Scientific Research

Biodegradable film based on the mucilage of Pansyrak flower modified with licorice and copper sulfate nanoparticles, investigation of physicochemical properties

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ABSTRACT	ARTICLE INFO

The aim of this study. The production of film was from the mucilage of the Paneerak flower modified with licorice (0, 3, 6%) and copper sulfate nanoparticles (0, 2, 4%). It was used to study the properties of thickness, humidity, solubility, permeability to water vapor, color and mechanical properties of the films. The results show that with the increase of licorice and copper sulfate nanoparticles, the thickness of the film increases. Humidity, water vapor permeability and solubility of the film decrease with the increase of copper sulfate nanoparticles and increase with the increase of licorice. Also, with the increase of licorice concentration, the color indices a* increased and the brightness of the layers decreased significantly. The results of the mechanical test showed that with the increase of licorice and copper sulfate nanoparticles, the tensile strength decreased and with the increase of copper sulfate nanoparticles, the elongation at the breaking point increased significantly. Final conclusion: Addition of licorice root and copper sulfate nanoparticles to edible films based on the mucilage of paneerak flower improved the thickness and increased length at the breaking point of the films, and also weakened the tensile strength, humidity, permeability to water vapor and brightness.

1- Introduction

Nowadays, the increase of non-degradable waste and the problems of recycling synthetic packaging materials have prompted researchers to make more efforts to obtain suitable biodegradable materials for packaging [1]. Using biodegradable films can reduce waste disposal problems. The repeatability and edible nature of these films make them suitable for food and non-food packaging. Edible films and coatings can prevent microbial contamination caused by food spoilage. In addition, nanomaterials and food additives such as food additives, antimicrobial antioxidants and dyes can be added to food to expand their applications [2]. Mucilage is a hydrophilic substance, that is, it is either soluble or swellable in water. Mucilages are abundantly found in different parts of plants, such as leaf cells, seed coat, root, bark, and the middle parts of plants. Therefore, natural mucilage has a wide range of acceptability due to its easy availability, biocompatibility, low cost, non-toxicity, and minimal irritation, and is therefore more preferred than artificial mucilage [3]. The leaves of the cheese plant are rich in energy and contain carbohydrates, proteins, reducing sugars and fats. The biological activity of the leaves of this plant can be due to the presence of antioxidant compounds such as: phenolic compounds, vitamins C, E and A, carotenoids, mucilage (vegetable glaze), coumarins, terpenoids and pigments. Mucilages are carbohydrates with a very complex chemical structure and high molecular weight. These substances are amorphous mixtures of polysaccharides that combine with water to create a slimy and viscous substance. These substances dissolve in cold water. Licorice root has many compounds such as different sugars (up to 18%), flavonoids, sterols, amino acids, gum and starch, saponin oil essences. The main saponin (5-ring triterpene) is glycyrrhizic acid or glycyrrhizin with the formula C42H62O. It consists of two glucuronic units and one glyceretic acid molecule (aglycone). The root and rhizome of this plant is about 4000 They have been used medicinally for years and have been registered in the pharmacopoeia of countries such as America, China, and other countries. Licorice is used in traditional Asian and European medicine to treat gastritis, respiratory infections, and peptic ulcers. to be

In traditional Chinese medicine, it is also used in the treatment of hepatitis, tumor growth and heart diseases. In traditional Iranian medicine, licorice plant was used as a treatment for stomach inflammation and antitussive. Glycyrrhizic acid, with its ability to inhibit Helicopylori bacteria, is effective in the treatment of stomach ulcers, gastric mucosal problems, and reducing stomach acid [4]. Licorice also affects the body's endocrine system, and its use may reduce the amount of testosterone in the blood. Anticancer properties have also been reported for this plant [5]. Nanotechnology is one of the most important and fastest growing technology sectors. 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 nanoscale dimensions (between 1 and 100 nm) [6]. Nanocomposites are new alternatives to the traditional methods of improving the properties of polymers. Nanocomposites are currently used for non-alcoholic beverage and food packaging due to their improved thermal properties, strength and conductivity [7]. 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 of the film are other advantages of biopolymer nanocomposites. Metal salts such as cream, iron and copper not only cause variety of colors in dyeing, but also improve the light fastness and washability of many direct dyes. A lot of research has been done in improving the light fastness of direct dyes on textiles, and the use of metal salts, including copper sulfate, is of great commercial importance. In addition, copper sulfate is used as a disinfectant against fungal infections and

to treat bacterial infections [8]. According to the investigations, there has been no research on the effect of licorice root and copper sulfate nanoparticles on edible films prepared from the mucilage of Paneerak flower. The purpose of this research is to investigate the effect of licorice root and copper sulfate nanoparticles in different concentrations on the physical and chemical properties of edible films prepared based on the mucilage of Paneerak flower.

