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Research Article

New electrospun nanofiber based on grass pea protein isolate (*Lathyrus sativus* L.) for the food and biomedical applications

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ABSTRACT- In the present study, new protein nanofibers were produced from grass pea (*Lathyrus sativus* L.) protein isolate and polyvinyl alcohol. Different ratios of grass pea protein isolate and polyvinyl alcohol (100:0, 80:20, 60:40, 40:60, 20:80, and 0:100) were mixed, and the nanofibers were produced by the electrospinning process. First, the properties of spinning solutions, including viscosity, electrical conductivity, and surface tension were examined, then the morphological, thermal, and mechanical properties of the electrospun nanofibers were investigated. By increasing the amount of grass pea protein isolate, the viscosity decreased, but the electrical conductivity and surface tension increased from 683 to 1108 µS/cm and 52 to 76 mN/m, respectively. SEM image analysis showed that the nanofibers containing up to 60% protein content had a bead-free and uniform structure with an average diameter of 138.43 nm. The FTIR analysis results suggested the effective fabrication of the hybrid nanofibers. The XRD patterns indicated a reduction in the crystallinity of composite nanofibers compared to polyvinyl alcohol. These new electrospun nanofibers have the potential to create films incorporating bioactive compounds, acting as functional food products.

INTRODUCTION

Biodegradable materials are sustainable options for packaging, and their eco-friendly nature has made them a popular choice for food and non-food applications (Sharif et al., 2021). In recent years, the nanofibrous mats have gained significant attention as packaging materials due to their high surface-to-volume ratios, skill in controlling pore size, surface energy, barrier properties, antimicrobial activity, mechanical strength, and primarily for the encapsulation of active agents for their application as active food packaging materials. Electrospinning is a convenient and affordable technique for producing fiber mats for various applications (Aghababaei et al., 2024; Aman Mohammadi et al., 2024). Through electrospinning, fibrous structures with specific arrangements and structural integrity can be created using a wide variety of natural and synthetic polymers. Due to the unique functional properties of proteins, they are used as essential components in many food systems (Balandrán-Quintana et al., 2019; Rostami et al., 2023). Biomaterials derived from proteins are highly popular due to their favorable biophysical and biochemical properties. Proteins are known for being easy to extract and purify, biocompatible, and non-toxic (Kumar et al., 2019). Grass pea (*Lathyrus sativus* L.), a plant from the legume family, is grown for human and animal feed in Asian countries as an annual plant. The grass pea seeds contain 20-30% protein with low sulfur amino acids and rich in lysine (Ebrahimi et al., 2016). Besides being nutritional, leguminous proteins possess functional properties that enable their use in a broad range of food products (Boye et al., 2010). Protein functionality can be affected by the protein source, techniques employed for defatting, protein extraction, and drying (Feyzi et al., 2018). The unfolding of proteins is essential for successful electrospinning. Consequently, globular proteins pose challenges for electrospinning due to their strong hydrophobic and ionic interactions, hydrogen bonds, intricate network structure, and low charge density. Several strategies can be employed to enhance the spinnability of proteins. One of the most efficient approaches involves blending proteins with easily spun polymers such as polyvinyl alcohol (PVA) (Aguilar-Vázquez et al., 2020).

PVA is a thermoplastic polymer known for its semi-crystalline structure, biocompatibility, and hydrophilic properties. It is commonly mixed with other polymers to create electrospun fiber mats for a variety of applications in the biomedical and food industries. The utilization of this approach will enhance the mechanical properties, stabilize the electrospinning process, and sometimes enhance the biocompatibility of the nanofibers (Mahmud et al., 2018).

