ELECTROSPUN ZEIN NANOFIBERS FOR FOOD AND PACKAGING APPLICATIONS

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ABSTRACT – The objective of this work was to produce zein nanofiber by an electrospinning technique. Apparent viscosity and electrical conductivity of zein polymeric solutions, prepared by dissolving zein in ethanol (70% v/v) were evaluated. The morphology of the electrospun fibers was evaluated by scanning electron microscope and size distribution. Results show that increasing zein concentration increased apparent viscosity and increased the average diameter of the fibers. Changing the concentrations of zein did not affect the electrical conductivity of the solutions. Fibers prepared with the lowest zein concentration had many beads and the smallest average fiber diameter, while fibers prepared from zein solution at the highest concentration had ribbon-like shapes with the highest average diameter. The fibers electrospun from 30% zein had greater uniformity and average diameter of 417 nm. These nanofibers, in the form of nonwoven membranes, potentially can be used for encapsulating compounds for food and active food packaging applications.

KEYWORDS: active packaging; electrospinning; zein, nanofibers.

1. INTRODUCTION

In recent years, the production of polymeric nanofibers by electrospinning technique has been widely investigated. Electrospinning is a technique that produces ultrafine polymeric fibers, under electric field with high voltage, that are collected as nonwovens. During a typical electrospinning process, a polymeric solution is placed in a syringe coupled to the pump to control the solution fee rate. A stainless steel needle, which serves as a spinneret, is to the positive electrode of a DC power source, while a metal collector is negatively charged or grounded.

Several parameters influence the formation of fibers by electrospinning, such as voltage, distance between needle tip to collector, polymeric solution viscosity and conductivity, environmental conditions (relative humidity, temperature), among others (Fabra et al., 2013). Depending on the electrospinning process parameters used, fiber/capsule morphology, size and uniformity are being affected. Nanofibers or nanocapsules can be used as protective material of antioxidants and antimicrobials for food and active and/or intelligent packaging development, in addition to its wide application in the area of drugs and tissue engineering (Bhardwaj & Kundu, 2010; Bhushani & Anandharamakrishnan, 2014).

In this study, zein polymer has been studied in this work for producing fibers by electrospinning technique. Zein is a protein present in corn with hydrophobic characteristics due to the
presence of apolar amino acid residuals such as leucine, proline and alanine. This protein has good elasticity and capacity of film formation, potentially useful in food packaging (Neo et al., 2013), as well as being used, as a renewable and biodegradable material in various applications (Mori et al. 2014). Many studies have demonstrated the effectiveness of zein as the encapsulating bioactive compounds by electrospinning (Moomand & Lim, 2014).

This study aimed to produce zein nanofibers by an electrospinning technique using different zein concentrations using ethanol (70% v/v) as solvent and evaluate their physical and morphological properties. These fibers potentially can be used as encapsulants of bioactive compounds in food and active packaging applications.

2. MATERIAL AND METHODS

2.1. Material

The zein was from Sigma-Aldrich and ethanol 99.5% P.A. was purchased from CRQ Products Chemicals LTD.

2.2. Electrospinning solutions preparation

Polymeric solutions containing 20, 30 and 40% (w/v) of zein were prepared using aqueous ethanol (70% v/v) solution. The polymeric solutions were submitted the magnetic stirring for 1 h.

2.3 Apparent viscosity and electrical conductivity of solutions

The apparent viscosity of the solutions was evaluated in Brookfield Digital viscometer (Model DV - II, USA). The solutions (± 9 mL) were placed in a stainless steel container of viscometer and this was coupled to the equipment using a nº. 18 spindle (Neo et al., 2013).

The electrical conductivity of the solutions was determined using a conductivity meter (CON500) expressed in μS.cm⁻¹ (Wen et al., 2016). All measurements were made at room temperature (± 23 °C) and in triplicate and the results were analyzed by analysis of variance (ANOVA).

