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Preparation of antioxidant and antibacterial film based on mucilage of Panerak flower modified with licorice root and copper sulfate nanoparticles

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ABSTRACT

The aim of this research was to produce a film based on the mucilage of Paneerak flower modified with licorice root and copper sulfate nanoparticles. The D-optimal statistical scheme was used to study the antioxidant, antimicrobial, X-ray diffraction (XRD), Fourier transform infrared (FTIR), scanning electron microscopy (SEM) and thermal decomposition (DSC) properties of the films. The results showed that with the increase of licorice root and copper sulfate nanoparticles, the antioxidant activity of the film increased significantly ($p<0.05$). The results of the antimicrobial activity of the prepared films showed that the addition of licorice and copper sulfate nanoparticles caused the antimicrobial activity of the film against Escherichia coli and Staphylococcus aureus. X-ray diffraction analysis shows that copper sulfate nanoparticles are physically combined with the mucilage polymer of Paneerak flower and it reduces the crystal structure.Fourier transform infrared (FTIR) results confirm the physical presence of copper sulfate nanoparticles in the polymer matrix. FTIR results also showed weak electrostatic interactions between film components and composites. The results of the scanning electron microscope (SEM) show that the surface of the films containing copper sulfate nanoparticles and licorice root are more heterogeneous than the mucilage of Paneerak flower. Shirin-bian and copper sulfate nanoparticles were able to delay the thermal decomposition of the mucilage of Paneerak flower and increase the thermal stability of the mucilage film. Finally, based on the results, the addition of copper sulfate nanoparticles and licorice root to edible films based on the mucilage of paneerak flower improved antimicrobial, antioxidant, DSC and SEM.

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 resources, they have high recycling capability, they have mixing capability and they are biodegradable [1]. 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 [2].The Malva parviflora plant with the scientific name (Malva parviflora L) from the Malvaceae family is a herbaceous plant of one or more years that is native to North Africa, Europe and Asia. . 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. Mucilage forms Mucilages are carbohydrates with a very complex chemical structure and high molecular weight. These substances are amorphous mixtures of polysaccharides that form a slimy and sticky substance with water. and form a gel substance and make colloidal solutions dissolved in warm water [3].Also, due to the presence of active compounds such as flavonoids, the extract of this plant has antibacterial properties and causes the reduction or non-growth of all types of bacteria such as Escherichia coli, Staphylococcus aureus, Bacillus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Salmonella [4] .The use of edible cheese films is limited due to inherent sensitivity to humidity and poor mechanical properties, especially in humid environments. One of the methods presented to overcome the mentioned limitations is the use of polymers for the purpose of film formation, in this way, the best functional characteristics of each composition can be used. Nanocomposites are polymers in which different organic or inorganic compounds with different planar and spherical shapes are used as fillers in nano dimensions [5]. Films obtained from the combination of nanomaterials and biopolymers, or the so-called biopolymer nanocomposites, show more desirable practical 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. Such as different sugars (up to 18%), flavonoids, sterols, amino acids, gum and starch, oil essences, saponins [6]. 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) [7]. Nanocomposites are new alternatives to the traditional methods of improving the properties of polymers.Nanocomposites are currently used for packaging non-alcoholic beverages and food due to their improved thermal properties, resistance and conductivity [8]. Nanocomposites are polymers in which various organic or inorganic compounds with various 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 desirable practical 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 [9]. 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 modified cheese flower was purchased from Attari (Ajabshir), copper sulfate nanoparticles with a purity of 99% from the company brand (Germany) and licorice root were purchased from Attari (Ajabshir). Sodium nitrate, 99% methanol, glycerol, silica gel, and other chemicals were purchased from Merck (Germany) and Sigma-Aldridge (USA) brands and were used without further purification.

2-2- Method of preparation of mucilage from modified Paneerak flower

Paneerak flower was mixed with distilled water at a ratio of 1 to 20 and was first ultrasonicated at 20°C and then placed on a magnetic stirrer at 50°C for 2 hours. Then, in order to separate the mucilage from the flower cheese, 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 degrees Celsius and stored in a zipped bag [10].