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Based on our knowledge, grass pea protein isolate (GPPI) has not been used in the production of nanofibers through electrospinning. GPPI nanofibers offer the potential to be utilized as nanocarriers for food packaging and enhance the nutritional quality of food products. In addition, these nanofibers are suitable for drug delivery, tissue scaffolding, and antibacterial uses. To create an eco-friendly structure, we developed and characterized electrospun GPPI fibers with great potential to be used in medical applications and packaging materials. The properties of polymer solutions (viscosity, electrical conductivity, and surface tension) and the characteristics of nanofibers, SEM (scanning electron microscopy), FTIR (Fourier transform infrared analysis), XRD (X-ray diffraction), thermal properties (DSC; differential scanning calorimetry), and mechanical properties were investigated.

MATERIALS AND METHODS

Materials

The grass pea seeds were purchased from a shop located in Shiraz, Iran. Pure ethanol was obtained from Pars Alcohol Company (Eghlid, Iran). Glacial acetic acid, polyvinyl alcohol, NaOH, and HCl were procured from Sigma-Aldrich. All chemicals were utilized without additional purification and were of analytical grade.

Preparation of GPPI

GPPI was obtained through an alkaline extraction/acid precipitation method. Initially, grass pea seeds were ground and sieved (mesh 60). The resulting flour was mixed with distilled water and NaOH 1 M to adjust the pH to 9.5. After centrifugation at 10,000 × *g* for 10 min, the undissolved matter was decanted, and the supernatant was acidified to pH 4.5 with HCl 1 M. After being washed twice with distilled water and recentrifuged under the same conditions, the pellet's pH was adjusted to 7 by introducing a 1 M NaOH solution and then freeze-dried (Feyzi et al., 2018).

Preparation of electrospinning solutions

A 7% (w/v) PVA solution was created by dissolving PVA in acetic acid 50% (w/w) and stirring at 85 ˚C for 2 hours. Subsequently, a 7% (w/v) GPPI solution was prepared by dissolving 7 gr GPPI powder in acetic acid 50% (w/w) and stirring for 1 h at 80 ˚C. Various ratios of GPPI:PVA blend solutions (100:0, 80:20, 60:40, 40:60, 20:80, and 0:100) were then produced by stirring for 30 min at room temperature to achieve a homogenous solution.

Solution characterization

The rheological behavior, surface tension (ST), and electrical conductivity (EC) of the various GPPI:PVA solutions were determined through a rheometer (Rheometer mcr 302 Anton Paar, Austria), a tensiometer (Krüss K100 Tensiometer, Hamburg, Germany), and an electrical conductivity meter (Consort C933, Turnhout, Belgium), respectively, before the electrospinning process (Goudarzi et al., 2023; Souri et al., 2023).

Electrospinning of solutions

An electrospinning machine (side-by-side electrospinning unit, dual pump, ES2000, Iran) was used to conduct the electrospinning process. A 2.5-mL plastic syringe with a 23-gauge metal needle was filled with the polymer solution, and electrospinning was carried out under specific conditions (flow rate: 0.5 mL/hour, voltage: 20 kV, and needle-to-collector distance = 15 cm; at room temperature).

Characterization of electrospun fibers

SEM

A scanning electron microscope (TESCAN-Vega 3, Brno, Czech Republic) was used to investigate the morphology of electrospun nanofibers. Fibers were positioned on the horizontal section and fixed in place using conductive glue. Before testing, the samples underwent a gold coating to increase their conductivity. The average diameter of the fibers was measured using image analysis software (Version 5.3.5, Ostend, Belgium) (Sharif et al., 2021).

FTIR and XRD

The functional groups and structural conformations of the samples were investigated using a FTIR spectrometer (Tensor II, Bruker, Germany) with a range of 4000-400 cm^{-1}. The XRD was used to investigate the crystallinity of the samples, employing Cu Kα radiation within the range of 10–70˚ (2θ) (Hajjari et al., 2023).

DSC

The thermal properties of the fibers were investigated using Differential Scanning Calorimeters (DSC-131 evo, Setaram, France). The samples were subjected to a gradual temperature increase from 25 to 400 ˚C, with a heating rate of 10 ˚C/min (Sharif et al., 2019).

