Iranian journal of food science and industry, Number 147, Volume 21, May 2024

Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Production of alfalfa seed mucilage film with nanoparticles of Smyrnium cordifolium Boiss and milk thistle seed oil and physical and chemical properties

Fatemeh Khakpour [1*], Zhiyar Heydari [2], Sajad Pirsa [3]

1- Master's student, Department of Food Science and Industry, Faculty of Agriculture, Urmia University, Urmia, Iran.
2- Master's student, Department of Food Science and Industry, Faculty of Agriculture, Urmia University, Urmia, Iran.
3- Department of Food Science and Industry, Faculty of Agriculture, Urmia University, Urmia, Iran

ARTICLE INFO

Article History:
Received:2023/10/4
Accepted: 2024/2/3

Keywords:

Edible film,
mucilage,
Avondol nanoparticles,
milk thistle oil
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DOI: **10.22034/FSCT.21.147.100.**

*Corresponding Author E-Mail:
sevdakhakpour1@gmail.com

ABSTRACT

The aim of this research was to produce a film based on alfalfa seed mucilage with avondol nanoparticles and milk thistle oil. A composite film of alfalfa seed mucilage with avondol nanoparticles (0, 2, 4%) and milk thistle seed oil (0, 1, 2%) was prepared. . The physicochemical properties of the prepared films were investigated. According to the obtained results, adding Avondol nanoparticles and milk thistle seed oil increases the thickness and antioxidant. But increasing the amount of Avondol nanoparticles and milk thistle essential oil in the film decreased the humidity, solubility and water vapor permeability of the films. X-ray diffraction analysis showed that the Avondol nanoparticles were physically incorporated with alfalfa seed mucilage polymer. The results of scanning electron microscope (SEM) showed that the surface morphology of nanocomposite film is heterogeneous compared to alfalfa seed mucilage. Finally, based on the results, adding avondol nanoparticles and milk thistle oil to edible films based on alfalfa seed mucilage improved the thickness, antioxidant and SEM, but weakened the moisture, solubility and permeability to water vapor.

1. Introduction

In recent years, research related to packaging has paid much attention to biodegradable films produced from natural biopolymers. The use of these edible films has a significant impact on the environment. Because natural biopolymers are completely compatible with the environment, they are supplied from renewable sources, they have high recycling capability, they have mixing capability and they are biodegradable [1 2]. On the other hand, due to constant concerns in the field of preventing chemical spoilage and especially microbial spoilage in food, the tendency to use active packaging has increased. The use of food films and coatings containing antimicrobial substances has shown that these coatings can be an effective way to protect food against spoilage microbial agents and reduce the risk of the growth of pathogenic agents [3 4].Nanotechnology is one of the most important and fastest growing sectors of advanced technology. Products containing nanoparticles can be used in various industrial, medical, personal and military applications. Nanocomposite is a composite material in which at least one of its phases has nano dimensions (between 1 nm and 100 nm). Nanocomposites are currently used for packaging non-alcoholic beverages and food due to their thermal properties, improved strength and conductivity [5 6]. Nanocomposites are polymers in which different organic or inorganic compounds with different planar and spherical shapes are used as fillers in nano dimensions. Films obtained from the combination of nanomaterials and biopolymers, or the so-called biopolymer nanocomposites, show more favorable functional properties, the most important of which are increased mechanical resistance and reduced permeability to water vapor.Increasing resistance against the penetration of gases, increasing the efficiency of the film as active packaging, increasing the heat resistance of the packaging material, creating transparency and improving the appearance properties. Mucilages have a wide range of applications: in food and nutraceuticals as structuring, gelling, texture, and film forming, in pharmaceuticals as binders and disintegrants for drug delivery systems, and in cosmetics as stabilizers. They have also attracted a lot of interest in the textile and paper industries and they can be used in the production of paints [12 11]. Alfalfa (Medicago sativa L.) is a herbaceous and perennial forage legume. All over the world alfalfa is known for its high nutritional value as fodder and animal feed, as well as for its sustainable character, excellent adaptability to extreme weather conditions and environmental flexibility, for example, alfalfa to soil conservation, nitrogen fixation , reducing soil pollutants, reducing air pollutants, carbon dioxide deposition, etc. helps [13].A protein material with the composition of essential amino acids similar to that of soy protein concentrate and 17-27% of soluble and insoluble dietary fibers [9] In addition, a set of micronutrients including carotenoids, tocopherol, polyphenols, saponins and vitamins from B complex have been identified [14]. The therapeutic effects of St. John's wort are closely related to the presence of flavonoid, a complex called silymarin, consisting of a mixture of silybin A and B, isosilybin A and B, silycristine and silydianin [15] is formed from the whole plant for medicinal purposes for kidney treatment, Spleen, liver and gall bladder diseases are used [16] Silymarin also showed good antioxidant, anti-inflammatory and anti-fibrotic links.It was found to stimulate protein biosynthesis, increase lactation, and immunomodulatory activity. Furthermore, silymarin inhibits cell growth, DNA synthesis, and other mitogenic signals in human prostate, breast, and cervical cancer [17]. Smyrnium cordifolium Boiss is a medicinal plant belonging to the Apiaceae family and among the native plants of Iran, which grows well in the heights and slopes of the Zagros mountain range in the western regions of Iran [18]. Sesquiterpenes, monoterpenes and flavonoids are among the main compounds found in the essential oil of this plant species [19]. The purpose of this research is to investigate the effect of Avondol nanoparticles and milk thistle oil in different concentrations on the physical and chemical properties of edible films prepared based on alfalfa seed mucilage.

