**Electrospun β -carotene-loaded SPI:PVA fiber mats produced by emulsion-
electrospinning as bioactive coatings for food packaging**

Graziella Pinheiro Bruni^{a,b}, Jean Paulo de Oliveira^{a,b}, Laura G. Gómez-Mascaraque^b, Maria
José Fabra^b, Vilásia Guimarães Martins^c, Elessandra da Rosa Zavareze^a, Amparo López-
Rubio^{b*}

^a Department of Agroindustrial Science and Technology, Federal University of Pelotas,
96010-900, Pelotas, Brazil.

^b Food Safety and Preservation Department, IATA-CSIC, Avda. Agustin Escardino 7, 46980
Paterna, Valencia, Spain.

^c Laboratory of Food Technology, School of Chemistry and Food, Federal University of Rio
Grande, Av. Italia, km 8, CEP 96203-900, Rio Grande, RS, Brazil.

*Corresponding author: Amparo Lopez-Rubio

E-mail: amparo.lopez@iata.csic.es

Abstract

In this work, emulsion electrospinning was used to develop an active internal coating for food packaging applications. Specifically, the antioxidant molecule β -carotene, was encapsulated in a mixture of soy protein isolate (SPI) and polyvinyl alcohol (PVA) which was directly electrospun onto a polyhydroxybutyrate-co-valerate (PHB92/PHV8) film. An annealing treatment was applied to improve the adhesion of the electrospun mat onto the packaging film, which contributed to modulate the release of the active compound. Stable SPI:PVA emulsions (with a 50:50 ratio) were developed using soybean oil (SBO) as carrier of the hydrophobic β -carotene. The encapsulation efficiency of β -carotene in the electrospun SPI:PVA fibers was $65.0\% \pm 2.6\%$, being $51.4\% \pm 0.9\%$ effectively incorporated into their cores. Finally, the in-vitro release assay of the antioxidant in soybean oil, which simulates fatty foods, demonstrated that the heat treatment (annealing) contributed to a slower and more sustained released of the bioactive compound.

Keywords: Soy protein isolate; Polyvinyl alcohol; Active food packaging; polyhydroxyalkanoates; electrohydrodynamic processing

1. Introduction

There is increased interest in the development of active antioxidant packaging materials for improving the quality and safety of food products. It is known that the direct application of antioxidant compounds onto the food surface can be inefficient because of their rapid diffusion within the bulk of food. In this regard, the incorporation of antioxidants into packaging structures could improve their activity, maintaining an optimal effect during food storage due to the sustained release of the antioxidant molecules (Atay et al., 2018; Desai and Park, 2005).

Furthermore, in line with the current environmental concerns related to the massive use of plastics, the use of biopolymers for food packaging applications is gaining attention. Among them, polyhydroxyalkanoates (PHAs) are synthesized by a wide range of microorganisms which have recently attracted much attention since they can be produced in large scale, having interesting thermal, barrier and mechanical properties for food packaging applications. However, the high temperatures involved during industrial processing of these biopolymers (like melt blending or extrusion processes) prevent the direct incorporation of the antioxidant molecules within the packaging structures, as these bioactive compounds are normally very temperature sensitive. As a strategy for antioxidant incorporation within the packaging structure, the use of electrohydrodynamic processing to develop an inner packaging coating is an interesting approach. Several researchers have recently reported the potential of the electrospinning technique to produce biodegradable multilayer structures showing that, the use of this processing method was crucial to attain a good adhesion between the different materials used in the multilayer structures like hydrophilic natural biopolymers (i.e. proteins) and hydrophobic biopolyester layers (such as polyhydroxyalkanoates –PHAs) (Alehosseini et al., 2019; Tampau et al., 2018; Deng et al., 2018; Fabra et al., 2014). In this way, electrospinning has also been used for the

incorporation of active fiber-base mats as coating of the packaging material, leading to bioactive layers (Fabra et al., 2014). However, little information is available about how the modification of the structure of the fibers can impact bioactive release.

An interesting group of bioactive compounds of relevance for food applications is carotenoids. Carotenoids are a group of lipophilic compounds that provide several benefits to human health and contribute to the yellow -red color in many foods. In particular, β -carotene provides protection to the human body by reducing the risk of some types of cancer as well as heart disease (Rao and Rao, 2007), and it also presents an important role as an antioxidant in food products (Hou et al., 2012). Endogenous carotenoids in food are generally stable. However, their application as food additives is limited due to their low solubility in water and instability in the presence of heat, light, oxygen and they are prone to chemical degradation during food processing and storage (Xianquan et al., 2005).

Therefore, in an effort to reduce the losses in antioxidant activity during the development of the packaging material, incorporation of the carotenoids within the electrospun fiber structures is a feasible route to stabilize and control their release from the bioactive packaging structures (Fabra et al., 2014).

Due to the lipophilic nature of β -carotene, the emulsion electrospinning technique is here proposed as a feasible route to encapsulate the carotenoid in hydrophilic fiber-forming materials (Babitha et al., 2017; Gómez-Mascaraque et al., 2017). This technique is based on the electrohydrodynamic processing of stable biopolymeric emulsions. Specifically, the feed emulsion is subjected to a high voltage electric field, producing a charged biopolymer jet which is ejected from a conductive capillary through which it is pumped, to a grounded collector. In this case, β -carotene was microencapsulated through emulsion electrospinning and, for this purpose, proteins are particularly interesting matrices for emulsion electrospinning, since their amphiphilic nature allow their use as emulsifiers. Furthermore,

soy bean oil (SBO) was used as a carrier oil to dissolve the hydrophobic antioxidant compound and oil-in-water (O/W) emulsions were prepared using a high-speed homogenization process and a sonication process.

Soy protein isolate (SPI) is mainly composed of proteins isolated from soybean and it is widely used in the food industry for its functional properties (Silva et al., 2018). SPI is here proposed as an excellent carrier material due to the well-known emulsifying and stabilizing properties when O/W emulsions are formed (McClements and Li, 2010). However, SPI is a globular protein with low solubility in organic solvents and poor mechanical properties, which complicates fiber formation through electrospinning (Cho et al., 2012). An option to develop encapsulation structures using SPI is to blend this protein-rich ingredient with a fiber forming biopolymer, such as polyvinyl alcohol (PVA), which is a biodegradable polymer widely used in the field of biomedicine (Cho et al., 2012).

Previous literature works have reported on the development of hybrid SPI:PVA electrospon fibers (Cho et al., 2012; Fang et al., 2016; Zhang et al., 1999). However, the ability of these fibers for β -carotene encapsulation and the use of these fibers as packaging coatings has not been explored. Moreover, the potential modification of the crystalline structure of the electrospun fibers through annealing (heating between the glass transition temperature and the melting temperature of the fibers) and its effect on bioactive release was also attempted in this work. Thus, the aim of this work was to develop bioactive coatings for food packaging applications consisting of SPI and PVA blends containing β -carotene and modifying the bioactive release properties through annealing the electrospun fibers.