Mechanical properties and thickness

A texture analyzer (TA-XT2, Stable Microsystems, Surry, UK) was used to measure the tensile strength (TS) and elongation at break (EB) of nanofiber mats. The thickness of the fibers was measured by a micrometer (Mitutoyo No. 293–766, Tokyo, Japan) at three different positions of each sample, and their mean values were used as the overall thickness (Yao et al., 2022).

Statistical analysis

IBM SPSS Statistics 27 (SPSS Inc., Chicago, IL) was used for data analysis, which included analysis of variance (ANOVA) and Duncan's multiple range test (P values < 0.05).

RESULTS AND DISCUSSION

Solution Characteristics

As presented in Fig. 1, PVA showed Newtonian behavior. The inclusion of GPPI reduced the viscosity of solutions. In all samples, except for pure PVA, the apparent viscosities exhibited a decreasing trend as the shear rate

increased. The EC and ST values for different GPPI:PVA ratios are presented in Table 1. Successful polymer electrospinning and the formation of a Taylor cone require an appropriate viscosity level. High viscosity causes drying of the polymer solution in the needle tip, and low viscosity prevents the continuous production of fibers (Bombin et al., 2020). The solutions exhibited EC ranging from 580 ± 3.45 to 1283 ± 7.63 (μS/cm^{-1}). An increase in EC was observed with the rise in the GPPI ratio in the solutions (P < 0.05). The electrical conductivity of polymer solutions is another crucial factor in electrospinning, as it directly impacts the formation of the jet. The rise in electric charge within the solution leads to an increase in tension within the electric field, resulting in the formation of more uniform fibers. As electrical conductivity rises, the repulsion between charged particles also increases, leading to favorable conditions for fiber formation (Raghavan et al., 2012; Vega-Lugo & Lim, 2012). The surface tension showed a significant increase as the amount of GPPI in GPPI: PVA mixtures increased. The surface tension value for GPPI: PVA with a ratio of 0:100 was 46.22 ± 4.31 mN/m, whereas the highest value of 91.39 ± 5.06 mN/m was recorded for the 100:0 ratio. The solution's surface tension plays a crucial role in electrospinning, and the process starts when the internal electrostatic repulsions exceed the surface tension of the molecules. The decrease in surface tension enhances the interaction between polymer and solvent molecules, leading to the creation of a smooth fiber (Azizi et al., 2023; Zaitoon & Lim, 2020). Similar results were identified in the solutions of bean protein/PVA (El Halal et al., 2019) and sunflower seed protein/PVA (Shanesazzadeh et al., 2018).

