



## Studying the production of composite nanofibers from the hydrocolloid compounds of *Salvia macrosiphon L.* seeds by electrospinning method in order to cover vitamin D3

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### ABSTRACT

Advancement in the production processes of nanostructures with appropriate formula characteristics provides the production of stable nanoparticles with the ability to be used in the food industry. Microencapsulated bioactive compounds can be integrated into electrospun fibers to achieve greater stability of nanoparticles against heat and light, which leads to increased storage time. In the present study, composite nanofiber layers were made from mucilage extracted from *Salvia macrosiphon L.* seeds using electrospinning. The nanocomposite of nanofibers was prepared from polyvinyl alcohol/rice bran protein isolate/ *Salvia macrosiphon L.* seed mucilage in different ratios. Then the morphology and FTIR spectroscopy were investigated. The average diameter of the produced nanofibers is about 40 nm and the coefficient of variance is 13%, which showed that the diameter of the fibers is relatively uniform. Increasing the concentration of the mucilage solution and the constant percentage of polyvinyl alcohol significantly increased the diameter of the nanofibers. In the next step, vitamin D3 was encapsulated in polyvinyl alcohol nanofibers and rice bran protein concentrate. FTIR results confirmed the presence of vitamin D3 in the prepared nanofibers. At higher concentrations of phenolic compounds, with the increase in the number of hydroxyl groups of aromatic rings in the reaction medium, the inhibitory power of mucilage free radicals increased. The composition of nanofibers in the spectroscopic graphs showed that there are two strong peaks in the range of 1454 and 1743 CM-1 from vitamin D3 in the nanocomposite and microcoated samples which show the stretching vibrations related to the C=C group in the aromatic rings of phenolic compounds. Based on the findings, bioactive compounds to increase access to vitamin D3 can be enclosed in electrospun nanofibers of *Salvia macrosiphon L.* mucilage/polyvinyl alcohol/rice bran protein concentrate.

## 1- Introduction

In recent years, nanotechnology has been recognized as one of the advanced research and application fields in the world [1]. Advances in the production processes of nanostructures and nanomaterials with appropriate formula characteristics provide the production of stable nanoparticles with the ability to be used in the food industry and related industries [2, 3]. Nanofibrous structures have a special place, when the diameter of polymer fibers is reduced from micrometer to nanometer, interesting properties appear in them, which can be attributed to the increase of the surface-to-volume ratio, increased flexibility in surface functional groups, and performance. Excellent mechanical properties such as hardness and tensile strength [4, 5]. The electrospinning process involves applying high voltage to polymers in solution or melt, which leads to the spinning of micron or even nanometer-sized fibers, and biodegradable polymer materials such as proteins and carbohydrates are used in the microencapsulation of bioactive compounds [6, 7]. Electropinning is a simple and versatile technique and bioactive compounds can be incorporated into electrospun fibers. These nanofibers have useful properties such as high porosity and specific surface area. including high-performance microcoating, stability and stability of microcoated materials, greater stability against heat and light, which leads to increased storage time and protection of bioactive materials from chemical degradation [8, 9]. The stability of emulsions is an important part of emulsion electrospinning, emulsions tend to break over time, therefore, emulsifier must be added to increase stability. One of the common emulsifiers are surfactants, which prevent the accumulation of droplets by increasing repulsive forces. Therefore, electrospinning with microemulsions can be a significant advantage for new product development with improved functions [10, 11]. Natural polymers have gained a lot of attention to nanofibers due to their biodegradability and

better biocompatibility and non-toxicity. Active antibacterial has been used for food and drug carriers [12, 13, 14]. Zhang *et al.*, 2020 After adding glycerol monolaurate to the electrospinning solution of wheat gluten, the mixture was electrospun. The obtained films had good water stability and antibacterial activity and were suitable as antibacterial food packaging materials. Polyvinyl alcohol (PVA) is known as a very suitable polymer raw material for electrospinning due to its non-toxic nature, excellent solubility in water and complete biodegradability. Consequently, it is often incorporated into electrospinning solutions as a spinning agent to enhance the solution spinning properties and the mechanical properties of the nanofiber layers [2]. Aziz *et al.*, 2019 have successfully prepared nanofiber (polyvinyl alcohol/wheat gluten) PVA/WG films that can be used to load drugs [15]. To overcome these limitations, transverse connection techniques can be used. Chemical and enzymatic cross-linking methods are commonly used, but produce toxic residues, thus limiting their implementation in the field of active food packaging [16]. Hence, adopting a non-toxic and simple cross-linking method was considered, by adding a reducing sugar (eg, glucose) to the electrospinning solution, And then placing the resulting electrospun film at high temperature to create cross-linking, this reaction occurs between the electrophilic carbonyl group of the reducing sugar and the adprotonated amine group in a protein. This changes the conformation and interaction of the protein and leads to Maillard cross-linking [4, 17]. In another study Deng *et al.*, 2019 Glucose/gelatin/zein was placed at a temperature of 140 °C for 3 hours, and showed that the water stability of gelatin nanofibrous layers added with glucose could be increased up to 14 days after Maillard cross-linking [4]. Hydrocolloid materials and natural phenolic extracts are antibacterial and antioxidant materials that are usually used in food packaging to improve safety and reduce food spoilage [18, 19]. Eggs *Salvia macrosiphon L.*, which is also known by the local name "Brviz",

