroughly mixed. For better dissolution, homogenization of the solution was done using a high-speed mixer (4000 rpm) for 3 minutes. The pH of the solution was adjusted to 8 using 2 normal soda and the solution was heated at 70°C for 15 minutes and then cooled to room temperature. Nanoparticles of montmorillonite (nanoclay) were also added to the samples in concentrations of zero, 0.5 and 1%. After adding different concentrations of essential oil (0, 250 and 500 ppm), finally 20-25 grams of this solution was poured into 10 cm diameter plates by casting method and left for 24 hours in It was dried at room temperature [9].

### 2-2- Experiments

#### 2-2-1- Determination of film thickness

A micrometer with an accuracy of 0.01 mm was used to determine the thickness of the films. Measurements were made at 5 different points of the film and then their average was calculated. The calculated average thickness was used to determine tensile strength and permeability to water vapor [10].

#### 2-2-2- Moisture content of films

In order to measure the moisture content, first the film samples were conditioned in a desiccator containing magnesium nitrate for 48 hours. Moisture determination was done in three replicates. The amount of moisture was calculated according to the following equation [11].

Equation 1-2

$$MC_{wb} = \left(\frac{m_1 - m_2}{m_1}\right) \times 100$$

$MC_{wb}$: moisture percentage based on relative weight

$m_1$: sample weight before drying in grams

$m_2$: sample weight after drying in grams

#### 2-2-3- Solubility in water

To measure the solubility, after weighing, pieces of the film were dried at $105^{oC}$ for 6 hours and then weighed ($M_1$). In the next step, the film was immersed in 50 ml of distilled water and for It was stirred for 6 hours at $25^{oC}$ with 250 rpm. Then the solution was passed through Whatman No. 4 filter paper and finally its weight ($M_2$) was obtained after drying in an oven [11].

The solubility percentage was calculated using the following equation:
Equation 2-2

$$Solubility\ (\%) = \left(\frac{M_1 - M_2}{M_1}\right) \times 100$$

**2-2-4- Water Vapor Permeability(WVP)**

Water vapor transmission was measured according to the ASTM E96-92 method used in the research of Casarigo et al. (2009). For this purpose, special falcons with a diameter of cm 2 were used. 10 ml of distilled water was poured into the falcons. A piece of cut film was placed on the Falcon cap and the vial was closed. The amount of water vapor transferred from the films was determined from the weight loss of the falcon. Water Vapor Transmission Rate (WVTR) and Water Vapor Permeability (WVP) It was calculated according to the following equations [12].

Equation 2-3

$$\mathrm{WVTR} = \frac{Slope}{A}$$

Equation 2-4

$$\mathrm{WVP} = \frac{(\mathrm{WVTR} \times \mathrm{L})}{\Delta \mathrm{P}}$$

WVTR: water vapor transfer rate ($Kg/m^2$ s)
L: film thickness (m)
ΔP: relative water vapor pressure difference in pascals between the two sides of the film
A: Film surface ($m^2$)

**2-2-5- Determination of antioxidant properties**

The ability to lose hydrogen atoms by phenolic compounds or the degree of decolorization of violet 2 and 2-diphenyl-1-picryl-hydrazyl ethanolic extracts of films was measured. In this test, DPPH was used as a stable radical compound. In order to determine the antioxidant capacity of the films through free radical scavenging power (DPPH), the method of Byun et al. (2010) was used [13].

Equation 2-5

$$\mathrm{DPPH}_{\text{scavenging activity}}\ (\%) = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

$Abs_{control}$: absorption rate of the control sample
$Abs_{film\ sample}$: absorption rate of sample containing nanoparticles and essential oil

**2-2-6- Measuring the surface color of films**

To measure surface color using a colorimeter (Colorimeter Minolta model CR-410 Japan). The results include the size of three dimensions of color with quantitative indices L*, a* and b*, which respectively represent brightness (from L=0 for black to L=100 for white), green to red (a=-60 for green and a=60 for red) and blue to yellow (from b=-60 blue and b=60 for yellow). According to the following equations, the total color difference (ΔE), whiteness factor (WI), Chroma (C*) and yellowness factor (YI) of the films were calculated respectively [14].

Equation 2-6

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Equation 2-7

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

Equation 2-8

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

Equation 2-9

$$\mathrm{YI} = \frac{42.86 \times b^*}{L^*}$$

**2-2-7- Transmittance**

The barrier property against visible light in the films was measured using the method of Hah and Floros (1997) at the determined wavelength. The piece of film was placed in the transparent wall inside the quartz cell of the device and the absorption rate of the sample was read. The amount of light transmission was calculated from the equation 2-10 [15].

Equation 2-10

$$Opacity = \frac{A600}{X} \times 100$$

In this equation, A600 is the amount of absorption at the wavelength of 600 nm and X is the average thickness of the film in millimeters.

**2-2-8- Measuring the mechanical properties of films**

The stress-strain test is one of the most widely used tests for determining the mechanical behavior of films. Tensile tests were measured using a texture tester and according to the standard (22-91) [ASTM D882]. The measurement factors include Ultra Tensile strength (UTS) and Strain to Break (STB) [14].