2-3- Preparation of composite film

First, 2 grams of mucilage of Panerak 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 %) (w/w) and licorice (0, 3, 6 %) (w/w) were dissolved in 20 ml of distilled water and added to the mucilage solution became. After adding 30% glycerol to the solution, the pH was adjusted using 0.1 normal 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 [11].

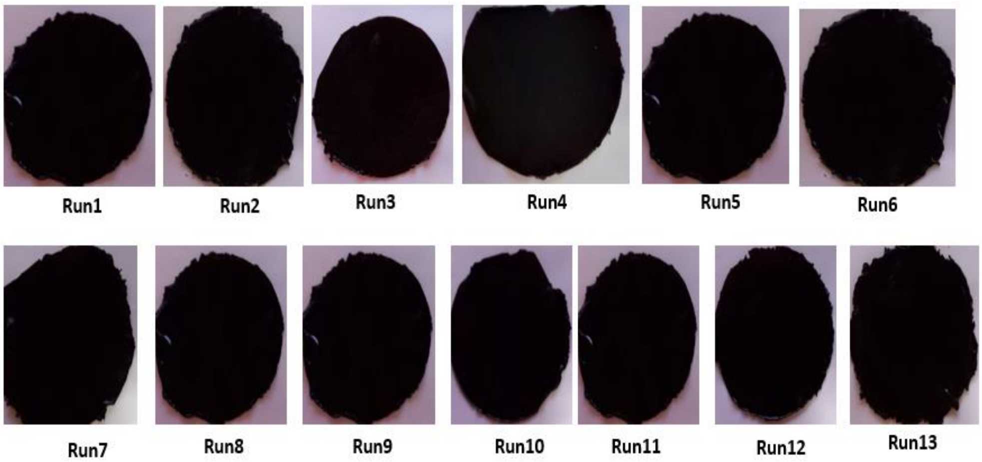

Fig 1: An image of the prepared films

2-4- Tests to check the properties of the prepared films

2-4-1- Antioxidant property

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] methanolic solution. The mixture was well dissolved in a vortex at 2000 rpm for 1 minute. After 30 minutes of storage in a dark place, the absorbance at 517 nm was calculated using a T60 UV-Visible spectrophotometer (Spectrophotometer made in Germany) with the following formula [11].

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

Ab: Absorption rate of the control sample

As: sample absorption rate

2-4-2- Investigation of antimicrobial properties

In the agar diffusion method, films with a diameter of 15 mm were cut into disks and placed on Mueller Hilton agar culture medium containing Escherichia coli and Staphylococcus aureus. Then the plates were kept in a greenhouse at 37°C for 24 hours. Then the diameter of the lack of growth was measured with a caliper [11].

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

X-ray diffraction (XRD) is used to investigate the crystal structure of films. The composite film was done. XRD diffraction patterns (X'Pert Pro Panalytical Netherlands) were obtained through a diffractometer using Cu Ka radiation (1.54 Å) in the range of 2θ=40-40 and a scan step time of 53 seconds [11].

2-4-4- Fourier transform infrared spectroscopy (FTIR)[2]

The changes of functional groups were investigated with the help of infrared Fourier transform spectroscopic device. In the resulting spectrum of the material, the functional groups in the chemical structure appeared as peaks in specific areas. The spectrum of FTIR (Spectrum Two, Perkin Elmer) made (USA) was checked in the transmission mode using a spectrophotometer in the range of 4000-500 cm-1 wave number and 0.5 cm-1 resolution [11].

2-4-5- Scanning electron microscope (SEM)[3]

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

1. 2-2-Diphenyl-1-picrylhydrazyl

2.Transform infrared spectroscopy

3- Scanning electron microscope

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 [11].