2- Materials and methods

2-1- Materials

The stem and leaves of the Avondol plant and the seeds of the thistle plant were collected from the mountains. Edible alfalfa seeds were bought in Urmia market. Glycerol with a purity of 99.5% was purchased from Merck. Calcium sulfate, potassium sulfate, methanol, Mueller Hilton culture medium were purchased from Merck (Germany).

2-2- Nanoparticle extraction from Avondol plant

First, the stem and leaves of the Avondol plant were collected from the heights of the mountains and then dried for four days in the shade and then crushed using a mill and then sieved using a micrometer sieve and then for six hours in a planetary mill. E was set at 6000 rpm. After every hour and ten minutes, the rotation of the planetary mill stopped. And finally, the powdered nanoparticles are stored in a black container.

2-3- Production of films

First, alfalfa seed mucilage was poured into 80 ml of distilled water and stirred using a magnetic stirrer at a temperature of 70 degrees Celsius and a speed of 500 rpm. 2%) was dissolved in 20 ml of distilled water and added to alfalfa seed mucilage solution. After adding glycerol to the solution, pH was adjusted using NaOH solution. The solution was poured into the Falcon and centrifuged. The resulting supernatant solution was poured into the plate and after 48 hours, the films were dried at room temperature, then the dried films were kept in zipped bags [20].

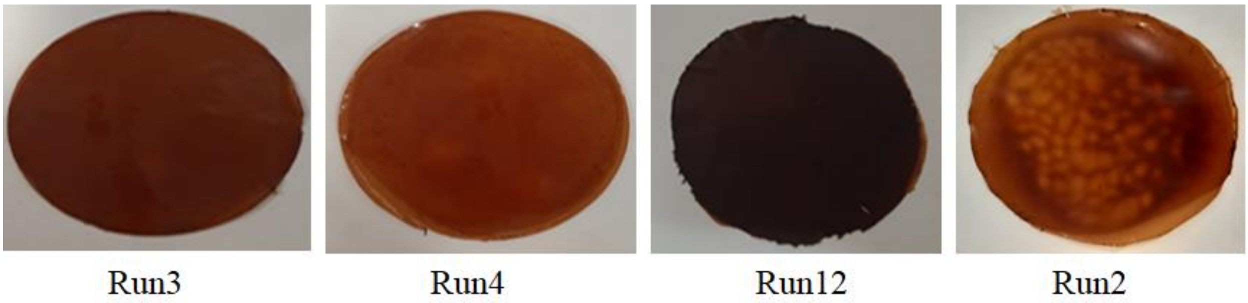

Fig 1: An image of the produced films

2-4- Characteristics of films

2-4-1- Film thickness

The thickness of the prepared films was measured using a digital micrometer with an accuracy of 0.001 mm. At least 5 different points in each film were measured and the average numbers obtained were reported and used in different experiments [20].

2-4-2- Humidity measurement

To check the moisture level, first, the films were cut into 3x3 cm2, weighed with a scale (initial weight) and placed in a desiccator containing silica gel for 24 hours. Then the films were weighed again (final weight). Then, using the following formula, the amount of moisture was obtained [21].

$$Moisture(\%) = \frac{Wi - Wf}{Wi} \times 100$$

In this regard, Wi is the initial weight and Wf is the final weight

2-4-3-Measurement of antioxidant properties

25 mg of each film was dissolved in 4 mL of water for 2 min. Then 2 ml of the film extract solution was mixed with 0.2 ml of 1 mM DPPH[1] methanol solutions. The mixture was well dissolved in a vortex at 300 rpm for 1 minute. After 30 minutes of storage in a dark place, the decrease in absorbance at 517 nm was calculated using spectrophotometry with the following formula [22].

$$A\ (\%) = \frac{Ab - As}{Ab} \times 100$$

Ab: Absorption rate of the control sample
As: sample absorption rate

2-4-4- Solubility in water

To measure the solubility in water, first each film sample (3x3 cm2) was weighed and placed in a desiccator and then accurately weighed by a digital scale. Then, the films were placed in a

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[1] - 2-2-Diphenyl-1-picrylhydrazyl

flask for 6 hours, in which 50 ml of deionized water was poured and gently stirred every 20 minutes, and then the solution was filtered through a filter. In the next step, the filter paper with the film was kept in a 40 degree Celsius oven for 24 hours and weighed again [20].

$$Solubility\ (\%) = \frac{M1 - M2}{M1} \times 100$$

M1 is the initial weight of the sample and M2 is the weight of the sample after drying.

