Electrospinning of glutelin-hordein incorporated with *Oliveria decumbens* essential oil: Characterization of nanofibers

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**ARTICLE INFO**

**Keywords:**
Electrospinning
Glutelin
Hordein
Nanofiber
*Oliveria decumbens* essential oil

**ABSTRACT**

In this study, electrospinning of hordein and glutelin extracted from barley was carried out. Different ratios of the glutelin-hordein blends (25:75, 30:70, 35:65) were tested and the operation parameters including voltage, ejection flow rate and needle-to-collector distance were optimized. According to the scanning electron microscope images, the glutelin-hordein 25:75 blend generated at the voltage of 15 kV, the needle-to-collector distance of 150 mm and the ejection rate of 1 mL/h was selected for the fabrication of uniform nanofibers. The apparent viscosity at the ejection point was decreased with increasing the glutelin concentration from 25 to 35 %. Moreover, the *Oliveria decumbens* essential oil (ODEO) with different loading concentrations (2–4 % (v/v)) was incorporated into the protein blend. Fourier-transform infrared spectra demonstrated the occurrence of the interactions of proteins the ODEO. The encapsulation efficiency of ODEO in the nanofibers was 79.30 %. The presence of ODEO led to inhibition the growth of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* in a synthetic medium. The optimal nanofibers showed high antioxidant activity. The results herein showed the possibility of the production of electrospun nanofibers using barley proteins with promising (bio)functionalities for the active food packaging applications.

1. Introduction

Electrospinning has emerged as a simple, cost effective, and versatile electrohydrodynamic technique which is used for the fabrication of micron-, submicron- and nano-scale fibers from different polymer suspensions. Electrospun fibers are formed using the application of a high-voltage electrostatic field between a jet of a polymer suspension and a collector plate with opposite charges [1]. These fibers may possess several unique structural and functional properties including high porosity with small pore size, large surface area-to-volume ratio, high gas permeability and promising mechanical properties [2]. The electrospinning technique is influenced by numerous factors associated with the properties of the polymer suspension (e.g., viscosity, electrical conductivity), operating parameters (e.g., voltage, flow rate, distance) and experimental conditions (e.g., relative humidity, temperature) [3, 4]. The electrospun fibers have been widely employed across various fields such as drug delivery systems, membrane technology, protective clothing, mass spectrometry, electronics and tissue engineered scaffolds [5–7]. Electrospinning has also been employed in the food industry, e.g., for enzyme immobilization, active food packaging and filtration membranes [8,9]. Additionally, electrospun nanofibers have been recently emerged as novel carriers to encapsulate and deliver bioactive compounds extracted from the plant sources [10]. It is well documented that the incorporated bioactive compounds in ultrafine electrospun fibers can significantly enhance their functionality as a result of nano-scale advantages [11].

Spinnable polymers are good candidates as carriers for electrospinning of other compounds [12]. Electrospinning of renewable and biodegradable natural biopolymers is a newly emerging area and the existing research studies are focused to pave the ways for its potential industrial applications [13]. Among different types of biopolymers, proteins have attracted more attention due to their specific characteristics and also their important roles in the human body [14,15]. However, the electrospinning of proteins is a challenging process, mainly due to their complex secondary and tertiary structures. Furthermore, globular proteins are unable to effectively entangle during the spinning...
process as a result of their poor internal interactions. This challenge may be overcome by fully dissolving the protein in a random coil conformation or by combining them with other polymers having proper spinnability [16].

Barley (Hordeum vulgare L.), as one of the most important cereals in the world with global production of approximately 150 Mt/year, is widely used for the animal feed and in malting industry. The whole grains of barley have a protein content of 8–13 wt% [17,18]. These proteins are categorized according to their solubility into four groups: albumins, globulins, glutelins and hordeins. Hordein is an alcohol-soluble protein fraction belonging to prolamins accounting for up to 50 wt% of the total barley proteins. Hordeins and glutelins are mainly found in the starchy endosperm and together contribute up to 70–90 wt% (on a dry weight basis) of the the overall barley proteins [19,20].

Oliveria decumbens essential oil (ODEO) is a volatile compound with high antimicrobial and antioxidant activities due to the presence of high phenolic content, thymol, carvacrol and their synergistic effect [21]. The non-polar organic compounds in thymol exhibited a high solubility and permeability into the outer membrane of the bacterial cell, resulting in cell membrane damage and bacterial growth reduction [22]. Nevertheless, high volatility of ODEO and its poor water solubility and stability restrict its widespread applications in the food and pharmaceutical formulations. Encapsulation of essential oil into electrospun nanofibers is a promising strategy for improving its stability, sustained release to the food surface and biofunctionalities [23,24].

