Electrospinnability study of pea (*Pisum sativum*) and common bean (*Phaseolus vulgaris* L.) using the conformational and rheological behavior of their protein isolates.

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Abstract

Pea protein isolate (PPI) and bean protein concentrate (BPC) were evaluated as fiber-forming vegetal source materials through electrospinning using various solvents. The effects of hexafluoroisopropanol (HFIP), trifluoroethanol (TFE), trifluoroacetic acid (TFA), formic acid (FA) and water on rheological and conformational properties of the protein solutions were determined. The morphology and molecular organization of the electrospun structures were studied. All PPI and BPC solutions displayed pseudoplastic behavior. Circular dichroism spectroscopy revealed that β -type turns and β -sheets were the dominant protein conformations in water, HFIP, and TFE. After electrospinning, most of the solutions afforded beads. Fiber-like morphologies were only obtained when BPC was dissolved in HFIP. BPC demonstrated better performance in the electrospinning process than PPI. Denaturation of the protein isolates was not sufficient to form fibers, the viscosity of the solution as well as the vapor pressure of the solvents played an important role in defining the morphology.

Key words: Electrospinning; pea protein isolate; common bean protein isolate; rheology

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42 **1. Introduction**

43 In recent years, the development of nanotechnology has fostered the interest on the use of
44 electrospinning for the preparation of polymeric nanofibers [1]. The need of biodegradable
45 and biocompatible nanomaterials of renewable nature has directed the research interest on
46 biopolymers such as proteins and polysaccharides. Animal and vegetable proteins have been
47 used to produce electrospun fibers; but vegetable proteins offer advantages like low cost and
48 high availability. Among legumes are peas and beans, which are recognized as good sources
49 of protein.

50 Commercial pea protein isolate (PPI) is obtained by wet processing, in which proteins are
51 isolated by a selective precipitation step at the isoelectric point and a final protein content of
52 85% is usually obtained. Most of pea proteins are storage proteins, being globulins the most
53 abundant (66%) [2]. Among these globulins, legumin (11S), a protein with hexameric
54 quaternary structure and a molecular weight around 320-380 KDa, and vicilin (7S), a protein
55 with trimer quaternary structure with 150-180 KDa are the predominant ones. Pea protein
56 aqueous solutions on the other hand, have been characterized to study its functional
57 properties such as foaming, gelling, and particle aggregation [3–6].

58 Bean protein concentrate (BPC) is characterized by its well-balanced amino-acid content.
59 BPC is rich in essential amino acids like lysine, tyrosine, and phenylalanine. Phaseolin is the
60 major storage protein in bean seeds, and represents about 30–50% of total bean protein. It is
61 a glycosylated protein with a trimer quaternary structure. Lectin proteins (lectin,
62 phytohemagglutinin E and L, α -amylase inhibitors and arcelins) and protease, trypsin and
63 Bowman–Birk inhibitors are other important proteins found in beans [7]. Functional
64 properties of bean protein such solubility, foaming and emulsifying capacity from flours
65 [8,9], bean protein isolates [10] and bean proteins fractions [11] have already been evaluated.
66 Vegetable proteins from zein [12] and soy [13] have demonstrated to produce solutions with
67 viscosity, electric conductivities and surface tension values that readily allow electrospun
68 fiber production. Protein unfolding is acknowledged as a requisite for electrospinning and
69 therefore, the solvent needs not only to solvate the protein structure but to induce unfolding.
70 In this way, globular proteins are notoriously difficult to be electrospun due to high

hydrophobic and ionic interactions [14], hydrogen bonds [15], complex network structure [16], and low charge density [17]. One approach to overcome these drawbacks is to blend the proteins with easily spinnable polymers such as PEO, PVA, and pullulan as in the case of pea and amaranth protein isolates [1,16,18,19]. It has been suggested that chains from spinnable polymer structures form an intertwine network containing the 'non-electrospun' polymers, thereby providing a mixed system that can be successfully electrospun [20,21].

In this context, successful processing of protein solutions into fiber through electrospinning requires a smart solvent selection. The solvent-polymer interactions have a critical influence not only on the viscoelasticity and the minimum critical polymer concentration of the solution required for electrospinning, but also on the diameter, crystallinity, resistance to tension, aspect ratio and morphology of the fibers [22]. Therefore, there is a need for a better understanding of the vegetable protein behavior in different solvents. It has been observed for example that aggregation of denatured proteins in aqueous-based media precluded fiber formation through electrospinning [23].