2. Materials and methods

2.1 Materials

Soy protein isolate (SPI) was kindly donated by The Solae Company (Switzerland) and polyvinyl alcohol (PVA) was purchased from *Plásticos Hidrosolubles S.L.* (Valencia, Spain). Dimethylformamide (DMF), sodium hydroxide (NaOH), hydrochloric acid (HCl), soybean oil (SBO), Triton™ x-100 and β -carotene were obtained from Sigma-Aldrich (Madrid, Spain). The plastic packaging film of the copolymer polyhydroxybutyrate/polyhydroxyvalerate with 8% valerate content (PHB92/PHV8, thickness of 0.025 mm) was obtained from Goodfellow Cambridge Limited (Huntingdon, England).

2.2 Preparation of SPI and SPI:PVA aqueous dispersions

Initially, the best conditions for SPI electrospinning were evaluated. Aqueous solutions containing 11% (w/v) of the soy protein ingredient were prepared at room temperature (20 ± 2 °C) by dispersing the required amount of the SPI powders into distilled water. One of the solutions was used as prepared (control one) and the other three SPI dispersions were denatured by using different procedures: i) 30 min at 100 °C (Pérez-Masiá et al., 2014), ii) pH 11.0, using a 2 M NaOH solution (Cho et al., 2012) and iii) pH 2.0, using a solution of 1M HCl and, subsequently, heating the SPI dispersion at 80 °C for 40 min (Romani et al., 2018). pH 2 was selected based on previous experiments carried out in a wide range of pH where the denaturation of the protein was confirmed by DSC.

Later on, the processed solution with the best performing behavior, i.e. the one prepared at pH 2.0, was mixed with an aqueous PVA solution (also prepared at 11% w/v in distilled water at 95°C until completely dissolved) at different SPI:PVA ratios (25:75, 50:50, 60:40 w:w). A nonionic surfactant, Triton™ x-100, was added to the different solutions at 0.5% w/v in order to reduce the surface tension of the formulations, facilitating their subsequent processing through electrospinning.

2.3 Preparation of β -carotene-SPI:PVA emulsions

In order to prepare the O/W emulsions, β -carotene was first dissolved in SBO under magnetic stirring by heating at 90°C for 5 min (Gómez-Mascaraque et al., 2017) and then mixed, under magnetic stirring for 30 °C at room temperature, with the SPI:PVA dispersions previously prepared at pH 2 which presented better electrospinnability (the selected SPI:PVA ratio was 50:50 ratio). Three different homogenization procedures were evaluated for preparing the β -carotene-containing emulsions: I) Homogenization at 11000 rpm during 5 min with an IKA T-25 Digital ULTRA-TURRAX® equipped with a S 25N– 25F dispersing element with a stator diameter of 25 mm (IKA, Germany); II) Ultrasound homogenization at a power of 80 W for 2 min at a frequency of 24 kHz in pulse mode (50% active cycle) using an UP-400S ultrasound equipment (Hielcher GmbH, Germany). An ice bath was used in this case to avoid excessive heating of the sample; and III) A combination of the two previous methods, i.e. the dispersions were first homogenized using the procedure I (high-speed homogenization –HSH) and subsequently, the ultrasound (US) treatment described in II was applied.

The final concentration of SBO that provided stable emulsions was 5 % (w/w) and the amount of β -carotene in the final solutions was 5% (w/w) with respect to the mass of both biopolymers.

2.4 Characterization of the feed formulations

The electrical conductivity of the solutions was measured using a conductivity meter XS Con6 (Labbox, Barcelona, Spain) at room temperature ($22 \pm 2^\circ\text{C}$). The surface tension was measured using the Wilhemy plate method in a tensiometer (EasyDyne K20, Krüss GmbH, Germany). The rheological behavior of the SPI-based solutions was studied using a rheometer (AR-G2 TA Instruments, USA), with a parallel plate geometry following the

method described by Gómez-Mascaraque et al., 2015. Briefly, the shear stress (σ) was measured as a function of the shear rate (γ) from 0 to 200 s⁻¹ during 15 min at 25 °C using a stainless steel plate with a diameter of 60 mm and a gap of 0.5 mm. The power law model (Equation 1) was applied to determine the consistency index (k) and flow behavior index (n). Apparent viscosities were determined at 100 s⁻¹. All measurements were made at least in triplicate.

$$\sigma = k \cdot \gamma^n \quad \text{Eq. (1)}$$

The gravitational stability of the β -carotene-SPI:PVA emulsions was analyzed by the creaming index method (Surh et al., 2006). To this end, emulsions were introduced in sealed tubes and stored for different times (0, 5 and 24 h) at room temperature (22 $\pm$ 2 °C). When the phase separation occurred, the height of the cream layer (H_c, phase rich in oil) and the total height of each emulsion in the tube (H_e) were measured, and the creaming index (CI) was calculated according to Equation 2.

$$CI = 100 \times \frac{(H_e - H_c)}{H_e} \quad \text{Eq. (2)}$$

The emulsions were also visualized from microscopic images using a digital microscopy system (Nikon Eclipse 90i) fitted with a 12 V, 100 W halogen lamp. Digital images were taken using an Eclipse 90i Nikon microscope (Nikon corporation, Japan) equipped with 5-megapixels cooled digital color microphotography camera Nikon Digital Sight DS-5Mc. Acquired images were processed by using Nis-Elements Br 3.2 Software (Nikon corporation, Japan).

2.5 Electrohydrodynamic processing of the solutions

The different solutions were processed using the electrospinning technique at room temperature. The electrospinning apparatus, equipped with a variable high-voltage 0-30 kV power supply, was supplied by Spraybase® (Dublin, Ireland). The solutions were electrospun under a steady flow-rate using a stainless-steel needle placed towards a metallic plate used as collector. The needle was connected through a polytetrafluoroethylene wire to the polymer solution kept in a 5 mL plastic syringe, which was disposed vertically lying on a digitally controlled syringe pump. The electrospinning conditions were the following: 0.15 mL/h flow-rate, 15 kV voltage and 17 cm tip-to collector distance for SPI and SPI:PVA dispersions.

The β -carotene-containing SPI:PVA emulsions were either electrospun onto the collector or directly onto the PHB/PHBV film attached to the collector. The emulsion-electrospinning conditions were 0.15 mL/h flow-rate, 18 kV voltage and 20 cm tip-to collector distance with a deposition time of 5 hours. In this case, an additional annealing step was applied in some of the electrospun fibers, placing them between two Teflon layers and compressed in a hot press (Carver 4122, USA) at a temperature below the melting temperature of PVA for 1 min (without pressing) to promote PVA crystallinity formation.

2.6 Morphological characterization of the materials

The morphology of the isolated electrospun fibres and those coated onto the PHB/PHBV films was analyzed using a scanning electron microscope (SEM, Hitachi S-4800). An accelerating voltage of 10 kV and a working distance of 8-10 mm were used. The samples were previously sputter-coated with gold-palladium mixture under vacuum prior to examination.

