



Electrospun Ultrafine Fibers from Black Bean Protein Concentrates and Polyvinyl Alcohol

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Abstract

In this study, ultrafine fibers were produced from black bean protein concentrates (BPCs) and polyvinyl alcohol (PVA) by electrospinning. The BPC was denatured under acidic (pH 2) or basic (pH 11) conditions. Polymer solutions containing different PVA concentrations (11% or 21%, w/v) and different BPC: PVA ratios (50:50 or 75:25, v/v) were used for fiber production. The electrical conductivity and rheological properties of the fiber-forming solutions were evaluated, as well as the morphology, size distribution, infrared spectrum, and thermal properties of the electrospun fibers. The fibers showed a homogeneous morphology and diameters ranging from 115 to 541 nm. Fibers from the solution containing BPC denatured at pH 11, 11% PVA, and 75:25 (v/v) BPC: PVA presented the lowest diameter, and those from BPC denatured at pH 2 had less beads than the fibers obtained from BPC denatured at pH 11. The solution formulation affected the thermal properties of the fibers, with weight loss increases ranging from 39.0% to 60.9%. The polymeric solutions containing PVA and BPC (whether denatured under basic or acidic conditions) resulted in ultrafine electrospun fibers with highly favorable characteristics that could potentially be used for the encapsulation of bioactive compounds and food applications.

Keywords Electrospinning · Black bean · Denaturation · Morphology

Introduction

According to the Food and Agricultural Organization, pulses are dry seeds of annual leguminous plants belonging to the Fabaceae (or Leguminosae) family, which covers 11 primary pulses: dry beans, dry broad beans, dry peas, chickpeas, dry cowpeas, pigeon peas, lentils, Bambara beans, vetches, lupins, and/or minor pulses [1]. In Brazil, the common beans (*Phaseolus vulgaris* L.) from both the carioca and black groups make up the main pulse crops. The protein content of black beans is approximately 20–25%, which makes these

grains an attractive source of proteins for extraction and modification [2].

Proteins can be used to produce fibers via the electrospinning process [3–5]. This method of achieving fibers with distinct diameters and morphologies is made possible by controlling the process parameters, such as applied voltage, flow rate, and spinneret-to-collector distance, as well as the fiber-forming solution parameters (i.e., concentration, viscosity, and electrical conductivity) [6–8]. The solutions to be electrospun are prepared as a polymer–solvent system that is pumped at a controlled flow rate from a syringe to which a high voltage is applied. Electrostatic forces pull the solution away from a positively charged spinneret needle into a Taylor cone formation that is drawn to a grounded stainless-steel collector. As this process occurs, the solvent evaporates, leaving a mat of fibers in the collector [6, 9].

Among the polymers most used to produce electrospun fibers, synthetics such as polyethylene glycol, poly(lactic acid), nylon 6.6, polyvinyl alcohol (PVA), and polyvinyl chloride, and natural polymers such as protein, starch, modified starch, and gums (xanthan, guar, and alginate) can be highlighted [10–14]. The solution conditions, such as pH,

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ionic strength, temperature, and protein concentration, can control the solubility of the proteins by influencing the protein–water and protein–protein interactions [3, 15]. A solution with a pH that is moderately below the isoelectric point or in an alkaline range results in high proteins solubility [16]. The electrospinning of aqueous solutions of proteins in their native or denatured state is not an effective process, and these solutions have relatively low electrospinnability [17]. In this case, the addition of carrier polymers can facilitate fiber formation. Vega-Lugo and Lim [18] evaluated the electrospinnability of soy protein solutions using poly (ethylene oxide) (PEO) as carrier polymer. The authors reported that the successful production of electrospun fibers is affected by the protein solution properties as surface tension, viscosity and electrical conductivity, which are not ideal to produce the needed chain entanglement for fiber formation. Thus, even in the protein denatured form, neat protein solutions present low electrospinnability. This can be overcome with the incorporation of a carrier polymer, hence the protein interacting synergistically with the polymer, enhancing its' electrospinnability due to a higher chain entanglement [18]. PVA can be used as carrier polymer and has been reported in the literature to enhance the electrospinnability of fiber-forming solutions, producing fibers with highly favorable morphologies and other properties, furthermore PVA is a biodegradable and biocompatible polymer [13, 19].

There are currently no published studies regarding the use of bean protein concentrates for fiber formation by electrospinning. Therefore, the use of bean protein combined with PVA provides a biodegradable material with great properties (e.g. great morphology, low diameter and thermal stability) promisor for various applications. The main objective was to evaluate fiber formation by the electrospinning of different concentrations of denatured black bean protein concentrates (BPCs) under acidic (pH 2) or basic (pH 11) conditions and with the addition of a carrier polymer.

Material and Methods

Materials

Black bean grains were obtained from a local market in Pelotas, Rio Grande do Sul, Brazil. The chemical composition of the grains was protein 23.1%, lipids 1.2%, ash 4.3%, starch 48.6%, and fibers 22.8% (w/w). All chemicals used were of analytical grade. The PVA was obtained from Sigma-Aldrich.

Bean Protein Concentrate Preparation

The BPC was prepared according to the method described by Carrasco-Castilla et al. (2012) [20], with slight modifications. The bean flour was prepared in a laboratory mill. For protein

extraction, the flour was suspended in distilled water (1:10), the pH was adjusted to 9.5 with NaOH ($1 \text{ mol}\cdot\text{L}^{-1}$), and the solution was agitated for 30 min at 35 °C. Thereafter, the material was centrifuged for 30 min at 10,000 rpm and the pH of the supernatant was adjusted to 4.5 with HCl ($1 \text{ mol}\cdot\text{L}^{-1}$). Then, it was centrifuged under 12,000 rpm and the precipitate was collected and kept at $-80 \text{ }^\circ\text{C}$ until lyophilization.