The electrospinning of vegetable proteins without the presence of easily spinnable polymers is a very complex process that is not clearly understood and therefore, the aim of this work was to investigate the effect of the most common solvents used in the electrospinning process on the conformation and rheological properties of two protein isolates from bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum*) and evaluate their performance to obtained electrospun structures.

2. Experimental section

2.1 Materials

Common bean (*Phaseolus vulgaris* L.) cultivar Bayo Madero, donated by the National Institute of Agricultural and Livestock Forestry Research (INIFAP, Mexico), was used as a raw material. Pea protein isolate (PPI) NUTRALYS® S85 with 85 % of protein content was donated from Roquette (Frères S.A., Lestren, France). Trifluoroacetic acid (TFA) was supplied by Merck, formic acid (FA), trifluoroethanol (TFE) and hexafluoroisopropanol

(HFIP) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH) and HCl was purchased from J.T.Baker®. All chemicals and solvents were reagent grade.

2.2 Methods

2.2.1 Preparation of bean protein concentrate (BPC)

Bean protein concentrate (BPC) was prepared using the method reported by [24]. Briefly, the beans were soaked in water for 16 h, the hull was manually removed, and cotyledons were dried at 40 °C for 12 hours. After this step, the beans were ground in a commercial grinder to produce flour with a particle size smaller than 20 mesh. The flour was resuspended in water (1g/ 10 ml bean/water ratio), the pH was adjusted to 8.0 with 0.1 M NaOH and the protein extraction was carried out at 35 °C under stirring for 1 h. The mixture was centrifuged at 5000 g for 15 minutes at 4 °C. The precipitate was re-extracted under identical conditions in order to maximize the yield and both extracts were combined. Subsequently, the pH was adjusted to 4.3 with HCl to precipitate the proteins, followed by centrifugation at 10000 g for 20 min at 4 °C. The supernatant was discarded and the pellet was lyophilized. Finally, the BPC was stored at -20 °C until further use.

2.2.2 Characterization of BPC

The protein content and SDS-PAGE electrophoresis under reducing and non-reducing conditions were conducted according to the method reported by Aguilar-Vazquez *et al.*, [19].

2.3 Preparation and characterization of protein solutions

Solutions containing 10 wt./v % of PPI and BPC in the solvents: deionized water, HFIP, TFE, TFA and AF were prepared and maintained under magnetic agitation during 24 h.

2.3.1 Rheological behavior

The rheological behavior of the solutions was studied using a Rheometric Scientific ARES apparatus equipped with a Couette accessory that consists of two concentric tubes with the following dimensions: 23 mm (inner diameter), 25 mm (outer diameter) and 20 mm height. This geometry allows the use of 5 ml of solution. The shear stress (τ) was measured as a function of the shear rate from 0 to 180 s⁻¹. All measurements were made in duplicate at 25

° C. The apparent viscosity (η) was calculated following the Ostwald de Waele model, also known as power law model (see Eq. 1), which was applied to determine the consistency index (k) and the flow behavior index (n). Apparent viscosities were determined at 100 s⁻¹.

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

2.3.2 Circular dichroism

Circular dichroism (CD) spectroscopy was used to provide information on the secondary structure of the proteins. The protein powders were dissolved in water, HFIP, TFE, TFA and AF at a concentration of 0.4 mg/mL and 1.2 mg/ml for the measurements in the UV-far region (205-250 nm) and in the UV-near region (250-320 nm), respectively. Circular dichroism spectra were obtained in a Bio-Logic Science Instruments MOS-500 spectrophotometer at 25 ° C using a 1 cm long quartz cell. The spectra were corrected for baseline by subtraction of specific spectra of protein-free samples. The data points for the spectra were recorded with a bandwidth of 1 nm.