To date, only a few proteins have been electrospun into nanofibers using solvents acceptable in the food industry, e.g., ethanolic solution of zein and warm water solution of gelatin [25]. To our knowledge, this is the first report associated with the fabrication of food grade hordein-glutelin electrospun nanofibers incorporated with ODEO for active biopolymer-based packaging purposes. The development of uniform electrospun matrices from barely proteins may be useful to facilitate the incorporation of natural antioxidant and antimicrobial agents in active packaging. This may enhance the safety and the quality and extend the shelf-life of different food products. Moreover, the encapsulation of bioactive compounds in electrospun fibers is documented to improve their stability and bioavailability. To this end, the suspension dependent parameters (polymer concentration, viscosity and electrical conductivity) and the operating conditions (needle-to-collector distance, ejection flow rate and voltage) were optimized during the electrospinning of hordein and glutelin. The hypthesis is that by blending a non-electrospinnable protein (glutelin) with an electrospinnable protein (hordein), a new set of interactions between these proteins will be occurred, which may result in a high electrospinnable proteinaceous suspension with new biofunctional properties, e.g., high antioxidant and antimicrobial activities. Furthermore, different concentrations of ODEO were encapsulated into the nanofibers at the optimum electrospinning conditions. The resultant nanofibers were characterized in terms of chemical structure, morphological properties and biological activities.

2. Materials and methods

2.1. Materials

Barley grains were provided from the School of Agriculture of Shiraz University (Shiraz, Iran). Hexane, ethanol, sodium hydroxide (NaOH), acetic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and dimethyl sulfoxide (DMSO) were purchased from Merck Company (Darmstadt, Germany). Oliveria decumbens plant was collected from Kazerun (Fars province, Iran) and dried under the shade at the laboratory condition on a large screen tray at 25 °C. Other chemical reagents were of analytical grade.

2.2. Proteins isolation from barley grains

Barley grains were pearled using a laboratory pearling machine to separate the outer layers of the grain with pearling efficiency of 27 %. This level of pearling was found to be efficient in removing most of the outer layers. The pearled grains (PG) were grounded to pearled grain flour (PGF) using the laboratory milling equipment. The PGF was defatted with hexane at a PGF to hexane ratio of 1:7 (w/w). The defatted PGF was separated from the hexane using a centrifugation (Froilabo, SW14R model, France) at 9000 g at 25 °C for 10 min [26]. The PGF samples were air-dried under a fume hood overnight to remove the residual hexane, packed in the polyethylene bags, then stored at 4 °C for further analyses.

To isolate the hordein fraction, PGF was treated with an ethanol solution (50 %, v/v) at a ratio of 1:6 (v/w) at 60 °C for 2 h. The supernatant was collected through centrifugation and subjected to cold precipitation at 4 °C for at least 12 h in order to isolate the hordein fraction. The crude hordein extract was then isolated from the solvent using centrifugation (9000 g, 4 °C, 10 min). To extract the glutelin fraction, the residue obtained following the extraction of hordein was separated by further centrifugation (9000 g, 25 °C, 10 min) and suspended with 0.5 M NaOH for 30 min at 25 °C. The insoluble solids and the supernatants were separated using centrifugation as above-mentioned. The collected supernatants were adjusted to pH 5.0 using 0.5 M HCl followed by 30 min holding at 25 °C to precipitate the proteins. Protein isolates were then obtained by centrifugation. The isolated protein fractions were lyophilized and the dried powders were stored in the plastic bags at 4 °C before further analysis. The protein content of each isolated protein fraction was determined using a standard micro Kjeldahl method [26].

2.3. Molecular weight (MW) profile

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to obtain the MW of barley protein fractions (hordein and glutelin) [27]. Each isolated protein (30 µL) was mixed with a loading buffer consisting 0.125 M Tris-HCl, pH 6.8, 4 % (w/v) SDS, 20 % (v/v) glycerol, 0.5 % 2-mercaptoethanol and 1 % (w/v) bromophenol blue. The samples were heated at 95 °C for 5 min followed by cooling down to room temperature. Afterward, 15 µg of protein suspension was loaded on 12 % SDS gel and electrophoresis was performed at a constant voltage of 80 V for 2 h. Staining was performed using Coomassie brilliant blue-R-250.

2.4. Preparation of electrospun nanofibers

A 450 mg of different glutelin-hordein blends (0:100, 25:75, 30:70, 35:65 and 100:0 (w/w)) was dissolved in 3 mL acetic acid (100 %). The dispersions were left at room temperature under constant stirring for 24 h. The prepared dispersions were then subjected to ultrasound (100 W, 10 s) followed by heating at 80 °C for 10 min to form a homogenous mixture. A 10-ml disposal plastic syringe was filled with the protein suspension of glutelin-hordein and processed using an electrosprinning apparatus (spinner-3X-Advance, ANSTCO, Tehran, Iran). The positive electrode of the power supply was connected to the needle, while that of the negative electrode was connected to a collector. The aluminum foil was wrapped around the collector to enable the evaporation of any residual solvent. Different operating parameters including voltage (12 and 15 kV), flow rate (0.5 and 1.0 mL/h) and tip-to-collector distance (100 and 150 mm) were initially examined to find the optimum setup.

