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Study of electrospun composite nanofibers based on quince (*Cydonia oblonga*) seed hydrocolloid for microencapsulation of vitamin D₃ and *Bacillus coagulans*

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ABSTRACT

In this study was to develop and optimize electrospun composite nanofibers based on quince seed mucilage, polyvinyl alcohol (PVA), and rice bran protein concentrate (RPC) for the simultaneous microencapsulation of vitamin D₃ and the probiotic bacterium *Bacillus coagulans*. After investigating different component ratios and processing conditions, a formulation containing 4% mucilage, 4% PVA, and 4% RPC was selected as the optimal composition, resulting in uniform, bead-free nanofibers with an average diameter of 310 ± 18 nm. FTIR analysis confirmed the presence of hydrogen bonding and effective intermolecular interactions among the polymers. SEM observations indicated that vitamin D₃ and *B. coagulans* were uniformly distributed within the nanofibrous structure. Microencapsulation efficiency results showed encapsulation yields of 87.6% for vitamin D₃ and 92.3% for *B. coagulans*. Release studies under simulated gastric conditions (pH 2.1) demonstrated that the nanofibrous system provided a gradual and controlled release of the bioactive compounds over 180 minutes, while effectively preventing vitamin degradation and reducing bacterial viability loss under acidic conditions. Stability tests conducted over 20 days the produced nanofibers retained more than 80% of vitamin D₃ and 90% of viable *B. coagulans* cells, whereas a significant reduction was observed in the free forms ($p < 0.05$). Overall, the developed electrospun nanocomposites, with their stable structure, biocompatibility, and high protective performance, show strong potential as novel and efficient carriers for the microencapsulation of bioactive compounds in food and pharmaceutical applications.

1- INTRODUCTION

As one of the most advanced fields of modern science, nanotechnology has significantly impacted the structure, stability, and biological functionality of food and pharmaceutical compounds. The development of nanostructures such as nanoparticles and nanofibers characterized by high surface-to-volume ratios, surface versatility, and favorable mechanical properties, has enabled the loading and controlled release of bioactive compounds, thereby enhancing their stability and bioavailability [1, 3]. In this context, electrospinning has emerged as an efficient and simple technique requiring no harsh processing conditions such as high temperatures or toxic solvents that holds a special place in the microencapsulation of sensitive compounds. By utilizing biocompatible polymers such as proteins and polysaccharides, this method produces uniform, stable nanofibers capable of protecting bioactive compounds against degradation caused by light, heat, and environmental conditions [4, 6]. In recent years, special attention has been focused on the use of natural hydrocolloids in the development of biocompatible nanofibers. Quince seed is a rich source of bioactive compounds with antioxidant, antibacterial, and anti-inflammatory properties, finding widespread application in traditional medicine as well as the food and pharmaceutical industries [7, 8]. The extraction of mucilage from quince seeds has emerged as a sustainable and biocompatible alternative to commercial thickeners and texturizers, and it can be utilized in the production of electrospun nanofibers. Combining this mucilage with polymers such as polyvinyl alcohol and rice bran protein concentrate provides a suitable matrix for producing uniform, biocompatible fibers that are effective for loading bioactive compounds [6, 9]. Alongside the use of polymer matrices, the encapsulation of valuable bioactive compounds is of particular importance. Vitamin D₃ is essential for bone health and immune system function; however, it exhibits limited stability due to its high sensitivity to light and heat. Electrospinning technology can enhance the vitamin's stability while enabling its gradual release under gastrointestinal conditions [10, 11]. Furthermore, probiotics particularly spore-

forming species such as *Bacillus coagulans* have attracted significant attention from researchers due to their high resistance to adverse processing and gastrointestinal conditions. This facultative anaerobic, catalase-positive bacterium is capable of producing lactic acid and plays a pivotal role in extending shelf life, improving gut health, and developing functional products [12, 13]. Numerous studies have been conducted on the production of electrospun nanofibers containing bioactive compounds. For instance, Song et al. (2022) succeeded in improving the quality and shelf life of food products by producing collagen/zein layers containing gallic acid [14]. Furthermore, Montano et al. (2014) produced nanofibers with antioxidant and antimicrobial properties by incorporating silver nanoparticles and vitamin E into a polylactic acid matrix, which completely inhibited the growth of pathogenic bacteria [15]. Recent studies have also confirmed the use of fibers containing natural antioxidants and plant proteins to enhance the controlled-release efficiency of bioactive compounds. Xu et al. (2012) conducted research on the preparation and properties of electrospun soy protein isolate/polyethylene oxide nanofiber membranes. They produced non-woven nanofibers by dissolving soy protein isolate and polyethylene oxide in HFIP solvent and employing the electrospinning technique. The results indicated that the majority of the nanofibers fell within the 200–300 nm diameter range, and that the soy protein isolate and polyethylene oxide were homogeneously dispersed at the nanoscale within the fibers, exhibiting high compatibility [16]. Despite these achievements, no comprehensive study has yet been reported on the development of seed-mucilage-based composite nanofibers for the simultaneous microencapsulation of Vitamin D₃ and *Bacillus coagulans*. Therefore, in the present study, biocompatible composite nanofibers were first prepared via electrospinning, and their efficacy in the microencapsulation and stability of Vitamin D₃ and *Bacillus coagulans* was subsequently evaluated.

2- Materials and Methods

2-1- Extraction of Quince Seed Mucilage

First, mucilage was extracted from quince seeds. To carry out this process, water was added to the quince seeds at a ratio of 20:1. The pH of the water was adjusted to the appropriate

level using 0.2 M sodium hydroxide (NaOH). Subsequently, to separate the mucilage from the seeds, the mixture was stirred for three hours at 1,500 rpm using a homogenizer (HD3200, Berli, Germany). Following the formation of swollen seeds and surrounding gel, the samples were centrifuged (Hettich-320R Universal, Germany) for 20 minutes at 4°C. The extracted mucilage was dried in an oven (Memmert UN55, Germany) at 50°C for 2 hours, then placed in polyethylene bags and stored in a cool, dry place until use [17].

2-2- Antioxidant assays of quince seed mucilage

2-2-1- Evaluation of free radical scavenging activity

Various concentrations (50, 100, 150, 200, 250, 300, and 350 µg/ml) of quince seed mucilage were prepared, along with 1 ml of 0.1 mM DPPH and 1 ml of methanol. After incubation at room temperature for 30 minutes, the absorbance of the DPPH-treated samples was measured at 517 nm against a blank using a spectrophotometer (Shimadzu UV/Vis-240 IPC, Japan) [18].

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

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

%RSD: Free radical scavenging percentage (percentage of antioxidant inhibition against free radicals)

A₀: Absorbance of the control (containing 1 ml of methanol and 1 ml of DPPH solution)

A: Absorbance of the sample (containing various volumes of quince seed mucilage or BHT, and DPPH)

2-2-2- Determination of Total Phenolic Content

The total phenolic content of the seed mucilage was determined using the Folin-Ciocalteu colorimetric method and expressed as gallic acid equivalents (Merck, Germany). For this purpose, standard gallic acid solutions were prepared at various concentrations (12.5, 25, 50, 62.5, 100, and 125 µg/ml) in a 60% solution. Then, 0.1 ml of each sample was transferred to a test tube, and 0.5 ml of 10% Folin-Ciocalteu

reagent solution was added. After 3 to 8 minutes, 0.4 ml of 7.5% sodium carbonate solution was added, and the absorbance was measured at a wavelength of 765 nm using a spectrophotometer. A mucilage sample (1 g) was dissolved in 60% methanol and analyzed using the same method. Finally, the sample's absorbance was plotted against the standard curve, and the total phenolic content was calculated as milligrams of gallic acid equivalent per gram [19].

2-2-3- Evaluation of the antimicrobial activity of the seed mucilage

The agar diffusion method was used to determine antimicrobial activity. Samples were formed into discs with a diameter of 10 mm using a mold. Prior to placing the discs on the culture medium surface, surface inoculation was performed using 100 µl of liquid culture medium containing approximately 10⁸ CFU/ml of each test bacterium. The discs were placed on Mueller-Hinton agar culture medium under strictly sterile conditions and subsequently incubated at 37°C for 24 hours. The difference between the diameter of the formed inhibition zones and the disc diameter was considered an indicator of antimicrobial activity. In cases where no zone was formed indicating the absence of antimicrobial activity the difference was considered zero [20].