2.7 Fourier transform infrared (FT-IR) analysis of the microstructures

Electrospun samples, unloaded and loaded with the β -carotene, were characterized using a spectrometer FTIR (Rheinstetten, Germany), equipped with an attenuated total reflection (ATR) accessory. Samples were placed on the ATR crystal for analysis at room temperature (22 ± 2 °C) and the scans were performed in the spectral range of 4000-500 cm^{-1} .

2.8 Thermal stability of the fibers

The thermal stability of fibers and the pure compounds (SPI, PVA, and β -carotene) was evaluated using a thermogravimetric analyzer (TA Instruments model Q500 TGA). The samples (approximately 8 mg) were heated in platinum capsules in the range of 30 to 600 °C, with a heating rate of $10\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ under dynamic air atmosphere.

2.9 Structural integrity of the electrospun fibers in water

The structural integrity of the electrospun fibers in aqueous media was also evaluated. To this end, 10 mg of the electrospun fibers were placed in vials containing 10 mL of distilled water. The structural integrity of fibers was visually evaluated in the period of 0 and 24 h.

2.10 Encapsulation efficiency

The encapsulation efficiency (MEE) of the samples loaded with β -carotene was performed according to Gómez-Mascaraque et al. (2017), with some modifications. The fibers (30 mg) were dissolved in a mixture of 20 mL of water: DMF (1:1, v/v) and centrifuged (4,000 rpm) to obtain aliquots of the supernatant for further UV-vis absorbance measurements at 418 nm. A calibration curve was obtained ($R^2 = 0.9981$) for β -carotene in the solvent mixture. The β -carotene content in the microstructures was interpolated from the linear calibration equation obtained. The MEE of the β -carotene loaded microstructures was calculated using Equation 3.

$$MEE (\%) = \frac{\text{Actual } \beta\text{-carotene content in the microstructures}}{\text{Theoretical } \beta\text{-carotene content in the microstructures}} \times 100 \quad \text{Eq (3)}$$

The content of β -carotene on the surface of the fibers was estimated according to Umesha et al. (2013) and Gómez-Mascaraque et al. (2017a). Briefly, 10 mg of fibers were resuspended in hexane (1 mg. mL⁻¹), vortexed for 5 s and immediately filtered using Whatman™ filter devices with 0.2 mm PTFE membranes (GE Healthcare Life Sciences, UK). The absorbance of the filtrate at 418 nm was measured as described above. A calibration curve was obtained ($R^2_{\text{hexane}} = 0.9969$) for β -carotene in hexane.

2.11 Differential scanning calorimetry (DSC)

In order to verify the denaturation of SPI, a differential scanning calorimetry (DSC) analysis was performed using a Perkin-Elmer DSC 8000 (Waltham, MA, USA). To this end, 2.5 mg of lyophilized protein solution and 0.75 μ L of 0.05 M phosphate-buffered saline (PBS) (pH 7.0) were mixed in aluminum pans. The pans were sealed and stored for 24 h for complete hydration of the sample before heating. The sample was heated from 20 to 110 °C at 5 °C/min, using an empty pan as a reference.

DSC of electrospun fibers containing β -carotene was also performed on the DSC 8000. To this end, small amounts (~5 mg) of dry materials were placed into aluminium pans and heated at scanning speed of 10 °C min⁻¹ from room temperature to 200 °C, using N₂ as the purging gas. An empty aluminium pan was used as reference and the melting endotherm was analyzed. Before evaluation, similar runs of an empty pan were subtracted from the thermograms. The DSC equipment was calibrated using indium as a standard.

2.12 In vitro release assays of β -carotene

The *in vitro* release profile of β -carotene was performed according to Costamagna et al. (2017). Soybean oil was chosen as the simulant medium of fatty foods according to the

Commission Regulation UE (2011) number 10/2011/EC. 10 mg of fibers with and without applying a post-spinning annealing process were deposited in a recipient with ~14 mL of soybean oil. The β -carotene concentration in the release medium was estimated by measuring the absorbance of the supernatant at 418 nm using a NanoDrop Spectrophotometer (ND1000, Thermo Fisher Scientific, USA) after selected time periods. The obtained data were used to determine the total amount of β -carotene released from the microstructures at each time point.

2.13 Statistical analysis

The statistical analysis of data was carried out by means of StatGraphics Plus version 5.1 (Statistical Graphics Corp.) through the analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) was used at the 95% confidence level for multiple comparison tests

3. Results and discussion

3.1 Characterization of denatured protein solutions

One of the key aspects for producing SPI electrospun fibers is the denaturation of the protein (Mendes et al., 2017). Thus, the denaturation efficiency of SPI dispersions prepared by the methodologies previously described was firstly evaluated and compared to the native SPI. Figure 1 displays the DSC thermograms of the SPI based dispersions where two endothermic peaks, at ~96 and 117 °C, respectively, were clearly observed in the native SPI (pH ~ 6.5), being ascribed to the denaturation of 7S and 11S globulins (Sousa et al., 1995) during the heating process. This fact denotes that the commercial SPI protein was not denatured in the original form, as expected. In contrast, thermograms of SPI dispersions subjected to the acid,

basic or heating processes, did not show any endothermic peaks (Figure 1), indicating an effective denaturation of the samples in the various conditions tested.

Figure 1. DSC thermograms of the native protein and denatured protein using basic conditions (pH 11), acid conditions (pH 2) or a heat treatment.

It is well-known that the electrospinnability of the biopolymer solutions strongly depends on the solution properties (viscosity, surface tension and electrical conductivity), which play an important role in determining the better conditions from which continuous fibers can be obtained. The properties of the SPI-based dispersions are compiled in Table 1, while Figure 2 show the corresponding SEM images of the obtained structures.

Figure 2. SEM images of electrospayed structures obtained from solutions of native SPI (A), SPI denatured at pH 11 (B), SPI denatured at pH 2 (C), SPI denatured using heat (D). A concentration of 11% w/v of SPI was used in all the formulations.

325 **Table 1.** Surface tension, electrical conductivity and rheological behavior of the protein solution and soybean oil/protein emulsions.