2.4 Electrospinning

The experimental set-up used for conducting electrospinning included a high-voltage power supply (Acopian P030HPI-230, USA), and a digitally controlled syringe pump (KDS 100, KD Scientific). During electrospinning, a positive high voltage was applied at the tip of a syringe needle (inner diameter of 0.9 mm). The samples were collected on an electrically grounded metal plate. The effect of the process variables on fiber formation was evaluated by a 2³ experimental design, being the factors studied: voltage (15 and 22 kV), flow rate of the polymeric solution (0.2 and 0.4 mL/h), and tip-to-collector distance (10 and 15 cm). The electrospinning process was conducted under a relative humidity < 50% and a temperature ≤ 25 °C. All experiments were made in triplicate.

2.5 Characterization of electrospun fibers

2.5.1 Field emission scanning electron microscopy (FESEM)

The morphology and surface characteristics of the electrospun samples were analyzed using a JSM-7610F apparatus to carry out FESEM (Field Emission Scanning Electron Microscopy). The samples were collected in gold-coated silicon wafers and dried at 40 °C for 4 hours. Then, the wafers with the samples were coated with gold again.

2.5.2 Attenuated total reflectance infrared (ATR-FTIR) spectroscopy

The samples resulting from the electrospinning process were dried at 40 °C for 4 hours and characterized by Fourier transform infrared (FTIR) spectra using a spectrometer equipped with an ATR accessory with a diamond tip (ATR-FTIR Perkin Elmer Spectrum 100). Scans were taken over a spectral range of 4000–600 cm⁻¹ with 4 cm⁻¹ wavenumber resolution and an average of 16 scans. Data transformation, deconvolution and peak-separation analysis of the amide I band (1700 cm⁻¹ to 1600 cm⁻¹) were processed using the OriginPro8.5 software (OriginLab Corporation, Northampton, MA01060, USA).

3. Results and discussion

3.1 Protein content and molecular weight

The protein content of BPC was 71 ± 3% and Fig. 1 shows the electrophoretic profile of the BPC under non-reducing (lane 2) and reducing (lane 3) conditions. The gel showed the presence of 9 bands with molecular weights ranging from 12 to 97 kDa, corresponding to lipxygenase (97 kDa), phaseolin (43, 53 kDa), arcelin-4 (29 kDa), trypsin inhibitor (20 kDa) and α amylase inhibitor (18 kDa) [25]. As reported, the electrophoretic profile of BPC under reducing and non reducing conditions was very similar [26, 27]. For PPI a protein content of 84 ± 3 % with the presence of lipxygenase (97 kDa), conviciline (70 kDa), legumin (50 kDa), α-legumin (38-40 kDa), β-legumin (19-22 kDa), and viciline (33 kDa) has been reported [19]. The BPC and PPI protein content values are in agreement with reported values and it is known that the remaining percentage amounts are fiber and starch [6].

Figure 1. SDS-PAGE profiles of bean protein concentrate (BPC) under non reducing (lane 2) and reducing (lane 3) conditions. Lane 1 represents molecular weight marker.

3.2 Protein solutions

As it was previously mentioned, the solvent plays a vital role in electrostatic processes since the solution properties (polymer conformation, concentration, viscosity and evaporation rate) strongly affect fiber formation. Generally, homogeneous solutions and completely dissolved materials are necessary for stable electrospinning [1,28]. The solubility of the proteins was determined visually. If after the time of agitation there was phase separation or presence of particles, it was considered that the protein was not soluble in the solvent. Table 1 shows the physical properties of the studied solvents. BPC and PPI at 10 wt./v % were dissolved in water, HFIP, TFE, TFA and FA, which are classified as polar protic solvents that have a strong ionizing power and produce the cleavage of protein intramolecular interactions [29]. Acetic acid was also tested but phase separation was observed and therefore, this solvent was discarded for subsequent studies.

Table 1. Physical properties of the solvents [30].