2- Materials and methods

2-1- Materials
The mucilage was extracted using the modified fennel flower, copper sulfate nanoparticles with a purity of 99% and licorice. Was used. Sodium nitrate, 99% methanol, glycerol, silica gel and other chemicals and solutions were obtained from Merck (Germany) and Sigma-Aldridge (USA) and were used without further purification.

2-2- The method of preparing mucilage from Panerak flower
Paneerak flower was mixed with distilled water at a ratio of 1:20 and first exposed to ultrasound at a temperature of 20°C and then placed on a magnetic stirrer at a temperature of 50°C for 2 hours. Then the entire contents were passed through a fabric filter. The remaining flowers on the fabric filter were again mixed with a smaller proportion of distilled water and after stirring for 1 hour, it was passed through the fabric filter. Then the mixture obtained from the previous step was centrifuged for 10 minutes at a speed of 4000 rpm. The mucilage obtained from the previous step was dried using an oven at a temperature of 40 °C and stored in a zipped bag [9].

2-3- Preparation of composite film
First, 2 grams of mucilage of Paneerak flower was mixed in 80 ml of distilled water using a magnetic stirrer at a temperature of 70 degrees Celsius and a speed of 500 rpm. Different percentages of copper sulfate nanoparticles (0, 2, 4 %) and licorice root (0, 3, 6 %) were dissolved in 20 ml of distilled water and added to the mucilage solution. After adding 10% glycerol to the solution, pH was adjusted to 7.5 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 separated and stored in zipped bags [10].
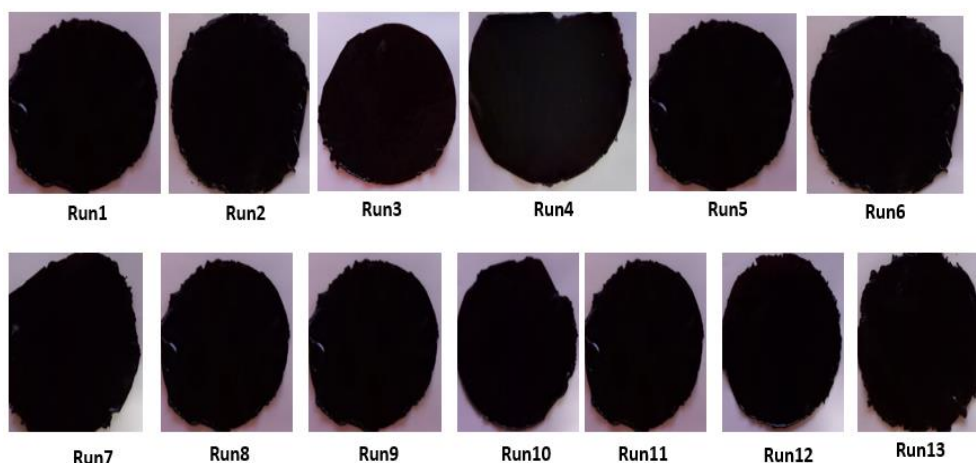

Fig 1: An image of the prepared films

2-4- Features of the film

2-4-1- Thickness
The thickness of the prepared films was measured using a digital micrometer with an accuracy of 0.001 mm. At least five different points in each film were measured and the average numbers obtained were reported and used in different experiments.

2-4-2- Moisture

To check the humidity, first, the films were cut into 3 x 3 cm square dimensions and weighed (digital scale with an accuracy of 0.001 g, Sartori, Germany) (initial weight) and placed in a dryer containing silica gel for 24 hours. They gave. The films were then reweighed (final weight). Then, using the following relationship, the amount of moisture was obtained [10].

Moisture(%) = (Wi- Wf)/Wi x 100

where Wi is the initial weight and Wf is the final weight

2-4-3- Solubility

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 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 the oven at 40 degrees Celsius for 24 hours and weighed again [10].

Solubility (%) = (M1-M2)/(M1) ×100

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

2-4-4- Water vapor permeability (WVP)

Permeability is defined as the passage of molecules through the film and was measured at 25°C and 50% relative humidity gradient according to the ASTM E96-05 method. Films of specific thickness were placed in 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 has 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 were weighed in containers to remove moisture, and then a piece of film was placed inside the lid and closed on the vial. 1 liter of distilled water at 23°C and then the containers were weighed every 24 hours for one week. WVP was calculated based on the following [10]

WVP=(WVTR×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%).

2-4-5- Color characteristics

Color parameters (L, a, b) were measured using a laboratory system (Colorimeter Minolte CR-400) [11]. In order to calibrate the device, a standard white screen was used to measure the color of the films. The factors determined in this device include L or film brightness (0 to 100), a green-red (-80 to 100) and b yellow-blue (-80 to 70) [10].

2-4-6- Mechanical properties

Tensile test was done with tissue analysis machine. The force was 200 newtons, the distance between the two jaws of the machine was 40 mm, and the speed of movement was 50 mm/min. Then, the tensile properties of the layers, including tensile strength (TS) and percentage of elongation at break (%E) were evaluated based on the ASTM D882-12 standard method [10].