2.5. Effects of protein suspensions parameters on electrospinning

2.5.1. Apparent viscosity and rheological properties

The apparent viscosity of the polymeric suspensions was determined according to Kurd et al. [14] using a rotational Brookfield viscometer (Brookfield Model DV-III + Pro, Brookfield, MA, USA) equipped with CPS1 spindle at 25 °C. The shear rates were within a range between 0 to 200 s⁻¹ over 120 s. The apparent viscosity (η, expresses as Pa s) at the
ejection point was calculated using the Eq. (1).

\[ \eta = K \gamma^{n-1} \]  

(1)

where \( K \) is the consistency index (Pa s\(^n\)), \( n \) is the flow behavior index and \( \gamma \) is the shear rate (s\(^{-1}\)).

The maximum shear rate which is used for power-law materials in tubular geometry was obtained from the Eq. (2):

\[ \gamma = \left( \frac{3n + 1}{4n} \right) \left( \frac{4Q}{\pi R^3} \right) \]  

(2)

where \( Q \) represents the volumetric flow rate of the material and \( R \) corresponds to the radius of the tip, where the polymer suspensions sprayed into the electric field (0.5 × 10\(^{-2}\) m). The flow rate was adjusted to 0.5 and 1.0 mL/h for all polymer suspensions. Also, the flow rates of 1.5 and 2.0 mL/h were used only for the selected polymer suspensions arising from the optimization experiment.

2.5.2. Electrical conductivity

The conductivity of protein suspensions (mS/cm) was measured with a digital conductivity meter (Ohaus, Starter 300, Switzerland) at 25 °C using a method adopted from Moomand and Lim [28].

2.6. Morphological characterization

The microstructure of the electrospun nanofibers was studied using a scanning electron microscope (SEM) (TESCAN-Vega3, Czech Republic) after sputter coating with a thin layer of gold using a gold sputter coater (Q 150R-ES; Quorum Technologies, Laughton, UK) under a high vacuum. The average nanofibers diameter was determined using an image processing software (Digimizer, MedCalc Software, Ostend, Belgium) [29].

2.7. Essential oil extraction

A sample of *Oliveria decumbens* plant (40 g) was submitted to conventional hydrodistillation in a Clevenger-type apparatus containing 500 mL of distilled water for 3 h. An electromantle heater (335 W; EM2000/C, Electrothermal Engineering Ltd., Stone, UK) was used to hold the samples at the boiling point. The extracted ODEO was dried over anhydrous sodium sulfate and stored in airtight-sealed glass vials at 4 °C in the darkness until further use [30].

2.8. Incorporating ODEO into the protein suspension

A 450 mg of glutelin-hordein blend (25:75 (w/w)) was dissolved in 3 mL acetic acid. After that, 0.12, 0.18 and 0.24 mL of a mixture of ODEO and ethanol (1:1) was supplemented into the protein suspension to reach the final concentrations of 2, 3 and 4 % (v/v) of ODEO in the protein suspension. Then, the electrospinning was performed at optimum process conditions as outlined in Section 2.4. Furthermore, the apparent viscosity, electrical conductivity and rheological properties of protein suspensions containing ODEO as well as the morphological characteristics of the corresponding films were investigated.

2.9. Encapsulation efficiency and loading capacity

The encapsulation efficiency (EE) and the loading capacity (LC) of ODEO were calculated by measuring the free surface (non-encapsulated) ODEO [91,32]. To extract free surface ODEO, 1 mL of ethanol (70 %) was added to 5 mg ODEO-loaded glutelin-hordein electrospun nanofiber. After gentle shaking, the mixture was centrifuged (4000 g, 2 min, room temperature) and the absorbance of the supernatant was determined using a UV–vis spectrophotometer (Rayleigh, UV9200, China) at a wavelength of 274 nm [33]. Then, the remaining pellet (loaded nanofiber) was washed (three times) with 1 mL of ethanol to ensure that ODEO was not detected in the supernatant. The supernatants were collected and subsequently, the ODEO content entrapped in nanofiber was determined by measuring the absorbance at a wavelength of 274 nm. Since electrospun fiber was partially dissolved in ethanol, the fiber without ODEO was tested as a control. Total ODEO was considered to be equal to the sum of the free surface and the entrapped ODEO. It should be noted that a low level of ODEO was degraded during the preparation and electrospinning process. The standard solutions were prepared by dissolving different concentrations of ODEO (150, 100, 50, 25, and 10 mg/L) in ethanol. The EE and LC values were calculated according to the Eqs. (3–5):

\[ EE(\%) = \left( \frac{\text{Experimental total ODEO} - \text{Free surface ODEO}}{\text{Experimental total ODEO}} \right) \times 100 \]  

(3)

\[ \text{Theoretical LC(\%)} = \left( \frac{\text{Initial ODEO amount}}{\text{Total mass of electrospun nanofiber}} \right) \times 100 \]  

(4)

\[ \text{Effective LC(\%)} = \left( \frac{\text{Experimental total ODEO} - \text{Free surface ODEO}}{\text{Total mass of electrospun nanofiber}} \right) \times 100 \]  

(5)

2.10. Fourier Transform Infrared (FTIR) spectroscopy

FTIR (FTIR-8400S, Shimadzu Corp., Kyoto, Japan) was used to characterize the chemical structures of hordein, glutelin and the selected hordein-glutelin blend nanofibers. All spectra were recorded in a range of 4000–400 cm\(^{-1}\) with a resolution of 2 cm\(^{-1}\) [23].