2.4. Electrospinning nanofibers production

The zein solutions (20%, 30% and 40%) were electrospun at 1 mL.h⁻¹ flow rate controlled by an infusion pump (Model k100, Kd ScientificInc., Holliston, MA, USA) using a 3 mL syringe with a 0.7 mm blunt end needle spinneret. The positive electrode of a direct current power supply was connected to the spinneret (INSTOR – Projetos & Robótica) and kept at 18 kV. A negative electrode was connected to the target covered with aluminum foil, placed horizontally to the pump to a needle spinneret with 15 cm distance of the target and voltage of 18 kV. All electrospinning experiments were carried out at 23 ± 2 °C under 58% RH with the aid of an air conditioner and dehumidifier.

2.5. Nanofibers characterization

The nanofibers morphology was evaluated by a scanning electron microscope (SEM) (Shimadzu SSX-550) with a 10 kV acceleration voltage. The average diameter and diameter
distribution of nanofibers with different zein concentrations were evaluated from SEM images using 50 measures through the ImageJ software (Mori et al., 2014).

3. RESULTS AND DISCUSSION

3.1 Apparent viscosity and electrical conductivity of the polymeric solutions

Apparent viscosity and electrical conductivity of zein solutions aqueous ethanol (70% v/v) at different concentrations (20, 30 and 40%; w/v) are summarized in Table 1. The viscosity of the solutions increased with increasing zein concentration (Table 1). These results are accordance with those reported by Neo et al. (2012), who studied the influence of polymeric solution in the production of zein fibers and observed that there was increase in the solution viscosity as polymer concentration increased. They reported that at 15% zein concentration, it was not possible to form fibers due to low solution viscosity (22.1 mPa), forming only microspheres. However, with a zein concentration of 35%, thick fibers were observed due to high solution viscosity (358.0 mPa). Solution viscosity played an important role in fibers formation during electrospinning due to the entanglement between polymer chains essential for stabilizing the electrified jet, preventing the formation of beads. Bhardwaj and Kundu (2010) summarized in their review the effects of viscosity polymer concentration on fibers morphology. Increasing solution viscosity tends to prevent the formation of beads, while increase in fiber diameter. The extent of chain entanglement should be enough to maintain the level of solution viscosity to produce a uniform jet during electrospinning and restrain effects of surface tension, which plays a significant role in beads formation on electrospun nanofiber.

Dhanalakshmi et al. (2015) reported that optimal viscosity is essential for ensuring the stability of solution jet during the electrospinning process. Low viscosity tends to produce beads and not fibers, and vice versa. In the present study, the most optimal condition for the formation of zein nanofibers was 30% polymer concentration with a viscosity of 123.4 cP (Table 1).

The electrical conductivity also plays a key role in the formation of fibers by electrospinning. It has been found that increasing electrical conductivity of the solution, significant decreased the diameter of the electrospun nanofibers (Bhardwaj & Kundu, 2010). Increasing zein concentration did not affect the electrical conductivity of the polymeric solution (Table 1). Generally, dissolved proteins in aqueous solution produce ions, which are responsible for the electrical conductivity of the solution (Ramakrishna et al., 2005). Electrospun nanofibers with the smallest fiber diameter can be obtained with the highest electrical conductivity (Bhardwaj & Kundu, 2010). In view of this, the increase in protein concentration can increase the electrical conductivity of the solution, however, this behavior was not observed in the present study.

Ramakrishna et al. (2005) reported that to start the process of electrospinning, the polymeric solution must have sufficient static charge build up to establish the repulsive force that must exceed the surface tension of the polymer solution. Furthermore, the subsequent stretching of electrospinning jet is also dependent on the ability of solution to carry charges.
Table 1 - Apparent viscosity and electrical conductivity of zein solutions in different concentrations.