2-4-5-Measurement of film permeability

Permeability is defined as the passage of water molecules through the film and was measured at a temperature of 25 degrees Celsius and a relative humidity gradient of 50% according to the ASTM E96 05 method. Films with a specific thickness were kept at 50% relative humidity and 25°C temperature for 48 hours before testing. A vial with a diameter of 2 cm and a height of 10 cm, which had a hole with a diameter of 8 mm, was used to measure the permeability to water vapor. For this purpose, 3 grams of calcium sulfate was weighed in the containers to remove moisture, and then a piece of film was placed inside the cap and closed on the vial. Next, the dishes were weighed and placed in a desiccator containing 1 liter of distilled water at a temperature of 23 degrees Celsius, and then the dishes were weighed every 24 hours for a week. Permeability to water vapor was calculated according to the following equation [20].

$$WVP = \frac{WVTR \times T}{P(R_1 - R_2)}$$

where WVTR is the constant water vapor transfer rate (g/m2.h), T is the film thickness (mm), P is the partial water vapor pressure at 25 degrees Celsius (2.642 kPa), R1 is the relative humidity in the desiccator (100%) and R2 The relative humidity in the container is (0%).

2-4-6- Scanning electron microscope (SEM)[2]

Morphology was examined using Leo 1430VP (Germany) scanning electron microscope. The film was glued on the aluminum base with the help of silver glue. For better conductivity during photography, the samples were covered with a thin layer of gold (thickness about 5 to 6 nm) for five minutes. Imaging of the samples was done with an accelerating voltage of 30 kV and a magnification of 10,000 times. Then the average diameter was calculated with the software [20].

2-4-7- X-ray diffraction (XRD)

X-ray diffraction (XRD) (X'Pert Pro Panalytical Netherlands) was performed to investigate the physical state of nanoparticles inside the composite film. XRD diffraction patterns were obtained through a diffractometer using Cu Ka radiation (1.54 Å) in the range of 2θ=40-40 and the scan step time was 53 seconds [20].

2-5- Statistical analysis

In this study, the statistical method of the response surface and the central composite statistical design were used to investigate the effect of two variable factors, the percentages of avondol nanoparticles and milk thistle essential oil, on the physicochemical properties of the prepared films. The statistical analysis of the data was done at the probability level of 95% using Design Expert-10 software.

Table 1: Produced films

Film	A nanoparticles (%)	B: Essential oil (%)
F1	2	**1**
F2	4	**2**
F3	0	**0**
F4	0	**2**
F5	2	**1**
F6	4	**1**
F7	2	**0**
F8	2	**1**
F9	2	**1**
F10	0	**1**
F11	2	**1**
F12	4	**0**
F13	2	**2**

3- Results and Discussion

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[2] - Scanning electron microscope

3-1- Film thickness

Thickness is one of the important factors of edible films, it directly affects the biological characteristics and shelf life of the packaging product. The thickness of edible films affects the mechanical properties and permeability to different gases, hence it is very important. Thickness measurement is also important to measure the uniformity of films. Thickness changes cause problems in the mechanical performance of films and changes in permeability characteristics. As it is clear in Figure 1-3-, Avondol nanoparticles and milk thistle essential oil increased the thickness of the film. The presence of these two substances has caused a significant increase in the thickness of the film compared to the pure alfalfa seed mucilage film sample ($p<0.05$), which is consistent with the results of Davachi et al. [23].The reason for the increase in the thickness of the films with the addition of Avondol nanoparticles and milk thistle essential oil is the increase of the dry matter of the films as well as the absorption of water in the single layer area by this hydrocolloid compound, so that less moisture is removed from the films during drying and the total These changes increase the thickness of production films [24].

