

CHAPTER 1

INTRODUCTION

1.1 Rationale for the Research

Technological developments are accompanied by an increase in various material needs, both as raw materials and as intermediate products. One of the most needed materials today is cellulose-based products such as nanocellulose and various derivatives. Although it had decreased in the 20th century, the use of cellulose is now rising, owing to its natural abundance, renewability, biodegradability, and inherent biocompatibility, especially with the development of nanotechnology and characterization techniques such as isolating, modifying, and characterizing cellulose (Jiang et al. 2021).

Cellulose has been known for a long time—around 150 years ago—as a renewable and biodegradable polymer (Börjesson and Westman 2015). Current technological developments have succeeded in changing or modifying cellulose polymers into various derivative products, and these products are widely used in various applications such as coatings, films, membranes, new building materials, drilling techniques, pharmaceuticals, and food products (Börjesson and Westman 2015). In general, cellulose is one of the main components of lignocellulosic, which is mostly part of the plant cell wall along with other components such as hemicellulose, lignin, pectin, and wax (Mulyadi 2019). The composition of cellulose can reach one-third of the plant tissue and is the main component of several natural fibers such as cotton, flax, hemp, jute, and others (Moran et al. 2008). Apart from plant tissue sources, cellulose can also be obtained from other sources such as tunicates, algae, and bacteria. According to Kargarzadeh et al. (2017), several sources of cellulose other than

plants are (1) tunicates, namely a group of invertebrates from marine animals, especially from the subphylum of *Tunicate* group; (2) several types of algae such as green, red, gray, and brown algae; and (3) several species of bacteria that can produce cellulose, such as the bacteria *Gluconacetobacter xylinus*.

Bacteria may create cellulose in a fermentation process, such as when making kombucha. Kombucha is a fermented beverage with a long list of health advantages. As a by-product of kombucha production, a white pellicle layer (cellulose) known as bacterial cellulose (BC) is produced. Bacteria like *Acetobacter xylinum* can produce BC during their metabolism (Jasmania 2018). BC has a unique structure, a better degree of purity, and a higher mechanical property, to name a few advantages (Klemm et al. 2005). The unique characteristics and its potential application are the advantages of BC such as biodegradability and high purity (Ludwicka et al. 2020), high flexibility, high water holding capacity, hydrophilicity, high crystallinity, moldability, mechanical stability, etc. (Gorgieva and Trček 2019; Ullah et al. 2019; Lin et al. 2020; Choi et al. 2022).

BC has been used for many applications, including in the food industry. In the food industry, cellulose can be utilized as a low-calorie carbohydrate alternative, thickener, flavor carrier, and suspension stabilizer (Peng et al. 2011). A study reported as well on the application of BC in food industries such as food packaging, dietary food, thickening agents, and traditional desserts like nata de coco (Reiniati 2017), fat replacers, meat analogs, stabilizers of Pickering emulsions (PEs), rheology modifiers, and immobilizers of probiotics and enzymes (Azeredo et al. 2019a). Nanocellulose can extend food shelf life and improve food quality by acting as a carrier for active ingredients such as antioxidants and antimicrobials (Peng et al. 2011). So, in the nutraceutical product, there are such things as an emulsifier, the immobilization of bacterial and mammalian cells, the immobilization of enzymes, and the immobilization of bioactive agents (Khan et al. 2018).

BC has been produced and marketed commercially. One biotechnology company, *Polybion*, has completed the construction of the world's first BC biomanufacturing factory (www.prnewswire.com 2022). Even though it has been manufactured commercially, the synthesis of BC is claimed to be expensive due to the use of expensive medium components (Revin et al. 2018; El-Gendi et al. 2022; Kamal et al. 2022). Some studies have been reported about the effort of using low-cost substrates for BC production, such as agricultural waste or residual biomass (Waghmare et al. 2018; Akintunde et al. 2022) and industrial waste and by-product streams (Tsouko et al. 2015).

In the kombucha production process, BC is a by-product that has not been used optimally so far. This by-product can increase its value by being made into an intermediate product or a final product. Apart from only producing BC as a by-product, the kombucha fermentation method is actually very possible to apply to produce BC as the main product. BC production by applying this method can be an alternative for producing BC with low production costs. The substrate used in BC production media through kombucha fermentation generally only consists of sugar (sucrose), tea, water, and the addition of microbial cultures.

Thailand is well known for producing tea (Pongpruttikul and Yamsa-ard 2022). Thailand produces some types of tea, which are classified into four major groups based on the production methods and product used: green tea, black tea, oolong tea, and chewing tea (P. Winyayong 2007). Different types of teas show different nutritional or compositional values, such as protein, water content, ash, volatiles, caffeine, and minerals (Czernicka et al. 2017). As it is known, production of BC is affected by the nutritional value of the medium; thus, different types of tea may affect the yield and characteristics of BC. Therefore, an investigation of the effect of the tea on the yield and characteristics of BC is necessary. Once the tea is selected, it must also be selected for other factors such as the best carbon source, additives, initial pH of the medium, tea concentration, fermentation time, and cultivation technique. To get the optimal

conditions for BC production, the medium composition and environmental conditions must also be optimized.

Optimization of BC production is essential to improve yield and process efficiency, particularly when using alternative substrates or modified culture conditions. Response Surface Methodology (RSM) is a widely used statistical approach that enables the evaluation of multiple variables and their interactions in a systematic and efficient manner. Several previous studies have successfully applied RSM to optimize BC production, such as from agro-industrial waste materials (Singh et al. 2017), waste fig substrates (Yilmaz and Goksungur 2024), and by modifying the composition of the Hestrin–Schramm (HS) medium to enhance culture performance (Aswini et al. 2020). In this study, RSM was employed to optimize the production conditions of BC, based on selected key factors known to influence microbial cellulose synthesis.

