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UNLOCKING THE POTENTIAL: PITANGA FOR HIGH-YIELD AND LOW-COST BIOCELLULOSE PRODUCTION

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ABSTRACT

Recent advancements in biotechnology have instigated interest in the potential of bacterial nanocellulose (BNC) as a versatile material with superior properties compared to plant cellulose. However, the widespread application of BNC has been hindered by its high production costs, prompting the exploration of alternative, cost-effective culture media. Pitanga (*Eugenia uniflora*), a native Brazilian fruit, presents itself as a promising candidate due to its abundance of carbon sources and various organic compounds. Despite its potential, pitanga pulp has remained largely unexplored as a culture medium for BNC biosynthesis. The aim of this study is to develop a novel process for the production of BNC using pitanga pulp, thus offering a sustainable and economical alternative. BNC membranes using only pitanga pulp as culture media, retained key properties, such as high water retention capacity of around 98% and high porosity reaching 73%, mechanical strength, and preserved structural chemical composition. Thus, this study points to an innovative approach to producing BNC from a native Brazilian fruit, without adding other complex components to the media, guaranteeing a high yield and low cost, opening the way for broader applications of BNC in various industries.

Keywords: Bacterial cellulose. culture media. Myrtaceae. *Komagateibacter xylinus.* native fruits

1 INTRODUCTION

Cellulose stands as Earth's most abundant polymer, boasting a pivotal role in the global market [1]. Traditionally sourced from plants, it serves as a fundamental raw material across diverse industries. However, the chemical purification processes involved in its extraction often yield environmentally harmful effluents, undermining its renewable status [2]. In response to this challenge, bacterial nanocellulose (BNC) emerges as a promising alternative. Primarily synthesized by the species *Komagataeibacter xylinus*, BNC exhibits superior production capabilities from various carbon and nitrogen sources, offering a sustainable solution to mitigate environmental impacts [3–5].

BNC has numerous advantages, but the high cost of the fermentation medium, which comprises nearly 30% of the total production cost, has limited its industrial applications [6,7]. Conventionally, the standard medium employed for BNC production is the Hestrin Schramm (HS) complex medium, composed of glucose, yeast extract, peptone, disodium phosphate and citric acid [8]. However, the inclusion of costly components such as yeast extract and peptone significantly increase the production costs [9]. In this sense, many studies have focused on alternative sources for the culture medium in BNC production. The overarching objective is to curtail production expenses, increasing BNC yields, and repurpose agricultural and agro-industrial waste [10].

Fruit-based culture media present a promising alternative for the production of BNC, leveraging their rich content of sugars and bioactive compounds to facilitate BNC synthesis [11,12]. In Brazil, the Myrtaceae family's fruits hold significant economic potential, with many species, such as pitanga (*Eugenia uniflora*), finding application in the food industry [11]. Pitanga, renowned for its abundance of bioactive compounds and high antioxidant potential, serves as a valuable carbon source for BNC production. However, its perishable nature limits its direct commercial use.

In this sense, an innovative approach was proposed in this study, to evaluate the potential of a culture medium based on pitanga pulp for BNC synthesis. In addition to enhancing the economic prospects of native fruits, the production of BNC-based materials with fruit-derived properties into the BNC matrix could be an attractive alternative for the development of novel products with enhanced functionalities and applications.

2 MATERIAL & METHODS

The pitanga fruit (*Eugenia uniflora*) was obtained from the orchard of the Universidade Tecnológica Federal do Paraná - Dois Vizinhos Campus, where it underwent meticulous sanitization and pulping processes. The medium formulation was prepared with 500 g of fruit pulp with 1 L of distilled water, followed by liquefaction [12,13].Subsequently, the mixture was centrifuged for 15 min at 4,000 rpm. The resulting filtrate was passed through a mesh with a pore diameter of 1 mm before being subjected to autoclaving [12].The culture medium derived from the pitanga fruit pulp was stored at 7°C for further analysis.