2-5- Statistical study

In this study, response surface statistical method and central composite statistical design were used to investigate the effect of two variable factors licorice and copper sulfate nanoparticles 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: Table of prepared films

Film	A: CuSO4 NP (%)	B: licorice (%)
F1	2	3
F2	4	6
F3	0	0
F4	0	6
F5	2	3

F6	4	**3**
F7	2	**0**
F8	2	**3**
F9	2	**3**
F10	0	**3**
F11	2	**3**
F12	4	**0**
F13	2	**6**

3. Results and Discussion

3-1- thickness

The 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 the packaging films affects the mechanical properties and permeability to different gases, and for this reason, it is very important. . According to figure (1-3), with the increase of licorice up to (6%) and copper sulfate nanoparticles up to (4%), the thickness increased significantly (p<0.05). The overall average film thickness values are 0.2-0.3 mm. The reason for the increase in the thickness of the films with the addition of licorice and copper sulfate nanoparticles 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 sum of these The changes cause an increase in the thickness of the production films [13]. which is consistent with the results of Davachi et al. [14].
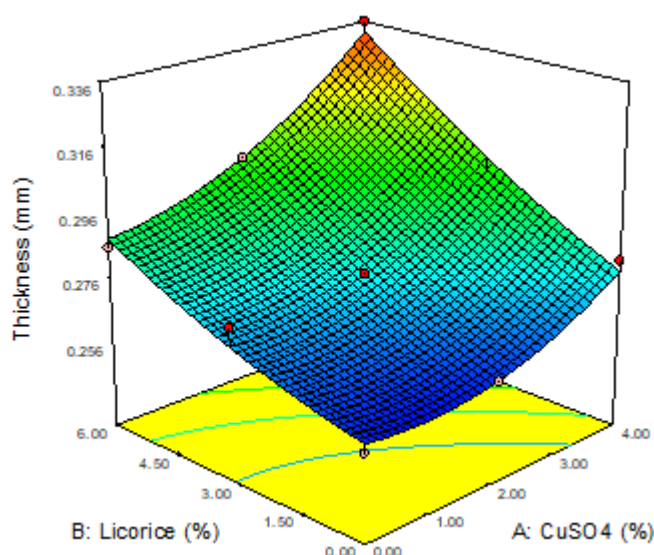
Thickness (mm)= 0.277 +0.016 * A +0.021 * B +0.006 *A*B +0.008 * A^2 +0.003 *B^2

(R^2 =0.977 ;   AdjR^2 =0.961)

A: CuSO4   B: Licorice

Figure 3-1: Three-dimensional figure of the thickness of the mucilage film of the flower modified with licorice and copper sulfate nanoparticles.

3-2- Moisture

The effect of licorice and copper sulfate nanoparticles on humidity is shown in Figure 2-3. Mathematical equation (2) shows the relationship between independent variables and humidity and regression coefficients. Solubility refers to the resistance of layers to water and is an important factor for polymers used in food packaging with high water activity or when the layers are in direct contact

with water, as well as in films that act as food preservatives. Additives on the solubility of films depend on their type, concentration, hydrophobicity and hydrophilicity indices, and it is expected that hydrophilic compounds increase the solubility of films and aqueous compounds. [15]. The polymer used in food packaging should be as resistant to moisture as possible to prevent the appearance of undesirable properties caused by moisture penetration into food. According to Figure (2-3), with the increase of licorice, the humidity increased significantly ($p<0.05$), but with the increase of copper sulfate nanoparticles, the humidity decreased significantly. The amount of moisture absorption depends on the amount of empty spaces available for penetration Water molecules and the degree of hydrophilicity of the polymer depend. Licorice is naturally hydrophilic and increases the amount of moisture absorption in the film [16]. They have hydroxyl groups in their structure and therefore are considered hydrophilic materials. The creation of strong hydrogen bonds between the cheese creates a very

Moisture (%)= 18.83 -7.52 *A +3.16 *B

($R^2 =0.807$; $AdjR^2 =0.769$)