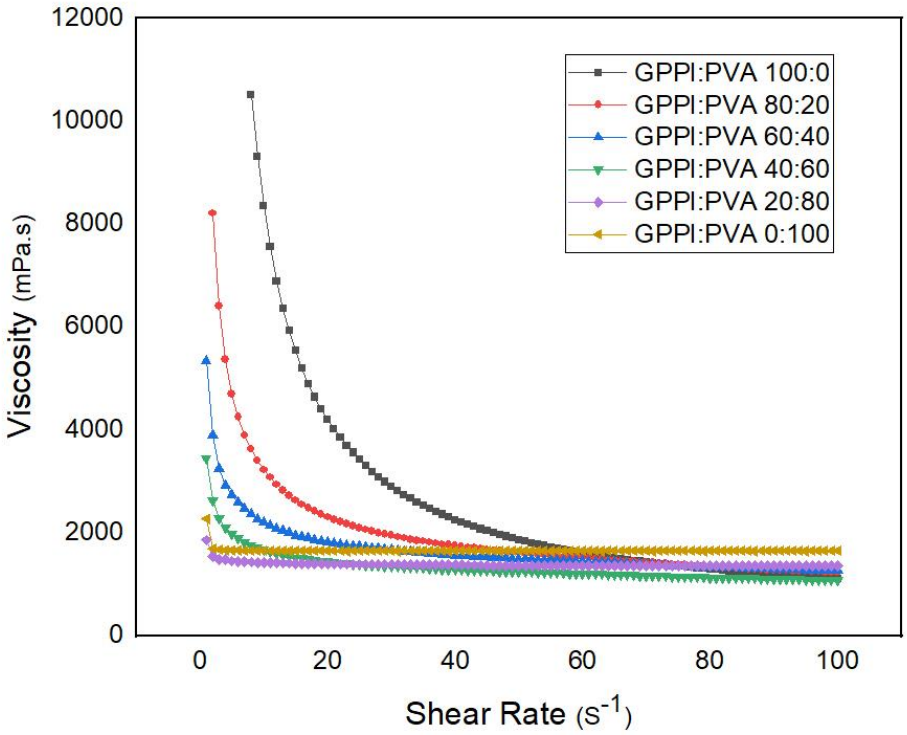

Fig. 1. Apparent viscosity/shear rate. PVA: Polyvinyl alcohol; GPPI: Grass pea protein isolate.

Table 1. The effect of different ratios of GPPI:PVA on the electrical conductivity and surface tension of spinning solutions

Polymeric solution (GPPI:PVA)	Conductivity (μS/cm)	Surface tension (mN/m)
100:0	1283.33 ± 7.63^{a}	91.39 ± 5.06^{a}
80:20	1108.33 ± 7.63^{b}	76.87 ± 1.26^{b}
60:40	907.10 ± 6.35^{c}	63.67 ± 4.08^{c}
40:60	817.36 ± 13.06^{d}	57.38 ± 3.64^{cd}
20:80	683.50 ± 10.73^{e}	52.79 ± 5.33^{de}
0:100	580.40 ± 3.45^{f}	46.22 ± 4.31^{e}

PVA: Polyvinyl alcohol; GPPI: Grass pea protein isolate. Different letters on the same column exhibited significant differences (P < 0.05).

SEM

The SEM micrographs (Fig. 2) showed that the GPPI:PVA ratio had significant effects on the morphology of nanofibers. The optimum combination of GPPI and PVA, leading to the bead-free nanofibers, was GPPI:PVA with a ratio of 60:40. Exceeding 60% protein in the polymeric composites caused the formation of beads and discontinuous electrospinning. The analysis of nanofiber diameter is critical in determining the uniformity of the fibers (Maftoonazad et al., 2019). Mean fiber diameters for GPPI:PVA blends (0:100, 20:80, 40:60, 60:40, and 80:20) were determined to be 227.31, 181.09, 149.18, 138.43, and 85.21 nm, respectively. As the amount of GPPI increased, the average diameter of the fibers decreased. The decrease in fiber diameter by increasing GPPI ratio may be due to the decrease in solution viscosity and increase in solution conductivity. These factors increase with the stretching of the polymer jet during electrospinning, resulting in thinner fibers. Similarly, in another study, it was reported that the diameter of electrospun fibers containing higher ratios of soy protein is smaller compared to those with lower protein ratios. Electrical conductivity and solution viscosity are key factors that directly affect the configuration and appearance of fibers (Gutschmidt et al., 2021). Although the GPPI solution exhibited high electrical conductivity, it could not be spun into a fiber in its pure form.