Equation 2-11

$$UTS = \frac{Maximum\ Load}{Cross\ sectional\ area\ of\ samples}$$

Equation 2-12

$$\%\ STB = \frac{Elongation\ at\ breaking\ point}{original\ length}$$

In these relationships, Maximum Load is the maximum force applied to the film in Newtons, Cross sectional area is the original cross-sectional area of the film in square meters, Elongation at breaking point is the amount of expansion until the moment of rupture in millimeters, Original length is the initial length of the two-jaw sample. In terms of millimeters, UTS is the amount of tensile strength in megapascals and STB is the amount of elongation to breaking point in percent.

**2-2-9- infrared spectroscopy (FT-IR)**

For IR spectroscopy, thin tablets with a thickness of less than one millimeter from mixing and grinding the film sample coated with dry potassium bromide at a ratio of 1:20 and applying a pressure of about 60 Kpa for 10 minutes in the device Tablets were prepared and the transmission spectrum of the samples was analyzed in the wave number range of 400 to 4000 $cm^{-1}$ with a resolution of 0.5 $cm^{-1}$ in the device (Spectrum Two, Perkin Elm) of the Chemistry Department of Urmia University.

**2-2-10- Scanning Electron Microscope (SEM)**

The microstructure of the produced films was investigated using the Tescan Vegan-3 scanning electron microscope available in Tehran Metallurgy Research Institute. Imaging of the samples was done with a scanning electron microscope using 20 kW and at a magnification of 20 $\mu m$ [12].

**2-3- Statistical Analysis**

The statistical population includes 9 soy protein isolate films containing montmorillonite nanoparticles in different concentrations (0, 0.5 and 1%) and *Salvia officinalis* essential oil with different concentrations (ppm 500, 250 and zero). All tests were performed in triplicate. Data analysis and evaluation (ANOVA) was performed in the form of a completely random design using Design Expert software at a probability level of 0.01% ($P< 0.0001$).

## 3- Results and Discussion

**3-1- Thickness**

Thickness is one of the important factors in films and it directly affects the characteristics of permeability to water vapor and oxygen, mechanical properties, and thus the biological characteristics and shelf life of the packaged product. According to the results of the data obtained from the thickness of the produced films in Figure1, the difference between the thickness of different treatments was significant ($P<0.0001$). Compared to other samples, the sample of pure soy protein isolate film had the lowest thickness (0.143 mm) among the produced films. Among the samples without essential oil, with the addition of different percentages of nanoclay to the composition of the protein film, the thickness of the samples increased significantly ($P<0.0001$). The reason for the increase in the thickness of the films with the addition of nanoclay can be the increase of the dry matter of the films as well as the absorption of water in the single-layer region by the hydrogen compound, so that less moisture is removed from the films compared to the control sample during drying, and the sum of these The changes increase the thickness of the produced films [16]. The increase in the percentage of essential oil in the composition of protein films without nanoclay has also increased the thickness, but this increase is more evident in the group of samples with nanoclay and without sage essential oil. The simultaneous application of sage essential oil and nanoclay caused a significant increase in the thickness of the produced samples (P<0.0001). Thus, the film sample containing 1% nanoclay and 1% sage essence had the highest thickness (0.213 mm) among all production samples. The results were consistent with the results of Pirouzifard et al. (2020) regarding the effect of *Salvia officinalis* essential oil on increasing the thickness of potato starch-based production films [17]. The results of the research of Ghadermarzi et al. (2015) and Salarbashi et al. (2016) are contrary to the results of this research. In the investigation of the physicochemical and microbial properties of the films prepared containing nano titanium oxide based on soybean flour polysaccharide, they reported that the thickness of the films decreases with the increase in the amount of nano titanium oxide [18, 19].

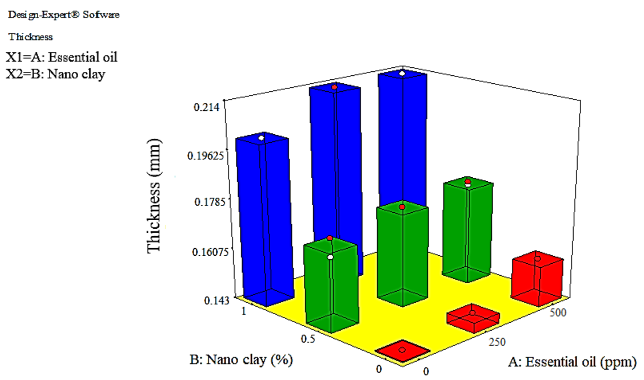


Fig 1: Influence of Nanoclay and *salvia officinalis* essential oil on thickness of films