326 HSH= High-speed homogenization; US= Ultrasonication.

327 Different letters (a-d) within the same column indicate significant at $p < 0.05$ among the samples.

328

Solution	Surface tension (mN.m ⁻¹)	Electrical conductivity (mS.cm ⁻¹)	Rheological behavior	μ (Pa.s) ($0 \leq \dot{\gamma} \leq 200$ s ⁻¹)	n	K(Pa.s) ⁿ	r ²	η (Pa.s) ($\dot{\gamma} = 100$ S ⁻¹)
Protein								
native	34.20 ± 0.32c	1.40 ± 0.09c	Pseudo-plastic		0.65 ± 0.02b	0.44 ± 0.04c		0.47 ± 0.06b
heating	51.40 ± 0.74a	1.20 ± 0.03d	Pseudo-plastic		0.77 ± 0.02a	0.61 ± 0.07b		0.43 ± 0.04b
pH 11	52.90 ± 0.99a	1.54 ± 0.04c	Pseudo-plastic		0.43 ± 0.03b	3.72 ± 2.02a		4.65 ± 0.07a
pH 2	33.40 ± 1.04c	4.56 ± 0.04a	Pseudo-plastic		0.74 ± 0.01a	0.46 ± 0.05c		0.40 ± 0.07b
PVA	44.80 ± 1.00b	2.6 ± 0.0b	Newtonian	0.07 ± 0.04a			0.9964	
Emulsion								
HSH	34.60 ± 2.19a	4.10 ± 0.03a	Newtonian	0.07 ± 0.02b			0.9952	
US	34.20 ± 1.41a	4.13 ± 0.03a	Newtonian	0.08 ± 0.01a			0.9953	
HSH + US	34.30 ± 0.40a	4.02 ± 0.04a	Newtonian	0.05 ± 0.01c			0.9965	

From the results obtained, it was clearly observed that neither the surface tension nor the electrical conductivity explained the differences observed in the morphology of the structures obtained. Surface tension is a crucial parameter for the process, since high surface tension values could overcome the electrostatic forces generated by the high voltage applied and the electrical conductivity of the solution, thus, hindering the Taylor cone formation and the subsequent electrospinning process (Bhardwaj and Kundu, 2010, Fong et al., 1999, Zuo et al., 2005, Gómez-Mascaraque et al., 2017a]. It was interesting to note that while the SPI dispersions denatured by heating or using basic pH presented surface tension values ~ 50 mN.m^{-1} , both native SPI dispersions and those prepared at pH 2.0 showed much lower surface tension values (around 30 mN.m^{-1}), which would facilitate the processing of the solutions through electrospinning. In general, not significant differences were observed in the electrical conductivity except in SPI dispersion prepared at pH 2.0 which showed significantly higher values probably due to the use of HCl which dissociates in water.

As expected, a shear thinning behaviour (pseudoplastic) was observed for all SPI dispersions (Liu et al., 2017). Flow and consistency indexes, together with the apparent viscosity values at a shear rate of 100 s^{-1} are also gathered in Table 1 and the values of the correlation coefficient were in all cases around 0.99. The first clear observation was that only SPI dispersions denatured using basic pH conditions were significantly more viscous, being approximately 10 times higher, with a more pronounced pseudoplastic behaviour according to the lower n value and giving raise to agglomerated structures (Figure 2). These differences could be explained the different arrangement of protein complexes in basic and acid media as previously reported. In protein solutions at basic pH conditions, mainly hydrophobic interactions and hydrogen bonds are responsible of protein conformation, while at acid pH, the disulfide binding and noncovalent interactions play a crucial role in structure formation (Romani et al., 2018).

The observed differences in solution properties were responsible for the better spinnability of the SPI denatured in acidic conditions. In fact, the protein solutions denatured through heat and basic pH were rather unstable during the electrospinning process, with a constant solution dropping. It is interesting to note that electrospinning of aqueous biopolymer solutions is sometimes favoured when decreasing chain length as previously observed also for polysaccharides (Pérez-Masiá et al., 2014), and the acid conditions applied for denaturing SPI, could have caused a decrease in chain length because of a hydrolysing effect of the acid conditions. Therefore, for subsequent experiments, the SPI denatured at acidic conditions was selected.

3.2 Selection of SPI:PVA ratio for coating formation through electrospinning

As the objective of this study was to develop inner active packaging coatings through electrospinning, and the SPI solutions only gave rise to capsule-like morphologies, polyvinyl alcohol (PVA) as a fiber-forming biopolymer was used to favour coating formation. Initially, different SPI: PVA ratios were evaluated aiming at incorporating the maximum amount of SPI in the fibers. Figure 3 shows the morphology of the electrospun fibers prepared with different SPI: PVA ratios (0:100, 25:75, 50:50 and 60:40).

As observed from Figure 3, increasing the amount of SPI in the solutions led to increased presence of beads in the electrospun fibers. From the different ratios evaluated, the most stable one in terms of solution dropping was the 50:50 ratio, which in spite of displaying beads at the microscale, it formed a continuous fiber mat when directly electrospun onto either the collector or the PHB92/PHV8 film and, thus, this blend ratio was selected for subsequent formulations. Similarly, Cho et al. (2012) observed bead formation in SPI:PVA electrospun fibers prepared at a 50:50 ratio and by denaturing the protein at basic conditions.

Figure 3. SEM images of SPI:PVA electrospun fibers with varying composition: (A) 0:100; (B) 25:75; (C) 50:50; and (D) 60:40.

3.3 Characterization of the O/W emulsions

The 50:50 SPI:PVA ratio was selected to develop the emulsions with β -carotene as an strategy to incorporate the hydrophobic bioactive within the hydrophilic fibers. With these aim, O/W emulsions were prepared by three different methodologies and the properties of the emulsions for electrospinning (surface tension, conductivity and rheological properties) were characterized. The surface tension and electrical conductivity (Table 1) of the emulsions of SPI: PVA, regardless of the emulsification method were similar, and the surface tension did not exceed the value of 50 mN.m^{-1} , which could compromise the electrospinning process as previously mentioned. In relation to the rheological properties,

the emulsions showed a Newtonian behavior. Although SPI has a pseudoplastic behavior, blending with PVA changes it to Newtonian, as previously observed for polycaprolactone (PCL) with PVA blends (Pal et al., 2017) and, thus, if further diluted in emulsion form, this rheological behavior was expected.

According to McClements et al., (2007), gravitational separation is one of the most common forms of instability in emulsions, giving raise to a “cream” layer. Creaming is the upward movement of droplets due to their lower density than the surrounding liquid. Edible oils have lower densities than liquid water, so creaming is commonly observed in O/W emulsions. Therefore, because β -carotene has a hydrophobic character and was solubilized in the oil phase of the emulsion, the gravitational stability of the various emulsions prepared was evaluated as emulsion stability is a key aspect for stable electrospinning, as it would otherwise affect fiber formation.

The gravitational stability, as well as the creaming index values of the emulsions are shown in Table 2. The SPI: PVA emulsions prepared with HSH and the combination of the methods (HSH + US) after 24 hours were not stable (Table 2). The treatment of HSH is a methodology with high shear stress (Trujillo-Cayado et al., 2017), which may have caused physical disintegration of emulsion droplets of the protein when used alone or when combined with the US.

In contrast, the SPI: PVA emulsion prepared by sonication did not show any phase separation after 24 hours, guaranteeing gravitational stability during the electrospinning process. The excellent gravitational stability of the emulsion prepared by sonication can be also partially attributed to the presence of SPI in the formulation, as the protein has good emulsifying properties, ascribed to its amphiphilic character, which favors a faster adsorption of the protein at the oil-water interface through the non-polar amino acids of the protein chains (Gómez-Mascaraque et al., 2017a).