Solvent	MW	Boiling point (°C)	ϵ at 20°C	Dipole	Electrical conductivity at 25 °C (S/m)	Surface tension at 20 °C (mN/m)	Viscosity at 25 °C (mPa.s)	Vapor pressure atm. at 25°C
Acetic acid	60	118	6.2	1.7	6×10^{-7}	24.7	1.13	0.020
Formic acid	46	100.8	58.5	1.41	6.4	37.67	1.78 (20 °C)	0.056
Water	18	100	79.7	1.87	5.5×10^{-6}	72.75	0.89	0.031
HFIP	168	58.2		2.05	-----	16.4	1.021	0.158
TFE	100	78	8.55	2.52	-----	21.11	1.73	0.068
TFA	114.023	72.4	8.55	2.26	-----	-----	0.93	0.128

3.3 Protein Conformation

3.3.1 Far and near UV

Circular dichroism (CD) was used to study the secondary structure (α -helices, β -sheets, turns and random coil) of protein isolates when dissolved in the studied solvents. CD is an absorption spectroscopy technique based on the change of molecular electronic configuration from a ground state to an excited state due to the absorption of polarized electromagnetic radiation. In this way, proteins show characteristic absorptions corresponding to the peptide bond (absorptions at wavelength smaller than 240 nm), side chains of aromatic amino acids (absorption in the range of 260 to 320 nm) and disulfide bonds (weak broad absorption bands centered around 260 nm) [31]. Fig. 2a shows the far UV CD spectra of PPI dissolved in water, HFIP and TFE. The CD spectra of PPI solutions in TFA and FA could not be obtained due to solvent interference [32]. The PPI aqueous solution shows a prominent negative peak at 230nm with a broad shoulder extending from 235 to 260 nm. These results suggest that β -type turns are predominant in PPI [33]. A similar CD spectrum was obtained when PPI was dissolved in HFIP but the magnitude of the corresponding signals was small, indicating that HFIP induces unfolding in the PPI; thus reflecting an increased conformational flexibility [37]. When using TFE as a solvent on the other hand, two prominent negative bands at 219 and 233nm were observed; suggesting that β sheets dominate the conformation of PPI.

For the aqueous solution of BPC (Fig. 2b) a negative peak at 236 nm and a broad shoulder extending from 238 to 262 nm were observed. As in the case of PPI, β -type turns are the dominant conformation. A similar CD spectrum was obtained when BPC was dissolved in TFE, with a smaller magnitude of the corresponding peaks, suggesting higher conformational flexibility than that of BPC dissolved in water. When HFIP was used, BPC had a negative peak located at 224 nm, suggesting once again that β -sheet is the predominant conformation. It is important to note that there are few studies on CD of protein isolates, as most of the existing ones focus on the study of pure proteins. The CD results of the protein isolates of this work, show the overall behavior of the protein mixture conformation. CD studies of phaseolin for instance, the major protein in BPC, demonstrated that when it is dissolved in aqueous solution at pH = 7, highly ordered structures including α -helices and β turns are dominant [34].

Figure 2. Circular dichroism spectra of the protein isolate solutions. Far UV: a) PPI dissolved in water, HPFI and TFE, b) BPC dissolved in water, HPFI and TFE. Near UV: c) PPI-water, d) PPI-HPFI and e) PPI-TFE, f) BPC- water, g) BPC- HPFI and h) BPC-TFE.

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236 The tertiary conformations of the protein isolates were analyzed by UV-near CD. The
237 corresponding spectra of PPI and BPC in water showed great similarity (Fig. 2c,f). The
238 presence of small shoulders located at 268 and 270 nm, can be attributed to residues of
239 phenylalanine (Phe), and the small shoulder observed at 298 nm can be attributed to
240 tryptophan (Trp). The small intensity of the bands on the other hand, suggests that the
241 aromatic amino acids are not exposed because the proteins of PPI and BPC are essentially
242 folded.

243 Figures 2d and 2e show the CD spectra of PPI dissolved in HFIP and TFE. For HFIP, positive
244 peaks attributed to Phe can be observed at 255 nm. When TFE was used, the PPI CD spectrum
245 consisted of a positive peak at 258 nm, followed by negative peak in the Tyrosine (Tyr)
246 region (285 nm) and the presence of a shoulder at 305 nm corresponding to Trp.

247 Figures 2g and 2h show the CD spectra of BPC dissolved in HFIP and TFE, respectively.
248 BPC in HFIP showed a positive peak at 262 nm corresponding to Phe, and two negative
249 peaks in the region corresponding to Tyr and Trp. In the case of BPC dissolved in TFE, the
250 spectra showed a negative peak at 257 nm corresponding to Phe and the presence of a
251 shoulder in the region of 295 to 303 nm corresponding to Trp.