### 3-2- Moisture content

The addition of nanoclay by creating hydrogen and covalent bonds with the protein network has reduced the free hydrogen groups available to form hydrophilic bonds with water and ultimately reduced the water activity and moisture content of protein films [20]. According to the results of Figure 2, by adding nanoclay to the control film and increasing the added percentage along with essential oil, the moisture level of the films decreased. This decrease in humidity has increased more in the samples containing essential oil. The cause of this phenomenon is the increase in the amount of solids and the repellent effect of the fatty acids in the essential oil by covering the active groups of the protein, causing them to be removed from their reach and therefore preventing hydrogen bonds between water molecules and those groups [ 21]. According to the obtained results, the effect of essential oil on the amount of moisture was significant, which is probably due to the fact that the essential oil is hydrophobic and prevents the absorption of more moisture. The essential oil combined with the film prevents the absorption of high moisture by the glycerol combined with the film due to the hydrophobic property of the essential oil, while the glycerol in the film without essential oil can absorb the maximum moisture and swell without any obstacles. and increase the thickness of the film [18]. The addition of nanoclay through covalent hydrogen bonds with the protein network has reduced the free hydrogen groups available to form hydrophilic bonds with water and ultimately reduced the aqueous activity and moisture content of protein films [20]. The results are consistent with the research results of Salarbashi et al. (2016) who investigated the characteristics of the film containing nano titanium oxide based on soybean flour polysaccharide and reported that by adding nano titanium oxide to the composition of the film, the amount of moisture in them significantly decreased [19]. The result of the effect of essential oil on reducing the moisture content of films was consistent with the results of similar research by researchers [17, 22].

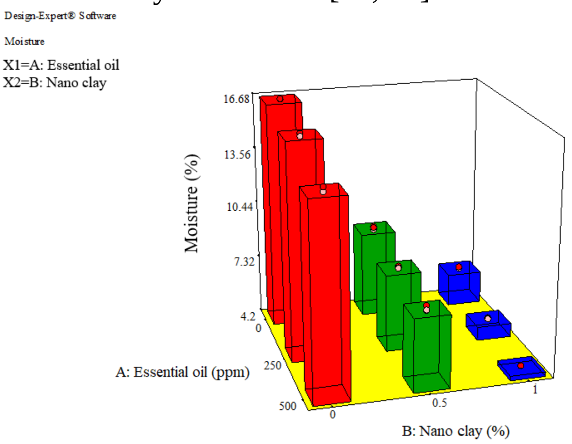


Fig 2: Influence of Nanoclay and *salvia officinalis* essential oil on Moisture of films

### 3-3- Solubility

Solubility in water can be one of the most important properties for edible films due to its resistance to water, especially in humid environments. The solubility of films in water determines the release of antioxidant and antimicrobial compounds from active films when they are used to cover food [23]. The solubility of produced films in water is presented in Figure 3. The reason for this decrease in solubility between the samples with nanoclay without essential oil can be the increase in the inhibitory properties of soy protein due to the addition of nanoclay due to the disc structure like nanoclay or the interactions caused by the formation of bonds between nanoparticles and soy protein and the stabilization of the film structure in The result stated. In other words, water is not able to sufficiently break the hydrogen bond between the layers of nanoclay and soy protein, which reduces the solubility in nanocomposite. With the addition of *salvia officinalis* essential oil to the structural composition of the film, we see a significant decrease in the solubility of the samples. The reason for this can be the creation of transverse links between the content of essential oil and soy protein isolate. The transverse connections created are very effective in reducing the solubility of the film and producing a water-resistant film. The creation of these connections leads to the reduction of free hydroxyl and amine groups in the film network. Based on this, it can be said that the addition of plant essential oil to the protein film matrix reduces the amount of hydrogen bonds between water molecules and the functional groups of polymer chains, and ultimately, the reduction of hydrogen bonds leads to a decrease in the moisture content of films containing essential oils. became [24]. Similar results have been reported from the studies of other researchers regarding the effect of nanoparticles and different essential oils and increasing their concentration used in the composition of production films on reducing the solubility of films [24, 25].

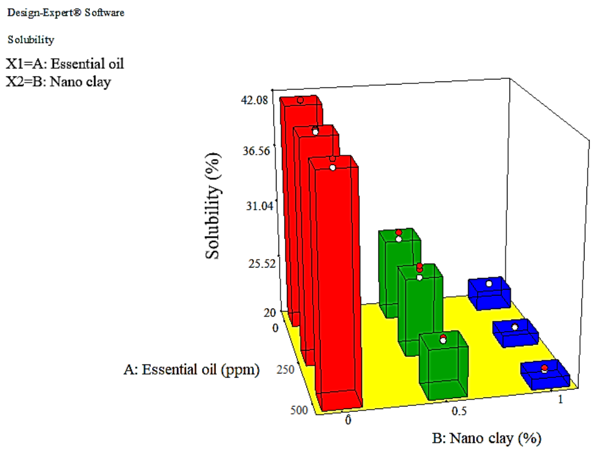


Fig 3: Influence of Nanoclay and *salvia officinalis* essential oil on Solibility of films