Electrospinning Process

Preliminary tests were performed to determine the optimal amount of BPC to use in proportion to the PVA for the fiber-forming solution. As a neat polymer in solution, BPC was not able to produce electrospun fibers, and instead formed only droplets in the stainless-steel collector. Thus, PVA at 11% or 21% (w/v) was used as a carrier in different ratios with BPC (i.e., BPC: PVA ratios of 50:50 and 75:25, v/v). The optimal PVA concentrations were also determined in preliminary tests. The BPC was prepared by adding 11 g of the lyophilized powder into 100 mL of distilled water. After its dissolution, the protein was denatured under acidic conditions at pH 2 or basic conditions at pH 11. The PVA solution was prepared by adding 11 or 21 g of the polymer to 100 mL of distilled water. The fiber-forming solutions were then prepared by stirring the various mixtures of BPC and PVA solutions on a magnetic stirrer (Model 752/6, FISOTOM, Brazil) for 30 min. In all solutions, Tween 80 was added to decrease the surface tension. Fibers electrospun from solutions with PVA 11% were prepared as a control. Because the PVA concentration of 21% had a high viscosity, it could not be loaded into the syringe for fiber production. The electrospinning process was carried out by drawing the fiber-forming solutions into a 3-mL syringe with a stainless-steel needle of 0.7 mm. A syringe infusion pump (Model 100, KD Scientific, England) was used to maintain a steady flow rate of $1 \text{ mL}\cdot\text{h}^{-1}$. The DC power supply for applying the tension (INSESHV30, INSTOR, Brazil) was set at 23 kV. A grounded stainless-steel collector plate was positioned at 20 cm away from the tip of the needle. The fibers were electrospun at $23 \pm 2 \text{ }^\circ\text{C}$ and 40–50% relative humidity.

Electrical Conductivity and Rheological Properties of the Fiber-Forming Solutions

The electrical conductivity was analyzed with a portable conductivity meter (Model mCA 150P, MS TECNOPON, Brazil) and expressed in units of $\mu\text{S}\cdot\text{cm}^{-1}$. The rheological properties were measured using a digital rheometer (Model DV-II, Brookfield, USA). Approximately 1 mL of the fiber-forming solution was placed in a stainless-steel recipient coupled to the equipment and a $n^\circ 18$ spindle was used. The power law model (Eq. (1)) was applied to determine the viscosity (μ) and flow behavior index (n). The apparent viscosity (η_{ap})

was determined at $\dot{\gamma} = 100 \text{ s}^{-1}$. The analyses were carried out under ambient temperature ($23 \pm 2 \text{ }^\circ\text{C}$) and at least in triplicates.

$$\sigma = k\dot{\gamma}^n \quad (1)$$

Morphology and Size Distribution of the Electrospun Fibers

The morphology of the electrospun fibers was analyzed by scanning electron microscopy (SEM) using a JSM-6610LV microscope (Jeol, USA) at a voltage acceleration of 15 kV. A small portion of the fibers was placed in a stub and sputter coated with gold before the analysis [8]. On the basis of 60 randomly chosen fibers in the SEM images, the average diameter and the diameter distribution of the fibers were evaluated using ImageJ software.

Fourier-Transform Infrared Spectroscopy Analysis of the Electrospun Fibers

The functional groups of the electrospun fibers and their constituents (i.e., BPC, PVA, and Tween) were analyzed by Fourier-transform infrared (FTIR)-attenuated total reflection (ATR) spectroscopy using an IR Prestige-21 device (Shimadzu Corp., Japan). The spectrum was obtained from an average of 60 scans for each sample at a resolution of 4 cm^{-1} at room temperature ($23 \pm 2 \text{ }^\circ\text{C}$).

Thermogravimetric Analysis of the Electrospun Fibers

The thermal stability of the electrospun fibers and their constituents was evaluated by thermogravimetric analysis (TGA), using a TA-60WS workstation (Shimadzu, Japan), and by derived thermogravimetry (DTG). The samples ($\sim 5 \text{ mg}$) were heated in platinum capsules at a range of $30\text{--}600 \text{ }^\circ\text{C}$, with a heating rate of $10 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ and nitrogen flow of $50 \text{ mL}\cdot\text{min}^{-1}$. An empty platinum capsule was used as the reference.

Statistical Analysis

The data were analyzed using Tukey's test at a 5% level of significance by analysis of variance.

Results and Discussion

Electrical Conductivity and Rheological Properties of the Fiber-Forming Solutions

The electrical conductivity and rheological properties of the fiber-forming solutions containing BPC (denatured at pH 2 or

11) and PVA (11% or 21%, w/v) at different ratios (BPC:PVA at 50:50 or 75:25) are shown in Table 1. The electrical conductivity of the fiber-forming solutions ranged from 1411 to 2324 $\mu\text{S}\cdot\text{cm}^{-1}$, where there was no statistically significant differences among the solutions with the same BPC:PVA ratio ($p < 0.05$). There was an increase in the electrical conductivity with the increase in BPC ratio (75:25) in the solution ($p < 0.05$). This increase could be related to the high electrical conductivity of the BPC, which was approximately 10 times higher than that of the PVA solutions. Shanesazzadeh et al. [21] studied the electrical conductivity of solutions of sunflower protein isolate with PVA and also observed high values with the increase of protein in the solution.