2-3- Extraction of rice bran protein concentrate

100 g of defatted rice bran was mixed with distilled water at a 1:1 (w/w) ratio. The pH of the mixture was adjusted to 11.1 using a 1.1 M sodium hydroxide solution, and the mixture was stirred at room temperature for one hour. Finally, the mixture was centrifuged at 500 rpm for ten minutes. The pH of the supernatant (the liquid obtained after centrifugation) was adjusted to 4.1 using hydrochloric acid, and the mixture was stirred for half an hour. Subsequently, the supernatant was stored at 4°C overnight to allow the proteins to precipitate. The precipitate was carefully separated, and the precipitated proteins were washed with distilled water and neutralized [21].

2-4- Solution Electrospinning

In this study, solution electrospinning was performed using the compositions specified in Table 1 under controlled environmental and

instrumental conditions. Environmental conditions included a relative humidity of 30%, while instrumental parameters comprised a voltage of 12–20 kV, a solution feed rate of 0.125–0.5 ml/h, and a needle-tip-to-collector distance of 9–15 cm. To determine the optimal conditions, two parameters were kept constant in each run while the third was varied within the specified range. To prepare the solutions, specific amounts of quince seed mucilage were dissolved in 3 ml of distilled water. Subsequently, 4% polyvinyl alcohol and 4% rice bran protein concentrate were added to the

mixture, which was then stirred at room temperature for 15 hours to obtain a homogeneous solution. To ensure greater homogeneity, the solution was treated using an ultrasonic device equipped with a titanium probe (HD3200, Berlin, Germany). The prepared solution was transferred into 5 ml syringes, and nanofibers were produced using an electrospinning apparatus (Full Option Lab 2ESII-II, Iran) at a positive voltage of 18 kV, a needle-tip distance of 12 cm, and a collector drum speed of 500 rpm [22, 23].

Table 1. Solution samples for electrospinning.

Treatment	Quince seed mucilage (% w/v)	Polyvinyl alcohol (% w/v)	Protein concentrate (% w/v)
A	0.1	4	4
B	0.3	4	4
C	0.5	4	4
D	0.7	4	4

In this experiment, the nanofiber structure was examined using a scanning electron microscope (TESCAN Vega3, Czech Republic/China). Following imaging, the diameters of the produced fibers were measured using software, and the samples exhibiting the best morphology and the fewest beads were selected for the subsequent stage (microencapsulation).

2-5- Preparation of the probiotic bacterium *Bacillus coagulans*

The bacterium *Bacillus coagulans* was purchased in pure, lyophilized form from the Iranian Research Organization for Science and Technology (IROST) and activated on NYSM agar medium at 37°C for 24 hours. Subsequently, a colony from the NYSM culture medium was inoculated into 500 ml of liquid NYSM and incubated at 37°C for 48 hours with agitation at 250 rpm. The bacterial suspension was harvested three times by centrifugation (Hettich-320R Universal, Germany) at 3000 ×g for 20 minutes, washed with sterile peptone water, and stored in a refrigerator until use [24].

2-6- Microencapsulation

Table 2- Encapsulated with nano-composite treatments containing mucilage Queen seed, vitamin D₃ and *Bacillus coagulans*

To prepare the electrospinning solution containing quince seed mucilage, rice bran protein concentrate, polyvinyl alcohol, *Bacillus coagulans* bacteria, and vitamin D₃, the base components comprising the mucilage, protein concentrate, and polyvinyl alcohol were first mixed according to the established formulations (Table 2). Vitamin D₃ was added to 1.5 ml of water at a 1:4 ratio, and the resulting solution was stirred for 45 minutes to ensure the vitamin was completely dispersed within the polymer structure. Subsequently, specific amounts of *Bacillus coagulans* bacteria were added to the solution in accordance with the experimental treatments. The final solution was subjected to gentle stirring at ambient conditions until complete homogeneity was achieved. The prepared solution was drawn into 5 ml syringes, and the electrospinning process was carried out for 2 hours under optimized voltage and conditions to produce nanofibers containing the microencapsulated composite. Treatment A was considered the control without additives, whereas treatments B and C contained specific amounts of vitamin D₃ and *Bacillus coagulans* bacteria [24].

Treatment	The amount of <i>Bacillus coagulans</i> bacteria (%)	Vitamin D ₃ (%)	Quince seed mucilage (% w/v)	Polyvinyl alcohol (% w/v)	Rice bran protein concentrate (% w/v)
A	0.00	0.00	0.50	4.0	4.0
B	0.10	0.10	0.50	4.0	4.0
C	0.50	0.10	0.50	4.0	4.0
D	0.50	0.05	0.50	4.0	4.0
E	0.00	0.10	0.50	4.0	4.0
F	1.00	0.10	0.50	4.0	4.0

Microencapsulated nanocomposite treatments contained Quince seed mucilage, rice bran protein concentrate, polyvinyl alcohol, and varying amounts of vitamin D₃ and *Bacillus coagulans* bacteria. Treatment A was considered a negative control without active agent, and treatments B to F contained varying amounts of vitamins and probiotics.

2-7- Particle Size Determination

The mean particle size and span index of the electrospun microencapsulated particles were determined based on the particle size distribution using a particle size analyzer (SLS SALD 2101, Shimadzu, Japan). Particle size measurements were performed 24 hours after production, following storage of the samples at ambient temperature (25°C). The average particle size was determined based on the mean volumetric diameter [25].

Volume-mean diameter (mean equivalent diameter) or D_v :

$$D_v = \left(\frac{\sum N_i d_i^3}{\sum N_i} \right)^{1/3}$$

N_i : number of particles, d_i : mean particle diameter; the particle size distribution was also calculated using the following equation:

$$\text{Span} = \frac{D(90\%) - D(10\%)}{D(50\%)}$$

$D(90\%)$: The diameter below which 90% of the total particle volume in the system lies.

$D(50\%)$: The diameter below which 50% of the total particle volume in the system lies (median diameter).

$D(10\%)$: The diameter below which 10% of the total particle volume in the system lies.

2-8- Nanofiber morphology using electron microscopy

The samples were first sputter-coated with gold for 3 minutes using a sputter coater (Model DSR1, Nanostructured Coatings Co., Iran). Subsequently, images were obtained using a

scanning electron microscope (Model TESCAN Vega3, Czech Republic) [26].

2-9- FTIR Spectroscopy Test

To perform the FTIR test (PerkinElmer Spectrum Two model, USA), thin sample pellets with a thickness of less than one millimeter were prepared. These pellets were obtained by mixing the sample with potassium bromide at a 20:1 ratio, grinding the mixture, and drying it using a freeze-dryer (Christ Alpha 1-4 LD Plus model, Germany). Subsequently, the final pellets were produced by applying a pressure of approximately 60 kPa for 10 minutes using a pellet press. The transmission spectra of the samples were analyzed within the wavenumber range of 400–4000 cm⁻¹ with a resolution of 5 cm⁻¹ [27].

2-10- Determination of the encapsulation efficiency of Vitamin D₃ and *Bacillus coagulans* in composite nanofibers

To determine the encapsulation efficiency of Vitamin D₃, 1 ml of each encapsulated nanofiber sample was centrifuged at 13,000 rpm for 30 minutes to separate the free and entrapped components. Subsequently, the supernatant was filtered through a 0.22 µm syringe filter to remove remaining coarse particles and fibers. To measure the free vitamin content, 40 µL of the filtered phase was diluted with methanol to a final volume of 2 ml, and its absorbance was measured at a wavelength of 275 nm using a spectrophotometer (Shimadzu UV/Vis-240 IPC, Japan) [28]. Microencapsulation efficiency was calculated using the following equation:

$$EE\% = \left(\frac{\text{Total } D_3 - \text{Free } D_3}{\text{Total}} \right) \times 100$$

Total D₃: Total amount of vitamin D₃ added to the solution prior to electrospinning

Free D₃: Amount of free vitamin in the supernatant phase after centrifugation

EEVitD₃%: Encapsulation efficiency percentage of vitamin D₃ in the produced nanofibers

To determine the bacterial encapsulation efficiency, the samples were dissolved in phosphate buffer solution (pH 7.4) following electrospinning and cultured using the serial dilution method. Colony counting was performed after 24 hours of incubation at 37°C, and the results were expressed in CFU/ml [24].