### 3-4- Permeability to water vapor

The degree of permeability to water vapor in edible and biodegradable films is related to the type of their application; So that it is not possible to pack every type of food with the same polymer. Particle size is one of the influencing factors on the amount of WVP of edible and biodegradable films [26]. According to the results of the analysis of the water vapor permeability index of the produced films in Figure 4, the reason for the decrease in water vapor permeability due to the addition of nanoclay to the structure of the films is that in these concentrations, the nanoprotein chains have covalent and hydrogen bonds with each other. And the proper dispersion of nanoparticles in the matrix of the protein film creates a twisted path for water molecules to pass through, and on the other hand, the path of water is blocked and the penetration of water vapor is limited throughout the desired coating film [27]. Also, it seems that the observed reduction in water vapor permeability in films with a higher percentage of nanoclay is due to the presence of nanoclay with a high ratio, which are uniformly dispersed in the polymer matrix. By adding different amounts of *salvia officinalis* essential oil to the composition of the films, a significant decrease in the WVP index of the samples was seen. In explaining the reason for this decrease, it can be stated that sometimes polymer chains cause a decrease in polymer cracks, therefore, by decreasing the diffusion of water through the interspace of protein chains, it causes a decrease in permeability. This issue can be explained by the formation of the fat interaction network in the structure of the protein film [28]. Atares et al. (2010) by adding essential oils of ginger and cinnamon to soy protein isolate film, concluded that the essential oil does not cause significant changes in the permeability of the films [29]. Other researches show the reduction of water vapor permeability of bio-based films due to the addition of essential oils. Researchers have mentioned the reason for the decrease in permeability due to the repulsive effect of non-polar compounds in essential oils on water molecules [17, 30].

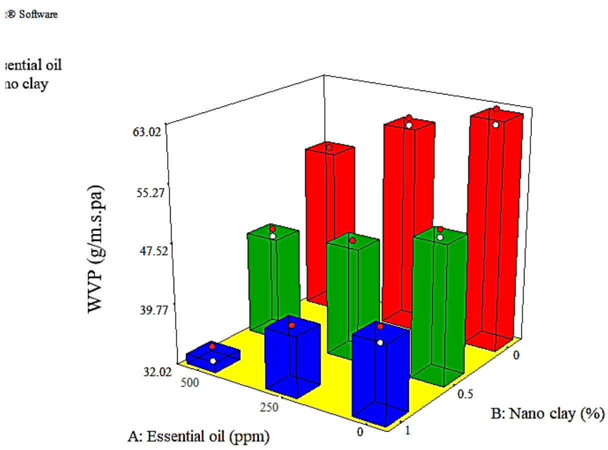


Fig 4: Influence of Nanoclay and *salvia officinalis* essential oil on WVP of films

### 3-5- Antioxidant property

The amount of total phenolic compounds is an indicator of the antioxidant power of the plant. *salvia officinalis* has higher phenolic compounds than many other medicinal plants such as chamomile, marigold, rhubarb and lavender [31]. In *salvia officinalis* essential oil, there are oxygenated monoterpenes with an amount of nearly 60%, followed by oxygenated hydrocarbons with an amount of about 20%, and the most important compounds of these groups are alphatogen, camphor, and viridiflorol. , borneol, 1 and 8-cineol, betatogen and bornel acetate, the amount of these compounds will have different antioxidant power in different conditions [32]. The results of investigating the antioxidant properties of the films are presented in Figure 5. Adding different percentages of nanoclay to the composition of soy protein isolate film caused a significant increase in the antioxidant content of samples without essential oil, and this increase among the samples without nanoclay with the addition of higher concentrations of essential oil in the composition of the film It is much more obvious. In the combined samples of nanoclay and essential oil, a significant increase in the amount of antioxidant content of the samples can be seen, which indicates the intensification of the antioxidant content of the samples due to the simultaneous presence of nanoparticles and essential oil (sample $N_1E_{500}$ with 32.88% had the highest antioxidant content among the samples). The results of other research also indicate an increase in antioxidant power due to the addition of essential oils and plant extracts to the protein and carbohydrate matrix of films [17, 22, 27, 33].

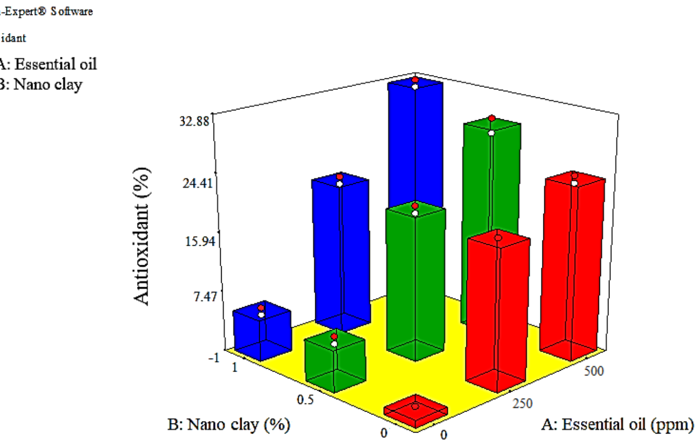


Fig 5: Influence of Nanoclay and *salvia officinalis* essential oil on Antioxidant property of films