252 Therefore, CD results indicate that when both, PPI and BPC, are dissolved in HFIP and TFE,
253 the acid environment promotes the unfolding of the proteins. In addition, it is important to
254 point out that there are substantial differences in the arrangement, proximity and exposure of
255 aromatic amino acids between PPI and BPC proteins.

256

257 *3.4 Rheological behavior*

258 The rheological behavior of the solutions was evaluated in order to understand its effect on
259 the electrospinning process. Fig. 3 shows the flow curves of the protein solutions. These
260 curves represent the effect of the shear rate ($\dot{\gamma}$) on the value of the shear stress (τ), which
261 reflects a pseudoplastic behavior that was observed for all solutions. Solutions of PPI
262 dissolved in water, TFE and FA presented higher values of τ than the corresponding BPC
263 solutions; while in HFIP an opposite behavior was observed. This result is in agreement with
264 the major conformational change observed in DC for BPC when dissolved in HFIP.

Figure 3. Flow curves of PPI and BPC solutions: a) Water, b) HFIP, c) TFE and d) FA.

The rheological data were fitted to the Ostwald de Waele model (Eq. 1). Table 2 shows the apparent viscosity values (η) at a shear rate of $100 s^{-1}$, consistency (k) and flow (n) indexes, being the values of the correlation coefficient around 0.99.

For PPI and BPC dissolved in water, TFE and FA, the PPI solutions exhibited higher η than the BPC solutions. When HFIP was used, the highest viscosity values were observed and BPC showed higher η value than PPI (16.31 and 28.78 Pa.s, respectively). High viscosity of the polymer solutions correlates with strong intermolecular interactions between polymer chains, and then HFIP promotes higher BPC protein chains intermolecular interactions than PPI, thus it is expected that HFIP favors the electrospinning process.

The k values on the other hand, describe the reduction of the viscosity with the increase in the shear rate. The aqueous solution of PPI and BPC presented low viscosity at the different shear rates, while the HFIP solutions of PPI and BPC presented a greater reduction of viscosity with the increase in shear rate; thus indicating that there are less interactions between adjacent protein chains during the flow stage.

The value of n is an indicator of the degree of deviation from Newtonian fluid behavior ($n=1$), as n is closer to a value of 1 when the solutions are less pseudo-plastic. The electrostatics and fluid viscoelastic rheology of the polymeric solution govern the stretching of the electrically driven polymeric jet in the electrospinning process; thus influencing the morphology of the electrospun structures. For both, Newtonian and non Newtonian polymeric solutions, a critical concentration of polymer is needed to obtain fibers. Several examples of protein pseudo-plastic solutions have been reported to produce fibers from the electrospinning process, like 1.6 wt. % chitosan-PEO in acetic acid [35], and 91:9, 77:23, 66:34 wt. % cellulose acetate-egg albumin blend in acetic acid/formic acid [36].

Table 2. Apparent viscosity at a shear rate of 100 s^{-1} , power law flow behavior (n) and consistency (K) indexes of PPI and BPC solutions.

Sample	Solvent	η (Pa.s) ($\dot{\gamma} = 100 \text{ s}^{-1}$)	K (Pa.s)	N	R ²
PPI	Water	1.68 ± 0.00^A	0.05 ± 0.00	0.75 ± 0.00	0.99
BPC		$0.43 \pm 0.01^{a*}$	0.15 ± 0.00	0.24 ± 0.01	0.98
PPI	HFIP	16.31 ± 0.00^B	1.44 ± 0.01	0.53 ± 0.00	0.99
BPC		$28.78 \pm 0.00^{b*}$	0.88 ± 0.00	0.76 ± 0.00	0.99
PPI	TFE	5.75 ± 0.00^C	0.34 ± 0.00	0.61 ± 0.00	0.99
BPC		$3.41 \pm 0.00^{c*}$	0.36 ± 0.00	0.49 ± 0.00	0.99
PPI	FA	11.63 ± 0.02^D	1.05 ± 0.02	0.52 ± 0.00	0.99
BPC		$1.15 \pm 0.03^{d*}$	0.02 ± 0.00	0.88 ± 0.01	0.99

The results are expressed as the mean $\pm$ E.E of two independent experiments. Different uppercase and lowercase letters indicate significant difference ($\alpha < 0.05$) in the viscosity value (η) in the

different solvents. * indicates significant difference ($\alpha < 0.05$) between PPI and BPC using the Tukey test.