$$\text{Thickness (mm)} = 0.410 + 0.096 * A + 0.087 * B + 0.075 * A * B + 0.027 * A^{\wedge}2 + 0.025 * B^{\wedge}2$$

$$(R^2 = 0.973 ;\quad AdjR^2 = 0.954)$$

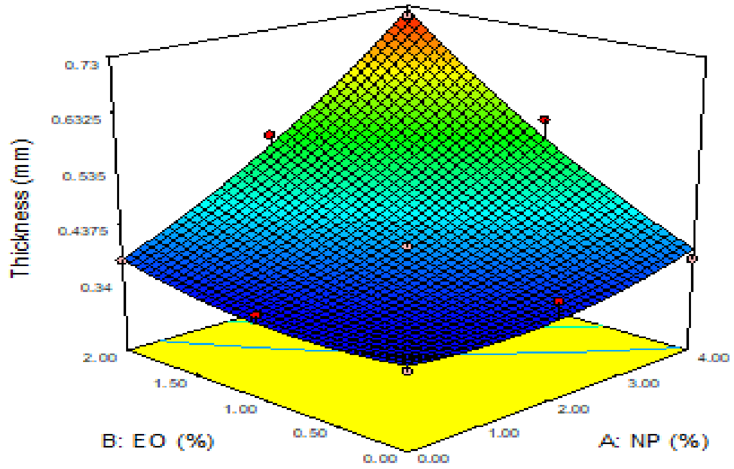

Figure 3-1: The three-dimensional figure of the film thickness of alfalfa seed mucilage with Avondol nanoparticles and milk thistle essential oil.

3-2- humidity

The effect of the percentage of Avondol nanoparticles and milk thistle essential oil on moisture content is shown in Figure 3-2. The mathematical equation shows the relationship between independent variables and humidity and regression coefficients. The amount of moisture absorption depends on the amount of empty spaces available for the penetration of water molecules and the degree of hydrophilicity of the polymer. Mucilages are naturally hydrophilic in nature and increase the amount of moisture absorption in the film. They have hydroxyl groups in their structure and are therefore considered hydrophilic. Moisture absorption of a film depends on the hydrophilic property of that polymer or biopolymer and then on the presence of holes and empty spaces between the chains. According to the three-dimensional shape of the moisture content of the film, alfalfa seed mucilage increases the moisture content of the film, but Avondol nanoparticles decrease the moisture content of the film.By creating electrostatic forces through oxygen atoms, Avondol nanoparticles cause the H and OH groups to engage the alfalfa seed mucilage chain and block the entry of H_2O molecules into the polymer structure and reduce the moisture content. Also, the nanoparticles of Avondol, by being placed in the empty spaces of the film, reduce the spaces necessary for the placement of water molecules. The polymer used in food packaging should be as resistant to moisture as possible to prevent the occurrence of undesirable properties caused by the penetration of moisture into the food. According to Figure 3-2, the increase of Avondol nanoparticles and milk thistle essential oil decreased the moisture significantly ($p<0.05$), which is consistent with the results of Fard et al. [25].By occupying the space between the polymers in the alfalfa mucilage water, Avondol nanoparticles and milk thistle essential oil do not allow water molecules to be trapped, and perhaps that is the reason why the moisture percentage of the film has decreased.

Moisture (%)= 19.787 -1.625 *A -3.547 *B (R^2 =0.916 ; AdjR^2 =0.899)

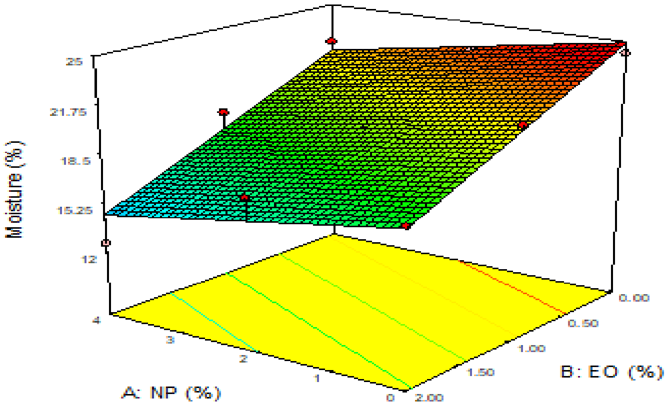

Figure 3-2: The three-dimensional figure of the moisture content of alfalfa seed mucilage film with Avondol nanoparticles and milk thistle essential oil.