2-4-6- Measurement of thermal properties (DSC)

DSC machine (Netzsch 200 F3 model made in Germany) was used in Urmia University to measure the thermal properties of the films. The accuracy of the device is 0.1 °C and it measures the transferred heat with an accuracy of 0.1 w/mg. The device was calibrated by indium and silver. Empty aluminum container was used as reference and nitrogen as atmosphere. Samples weighing 0.04 grams were heated at a speed of 22 Ċ/min in the temperature range of 0 to 240 degrees Celsius for 25 minutes. From the obtained thermal pattern, the melting temperature (Tm), the glass transition temperature (Tg) was determined [11].

2-5- Statistical study

In this study, the response surface statistical method and the central composite statistical design were used to investigate the effect of two variable factors, sugar and copper sulfate nanoparticles, on the physicochemical and structural 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

A : CuSO4 (%)	B: Licorice (%)
2	3
4	6
0	0
0	6
2	3
4	3
2	0
2	3
2	3
0	3
2	3
4	0
2	6

3. Results and Discussion

3-1- Antioxidant

The increase in the percentage of licorice and copper sulfate nanoparticles on the antioxidant property is shown in Figure 1-3. The mathematical equation shows the relationship between independent variables and antioxidant properties and regression coefficients. According to the figure below, with the increase of licorice and copper sulfate nanoparticles, the antioxidant properties increased significantly ($p<0.05$). Increasing the percentage of licorice and copper sulfate nanoparticles greatly increases the antioxidant property of the film. 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, considering the antioxidant structure of licorice, increasing the antioxidant properties of the film. It was expected with increasing licorice [12].Due to its high surface-to-volume ratio, copper sulfate nanoparticles can easily deactivate free radicals by reacting with free radicals. Hosseini et al. (2021) showed that the use of magnesium oxide nanoparticles in nanobiocomposite films increases antioxidant activity, which is consistent with the present results [13].

Antioxidant activity (%)= 9.504 +3.772 *A +4.096 *B -0.992 *A*B +2.474 *A^2 +2.442 *B ^2

(R^2 =0.8709; AdjR^2 =0.881)

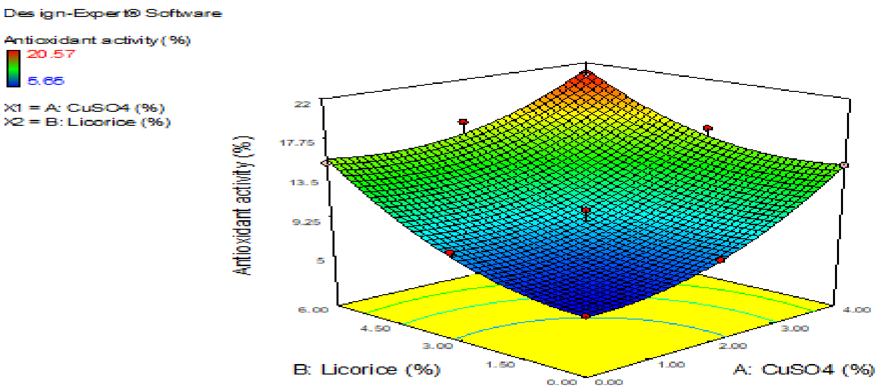

Figure 3-1: Counterplot of the antioxidant property of Panerak flower mucilage film modified with Shirin Bayan and copper sulfate nanoparticle