2.11. Antioxidant activity

The antioxidant activity of ODEO-loaded glutelin-hordein electrospun nanofibers was measured using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity assay [11]. The results were expressed by half maximal effective concentration (EC\(_50\)) value which was calculated from a linear regression of scavenging percentage plotted curves against sample concentration. Briefly, 50 mg ODEO-loaded glutelin-hordein electrospun nanofibers was dissolved in 10 mL DMSO solution. An aliquot (100 μL) of DMSO extract was mixed with 1 mL of 0.1 mM prepared DPPH\(^•\) in methanol and incubated at room temperature in the darkness. The optimal glutelin-hordein electrospun nanofiber without ODEO was also investigated as the control sample. After incubation for 30 min, the absorbance was recorded spectrophotometrically at a wavelength of 516 nm. The DPPH\(^•\) scavenging activity was calculated using the Eq. (6):

\[ \text{DPPH}^• \text{ scavenging activity(\%)} = \left( \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{{\text{Absorbance}_{\text{control}}}} \right) \times 100 \]  

(6)

2.12. Antibacterial activity

The disc diffusion assay was employed to evaluate the inhibitory zone of optimal glutelin-hordein/ODEO electrospun nanofibers. Bacterial cells of *Escherichia coli* (ATCC 35218), *Bacillus cereus* (PTCC 1154) and *Staphylococcus aureus* (ATCC 6538) were cultured for 24 h inside a shaker incubator (37 °C, 100 rpm). The inoculum was suspended in a phosphate buffer solution (PBS) to provide the approximate final density of 1 × 10\(^8\) colony forming units (CFU)/mL, according to the 0.5 McFarland turbidity standard. The control glutelin-hordein electrospun films and 3 % (v/v) ODEO-loaded glutelin-hordein electrospun films were aseptically cut into 1 × 1 cm\(^2\) disks and placed on plates containing Mueller Hinton agar that were previously seeded with 100 μL of an
overnight culture with the bacterial strains. Afterward, the plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zones (mm) [34].

2.13. Statistical analysis

All experiments were performed for at least two replicates and analyzed using one-way analysis of variance (ANOVA) using SAS software (version 9.1, SAS Institute Inc., Cary, NC). The obtained data were expressed as mean ± standard deviation (SD). Comparison between the mean values was carried out using Duncan’s multiple range tests at a confidence level of 0.05.

3. Results and discussion

3.1. Protein characterization

According to the Kjeldahl analysis, the protein contents of isolated hordein and glutelin powders were 92.16 ± 0.19 % and 91.01 ± 0.21 %, respectively. As shown in Fig. 1, the B hordeins and C hordeins appeared as broad bands at 30–45 and 50–60 kDa, respectively. However, it was expected that the D hordeins bands were identified at 80–90 kDa, no visible band on the SDS-PAGE pattern suggested that D hordeins were not extracted effectively when ethanol was used as the only extraction media. A similar observation was also reported by Wang et al. [35]. According to Fig. 1, the band associated with the B hordeins was more intense compared to the bands associated with the C fraction, indicating that a high portion of B hordeins was isolated from barley under alcoholic extraction media. The results were similar with Wang et al. [26] who observed the B subfraction as the main protein fraction of hordein, accounted for 70–80 % of the total protein, while the C hordein accounted for 10–20 % of the total proteins. It seems that A hordeins were not extracted from barley as no band was identified around 15 kDa. The barley glutelin fraction showed three major bands at MW of 45–55, 25–35 and <20 kDa. The band from 25 to 55 kDa may be a contamination of B-hordeins in the glutelin fraction during alkaline extraction. Overall, barley glutelin has not been explored as widely as hordein and hence information on its subunits is limited.

3.2. Protein suspensions properties

Table 1 represents the physical and rheological properties of protein suspension under various glutelin-hordein ratios. The highest electrical conductivity (1.87 ± 0.10 mS/cm) of protein suspension was achieved with the pure glutelin, while the lowest value (0.91 ± 0.08 mS/cm) was observed for the pure hordein. The conductivity increased significantly (p < 0.05) when the amount of glutelin increased up to 30 % in the proteins blend compared to the pure hordein. However, no significant differences (p > 0.05) were observed in the electrical conductivity with the increase in the glutelin content of the protein blend from 25 to 35 % (Table 1). Ghorani and Tucker [36] reported that the polymer concentration and the MW play critical roles in the electrical conductivity of the polymer suspensions. The electrical conductivity of a polymer suspension is generally associated with the presence of charge density on a jet. Therefore, a higher elongation of jet along its axis may result in a higher electrical conductivity, thereby nanofibers with smaller diameter sizes are obtained.

On the other hand, the polymer suspension containing the pure hordein had the highest viscosity while the suspension having the pure glutelin had the lowest viscosity. There were no significant differences (P > 0.05) with respect to the viscosities of protein blends and the pure protein suspensions (Table 1).