compact and strong structure that limits the absorption and penetration of water molecules into this structure. The absorption of moisture in a film is due to the hydrophilic property of that polymer or biopolymer and then to the existence of holes. and the void spaces between the chains depend. According to the three-dimensional shape of the moisture content of the film, licorice increases the moisture content of the film, but copper sulfate nanoparticles decrease the film moisture content. Copper sulfate nanoparticles create electrostatic forces through oxygen atoms. , engage the H+ and OH- groups with the cheese chain and block the entry of H2O molecules into the polymer structure. Reduces humidity. Also, by placing copper sulfate nanoparticles in the empty spaces of the film, they reduce the spaces required for the placement of water molecules. Due to the gelling properties of licorice and its ability to absorb water, by adding licorice, the film increases the ability to absorb and retain water in its structure [17]. It is consistent with the results of Naftchi et al. [18].
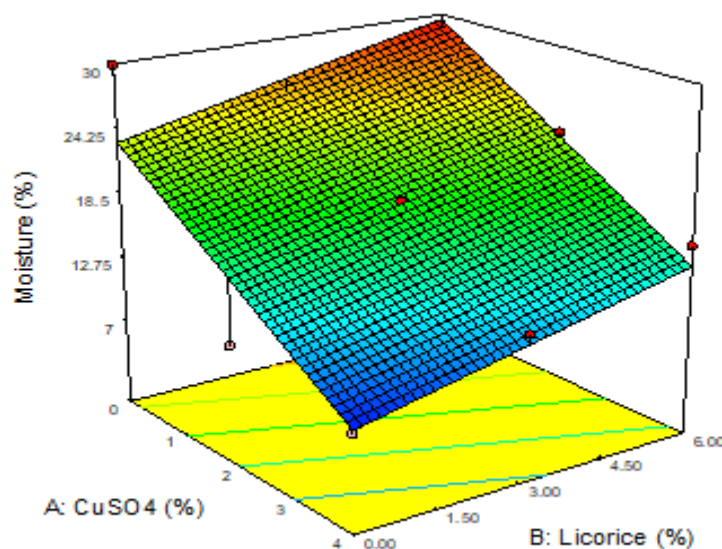

Figure 3-2: The three-dimensional shape of the moisture content of the mucilage film of the flower modified with licorice and copper sulfate nanoparticle.

3.3 Solubility
The effect of licorice percentage and copper sulfate nanoparticles on solubility is shown in

Figure 3-3. Mathematical equation (3) shows the relationship between independent variables and coefficients of solubility and regression.

As is known, water solubility is an important factor in defining possible applications for composite biopolymer films and food packaging. Most biopolymers in their natural state are sensitive to moisture and soluble in water, which can be reduced by various methods such as including fat components from food sources in the film, protein/fat composite, and using nanoparticles by creating crosslinks. Low solubility in water is a desirable feature for food packaging. Because packaging films with such characteristics can be resistant to conditions with high humidity. According to Figure 3-3, with the increase of licorice, the solubility increased significantly, but with the increase of copper sulfate, the

solubility of the film first increased and then decreased significantly ($p<0.05$). As can be seen from the results, the solubility of nanocomposite layers increases with the increase of licorice and the decrease of copper sulfate. Since mucilage is hydrophilic, it is soluble in water. Licorice is a hydrophilic substance and easily reacts with water and forms a gel, which is a suitable interaction with water, which causes dissolution by adding licorice. Also, the presence of hydroxyl groups and high solubility are attributed to hydrophilic softeners (glycerol) that have been added to the films to create sufficient flexibility [19]. Tang's results are consistent with these results [20].
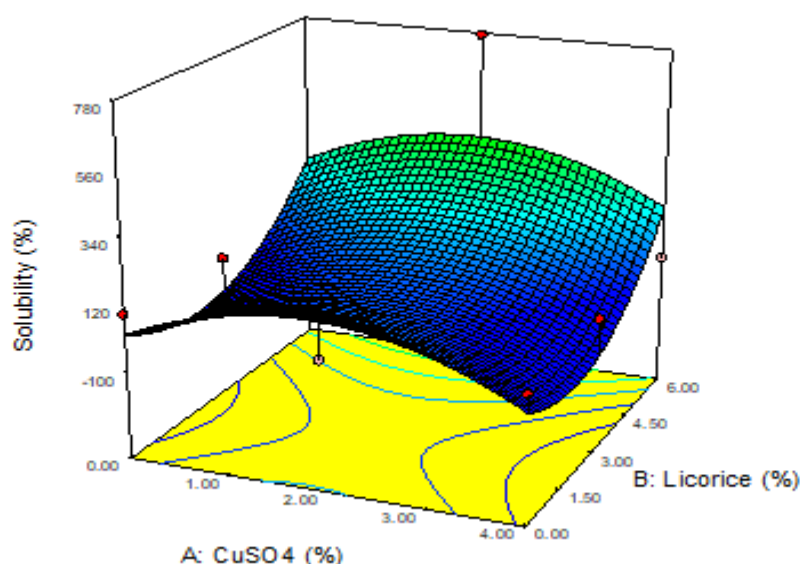
Solubility (%)= $112.053 -21.298 *A +124.401 *B +6.72 *A*B -164.547 *A^2 +178.062 *B^2$
($R^2 =0.457$; AdjR$^2 =0.069$)

Figure 3-3: The three-dimensional figure of the solubility of the mucilage film modified with licorice and copper sulfate nanoparticles.