refers to the eggs of the Meru tree. Egg hydrocolloid is recognized as a beneficial food source due to its nutrients such as protein, healthy fats, fiber, vitamins and minerals, and antibacterial and antioxidant properties [20]. In order to provide protein for the growing population and also to minimize the use of protein from animal sources, initiatives have been taken to find alternative methods of high quality protein from plant [21]. Among plant sources, rice protein consists of four main substances: albumins, globulins, glutelins and prolamins. Among them, glutelin is the main component of protein, which constitutes about 80% of the total protein in endosperm. In rice bran, water-soluble proteins such as albumin are the main proteins. Rice bran contains important amino acids arginine, threonine, glycine, cystine, valine, methionine, leucine, isoleucine, tyrosine, lysine, histidine and phenylalanine [22]. Today, the lack of micronutrients in human society is felt, paying attention to food enrichment Vitamin D3 is a compound of the vitamin D family that naturally converts 7-dehydrocholesterol in the skin to vitamin D3 upon exposure to sunlight. Research has shown that the cause of gastrointestinal problems, which include abdominal pain, constipation, diarrhea and swelling, is due to vitamin D deficiency [23]. In the upcoming research, with attention and advanced techniques to increase polymers with high quality properties, to produce composite nanofibers from the hydrocolloid compounds of mulberry seeds, Polyvinyl alcohol and rice bran protein concentrate have been treated by electrospinning in order to coat vitamin D3, and investigated the properties of produced nanofibers.

## 2-Materials and Methods

### 2-1-Raw material

The main raw materials used for the preparation of electrospun nanocomposite include *Salvia macrosiphon L.* seeds and rice bran from the local market (Kazron, Iran), polyvinyl alcohol with a molecular weight of 44.053 g/mol (Merck, Germany), and to extract wheat bran protein

concentrate, sodium hydroxide and hydrochloric acid (Merck, Germany) were prepared and purchased.

### 2-2- *Salvia macrosiphon L.* mucilage extraction

In order to extract mucilage, water was added to the seeds at a ratio of 20:1. The pH of the water was adjusted to a suitable value using 0.2 M NaOH. Then, in order to separate the mucilage from the seeds of *Salvia macrosiphon L.*, the mixture was stirred for 1.5 hours using a homogenizer mixer (Berli, HD3200, Germany) with a speed of 1500 rpm to separate the mucilage. With the formation of swollen grains and gel around them, the samples were placed in a centrifuge (Universal Hettich-320R, Germany) for 20 minutes at a temperature of 4 °C. The extracted mucilage was dried in the oven for 2 hours at 50°C, then placed in polyethylene bags and kept in a dry and cool place until use [24].

### 2-3-Evaluation of free radical inhibition

Different concentrations (50, 100, 150, 200, 250, 300, 350) of the mucilage compounds extracted from *Salvia macrosiphon L.* seeds along with 1 ml of (diphenyl-1-picrylhydrazyl free radical) DPPH 0.1 mM and 1 ml A liter of methanol was prepared. After 30 minutes at room temperature, the optical absorbance of the samples was read at 517 nm with DPPH against the blank by a UV-Vis device (Schimadzu UV/Vis-240 IPC, Japan) [25].

The percentage of free radical inhibition was calculated using the following formula:

$$\%RSD = (A_0 - A/A_0) \times 100$$

free radical inhibition percentage: (antioxidant inhibition percentage against free radicals)

A0: control absorption (which contains one ml of methanol and one ml of DPPH solution)

A: sample absorption (which contains different volumes of BHT and DPPH mucilage)

### 2-4-Measuring the content of total phenolic

The amount of total phenolic compounds was measured based on Folin-ciocalteu colorimetric method and in terms of gallic acid. Standard solutions with concentrations of 12.5, 25, 50, 62.5, 100 and 125 ppm of gallic acid (Merck, Germany) were prepared in 60% methanol solution, then 0.1 ml of each was added to The test tube was transferred and 0.5 ml of 10% solution of Folin-Siocaltio reagent (Merck, Germany) was added to them. After 3 to 8 minutes, 0.4 ml of 7.5% sodium carbonate solution was added to it. Then the tubes were kept at the laboratory temperature for 30 minutes and after that the amount of light absorption was measured by a spectrophotometer (Schimadzu

UV/Vis-240 IPC, Japan) at a wavelength of 765 nm. Then 1 gram of dried mucilage was dissolved in 60% methanol and the volume was increased to 10 ml, and the amount of total phenol was determined based on Folin-Siokaltio method. with the difference that instead of the standard solution, 0.1 ml of the mucilage solution was added, then the read absorption rate was placed in the standard graph. And in this way, the amount of total phenol of hydrocolloid compounds was obtained in terms of mg/ml equivalent of gallic acid [25].

#### 2-5-Investigation of antimicrobial activity

Examining the antimicrobial property by the disc diffusion method is classified as an agar diffusion method, because the plant compounds to be tested are spread from their reservoir through the agar medium seeded with the test microorganism. In general, the reservoir is a flat paper disk that is placed on top of the agar surface. If the tested plant compounds or isolated compounds are microbiologically active. After incubation, an inhibitory zone is formed around the filter paper disc. The diameter of the zone of inhibition correctly describes the antimicrobial power of herbal compounds or individual compounds. It should be noted that the disk diffusion method is not a suitable method for lipophilic extracts because the water-insoluble diffusion and its compounds from the filter paper disk to the agar medium are insufficient. Therefore, lipophilic compounds give negative results or smaller zones of inhibition with the disc diffusion method than other tests. Agar diffusion method was used to determine antimicrobial activity. The samples were converted into discs with a diameter of 10 mm using a mold. Before placing the disks on the surface of the culture medium, surface culture was performed using 100 microliters of liquid culture medium containing approximately  $10^8$  CFU/ml of each of the tested bacteria (gram-negative and gram-positive). Discs were placed on Mueller Hinton Agar culture medium under completely sterile conditions and it was kept in a incubate for 24 hours at a temperature of 37 °C. The difference in the diameter of the halos formed from the diameter of the discs was considered as an indicator of antimicrobial activity. In cases where halos are not formed, i.e. there is no antimicrobial activity, the area difference was considered to be zero [26].