2-11- Investigation of the release of microencapsulated compounds (Vitamin D₃ and *Bacillus coagulans*) in a simulated gastric system model.

To investigate the release behavior of the bioactive compounds (vitamin D₃), the samples were carefully detached from the aluminum collector surface and cut into pieces weighing approximately 10 mg. Each piece was placed in 25 ml of simulated gastric fluid, and the solution was continuously stirred using a magnetic stirrer at 100 rpm at room temperature.

A simulated gastric environment was prepared by combining 0.2 M hydrochloric acid and 0.2 M potassium chloride, adjusting the final pH to 1.2. At predetermined time intervals (0, 30, 60, 90, 120, and 180 minutes), a 3 ml aliquot of the solution was withdrawn and filtered through a 0.45 µm filter to remove fibrous particles. The absorbance of the filtered solutions at a wavelength of 282 nm was measured using a spectrophotometer (Shimadzu UV/Vis-240 IPC, Japan). The amount of released compound was calculated based on a vitamin D₃ standard curve [28], and the final release percentage was determined using the following equation:

Release percentage of microencapsulated compounds = (Amount of released microencapsulated compounds / Total amount of microencapsulated compounds) × 100

To investigate the release behavior of *Bacillus coagulans*, the microencapsulated nanofibers were cut into pieces weighing approximately 10

mg, and each piece was placed in 25 ml of simulated gastric fluid (pH 1.2) containing 0.2 M hydrochloric acid and 0.2 M potassium chloride. The samples were maintained at 37°C under magnetic stirring (100 rpm). At specific time points (0, 30, 60, 90, 120, and 180 minutes), a 1 ml aliquot of the solution was withdrawn and immediately neutralized with phosphate buffer (pH 7.4) to prevent cell death caused by the acidic environment. The solutions were then cultured using the dilution method, and after 24 hours of incubation at 37°C, viable colonies were counted and reported as CFU/ml [24].

2-12- Stability of microencapsulated compounds in nanofibers during storage

The stability of vitamin D₃ in the nanofibers was investigated during a 20-day storage period at 25 ± 1°C and 33% relative humidity. To determine the residual vitamin D₃ content, the vitamin was first extracted from the nanofiber structure; due to the sensitivity of vitamin D₃ to light and oxidation, all extraction steps were performed under low-light conditions. A specific amount of nanofibers was weighed and dissolved in a suitable organic solvent (96% ethanol). Subsequently, the sample was subjected to sonication (40 kHz frequency for 15 minutes) and centrifugation (6000 rpm for 10 minutes); the clear solution was then collected and passed through a suitable filter (syringe filter with a 0.45 µm pore size) to prepare it for analysis. Standard Vitamin D₃ solutions at various concentrations (5, 10, 20, 30, 40, and 50 µg/ml) were transferred into clean cuvettes, and their absorbance was recorded at a wavelength of 282 nm. A plot of absorbance versus concentration (µg/ml) was constructed. A linear relationship between absorbance and concentration, in accordance with the Beer-Lambert law, was established within this concentration range. Nanofibrous samples and fiber-free samples were analyzed on days 0, 7, 14, and 20. The amount of remaining vitamin at each time point was measured using a spectrophotometer at a wavelength of 282 nm.

$$D_3\% = Tc/0C \times 100$$

C₀: Initial Vitamin D₃ concentration on day zero; TC: Vitamin concentration at time t (days 7, 14, 20)

Remaining Vitamin D₃ (%): Percentage of Vitamin D₃ remaining relative to the initial amount

To evaluate microbial stability, nanofibrous and free samples containing *Bacillus coagulans* were stored for 20 days at $25 \pm 1^\circ\text{C}$ and 33% humidity. On days 0, 7, 14, and 20, samples were dissolved in phosphate buffer (pH 7.4) and then cultured on MRS agar medium using the serial dilution method. After 24 hours of incubation at 37°C , the number of viable colonies (CFU/ml) was counted [29].

2-13- Statistical Analysis

All data obtained from the experiments were analyzed based on a completely randomized design with three independent replicates. Statistical analysis was performed using SPSS software (version 26), and treatment means were compared using Duncan's test at the 5% probability level. Excel software was used to plot curves and graphs in order to visualize parameter trends and compare results. Results were reported as mean $\pm$ standard deviation.

3- Discussion and Results

3-1- Evaluation of DPPH free radical scavenging and total phenol content

The antioxidant activity of quince seed mucilage in the DPPH assay demonstrated that the percentage of free radical inhibition increased significantly ($p < 0.05$) with increasing sample concentration. This increase ranged from 22.1% at a concentration of 50 $\mu\text{g/ml}$ to 79% at 350 $\mu\text{g/ml}$, indicating a concentration-dependent behavior of the mucilage in scavenging free radicals. Antioxidant activity in plant extracts is typically associated with the presence of phenolic and polysaccharide compounds, which stabilize free radicals by donating electrons or hydrogen

atoms to them. This phenomenon is attributed to the chemical structure of the mucilage, which contains active hydroxyl groups and possesses a high capacity for hydrogen transfer. The trend of increasing radical inhibition in quince seed mucilage is similar to results reported for bioactive polysaccharides such as tragacanth gum and flaxseed mucilage, where previous studies have also observed a significant increase in antioxidant activity with rising concentrations [30, 32]. On the other hand, the total phenolic content in quince seed mucilage was determined to be 29.6 mg gallic acid equivalent per gram of dry mucilage, indicating a high concentration of phenolic compounds with reducing properties. Phenolic compounds are recognized as key contributors to antioxidant activity; due to their aromatic structure and hydroxyl (-OH) groups, they are capable of neutralizing free radicals through redox reactions. The positive correlation between total phenolic content and the percentage of free radical inhibition confirms that phenolic compounds are the primary factor responsible for the antioxidant properties of the mucilage. In addition to phenolic compounds, the three-dimensional polysaccharide structure of mucilage may also play a role in trapping free radicals and reducing their stability [33, 35]. Overall, the results of this study demonstrated that the seed mucilage possesses significant antioxidant potential and can serve as a natural, bio-based source of antioxidant compounds. The simultaneous presence of polyphenols and bioactive polysaccharides within the mucilage structure makes it a suitable candidate for use in producing edible films and coatings with antioxidant properties. Beyond extending the shelf life of food products, these characteristics also contribute to enhancing the nutritional and functional value of bio-based products.

Table 3-Examination of DPPH radical inhibition evaluation of the mucilage of Queen seed

Radical inhibition (%)	Concentration ($\mu\text{g/ml}$)
22.1 ± 2.0^a	50
34.8 ± 2.4^b	100
47.3 ± 2.7^c	150
58.6 ± 3.0^d	200
66.2 ± 2.6^e	250
73.1 ± 3.1^f	300

Radical inhibition (%)	Concentration (µg/ml)
79.0 ± 3.3 ^g	350

The results are presented as mean ± standard deviation for each treatment, and all experiments were performed in triplicate. Values with different capital letters are significantly different from each other at the 5% level.

Table 4- Examination of the total phenol of the mucilage of Queen seed

Sample	Total phenolic content (mg GAE/g)
mucilage of Queen seed (350 µg/ml)	29.6 ± 1.5 ^a
BHT (Standard)	32.1 ± 1.4 ^a

The results are presented as mean ± standard deviation for each treatment, and all experiments were performed in triplicate. Values with different capital letters are significantly different from each other at the 5% level.