3-4- Permeability to water vapor

The effect of licorice percentage and copper sulfate nanoparticles on water vapor permeability is shown in Figure 4-3. The following mathematical equation shows the relationship between independent variables and water vapor permeability and regression coefficients. As it is known, the materials used in the packaging must have a minimum water vapor permeability (WVP) to prevent the exchange of moisture between the environment and the food. Product packaged or covered with outer space due to the polar

nature of most of their constituent units, most polymer films are hydrophilic in nature and have high permeability to water vapor, which limits their use as packaging materials. According to Figure (3-4), with the increase of licorice, the permeability of the film to water vapor increased significantly, but with the increase of copper sulfate nanoparticles, the permeability of the film to water vapor increased. decreased significantly. Weak resistance to water vapor is one of the main defects of polysaccharide layers, and due to the hydrophilicity of polysaccharides, their layers have high permeability against water

vapor, and this sensitivity to moisture changes the functional properties of polysaccharide layers. have different environmental conditions and thus limit the use of these films in different conditions, especially in high relative humidity. When the nanoparticle is present in the polymer matrix, the water molecule has to follow a more complex path than in the pure polymer composition, thus

reducing the WVP [21]. Also, nanoparticles fill the empty spaces of the polymer layer and do not allow water molecules to pass through. Also, copper sulfate nanoparticles probably prevent the passage of water molecules by filling the polymer spaces and reduce the WVP. Licorice has gelling properties and this increases the permeability of the film to water vapor.
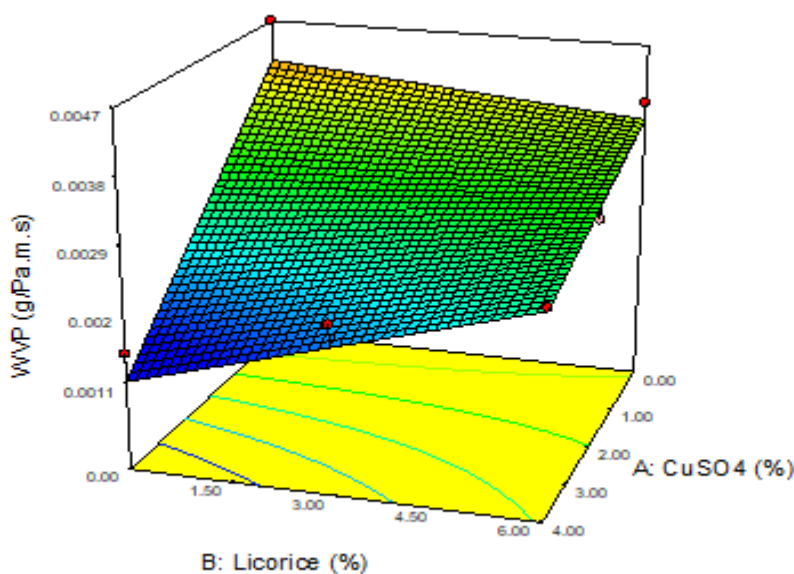
WVP (g/Pa.m.s)= 0.0028 -0.001 *A +0.0002 *B +0.0005 *A*B

$(R^2 =0.843;\ AdjR^2 =0.790)$

Figure 3-4: The three-dimensional figure of the water vapor permeability of the mucilage film of Panerak flower modified with licorice and copper sulfate nanoparticle.

3-5- (L, a, b)

The effect of licorice and copper sulfate nanorate on L,a,b is shown in Figure 3-5. The mathematical equation shows the relationship between the independent variables L, a, b and the regression coefficients. Research in the field of food color and factors affecting it is an important part of research in the field of food [22]. The color parameter L provides a measure of lightness/darkness. Color parameter a provides a measure of red/greenness, color parameter b provides a measure of yellowness/blueness. L values range from 0 to 100 as an indication of dark to light. The higher the amount of the compound, the brighter it is. According to the figures, with the increase of licorice and copper sulfate nanoparticles, the amount of L decreases. The

decrease of L is due to the purple licorice and blue colors of copper sulfate nanoparticles that decrease the brightness of the films. Based on numerous studies, there are different results related to the effect of different nanoparticles on the color indices of nanocomposite films, which can be due to the different properties and characteristics of nanoparticles. With the increase of licorice, the amount of a increased significantly (p<0.05). With the increase of copper sulfate nanoparticles, the amount of a decreased. The reason for the increase of factor a with the increase of licorice is because of the purple color of licorice. Also, reducing a with the increase of copper sulfate nanoparticles due to its blue color. LT is. With the increase of licorice, the amount of b did not change, but with the increase of copper sulfate nanoparticles, the amount of b

decreased significantly (p<0.05). The reason for this reduction is due to the blue color of