#### 2-6-Extraction of rice bran protein concentrate

To extract the rice bran protein concentrate, first, 100 grams of oiled rice bran using the Keldahl method, Then the dried rice bran was mixed with distilled water at a ratio of 1 to 1 (W/W). By using sodium hydroxide solution with a concentration of 1.1 M, the pH of the solution reached 1.1 and stirred for one hour at room temperature. The mixture was centrifuged (Universal Hettich-320R, Germany) in a centrifuge at 500 rpm/min for 10 minutes. The pH of the supernatant (liquid obtained after centrifugation) was raised to 1.4 using hydrochloric acid and stirred for half an hour. Then, together with the proteins, the supernatant was kept at a temperature of 4°C for one night to precipitate the proteins. The sediment layer was carefully separated from the top of the mixture and the precipitated proteins were washed with distilled water and neutralized [27].

#### 2-7-Solution electrospinning

In this experiment, electrospinning solution with different ratios of Table 1 in environmental conditions and different device conditions including voltage in the range of 12 to 20 kV, feeding rate of electrospinning solution in the range of 0.125, 0.5 ml/h and the distance between the needle tip and the surface of the nanofiber collector, It was investigated in the range of 9 to 15 cm [28]. In order to choose the optimal device conditions, the range of 9 cm to two fixed parameters was considered at each time and the third parameter was changed in the mentioned range. After electrospinning, all samples were examined by optical microscope (HUTACT-40X-2000X, Japan). In this way, the right conditions were determined to obtain fibers with the least knots and the most uniformity. In the first step, different contents of mucilage were dissolved with 3 mm of distilled water. Then 0.4 polyvinyl alcohol and 0.4 rice bran protein concentrate were stirred at room temperature for 15 hours. until a homogeneous solution was formed. Then, the solution was well homogenized with a titanium ultrasonic probe (Berlin, HD3200, Germany). The prepared solution was transferred into a 5 ml syringe and electrospun using an electrospinning machine (Full Option Lab 2ESII-II, Iran) with a positive voltage of 18 kV. The distance to the tip of the needle was considered to be 12 cm, and the speed of the collecting drum was 500 rpm.

**Table 1. Solution samples for electrospinning.**

Treatment	Mucilage (%)	Polyvinyl alcohol (%)	Protein Concentrate (%)
A	0.1	4	4

B	0.3	4	4
C	0.5	4	4
D	0.7	4	4

## 2-8-Encapsulate

At this stage, use different ratios according to Table 2 in environmental conditions, and immediately the resulting solution was electrospinning for 2 hours [29]. First, different concentrations of mucilage according to table 4-3 and vitamin in a ratio of 1 to

4 were added to 1.5 ml of water and placed on a stirrer for 45 minutes. so that the vitamin is placed in the structure of the polymer solution and finally it was pulled into the syringe and ready for electrospinning. Encapsulated treatment with vitamin D3 nanocomposite

**Table 2. Samples containing encapsulated composite compounds**

Treatment	Encapsulated with nano composite
Control	Polyvinyl alcohol 4% + rice bran protein 4% + 1% vitamin D3
A	Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.1% <i>Salvia macrosiphon L.</i> seed mucilage
B	Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.3% <i>Salvia macrosiphon L.</i> seed mucilage
C	Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.5% <i>Salvia macrosiphon L.</i> seed mucilage
D	Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.7% <i>Salvia macrosiphon L.</i> seed mucilage

## 2-9-Morphology of nano fibers using electrospinning

Since water-containing samples cause problems with outgassing in vacuum systems such as scanning electron microscope chambers, scanning electron microscope samples must be dry. It is also possible that the temperature of the surface of the sample may increase as a result of high-energy electrons hitting it, causing the phenomenon of evaporation and degassing, as well as increasing the pollutants in the chamber, and as a result, reducing the quality of the image. In addition, increasing the temperature of the sample surface can lead to sample damage. In addition to being dry, the scanning electron microscope sample must also be conductive to prevent the accumulation of electric charge on the surface of the sample due to the impact of the electron beam. For this purpose, non-conductive samples are covered with a thin layer of conductive material. By scanning electron microscope (TESCAN vega3, Czech Republic, China) To study the surface of nanocomposite samples containing

vitamin D3 with a power of 5 kw, small pieces of nanocomposite samples were attached to the aluminum support base with the help of silver glue and the bases were coated with gold (DSR1, Nanostructure Coating Company, Iran) for 5 minutes in a coating/spraying device and imaging was done in the magnification range of 20000 [30].

## 2-10-FTIR spectroscopy test

FTIR device (Spectrum Two, FTIR Perkin Elmer, USA) was used to perform the test. Thin tablets were obtained from the dried samples with a thickness of less than one millimeter and coated with potassium bromide in the tablet preparation machine and the transmission spectrum of the samples was analyzed in the range of 4000-4000  $\text{cm}^{-1}$  wave number and with 5  $\text{cm}^{-1}$  resolution [31].

## 2-11- Statistic analysis

In this research, data analysis was done using SPSS and Excel software. All the experiments were done based on completely randomized block design and in three replications. Means were compared with Duncan's test at the 5% level. Curves were drawn with Excel software.