3-3- Antioxidant

The effect of the percentage of Avondol nanodrate and milk thistle essential oil on the antioxidant property is shown in Figure 3-3. The mathematical equation shows the relationship between independent variables and antioxidant properties and regression coefficients. According to the figure below, with the increase of Avondol nanoparticles and milk thistle essential oil, the antioxidant properties increased significantly ($p<0.05$). Measuring DPPH radical inhibition is one of the reliable, accurate and easy methods that evaluates the antioxidant activity of plant extracts and essential oils. DPPH radical is a stable free radical with a central nitrogen atom that is reduced and produces a stable DPPH molecule. In the presence of antioxidants, it changes color from purple to yellow. The intensity of this reaction depends on the ability of the antioxidant to donate hydrogen. Finally, the reduction of color reduces the spectrophotometric absorption.The higher the amount of this variable in the film, the higher its desirability. The antioxidant property of milk thistle has been confirmed in various studies[26], some of the compounds of this plant have anti-cancer effects and some enzyme inhibition effects. The presence of fat-reducing compounds and flavonoids with strong antioxidant activity have been reported in this plant. Therefore, according to the antioxidant structure of milk thistle, An increase in the antioxidant property of the film was expected with the increase of milk thistle [27]. Due to its high surface-to-volume ratio, Avendol nanoparticles have the ability to react with free radicals and physically absorb them, and they can easily deactivate free radicals, which is consistent with the results of Fard et al.

Antioxidant activity (%)= 38.878 +5.408 *A +17.849 *B -3.099 *A*B +1,627 *A^2 -7.537*B^2

(R^2 =0.994; AdjR^2 =0.990)

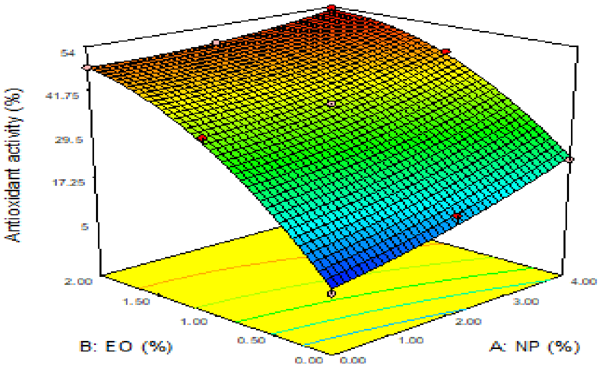

Figure 3-3: The three-dimensional figure of antioxidant property of alfalfa seed mucilage film with Avondol nanoparticles and milk thistle essential oil.

3-4- Solubility

The effect of the percentage of Avondol nanowire and milk thistle essential oil on solubility is shown in Figure 4-3. The mathematical equation shows the relationship between independent variables and solubility and regression coefficients. Solubility indicates the resistance of films against water and for polymers used in food packaging with high water activity or when films are in direct contact with water and also in films that act as food preservatives. is considered an important factor. Generally, the effects of additives on the solubility of films depend on their type, concentration, hydrophobicity and hydrophilicity indices, and it is expected that hydrophilic compounds increase film solubility and hydrophobic compounds decrease it [28].

According to Figure 4-3, the solubility of the film decreased significantly ($p<0.05$) with the increase of Avondol nanoparticles of milk thistle essential oil.As it can be seen from the results, the solubility of nanocomposite films decreases with the increase of Avondol nanoparticles and milk thistle essential oil. Milk thistle essential oil does not allow water molecules to be trapped by occupying the space between the polymers in alfalfa seed mucilage, and maybe that is the reason why Essential oil has reduced the solubility percentage of the film.

Solubility (%)= 32.406 -2.076 *A -5.029 *B
(R^2 =0.926; AdjR^2 =0.912)

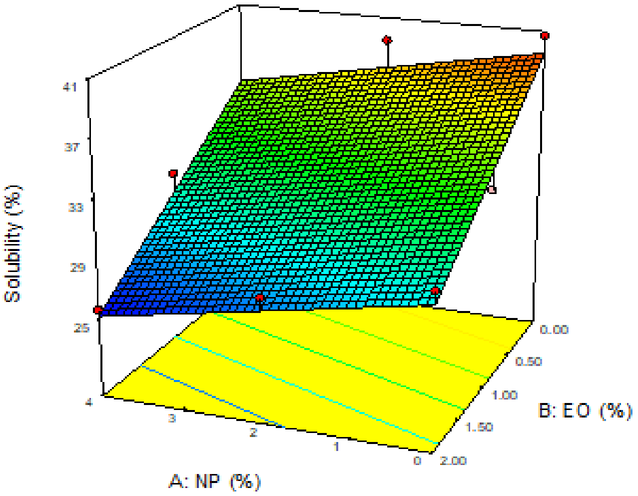

Figure 3-4: The three-dimensional figure of the solubility of alfalfa seed mucilage film with Avondol nanoparticles and milk thistle essential oil.