The electrical conductivity is a parameter that influences the charge density of the solution that is transported through the jet during the electrospinning process. As the charge density increases, the amount of beads becomes smaller and so too the fiber diameter [22]. According to Wen et al. [14], a high viscosity combined with a low electrical conductivity results in less stretching of the solution jet, thus producing thicker fibers.

The neat PVA 21% solution presented a higher electrical conductivity value than the neat PVA 11% solution (Table 1). However, the behavior was the opposite for the rest of the fiber-forming solutions analyzed ($p < 0.05$) when BPC was added, being the BPC:PVA solutions with PVA 21% with lower electrical conductivity than the ones with PVA 11%. This was likely because the solutions showed a behavior close to that of the neat BPC solutions, showing different behavior than the neat PVA solutions for this parameter.

The polymeric solutions showed different rheological behaviors; namely, Newtonian, pseudoplastic, or dilatant (Table 1). Rheological studies relate the shear rate to the shear stress. When this relation is linear, the fluid is Newtonian and the viscosity is constant and independent of the shear rate or stress applied. In several cases, this relation is not linear and the fluid is classified as non-Newtonian and further subclassified as pseudoplastic or dilatant. In pseudoplastic fluids, the viscosity decreases according to the increase in shear rate applied [23]. In dilatant fluids, the viscosity increases along with the increase in the strain rate. The fiber-forming solutions that presented a pseudoplastic behavior presented a flow behavior index (n) of less than 1, whereas the solution with dilatant behavior presented an n value of greater than 1, which were representative of pseudoplastic and dilatant behavior, respectively (Table 1).

The fiber constituents were evaluated separately, where the BPC in the basic condition (denatured at pH 11) presented a low viscosity and therefore its rheological parameters could not be measured and its rheological behavior was not determined. On the other hand, the BCP in the acidic condition (denatured at pH 2) demonstrated Newtonian behavior, whereas the solutions of neat PVA 11% and 21% presented

Table 1 Electrical conductivity and rheological parameters of the fiber-forming solutions from BPC in basic (pH 11) or acid (pH 2) conditions with PVA in the concentrations of 11 and 21% and in different BPC:PVA ratios

Constituents ¹			Electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	Rheological behavior	μ (Pa.s) ($0 \leq \dot{\gamma} \leq 100^{-1}$) ²	n	r^2	η_{ap} (Pa.s) ($\dot{\gamma} = 50 \text{ s}^{-1}$)
BPC pH 2			3016 ± 3^b	Newtonian	0.975 ± 0.161^e		0.9980	
BPC pH 11			3480 ± 15^a	–	–			–
PVA 11			369 ± 24^h	Newtonian	0.080 ± 0.00^e		0.9996	
PVA 21			470 ± 5^g	Dilatant		1.470 ± 0.006^a		3.274 ± 0.992^d
Fiber-forming solutions								
BPC pH	PVA (%)	BPC:PVA						
2	11	50:50	1595 ± 18^e	Newtonian	0.303 ± 0.002^e		0.9934	
		75:25	2236 ± 1^c	Pseudoplastic		0.650 ± 0.001^e		3.911 ± 0.137^{cd}
	21	50:50	1411 ± 3^f	Pseudoplastic		0.814 ± 0.001^d		0.065 ± 0.001^e
		75:25	1957 ± 9^d	Pseudoplastic		0.865 ± 0.001^b		9.854 ± 0.144^b
11	11	50:50	1649 ± 48^e	Newtonian	0.850 ± 0.009^e		0.9937	
		75:25	2324 ± 72^c	Newtonian	4.568 ± 0.199^c		0.9954	
	21	50:50	1444 ± 3^f	Pseudoplastic		0.842 ± 0.03^c		20.742 ± 0.019^a
		75:25	1987 ± 44^d	Newtonian	1.063 ± 0.013^e		0.9966	

Different letters in superscripts (a-h) indicate significant differences among the samples ($p < 0.05$)

¹ BPC Bean protein concentrate; PVA Polyvinyl alcohol

² Rheological parameters: μ viscosity; n flow behavior index; η_{ap} apparent viscosity

Newtonian and dilatant behaviors, respectively (Table 1). In the fiber-forming solutions with PVA 11% being constant in the BPC:PVA ratios, the highest BPC in the ratio (75:25) presented a high viscosity ($p < 0.05$), which occurred because the BPC showed higher viscosity in solution than PVA 11% did, thus contributing to the increase in solution viscosity as the BPC:PVA ratio increased.

Morphology and Size Distribution of the Electrospun Fibers

The electrospinning of aqueous solutions of proteins in their native or denatured state is not effective without the addition of a carrier polymer. This is because the protein molecules with a globular conformation do not present enough chain entanglement to form fibers [17]. For this reason, the use of auxiliary polymers in the fiber-forming solution is necessary for fiber formation by electrospinning [19]. Thus, we successfully electrospun fibers from BPC, denatured under both acidic (Fig. 1) and basic pH conditions (Fig. 2), through the addition of PVA.

There were several factors that could influence the electrospinning process, since a mixture of polymers was used. The electrospun fibers from BPC denatured at pH 2 showed uniform morphology and less beads (Fig. 1) compared with the fibers from BPC denatured at pH 11 (Fig. 2). The density of beads in the images was less visible for the fibers produced from BPC denatured at pH 2 with PVA 21% in both BPC:PVA ratios (Fig. 1c, d) and from BPC denatured

at pH 11 with PVA 21% in the BPC:PVA ratio of 50:50 (Fig. 2c). Thus, PVA 21% contributed to less bead formation than did PVA 11%, regardless of the pH condition used for BPC denaturation.