3-2- Antimicrobial

Antimicrobial properties of licorice film and copper sulfate nanoparticles are shown in Figure 3 2. As it is clear in the figure, the activity of Escherichia coli and Staphylococcus aureus decreases significantly ($p<0.05$) with the increase of copper sulfate nanoparticles and licorice root. The said films prevented the growth of Escherichia coli and Staphylococcus aureus. Staphylococcus aureus is one of the most important bacteria in food. Antibiotic resistance is one of the problems that is very important to eliminate these bacteria, so nanomaterials can be used as suitable materials to inhibit and eliminate these bacteria [14]. It is clear that by increasing the amount of licorice and copper sulfate nanoparticles, the diameter of the growth halo has increased.Therefore, it can be said that films supported with these compounds can act as an active packaging against microorganisms. In general, the inhibitory property of films containing licorice and copper sulfate nanoparticles against Gram-positive microorganisms (s. aureus) Gram negative types (E.coli) are more. The cause of this phenomenon is the difference in the cell wall structure of these microorganisms. The main composition of the cell wall of gram-positive bacteria is peptidoglycan with a small amount of protein; However, the cell wall of gram-negative bacteria is more complex despite being less thick and contains different polysaccharides, proteins and lipids in addition to peptidoglycan. Also, the cell wall of Gram-negative bacteria has an outer membrane that covers the outer surface of the wall. The combination of these factors increases the resistance of Gram-negative bacteria compared to Gram-positive bacteria [15].Also, nanomaterials delay bacterial cell adhesion and biofilm formation, which prevents a group of bacteria from stabilizing and multiplying [16]. Khakpour et al. (2023), the results of the investigation of antibacterial properties showed that increasing the concentration of chromium oxide nanoparticles and lycopene pigment increases the antibacterial property of composite samples, which is consistent with the present results [11].

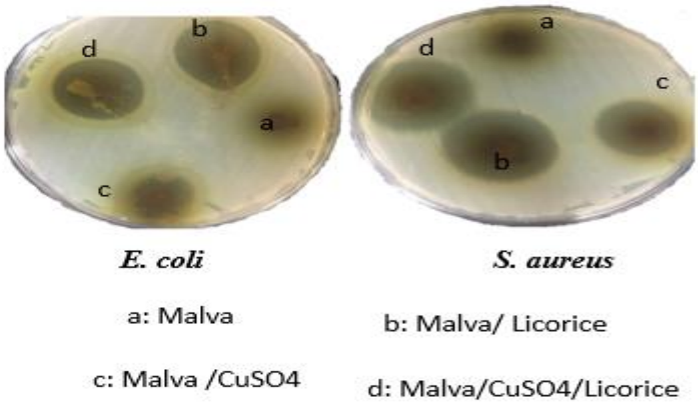

Figure 3-2: The diameter of the lack of growth halo (mm)

3-3- X-ray diffraction (XRD)

X-ray diffraction patterns were used in order to investigate the crystal structure of the mucilage film modified with licorice and copper sulfate nanoparticles. The spectrum related to the mucilage of pure Paneerak flower has three peaks. In the spectrum of Paneerak flower mucilage, the θ2 peaks observed at 28.6240°, 40.6818° and 50.0584 are specific to mucilage, and the degree of crystallinity of Paneerak flower mucilage is good, and the height of these peaks confirms the crystal structure of Paneerak flower mucilage. slow Therefore, it can be classified as a crystalline biopolymer. It has three peaks in the range related to the mucilage of Paneerak flower / licorice. θ2 peaks observed at 28.5763°, 40.5755° and 50.3322° are specific to licorice. Adding licorice root to the films, the intensity of the diffraction peak compared to the peak intensity of the mucilage film of paneerak flower (control film) changed a lot. has not done.There are three peaks in the related spectrum of Paneerak flower mucilage/copper sulfate nanoparticles. θ2 peaks observed at 30.0022, 30.0061 and 31.0047 are specific to copper sulfate nanoparticles, also these peaks are usually attributed to the amorphous phase of proteins, also the appearance of a peak at θ2 can be The reason is the crystalline part of the polymer. In this way and according to the shape, the structure of the film obtained from the mucilage of Panirak flower and added variables can be considered as semi-crystalline. There are three peaks in the mucilage spectrum of Paneerak flower / licorice / copper sulfate nanoparticles. θ2 peaks observed at 29.0022, 30.0061, and 32.0021 are specific to licorice and copper sulfate nanoparticles. This spectrum is largely similar to the spectrum of copper sulfate nanoparticles, which have a semi-amorphous structure.The results show that adding copper sulfate nanowire reduces the crystalline properties of the film. Maidanji et al. (2022) showed that adding TiO2-Ag to the layers reduces the crystalline properties of the film [17].

