
INCORPORATION OF PHENOLIC COMPOUNDS IN PHB NANOFIBERS WITH POTENTIAL ANTIBACTERIAL ACTIVITY

Suelen G. Kuntzler¹, Ana Cláudia A. Almeida¹, Jorge Lucas Da Maia¹, Jorge Alberto V. Costa² and Michele G. Morais¹

¹ Federal University of Rio Grande, College of Chemistry and Food, Laboratory of Microbiology and Biochemistry

² Federal University of Rio Grande, College of Chemistry and Food, Laboratory of Biochemical Engineering

E-mail: suelen_goettems@hotmail.com

ABSTRACT

Polyhydroxybutyrate (PHB) is a biopolymer synthesized by microalgae that can be applied in the production of nanofibers by electrospinning process. The microalga also produces phenolic compounds that present biological activities as antifungal, antibacterial, anti-inflammatory and antioxidant. The objective of the study was to produce PHB nanofibers with addition of microalgal phenolic compounds. PHB and phenolic compounds were extracted from the biomass of *Spirulina* sp. LEB 18. The polymer solutions were subjected to electrospinning. The development of the nanofibers was possible with the solution of 35% microalgal PHB, 2.4% NaCl and 1% phenolic compounds. The uniform and dropless nanofibers had an average diameter of 814 ± 91 nm with potential use in food packaging because they can improve the barrier properties in order to hamper the gases and vapors permeation, as well as contribute to the mechanical properties. In addition, phenolic compounds have potential antibacterial activity which suggests increases the shelf life of the food and gives greater safety to the consumer.

1. INTRODUCTION

Microalgae are autotrophic microorganisms that produce biopolymers such as polyhydroxybutyrate (PHB) and phenolic compounds. The biopolymer has characteristics of thermal degradation, melting

temperature and mechanical properties similar to polypropylene, and can act as an alternative to petrochemical plastics. PHB due to its biodegradability and biocompatibility with cells and tissues can be used in the development of nanofibers for application in food or medicine. Furthermore, microalgal phenolic compounds are promising for use in the present nanofibers as antibacterial activity against the microorganisms *Staphylococcus aureus* and *Escherichia coli* (Kuntzler, 2017). Polymeric nanofibers have important properties for food application because of their nanometric diameter, which improves mechanical properties such as elasticity and strength, high porosity and high surface area in relation to volume (Beachley; Wen, 2009). In this way, this study makes possible the development of an innovative product with potential application in food packaging, contributing to preserve the quality and extend the useful life of the products, not harming the environment like the current packaging of petrochemical origin. The objective of the study was to produce PHB nanofibers with addition of microalgal phenolic compounds.

2. METHODOLOGY

2.1. Extraction of PHB Using Chloroform

The extraction of PHB from *Spirulina* sp. LEB 18 biomass was performed according to Morais et al. (2015) (PHB1 treatment).

2.2. Extraction of PHB with 4% sodium hypochlorite Using Pre-treatment with Chloroform

Pre-treatment with chloroform was performed according with Morais et al. (2015) methodology. After the extraction of PHB from the biomass *Spirulina* sp. LEB 18 with sodium hypochlorite was also performed according to Morais et al. (2015) and the final precipitate was oven dried at 35 °C for 48 h (PHB2 treatment).

2.3. Extraction of phenolic compounds

The extraction of phenolic compounds from *Spirulina* sp. LEB 18 was performed using methodology of Souza et. (2011). Quantitative determination of phenolic compounds in the extract was performed according to the method described by Folín, Ciocalteu (1927).

2.3. Preparation of Polymer Solutions and Development of Nanofibers

The polymer solutions were prepared with 35% (w/v) of PHB1 and another with 30% (w/v) of PHB2 in chloroform (Morais et al., 2015). In the solution that formed uniform nanofibers, 2.4% (w/v) sodium

chloride (NaCl) and 1% phenolic compounds were added in chloroform. All solutions were shaken on magnetic stirrer (Fisatom, Brazil) for 16 h.

The polymer solutions were injected through the capillaries with a diameter of 0.45; 0.55; 0.70 and 0.80 mm. The voltage was studied varying from 15, 20 and 24.3 kV and the feed rate of the solution between 150 and 1150 $\mu\text{L/h}$. The distance from the capillary to the collector was set at 150 mm. All the tests were conducted at 20 °C and relative humidity of $60 \pm 1\%$.