In the size distribution analysis, the diameters of the electrospun fibers from BPC denatured at pH 2 were in the range of 145 to 541 nm (Fig. 1), whereas those of fibers from BPC denatured at pH 11 ranged from 115 to 428 nm (Fig. 2). Regardless of the BPC-denaturing pH condition, the fibers with PVA 21% presented larger diameters than did those with PVA 11%. This behavior can be related to the higher viscosity of the fiber-forming solutions with PVA 21% (Table 1). According to Huang et al. [24], one of the parameters that have a crucial influence on the diameter of fibers is the viscosity of the fiber-forming polymer solution or the concentration of polymers used in the matrix. These authors state that, in general, along with the increase in viscosity, the resistance of the solution to elongation in the electrospinning process occurs and, consequently, the fiber diameter increases. The increase in the polymer concentration results in polymer chemical chain bonds within the solution, which provide a uniform continuity of the jet during electrospinning [25, 26].

In the comparison of the electrospun fibers from BPCs denatured under the same pH conditions and in different BPC:PVA ratios, it was observed that those with the highest BPC ratio (75:25) had a lower mean diameter (Figs. 1b and 2b, d). This may be due to the higher electrical conductivity of the fiber-forming polymer solutions with a higher BPC ratio (Table 1). Generally, proteins dissolved in aqueous solution

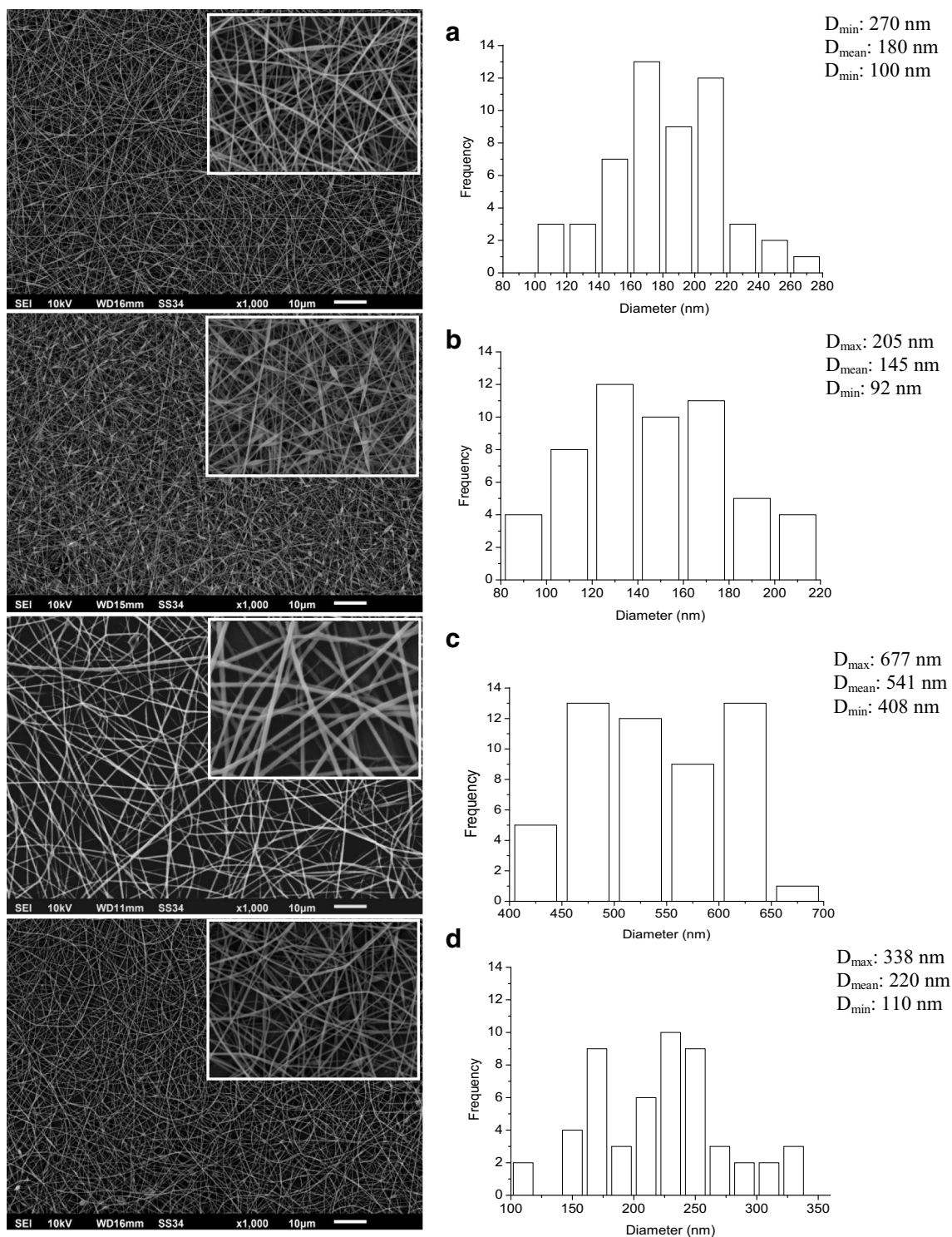


Fig. 1 Morphology and size distribution of the electrospun fibers from BPC denatured under acidic conditions at pH 2. **(a)** PVA 11%, BPC:PVA ratio 50:50; **(b)** PVA 11%, BPC:PVA ratio 75:25; **(c)** PVA 21%, BPC:PVA ratio 50:50; **(d)** PVA 21%, BPC:PVA ratio 75:25

produce ions, which are responsible for the electrical conductivity of the solutions. With the application of a high electric field, these charges are oriented to allow the jet to undergo a greater elongation, resulting in thicker segments and a decrease in the diameter of the electrospun fibers [26, 27].