For BNC production, the standard Hestrin Schramm (HS) medium was employed, with 20 g of glucose, 5.0 g of yeast extract, 5.0 g of peptone, 2.7 g of disodium phosphate (Na_2HPO_4) and 1.15 g of citric acid per liter of distilled water. The medium was sterilized in an autoclave for 20 min at 121°C [8].

To characterize the HS and pitanga media, total carbon and total nitrogen were determined using the Total Organic Carbon Analyzer, pH, phenolic compound content and antioxidant capacity using the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and FRAP (ferric reducing antioxidant power) assays [14–16].

For the production of bacterial nanocellulose (BNC), we utilized different plate sizes (6-, 12-, and 24-well plates) to characterize the membranes. Larger-scale production was carried out in 250 mL Erlenmeyer flasks, with the yield calculated accordingly. The membranes were inoculated with a 10% (v/v) pre-inoculum of *Komagateibacter xylinus* in the evaluated culture media and then incubated under static conditions in a BOD incubator at 30°C for 15 days. Following the incubation period, the produced membranes were removed and purified with a 0.1 M sodium hydroxide solution for 24 hours at 50°C, followed by seven washes in distilled water interspersed for 15 minutes at 50°C. Subsequently, the membranes were sterilized in an autoclave for 20 minutes at 121°C.

The yield was determined by considering the dry weight of the BNC membranes with the highest yield, with the analysis conducted in triplicate. To estimate the production cost of BNC membranes, the price of the components of the HS media and the average price of pitanga on a laboratory scale were calculated. The estimated cost of the media was related to the production of BNC in terms of the wet weight of the membranes produced under the six evaluated conditions. The membranes produced were assessed for their water retention capacity (WRC), membrane porosity, scanning electron microscopy (SEM), mechanical properties - stress-strain tests and Young's modulus and Fourier transform infrared spectroscopy (FTIR).

3 RESULTS & DISCUSSION

BNC membranes produced in the erlenmeyer flask using pitanga pulp medium (BNC-P) exhibited around 38.10±1.5 g (wet weight) and a thickness of 9.0±0.6 mm, while BNC membranes produced in HS medium (BNC-H) showed around 40.3±1.3 g (wet weight) and a thickness of 9.3±0.5 mm. Figure 1 ive medium using only pitanga fruit, without the use of other components in the medium, the yield result was extremely interesting and unexpected.