3-2- Antimicrobial results of quince seed mucilage

Antimicrobial activity test results demonstrated that quince seed mucilage exerts a significant inhibitory effect on the growth of *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria. The diameter of the inhibition zone was measured at 19.5 mm for *Staphylococcus aureus* and approximately 16.8 mm for *Escherichia coli*; compared to the positive control (standard antibiotic), these results showed a significant difference at the 5% level ($p < 0.05$). These results indicate that the compounds present in quince seed mucilage particularly phenolic compounds and active polysaccharides—can inhibit bacterial growth by disrupting the cell membrane or altering bacterial cell wall permeability [36, 37]. When comparing the two types of bacteria, the inhibitory activity of the mucilage was greater against *Staphylococcus aureus* than against *Escherichia coli*. This difference may be attributed to the distinct cell wall structures of the bacteria. Gram-positive bacteria possess a thick peptidoglycan layer that interacts more extensively with the phenolic compounds and charged polysaccharides of the mucilage, leading to membrane permeability and cell death; in contrast, Gram-negative bacteria exhibit greater resistance to the penetration of natural compounds due to the presence of a lipopolysaccharide layer in their

outer membrane [36]. The results of this study align with the findings of previous research including studies by Mishra et al. (2023) on tamarind and fenugreek mucilage, and Pirsá et al. (2023) on garden cress seed mucilage—which demonstrated the effect of plant secondary metabolites on inhibiting bacterial growth [38, 39]. Similar results have also been reported, in which polyphenol extracts from various plants demonstrated higher efficacy against Gram-positive bacteria [40, 41]. In addition to the presence of phenolic compounds, the antimicrobial activity of the seed mucilage may be attributed to acidic polysaccharides and anionic groups such as carboxyl and hydroxyl that interact with the positively charged bacterial cell surface, leading to membrane structural disruption, leakage of cellular contents, and ultimately the inhibition of microbial growth. Furthermore, the gel-like structure of the mucilage may impede nutrient transport into the cell by forming a physical barrier on the bacterial surface [42, 43]. Overall, the results demonstrated that the seed mucilage possesses significant potential for use as a natural antimicrobial agent in bio-based packaging systems, edible films, and food preservation products. This attribute, combined with its antioxidant properties, offers dual functionality in maintaining food quality and extending shelf life.

Table 5 - Examination of the average diameter of the halo of non-growth (disc diffusion) of mucilage Queen seed on Gram-positive and Gram-negative bacteria

Bacteria	Diameter of the growth inhibition zone (mm)	Positive control (Antibiotic)	Negative control (no sample)
<i>Escherichia coli</i> (Gram-negative)	16.8 ± 0.8 ^b	22.5 ± 0.9 ^a	0.0 ± 0.0 ^c
<i>Staphylococcus aureus</i> (Gram- positive)	19.5 ± 0.8 ^b	25.3 ± 1.0 ^a	0.0 ± 0.0 ^c

The results are presented as mean ± standard deviation for each treatment, and all experiments were performed in triplicate. Values with different capital letters are significantly different from each other at the 5% level.

3-3- Optimization of formulation parameters and selection of a suitable structure for the microencapsulation of Vitamin D₃ and *Bacillus coagulans*

In the first stage, the electrospinning process conditions were investigated to determine the optimal parameters, based on the treatments listed in Table 2. Results from microscopic images revealed the samples that exhibited the most uniform fiber distribution, the smallest mean diameter, and the fewest structural beads. Therefore, this formulation was selected as the optimal composition and was subsequently used for the encapsulation of vitamin D₃ and *Bacillus coagulans* bacteria (Table 2). Furthermore, an investigation into various electrospinning process conditions revealed that key parameters including applied voltage, needle-to-collector distance, and solution feed rate significantly influence the morphology, uniformity, and diameter of the nanofibers. Increasing the voltage from 12 to 18 kV enhanced the electrostatic force relative to surface tension, thereby reducing fiber diameter and improving fiber uniformity. Consequently, in treatment D, fiber uniformity was enhanced

by reducing the oil phase [44]. However, applying a higher voltage (20 kV) caused jet instability and the formation of localized beads, resulting from electric field fluctuations and sudden charge discharge. On the other hand, setting the needle-to-collector distance at 12 cm created balanced conditions, allowing the solution sufficient time for solvent evaporation and the formation of coherent fibers. Furthermore, an injection rate of 0.5 ml/h ensured flow stability and reduced the likelihood of forming non-uniform droplets at the needle tip. Overall, optimal conditions comprising a voltage of 18 kV, a distance of 12 cm, and an injection rate of 0.5 ml/h, combined with a formulation of 4% polyvinyl alcohol, 4% rice bran protein concentrate, and a medium concentration of quince seed mucilage resulted in the production of uniform, bead-free nanofibers with an average diameter of approximately 310 ± 18 nm. These findings are consistent with previous reports [45–47]. They underscore that precise control of process parameters plays a decisive role in achieving stable nanofibrous structures capable of effectively loading bioactive compounds.

Table 6 - Compositions and optimal conditions for nanocomposite electrospinning

Treatment	Voltage treatment (KV)	Needle-to-collector distance (cm)	Injection rate (ml/h)	Drum speed (rpm)	Average fiber diameter (nm)	Fiber uniformity	Presence of knots (Beads)	Final evaluation
A	12	9	0.125	300	540 ± 32	Low	High	Inappropriate
B	14	10	0.25	400	470 ± 28	Moderate	Moderate	Acceptable
C	16	12	0.25	500	390 ± 25	High	Low	Suitable
D	18	12	0.5	500	310 ± 18	Very high	Very low	Best conditions

The results are presented as mean ± standard deviation for each treatment, and all experiments were performed in triplicate. Values with different capital letters are significantly different from each other at the 5% level.

3-4- Results for mean particle size and polydispersity index of microencapsulated nanocomposites

According to the results presented in Table 7, the mean particle size of the microencapsulated nanocomposites was influenced by the presence

of vitamin D₃ and *Bacillus coagulans* bacteria, showing a significant increase. The smallest particle size was observed in the control sample (A), whereas the addition of vitamin D₃ (B) and particularly the bacteria at higher concentrations (C, D, and F) resulted in a gradual increase in particle diameter. This trend can be attributed to the cumulative effects of the bio-additives; specifically, the vitamin and bacterial particles acted as nucleation sites during the electrospinning process and, by attracting more of the polymer matrix, led to an increase in particle diameter [48]. Furthermore, the span index changed with increasing active ingredient loading, and particle uniformity decreased. The lowest span value was observed in the control sample and the treatment containing only vitamin D₃ (B), indicating a more uniform particle distribution. In contrast, the presence of bacteria at higher dosages led to an increase in Span, resulting in greater heterogeneity in particle size distribution. This phenomenon can be attributed to changes in the

physicochemical properties of the electrospinning solution such as viscosity and surface tension induced by the presence of biological components, which affect jet stability and the rate of solvent evaporation [49, 50]. Overall, the results demonstrated that while the simultaneous loading of vitamin D₃ and probiotic bacteria leads to increased particle size and heterogeneity, it can offer enhanced functional benefits regarding the release of bioactive compounds. These findings align with similar studies on the electrospinning of drugs and probiotics, which have reported that increasing the loading of active agents generally results in larger particle sizes and reduced distribution uniformity [51, 52]. These findings align with previous studies on the microencapsulation of bioactive compounds and underscore the importance of selecting optimal formulation component ratios to achieve stable and uniform particles [53].

Table 7- Average particle size and dispersion index (Span) of microencapsulated nanocomposites containing mucilage from soybeans, vitamin D₃, and *Bacillus coagulans*

Treatment	Particle size (nm)	Span
A	285.4 ± 6.2 ^c	1.21 ± 0.03 ^c
B	302.7 ± 8.5 ^b	1.18 ± 0.04 ^c
C	315.9 ± 9.1 ^b	1.26 ± 0.05 ^b
D	341.6 ± 7.4 ^a	1.34 ± 0.04 ^a
E	329.2 ± 6.7 ^{ab}	1.29 ± 0.03 ^{ab}
F	358.3 ± 10.2 ^a	1.38 ± 0.06 ^a

The results are presented as mean ± standard deviation for each treatment, and all experiments were performed in triplicate. Values with different capital letters are significantly different from each other at the 5% level. Treatment A (control): Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate without the addition of vitamin D₃ and *Bacillus coagulans*. Treatment B: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 0.1% *Bacillus coagulans*. Treatment C: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 0.5% *Bacillus coagulans* bacteria. Treatment D: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 1% *Bacillus coagulans* bacteria. Treatment E: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol and 4% rice bran protein concentrate with 0.01% vitamin D₃, no bacteria. Treatment F: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 2% *Bacillus coagulans* bacteria (high dose of bacteria).