### 3-6- Colorimetric

Table 1 shows the parameters L*, a*, b*, WI, YI, C* and ΔE measured for the samples. The a* index indicates the degree of redness-greenness of the samples, so that positive numbers indicate redness and negative numbers indicate greenness of the samples. In the same way, the b* index indicates the yellow-blue color of the samples, and the negative b* value indicates the presence of blue undertone and the positive b* indicates the yellow undertone in the samples. The a* values obtained for all the samples were negative values, which, according to the explanations provided, indicate the green color in the samples. *salvia officinalis* essential oil has a pale yellow color, which by adding different concentrations of this essential oil to the composition of the films and the synergistic effect of the color of this essential oil with nanoclay, the whiteness coefficient and transparency of the samples containing the essential oil It has decreased significantly to the samples without essential oil. In general, with the addition of nanoclay, the factor L* and b* has increased. As can be seen, the decrease in the amount of L factor was not significant, which could be due to the hydrophilic property of nanoclay and its compatibility with the protein network [24]. Overall color difference (ΔE) is a measure to measure the transparency of films. The reduction of factor a* and the increase of factor b indicate the increase of green and yellow color in the resulting nanocomposites. Also, the increase of nanoclay increased ΔE and the yellowness index (YI) and decreased the whiteness index (WI). Zolfi et al. (2014) who investigated the effect of montmorinolite on the characteristics of kefir-whey protein composite film, reached similar results. have found [34]. Chroma (C*) is a measure of the difference of a color from gray and is defined as a measure of purity. The calculation of the amount of chroma in the samples indicates the highest amount of color purity in the film samples containing N1E500 as 28.57 and the lowest amount of chroma in the control sample as 15.35. By preparing active films based on carboxymethyl cellulose-chitosan-oleic acid containing ginger essential oil, Noshirvani et al (2018) reported that the addition of ginger essential oil caused a significant decrease in brightness and an increase in the yellowness and greenness of the films. Also, the degree of color purity (chroma index) increased significantly by adding ginger essential oil and increasing its concentration, which indicates the effect of ginger essential oil on the color of carboxymethyl cellulose-chitosan-oleic acid film [35]. The results of this research are consistent with the results of Qadimerzai et al., who investigated the effect of *salvia officinalis* essential oil on edible HPMC films [18].

Table 1: Colorimetric data (L*, a*, b*, WI, C*, YI, ΔE) analysis of biodegradable films

| Sample | L* | a* | b* | C* | WI | YI | ΔE |
|---|---|---|---|---|---|---|---|
| $N_0E_0$ | $59.63^a$ | $-1.419^g$ | $13.017^i$ | $15.351^i$ | $65.403^a$ | $4.006^i$ | $61.473^a$ |
| $N_0E_{250}$ | $78.107^c$ | $-1.651^h$ | $14.604^h$ | $16.413^h$ | $64.364^c$ | $9.722^h$ | $63.516^c$ |
| $N_0E_{500}$ | $78.964^b$ | $-1.995^i$ | $14.890^g$ | $16.973^g$ | $64.994^b$ | $10.481^g$ | $63.842^b$ |
| $N_{0.5}E_0$ | $77.171^d$ | $-4.375^d$ | $26.410^f$ | $27.527^f$ | $59.166^d$ | $31.682^f$ | $80.517^d$ |
| $N_{0.5}E_{250}$ | $76.351^e$ | $-4.627^e$ | $26.607^e$ | $27.892^e$ | $58.514^e$ | $33.005^e$ | $80.680^e$ |
| $N_{0.5}E_{500}$ | $76.618^f$ | $-4.831^f$ | $26.981^d$ | $27.907^d$ | $57.755^f$ | $34.207^d$ | $80.794^f$ |
| $N_1E_0$ | $80.207^g$ | $-5/116^c$ | $23.019^c$ | $28.146^c$ | $54.110^g$ | $44.155^c$ | $83.251^g$ |
| $N_1E_{250}$ | $81.318^h$ | $-5.326^b$ | $24.163^b$ | $28.296^b$ | $54.376^h$ | $46.362^b$ | $84.559^h$ |
| $N_1E_{500}$ | $81.956^i$ | $-5.710^a$ | $24.876^a$ | $28.571^a$ | $53.101^i$ | $48.172^a$ | $84.847^i$ |

Different letters in each column indicate the significance of the differences ($P<0.0001$)

## 3-7- Transparency and light transmission

Light radiation is one of the most important factors of food spoilage, some products are so sensitive to light that their color, smell and taste undergo drastic changes with minimal oxidation resulting from the effects of light. The main challenge for such products is to protect the packaged contents from light entering from different sources [36]. In the examination of the level of transparency of the films, the control sample had a significant difference compared to the other samples. The transparency of the films is affected by the thickness of the films. According to Figure 6, the control sample with the lowest amount of thickness had high transparency and less turbidity than other samples. The transparency of nanocomposite films decreased significantly with increasing amount of nanoclay. This decrease in transparency is more evident among the samples with higher percentage of nanoclay and essential oil. The matte and non-transparent appearance of biodegradable films containing nanoclay blocks the passage of light through the film network, and as a result, reduces the transparency of composite films containing nanoclay. The results of this research were consistent with the results of Sothornvit et al. (2009) [24].