2.4. Nanofibers Morphology and Solution Viscosity

The images and thirty measurements of nanofiber diameters with and without the phenolic compounds were obtained by scanning electron microscopy (SEM) (Jeol JSM-6610 LV, Japan). The viscosities of the polymer solutions were determined by means of a rheometer (Brookfield DV-III Ultra Programmable Rheometer, USA) from 0.5 ml samples of each solution.

3. RESULTS AND DISCUSSION

The formation of continuous nanofibers was possible using 35% PHB1 polymer solution under electrospinning conditions as diameter of 0.45 mm, feed rate of 150 $\mu\text{L/h}$ and electric potential of 15 kV. After defining the process conditions, was added 2.4% (w/v) of NaCl in the polymer solution, which reduced and uniformized the diameters of the nanofibers resulting in 814 ± 91 nm, due to the conductive character that this salt provides to the solution polymer promoting greater elongation of the nanofibers during the electrospinning process. In the solution, was also added 1% (w/v) of phenolic compounds were added, in which there was no increase in nanofiber diameter (810 ± 85 nm) (Figure 1).

In the studies performed with PHB2 solution and the electrospinning conditions described in the methodology, only the formation of nanofibers with droplets was obtained. This may be related to the concentration of polymer solution 30% (w/v) which affects the elongation of the jet during the process forming the chain break. These fragments cause the formation of droplets or nanofibers with droplets. In addition, PHB extraction method that involves the sodium hypochlorite reagent damages the polymer chains, breaking them into monomers, which makes it impossible to apply this biopolymer to electrospinning.

Solutions of 35% PHB1 and 35% PHB1 with 1% phenolic compounds showed viscosity of 2.5 and 2.4 Pa.s. Solution viscosity is a parameters that influences the determination of the diameter and uniformity of the nanofibers. High viscosities cause electrospinning process interruption, making it

difficult to eject the polymer solution jets and low viscosities result in the formation of drops or nanofibers with drops. Then, a suitable viscosity value or considered ideal for formation and uniform nanofibers is required (Venugopal et al., 2008).

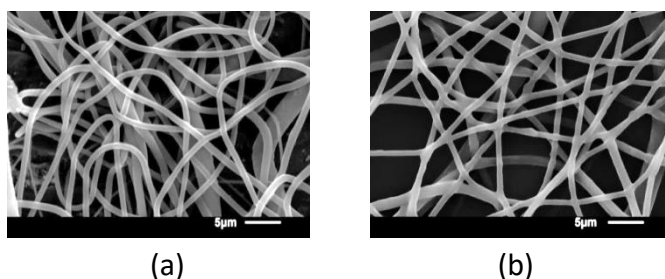


Figure 1. Nanofibers developed from 35% PHB1 and 2.4% NaCl (a) and 35% PHB1, 2.4% NaCl and 1% phenolic compounds.

4. CONCLUSION

The development of nanofibers was possible with solution 35% microalgal PHB, 2.4% NaCl and 1% phenolic compounds resulting in mean diameter of 810 ± 85 nm. Incorporation microalgal phenolic compounds confer the nanofibers antibacterial property and potential application as food packaging to increase the shelf life of the products.

5. REFERENCES

- Beachley, V., Wen, X., 2009. Effect of electrospinning parameters on the nanofiber diameter and length. *Mater. Sci. Eng.* 29.
- Folin, O.; Ciocalteu, V., 1927. On tyrosine and tryptophane determinations in proteins. *J. Biol. Chem.* 73.
- Kuntzler, S. G., 2017. Antimicrobial nanofibers for food application. Dissertação (Mestrado em Engenharia e Ciência de Alimentos) – Universidade Federal do Rio Grande. Rio Grande, 2017.
- Morais, M. G., Stillings, C., Dersch, R., Rudisile, M., Pranke, P., Costa, J. A. V., Wendorff, J., 2015. Extraction of poly(3-hydroxybutyrate) from *Spirulina* LEB 18 for developing nanofibers. *Polímeros* 25, 2.
- Souza, M. M., Prietto, L., Ribeiro, A. C., Souza, T. D., Furlong, E. B., 2011. Assessment of the antifungal activity of *Spirulina platensis* phenolic extract against *Aspergillus flavus*. *Ciênc. Agrotec.* 35.
- Venugopal, J., Low, S.; Choon, A.T., Ramakrishna, S., 2008. Interaction of cells and nanofiber scaffolds in tissue engineering. *J. Biomed. Mater. Res.* B 84, 34–48.