FTIR

The FTIR spectra of fibers are depicted in Fig. 3a. The PVA spectra displayed peaks at 3344, 2938, 1248, and 1091 cm^{-1}. The FTIR spectra of PVA displayed a peak at 3326 cm^{-1} ascribed to the vibrations of O-H stretching, while the bands seen at 2938 cm^{-1} are linked to the vibrations of C-H stretching. The peaks noted at 1248 and 1091 cm^{-1} are allocated to the stretching vibrations of carbon-hydrogen (C-H) and carbon-oxygen (C-O) bonds (Ansarifar et al., 2022). On the other hand, the FTIR spectrum of pure GPPI showed distinct peaks at 3278, 2921, and 1644 cm^{-1}, along with peaks at 1531 and 1396 cm^{-1}. The shoulder at 2921 cm^{-1} corresponds to the stretching vibrations of CH and NH_2, while the absorption at 1644 cm^{-1} is attributed to the amide I carbonyl group (C=O). The characteristic peak at 1531 cm^{-1} indicates the bending movement of N-H groups and the stretching motion of C-N groups in amide II. A band at 1396 cm^{-1} signifies the movement or vibration of the amide III region (Ebrahimi et al., 2016). Notable bands were detected between 3500 and 3000 cm^{-1}. The hybrid nanofibers exhibited a shift of the amide II and III peaks to higher wave numbers, indicating successful mixing and interaction between GPPI and PVA (Shanesazzadeh et al., 2018). The bands between approximately 3000 and 3500 cm^{-1} are significant as they correspond to the stretching of C-H bonds in alkyl chains. Moreover, the presence of asymmetric C-O, C-O-C, and CH_2 stretching groups is confirmed by the relatively strong bands observed between 1400 and 1100 cm^{-1} (Maftoonazad et al., 2019). The findings of this study align with the results reported by Maftoonazad et al., 2019 (Maftoonazad et al., 2019) and Fang et al., 2016 (Fang et al., 2016).