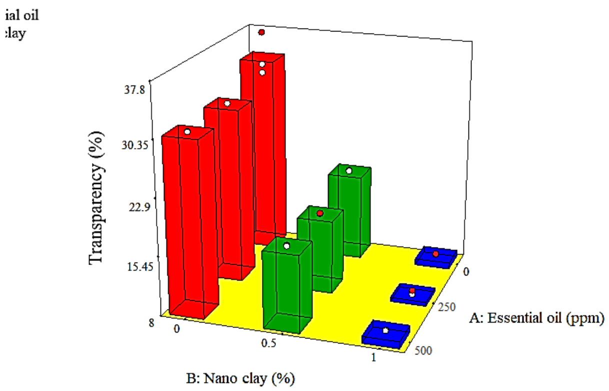


Fig 6: Influence of Nanoclay and *salvia officinalis* essential oil on Transparency of films

## 3-8- mechanical properties

The results related to the percentage of elongation and tensile strength of the films in Table 2 show an increase in TS and a decrease in the elongation at the breaking point due to the addition of nanoclay and sage essence into the protein matrix. It has less elasticity than nanocomposite. The highest value of TS corresponds to the sample $N_1E_0$ and the highest percentage of elongation at the breaking point corresponds to the control film ($N_0E_0$). In general, with the addition of different percentages of nanoclay to the composition of the film, we see a significant increase in the tensile strength index of the samples. This increase is more evident in the sample without *salvia officinalis* essential oil. It can be said that the addition of essential oil has reduced the tensile strength of the samples in comparison with the state without essential oil. In general, the presence of essential oil causes the structural density to be disrupted and the tensile strength of the samples containing essential oil to decrease. Based on the research done by different researchers, adding essential oil compounds to different resins changes the mechanical properties of the films produced from these resins due to changes at the molecular level. These changes are due to the destruction of the film matrix, in most cases, in the direction of reducing the resistance of films against stretching [37]. Ghadermarzi et al. (2015) reported that the tensile strength of HPMC film decreased by about 3 times and Young's modulus decreased by more than 2 times due to the addition of *salvia officinalis* essential oil to HPMC film. These researchers stated that when the concentration of essential oil is high, weak reactions occur between polymer and essential oil, and this causes a decrease in tensile strength and percentage of strain up to the breaking point of the polymer strands, while in the concentration at lower values, this happens less often [18]. Pirouzifard et al. (2020) in evaluating the mechanical properties of edible films based on potato starch stated that the addition of *salvia officinalis* essential oil caused a significant decrease in the percentage of stretch and tensile strength of the films compared to the control sample. [17]. The increase in stiffness of the films indicates the strengthening effect of nanoparticles and evidence of the brittle behavior of the nanocomposite due to the addition of fillers to the nanocomposites. The increase in tensile strength may be related to the inherent strength and stiffness of the nanoclay chain, the uniform distribution of nanofillers in the protein matrix bed, and the high compatibility between the nano particles and the protein network due to the high surface area and the interactions created between the nanoclay and the protein network. And their reduction at higher levels can be due to the possible accumulation of clay nanoparticles and the lack of uniform distribution of nanoparticles in the protein substrate [14]. The increase in TS of the film due to the addition of MMT can be attributed to several factors, including the uniform dispersion of MMT nanoparticles in the soybean protein matrix, the strong interaction between the soybean pezotein isolate and the wide surface of layered silicate nanoclay through hydrogen or ionic bonds and the strengthening effect. The ductility of nanoclay was attributed to the effective stress transfer through the interface to nanoclay sheets. A similar behavior has been reported regarding the effect of adding nanoparticles to other polymer nanocomposites such as starch, agar, and soy protein [5, 38, 39].

Table 2: Data analysis of mechanical properties of biodegradable films

| Sample | Tensile Strength (MPa) | Gradient (kg.s) | Strain to Break (%) |
|---|---|---|---|
| $N_0E_0$ | 5.037[d] | 0.209[d] | 22.325[a] |
| $N_0E_{500}$ | 6.137[c] | 0.265[c] | 20.417[b] |
| $N_{0.5}E_{250}$ | 8.452[a] | 0.295[b] | 12.639[c] |
| $N_1E_0$ | 8.637[a] | 0.308[a] | 8.527[d] |

Different letters in each column indicate the significance of the differences (P<0.0001)