### 3-Discussion and results

#### 3-1- Evaluation of DPPH free radical scavenging and total phenol of *Salvia macrosiphon L.* mucilage

DPPH free radical in hydrocolloids as a class of water-soluble polymeric materials was studied and evaluated. Hydrocolloids include polysaccharides and proteins that have significant biological and antioxidant properties. In addition, these polysaccharides can increase the content of superoxide dismutase, which supports the antioxidant mechanism [32]. In Tables 3 and 4, the DPPH free radical inhibition evaluation of the total phenol of Mero mucilage was investigated. The results of investigating the antioxidant activity by DPPH free radical reduction method in six different concentrations are shown. Evaluation of free radical scavenging activity, DPPH showed that increasing

concentration had a significant effect on free radical scavenging. It means that the free radical scavenging activity of different concentrations of mucilage depends on the concentration, and with the increase of mucilage concentration, the amount of free radical scavenging increased [33]. A factor called IC<sub>50</sub> was used to compare mucilage free radical inhibition activity. According to the definition of IC<sub>50</sub>, it is said that the concentration of the extract in which half of the free radicals are inhibited. This value was observed for DPPH free radical at a concentration of 250 ppm (73.08) from mucilage. Due to the variety of compounds found in different plants, several methods are usually used to evaluate antioxidant activity. The results of various studies have shown that protein and polysaccharide hydrocolloids have antioxidant power and the ability to reduce free radicals [34, 35].

**Table 3. Evaluation evaluation of DPPH radical inhibition in the extracted mucilage of *Salvia macrosiphon L.* seed**

Concentration µg/ml	Control absorption	<i>Salvia macrosiphon L.</i> mucilage	RSA %	IC <sub>50</sub> (mg/ml)
50	0.52	0.312	40.00	2.34
100	0.52	0.291	44.04	7.73
150	0.52	0.228	56.15	13.12
200	0.52	0.180	65.38	18.51
250	0.52	0.140	73.03	23.90
300	0.52	0.075	85.58	29.29
350	0.52	0.035	93.27	34.68

*Salvia macrosiphon L.* seed mucilage contains phenolic compounds. The phenolic content of the gum was measured with a Vis-UV spectrophotometer. The obtained results are reported in Table 4. Phenolic acids and polyphenolic derivatives are among the compounds present in this mucilage. These compounds have significant antioxidant activity due to their hydrophilic structure. Also, the amount of phenolic compounds in seed mucilage was about 10 (mg/µg) equivalent to gallic acid. This value indicates the presence of a significant level of phenolic compounds in the mucilage, which can be used as a rich source of antioxidants [36]. The amount of phenolics in food varies depending on the type of food, cultivation method, growing season, weather conditions and other factors. The inhibitory power of phenols is due to the hydroxyl groups in their molecules [37]. Increasing the concentration of phenolic compounds directly increases the ability of various extracts and hydrocolloids to inhibit free radicals. At higher

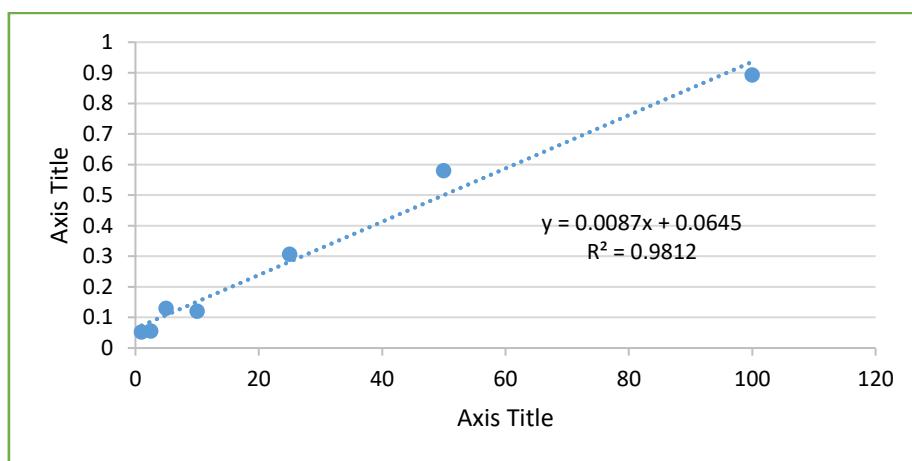
concentrations of phenolic compounds, due to the increase in the number of hydroxyl groups of the aromatic rings of phenolic compounds in the reaction medium, the probability of giving hydrogen to free radicals increases, and as a result, the inhibitory power of mucilage increases [38]. Petriccione et al. (2015) The effect of chitosan fruit coating in delaying the quality and nutritional traits of three strawberry cultivars named "Kandonga", "Junica" and "Sabrina" and also the effect of chitosan on antioxidant enzymes were investigated. Physicochemical properties (weight loss, soluble solid content and titratable acidity) and nutrients (total polyphenol, anthocyanin, flavonoid, ascorbic acid content and antioxidant capacity) along with catalase, ascorbate peroxidase, polyphenol oxidase, guaiacol peroxidase and lipoxygenase activity were evaluated. Chitosan treatment significantly reduced water loss and delayed qualitative changes in color, titratable acidity and ascorbic acid content. In addition, changes in total polyphenol, anthocyanin and flavonoid content and antioxidant capacity of

strawberry fruits coated with chitosan were delayed. Chitosan coating increased the activity of some

antioxidant enzymes and prevented browning of meat [39].