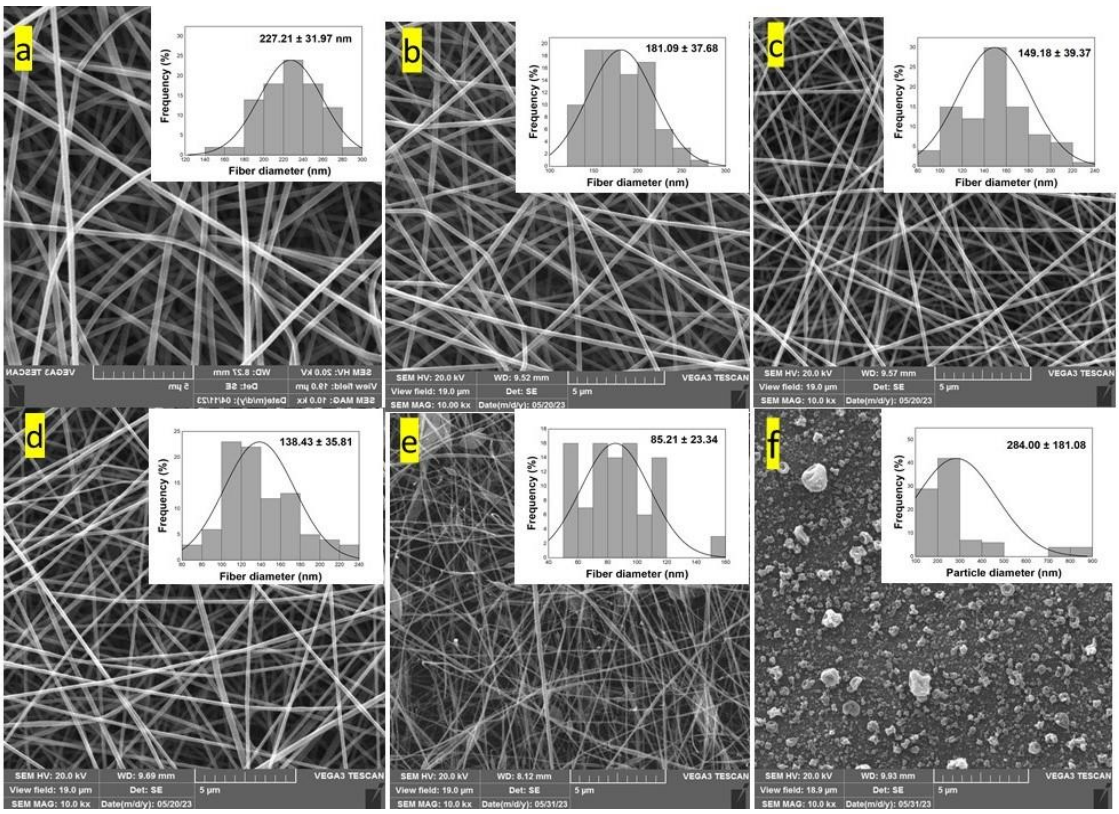

Fig. 2. SEM image of GPPI:PVA (a) 0:100, (b) 20:80, (c) 40:60, (d) 60:40, (e) 80:20, and (f) 100:0. PVA: Polyvinyl alcohol; GPPI: grass pea protein isolate

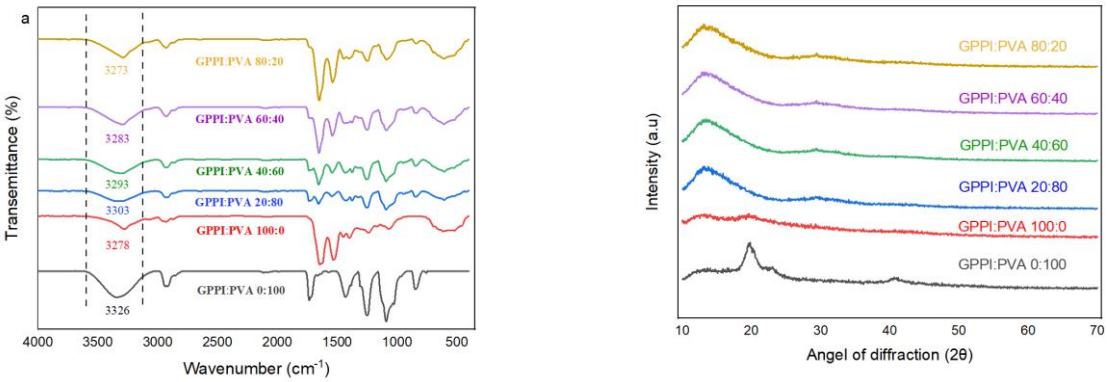

Fig. 3. (a) FTIR spectra and (b) XRD patterns of the different ratios of GPPI:PVA, PVA: Polyvinyl alcohol, GPPI: grass pea protein isolate.

XRD

Fig. 3b shows the XRD diffraction patterns of PVA, GPPI, and GPPI:PVA nanofibers. Peaks located at approximately 14.4˚ and 19.4˚, as well as at 40.7˚, are detected in the diffraction pattern of PVA, suggesting a partial crystalline structure. The crystalline nature of PVA is a result of the strong intermolecular interactions found within the PVA polymer network (Dey et al., 2020). On the other hand, the GPPI pattern displayed two significant peaks at angles of 13˚ and 20˚, representing the α-helix and β-sheet structures. When GPPI is blended with PVA, the XRD spectrum shows a prominent peak at 13˚. The GPPI:PVA physical mixture exhibits a sharp peak; the composite polymer shows a reduction in crystallinity. However, the XRD pattern of electrospun fibers showed broader peaks and reduced angular displacement, suggesting a decrease in crystallinity. This decrease may be due to the intermolecular interaction between GPPI and PVA, possibly resulting from intermolecular and intramolecular hydrogen bonding (Chanda et al., 2018). These findings were in line with the earlier investigations that the electrospinning process could inhibit the crystallization and facilitate the formation of amorphous structures in polymers (Fang et al., 2016; Wen Jia et al., 2020).