Table 2. Gravitational stability of the various emulsions prepared: images of the emulsions after storage for 0, 5 and 24 h and values of their creaming index (CI).

Procedures	Emulsion SPI:PVA			Emulsion PVA		
	Time (h)			Time (h)		
	0	5	24	0	5	24
High-speed homogenization (HSH)
CI (%)	100.0	92.3	80.0	100.0	61.5	84.6
Ultrasonication (US)
CI (%)	100.0	100.0	100.0	100.0	53.8	84.3
High-speed homogenization + Ultrasonication (HSH +US)
CI (%)	100.0	100.0	84.6	100.0	38.4	69.2

Moreover, according to Yang et al. (2018), the ultrasound process significantly increases the hydrophobicity of the surface as it exposes the hydrophobic regions of SPI after the cavitation process. In fact, there was an increase in emulsion stability due to the incorporation of SPI, since formulations only containing PVA as a biopolymer matrix in the hydrophilic phase were not stable. As observed in Table 2, neither of the processing methods used led to stable emulsions when no SPI was present.

The optical microscopy images of the different emulsions are shown in Figure 4. The emulsion prepared by US (Figure 4B) showed some larger but individualized droplets. On the other hand, the emulsions prepared by HSH (Figure 4A) and the combined US + HSH method (Figure 4C) presented small agglomerated droplets, suggesting that the HSH process may have promoted flocculation among the oil droplets.

According to McClements et al. (2007), flocculation is the process whereby two or more droplets associate with each other while maintaining their individual integrity. This phenomenon is observed when the attractive interactions among droplets dominate the long-range repulsive interactions, but not the short-range repulsive ones. Hence, the droplets remain in close proximity to each other (flocculate), without coming close enough together to merge into each other (coalesce). The macroscopic properties of emulsions, such as appearance and sensory perception, are strongly influenced by the extent and nature of flocculation.

Previous works dealing with SPI emulsions already showed that high-speed homegenization did not lead to stable emulsions, while the reduction in droplet size induced by the sonication process, had a positive effect on SPI emulsion stability, avoiding the creaming phenomenon for at least 48 hours (McClements et al. 2007).

458

459 **Figure 4.** Optical microscopy images of SPI: PVA emulsions prepared by: (A) HSH; (B)

460 US; (C) HSH+US and PVA emulsions prepared by (D) HSH; (E) US; and (F) HSH+US.

461 SPI: soy protein isolate; PVA: polyvinyl alcohol; HSH: High-speed homogenization; US:

462 Ultrasonication.

463

Figure 4 also shows that, after 5 hours of preparation, the emulsions prepared only using PVA, despite of the emulsification method, presented massive flocculation, explaining the lack of gravitational stability observed in Table 2. This result indicates that the emulsions prepared with only PVA would not be suitable for the encapsulation of β -carotene. According to Gómez-Mascaraque et al. (2017a), the large emulsification instability and the subsequent phase separation during the electropinning process significantly decrease microencapsulation efficiency.

3.4 Morphological, thermal characterization and stability of the electrospun microstructures

The morphology and particle size distribution of the microstructures obtained by electrospinning the selected loaded β -carotene emulsion are shown in Figure 5.

Figure 5. SEM image (A) and particle size distribution (B) of the β -carotene loaded SPI:PVA (50:50) electrospun fibers.

The microstructure of the electrospun mat was characterized by beaded fibers. The PVA acted as a carrier polymer and aided in the formation of fibers since, in general, it is not

possible to obtain pure SPI fibers, given the globular nature of the protein and the difficulties found for attaining sufficient molecular cohesion for fiber formation even after protein denaturation (Cho et al., 2012). During the removal of the material deposited in the electrospinning collector, the formation of a continuous film was observed, which was the characteristic of interest for coating development.

Regarding the structural stability of the fibers in water, the influence of SPI incorporation within the fibers was also assessed. Figure 6 shows that, while pure PVA fibers completely loss their structure after 24 h in contact with water, the hybrid SPI:PVA fibers only partially dissolved in water, remaining most of the β -carotene encapsulated within the structures as suggested from the coloured structures observed in Figure 6C. This fact shows that the blend of SPI and PVA, besides aiding in fiber formation, also altered its water solubility properties. According to Chen et al., (2018) denaturation decreases the water solubility of proteins, since these have their conformational state and their intramolecular interactions altered. Since the application of the fibers made with SPI and PVA is intended for the production of coatings for active food packaging applications, the decrease in water solubility is shown as a positive factor, which will allow for a more gradual release of the bioactive into the food product.

Figure 6. SPI:PVA fibers and PVA fibers with β -carotene (0 hour) (A-B) and after 24 hours (C-D), respectively.

In order to verify the thermal stability of β -carotene in hybrid fibers, derivative thermogravimetric (DTG) curves were obtained. Figure 7 shows the derivative thermogravimetric (DTG) curves of the β -carotene-loaded hybrid fibers and those from the pure individual fiber constituents. The active coating showed four thermal degradation stages, which probably correspond to the thermal degradation of the individual compounds present in the fibers. However, it is difficult to unambiguously ascribe each of the peaks to a specific component, although in general a shift towards higher temperatures of the maximum degradation rates is observed, pointing out to enhanced thermal stability of the hybrid material, probably as a consequence of component interactions.

Figure 7. First derivatives (DTG) of the β -carotene loaded electrospun fibers, pure PVA, pure β -carotene and SPI denatured at pH 2.

3.5 Fourier transform infrared (FT-IR) analysis of the active electrospun coating

The FTIR spectra of the electrospun fibers loaded with β -carotene and of each individual component are presented in Figure 8.

Figure 8. FT-IR spectra of: (A) β -carotene loaded SPI:PVA (50:50) electrospun fibers; (B) pure PVA; (C) soybean oil; (D) pure β -carotene; and (E) SPI denatured at pH 2.

The IR spectrum of the microstructure loaded with β -carotene presented bands related to its precursors. In this spectrum, characteristic bands of SPI were identified at 3288 cm^{-1} for amide A (N-H stretching), 1645 cm^{-1} referring to amide I (C=O stretching), 1536 cm^{-1} referring to amide II (N-H bending and stretching) and 1240 cm^{-1} referring to amide III (C-N stretching) (Gómez-Mascaraque and López-Rubio, 2016). A band at 1735 cm^{-1} referring to C = O groups of soybean oil triglycerides (Gómez-Mascaraque et al., 2017a, Pérez-Masiá et al., 2015) and at 955 cm^{-1} corresponding to the C=C group of the carotenoids (Gomez-Mascarque et al., 2017a, Rubio-Diaz et al., 2011) were observed. No significant band displacements were observed in the hybrid fibers, just a slight shift towards greater

wavenumbers of the amide II band, which could indicate some interactions between the protein and the PVA through hydrogen bonding.