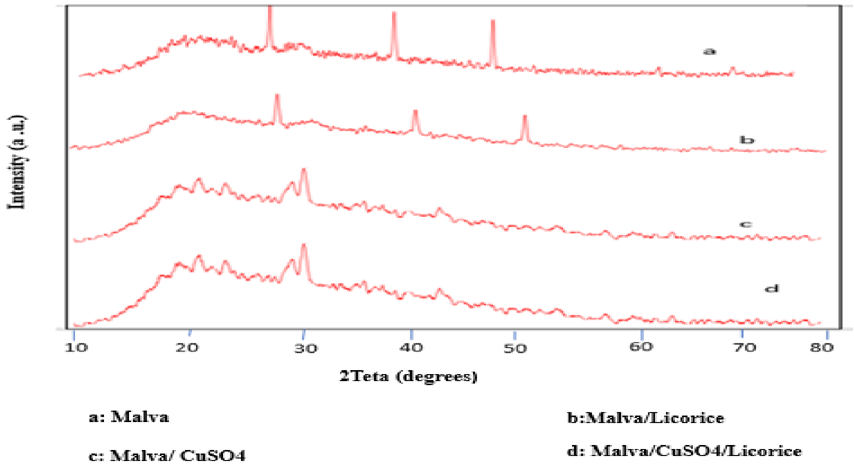

Figure 3-3: X-ray diffraction spectrum (XRD) of the mucilage films of Panirak flower with Shirin-Bian and copper sulfate nanoparticles

3-4- February infrared (FTIR)

In order to investigate the new links in the production films and to study the characteristics of the peaks related to the mucilage of the oleander modified with licorice and copper sulfate nanoparticles, the FTIR spectrum is shown in Figure 3-4. The FTIR spectrum of carbohydrates is used to determine their structural features and makes it possible to identify the main chemical groups in polysaccharides (the position and strength of bonds are specific for each polysaccharide). As can be seen, the observed peaks in the range of 3200 to 3600 cm 1 are related to the stretching vibration of the O H (hydroxyl) bonds that exist in the pyranose ring. And the peaks in the range of 2900 cm 1 are assigned to the presence of symmetric stretching vibrations of CH2. The peak in the range of cm 1,596 to cm 11,600 is related to the NH bending vibration of type one and two amide bonds, while the absorption at cm 1,220 to cm 11,461 is due to the stretching vibration of C N and NH amide type III bonds.Other peaks observed in the FTIR spectrum are assigned to the presence of bending vibrations of 1070 cm CH2 O CH2 (bending vibration of amide bond) NH in the range of 1700 cm [18]. As it is known, the spectra of Panirak flower mucilage are not much different from its composites, because the functional groups in copper sulfate nanowires are similar to Panirak flower mucilage and therefore their peaks have overlapped with the peaks of Panirak flower mucilage and with Paying attention to the fact that the percentage of copper sulfate nanoparticles used in the structure of the mucilage film was not high, there was no noticeable change in the intensity of the peaks that appeared. The point that should be noted is that by adding licorice to the structure of the mucilage film, the wave number of the appeared peaks has been shifted, and this shift of the peaks indicates the creation of electrostatic interactions between the polymer chains of the mucilage of the mucilage and the licorice. It is an expression.(Karimi Thani et al., 2021) showed that in the composite film of potato starch/apple skin pectin/Shirazi thyme essential oil and zirconium oxide nanoparticles, there are electrostatic interactions between the composite components. Their research results confirm the results of the current research. slow [19].

