SUMMARY

of the doctoral thesis titled:

OBTAINING NANOCELLULOSE FROM KOMBUCHA CULTURES AND USING IT FOR MUCOADHESIVE FORMULATIONS

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KEYWORDS: nanocellulose, Kombucha, mucoadhesive formulations, plant extracts

Cellulose produced by symbiotic cultures of bacteria and yeasts (SCOBY, Symbiotic Culture of Bacteria and Yeasts), also known as Kombucha, is a biopolymer produced in the form of a film/membrane by acetic bacteria in SCOBY. The cellulose film/membrane from Kombucha cultures is a source of nanocellulose with superior properties compared to that obtained from plants. Nanocellulose is one of the most studied nanostructures due to its interesting properties: high mechanical strength, very large specific surface area, chemical stability, hydrophilicity, crystallinity, transparency, biocompatibility, electromagnetic sensitivity, proton conductivity, high surface chemical reactivity, and availability—cellulose being the most abundant renewable material. These properties make nanocellulose interesting for various applications in different fields. In the biomedical/pharmaceutical field, nanocellulose has multiple uses—dressings, ultrafiltration sterilization devices, controlled drug release systems, cell culture support, tissue regeneration, or 3D/4D bio-printing. In the food industry, nanocellulose is a functional food ingredient, food stabilizer, or additive in nanocomposites with various properties for food packaging.

The main goal of this work was to obtain nanocellulose from Kombucha cultures and use it for mucoadhesive formulations by utilizing some by-products from the bioeconomy and the use of SCOBY consortia. Specifically, the aim was to develop a technology for the industrial valorization of agro-industrial by-products to obtain nanocellulose for mucoadhesive formulations, alongside the production of nutraceuticals.

The research focused on achieving the following specific objectives:

1. Optimization of the biosynthesis process of Kombucha cellulose membranes, along with functional food/supplement products, by supplementing the cultivation medium with plant extracts and obtaining nanocellulose from cellulose membranes;
2. Development of mucoadhesive formulations for maintaining and rebalancing vaginal microflora and their physicochemical characterization;
3. Determination of the biological properties of the developed mucoadhesive formulations.

The theoretical part of the thesis consists of a literature review structured into three chapters concerning: (i) the sources and specific properties of nanocellulose and its potential applications; (ii) mucoadhesive formulations and their role; and (iii) the advantages of using bacterial nanocellulose for mucoadhesive formulations. The first chapter of the theoretical part describes three types of cellulose (microfibrillated cellulose, nanocrystalline cellulose, and bacterial cellulose), their sources and specific properties, and the potential to use Kombucha membranes as a source of bacterial nanocellulose. This

chapter also presents the uses of nanocellulose in various applications, including the plastics industry (creating composites and reinforcement materials), biomedical applications, the paper and packaging industry, and the electronic components industry. The second chapter discusses mucoadhesive formulations, describing mucous membranes and mucoadhesion, the interactions between mucoadhesive formulations and mucous membranes, and three types of mucoadhesive formulations: nasal, vaginal, and oral. The third chapter focuses on the advantages of using nanocellulose for mucoadhesive formulations.

The research section of the thesis is structured into three experimental chapters corresponding to the three specific objectives of the thesis. The first experimental chapter focused on optimizing the process of obtaining Kombucha cellulose membranes (and subsequently nanocellulose), along with functional food/supplement products, by supplementing the cultivation medium with quince leaf extracts. It was demonstrated that quince leaf extract can increase the crystallinity of the initial membrane and the bacterial nanocellulose obtained after microfluidization, while improving the interaction with mucin. Quince extract induces a more interconnected fibrillar structure of nanocellulose compared to the control. These changes are most likely due to modifications in the SCOBY consortium, where aerobic bacteria (acetic bacteria), anaerobic/microaerophilic bacteria (lactic bacteria), and yeasts fermenting carbohydrates coexist.

The second experimental chapter aimed to develop mucoadhesive formulations and characterize their physicochemical properties. This chapter involved scaling up Kombucha cultivation with quince leaf extract, developing new mucoadhesive formulations for maintaining and rebalancing vaginal microflora, ultrastructural characterization of hydrogels (using various microscopy techniques), physicochemical characterization by Fourier-transform infrared spectroscopy, X-ray diffraction, viscosity and mucoadhesion evaluation through rheological analyses, and quantitative determination of the mucoadhesion of mucoadhesive hydrogel systems. Three hydrogels were formulated, including two binary hydrogels (H1 and H2), based on never-dried bacterial nanocellulose and Poloxamer 407, and one ternary hydrogel (H3), made from bacterial nanocellulose, Poloxamer 407, and chitosan. The three hydrogels were loaded with plant extracts to create biocompatible mucoadhesive systems with antimicrobial activity, promoting the development of probiotic bacteria.

Chapter 3 presents the results of tests determining the biological properties of the developed mucoadhesive formulations. Following the characterization of the three hydrogels, it was found that hydrogel H2 was the most promising formulation, showing positive results in all aspects, including L929 cell proliferation, prebiotic effects, antimicrobial activity, and an optimal sol-gel transition temperature.

The final conclusions and perspectives chapter summarizes the conclusions of the studies conducted. The addition of antioxidant plant extracts improves several characteristics of nanocellulose produced from Kombucha membranes that are crucial for mucoadhesiveness. The resulting nanocellulose, maintained in a state of maximum swelling (never-dried), forms stable hydrogels with semi-natural (chitosan) or synthetic (Poloxamer 407) polymers. The formed hydrogels have good compatibility and a phase transition that favors application, being highly viscous at room temperature and less viscous after application to mucous membranes at body temperature. Adhesion to mucin and cytocompatibility of the hydrogels formed with nanocellulose produced from Kombucha membranes biosynthesized on media supplemented with antioxidant plant extracts are high. The resulting hydrogels also have a prebiotic effect, stimulating the growth of beneficial bacteria, and antimicrobial activity, blocking the growth of pathogenic bacteria.

The study's perspectives are described in direct relation to the two areas where original contributions were made: (i) optimizing/directing the biosynthesis of bacterial cellulose membranes in Kombucha cultures by modulating the antioxidant activity of the cultivation medium and (ii) using nanocellulose maintained in a state of maximum swelling (never-dried) as a multifunctional formulation ingredient, also enhancing the effects of bioactive components in various adhesive formulations.