### 3-9- Infrared spectroscopy

The frequency of electromagnetic radiation in the infrared region (IR) corresponds to the natural vibration frequency of the atoms of a bond, and after the absorption of infrared waves in a molecule, it creates a set of vibrational movements in it, which is the basis of infrared spectroscopy. forms [40]. Figure 7 shows the spectra of pure soy protein isolate film and films containing nanoclay and *salvia officinalis* essential oil. The spectra obtained from the control sample are similar to other production films and have differences only in a few parts, which indicate the creation of new bonds due to the different compounds used in the structure of the films. that production peaks have been shifted to other wavelengths. All three investigated film samples have common peaks in the wavelengths of 746.746 $cm^{-1}$ to 1652.70 $cm^{-1}$, which indicate the presence of strong C-Cl, C-Br bonds, strong C-O-C stretching bonds and C-OH is in the structure of soy protein isolate. This set of peaks can be attributed to stretching hydrogen bonds and bending hydroxyl groups in the nanoclay structure. These changes in the spectra of the samples containing nanoclay are probably due to the reaction between the internal O-H stretching bands and the O-H groups present in the isolated carboxyl groups of soy protein with the surface groups of nanoclay [41]. The peaks created in the wavelength range of 1502/64 $cm^{-1}$ in samples b and c indicate the presence of weak aromatic C=C bonds and C=O amide bonds due to the presence of *salvia officinalis* essential oil in the structural composition of these films. be These groups are related to the compounds in the essential oil and the changes in the vibrations of protein compounds and indicate the stretching C-O bonds in soy protein and structural vibrations in nanoclay. Noshirvani et al. (2018) by investigating the active films based on carboxymethylcellulose-chitosan-oleic acid containing ginger essential oil, reported that by adding essential oil to the film substrate, the peaks related to amine and hydroxyl groups at different wave numbers compared to The witness film appeared, which indicates the establishment of hydrogen bonds between the polymer substrate and ginger essential oil. These researchers stated that the intensity of the peaks related to symmetric and asymmetric methylene in CH2 and CH3 groups, which are related to fats, also increased after adding ginger essential oil, which indicates an increase in the hydrophobicity of the films with adding ginger essential oil [35]. In total, the spectrophotometric spectrum of the composite film showed the effect of nanoclay and essential oil on the position, width and intensity of the spectra related to protein-nanoclay interactions.

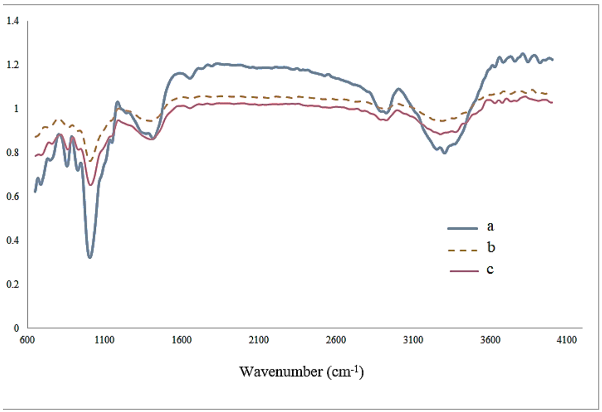


Fig 7: Infrared spectroscopy of Biodegradable films ((a) Soy Protein Isolate without Nano-clay and *salvia officinalis* essential oil, (b) Soy Protein Isolate containing 0.5% Nano-clay and 250 ppm *salvia officinalis* essential oil, (c) Soy Protein Isolate containing 1% Nano-clay and 500 ppm *salvia officinalis* essential oil)

### 3-10- Scanning Electron Microscope

SEM test is used to observe and evaluate the morphology of films and polymers. The microscopic image (SEM) taken from the surface of the produced films is shown in Figure 8. The surface of the control film is almost smooth and the small amount of roughness and bumps that are observed on the surface of the control film are most likely due to The presence of protein granules [42]. The appropriate density of the Shahid film is due to the establishment of strong connections between the hydrophilic soybean protein isolate compounds when the films dry. By adding nanoclay to the protein network, the surface of the composite is uneven and It becomes rougher and their density decreases. In sample b (0.5% nanoclay and 250 ppm of *salvia officinalis* essence), with the increase of nanoclay, the smoothness of the network is lost and more non-uniformity is observed compared to the control film. This non-uniformity leads to the formation of silicate plates on the film with the increase of nanoclay to 1%. The cracks and holes observed in the samples of films b and c may be caused by the decrease in force. between the surface of the matrix and the nanoclay, there is a lack of uniform distribution on the surface of the network and a reduction in the adhesion between the nanoclay and the protein network [43]. By adding different concentrations of *salvia officinalis* essential oil to the films, the surface of the films compared to the control sample, It has become smoother and smoother and the amount of cracks and gaps on the surface of the film has been significantly reduced, which is one of the reasons for this, we can point to the oily structure of the essential oil, which The reason for being hydrophobic is that it covers the surface of the film and causes the gaps to close. According to Figure 8, this decrease in the amount of cracks is more evident in sample c (1% nanoclay and 500 ppm *salvia officinalis* essential oil). Song et al. (2013), stated that the addition of clay nanoparticles to protein films causes the nanocomposite films containing clay nanoparticles to have more non-uniformity than the control film, and the reason for this is the placement of nanoparticles in the network. They expressed protein conjugation and destruction of network connectivity [20].