**Table 4. Examination of the total phenol of extracted *Salvia macrosiphon L.* mucilage**

Factor	The amount of
Total phenol	10.24 $\mu\text{g/ml}$



**Figure 1. Absorption diagram of the calibration of mucilage extracted from *Salvia macrosiphon L.* seed based on gallic acid ( $\mu\text{g/ml}$ )**

### 3-2-Antimicrobial properties of *Salvia macrosiphon L.* mucilage

The diameter of the halo of non-growth (halo of inhibition) by diffusion method using disc for Gram-positive and Gram-negative bacteria is shown in Table 5. A significant difference was observed in the mean diameter of the halo of non-growth of Gram-negative and Gram-positive bacteria in the treatments ( $p \leq 0.05$ ). In the examination of gram-negative bacteria, the diameter of non-growth halo for control and *staphylococcus* samples was determined to be 23 and 8 mm, respectively. Also, in the examination of gram-positive bacteria, the diameter of the halo of non-growth for the control sample and *Escherichia coli* showed 15 and 3 mm, respectively. The results of these studies show that the mucilage of *Salvia macrosiphon L.* seed has specific antimicrobial effects and has shown greater sensitivity on *staphylococcus*. The reason for the high sensitivity of Gram-positive bacteria compared to Gram-negative bacteria can be due to the lack of

lipopolysaccharide cell wall in Gram-positive bacteria [40]. In gram-negative bacteria, the cell wall prevents active compounds from entering the cytoplasmic membrane, due to the presence of surrounding external membranes, the cell wall in gram-negative bacteria is less sensitive than gram-positive bacteria. The outer membrane of Gram-negative bacteria prevents the diffusion of lipophilic substances through the lipopolysaccharide covering layer. In gram-positive bacteria, direct contact of hydrophobic compounds with bilayer phospholipids takes place. This contact is where the hydrocolloid compounds take effect. This effect can occur in the form of increasing the permeability of ions and vital compounds of the cell or leakage of the vital compounds of the cell or disable the bacterial enzyme system [41]. In the study of Safari et al., 2015 and Sakti et al., 2012, it has been shown that the extraction of microbial secondary metabolites causes antimicrobial effect in plants [41, 42], so the current research is consistent with previous researches.

**Table 5. Investigating the antimicrobial properties of the mucilage of *Salvia macrosiphon L.* seed using the average diameter of the halo of non-growth (disc diffusion).**

Factor	Treatment	The diameter of the aura of lack of growth (mm)
Gram positive bacteria	Control	23.00 $\pm$ 1.00 <sup>A</sup>

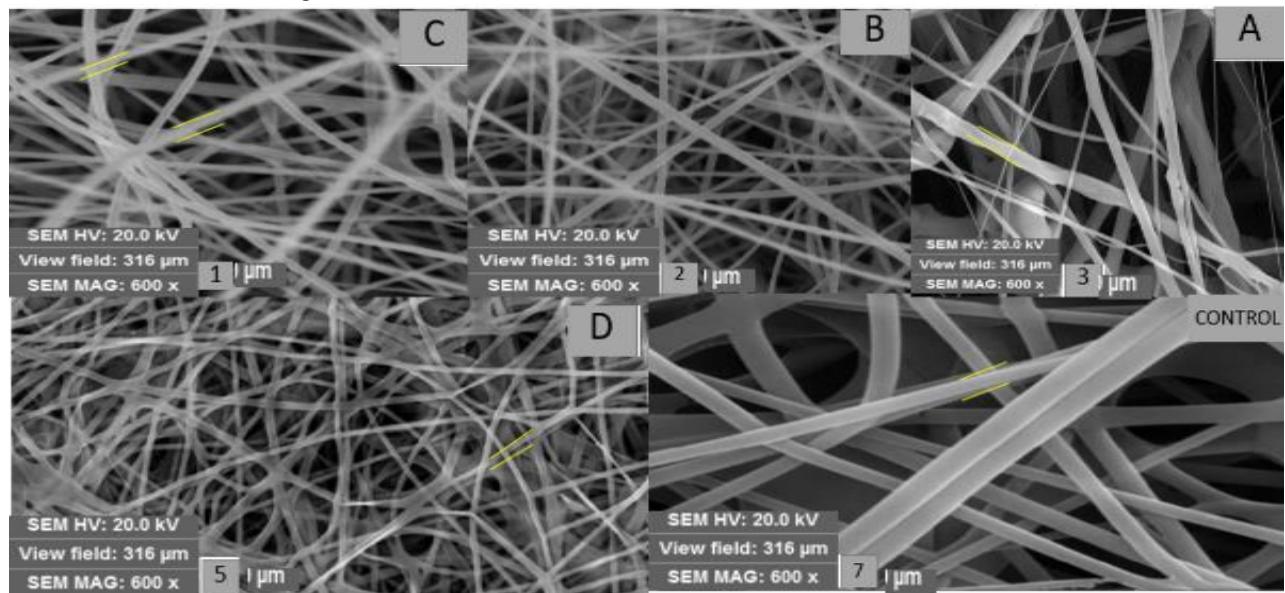
Gram negative bacteria	<i>Staphylococcus</i>	8.00±0.2 <sup>B</sup>
Control		15.00±0.5 <sup>A</sup>
	<i>Staphylococcus</i>	3.00±0.2 <sup>B</sup>

The results are presented in the form of average standard deviation for each treatment, all experiments were done in three repetitions. Values with different capital letters are significantly different from each other at the 5% level.