Moreover, bands at 2923 cm^{-1} referring to C-H from alkyl groups and 1730 cm^{-1} band referring to C=O and C-O from carbonyl group of PVA (Rezaee et al., 2017) were observed. In the spectra of the β -carotene-containing fibers, a band at 1446 cm^{-1} band was observed which could be due to the presence of the SBO which was the carrying oil used to encapsulate the antioxidant.

3.6 Microencapsulation efficiency (MEE)

The β -carotene entrapped in the SPI: PVA fibers was $65.0 \pm 2.6\%$, where $13.6 \pm 0.9\%$ of this was on the surface of the fibers and, thus, the percentage of carotenoid effectively microencapsulated in the structures was $51.4 \pm 0.9\%$. Gómez-Mascaraque et al. (2017a) reported a value of 34.7% of MEE of β -carotene, using zein as the matrix. According to Donhowe et al. (2014), the MEE of the β -carotene encapsulated in chitosan-alginate matrices was 54.7%. Ge et al. (2015) reported a MEE of 34.2%, in which self-assembled chitosan-based micelles were used for encapsulation of β -carotene. Jain et al. (2016) found a higher value of MEE (79.3%) of β -carotene using casein and tragacanth gum to encapsulate the β -carotene by a coacervation method. Therefore, the strategy developed in this work, which consisted on producing SPI: PVA fibers through emulsion electrospinning was efficient for β -carotene encapsulation.

Several factors can affect the encapsulation of bioactives. According to Gómez-Mascaraque et al. (2017a), that used zein and whey protein as encapsulation matrices for β -carotene, the main factor that affected microencapsulation efficiency through electrospraying was the gravitational stability of the feed emulsions. Kong et al. (2018) used an amylose complex as the encapsulation matrix for β -carotene and reported that effective encapsulation depended

on the emulsifying effect of surfactants to dissolve β -carotene. Therefore, the encapsulation efficiency of β -carotene depends on the emulsification procedure and the matrix into which it is incorporated.

3.7 Morphological characterization of the active coating and tuning of the release properties

The objective of this work was to develop a formulation that could be used as an internal coating of food biopackaging structures and, for that means, the coating was directly collected onto a polyhydroxyalcanoate (PHA) film (specifically a PHB92/PHV8 copolymer film). Moreover, in order to favor adhesion of the coating to the base PHA and to modulate the release of the active carotenoid compound, an annealing treatment was applied as specified in the methods section. This annealing treatment, which has been previously observed to increase the crystallinity of polymers (López-Rubio et al., 2003), was expected to change the crystallinity of the PVA component of the fibers, thus, affecting the release of the hydrophobic compound.

Figure 9 shows the SEM images of the cross-section and surface of the PHA film with the SPI-PVA coating before (Figures 9A and 9B) and after the annealing treatment (Figures 9C and 9D). As observed from the SEM images, the annealing treatment led to coalescence and improved adhesion of the fibers onto the PHA material and, even though a fiber-like structure could still be observed, they appear practically fused (see Figure 9D). Annealing processes have also been previously applied to fiber mat structures to promote fusion among fibers and increase the structural integrity of the membrane (Kaur et al., 2011), and also to improve adhesion to the supporting polymer (Li et al., 2013).

Figure 9. SEM images of cross-section (A, C) and surface (B, D) micrographs of the coating without annealing (A, B) and after annealing (C,D).

The in-vitro release assay was performed from the fiber coating with and without post-spinning heat annealing (Figure 10). A soybean oil release medium, which simulates fatty foods, was selected due to solubility of β -carotene in this medium. As observed from the figure, release of the hydrophobic compound from the matrix which was not annealed occurred during the first 5 hours of the experiment. However, a slower and more sustained release took place from the annealed fibers, suggesting that this treatment effectively favours a more controlled release of the bioactive. This can be ascribed to the partial collapse of the fibers after the annealing process, decreasing the porosity of the matrices and also due to

PVA crystallites formation during the annealing step. Similarly, Moreno et al. (2019) have also recently reported a controlled release of bioactive extracts encapsulated into electrospun zein fibers after they were chemically cross-linked with glutaraldehyde, indicating that changes in the morphology of the fibers exert an active role in the release profile.

DSC experiments of the fibers before and after the annealing process were carried out and the DSC curves are shown in Figure 11.

Figure 10. β -carotene release profile from electrospun fiber without (a) and with annealing (b), in a fat food simulant.

Apart from the broad peak (around 110 °C) ascribed to evaporation of remaining water in the fibers, the thermal transition observed at higher temperatures which may be related to the melting of PVA crystals, indicated that the annealing process effectively promoted crystalline growth in the materials, as a shift in the melting temperature from 175 to 180°C

is clearly observed (see arrows), indicating bigger or more perfect crystals which apparently have an effect on the release properties from the active coatings.

Figure 11. DSC of the SPI:PVA fibers with (a) and without annealing (b). Arrows point out to the melting of the PVA crystals.

4. Conclusion

The present work shows the convenience of the emulsion electrospinning technique for developing bioactive inner packaging layers as carriers of β -carotene (used as a model hydrophobic bioactive compound) for food packaging applications. The electrospinning feed solutions were initially optimized in order to obtain good fiber mats to facilitate the formation of a continuous coating onto a PHA-based film (specifically a PHB92/PHV8 copolymer film).

SPI solutions denatured using acid conditions showed lower surface tension and viscosity but higher electrical conductivity, which rendered them with better spinnability than their counterparts solutions prepared by heat or basic denaturation processes. However,

continuous fibers were not obtained with none of the SPI-based solutions and, thus, PVA (a fiber-forming biopolymer) was incorporated into SPI solutions denatured at acid conditions as a strategy to favour coating formation. Emulsions were prepared from the SPI:PVA mixture with the most stable feed solution to form electrospun fibers (50:50 ratio), using SBO as carrier oil for the antioxidant β -carotene, and the emulsions were directly electrospun on a PHA-based film. An annealing process was used to favour the adhesion of the coating onto the PHA-based film and to modulate the release of the β -carotene. The bioactive electrospun fibers showed high encapsulation efficiency ($65.0\% \pm 2.6\%$), being $51.4\% \pm 0.9\%$ effectively incorporated within their cores. Finally, the in-vitro release assay performed in a soybean oil (which simulates fatty foods) evidenced that the heat treatment (annealing) promoted a slower and more sustained release of the bioactive in the release medium. Therefore, this proof-of-concept study demonstrates that annealed electrospun coatings prepared by emulsion electrospinning can play an important role in the design of novel bioactive packaging materials incorporating antioxidant hydrophobic substances of interest in food packaging applications.