Once the optimum production conditions were achieved, the next phase of this study focused on modifying BC. Although BC is a highly pure, biocompatible, and biodegradable biopolymer with excellent native properties, its structure often requires further modification to meet specific functional and industrial demands. Various physical, chemical, and enzymatic techniques have been explored to tailor its morphology and performance. Among these, high-pressure homogenization (HPH) and, in particular, high-pressure microfluidization (HPM) are effective physical methods used to reduce fiber size and alter surface characteristics, thereby improving material functionality (Wang et al. 2015; Mert 2020). These treatments can significantly influence properties such as crystallinity, surface area, and mechanical strength, with HPM offering particular promise, though its application to BC remains relatively underexplored.

In this study, HPM was employed as a mechanical modification method with the aim of investigating how the structural changes in BC affect its performance when incorporated into a food matrix. The resulting BC nanofibers (BCNFs) were integrated into a jelly candy (JC) formulation to assess their influence on product characteristics

such as color and texture. In addition, the jelly candies were analyzed to evaluate the BCNFs' potential role in preserving the bioaccessibility of bioactive compounds—specifically total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity—during simulated gastrointestinal digestion. This approach enabled a deeper understanding of how HPM-treated BC behaves when mixed into food systems, highlighting its potential as a functional ingredient.

1.2 Hypothesis of the Research

This study aims to explore the following hypothesis:

- 1) The use of different types of tea, carbon source combinations, and variations in additives such as ethanol, yeast extract, vitamin C, soy protein isolate, and puree coffee will affect the yield and characteristics of the BC produced.
- 2) The use of different pH, harvesting times, tea concentrations, and cultivation methods will affect the yield and characteristics of the BC produced.
- 3) Response surface methodology (RSM) can be used for optimization of the best formulation to increase the yield of BC production.
- 4) The treatment methods of the high-pressure microfluidizer will result in different characteristics of the BCNFs produced.
- 5) BC can be used in the formulation of jelly candy (JC) and can affect its properties.

1.3 Objective of the Research

The research aims to achieve the following objectives:

- 1) To obtain the best types of tea, carbon sources, and additives on the yield of BC production.

- 2) To obtain the best pH of culture medium, harvesting time, tea concentration, and cultivation methods on the yield BC production.
- 3) To obtain the optimum condition for BC production using response surface methodology (RSM).
- 4) To obtain BCNFs from BC under different treatments of high pressure microfluidizer.
- 5) To develop a jelly candy (JC) formulation containing BC and bioactive ingredients

1.4 Expected Results of the Research

The expected results from this study are:

- 1) Knowledge on the best type of tea, carbon source combination, and additive type for the yield and characteristics of produced BC.
- 2) Knowledge on the effects of pH, harvesting time, tea concentration, and cultivation methods on the yield and characteristics of the produced BC.
- 3) Obtaining the best formulation for increasing the yield of BC using response surface methodology (RSM).
- 4) Knowledge on the effect of high-pressure microfluidizer treatment on the characteristics of BCNFs.
- 5) A prototype of a jelly candy product containing BC and bioactive compounds.

1.5 Scope of the Research

BC was produced using the kombucha fermentation method. The microbial starter used in this study was a symbiotic culture of bacteria and yeast (SCOBY) purchased online from Neo Cold Brew Thailand, a local supplier of kombucha cultures

and related products. The SCOBY was used directly after purchase and regenerated through standard cultivation methods, without further microbial identification.

Several experiments were conducted to investigate the effects of different types of tea, additives, carbon source combinations, initial pH levels, fermentation time, tea concentrations, and cultivation methods on the yield and characteristics of BC. Optimization of BC production was performed using response surface methodology (RSM) to determine the best production conditions based on these variables.

After optimization, BC was produced under the best conditions and further used to study the effects of mechanical treatment for the production of bacterial cellulose nanofibers (BCNFs). BCNFs were obtained from BC pulp using a high-pressure microfluidizer method, and their characteristics were examined. The BC pulp and BCNFs were first applied in the development of a JC formulation to identify the best texture and color properties, aiming to achieve a texture similar to the control sample. The optimized JC formulation was combined with selected bioactive ingredients to assess the role of BC as an effective delivery carrier. Subsequently, simulated gastrointestinal digestion was conducted to evaluate the impact of digestive processes on the bioaccessibility of bioactive compounds within the JC matrix.

1.6 Organization of the Research

The research comprises seven (7) chapters, structured as follows:

- 1) Chapter 1: Research Principles and Justification, including Background Information, Research Hypothesis, Research Objectives, Expected Results of the Research, and Research Scope.
- 2) Chapter 2: Literature Review including overview of the Kombucha (Tea in Thailand and Kombucha fermentation), BC (BC biosynthesis, BC production methods, Factors affecting BC production, Production of BCNFs, and Application of BC in nutraceutical), and Response Surface Methodology (RSM).

- 3) Chapter 3: Investigation of Factors Affecting Bacterial Cellulose Production and Characteristics from Thai Tea Kombucha, including Tea Varieties, Carbon Sources, Additives, Initial pH, Harvesting Time, Tea Concentration, and Cultivation Methods
- 4) Chapter 4: Study to optimize BC production from Thai red tea kombucha using central composite design (CCD) in response surface methodology (RSM)
- 5) Chapter 5: Study to explore the effect of High-Pressure Micro-fluidization on the Characteristics of BC Produced from Thai Red Tea Kombucha
- 6) Chapter 6: Study to explore the effect of high-pressure micro-fluidization on the characteristics of BC produced from Thai red tea kombucha
- 7) Chapter 7: Conclusion and Recommendation

1.7 References

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