The K value, n value and the maximum shear rate at different feeding rates are represented in Table 1. The n value was <1 for all protein

Table 1

| property                  | Glutelin:Hordein | 0:100 | 25:75 | 30:70 | 35:65 | 100:00 |
|---------------------------|------------------|-------|-------|-------|-------|--------|
| Conductivity (mS/cm)      |                  |       |       |       |       |        |
|                           |                  | 0.91± | 1.08± | 1.17± | 1.19± | 1.87±  |
|                           |                  | 0.08± | 0.17± | 0.10± | 0.03± | 0.10±  |
| Viscosity (Pa/s)          |                  | 0.52± | 0.49± | 0.43± | 0.35± | 0.29±  |
|                           |                  | 0.04± | 0.00± | 0.06± | 0.08± | 0.18±  |
| n                         |                  | 0.28± | 0.71± | 0.63± | 0.83± | 0.08±  |
| K (pa s⁻¹)                |                  | 0.03± | 0.00± | 0.00± | 0.01± | 0.01±  |
| Shear rate (s⁻¹; 0.5 mL/h)|                  | 2.36± | 1.56± | 1.63± | 1.63± | 1.63±  |
|                           |                  | 0.14± | 0.00± | 0.01± | 0.01± | 0.01±  |
| Apparent viscosity (Pa; 0.5 mL/h) | 3.11± | 0.26± | 0.00± | 0.03± | 0.08± |
| Apparent viscosity (Pa; 1 mL/h) | 1.88± | 0.12± | 0.00± | 0.02± | 0.02± |

* Values represent mean ± SD (n = 3). For each characteristic, means with different small letters within a row and different capital letters within a column are significantly different (p < 0.05).

![Fig. 1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) image of hordein and glutelin fractions of barley. Staining was performed using Coomassie brilliant blue-R-250.](image-url)
blends, indicating a non-Newtonian behavior of these solutions. The viscosity of protein suspension decreased by increasing the flow rate from 0.5 mL/h (shear rate = 2.36 ± 0.14 s⁻¹) to 1 mL/h (shear rate = 4.71 ± 0.27 s⁻¹) for glutelin-hordein blend at a ratio of 25:75. This decreasing trend in viscosity at a higher flow rate was also observed for other blend suspensions. Generally, the viscosity of the protein suspension is considered as a proper indication of the spinnability, size and morphology of the final electrospun nanofibers.

Table 1 demonstrated that the apparent viscosity at the ejection point of protein suspensions decreased with increasing the glutelin concentration from 25 to 35 % in the blend at both applied flow rates. It was observed that decreasing the apparent viscosities caused a reduction in the viscoelastic forces. Accordingly, the applied voltages were higher than viscoelastic forces, resulting in the lower average diameter sizes of the electrospun nanofibers.

3.3. Process parameters and the morphology of nanofibers

According to Table 2, electrospinning process led to a beaded structure in the electrospun nanofibers fabricated at both glutelin-hordein ratios of 30:70 and 35:65. In contrast, the structure of electrospun nanofibers processed at a glutelin-hordein ratio of 25:75 appeared to be more uniform with a limited number of beaded nanofibers. Therefore, an increase in glutelin concentration from 25 to 35 % in the proteins blend suspension resulted in the formation of a beaded structure nanofibers. However, the average diameter sizes of the nanofibers showed a decreasing trend at a higher concentration of glutelin in protein suspension. With the increase of glutelin concentration in the protein blend, a proper viscosity was achieved to have an ideal jet formation, leading to a smaller diameter. However, the electrospinning of low viscoelastic protein suspension led to a beaded fiber structure [37].

Table 2 clearly represents the effect of electrospinning parameters on the spinnability and the uniformity of the electrospun fiber films. At all glutelin-hordein ratios, an increase in the voltage under a constant flow rate and a certain needle-to-collector distance resulted in a decrease of nanofibers diameter. The higher applied voltage led to a greater protein suspension stretching during the electrospinning due to overcoming the protein droplets surface tension. This attributed to the charge repulsion within the polymer jet, resulting in the formation of electrospun fibers with smaller diameters. According to the results herein, a 15 kV was selected as an optimum applied voltage.

As presented in Table 2, the average diameter sizes of the fibers decreased significantly with increasing the needle-to-collector distance from 100 to 150 mm under constant values of applied voltage, flow rate and glutelin-hordein ratio. A possible explanation for this observation is that the needle-to-collector distance has a direct influence on the jet flight time. An increase in this distance increased the flight time and also the solvent evaporation time which consequently led to the formation of narrower fibers [38]. To this end, a 150 mm distance between the needle and the collector was selected for further experiments to obtain a uniform fiber morphology and fiber diameter. The flow rate of the polymer suspension is another important parameter during the electrospinning process which may affect the morphology of the electrospun nanofibers. At constant values of applied voltage, needle-to-collector distance and glutelin-hordein ratio, the diameter of the nanofibers decreased with the increase of flow rate from 0.5 to 1.0 mL/h (Table 2). Therefore, the applied flow rate value of 1.0 mL/h was lower than the critical value which could lead to bead formation or increase in the nanofiber diameter [39].