3-5- Efficiency results of encapsulating Vitamin D₃ and *Bacillus coagulans* in composite nanofibers

Results regarding microencapsulation efficiency indicated that different formulations significantly affected the encapsulation levels of Vitamin D₃ and *Bacillus coagulans* within the composite nanofibers ($p < 0.05$). Overall, increasing the bacterial content and the

simultaneous presence of Vitamin D₃ led to improved microencapsulation efficiency. The highest encapsulation efficiency for vitamin D₃ was observed in treatment D ($84.37 \pm 0.25\%$), which consisted of a combination of quince seed mucilage, polyvinyl alcohol, rice bran protein concentrate, and a moderate amount of bacteria. This enhancement can be attributed to hydrogen bonding and electrostatic interactions

between the hydroxyl groups of the mucilage and the polar groups of vitamin D₃; these interactions promote greater stability during the electrospinning process and reduce the leakage of the vitamin from the polymer matrix [54–56]. On the other hand, the encapsulation efficiency of *Bacillus coagulans* also increased significantly in the treatments containing the bacteria, particularly in treatment F, which had the highest bacterial concentration. The increased yield in this treatment is likely attributed to the formation of a denser nanofiber network and a high-microviscosity environment, which protect the bacteria against shear stresses and dehydration during electrospinning [57]. The presence of quince seed mucilage a natural hydrocolloid plays a significant role in the stability and survival of bacterial cells by forming a gel film and entrapping the cells [58]. The results of this study align with previously reported findings, which indicate that the use of biopolymer matrices such as polyvinyl alcohol and plant mucilages is effective in enhancing the microencapsulation efficiency of bioactive compounds and increasing the stability of microorganisms under processing conditions. In particular, combining biopolymers with

plant-based protein sources such as rice bran protein enhances molecular adhesion and reduces the leakage of active compounds [13, 59]. In a study conducted by Raghavan et al. (2020), the encapsulation of the probiotic yeast *Saccharomycopsis fibuligera* VIT-MN04 using the electrospinning technique was investigated to facilitate its passage through the gastrointestinal tract. Wheat bran fiber, exopolysaccharide, and 5% polyvinylpyrrolidone were used as the electrospinning materials in this study. The results demonstrated that encapsulation efficiency reached a maximum of 100%, while tolerance to gastrointestinal conditions reached 97% [60]. Overall, it can be concluded that the composite nanofiber structure produced via electrospinning using an optimal blend of quince seed mucilage, polyvinyl alcohol, and rice bran protein provides a stable and efficient matrix for the simultaneous microencapsulation of Vitamin D₃ and *Bacillus coagulans*. This approach not only enhances entrapment efficiency but also holds significant potential for food and pharmaceutical applications involving the controlled release of bioactive compounds.

Table 8- Encapsulation efficiency results of containing mucilage Queen seed, vitamin D₃ and *Bacillus coagulans*

Treatment	Vitamin D ₃ microencapsulation efficiency (EE%)	Bacterial microencapsulation efficiency (Log CFU/ml)
A	0.00 ± 0.00 ^e	0.00 ± 0.00 ^d
B	78.54 ± 2.14 ^b	0.00 ± 0.00 ^d
C	81.62 ± 1.98 ^{ab}	6.82 ± 0.15 ^c
D	84.37 ± 2.25 ^a	7.35 ± 0.21 ^b
E	0.00 ± 0.00 ^e	7.28 ± 0.18 ^b
F	79.43 ± 2.41 ^b	7.89 ± 0.22 ^a

The results are presented as mean ± standard deviation for each treatment, and all experiments were performed in triplicate. Values with different capital letters are significantly different from each other at the 5% level. Treatment A (control): Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate without the addition of vitamin D₃ and *Bacillus coagulans*. Treatment B: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 0.1% *Bacillus coagulans*. Treatment C: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 0.5% *Bacillus coagulans* bacteria. Treatment D: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 1% *Bacillus coagulans* bacteria. Treatment E: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol and 4% rice bran protein concentrate with 0.01% vitamin D₃, no bacteria. Treatment F: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 2% *Bacillus coagulans* bacteria (high dose of bacteria).

3-6- Gastric release results for the microencapsulated nanocomposite

The results regarding the release of Vitamin D₃ and *Bacillus coagulans* bacteria from composite nanofibers containing seed mucilage demonstrated that the release behavior of the bioactive compounds depends significantly on the formulation type and the amount of added probiotic ($p < 0.05$). No release was observed in the control treatment, which lacked bioactive compounds, whereas a gradual and controlled release pattern occurred in the treatments containing vitamin D₃ and bacteria. This phenomenon indicates that the nanofibrous matrix composed of quince seed mucilage, polyvinyl alcohol, and rice bran protein concentrate created a semi-permeable, porous environment that facilitates the gradual transfer of bioactive compounds into the simulated gastric environment [61, 62]. Among the samples, treatments D and F exhibited the highest release rates for Vitamin D₃ (86.0%) and *Bacillus coagulans* (94.6%), respectively, after 180 minutes. Increasing the bacterial content within the nanofiber structure resulted in the formation of finer pores in the matrix and enhanced permeability, thereby leading to a marked increase in the release rate of the compounds. However, at higher concentrations, a phenomenon of rapid initial release was observed attributed to the instability of the nanofiber structure and the accelerated degradation of the polymer matrix a finding also reported in similar studies [57, 63]. The overall release pattern exhibited two distinct phases: the first phase (0 to 60 minutes) corresponded to the rapid release of surface-associated compounds, while the second phase (60 to 180 minutes) represented the gradual release of

compounds entrapped within the inner layers of the fibers. This behavior aligns well with first-order and Higuchi kinetic models, indicating a diffusion-controlled release mechanism. The incorporation of seed mucilage into the nanofiber structure owing to its hydrophilic properties and ability to form gel networks retarded the rapid penetration of the acidic medium, thereby enhancing the stability of Vitamin D₃ and the survival of *Bacillus coagulans* under simulated gastric conditions. In a study by Yazdanpanah and Ghorbaninejad (2025), previous research has also demonstrated that microencapsulated nanocomposite systems can facilitate the controlled and gradual release of vitamins under simulated gastric conditions, a finding consistent with the results of the present study [64]. Furthermore, the use of biocompatible polymer matrices in electrospinning systems reduces the degradation of sensitive compounds in acidic environments and enhances the survival of probiotics during passage through the stomach [65, 66]. Overall, it can be concluded that electrospun nanofibers based on quince seed mucilage and rice bran protein concentrate serve as an efficient system for the microencapsulation and controlled release of vitamin D₃ and *Bacillus coagulans*, and are capable of significantly enhancing the stability of these bioactive compounds in the acidic environment of the stomach. These characteristics enhance the potential for utilizing these nanostructures in the production of functional foods and oral drug delivery systems characterized by high efficiency and optimal stability [67, 68].