3.5 Electrospinning process and morphology

Table 3 shows the results of the experimental design used to study the effect of the process variables on fiber formation. Several experiments were selected as representative samples. As expected, the solvent has a major impact on fiber formation, while the different process variables considered in the study are not determinant. The development of electrospun structures from the aqueous solutions was not possible as only drops were collected; the result is consistent with the low viscosity of the protein aqueous solutions, as well as the folded protein conformation. HFIP solutions on the other hand, gave rise to few PPI beaded fibers (Fig. 4a) and BPC fibers (Fig. 4b). Considering that HFIP is the tested solvent with the highest vapor pressure, that protein isolates solutions exhibited the highest viscosity, and that the dissolved protein isolates exhibited conformational flexibility, it was expected that HFIP solutions afforded electrospun fibers [37]. HFIP has been considered as an ideal solvent for electrospun proteins; however, for the electrospinning of legume protein isolates, the use of a carrier polymer to generate fibers has been found to be necessary [16, 46]. TFE is an organic solvent that has been used to electrospun globular proteins [39,40], here the electrospinning process yielded mostly beads (Fig. 4 c-d) which can be attributed to the conformation of β sheets in PPI and β turns in BPC, which were observed in CD results and in the pseudo-plastic behavior for both PPI and BPC.

Figure 4. SEM images of electrospun samples a) PPI HFIP (0.4 mL/h, 15 kV and 15 cm of distance), b) BPC HFIP (0.4 mL/h, 15 kV, 10 cm), c) PPI TFE (0.4 mL/h, 15 kV, 15 cm), d) BPC TFE (0.4 mL/h, 15 kV, 10 cm), e) PPI TFA (0.4 mL/h, 22 kV, 10 cm), f) BPC TFA (0.4 mL/h, 15 kV, 15 cm), g) PPI FA (0.2 mL/h, 22 kV, 10 cm) and h) BPC FA (0.4 mL/h, 22 kV, 15 cm).

326 **Table 3.** Results of electrospinning process.

Experiment	Flow rate (mL/h)	Distance (cm)	Voltage (kV)	HFIP		TFE		TFA		FA	
				PPI	BPC	PPI	BPC	PPI	BPC	PPI	BPC
1	0.2	10	15	B+F	B	B	B	B+F	B	B	B
2	0.2	10	22	B+SF	B	B	B	B	B+F	B	B+F
3	0.4	10	15	B+SF	F	B+D	B	B	B	B+F	B
4	0.4	10	22	B+SF	B+SF	B+D	B+D	B	B+F	B	B+BF
b	0.2	15	15	B+F	B	B	B	B	B	D	B+D
6	0.2	15	22	B+SF	B+F	B+F	B	B+F	BF+D	D	B+F
7	0.4	15	15	BF	B	B	B	B	B+F	D	B+F
8	0.4	15	22	B+SF	B+SF	B	B	B	B	D	B+F

327 B: beads, BF: beaded fibers, D: drops, F: fibers, SF: short fibers.

TFA is a good hydrogen donor solvent, which increases hydrogen bonding between proteins and peptides in solution. In fact, it has been reported that this solvent allows protonation of proteins and, thus, COOH groups form strong hydrogen bonds with the solvent allowing the interruption of intramolecular interactions [37], then the unfolding of the protein will promote electrospun fiber formation. Here, the electrospinning of PPI and BPC solutions mostly produced beads and beads mixed with few short fibers, respectively.

When FA was used, most of PPI solution resulted in drops (Fig. 4g) but BPC produced beads with fibers (Fig. 4h). Results of BPC in FA were similar to the results reported for amaranth protein isolates dissolved in FA [50].

In general, BPC solutions exhibited better performance to obtain electrospun structures than PPI solutions. HFIP and TFE solutions promoted fibers and beads formation, respectively; while TFA and FA for BPC solutions afforded beads with short fibers.