copper sulfate nanoparticles [23].
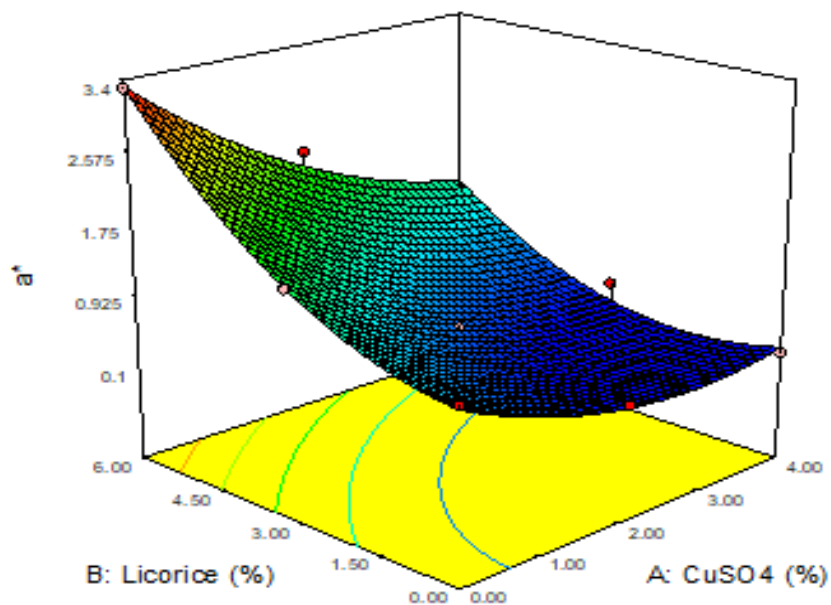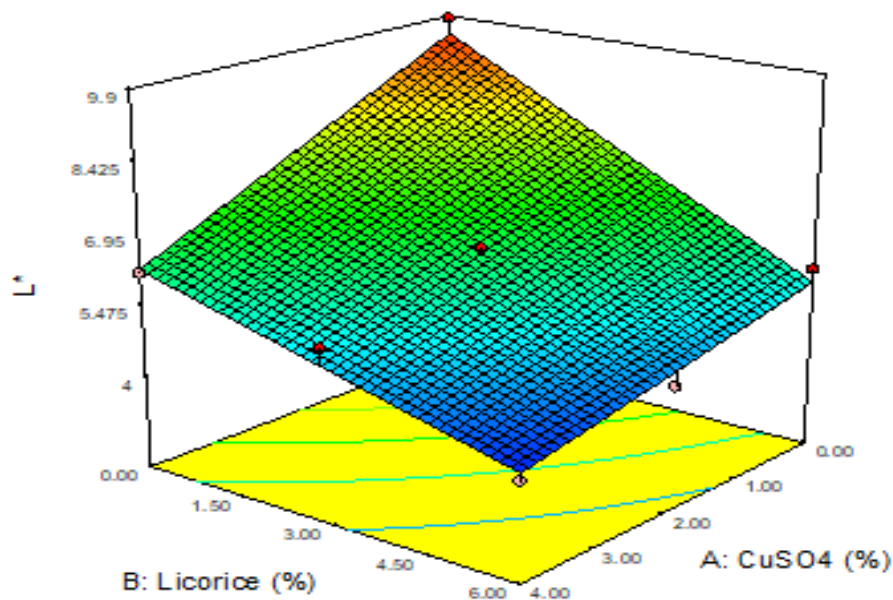
$L* = 6.398 -1.147 *A -1.486 *B +0.475 *A*B$

$(R^2 =0.958; \text{AdjR}^2 =0.944)$

$a* = 0.607 -0.652 *A +0.839 *B -0.352 *A*B +0.356 *A^2 +0.518 *B^2$

$(R^2 =0.988; \text{AdjR}^2 =0.980)$

$b* = 0.145 -3.135 *A -0.028 *B +0.323 *A*B +2.605 *A^2 +0.741 *B^2$
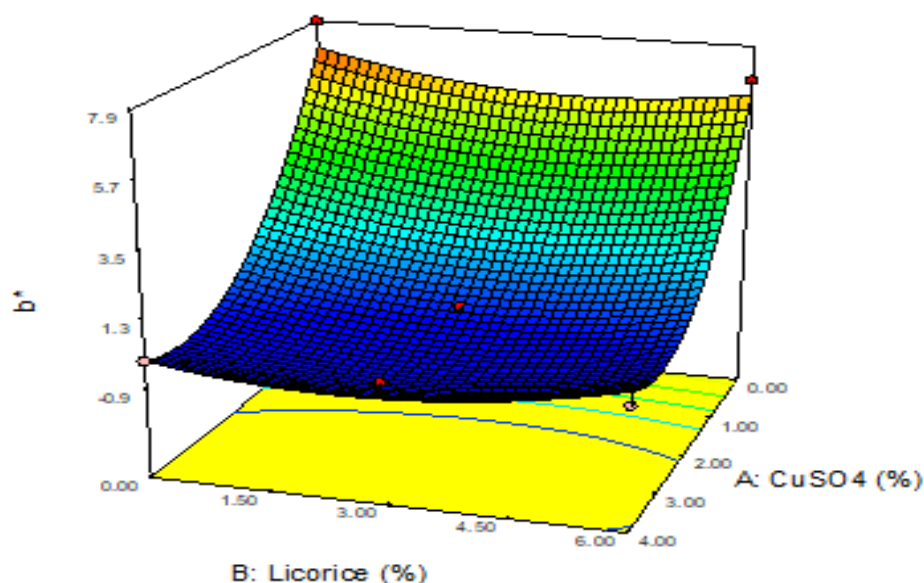
$(R^2 =0.954; \text{AdjR}^2 =0.917)$