As presented in Figs. 2 and 3, with increasing the flow rate from 0.5 to 1.0 mL/h, the average diameter size of the nanofibers decreased from 886 to 587 nm. However, as the flow rate increased from 1.0 to 2.0 mL/h, the average diameter size of the nanofibers increased to 1313 nm. This may be associated with the fact that the flow rate over the critical value resulted in incomplete evaporation of the solvent during air flight between the needle and the collector plate [36]. The large suspended droplet at the endpoint of the needle tip at a high flow rate provided limited time for the solvent drying, leading to the high diameter nanofibers on the collector surface [40]. Moreover, this result may be attributed to the fact that the viscous forces had stronger effects compared to the electric forces during the electrospinning process. These results were in accordance with previous reports which demonstrated that the ultimate effect of the flow rate on the fiber properties during the electrospinning process is not only important for the jet velocity, transfer rate of solution and evaporation of the solvent but also can determine the viscoelastic forces, thus lower flow rates are more desirable for the solvent.

Table 2

| Physical property | Average diameter of fiber (nm) | Voltage (kV) | Flow rate (mL/h) | Needle-to-collector distance (mm) | Glutelin: Hordein ratio | Number |
|-------------------|-------------------------------|-------------|-----------------|----------------------------------|------------------------|--------|
| Uniform fiber     | 1255 ± 53                      | 12          | 0.5             | 100                              | 25:75                  | 1      |
| Uniform fiber     | 1120 ± 44                      | 12          | 0.5             | 150                              | 25:75                  | 2      |
| Uniform fiber     | 1127 ± 47                      | 12          | 1               | 100                              | 25:75                  | 3      |
| Uniform fiber     | 853 ± 29                       | 12          | 1               | 150                              | 25:75                  | 4      |
| Uniform fiber     | 991 ± 46                       | 15          | 0.5             | 100                              | 25:75                  | 5      |
| Uniform fiber     | 886 ± 31                       | 15          | 0.5             | 150                              | 25:75                  | 6      |
| Uniform fiber     | 897 ± 40                       | 15          | 1               | 100                              | 25:75                  | 7      |
| Uniform fiber     | 587 ± 26                       | 15          | 1               | 150                              | 25:75                  | 8      |
| Beaded fiber      | 872 ± 41                       | 12          | 0.5             | 100                              | 30:70                  | 9      |
| Beaded fiber      | 859 ± 41                       | 12          | 0.5             | 100                              | 30:70                  | 10     |
| Beaded fiber      | 594 ± 28                       | 12          | 1               | 100                              | 30:70                  | 11     |
| Beaded fiber      | 566 ± 29                       | 12          | 1               | 150                              | 30:70                  | 12     |
| Beaded fiber      | 750 ± 30                       | 15          | 0.5             | 100                              | 30:70                  | 13     |
| Beaded fiber      | 720 ± 31                       | 15          | 0.5             | 150                              | 30:70                  | 14     |
| Beaded fiber      | 542 ± 29                       | 15          | 1               | 100                              | 30:70                  | 15     |
| Beaded fiber      | 505 ± 18                       | 15          | 1               | 150                              | 30:70                  | 16     |
| Beaded fiber      | 593 ± 28                       | 12          | 0.5             | 100                              | 35:65                  | 17     |
| Beaded fiber      | 542 ± 25                       | 12          | 0.5             | 150                              | 35:65                  | 18     |
| Beaded fiber      | 540 ± 25                       | 12          | 1               | 100                              | 35:65                  | 19     |
| Beaded fiber      | 473 ± 17                       | 12          | 1               | 150                              | 35:65                  | 20     |
| Beaded fiber      | 532 ± 25                       | 15          | 0.5             | 100                              | 35:65                  | 21     |
| Beaded fiber      | 520 ± 24                       | 15          | 0.5             | 150                              | 35:65                  | 22     |
| Beaded fiber      | 558 ± 29                       | 15          | 1               | 100                              | 35:65                  | 23     |
| Beaded fiber      | 456 ± 18                       | 15          | 1               | 150                              | 35:65                  | 24     |

* Values of average diameter represent mean ± SD (n = 3). Different small letters indicate a significant difference (p < 0.05).
evaporation [16]. Low flow rate may increase the apparent viscosity because of the dominant effect of the viscoelastic forces over the electric forces, leading to a larger fiber diameter and beaded structure (Fig. 3a–d). Therefore, it can be concluded that a higher applied voltage is required when a lower flow rate is used during electrospinning [36]. According to these observations, a flow rate of 1 mL/h was selected as the optimum flow rate for further analysis.

3.4. Effect of ODEO loading on the electrospun fibers

3.4.1. Physical and rheological properties of protein suspensions

The incorporation of ODEO in the protein suspensions from 1 to 4% had no significant changes (p > 0.05) in the electrical conductivity and the viscosity (Table S1 in Supplementary Material). Moreover, the determination of flow behavior index (n < 1) was also confirmed that the presence of ODEO did not affect the non-Newtonian shear thinning behavior of the protein blends (Table S1 in Supplementary Material).