There are studies exploring the fiber production by electrospinning using proteins combined with carrier polymers as soy protein/PEO/zein, soy protein isolate/PVA and alginate/soy protein isolated [19, 28, 29]. These studies reported the important use of proteins for their desirable properties as

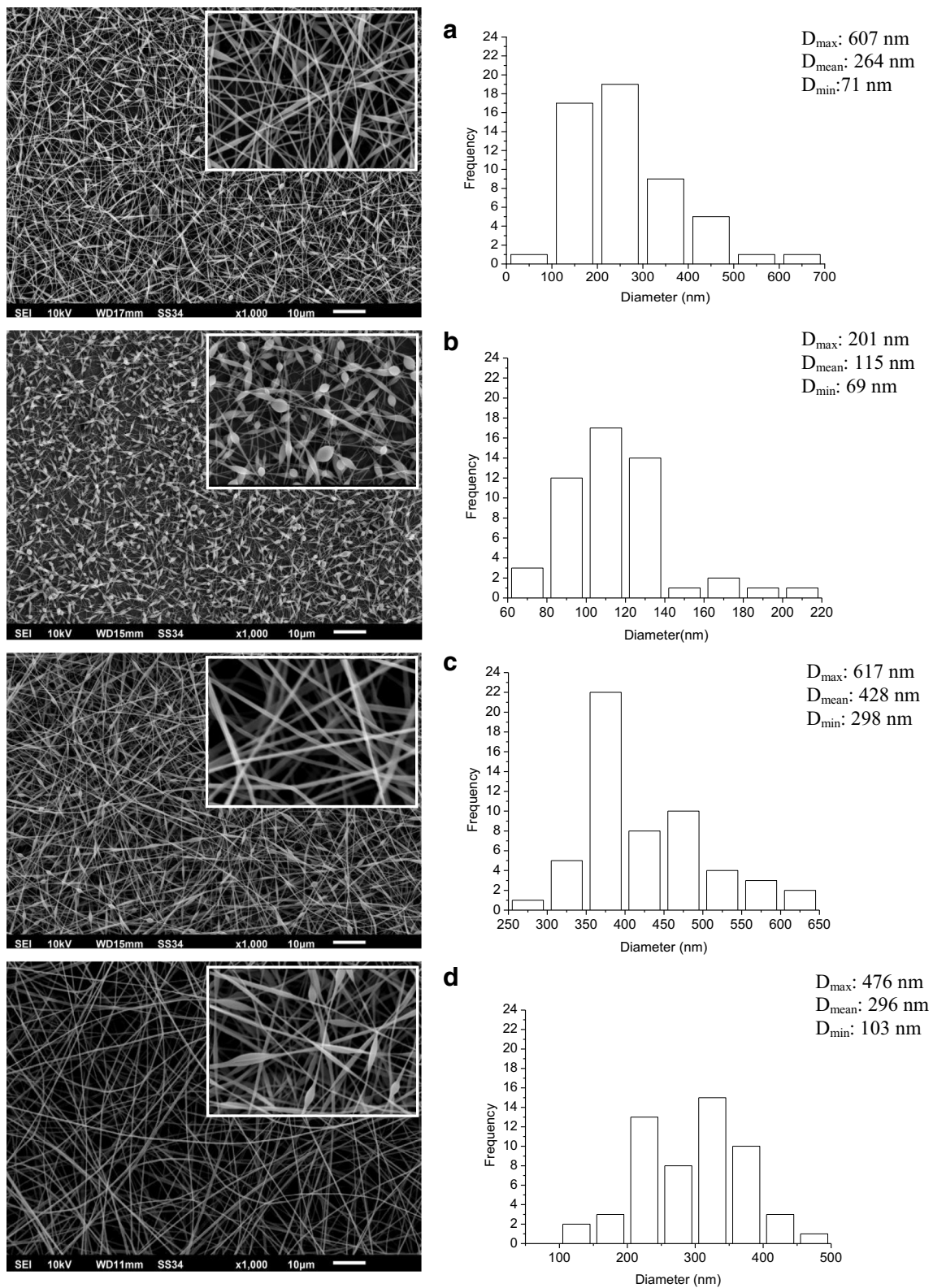


Fig. 2 Morphology and size distribution of the electrospun fibers from BPC denatured under basic conditions at pH 11. (a) PVA 11%, BPC:PVA ratio 50:50; (b) PVA 11%, BPC:PVA ratio 75:25; (c) PVA 21%, BPC:PVA ratio 50:50; (d) PVA 21%, BPC:PVA ratio 75:25

biodegradability, biocompatibility and functional properties as a great matrix for the encapsulation of bioactive compounds

and controlled release. Although, proteins present a complicated polyelectrolyte structure preventing the fibers production

without a carrier polymer incorporation in the fiber-forming solution. The fibers produced in the present study used as polymer matrix bean protein concentrate, which, to the best of our knowledge, has not been used for this end. Thereby, the great morphology and low diameter obtained for this new biomaterial can target the high demand for innovative nanomaterials.

Fourier-Transform Infrared Spectroscopy Analysis of the Electrospun Fibers

FTIR-ATR spectroscopy was used to evaluate the fiber constituents (i.e., BPC, PVA, and Tween) and the interaction among them in the electrospun fibers under different conditions in the system (Figs. 3, 4 and 5) and vibration frequencies are presented in Table 2.