Table 9- Examination of the release of vitamin D₃ in the stomach and intestines of a microcoated nanocomposite containing mucilage Queen seed, vitamin D₃ and *Bacillus coagulans*

	A	B	C	D	E	F
Time (min)	(%)	(%)	(%)	(%)	(%)	(%)
0	0.0 ± 0.0 ^a	0.0 ± 1.2 ^a	0.0 ± 1.2 ^a	0.0 ± 1.1 ^a	0.0 ± 1.3 ^a	0.0 ± 1.2 ^a
30	0.0 ± 0.0 ^d	31.2 ± 1.0 ^c	37.6 ± 1.2 ^{bc}	43.5 ± 1.3 ^b	35.2 ± 1.1 ^{bc}	47.4 ± 0.8 ^a
60	0.0 ± 0.0 ^e	51.0 ± 1.3 ^d	59.4 ± 0.7 ^c	66.6 ± 1.0 ^b	56.3 ± 1.2 ^{cd}	71.1 ± 1.0 ^a
90	0.0 ± 0.0 ^e	63.7 ± 1.2 ^d	72.2 ± 1.0 ^c	79.0 ± 0.8 ^b	68.9 ± 0.9 ^c	83.0 ± 0.6 ^a
120	0.0 ± 0.0 ^e	71.8 ± 0.7 ^d	79.6 ± 0.9 ^c	85.5 ± 1.0 ^b	76.6 ± 1.4 ^c	89.0 ± 0.7 ^a

Time (min)	A (%)	B (%)	C (%)	D (%)	E (%)	F (%)
180	0.0 ± 0.0 ^e	79.9 ± 0.9 ^d	87.2 ± 1.2 ^c	92.0 ± 1.0 ^b	83.4 ± 0.8 ^{cd}	94.6 ± 0.9 ^a

The results are presented as mean ± standard deviation for each treatment, and all experiments were performed in triplicate. Values with different capital letters are significantly different from each other at the 5% level. Treatment A (control): Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate without the addition of vitamin D₃ and *Bacillus coagulans*. Treatment B: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 0.1% *Bacillus coagulans*. Treatment C: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 0.5% *Bacillus coagulans* bacteria. Treatment D: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 1% *Bacillus coagulans* bacteria. Treatment E: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol and 4% rice bran protein concentrate with 0.01% vitamin D₃, no bacteria. Treatment F: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 2% *Bacillus coagulans* bacteria (high dose of bacteria).

Table 10-Release of *Bacillus coagulans* in the stomach

Time (min)	A (%)	B (%)	C (%)	D (%)	E (%)	F (%)
0	0.0 ± 0.0 ^a	0.0 ± 1.2 ^a	0.0 ± 0.7 ^a	0.0 ± 1.2 ^a	0.0 ± 1.1 ^a	0.0 ± 1.2 ^a
30	0.0 ± 0.0 ^e	28.2 ± 1.4 ^d	32.5 ± 1.0 ^{cd}	38.8 ± 0.8 ^b	34.8 ± 0.7 ^c	42.9 ± 1.2 ^a
60	0.0 ± 0.0 ^e	45.7 ± 0.9 ^d	51.5 ± 0.8 ^c	59.5 ± 1.3 ^b	54.4 ± 0.8 ^c	64.4 ± 1.0 ^a
90	0.0 ± 0.0 ^e	56.5 ± 0.9 ^d	62.6 ± 1.1 ^c	70.5 ± 1.2 ^b	65.5 ± 0.6 ^c	75.1 ± 1.1 ^a
120	0.0 ± 0.0 ^e	63.2 ± 1.0 ^d	69.0 ± 0.9 ^c	76.3 ± 0.9 ^b	71.8 ± 1.2 ^c	80.6 ± 0.7 ^a
180	0.0 ± 0.0 ^e	71.4 ± 1.1 ^d	77.3 ± 1.2 ^c	83.0 ± 1.0 ^b	79.2 ± 1.1 ^c	86.0 ± 0.9 ^a

The results are presented as mean ± standard deviation for each treatment, and all experiments were performed in triplicate. Values with different capital letters are significantly different from each other at the 5% level. Treatment A (control): Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate without the addition of vitamin D₃ and *Bacillus coagulans*. Treatment B: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 0.1% *Bacillus coagulans*. Treatment C: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 0.5% *Bacillus coagulans* bacteria. Treatment D: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol, and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 1% *Bacillus coagulans* bacteria. Treatment E: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol and 4% rice bran protein concentrate with 0.01% vitamin D₃, no bacteria. Treatment F: Microencapsulated nanocomposite containing 0.5% mucilage from Quince seed, 4% polyvinyl alcohol and 4% rice bran protein concentrate with 0.01% vitamin D₃ and 2% *Bacillus coagulans* bacteria (high dose of bacteria).

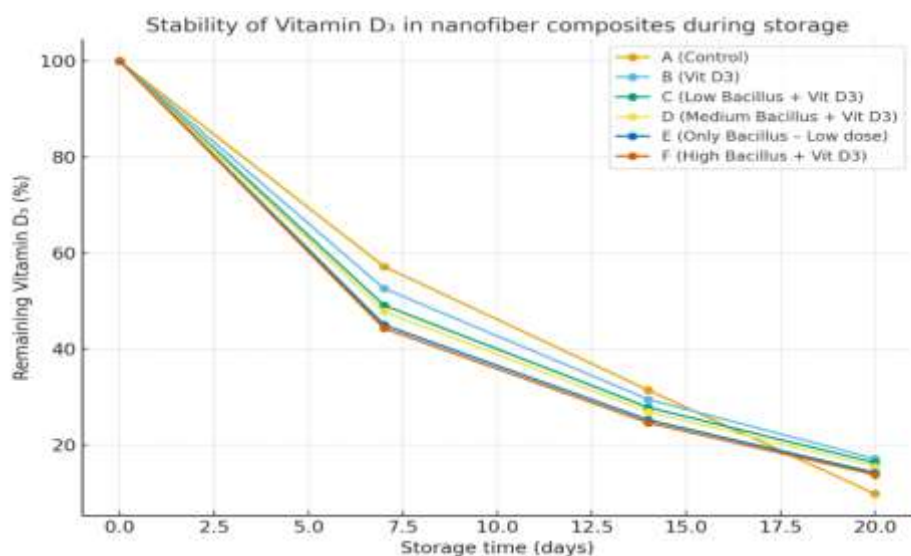
3-7- Investigation of Vitamin D₃ storage stability in microencapsulated nanocomposites

The results shown in Figure 1 demonstrated that microencapsulation within an electrospun matrix composed of quince seed mucilage, polyvinyl alcohol, and rice bran protein concentrate significantly protects vitamin D₃ and *Bacillus coagulans* cells during storage (25°C, 33% relative humidity). In the control treatment (A), which lacked the active

component/protective system, a rapid loss of the vitamin and a logarithmic decline in the bacterial population were observed; in contrast, the microencapsulated treatments (B–F) slowed this loss, resulting in a pattern of gradual decline with a shallower slope. This behavior can be explained by the formation of a low-mobility, glass-like microenvironment within the fibers and a hydrogen-bonding network among the polyvinyl alcohol, protein, and polysaccharide chains. The network restricts the permeation of oxygen and water vapor, thereby

reducing the release of reactive species and their accessibility to the vitamin. During the first seven days, the contribution of the surface/shallow region of the matrix predominated; however, from day 7 to 20, the rate of decline slowed significantly, indicating diffusion-controlled release and intra-matrix transport behavior consistent with first-order (and occasionally Weibull) kinetic models for microbial systems, and Higuchi-type diffusion models for molecular components [69]. A comparison of the treatments revealed that the presence of the vitamin alone (B) resulted in greater stability compared to the control; however, combining it with bacteria (C–F) offered an added advantage: at higher bacterial dosages (particularly F), microbial survival was maximized while vitamin stability remained favorable. This is likely due to cellular aggregation and protein-polysaccharide surface interactions, which create increased local network density and longer diffusion pathways for oxygen [70, 71]. Of course, an excessive

bacterial load can lead to the formation of micropores and slightly accelerate vitamin degradation; our data indicate that while treatment F ensures the best survival for *Bacillus coagulans*, treatment D offers a superior balance between vitamin stability and bacterial survival at the end of day 20 (stability exceeding that of B, C, and E, with survival rates close to those of F). Therefore, from a practical standpoint, treatment D is more advisable for preserving nutritional value and probiotic functionality during storage, whereas treatment F is preferable for formulations where maximizing probiotic survival is the priority. Previous studies have also shown that the use of microencapsulation systems can provide a similar controlled-release pattern by influencing the release rate and stability of vitamins, which aligns with the results of this study [71, 72].