### 3-3-Characteristics of electron microscope images of nanofibers before and after encapsulation

Using electron microscope images in different weight concentrations, mucilage nanofibers have been investigated. The images show that the fibers produced in different concentrations have a flat and uniform structure. The average diameter of these nanofibers is about 40 nm with a coefficient of variance of 13%, which indicates that the diameter of the fibers is relatively uniform. Flat nanofibers are produced due to the lack of solvent evaporation in the distance between the nozzle tip and the collector. Therefore, increasing the concentration of mucilage solution and constant percentage of polyvinyl alcohol significantly increases the diameter of nanofibers (control sample containing bran protein concentrate and polyvinyl alcohol). This increase in the diameter of nanofibers occurs due to the increase in the viscosity of the polymer solution [43]. Figure 1 also shows the effect of mucilage concentration on nanofiber diameter. By increasing the concentration of the mucilage solution, the diameter of the nanofibers increases significantly, and this increase occurs due to the increase in the viscosity of the polymer solution. The contact surface of the mucilage with the tip of the nozzle and the collector can also be effective. A high and uniform contact

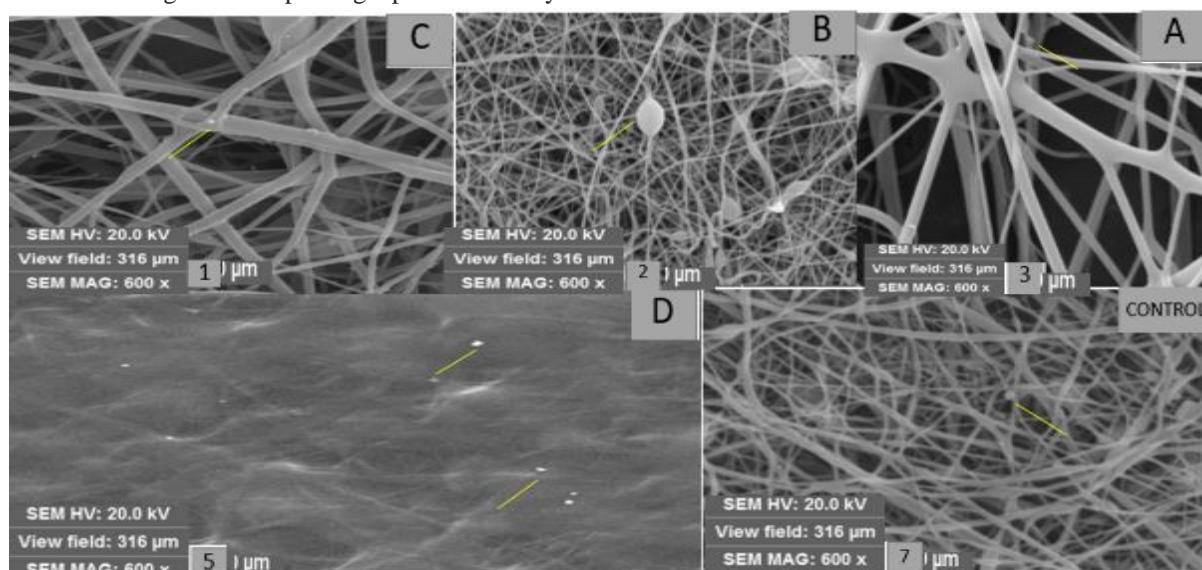
surface between the mucilage and the surface of the nozzle tip and collector can help to form flat nanofibers. Mucilage surface tension can also be effective in the formation of flat nanofibers. High surface tension can facilitate the formation of flat nanofibers. Chen et al, as a result of electrospinning experiments of collagen and chitosan in trifluoroacetic acid, reported that the average fiber diameter was between 200 and 250 nm [44]. They have also reported that the diameter of the fibers decreases with the increase in the amount of chitosan, but due to high viscosity in higher concentrations of chitosan, it was not possible to electrospin them. In this experiment, as the mucilage concentration increased, the viscosity of the polymer solution probably increased. This high viscosity can reduce the electric current and cause problems in the electrospinning of fibers. In other words, at higher concentrations of mucilage, the formed fibers are incapable of electrospinning due to high viscosity and electrical conditions. The best sample in terms of smaller diameter is sample B because if the amount of mucilage in the electrospinning solution is low or high, it prevents the formation of fibers on the surface. And this affects the properties of the tensile surface and electrical conductivity. For this reason, to form flat nanofibers with desirable properties, it may be necessary to choose samples with a more suitable diameter.



**Figure 1. Morphology of nanofibers produced from extracted mucilage of *Salvia macrosiphon L.* seeds in samples containing composite nanofibers (Control: Polyvinyl alcohol 4% + rice bran protein 4% + 1% vitamin D3, A: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.1% *Salvia macrosiphon L.* seed mucilage, B: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.3% *Salvia macrosiphon L.* seed mucilage, C: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.5% *Salvia macrosiphon L.* seed mucilage, D: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.7% *Salvia macrosiphon L.* seed mucilage).**

In Figure 2, electrospun nanofibers with different concentrations of mucilage and constant percentage of polyvinyl alcohol and protein isolate and vitamin D3 encapsulated substrate were observed in the sample containing 0.5 concentration of mucilage. Van Salk and colleagues 2014 used the composite made of zein and chitosan to micro-encapsulate alpha-tocopherol and reported the average diameter obtained between 500 and 700 nm [45]. They made a composite membrane of nylon-6/chitosan nanofibers with different weight ratio of nylon-6 to chitosan using electrospinning process. They

realized that the morphology and diameter of nanofibers are influenced by the concentration of the solution and the weight ratio of their constituents. Electron microscope images showed that by using nylon-6/chitosan mixture with different weight ratios, different fiber diameters are formed. According to the results, the nanofibers had an average diameter of 100 to 250 nm. The fiber diameter gradually decreases when the chitosan content increases, and when the chitosan content exceeds 31% by weight. that the present research is consistent with previous researches.