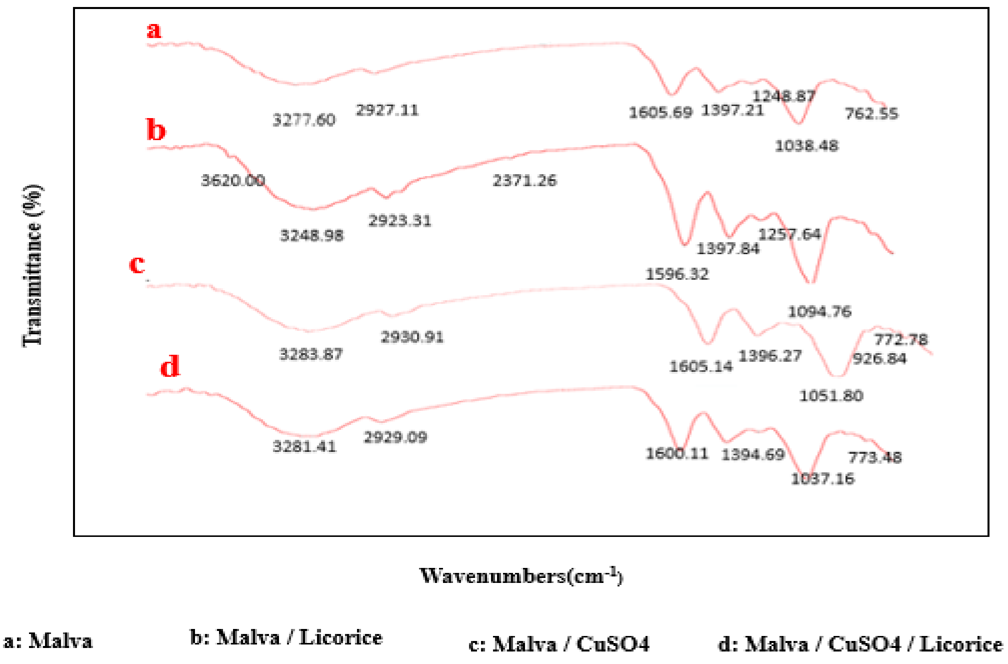

Figure 3-4: FTIR results of the mucilage film of Panax flower modified with Shirin-Bian and copper sulfate nanoparticles

3-5- Scanning Electron Microscope (SEM)

Scanning Electron Microscope (SEM) images of mucilage films modified with licorice and copper sulfate nanowires are shown in Figure 3-5. According to the observations obtained from the morphological images of the production films, the surface of the mucilage films of Paneerak flower is uneven, which is an indication of the irregular structure of their biopolymer matrix. And the surface morphology of the mucilage films with licorice are less heterogeneous than the mucilage of the mucilage, and this can be due to the hydroxyl groups that have good solubility, mucilage of the mucilage and licorice were evenly distributed in the polymer matrix But the interaction between them has not happened completely and all parts of the mucilage film are not saturated with licorice. Also, mass, aggregation and non-uniform distribution can be attributed to the presence of copper sulfate nanoparticles.They don't have proper dispersal properties in water environments and in some parts, accumulation has happened. Copper sulfate particles were spherically dispersed inside the film. Nanocomposite films with licorice and copper sulfate nanoparticles reduced the heterogeneity on the polymer surface, which is consistent with the results of Khakpour et al. (2023) modified starch/gum arabic nanocomposite film prepared with chromium oxide nanoparticles and lycopene pigment, investigation of physicochemical properties [11].

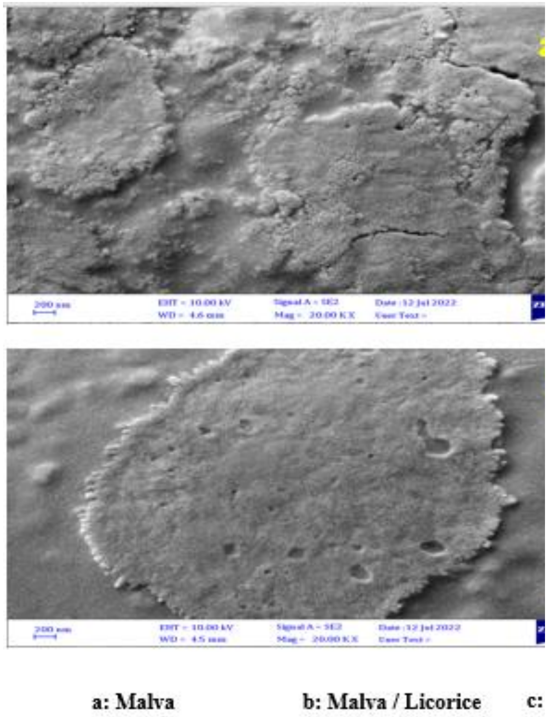

Figure 3-5: Scanning electron microscope images of mucilage films of Panerak flower modified with Shirin-Bian and copper sulfate nanoparticles