<table>
<thead>
<tr>
<th>Zein concentration (%)</th>
<th>Apparent viscosity (cP)</th>
<th>Electric conductivity (µS.cm⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>20</td>
<td>38.9 ± 2.3 c</td>
<td>205.0 ± 12.5 a</td>
</tr>
<tr>
<td>30</td>
<td>123.4 ± 5.7 b</td>
<td>192.3 ± 11.0 a</td>
</tr>
<tr>
<td>40</td>
<td>373.9 ± 7.0 a</td>
<td>203.6 ± 7.3 a</td>
</tr>
</tbody>
</table>

1 Different lowercase letters in the same column represent significant difference between the mean submitted to Tukey test at 5% significance.

3.2 Morphology and size distribution of nanofiber

Zein solutions, prepared in aqueous ethanol at all protein concentrations tested, produced nanofibers in the form of nonwoven membrane on the collector. The size and morphology of the zein material were dependent on the protein concentration used to prepare the zein solution (Fig. 1).

Figure 1 - Micrographs of zein nanofiber in concentrations of 20% (a) 30% (c) and 40% (e) of zein at magnification 1000x; and distribution of the nanofibers diameter elaborated with 20% (b) 30% (d) and 40% (f) of zein.

The nanofibers produced with 20% zein (Figure 1a) showed microspheres or beads, due to the low viscosity of the polymeric solution. On the other hand, increasing zein concentration to 40% (w/v) induced the formation ribbon-like fiber morphology, due to the high viscosity of the polymeric
solution, and very fast solvent evaporation. The morphology of fibers from 30% zein (Figure 1c) was cylindrical in shape, uniform, homogeneous, without beads Fernandez et al. (2009) analyzed the morphology of fibers with 33% zein concentration, also observed flattened and thin fibers. Neo et al. (2012) report that the morphology of fiber in the form of "tape" can be the collapse of the fibers due to the high volatility of ethanol in the zein solution. Some reports described that ethanol/water mixture solvent system results in ribbon-like morphology, due to the rapid skin formation and collapse of the fiber core because of the very fast evaporation of the solvent (Miyoshi et al., 2005; Selling et al., 2007; Torres-Giner et al., 2008).

Figures 1b, 1d and 1f shown the distribution of the diameters of zein fibers at 20, 30 and 40% zein concentrations, respectively. The fibers had the lowest average diameter at 20% zein (139 nm), followed by 30% zein (417 nm) and 40% zein (1587 nm). The polymer concentration favorable for fiber forming depends on the polymer structure and type, since the optimal concentration produces bead-free nanofibers and the smallest size possible with homogeous morphology. According to Huang et al. (2003) one of the most important parameters that influence the diameter of the fibers produced by electrospinning is the concentration of polymer in solution and its corresponding viscosity. Kayaci and Uyar (2012) reported the increase in bead-free nanofibers by increasing the polymer concentration, but fiber failed to form when the polymer concentration was too high.

The zein nanofibers have a good potential for food and active food packaging applications due to its high surface area contact (Kayaci & Uyar, 2012), allowing for controlled release of encapsulated functional components (Okutan et al., 2014), in addition to increase the stability of the encapsulated compound (Bhushani & Anandharamakrishnan, 2014).

4. CONCLUSIONS

Zein solutions, prepared in aqueous ethanol at all protein concentrations tested, exhibited electrospun behavior, producing nanofibers by electrospinning technique. The apparent viscosity of the solutions increased with the increase of polymeric concentration. The electrical conductivity of the polymeric solutions had no significant difference as zein concentration changed. The morphology and average diameter of the fibers were affected by different levels of zein concentration. Fibers produced with the lowest zein concentration showed beads and had the lowest average diameter. On the other hand, fibers with the highest zein concentration exhibited ribbon-like shape with the highest average diameter. The nanofibers produced with 30% zein, presented averaged diameter of 417 nm with improved uniformity, when compared with fibers prepared under other concentrations evaluated. These nanofibers have potential to use as encapsulants of compounds for food and active packaging development.

5. ACKNOWLEDGEMENTS

We would like to thank CAPES, CNPq, SCT-RS and to FAPERGS by project financing. To CEME-SUL by microscopy analyses and to University of Guelph, Canada, for technical support on electrospinning.
6. REFERENCES