Acknowledgments

We would like to thank CAPES (88887.124727/2014-00), CNPq (448800/2014-4), FAPERGS (16/2551-0000250-9), SCT-RS and *Pólo de Inovação Tecnológica em Alimentos da Região Sul*. M. J. Fabra is recipient of a Ramón y Cajal (RYC-2014-158) contract from the Spanish Ministry of Economy, Industry and Competitiveness (MINECO). The authors would also like to thank the Spanish MINECO project AGL2015-63855-C2-1-R for financial support.

References

693 Alehosseini, A., Gómez-Mascaraque, L. G., Martínez-Sanz, M., López-Rubio, A. (2019).
694 Electrospun curcumin-loaded protein nanofiber mats as active/bioactive coatings for food
695 packaging applications. *Food Hydrocolloids*, 87, 758-771.

696 Atay, E., Fabra, M. J., Martínez-Sanz, M., Gomez-Mascaraque, L.G., Altan, A., Lopez-
697 Rubio, A. (2018). Development and characterization of chitosan/gelatin electrosprayed
698 microparticles as food grade delivery vehicles for anthocyanin extracts. *Food*
699 *Hydrocolloids*, 77, 699-710.

700 Babitha, S., Rachita, L., Karthikeyan, K., Shoba, E., Janani, I., Poornima, B., Sai, K. P.
701 (2017). Electrospun protein nanofibers in healthcare: A review. *International Journal of*
702 *Pharmaceutics*, 90, 523- 52.

703 Bhardwaj, N., Kundu, S. C. (2010). Electrospinning: a fascinating fiber fabrication
704 technique. *Biotechnology Advances*, 28, 325-347.

705 Chen, X., Xu, X., Liu, D., Zhou, G., Han, M., Wang, P. (2018). Rheological behavior,
706 conformational changes and interactions of water-soluble myofibrillar protein during
707 heating. *Food Hydrocolloids* 77, 524-533.

708 Cho, D., Netravali, A. N., Joo, L. Y. (2012). Mechanical properties and biodegradability of
709 electrospun soy protein isolate / PVA hybrid nanofibers. *Polymer Degradation and*
710 *Stability*, 97, 747-754.

711 Commission regulation (EU). (2011). No 10/2011 of 14 January 2011 on plastic materials
712 and articles intended to come into contact with food. 10/2011/EC.

713 Costamagna, M. S., Gómez-Mascaraque, L. G., Zampini, I. C., Alberto, M. R., Pérez, J.,
714 López-Rubio, A., Isla, M. I. (2017). Microencapsulated chañar phenolics: A potential
715 ingredient for functional foods development. *Journal of Functional Foods*, 37, 523-530.

716 Deng, L., Kang, X., Liu, Y., Feng, F., Zhang, H. (2018). Characterization of gelatin/zein
717 films fabricated by electrospinning vs solvent casting. *Food Hydrocolloids*, 74, 324-332.

718 Desai, K. G. H., Jin Park, H. (2005). Recent developments in microencapsulation of food
 719 ingredients. *Drying Technology*, 3, 1361-1394.

720 Donhowe, E. G., Flores, F. P., Kerr, W. L., Wicker, L., Kong, F. (2014). Characterization
 721 and in vitro bioavailability of β -carotene: Effects of microencapsulation method and food
 722 matrix. *LWT- Food Science and Technology*, 57, 42-48.

723 Fabra, M. J., López-Rubio, A., Lagaron, J. M. (2014). Biopolymers for food packaging
 724 applications. *Smart polymers and their applications*, 476-509.

725 Fabra, M. J., Lopez-Rubio, A., & Lagaron, J. M. (2015). Effect of the film-processing
 726 conditions, relative humidity and ageing on wheat gluten films coated with electrospun
 727 polyhydrylicanoate. *Food Hydrocolloids*, 44, 292-299.

728 Fabra, M. J., López-Rubio, A., Lagaron, J. M. (2016). Use of the electrohydrodynamic
 729 process to develop active/bioactive bilayer films for food packaging applications. *Food*
 730 *Hydrocolloids*, 55, 11-18.

731 Fang, Q., Zhu, M., Yu, S., Sui, G. Yang, X. (2016). Studies on soy protein isolate/polyvinyl
 732 alcohol hybrid nanofiber membranes as multi-functional eco-friendly filtration materials.
 733 *Materials Science and Engineering: B*, 214, 1-10.

734 Fong, H., Chun, I., Reneker, D. H. (1999). Beaded nanofibers formed during electrospinning,
 735 *Polymer*, 40, 4585-4592.

736 Ge, W., Li, D., Chen, M., Wang, X., Liu, S., Sun, R. (2015). Characterization and antioxidant
 737 activity of β -carotene loaded chitosan-graft-poly (lactide) nanomicelles. *Carbohydrate*
 738 *Polymers*, 117, 169-176.

739 Gómez-Mascaraque, L. G., Lagarón, J. M., López-Rubio, A. (2015). Electrosprayed gelatin
 740 submicroparticles as edible carriers for the encapsulation of polyphenols of interest in
 741 functional foods. *Food Hydrocolloids*. 49, 42-52.

742 Gómez-Mascaraque, L. G., López-Rubio A. (2016). Protein-based emulsion electrosprayed
 743 micro-and submicroparticles for the encapsulation and stabilization of thermosensitive
 744 hydrophobic bioactives. *Journal of Colloid and Interface Science*, 465, 259-270.
 745 Gómez-Mascaraque, L. G., Pérez-Masiá, R., González-Barrio, R., Periago, M. J., López-
 746 Rubio, A. (2017a). Potential of microencapsulation through emulsion-electrospraying to
 747 improve the bioaccessibility of β -carotene. *Food Hydrocolloids*, 73, 1-12.
 748 Gómez-Mascaraque, L. G., Sipoli, C. C., de La Torre, L. G., López-Rubio, A. (2017).
 749 Microencapsulation structures based on protein-coated liposomes obtained through
 750 electrospraying for the stabilization and improved bioaccessibility of curcumin. *Food*
 751 *Chemistry*, 233, 343-350.
 752 Hou, Z., Zhang, M., Liu, B., Yan, Q., Yuan, F., Xu, D., Gao, Y. (2012). Effect of chitosan
 753 molecular weight on the stability and rheological properties of β -carotene emulsions
 754 stabilized by soybean soluble polysaccharides. *Food Hydrocolloids*, 26, 205-211.
 755 Jain, A., Thakur, D., Ghoshal, G., Katare, O. P., Shivhare, U. S. (2016). Characterization of
 756 microcapsulated β -carotene formed by complex coacervation using casein and gum
 757 *tragacanth*. *International Journal of Biological Macromolecules*, 87, 101-113.
 758 Kaur, S., Barhate, R., Sundarrajan, S., Matsuura, T., Ramakrishna, S. (2011). Hot pressing
 759 of electrospun membrane composite and its influence on separation performance on thin
 760 film composite nanofiltration membrane, *Desalination*, 279, 201-209.
 761 Kong, L., Bhosale, R., Ziegler, G. R. (2018). Encapsulation and stabilization of β -carotene
 762 by amylose inclusion complexes, *Food Research International*, 105, 446-452.
 763 Li, L., Hashaikeh, R., Arafat, H. A. (2013). Development of eco-efficient micro-porous
 764 membranes via electrospinning and annealing of poly (lactic acid). *Journal of Membrane*
 765 *Science*, 436, 57-67.