3-6- Thermal properties (DSC)

The thermal analysis of the nanocomposite films based on the mucilage of the Paneerak flower modified with licorice and copper sulfate nanoparticles is shown in Figure 6-3. Investigating the thermal properties of films such as the glass transition temperature (Tg) and melting temperature (Tm) of the film is important, because the films are soft and rubbery at temperatures higher than the glass transition temperature and at temperatures higher than Tg The reason for the increase in the space between the polymers is that the mobility of the polymers increases and as a result the permeability of the film increases, so the higher the Tg of a film, the packaging film can act as a protector in a wider temperature range. At the melting temperature, the polymer film becomes completely liquid and fluid [20]. In the present study, the thermal properties of the produced films have been evaluated in terms of parameters such as glass transition temperature (Tg) and melting temperature (Tm).The results show that Tg increases with the addition of copper sulfate nanoparticles and licorice, which confirms the reinforcing role of copper sulfate and licorice nanoparticles in making nanocomposite films in a wide temperature range [21]. The melting point temperature (Tm) of the characteristics of the crystalline regions and the glass transition temperature (Tg) depend on the characteristics of the amorphous regions, and these two temperatures do not necessarily increase or decrease together. According to the results, it was observed that by adding nanoparticles of copper sulfate and licorice, Tg and Tm increased in nanocomposite films. This can be attributed to the establishment of strong interactions between the hydroxyl groups of the matrix and the nanofiller, as a result of which, the mobility and flexibility of the mucilage strands of the oleoresin, copper sulfate and licorice nanoparticles in the crystalline and amorphous regions are reduced and The order of Tg and Tm increases [22].The studies of Swaroop and Shukla 2019), on the effect of adding magnesium oxide nanoparticles to increase the thermal stability of nanocomposite films, are consistent with the present study [23].

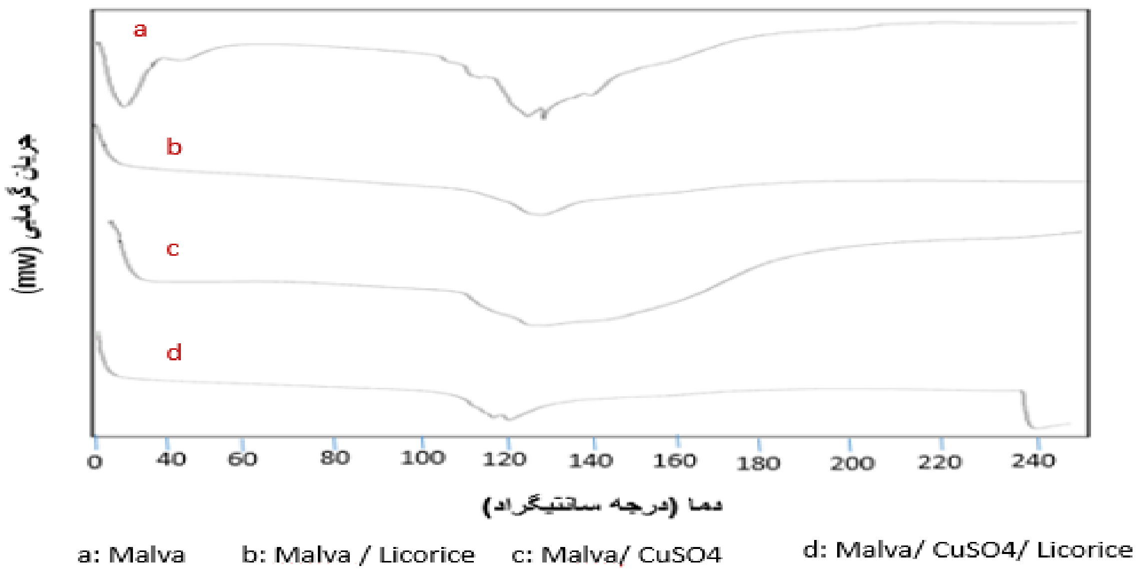

Figure 3-6: Measurement of the thermal properties of the mucilage films of the Paneerak flower modified with saffron and copper sulfate nanoparticles.