The spectra of the electrospun fibers from BPC denatured at pH 2 with PVA 11% or 21% (*w/v*) is presented in Fig. 4. Bands near 3200 cm^{-1} ($\nu\text{O-H}$ overlaid on $\nu\text{N-H}$) from amide A and near 2900 cm^{-1} ($\nu_{\text{as}}\text{CH}_2$; $\nu_{\text{s}}\text{CH}_2$) were found. According to the spectra in Fig. 4, a similar fingerprint region ($700 < \text{cm}^{-1} < 1100$) existed between the spectra in Fig. 4a, c with a BPC:PVA ratio of 50:50, and between those in Fig. 4b, d with a BPC:PVA ratio of 75:25. In the spectra of Fig. 4c that represent the electrospun fiber with PVA 21%, it was still possible to observe bands at 1720 and 840 cm^{-1} from CH vibrations, which were previously found in the PVA spectrum (Fig. 3b). This may be because the PVA was in a higher concentration and in a BPC:PVA ratio of 50:50. In the spectra in Fig. 4b, the vibration at 1398 cm^{-1} was related to Tween (Fig. 3c) residues. The band previously observed at 1520 cm^{-1} ($\nu_{\text{as}}\text{COO}^-$) in the BPC spectrum (Fig. 3a) and that previously observed at 1429 cm^{-1} ($\nu_{\text{as}} = \text{C-O}$) in the PVA spectrum (Fig.

3b) were displaced to a region with a higher wave number (maximum $\Delta\nu_{\text{as}} = 19\text{ cm}^{-1}$) in the fiber spectra. This suggested a hydrogen bond interaction between the BPC and PVA polymers, occurring between the carbonyl groups from BPC and alcohol hydroxyl groups from PVA.

Figure 5 shows the spectra of the electrospun fibers from the BPC denatured at pH 11 with PVA at 11% or 21% (*w/v*). The bands found were similar for all the spectra, being different only for a new band at 1401 cm^{-1} ($\nu_{\text{s}}\text{COO}^-$) in the spectra with a BPC:PVA ratio of 75:25 (Fig. 5b, d). This band could be characteristic of the increase in the pH through BPC denaturation in the basic condition. The active sites could be more exposed, resulting in the appearance of a different vibration or a displacement of the bands as compared with those in the spectra of electrospun fibers from BPC denatured at pH 2 (Fig. 4). The displacements found were at 1520 cm^{-1} (from BPC, with maximum $\Delta\nu = 23\text{ cm}^{-1}$) and 1251 cm^{-1} ($\nu\text{C}=\text{C}$, from PVA, with $\Delta\nu = 4\text{ cm}^{-1}$), suggesting the interaction between the functional groups from BPC and PVA. However, the disappearance of the PVA band in the region of 1429 cm^{-1} indicated that the interaction occurred with the hydroxyl group, presenting a complete incorporation of the groups from both electrospun fiber constituents.

On the basis of these FTIR-ATR results, the BPC and PVA presented good synergism in the interactions between the polymers. This was also observed in the SEM images (Figs. 1 and 2) from the formation of fibers with a homogeneous and well formed morphology. The great synergism between both polymers (BPC:PVA) also can be attributed to their rheological parameters since, when mixed together, the polymers molecules interacts forming chain entanglements making it possible the successful fiber formation.

Fig. 3 FTIR-ATR spectra of the electrospun fiber constituents: BPC (a), PVA (b), and Tween (c)

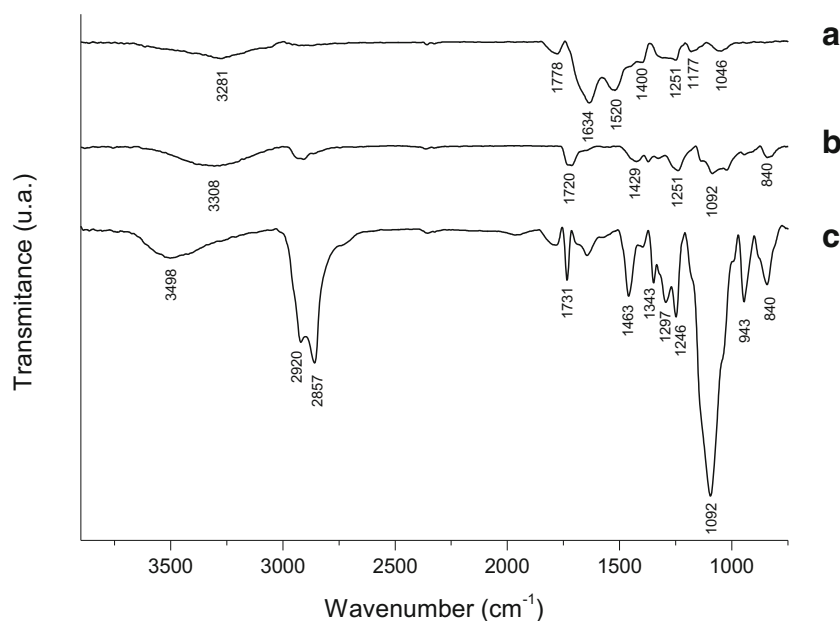
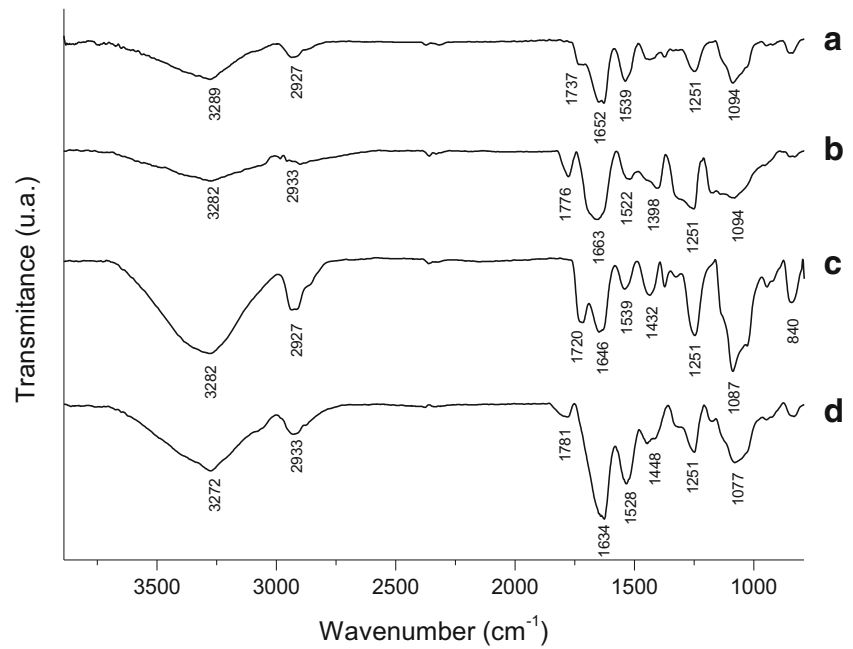