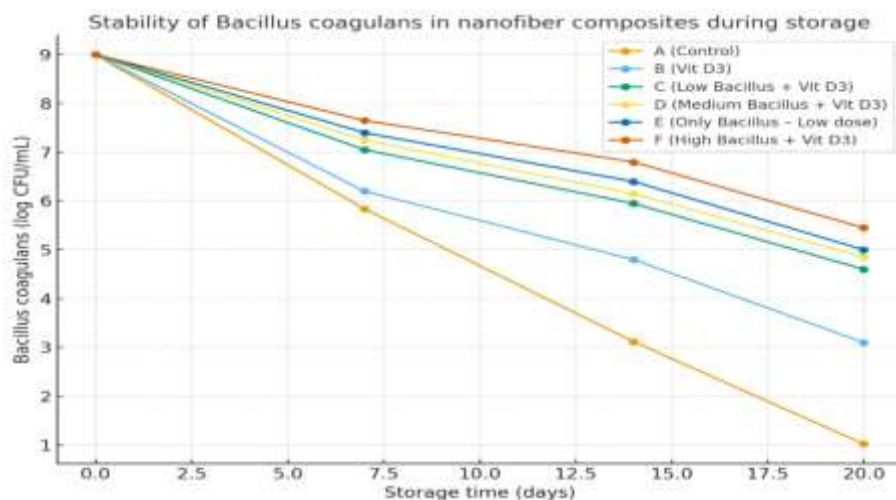


Figure 1- Storage results of vitamin D₃ of nanocomposite containing mucilage Queen seed, vitamin D₃ and *Bacillus coagulans*

3-8- Scanning Electron Microscopy Images

Scanning electron microscopy images of the electrospun nanofibers (Figure 2) revealed distinct differences in the surface structure of the various samples (A through F). The control sample (A) comprising the base polymer matrix (polyvinyl alcohol + rice bran protein concentrate + quince seed mucilage) exhibited uniform, smooth, and bead-free fibers with an average diameter of less than 300 nm, indicating optimal electrospinning conditions. In the samples containing vitamin D₃ (B) and those with a low probiotic concentration (C), the fiber surfaces remained predominantly smooth and continuous, although minor roughness and localized protrusions were observed [73, 74]. These irregularities stem from the presence of bioactive compounds within the fiber matrix. Although the presence of vitamin D₃ is not directly visible in the images, changes in the density and surface texture of the fibers confirm the penetration of the vitamin into the polymer structure [75]. This finding aligns with the results reported by Zhang et al. (2020) regarding the nano-encapsulation of fat-soluble vitamins. With an increase in the probiotic bacteria content (samples D and F), the average fiber diameter increased (to approximately 350–370 nm), and localized aggregates and protrusions appeared on the fiber surfaces. These changes are attributed to the presence of *Bacillus coagulans* cells within the polymer network, which altered the stability of the electrospinning jet and induced fiber non-uniformity [76, 77]. Similar

observations have also been reported, indicating that the presence of microorganisms within the nanofiber structure leads to surface roughness and microscopic aggregates [53, 56]. The greatest heterogeneity was observed in sample E (containing only probiotic bacteria, without vitamin D₃), where the fibers exhibited varying diameters and a relatively irregular structure. This indicates the direct impact of the high microbial concentration on jet stability and bead formation during the electrospinning process. Overall, electron microscopy results demonstrated that the addition of vitamin D₃ and *Bacillus coagulans* significantly influenced the morphological characteristics of the nanofibers, leading to increased diameter, surface roughness, and the formation of localized aggregates. However, the optimal formulation (Sample D) containing moderate levels of both bioactive compounds produced homogeneous, stable fibers with a uniform distribution, thereby providing the most suitable structure for the co-encapsulation of these bioactive agents [78]. Studies conducted by Golkar et al. (2019) investigated the production and characterization of electrospun nanofibers from *Plantago major* seed mucilage and polyvinyl alcohol. In this study, mucilage as a natural polymer and polyvinyl alcohol as a non-toxic expanding agent were combined to produce electrospun nanofibers. Scanning electron microscopy results indicated that the nanofibers produced using a 50/50 ratio of *Plantago major* and polyvinyl alcohol have an average diameter of 250 nm. This diameter demonstrates the impact of optimizing

parameters and polymer solution composition on the production of uniform nanofibers with controlled diameters [79]. Consistent with previous studies, the results of this research demonstrated that increasing the solution's viscosity and electrical conductivity can, to some extent, facilitate the production of

nanofibers with uniform diameters in the nanometer range, whereas unfavorable parameter adjustments may lead to increased diameters or structural defects in the nanofibers.

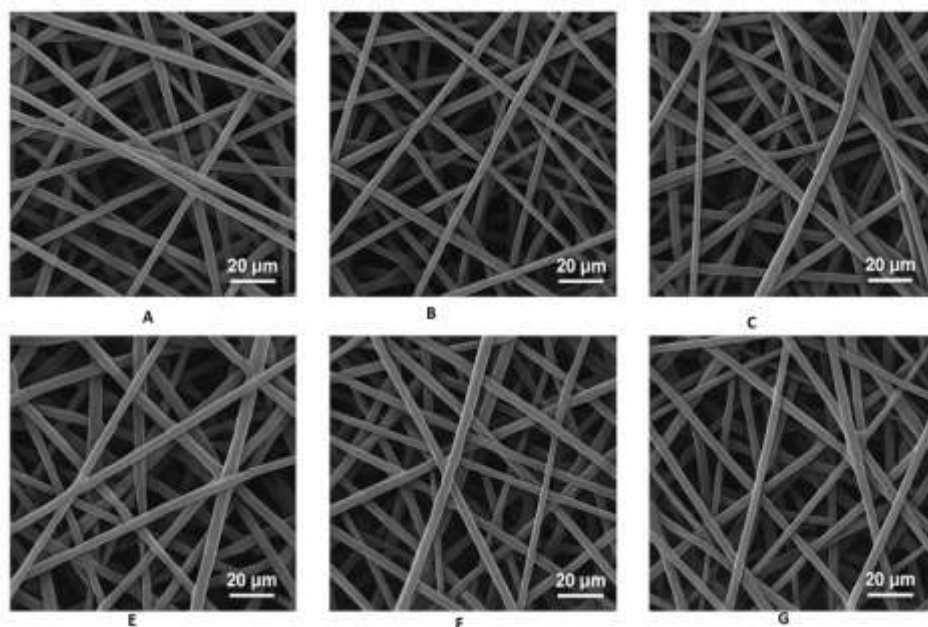


Figure 2- Morphology of nanofibers produced after microencapsulation containing mucilage to Quince seed, vitamin D₃, and *Bacillus coagulans*

3-9- FTIR Results

The FTIR spectra for the various treatments (A through F) (Figure 3) revealed a distinct absorption pattern for the major bands. The broad band in the 3600–3200 cm^{-1} range corresponds to O–H/N–H stretching vibrations; compared to the control (A), this band exhibited a slight decrease in intensity and a shift toward lower wave numbers in treatments B through F. This change stems from an increase in hydrogen bonding between the hydroxyl groups of the mucilage and polymer and the Vitamin D₃ and cellular components of *Bacillus coagulans*. C–H stretching bands in the 2920 and 2850 cm^{-1} range intensified in treatments containing the vitamin and bacteria; this is attributed to the increased contribution of lipophilic chains derived from these compounds [80]. Amide I and II bands associated with rice bran protein were also observed in the 1650–1540 cm^{-1} range; subtle changes in their intensity and position indicate a rearrangement of hydrogen and electrostatic bonds within the fiber matrix. The strong band at 1050 cm^{-1} , attributed to C–O/C–O–C vibrations of the mucilage,

intensified and broadened in the bacterial treatments (C through F) a phenomenon resulting from the presence of polysaccharides and sugars from the bacterial cell walls. Most importantly, the absence of new bands indicates that the microencapsulation process was primarily driven by stable physical interactions (hydrogen bonding and van der Waals forces) and that no destructive chemical changes occurred [19, 81]. Specifically, Treatment D (vitamin + moderate bacterial dosage) exhibited the most balanced changes and appears to have provided optimal conditions regarding chemical compatibility and structural stability. Treatment F (high bacterial dosage + vitamin) showed the greatest band broadening and intensity variations, indicating a more extensive and heterogeneous hydrogen-bonding network, which may correlate with a faster release of compounds. Therefore, FTIR not only confirms the successful co-loading of vitamin D₃ and *Bacillus coagulans* but also demonstrates that selecting appropriate levels of these components (similar to treatment D) can yield an optimal combination in terms of chemical

stability, controlled release, and the preservation of bioactivity [72, 82].