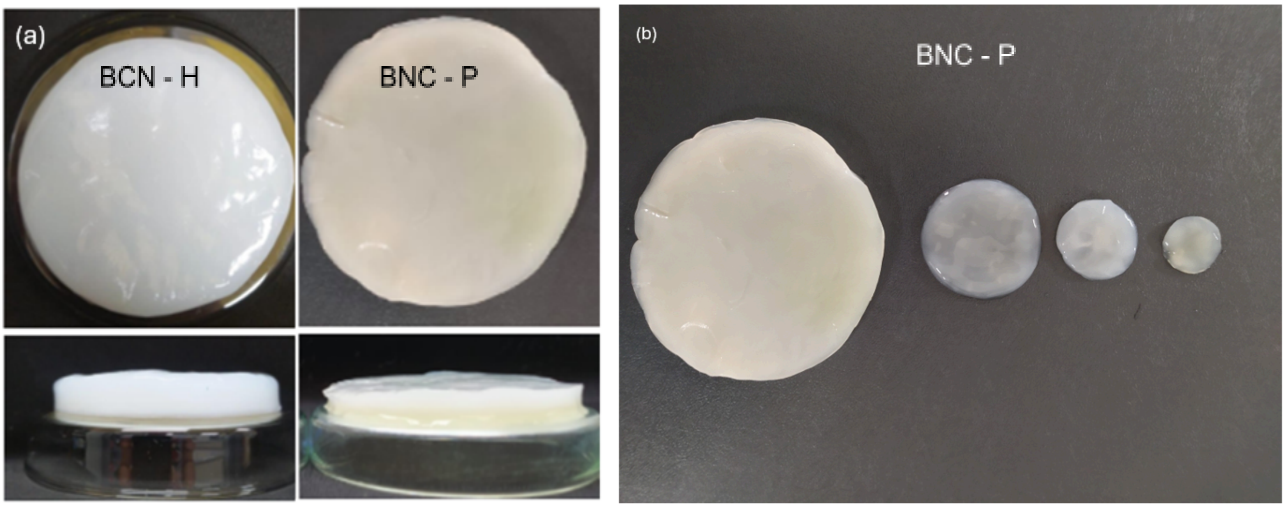

Figure 1 (a) Front and side view of BNC-H and BNC-P membranes (b) Different sizes of BNC-P membranes

The characterization of the HS and pitanga pulp media showed 23.66 g/L and 17.20 g/L of total carbon, respectively. Regarding total nitrogen, the HS medium contained 0.62 g/L compared to 0.11 g/L in the pitanga pulp medium. Despite the lower nitrogen content in the pitanga medium, it's noteworthy that this disparity did not significantly hinder the production of BNC membranes, indicating the prominent influence of the carbon source on bacterial growth and BNC production. Interestingly, despite its more acidic nature, with a pH of 5.80, the pitanga medium maintained favorable conditions for BNC production. This suggests the robustness of the bacterial strain in adapting to varying environmental factors and underscores the versatility of pitanga pulp as a potential medium for BNC synthesis.

The antioxidant capacity of the HS and pitanga pulp media was 1.46 mmol ET/L and 1.36 mmol ET/L, respectively for ABTS analysis; and 0.87 mmol Fe2+/L and 3.84 mmol Fe2+/L for FRAP analysis. These findings indicate that the h fruit pulp media exhibited notable antioxidant capacity through Folin-Ciocalteu, FRAP and ABTS methods, despite both media being subjected to a sterilization process in an autoclave at 121 °C for 20 min. Various parameters such as temperature, oxygen, lighting, pH and storage conditions can influence the degradation process of antioxidants [17].

Yield analysis was carried out using the dry weight of freeze-dried membranes produced in 250 mL Erlenmeyer flasks containing 100 mL of medium and incubated for 15 days. Accordingly, the yield of BNC-H was determined to be 5.96 g/L ± 0.07, while BNC-P exhibited a yield of 4.33 g/L ± 0.01. Notably, utilizing pure pitanga pulp medium allows for the BNC production in a wet state of approximately 381 g/L at an estimated average cost of R$18.02. Conservely, production with the traditional HS medium yields around 400 g/L, but at a higher estimated production cost of R$25.00 per liter of medium.

Overall, these results underscore the potential of producing BNC using pure fruit pulp without the need for chemical components from the traditional medium, offering a cost-effective, high-yield, and innovative approach.

Regarding membrane characterization, BNC-P membranes exhibited a water retention capacity (WRC) of 98.86±0.01%, while BNC-H membranes had a slightly lower water retention capacity of 98.56±0.04%. WRC correlates with the porosity of the BNC membranes, with BNC-P membranes displaying a porosity of 73.72±1.01%, compared to 73.36 ± 0.38% for BNC-H. Based on these results, BNC-P membranes maintained the excellent properties and characteristics, such as the porosity, which is important for the molecule incorporation (including drugs, nutrients and bioactive compounds) that provides the potential to develop new BNC-based materials [18]. In addition, porosity allows for cell growth, which has attracted a lot of attention in the field of tissue engineering [19].

4 CONCLUSION

This study has pioneered a novel approach to in situ BNC production process using pitanga pulp as a sole source, without peels and seeds. This strategy has not only facilitated robust bacterial growth but also yielded significant quantities of biocellulose surpassing yields achieved with the conventional HS medium. Remarkably, there is a dearth of prior records documenting the use of pure fruit pulp in BNC production at such impressive yields while preserving the physicochemical properties of BNC and lowering production costs. This breakthrough underscores the potential of pitanga pulp as a cost-effective and efficient medium for BNC synthesis, opening avenues for sustainable and economically viable biotechnological applications.

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