Fig. 4 FTIR-ATR spectra of the electrospun fibers from BPC denatured under acidic conditions at pH 2. **(a)** PVA 11%, BPC:PVA ratio 50:50; **(b)** PVA 11%, BPC:PVA ratio 75:25; **(c)** PVA 21%, BPC:PVA ratio 50:50; **(d)** PVA 21%, BPC:PVA ratio 75:25



Thermogravimetric Analysis of the Electrospun Fibers

TGA and DTG were conducted to investigate the thermal degradation of the electrospun fibers and their constituents (Table 3). The DTG curve is the first derivative of the TGA curve in the function of temperature. The BPC underwent decomposition in the temperature range of 218.0–352.9 °C (TGA and DTG curves of the constituents in supplementary material). According to Neo et al. [12] and Silva et al. [19], this is due to breakage of the peptide bonds. PVA was

decomposed in a distinct stage (327.9 °C) in a temperature range of 253.5–352.9 °C.

The weight losses of the fibers and their constituents were observed in a temperature range of 250–400 °C. The BPC presented a weight loss of 38.5%, showing higher stability in relation to PVA, which presented a weight loss of 61.0% (Table 3). Upon evaluation of the fiber decomposition, it was observed that the weight loss varied from 39.0% to 60.9% and was dependent on the fiber composition. The electrospun fibers from BPC denatured at pH 11 with PVA 21% in a

Fig. 5 FTIR-ATR spectra of the electrospun fibers from BPC denatured under basic conditions at pH 11. **(a)** PVA 11%, BPC:PVA ratio 50:50; **(b)** PVA 11%, BPC:PVA ratio 75:25; **(c)** PVA 21%, BPC:PVA ratio 50:50; **(d)** PVA 21%, BPC:PVA ratio 75:25

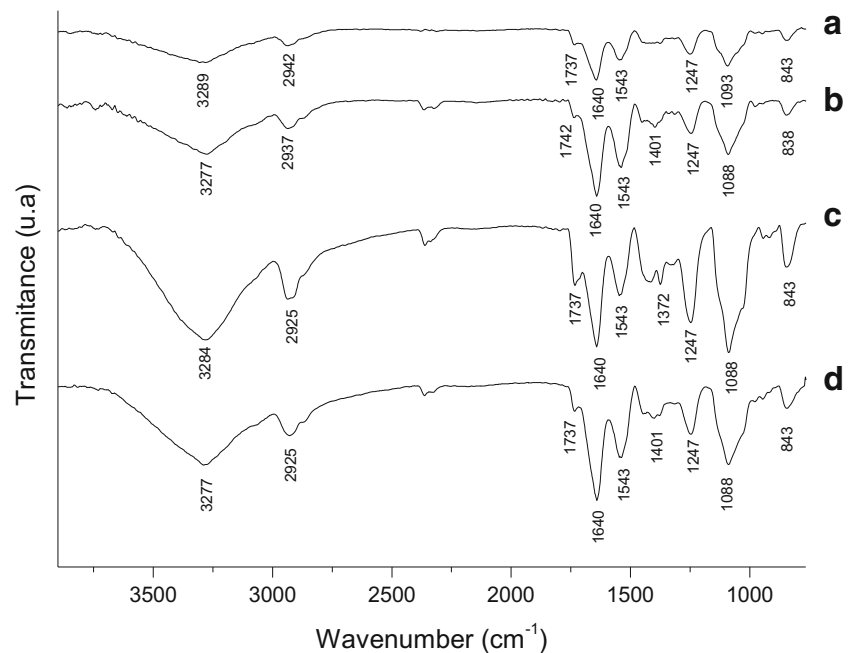


Table 2 Band positions (cm⁻¹) in the FT-IR spectra of the constituents and the electrospun fibers from BPC in basic (pH 11) or acid (pH 2) conditions with PVA in the concentrations of 11 and 21% and in different BPC:PVA ratios