**Figure 2. Morphology of nanofibers produced from extracted mucilage of *Salvia macrosiphon L.* seeds in samples containing encapsulated composite nanofibers (Control: Polyvinyl alcohol 4% + rice bran protein 4% + 1% vitamin D3, A: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.1% *Salvia macrosiphon L.* seed mucilage, B: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.3% *Salvia macrosiphon L.* seed mucilage, C: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.5% *Salvia macrosiphon L.* seed mucilage, D: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D3 + 0.7% *Salvia macrosiphon L.* seed mucilage).**

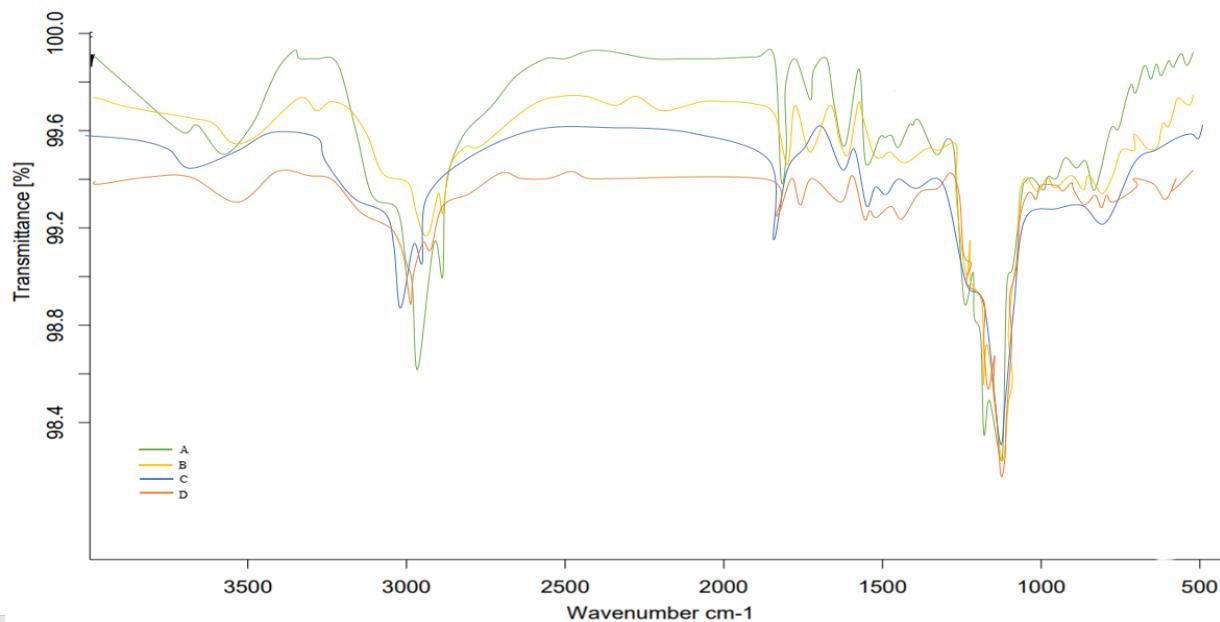
### 3-4-Characteristics of functional groups of nanofibers before and after encapsulation

FTIR spectroscopic analysis is one of the methods used to investigate noble groups in hydrocolloids. In this method, infrared light is passed through the sample and then collides with the interference solid material and is displayed in the form of an absorbed

and refracted spectrum [13]. The spectra obtained from the Fourier transform infrared spectroscopy system related to the nanoelectrophoresis hydrocolloid sample from mucilage under optimal conditions (power equal to 600 W, time equal to 4 minutes and pH equal to 0.1) are shown in Figure 3. The strong absorption area between 3500-3300 cm<sup>-1</sup> is related to the OH functional groups present in

different parts of the mucilage polymer structure. The peak appearing in the  $2122\text{ cm}^{-1}$  band is related to aliphatic H-C stretching vibrations, which includes bending and stretching vibrations of CH, CH<sub>2</sub> and CH<sub>3</sub> groups. Likewise, the band appearing at 1743 corresponds to CH-O<sub>3</sub> vibrations. Simon et al. (2014) concluded that the largest relative absorbance changes of gelatin scaffolds after Millard cross-linking were 1081 and 1035  $\text{cm}^{-1}$  (attributed to the C-O vibration of glucose). Then, the intensity of the features peaks at 109-1080  $\text{cm}^{-1}$  at 1080 and 112  $\text{cm}^{-1}$  [46]. Gadhav, Mahanwar & Gadekar (2019) also reported a similar result that the FT-IR profile of starch/PVA composite film shifted towards lower wavenumbers with increasing concentration [47]. Based on previous studies, electrospinning of composite solutions may lead to changes in the composition of the solution after encapsulation of electrospinning nanofibers. For this reason, the combination of nanofibers with spectroscopic diagrams shows that there are two strong peaks in the range of 1454 and 1743  $\text{cm}^{-1}$  from vitamin D<sub>3</sub> in nanocomposite and microcoated samples. which show the stretching vibrations related to the C=C group in the aromatic rings of phenolic compounds. The observed peaks in the