3.3 Characterization of electrospun samples

3.3.1 Attenuated total reflectance infrared spectroscopy (ATR-FTIR)

Fig. 5 A and B show the infrared spectra of the electrospun samples of PPI and BPC, in the five solvents under study. It is important to mention that for water, the drops that reach the collector were dried to obtain the IR spectra. The powders of PPI and BPC presented characteristic bands corresponding to the amide I (stretching of the C=O) and amide II (stretching of C—N and bending of N—H) located at 1633 and 1523 cm⁻¹ for PPI and 1635 and 1521 cm⁻¹ for BPC. For the electrospun samples, except for the sample where water was used, the amide I band was shifted to higher frequencies, being the highest displacement for the protein samples dissolved in TFA and FA (1650 and 1649 cm⁻¹, respectively). The amide band II was shifted at higher frequencies in all electrospun samples, moreover PPI samples showed higher shifts than BPC samples. The shifts of amide I band to higher frequencies for electrosprayed structures have been attributed to an increase in α -helical unfolded protein structures [41]. CD results evidenced that HFIP and TFE induces unfolding on the studied protein isolates; an interpretation that is in agreement with the observed displacements.

Figure 5. A) Spectra PPI: B) Spectra BPC: a) BPC powder, b) BPC-HFIP, c) BPC-TFE, d) BPC-Water, e) BPC-TFA, f) BPC-FA.

In this way, Amide I band is used to study protein secondary structure contribution, Table 4 and 5 shows the % of secondary conformations obtained from the deconvolution analysis of the Amide I band. When PPI was dissolved in water, a slight increase of β -turns and β -sheets and a decrease of α -helix, are observed. For BPC, opposite results were observed. Globular proteins mainly compose both protein concentrates but when dissolved in water, PPI structures are more compact than BPC structures. In general, compared with the protein isolates aqueous solutions, all the tested solvents decrease β -sheets %; thus conferring flexibility to the protein backbone.

The characteristic band corresponding to amide III was only observed in the samples where TFE and water were used as solvents. In general, the electrospun samples spectra were similar to those corresponding to PPI and BPC powdered samples. Residual solvent remains in the electrospun structures (see arrows Fig 5) and then, a treatment to remove the solvent is required.

Table 4. Deconvolution of amide I band IR absorption for PPI electrospun material.

Sample	% β -turn	% α -helix	% Random coil	% β -sheet
	1688 -1659 cm ⁻¹	1652-1651 cm ⁻¹	1645-1640 cm ⁻¹	1634-1615 cm ⁻¹
PPI	37.99	3.81	19.02	39.18
PPI-HFIP	38.96	1.64	21.87	37.53
PPI-TFE	43.02	1.90	28.07	27.01
PPI-WATER	40.25	1.60	15.40	42.75
PPI-TFA	36.54	6.57	24.21	32.68
PPI-AF	32.98	13.07	23.58	30.36

Table 5. Deconvolution of amide I band IR absorption for BPC electrospun material

Sample	% β -turn	% α -helix	% Random coil	% β -sheet
	1659 -1688	1651-1652	1640-1645	1634-1615
	cm ⁻¹	cm ⁻¹	cm ⁻¹	cm ⁻¹
BPC	38.06	1.56	16.06	44.33
BPC-HFIP	37.39	5.19	20.30	37.12
BPC-TFE	34.62	8.14	26.88	30.36
BPC-Water	30.82	6.67	20.88	41.63
BPC-TFA	35.90	12.17	23.42	28.50
BPC-AF	32.95	13.07	23.56	30.42

Conclusions

The conformation of the proteins and the viscosity of the solutions were affected by the solvents properties. Although the evaluated solvents denature in some grade the protein isolates, this effect was not sufficient to electrospun fibers; viscosity of the solution as well as the vapor pressure of the solvents, also played an important role in determining the morphology. While for PPI the poorest solvent to produce any electrospun structure was formic acid, for BPC, HFIP resulted in continuous fibers with some beads. Circular dichroism spectroscopy and FTIR results on the other hand, indicate that BPC presented more flexibility than PPI, which partially explains BPC performance for the production of electrospun structures. It is noteworthy that BPC electrospun structures were obtained for the first time. Since HFIP, TFA, and TFE are toxic solvents that remained in the electrospun structures, heat procedures are needed. BPC structures generated from formic acid solutions on the other hand, are the most convenient and could be improved by adding a surfactant to give rise to food and pharmaceutical applications.

Declarations of interest

None.

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