Constituents ¹		Electrospun fibers band positions (cm ⁻¹) ²								Assignment ³	
BPC	PVA	Tween	pH 2				pH 11				
			A	B	C	D	A	B	C		D
3281	3308	3498 2920 2857	3289 2927	3282 2933	3282 2927	3272 2933	3289 2942	3277 2937	3284 2925	3277 2925	$\nu(\text{OH})$; $\nu(\text{NH})$ $\nu_{\text{as}}(\text{CH}_2)$ $\nu_{\text{s}}(\text{CH}_2)$ $\nu(\text{C=O})a$
1778				1776		1781					$\delta(\text{CH})$; $\nu(\text{C=O})b$ $\nu(\text{CN})/\delta(\text{NH})c$
1634	1720	1731	1737	1633	1720	1634	1737	1742	1737	1737	$\nu_{\text{as}}(\text{COO}^-)$
1520		1463	1539	1522	1539	1528	1543	1543	1543	1543	$\delta_{\text{s}}(\text{CH}_2)$ $\delta(\text{OH})/\nu_{\text{as}}(\text{=CO})$
1400	1429				1432			1401		1401	$\nu_{\text{s}}(\text{COO}^-)$ $\delta_{\text{s}}(\text{CH}_3)$ $\nu_{\text{s}}(\text{COC})d$
		1343 1297		1398					1372		$\rho(\text{NH}_3)$; $\nu(\text{C=C})$ $\nu(\text{CCOO})/\delta(\text{COH})e$
1251	1251	1246	1251	1251	1251	1251	1247	1247	1247	1247	$\nu_{\text{as}}(\text{CCN})$ $\nu(\text{COC})$
1177											$\nu(\text{CO})$; $\nu(\text{OCC})f$
1046											$\nu(\text{CO})$
	1092	1092	1094	1087	1087	1077	1093	1088	1088	1088	$\gamma(\text{CH})$; $\nu_{\text{as}}(\text{COC})g$
	840	840			840		843	838	843	843	

¹ BPC Bean Protein Concentrate; PVA Polyvinyl alcohol² (A) PVA 11%, BPC:PVA ratio 50:50; (B) PVA 11%, BPC:PVA ratio 75:25; (C) PVA 21%, BPC:PVA ratio 50:50; (D) PVA 21%, BPC:PVA ratio 75:25³ ν - stretching, δ - in-plan bending, γ - out-off-plan bending, ρ - rocking, ν_{as} - antisymmetric, ν_{s} - symmetric, a - amide I, b - carbonyl groups, c - amide II, d - cyclic ether, e - alcohol and ester, f - aliphatic chain, g - cyclic chain

BPC:PVA ratio of 50:50 presented a weight loss similar to that of PVA. These fibers presented PVA bands in their FTIR-ATR spectra and the highest viscosity and diameter values, when compare with the other fibers produced. The rheological parameters of the solutions and the morphology of the fibers are directly related to its' thermal stability. For the fibers cited (BPC pH 11, PVA 21%, BPC:PVA 50:50), the interactions

between the polymers were not strong enough for the disappearance of the PVA band in the fiber spectra. In addition, the high viscosity of the solution provides resistance to elongation in the electrospinning process, hence the higher weight loss could be related to the rheological parameters of the fiber-forming solution. All the other fibers presented thermal stability values that were higher than that of the neat PVA. This

Table 3 Thermal gravimetric analysis (TGA) and its derivatives (DTG) of the constituents and electrospun fibers from BPC in basic (pH 11) or acid (pH 2) conditions with PVA in the concentrations of 11 and 21% and in different BPC:PVA ratios

Constituents			TGA ²			DTG	
			T _{di} (°C)	T _{dr} (°C)	Weight loss of 25–400 °C (%)	T _{d1} (°C)	
BPC ¹			218.0	352.9	38.5	318.1	
PVA			253.5	476.1	61.0	327.9	
Tween			360.6	454.8	34.3	415.1	
Electrospun fibers			T _{di} (°C)	T _{dr} (°C)	Weight loss of 25–400 °C (%)	T _{d1} (°C)	
BPC pH	PVA (%)	BPC:PVA					
2	11	50:50	227.5	374.1	40.2	329.6	
		75:25	234.0	393.7	45.4	324.2	
		21	50:50	298.6	411.9	39.0	377.0
			75:25	267.9	363.8	46.2	327.2
11	11	50:50	248.1	351.1	49.7	277.6	
		75:25	258.3	370.9	41.2	309.4	
		21	50:50	237.7	398.8	60.9	305.4
			75:25	248.1	355.5	47.0	297.3

behavior was in accordance with the interactions between BPC and PVA observed in the fiber spectra, which could be related to the enhanced stability of the fibers.

Conclusion

Fibers were successfully electrospun from BPC with the addition of PVA to the fiber-forming solution. The BPC concentration influenced the electrical conductivity and viscosity of the fiber-forming solutions, with higher concentrations increasing these solution parameter values. The solution parameters were not affected by the BPC-denaturing pH condition. The electrospun fibers showed homogeneous and uniform morphology, with those from BPC denatured at pH 2 showing less beads than the fibers from BPC denatured at pH 11. The thicker fibers (115 nm) were the ones from BPC denatured at pH 11 with PVA 11% addition (75:25).

The interactions between both polymers in solution were evidenced by FTIR-ATR analysis along with SEM of the fiber morphology. The electrospun fibers presented great thermal stability, with the weight loss of the fibers ranging from 39.0% to 60.9%, being affected by the differences in the formulations of the fiber-forming solutions. The present study showed that protein denatured under different pH conditions, combined with the addition of an auxiliary polymer (PVA) at various ratios in the fiber-forming solution, can yield fibers with distinct morphologies and thermal properties.

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