range of 2923 and 2854  $\text{cm}^{-1}$  also refer to the stretching vibrations of the H-C group of the methylene groups in the phenolic rings. The broad band centered at 3331  $\text{cm}^{-1}$  due to the presence of hydroxyl groups and vitamin D<sub>3</sub> shows the formation of (O=C) carboxyl group along with methyl bonds. The observed peaks at 1156 and 1087  $\text{cm}^{-1}$  are related to asymmetric O-C stretching vibrations and N-C stretching vibrations. Also, the peaks observed at 722 and 878  $\text{cm}^{-1}$  indicate the out-of-plane bending of the H-C bonds of the phenolic rings. In the graphs, the microcoated spectroscopy of vitamin D<sub>3</sub> also shows peaks in the ranges of 1458, 1517, 2854 and 3329  $\text{cm}^{-1}$ . These peaks are respectively related to H-O stretching vibrations, H-C stretching vibrations due to CH<sub>3</sub> groups, first-type amides, second-type amides, H-C bending and third-type amides. According to the existing compounds, in the range of 2000 to 2500  $\text{cm}^{-1}$ , peaks related to amide groups, aliphatic and aromatic C-H, and C=C alkenes can be observed, which have decreased over time .A similar finding was reported by Wang et al. (2015) [48].



**Figure 3. FTIR spectroscopy in the range of 400 to 4000  $\text{cm}^{-1}$  in the samples containing micro-coated composite nanofiber compounds. (A: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D<sub>3</sub> + 0.1% *Salvia macrosiphon* L. seed mucilage, B: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D<sub>3</sub> + 0.3% *Salvia macrosiphon* L. seed mucilage, C: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D<sub>3</sub> + 0.5% *Salvia macrosiphon* L. seed mucilage, D: Polyvinyl alcohol 4% + rice sauce protein 4% + 1% vitamin D<sub>3</sub> + 0.7% *Salvia macrosiphon* L. seed mucilage).**

#### 4-Conclusion

Advances in the production processes of nanostructures and nanomaterials with appropriate

formula characteristics provide the production of stable nanoparticles with the ability to be used in the food industry and related industries. Among them, nanofibrous films have more uniform diameter distribution and better orientation. *Salvia macrosiphon L.* seed mucilage showed great potential as a polymer for the production of capsules and fibers using electrospinning technique. Vitamin D3 was successfully encapsulated in high capacity capsules and fibers. Incorporation of mucilage of myrtle seed and/polyvinyl alcohol/rice bran carotene concentrate decreased the average diameter of the fibers and

increased the average diameter of the capsules. That capsules and fibers containing vitamins are practical materials for use in the food industry, especially those that are highly important in terms of phenolic and antioxidant compounds. Because vitamin degradation is reduced with microcoating. They show high antioxidant activity.

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## مطالعه تولید نانو الیاف کامپوزیت از ترکیبات هیدروکلوریک دانه *Salvia macrosiphon L.* به روش الکتروریسی به منظور ریز پوشینه کردن ویتامین D3

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### چکیده

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پیشرفت در فرآیندهای تولید نانوساختارها با خصوصیات فرمولی مناسب، تولید نانوذرات پایدار با قابلیت کاربرد در صنعت غذا را فراهم می‌سازد. می‌توان ترکیبات زیست فعال ریزپوشانی شده را در فیبرهای الکتروریسی ادغام کرد تا پایداری بیشتر نانوذرات در برابر حرارت و نور که منجر به افزایش زمان نگهداری است، محقق شود. در مطالعه حاضر، لیهای نانو الیاف کامپوزیت از موسیلاژ استخراجی از دانه *L. Salvia macrosiphon* با استفاده از الکتروریسی ساخته شدند. نانو کامپوزیت نانو الیاف از پلی وینیل کل/ایزوله *Salvia macrosiphon* پروتئین سبوس برنج / موسیلاژ دانه *L. Salvia macrosiphon* در نسبت‌های مختلف آماده شد. سپس مورفولوژی و طیف سنجی FTIR مورد بررسی قرار گرفت. متوسط قطر نانو الیاف تولیدی حدود ۴۰ نانومتر و ضریب واریانس ۱۳٪ است، که نشان داد قطر الیاف نسبتاً یکنواخت است. افزایش غلظت محلول موسیلاژ و درصد ثابت پلی وینیل کل باعث افزایش قطر نانو الیاف به طرز قابل توجهی شد. در مرحله بعد، ویتامین D3 درون نانو الیاف پلی وینیل کل و کنسانتره پروتئین سبوس برنج کپسوله شد. نتایج FTIR وجود ویتامین D3 را در نانو الیاف تهیه شده تایید کرد. در غلظت‌های بالاتر ترکیبات فلزی، با افزایش تعداد گروه‌های هیدروکسیل حلقه‌های آروماتیک در محیط واکنش، قدرت مهارکنندگی رایکال‌های آزاد موسیلاژ افزایش یافت. ترکیب نانو الیاف در نمودارهای طیف‌سنجی نشان داد که در نمونه‌های نانو کامپوزیت و ریزپوشانی شده، از ویتامین D3، دو پیک قوی در محدوده ۱۴۵۴ و ۱۷۴۳ بر سانتی متر وجود دارند که ارتعاشات کششی مربوط به گروه C=C در حلقه‌های آروماتیک ترکیبات فنولیک را نشان می‌دهند. بر اساس یافته‌ها، ترکیبات زیست فعال جهت افزایش دسترسی به ویتامین D3 را می‌توان در نانو الیاف الکتروریسی شده موسیلاژ *L. Salvia macrosiphon*/پلی وینیل کل/کنسانتره پروتئین سبوس برنج محصور کرد.