3-5- Permeability to water vapor

The effect of the percentage of Avondol nanoparticles and milk thistle essential oil on water vapor permeability is shown in Figure 5-3. The mathematical equation shows the relationship between independent variables and permeability to water vapor and regression coefficients. As it is known, the materials used in packaging must have a minimum permeability to water vapor (WVP) in order to prevent the exchange of moisture between the environment and the food. Packed or covered with an external atmosphere.Due to the polar nature of most of their constituent units, most biopolymer films are hydrophilic in nature and have high permeability to water vapor, and this limits their use as packaging materials, according to Figure 5-3, with the increase of nanoparticles Avondol and milk thistle essential oil decreased the permeability of the film to water vapor significantly ($p<0.05$), which is consistent with the results of Ghasemlou et al. [29].Weak resistance to water vapor is considered one of the main defects of polysaccharide films, and due to the hydrophilic nature of polysaccharides, their films have high permeability to water vapor, and this sensitivity to moisture causes changes in their application properties. Polysaccharide films change in different environmental conditions, and as a result, it limits the use of these films in different conditions, especially in high relative humidity.When the nanoparticle is present in the polymer matrix, a water molecule must follow a more complex path than the pure polymer composition, thus reducing the WVP [30]. Also, nanoparticles fill the empty spaces of the polymer film and do not allow water molecules to pass through. Avondol nanoparticles also probably prevent the passage of water molecules by filling the polymer spaces and reduce WVP.

WVP (g/Pa.m.s)= 0.003 -0.001 * B
(R^2 =0.911 ; AdjR^2 =0.848)

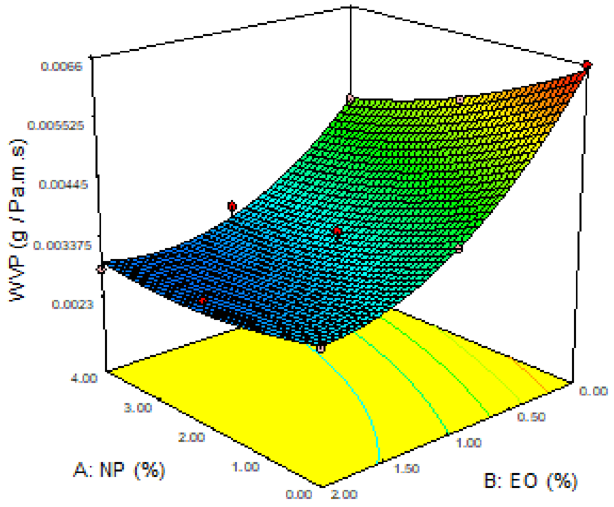

Figure 3-5: The three-dimensional figure of water vapor permeability of alfalfa seed mucilage film with Avondol nanoparticles and milk thistle essential oil.

3-6- Scanning Electron Microscope (SEM)

Using the images obtained from the electron microscope (SEM), useful information can be obtained about the uniformity of the composite film, the presence of voids, the level of dispersion of materials in the matrix, the presence of masses or the orientation of materials in the film substrate. The figure below shows SEM micrographs of alfalfa seed mucilage films with Avondol nanoparticles and milk thistle essential oil. The microscopic image shown of the surface of the production films in the form of alfalfa seed mucilage has more bubble-like shapes, cracks and cracks than other films. The slight roughness on the surface of the film and the ridges in some parts of the film are most likely due to the presence of impurities in the mucilage, also some cracks are probably caused by air bubbles created during the formation of the films [31].By adding milk thistle essential oil to the composition of the film, the surface of the resulting film has become smoother and the amount of cracks on the film surface has been significantly reduced, which indicates the proper combination of two polymers and the creation of proper bonds with each other. So that the least unevenness or roughness can be observed in the film, which can indicate the ability of proper combination and relative compatibility of the two polymers of alfalfa seed mucilage and milk thistle essential oil. And the surface morphology of alfalfa seed mucilage films and avondol nanoparticles is more lumpy compared to alfalfa seed mucilage. This effect of agglomeration, accumulation and non-uniform distribution of avondol nanoparticles used in the preparation of nanocomposite films can be attributed. They don't have proper dispersal properties in water environments and in some parts, accumulation has happened. Avondol nanoparticles were spherically dispersed inside the film.Nanocomposite films with Avondol nanoparticles and milk thistle essential oil reduced the mass on the polymer surface, these results are consistent with the results of Jigen et al [32].