766 Liu, P., Xu, H., Zhao, Y., Yang, Y. (2017). Rheological properties of soy protein isolate
 767 solution for fibers and films. *Food Hydrocolloids*, 64, 149-156.

768 López-Rubio, A., Lagarón, J. M., Giménez, E., Cava, D., Hernandez-Muñoz, P., Yamamoto,
 769 T., Gavara, R. (2003). Morphological alterations induced by temperature and humidity in
 770 ethylene-vinyl alcohol copolymers. *Macromolecules*, 36, 9467-9476.

771 McClements, D. J., Decker, E. A., Weiss, J. (2007). Emulsion-based delivery systems for
 772 lipophilic bioactive components. *Journal of Food Science*, 72, 109-124.

773 McClements, D. J., Li, Y. (2010). Structured emulsion-based delivery systems: Controlling
 774 the digestion and release of lipophilic food components. *Advances in Colloid and*
 775 *Interface Science*, 159, 213-228.

776 Mendes, A. C., Stephansen, K., Chronakis, I. S. (2017). Electrospinning of food proteins and
 777 polysaccharides. *Food Hydrocolloids*, 68, 53-68.

778 Moreno, M. A., Orqueda, M. E., Gómez-Mascaraque, L. G., Isla, M. I., & López-Rubio, A.
 779 (2019). Crosslinked electrospun zein-based food packaging coatings containing bioactive
 780 chilito fruit extracts. *Food Hydrocolloids*, 95, 496-505.

781 Pal, J. Wu, D., Hakkarainen, M., Srivastava, R. K. (2017). The viscoelastic interaction
 782 between dispersed and continuous phase of PCL/HA-PVA oil-in-water emulsion
 783 uncovers the theoretical and experimental basis for fiber formation during emulsion
 784 electrospinning. *European Polymer Journal*, 96, 44-54.

785 Pérez-Masiá, R., Lagaron, J. M., López-Rubio, A. (2014). Development and optimization of
 786 novel encapsulation structures of interest in functional foods through electrospraying.
 787 *Food and Bioprocess Technology*, 7, 3236-3245.

788 Pérez-Masiá, R., Lagaron, J. M., López-Rubio, A. (2014). Surfactant-aided electrospraying
 789 of low molecular weight carbohydrate polymers from aqueous solutions. *Carbohydrate*
 790 *Polymers*, 101, 249-255.

791 Pérez-Masiá, R., Lagaron, L. M., Lopez-Rubio, A. (2015). Morphology and stability of
 792 edible lycopene-containing micro-and nanocapsules produced through electrospraying
 793 and spray drying. *Food and Bioprocess Technology*, 8, 459-470.

794 Rao, A. V., Rao, L. G. (2007). Carotenoids and human health. *Pharmacological Research*,
 795 55, 207-216.

796 Rezaee, O., Chenari, H. M., Ghodsi, F. E., Ziyadi, H. (2017). Preparation of PVA nanofibers
 797 containing tungsten oxide nanoparticle by electrospinning and consideration of their
 798 structural properties and photocatalytic activity. *Journal of Alloys and Compounds*, 690,
 799 864-872.

800 Romani, V. P., Machado, A. V., Olsen, B. D., Martins, V. G. (2018). Effects of pH
 801 modification in proteins from fish (Whitemouth croaker) and their application in food
 802 packaging films. *Food Hydrocolloids*, 74, 307-314.

803 Rubio-Diaz, D. E., Francis, D. M., Rodriguez-Saona, L. E. (2011). External calibration
 804 models for the measurement of tomato carotenoids by infrared spectroscopy. *Journal of*
 805 *Food Composition and Analysis*, 24, 121-126.

806 Silva, F. T., Cunha, K. F., Fonseca, L. M., Antunes, M. D., El Halal, S. L. M., Fiorentin, Â.
 807 M., Zavareze, E. R., Dias, A. R. G. (2018) Action of ginger essential oil (*Zingiber*
 808 *officinale*) encapsulated in proteins ultrafine fibers on the antimicrobial control *in situ*.
 809 *International Journal of Biological Macromolecules*, 118, 107-115.

810 Sousa, M. I., Mitchell, J. R., Ledward, D. A., Hill, S. E., da Costa, M. L. B. (1995).
 811 Differential scanning calorimetry of lupin and soy proteins. *Zeitschrift für Lebensmittel-*
 812 *Untersuchung und –Forschung*, 201, 566-569.

813 Surh, J., Decker, E. A., McClements, D. J. (2006). Influence of pH and pectin type on
 814 properties and stability of sodium-caseinate stabilized oil-in-water emulsions. *Food*
 815 *Hydrocolloids*, 20, 607-618.

816 Tampau, A., González-Martínez, C., Chiralt, A. (2018). Release kinetics and antimicrobial
817 properties of carvacrol encapsulated in electrospun poly-(ϵ -caprolactone) nanofibres.
818 Application in starch multilayer films. *Food Hydrocolloids*, 79, 158-169.

819 Trujillo-Cayado, L. A., Alfaro, M. C, García, M. C., Muñoz, J. (2017). Comparison of
820 homogenization processes for the development of green O/W emulsions formulated with
821 N, N-dimethyldecanamide. *Journal of Industrial and Engineering Chemistry*, 46, 54-61.

822 Umesha, S. S., Monahar, B., Naidu, K.A. (2013). Microencapsulation of α -linolenic acid-
823 rich garden cress seed oil: Physical characteristics and oxidative stability. *European*
824 *Journal of Lipid Science and Technology*, 115, 1474-1482.

825 Xianquan, S., Shi, J., Kakuda, Y., Yueming, J. (2005) Stability of lycopene during food
826 processing and storage. *Journal of Medicinal Food*, 8, 413-422.

827 Yang, F., Liu, X., Ren, X. E., Huang, Y., Huang, C., Zhang, K. (2018). Swirling cavitation
828 improves the emulsifying properties of commercial soy protein isolate. *Ultrasonics*
829 *Sonochemistry*, 42, 471-481.

830 Zhang, Y. Ghasemzadeh, S., Kotliar, A. M., Kumar, S., Presnell, S., Williams, L. D. (1999).
831 Fibers from soybean protein and poly (vinyl alcohol). *Journal of Applied Polymer*
832 *Science*, 71, 11-19.

833 Zuo, W., Zhu, M., Yang, W., Yu, H., Chen, Y., Zhang, Y. (2005). Experimental study on
834 relationship between jet instability and formation of beaded fibers during electrospinning.
835 *Polymer Engineering & Science*. 45, 704-709.

836