DSC

Fig. 4 exhibits the DSC thermographs of pure PVA, GPPI, and the composite electrospun nanofibers. The thermograph of PVA exhibited three endothermic peaks at 57.82, 193.59, and 322.27 ˚C, which indicate the glass transition, melting, and decomposition, respectively (Moradinezhad et al., 2023; Shanesazzadeh et al., 2018). While GPPI showed a denaturation peak at 87.97 ˚C and a melting peak at 232.90 ˚C (Ghorani et al., 2020; Koosha et al., 2017). The composite nanofibers exhibited superior thermal stability compared to the pure PVA, and the increase in GPPI ratio in composite nanofibers enhanced their heat resistance. In the GPPI curve, a wide area ranging from 25 ˚C to 143.49 ˚C was identified, indicating the water loss and the glass transition temperature (Tg) due to the conversion from a triple helix to a random coil (Aguilar-Vázquez et al., 2018). The increased stability of nanofibers with higher protein content can be attributed to the hydrogen bonding interactions between the amino and carboxyl groups of GPPI and the hydroxyl groups of PVA (Aguilar-Vázquez et al., 2018). These findings are in agreement with the results reported by Shanesazzadeh et al. (2018) and Aguilar-Vázquez et al., for sunflower protein isolate/PVA and pea protein/pullulan, respectively (Aguilar-Vázquez et al., 2018; Shanesazzadeh et al., 2018).

Mechanical properties

Table 2 demonstrates the mechanical properties of nanofibers. During the electrospinning process, an increase in GPPI content in the solutions led to greater instability due to the jet branching, so the TS decreased by increasing GPPI. The highest TS (3.33 MPa) and EB (37.85%) were observed in the PVA. According to the findings of this study, the mechanical properties of fiber mats containing more than 40% GPPI were very weak, and the mechanical properties of GPPI:PVA nanofiber mats with a ratio of 80:20 could not be measured. The TS of pure PVA nanofibers and samples with a low GPPI ratio was relatively high. However, as the GPPI content increased, the TS of the nanofiber mats exhibited a consistent decrease because of the increased brittleness of the two polymer materials (Cho et al., 2012). Based on the results, the GPPI content plays a crucial role in determining the mechanical properties of GPPI:PVA hybrid fibers. Cho et al. (2012) observed a similar trend when soy protein isolate (SPI) content increased in the SPI/PVA hybrid nanofibers. Their observations indicated that with a higher SPI ratio, the mechanical strength of the mats declined. Apart from the material properties of the GPPI:PVA blends, the mechanical attributes of the nanofiber mats are influenced by various factors like the porosity and diameter of the nanofiber (Gutschmidt et al., 2021). In the present study, the high content of GPPI (60%) caused the production of nanofibers with unfavorable mechanical properties. It is possible that the unstable spinning process led to defects in the mats, which may have resulted in the GPPI:PVA nanofiber with a ratio of 60:40, showing lower failure strain and strength than the other mats. Similar results were found in the electrospinning of SPI/polyethylene oxide (Xu et al., 2012).