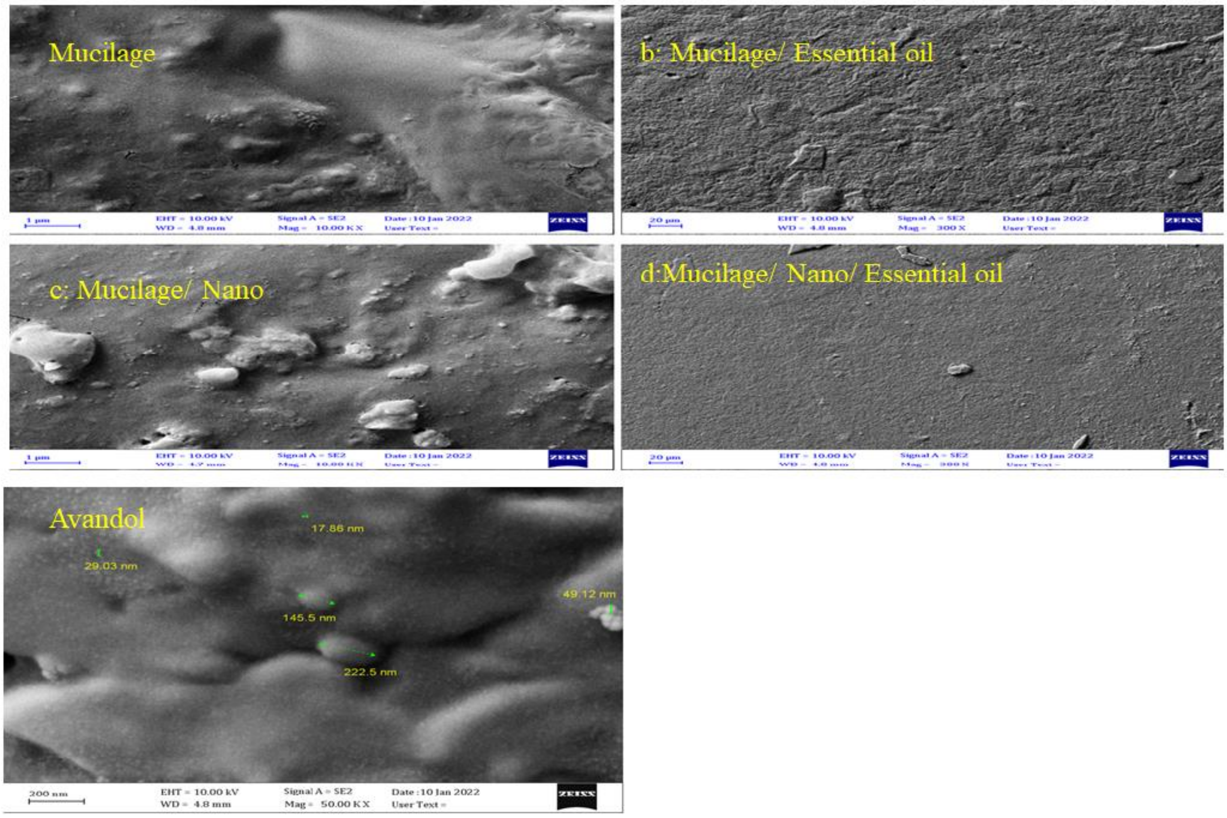

Figure 3-6: Scanning electron microscope images of alfalfa seed mucilage films with Avondol nanoparticles and milk thistle essential oil.

3-7- XRD

Figure 12-3 shows the X-ray diffraction pattern of alfalfa seed mucilage with avondol nanoparticles and milk thistle essential oil. All three materials have different degrees of crystallinity. Alfalfa seed mucilage is an amorphous polymer and it shows five diffraction peaks in the range of θ2 between 19, 20, 21, 24 and 26 degrees, which indicates the amorphous nature of this compound. Alfalfa seed mucilage and milk thistle essential oil have peaks at θ2 equal to 18, 20 and 22 degrees. This pattern shows the semi-crystalline structure of milk thistle essential oil. Alfalfa seed mucilage and Avondol nanoparticles have peaks in the θ2 range of 20 and 21 degrees. Alfalfa seed mucilage with Avondol nanoparticles and milk thistle essential oil has peaks at θ2 of 19, 20 and 21 degrees. This pattern indicates the crystallinity of the nanocomposite film. Also, a broad peak can be seen in the range of θ2 between 19-20 degrees, which confirms the presence of alfalfa seed mucilage.