3.4.2. Morphological properties

Fig. 3e–g represents the SEM images of electrospun fibers made from the glutelin-hordein (25:75) blends incorporated with different concentrations of ODEO. According to the average diameter sizes (Table S2 in Supplementary Material) and morphological properties of electrospun nanofibers, the presence of ODEO within the electrospun nanofibers did not lead to a significant change (P > 0.05) in the diameter. This finding was in accordance with the previous results regarding the insignificant effects of ODEO on viscosity and the electrical conductivity of ODEO-loaded protein suspensions. Nevertheless, the microstructure of electrospun nanofibers was directly affected by incorporating ODEO in protein blend suspension. Also, Hajjari et al. [41] reported the effect of cuminaldehyde encapsulation on the morphology of gliadin fibers. As shown in Fig. 3e–g, with increasing the ODEO concentration from 3 to 4%, non-uniform and highly beaded fiber structures were produced via electrospinning. This may be associated with the lower apparent viscosity of the protein suspension at high ODEO concentration (4 %) loading that contributes to lower viscoelastic forces (Table S1 in Supplementary Material). Under these circumstances, a higher tendency for the formation of beaded structures at an applied voltage of 15 kV was achieved. Therefore, the glutelin-hordein electrospun nanofiber incorporated with 3 % ODEO was selected for further analyses.

3.4.3. FTIR spectroscopy

FTIR spectroscopy provides an insight into the molecular changes which occurred within the chemical structure of nanofibers during electrospinning. According to Fig. 4, the hordein fraction spectrum exhibited a peak at 3395 cm⁻¹ which corresponded to OH⁻ stretching vibrations [42]. This was appeared at 3321 cm⁻¹ for glutelin. The stretch band of the OH⁻ characteristic of glutelin-hordein at 3333 cm⁻¹ decreased to 3327 cm⁻¹ for glutelin-hordein/ODEO nanofibers.

**Fig. 2.** Effect of ejection flow rate of protein suspension on the diameter of the nanofibers generated from the glutelin-hordein blend at a ratio of 25:75.

**Fig. 3.** Scanning electron microscopy (SEM) images of electrospun nanofibers of glutelin-hordein blend at a ratio of 25:75 using electrospinning process operated at a voltage of 15 kV, a needle-to-collector distance of 150 mm as affected by (a) 0.5, (b)1.0, (c) 1.5, and (d) 2.0 mL/h flow rates; electrospun nanofibers incorporated with (e) 2, (f) 3, and (g) 4 % Oliveria decumbens essential oil at the optimum electrospinning conditions.

**Fig. 4.** Fourier-transform infrared (FTIR) spectra of glutelin, hordein, glutelin-hordein, *Oliveria decumbens* essential oil (ODEO), and glutelin-hordein/3 % ODEO nanofibers.
Moreover, the intensity of OH— band was higher after incorporating ODEO which can be due to the formation of hydrogen bonds between hydroxyl groups of proteins and thymol and carvacrol in ODEO. In all spectrum, the bands from 2850 to 2990 cm⁻¹ can be assigned to CH— stretching. The higher intensity of the peak at 2924 cm⁻¹ in glutelin-hordein/ODEO spectrum compared to hordein-glutelin was attributed to increasing CH— stretching, suggesting the incorporation of ODEO into nanofibers. The band emerged at 1656 cm⁻¹ for the hordein, 1660 cm⁻¹ for the glutelin, 1657 cm⁻¹ for glutelin-hordein fiber, and 1658 cm⁻¹ for glutelin-hordein/ODEO were associated with the C=O stretching vibrations, CN— stretching vibrations and NH— bending (amide I) vibrations [43]. Moreover, the intensity of the proteins amide I band was reduced for electrospun fiber incorporated with ODEO as shown in Fig. 4. The amide II band was derived from N—H bending and CN— stretching vibrations with a peak at 1528, 1524, 1529 and 1530 cm⁻¹ for hordein, glutelin, hordein-glutelin and glutelin-hordein/ODEO nanofibers, respectively [44]. The maximum absorption peaks at 1426, 1444,1434 and 1426 cm⁻¹ can be assigned to the amide III bands of hordein, glutelin, glutelin-hordein, and glutelin-hordein/ODEO nanofiber, respectively [45]. These results indicated that the electrospinning process did not contribute to the alterations of the native chemical structure of the protein fractions examined herein.

Considering the ODEO spectrum in Fig. 4, the band at 3436 cm⁻¹ was attributed to the O—H stretching vibrations which are mainly related to the presence of thymol and carvacrol of ODEO. A band that appeared at 1619 cm⁻¹ over the ODEO spectrum indicated the presence of carbonyl group (C=O) and benzene ring (C=C) which could be associated with the components such as thymol, myristicin, terpine and carvacrol [46]. The peak appeared at 1438 cm⁻¹ was corresponded to the molecular vibration mode of the CH₂ deformation and asymmetrical CH₃ deformation in ODEO. The ring vibrational modes of thymol and carvacrol appeared at 810 cm⁻¹ [47] which was also observed at 808 cm⁻¹ in glutelin-hordein/ODEO spectrum. This is an indication for compatibility and the stability of the hybrid electrospun fibers containing ODEO, which may avoid phase separations in suspensions during electrospinning process. Since the main structure of glutelin-hordein/ODEO nanofiber is mainly composed of proteins mixture, its resulting spectrum was more consistent with glutelin-hordein spectrum compared to ODEO spectrum (Fig. 4).