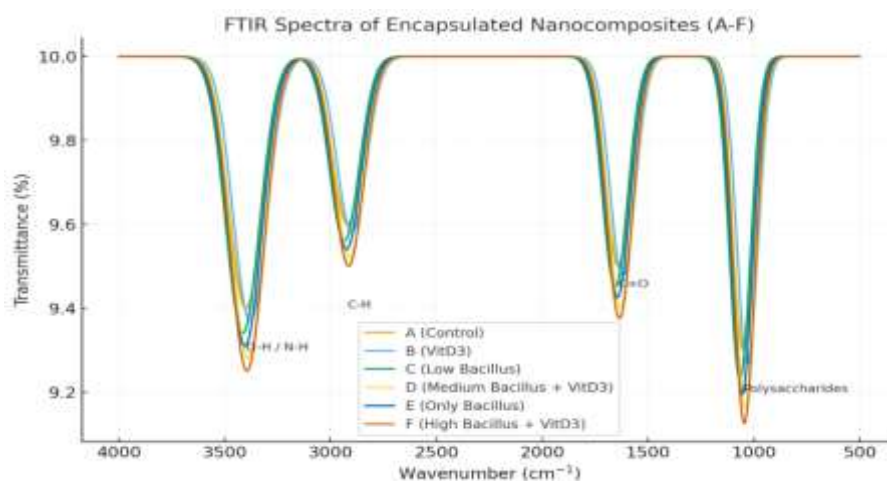


Figure 3- FTIR spectra of different treatments (A to F) containing mucilage to Quince seed, vitamin D₃, and *Bacillus coagulans*

4- Research Limitations

This study did not evaluate the performance of the nanofibers in an environment that fully simulates the gastrointestinal tract. Specifically, intestinal environmental conditions such as a more neutral pH and the presence of specific enzymes were not investigated. Furthermore, the mechanism and rate of Vitamin D₃ release within the intestinal environment (where the pH shifts gradually from acidic to alkaline) were not determined. This factor is crucial for the vitamin's effectiveness. There is no absolute certainty regarding the survival and continued activity of *Bacillus coagulans* bacteria after they pass through the challenges of the gastrointestinal tract (including acidity, bile, and competition with other microorganisms). Conducting in vitro studies using gastrointestinal simulators or in vivo studies on animal models is essential to verify the physiological function and the effectiveness of the microencapsulation system in vivo. The designed microencapsulation system has been evaluated solely under laboratory conditions and in isolation; it has not yet been applied to or tested on an actual food product (such as yogurt, a dairy beverage, or bread). The nanofibers and their contents (vitamins and bacteria) may interact with other food components such as fats, proteins, carbohydrates, and additives potentially compromising their stability and functionality. Conducting compatibility and

stability tests within various food matrices and evaluating the sensory characteristics of the final product are essential prerequisites for any industrial application.

5- Conclusion

The results of this study demonstrated that using a combination of quince seed mucilage (a natural polysaccharide with high biocompatibility), polyvinyl alcohol, and rice bran protein concentrate in the electrospinning process resulted in the formation of uniform, stable, and bead-free composite nanofibers. FTIR and scanning electron microscopy analyses indicated strong hydrogen bonding interactions between the functional groups of the polymers used, which enhanced network strength and increased the stability of the final structure. The simultaneous encapsulation of Vitamin D₃ and *Bacillus coagulans* within a nanofiber matrix resulted in a significant increase in encapsulation efficiency and controlled release of the bioactive compounds. Release tests in a simulated gastric environment demonstrated that formulations containing a moderate concentration of mucilage and a balanced protein-to-polyvinyl alcohol ratio (Treatment C) exhibited the best stability and release control. This characteristic was attributed to the formation of hydrogen bonds between vitamin D₃ and the polysaccharide chains, as well as the protective role of the protein layer against acidic conditions. Furthermore, the storage stability of the

nanofibrous samples over a 20-day period demonstrated that the seed mucilage composition not only prevented the degradation of vitamin D₃ but also maintained high viability of *Bacillus coagulans* compared to the free samples. The antioxidant and antimicrobial activities of the mucilage, combined with an optimized nanofibrous structure, played a significant role in protecting bioactive compounds. Overall, the results indicate that electrospun nanofibers based on quince seed mucilage, polyvinyl alcohol, and rice bran protein acting as a biocompatible, effective, and stable system possess great potential for the microencapsulation of sensitive compounds such as vitamin D₃ and probiotics; they can be utilized in the development of food and pharmaceutical products featuring enhanced functional properties and controlled release.

Data Availability

All data generated or analyzed during this study are included in this published article and its tables. Therefore, all information necessary to evaluate, interpret, and support the findings of this study is available to readers.

Conflict of Interest

The authors declare that they have no competing interests or conflicts of interest regarding the publication of this article.

Author Contributions

All authors contributed to the revision of the manuscript, approved the final version for publication, and agreed to be accountable for all aspects of the work.

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مطالعه نانوالیاف کامپوزیتی الکتروریسی شده بر پایه هیدروکلونید دانه به (Quince seed)

Cydonia oblonga جهت ریزپوشانی ویتامین D₃ و باکتری *Bacillus coagulans*

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در این پژوهش توسعه و بهینه سازی نانوالیاف کامپوزیتی الکتروریسی شده بر پایه موسیلاژ دانه به، پلی وینیل الکل (PVA) و کنسانتره پروتئین سبوس برنج (RPC) جهت ریزپوشانی همزمان ویتامین D₃ و باکتری پروبیوتیک *Bacillus coagulans* بود. پس از بررسی نسبت های مختلف اجزا و شرایط فرآیند، فرمولاسیون حاوی ۴٪ موسیلاژ، ۴٪ PVA و ۴٪ RPC به عنوان ترکیب بهینه انتخاب شد که منجر به تولید لیاف یکنواخت، بدون گره و با قطر میانگین 310 ± 18 نانومتر گردید. نتایج آزمون های FTIR وجود پیوندهای هیدروژنی و برهم کنش های بین مولکولی مؤثر بین پلیمرها را تأیید کرد. آزمون های SEM نیز نشان دادند که ویتامین D₃ و *B. coagulans* به صورت یکنواخت در ساختار نانوالیاف توزیع شده اند. بررسی کارایی ریزپوشانی نشان داد که بازده انکپسولاسیون ویتامین D₃ و *B. coagulans* به ترتیب ۸۷/۶٪ و ۹۲/۳٪ بود. آزمون رهایش در محیط شبیه سازی شده معده (pH ۱/۲) نشان داد که سیستم نانوالیافی توانست رهایش تدریجی و کنترل شده ترکیبات زیست فعال را در مدت ۱۸۰ دقیقه فراهم کند، در حالی که از تخریب ویتامین و کاهش زنده ماندن باکتری در شرایط اسیدی جلوگیری شد. نتایج آزمون پایداری طی ۲۰ روز نگهداری نشان داد که نانوالیاف تولید شده تا بیش از ۸۰٪ از ویتامین D₃ و ۹۰٪ از سلول های زنده *B. coagulans* را حفظ کردند، در حالی که در نمونه های آزاد کاهش معنی داری مشاهده گردید (در سطح ۵ درصد). نانوکامپوزیت های الکتروریسی شده حاصل، با ساختار پایدار، زیست سازگار و عملکرد حفاظتی بالا، می توانند به عنوان حامل های نوین و مؤثر برای ریزپوشانی ترکیبات زیست فعال در صنایع غذایی و دارویی مورد استفاده قرار گیرند.