Figure 3-5: L, a, b three-dimensional figure of mucilage film modified with licorice and copper sulfate nanoparticles

3-6- Mechanical properties of manufactured composites (tensile resistance, elongation percentage)

One of the most important features of a synthetic film is its mechanical properties. This feature is affected by the chain length and molecular weight of macromolecules, as well as the length and position of the side chains in it. These properties affect the biopolymer's ability to form intermolecular bonds. The more and stronger these bonds are, the greater the integrity of the structure and, as a result, the higher the mechanical resistance. Optimizing the mechanical properties of edible films is important from various aspects, including the high strength of the film, which prevents mechanical damage such as perforation due to stress on the packaging material, and as a result, its inhibitory properties are reduced. Better to retain gases and moisture, the high flexibility of the film makes it conform to the shape of the food item without breaking and can easily be used as a coating. Mechanical characteristics are among the important factors in choosing the type of food packaging. Packaging should be able to protect against physical stress and changing environmental conditions during storage until consumption. Tensile strength represents the maximum tensile stress a film can withstand, percent elongation represents the ability of a film to

stretch, it is the maximum change in length of a test specimen before breaking, and elastic modulus is a measure of film stiffness. Food packaging generally requires high stress with deformation based on intended use. TS is the maximum tensile stress that the sample experiences during the tensile test [24]. In Figure 6-3, the results of tensile strength and elongation percentage at the break point of the produced films can be seen, with the increase of licorice and copper sulfate nanoparticles, the tensile strength decreases significantly ($p<0.05$). The decrease in tensile strength with increasing licorice can be attributed to the gelling properties and strong hydrogen bonds between licorice chains. The further increase of nanoparticles due to the accumulation of nanoparticles leads to the weakening of mechanical properties because nanofillers have a high energy level and tend to create areas rich in nanofillers and weakness in the structural content of the polymer. Agglomeration of nanofillers reduces the positive effect of fillers in the polymer matrix and behaves in a way that causes defects in the increase of tension and final tearing of the films. Due to the very high specific surface area provided by nanoparticles, the surface interactions of the matrix filler play an essential role in the mechanical properties of nanocomposite films [25]. In high percentages, nanoparticles are dispersed in a larger volume of the polymer matrix and transfer the stress

concentration to a larger space, and as a result, more areas participate in the deformation process [26]. Distributed particles with proper bonding between the filler and the matrix allow the effective transfer of stress through the shear mechanism from the matrix to the particles. This can effectively increase the

efficiency of mechanical properties and increase the strength of nanocomposite films, but with the increase of copper sulfate nanoparticles, the elongation at the breaking point increased, but with the increase of licorice, the amount of elongation at the breaking point is constant.

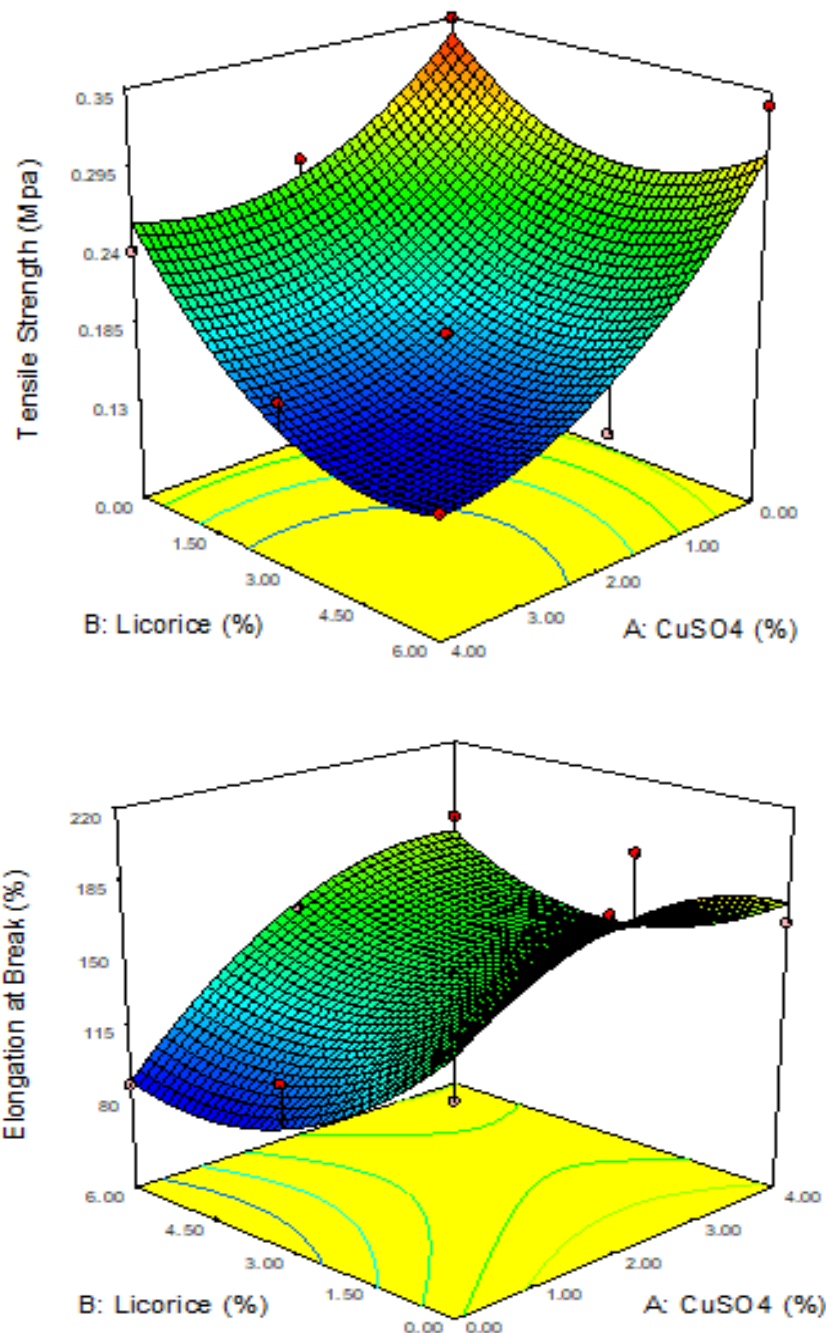Tensile strength (Mpa)= 0.175 -0.061*A -0.037*B -0.021*A*B +0.038*A^2 +0.047 *B ^2