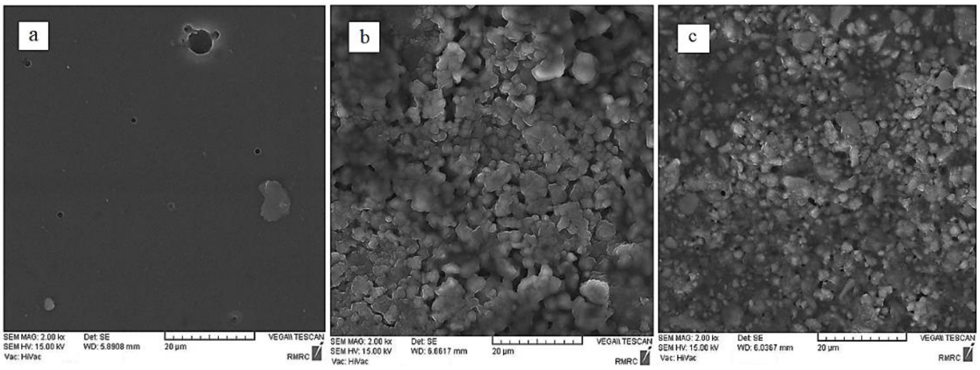


Fig 8: Scanning electron microscopy image of Biodegradable films ((a) Soy Protein Isolate without Nano-clay and *salvia officinalis* essential oil, (b) Soy Protein Isolate containing 0.5% Nano-clay and 250 ppm *salvia officinalis* essential oil, (c) Soy Protein Isolate containing 1% Nano-clay and 500 ppm *salvia officinalis* essential oil)

## 4- total resulting

Nano technology has shown great potential to provide important changes in the food packaging sector. The use of nano-enhancers of nano-clay will improve the overall performance of soy protein isolate and a factor to expand its use as biodegradable packaging. According to the results obtained from this research, the addition of small amounts of nanoclay and *salvia officinalis* essential oil to the protein network matrix significantly improved the physical and mechanical properties of the film. The proper interaction between the protein matrix, nanoclay and *salvia officinalis* essential oil was expressed as the main factor in reducing WVP and moisture content and increasing the tensile strength and reducing the elongation to breaking point of the soy protein isolate film. By increasing the percentage of nanoclay and the concentration of essential oil, the transparency of the films decreased under the influence of nanoclay concentration, and according to the SEM test results, the surface of the composite films became non-uniform. Examining the compatibility of *salvia officinalis* essential oil with soy protein isolate in order to produce bionanocomposite active film and antioxidant effects also resulted in satisfactory results and the combination of essential oil gave the films a favorable antioxidant power.

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**مقاله علمی_پژوهشی**

# بررسی ویژگی‌های عملکردی، مکانیکی و ساختاری فیلم زیست‌تخریب‌پذیر ایزوله پروتئین سویا حاوی نانورس (مونت موریلونیت) و اسانس مریم‌گلی

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**چکیده**

در این پژوهش فیلم‌های تولیدی بر پایه پروتئین سویا با دو متغیر نانورس در سه سطح (۰، ۰/۵ و ۱٪) و اسانس مریم‌گلی در سه سطح مختلف (۵۰۰ ppm، ۲۵۰ و ۰) تولید شده و تاثیر این دو متغیر بر خصوصیات عملکردی و مکانیکی و ساختاری فیلم‌های زیست‌تخریب‌پذیر تولیدی مورد بررسی قرار گرفت. نتایج نشان داد که با افزودن درصدهای مختلف از نانورس بر بستر فیلم پروتئین سویا، محتوای رطوبتی، میزان حلالیت، نفوذپذیری نسبت به بخارآب و شفافیت نمونه‌ها نسبت به نمونه شاهد به طور معنی‌داری کاهش یافت. این کاهش در شاخص‌ها با افزایش غلظت به کار رفته از اسانس مریم‌گلی مشهودتر بود. به طوریکه در بین نمونه‌های تولیدی، فیلم حاوی ۱٪ نانورس و ۵۰۰ ppm اسانس مریم‌گلی، دارای تراوایی نسبت به بخارآب معادل g/m.s.pa $۱۰^{-۱۱}$ × ۳۲/۰۲ بود. همچنین افزودن نانورس و اسانس مریم‌گلی سبب افزایش معنی‌داری در محتوای آنتی‌اکسیدانی نمونه‌ها شد و نمونه محتوی ۱٪ نانورس و ۵۰۰ ppm اسانس مریم‌گلی با ۳۲/۸۸٪ بیشترین محتوای آنتی‌اکسیدانی را داشت. بررسی نتایج طیف‌سنجی و ریزساختاری فیلم‌های بدست آمده نیز بیانگر تعامل مناسب بین نانوذره و اسانس با بستر پروتئین سویا و ایجاد پیوندهای قوی و جدید می‌باشد. نتایج بدست آمده نشان داد که افزودن نانورس و اسانس مریم‌گلی می‌تواند اثرات مثبتی بر خصوصیات فیزیکی و ساختاری فیلم ایزوله پروتئین سویا داشته باشد.