3.4.4. Encapsulation efficiency and loading capacity

The EE, theoretical LC, and effective LC values of glutelin-hordein/3%ODEO fibers were 70.82 ± 2.01 %, 16.60 % and 2.68 ± 0.08 %, respectively, suggesting that the ODEO has been effectively incorporated into the glutelin-hordein electrospun nanofibers. Therefore, glutelin-hordein electrospun complex can be proposed as a potential vehicle for the encapsulation and delivery of bioactive compounds. Similar to this finding, Rezaei et al. [48] reported the EE value of 68 %–75 % at different concentrations of vanillin (1, 2 and 3 %) in almond gum/polyvinyl alcohol composite nanofibers fabricated using the electrospinning process. Vafanella et al. [31] also demonstrated theoretical LC in the range of 20–40 % for thyme essential oil-loaded chitosan/gelatin nanofibers electrospun fibers. In general, different matrices and essential oil concentrations can also directly affect the final EE obtained in different studies. The contribution of various protein fractions in the nanofibers in this study could be a possible reason for achieving lower levels of LC and EE compared to those studies. In addition, the results of EE and LC in the present study were reported based on the experimental total amount of ODEO, and hence any degraded ODEO during the electrospinning process was excluded in the calculations herein.

3.4.5. Antioxidant activity

The antioxidant activities of glutelin-hordein/3%ODEO and glutelin-hordein nanofiber as control sample are presented in Fig. 5. The ODEO-loaded glutelin-hordein nanofibers exhibited a DPPH scavenging activity ~1.5 times greater than the control sample. This could be attributed to the presence of phenolic compounds such as thymol and carvacrol in ODEO [30]. The antioxidant activity of nanofibers may be due to the presence of peptides and amino acids which may be released from glutelin and hordein during the extraction process. In addition, the EC₅₀ measurements confirmed a strong antioxidant activity of glutelin-hordein/ODEO (2.32 mg/mL) compared to the glutelin-hordein nanofibers (3.57 mg/mL). Chanput et al. [49] showed the potential of antioxidant activity of the B, C and D hordeins fractions of barley grains.

3.4.6. Antibacterial activity

As shown in Fig. 6, glutelin-hordein nanofibers exhibited a significant antibacterial activity against B. cereus and S. aureus with inhibition zones of 12.00 ± 0.00 mm and 5.93 ± 0.12 mm, respectively, while no inhibition zone was observed for the plate associated with the growth of E. coli. The mechanism of the antibacterial activity of glutelin-hordein nanofiber was previously linked to the release of peptides during the extraction of glutelin and hordein fractions [50]. For glutelin-hordein nanofibers incorporated with 3% (v/v) ODEO represented high antibacterial activities against B. cereus, S. aureus, and E. coli with inhibition zones of 11.83 ± 0.29, 21.03 ± 0.15, and 36.00 ± 2.00 mm, respectively. This was mainly attributed to the presence of terpene constituents such as thymol and carvacrol in ODEO which can disrupt the structural integrity of bacterial phospholipid bilayer, leading to the death of the bacterial cell [34]. The mechanism of the antibacterial activity of glutelin-hordein nanofiber may be associated with the release of peptides during the extraction of glutelin and hordein [50].

4. Conclusion

Food grade hordein-glutelin electrospun nanofibers incorporated with ODEO for active packaging applications were successfully fabricated. The evaluation of protein suspension properties and electrospinning condition suggested that the glutelin-hordein ratio of 25:75, the voltage of 15 kV, the needle-to-collector distance of 150 mm and the electrospinning process yielded high entrapment efficiency of ODEO. FTIR spectroscopy confirmed the successful incorporation of ODEO in glutelin-hordein nanofiber structure. The insights achieved by this study delivered a better understanding of the fabrication of electrospun protein nanofibers. This study showed that by blending electrospinnable and non-
electrospinnable proteins from barely at an appropriate ratio, nanofibers with high functionalities can be produced. These electrospun nanofibers are promising for the applications in the active food packaging to provide additional safety/health promoting properties to the food products.

CRediT authorship contribution statement

Nafiseh Zahabi: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. Mohammad-Taghi Golmakani: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. Mahboubeh Fazaeli: Conceptualization, Validation, Resources, Data curation, Writing - review & editing, Visualization, Supervision. Fatemeh Ghiasi: Software, Investigation, Data curation, Writing - original draft. Mohammadreza Khalesi: Conceptualization, Validation, Resources, Data curation, Writing - review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgment

This research project was financially supported by Shiraz University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.colsurfb.2021.112058.

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Fig. 6. Antibacterial activity assay using a disc diffusion method for the different electrospun nanofibers. Panels (a), (d) and (g) represent cultured plates without exposure to electrospun films; Panels (b), (e) and (h) represent glutelin-hordein electrospun films; Panels (c), (f) and (i) represent glutelin-hordein/3% Oliveria decumbens essential oil electrospun films.
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