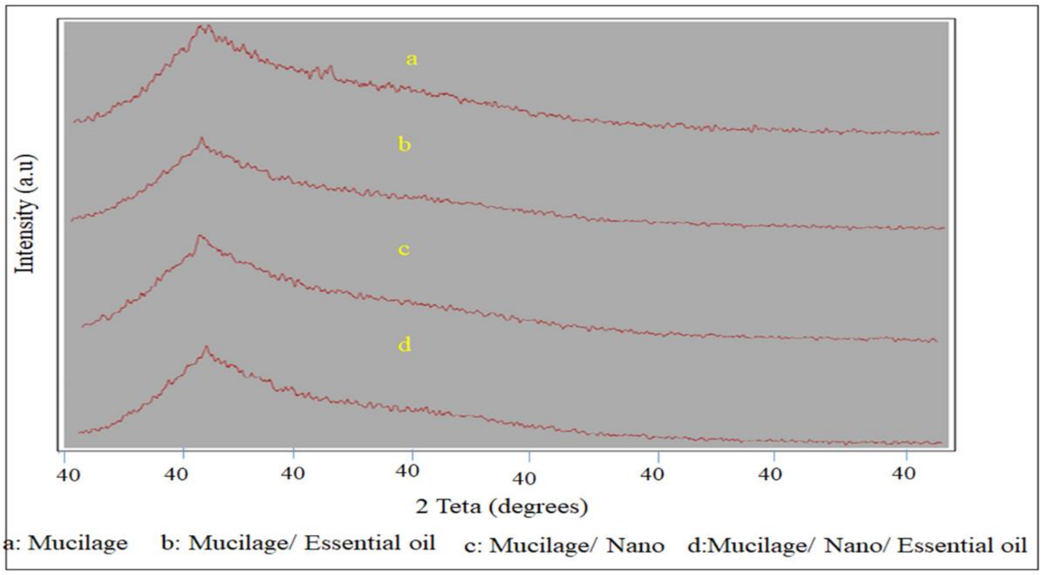

Figure 3-7: X-ray diffraction spectrum (XRD) of alfalfa seed mucilage films with Avondol nanoparticles and milk thistle essential oil.

4 – Conclusion

Despite all the advantages that alfalfa seed mucilage biopolymer has in the production of biodegradable films, but its poor mechanical properties and sensitivity to water are the main obstacles to the widespread use of this biopolymer in the packaging industry. For this reason, alfalfa seed mucilage film with avondol nanoparticles and milk thistle seed oil was used. The results showed that with the increase of Avondol nanoparticles and milk thistle seed oil, the thickness and antioxidant increased. Also, with the increase of Avondol nanoparticles and milk thistle essential oil, the humidity, solubility and permeability to water vapor decreased. Avondol nanoparticles did not change the physical properties of alfalfa seed mucilage and the physical presence of Avondol nanoparticles in the polymer matrix was confirmed. The surface morphology of nanocomposite films was more heterogeneous than alfalfa seed mucilage.

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مقاله علمی_پژوهشی

تولید فیلم موسیلاژ بذر یونجه با نانو ذرات گیاه آوندول (Smyrnium cordifolium Boiss) وروغن بذر گیاه خار مریم و بررسی ویژگی‌های فیزیکی و شیمیایی

فاطمه خاکپور[1]*، ژیار حیدری [2] ، سجاد پیرسا[3]

۱- دانشجوی کارشناسی ارشد گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه ارومیه، ارومیه، ایران

۲- دانشجوی کارشناسی ارشد گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه ارومیه، ارومیه، ایران

۳- استاد گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه ارومیه، ارومیه، ایران

اطلاعات مقاله	چکیده
تاریخ های مقاله : **تاریخ دریافت: ۱۴۰۲/۷/۱۲** **تاریخ پذیرش: ۱۴۰۲/۱۱/۱۴** **کلمات کلیدی:** فیلم خوراکی، موسیلاژ، نانوذرات آوندول، روغن خار مریم. DOI: **10.22034/FSCT.21.147.100.** مسئول مکاتبات: * sevdakhakpour1@gmail.com	هدف از این پژوهش تولید فیلم بر پایه موسیلاژ بذر یونجه با نانوذرات آوندول و روغن خار مریم بود فیلم مرکب موسیلاژ بذر یونجه با نانوذرات آوندول(۰، ۲، ٤ ٪) و روغن بذر خار مریم (۰،۱، ۲ ٪) تهیه شد. خواص فیزیکوشیمیایی فیلم‌ها تهیه شده بررسی شد. مطابق نتایج به دست آمده با افزودن نانوذرات آوندول و روغن بذر خار مریم باعث افزایش ضخامت و آنتی‌اکسیدانی می‌شود. ولی افزایش مقدار نانوذرات آوندول و اسانس خار مریم در فیلم باعث کاهش رطوبت، حلالیت و نفوذپذیری به بخار آب فیلم‌ها شد. تجزیه و تحلیل پراش اشعه ایکس نشان داد که نانوذرات آوندول به طور فیزیکی با پلیمر موسیلاژ بذر یونجه ترکیب شده‌اند. نتایج میکروسکوپ الکترونی روبشی (SEM) نشان داد که مورفولوژی سطح نانوکامپوزیت فیلم نسبت به موسیلاژ بذر یونجه ناهمگن است. در نهایت براساس نتایج حاصله، افزودن نانوذرات آوندول و روغن خار مریم به فیلم‌های خوراکی بر پایه موسیلاژ بذر یونجه سبب بهبود ضخامت، آنتی‌اکسیدانی و SEM ولی باعث تضعیف رطوبت، حلالیت و نفوذپذیری به بخار آب گردید.