4.Conclusion

Despite all the advantages that 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, 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 antioxidant content of the film increased. The addition of licorice and copper sulfate nanoparticles caused the antimicrobial activity of the film against Staphylococcus aureus and Escherichia coli. Copper sulfate nanoparticles and licorice root did not change the physical properties of Paneerak flower mucilage, and the physical presence of copper sulfate nanoparticles in the polymer matrix was confirmed. The surface morphology of the nanocomposite films was more heterogeneous than the mucilage of Paneerak flower.Licorice and copper sulfate nanoparticles increase the thermal stability of the mucilage film of Paneerak flower. In general, according to the investigations, the mucilage films containing copper sulfate nanoparticles and licorice root have the ability to be used as active packaging in the food industry.

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تهیه فیلم آنتی‌اکسیدانی و آنتی‌باکتریایی برپایه موسیلاژ گل پنیرک اصلاح شده با ریشه شیرین بیان و نانو ذرات سولفات مس

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چکیده

هدف از این پژوهش تولید فیلم بر پایه موسیلاژ گل پنیرک اصلاح شده با ریشه شیرین بیان و نانوذرات سولفات مس بود. طرح آماری D-optimal برای مطالعه خواص آنتی–اکسیدانی، ضدمیکروبی، پراش اشعه ایکس (XRD)، فوریه مادون قرمز (FTIR)، میکروسکوپ الکترونی رویشی (SEM) و تجزیه حرارتی (DSC) فیلم‌ها مورد استفاده قرار گرفت. نتایج نشان داد که با افزایش ریشه شیرین بیان و نانوذرات سولفات مس فعالیت آنتی‌اکسیدانی فیلم به طور معنی‌دار (p<۰/۰۵) افزایش یافت. نتایج فعالیت ضدمیکروبی فیلم‌های تهیه شده نشان داد که افزودن شیرین بیان و نانوذرات سولفات مس باعث فعالیت ضدمیکروبی فیلم بر علیه *اشریشیا کلی* و *استافیلوکوکوس اورئوس* گردید. تجزیه و تحلیل پراش اشعه ایکس نشان می‌دهد که نانوذرات سولفات مس با پلیمر موسیلاژ گل پنیرک به طور فیزیکی ترکیب می‌شود و باعث کاهش ساختار کریستالی می‌شود. نتایج تبدیل فوریه مادون قرمز (FTIR) حضور فیزیکی نانوذرات سولفات مس در ماتریس پلیمری را تایید می‌کند نتایج FTIR همچنین برهمکنش‌های الکترواستاتیک ضعیفی را بین اجزای فیلم و کامپوزیت‌ها نشان داد. نتایج میکروسکوپ الکترونی روبشی (SEM) نشان می‌دهد که سطح فیلم‌های حاوی نانوذره سولفات مس و ریشه شیرین بیان نسبت به موسیلاژ گل پنیرک ناهمگن‌تر هستند. شیرین‌بیان و نانوذرات سولفات مس توانستند تجزیه حرارتی موسیلاژ گل پنیرک را به‌تاخیر بیاندازند و باعث افزایش پایداری حرارتی فیلم موسیلاژ گردند. در نهایت براساس نتایج حاصله، افزودن نانوذرات سولفات مس و ریشه شیرین بیان به فیلم‌های خوراکی بر پایه موسیلاژ گل پنیرک سبب بهبود ضدمیکروبی، آنتی‌اکسیدانی، DSC و SEM گردید.