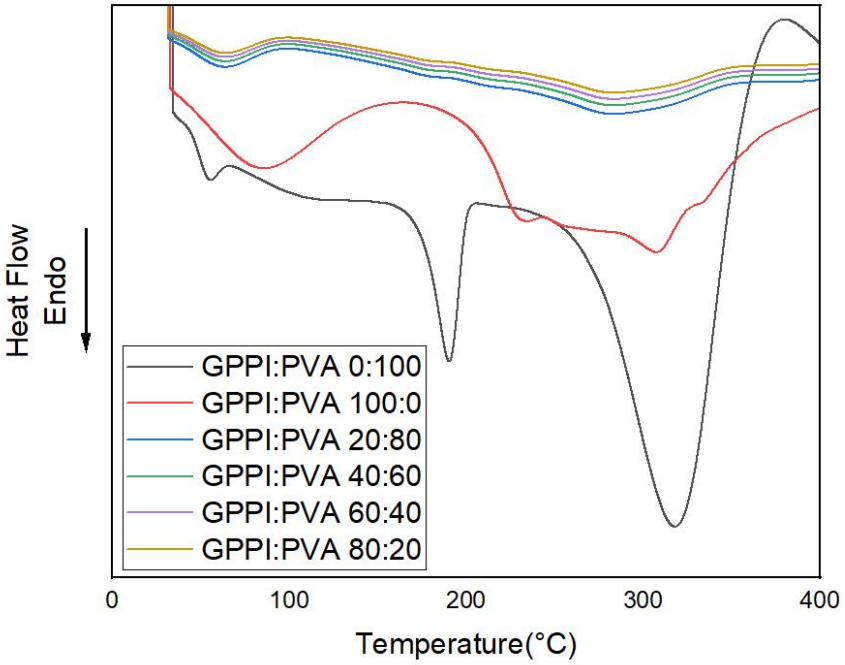

Fig. 4. DSC curves of the different ratios of GPPI:PVA. PVA: Polyvinyl alcohol; GPPI: grass pea protein isolate.

Table 2. The effect of different ratios of GPPI:PVA on thickness and mechanical properties of the nanofiber mats.

GPPI:PVA	Thickness (mm)	Tensile strength (MPa)	Elongation (%)
0:100	0.24 ± 0.01^{a}	3.33 ± 0.07^{a}	37.85 ± 2.89^{a}
20:80	0.22 ± 0.01^{b}	2.65 ± 0.12^{b}	31.69 ± 2.90^{b}
40:60	0.09 ± 0.01^{c}	0.88 ± 0.09^{c}	25.50 ± 1.82^{c}
60:40	0.04 ± 0.01^{d}	0.05 ± 0.01^{d}	11.21 ± 1.16^{d}
80:20	ND	ND	ND

PVA: Polyvinyl alcohol; GPPI: grass pea protein isolate. Different letters in the same column exhibit significant differences ($P < 0.05$).

CONCLUSION

A new protein nanofiber was created based on GPPI. Pure protein could not form fibers, but nanofibers containing 60% protein and 40% PVA had smooth surfaces without any beads or defects with an average diameter of 138 nm. By decreasing the ratio of GPPI in the mixed solutions, the electrical conductivity and surface tension showed a significant decrease and increase ($P < 0.05$), respectively. The FTIR analysis confirmed the interactions between GPPI and PVA components of the hybrid nanofibers. The nanofibers containing 80% GPPI were more heat-resistant compared to other nanofibers. Increasing the GPPI ratio resulted in a decrease in the mechanical strength of the composite nanofibers. GPPI can be used as a polymer for producing electrospun fibers, but it is essential to explore methods for enhancing the mechanical characteristics of the fibers in order to create stronger materials for food packaging applications.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Nasser Sedaghat, Marzieh Rezaei, and Sara Hedayati; Methodology: Sara Hedayati and Marzieh Rezaei; Software: Marzieh Rezaei; Validation: Nasser Sedaghat, Sara Hedayati, and Mohammad-Taghi Golmakani; Formal analysis: Marzieh Rezaei; Investigation: Marzieh Rezaei; Resources: Nasser Sedaghat, Sara Hedayati, and Mohammad-Taghi Golmakani; Data curation: Marzieh Rezaei; Writing—original draft preparation: Marzieh Rezaei; Writing—review and editing: Nasser Sedaghat, Sara Hedayati, and Mohammad-Taghi Golmakani; Visualization: Marzieh Rezaei; Supervision: Nasser Sedaghat and Sara Hedayati; Project administration: Nasser Sedaghat and Sara Hedayati; Funding acquisition: Nasser Sedaghat and Mohammad-Taghi Golmakani.

DECLARATION OF COMPETING INTEREST

The authors confirm that they do not have any competing financial interests or personal relationships that could have influenced the work presented in this paper.

DATA AVAILABILITY

Access to the data included in this study is available upon request from the corresponding author.

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