(R^2 =0.903; AdjR^2= 0.834)

Elongation at Break (%) = 144.697 +27.345 *A -16.543 *B +15.448 *A*B -25.544 *A^2 +27.965 *B ^2

(R^2 =0.836; AdjR^2 =0.719)

Figure 3-6: The three dimensional figure of tensile strength, the percentage of elongation of the mucilage film of Paneerak flower modified with Shirinbian and copper sulfate nanoparticles.

4- Conclusion

Despite all the advantages that mucilage biopolymer has in the production of biodegradable films. But the weak mechanical properties and its sensitivity to water are the main obstacles to the widespread use of this biopolymer in the packaging industry. For this reason, in this research, mucilage film modified with licorice and copper sulfate nanoparticles was produced. The results showed that with the increase of licorice and copper sulfate nanoparticles, the thickness of the film increased. Moisture, permeability and solubility decreased with increasing copper sulfate nanoparticles and increased with increasing licorice. With the increase of licorice and copper sulfate nanoparticles, the tensile strength decreased and with the increase of copper sulfate nanoparticles, the elongation at the breaking point increased dramatically. In general, according to the investigations, the mucilage films containing copper sulfate nanoparticles can be used as active packaging in the food industry.

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مقاله علمی‌ـ‌پژوهشی

فیلم زیست‌تخریب پذیر برپایه موسیلاژ گل پنیرک اصلاح شده با شیرین بیان و نانو ذرات سولفات مس: بررسی خواص فیزیکوشیمیایی

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اطلاعات مقاله	چکیده
	هدف از این پژوهش، تولید فیلم از موسیلاژ گل پنیرک اصلاح شده با شیرین‌بیان ۰، ۳، ۶ ٪ (وزنی/ وزنی) و نانوذرات سولفات مس ۰، ۲، ٤ ٪ (وزنی/ وزنی) بود. برای مطالعه خواص ضخامت، رطوبت، حلالیت، نفوذپذیری به بخار آب، رنگی و خواص مکانیکی فیلم‌ها مورد استفاده قرار گرفت. نتایج نشان می‌دهد که با افزایش شیرین‌بیان و نانوذرات سولفات مس، میزان ضخامت فیلم افزایش می‌یابد. رطوبت، نفوذپذیری بخار آب و حلالیت فیلم با افزایش نانوذرات
	سولفات مس کاهش و با افزایش شیرین‌بیان افزایش می‌یابد. همچنین با افزایش غلظت شیرین‌بیان، شاخص‌های رنگی a^*، افزایش یافته و روشنایی لایه‌ها به‌طور معنی‌داری کاهش یافت. نتایج بافت مکانیکی نشان داد که با افزایش شیرین‌بیان و نانوذرات سولفات مس، مقاومت کششی کاهش و افزایش نانوذرات سولفات مس ازدیاد طول در نقطه شکست به طور چشمگیری افزایش یافت. نتیجه‌گیری: افزودن ریشه شیرین‌بیان و نانوذرات سولفات مس به
	فیلم‌های خوراکی بر پایه موسیلاژ گل پنیرک سبب بهبود ضخامت و ازدیاد طول در نقطه شکست فیلم‌ها، همچنین موجب تضعیف مقاومت کششی، رطوبت، نفوذپذیری به بخار آب و روشنایی گردید.