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The Potential of Liquid Waste from the Fruit Preserves Production Process as a Low-cost Raw Material for the Production of Bacterial Cellulose

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ABSTRACT

The liquid waste from the production of fruit preserves was used as an alternative carbon source to replace sugar in the traditional Hestrin-Schramm (HS) and coconut water media (CM) and reduce the cost of bacterial cellulose (BC) production. The sugar components of liquid wastes from preserved tamarind (LWT) and preserved mango (LWM) were characterized, and the total sugars were between 237.50 g/L and 231.90 g/L. The effects of the nutrients in the media with LWT and LWM on the production of BC by *Acetobacter xylinum* were determined. The result showed that *A. xylinum* could grow and produce BC in the media with liquid waste. The highest concentration of BC, 6.60±0.04 g/L, was obtained from the medium containing 25% (v/v) LWM. In a medium containing LWT, *A. xylinum* produced a maximum BC of 5.50±0.30 g/L when 12.5% (v/v) LWM was added. However, when the structure and physical properties of the BC from the liquid waste were characterized, it was similar to BC from the HS medium and CM medium without liquid waste.

Keywords: Bacterial cellulose, fruit preserve process, liquid waste, low-cost carbon source, mango, tamarind

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INTRODUCTION

Bacterial cellulose (BC) is the fiber produced by microbes, particularly by acetic acid bacteria, such as *Acetobacter xylinum* (Aswini et al., 2020; Kongruang, 2008) and the fiber component is the bound cellulose

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structure. However, BC differs significantly from plant cellulose because BC does not contain lignin or hemicellulose (Klemm et al., 2001; Rahman & Netravali, 2016). In the past, BC was produced using raw food materials with additional ingredients, resulting in cellulose with better mechanical properties, such as high-water storage capacity or expansion ability. The best-performing BC can be used to develop many products, such as packaging, artificial skin, film, and stabilizer (Cacicedo et al., 2016; Esa et al., 2014; Ruka et al., 2014). Currently, the production of BC needs to be augmented.

In general, BC fiber may be produced by the cultivation of bacteria through a fermentation process. Coconut water has been used as a carbon source for microbial growth to produce cellulose fibers. Currently, coconut water is of greater value. It has higher market demand because it is a food and can be applied to cosmetic products with high-value BC because the competition for raw materials is increasing. However, the current price of coconuts is increasing due to increased demand, which affects the cost of BC produced from coconut water. Many reports have found that BC can be produced from many raw agricultural materials (Akintunde et al., 2022; Azeredo et al., 2019), such as pineapple juice, coconut milk, and skim milk, can produce a BC product, but the odor is significantly down to the raw material to the product. Consequently, the raw materials that produce BC are diverse and can be found local to production (Costa et al., 2017; Thongwai et al., 2022), so it is important to consider the nutrients and the appropriate state for microbial development.

This study focused on liquid waste from the fruit preservation process. The fruit preservation process is a sweet fermentation (called “Chair Im”). After fermentation, most of the ingredients in the fruit are sugars; the liquid waste contains lactic acid (Olszewska-Widdrat et al., 2020). If waste contaminates rivers or reservoirs, it will result in an algal boom or eutrophication because liquid waste is a source of energy for environmental microorganisms (Vollstedt et al., 2020). The purpose of this study was to evaluate the liquid waste from the fruit preservation process in the production of BC. The result of the study is the feasibility of applying this waste or other local waste products to the continuous production of BC.

MATERIALS AND METHODS

Preparation of Inoculum

A bacterial strain of *Acetobacter xylinum* was obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Thailand. *Acetobacter xylinum* was transferred to Hestrin-Schramm medium (HS) (Hestrin & Schramm, 1954; Nguyen et al., 2022), which contained 20 g of glucose (SCHARLAU, Spain), 5 g of peptone (SCHARLAU, Spain), 5 g of yeast extract (SCHARLAU, Spain), 2.7 g of disodium hydrogen phosphate (Na_2HPO_4) (QRëC™, New Zealand), and 1.5 g of citric acid (SCHARLAU, Spain) per 1 liter (L) of media. The pH of the medium was adjusted to 4.2 and incubated at 30 °C for 3 days. After that, 10% (v/v) of the culture was transferred to a fresh HS medium within 48 hours of incubation. Then, the culture was used as the initial inoculum for BC production.

Production of BC

The liquid waste from the fruit preservation process consisted of waste from tamarind preserves (LWT) and mango preserves (LWM) was used throughout this study, presented in Figure 1. The raw material was collected from the Talad Thai market area of Pathum Thani Province, Thailand. The liquid waste from the fruit preserve process was first filtered through a cotton sheet, and some physicochemical properties of the liquid waste were characterized. Then, high-performance liquid chromatography (HPLC) used the supernatant for sugar content analysis. The total soluble solid content was measured using a refractometer, while the pH was measured with a pH meter. Then, the sample was kept at 20 °C to prevent microbial contamination. It was left at room temperature and sterilized before use.

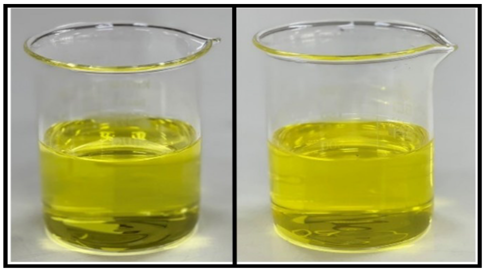

(A) (B)

Figure 1. Liquid waste from mango (A) and tamarind (B) preservation processes

The HS medium contains free sucrose: the addition of solid fruit waste compensates for the sucrose component in the experimental medium. The BC production of the medium utilizing liquid waste as a carbon source is compared with the BC production of the HS medium and the coconut medium (CM). The components and concentrations of the media containing LWT and LWM are presented in Table 1. After preparation, the medium was sterilized in an autoclave at 121 °C for 15 min, 10% (v/v) inoculum was added, covered with Whatman No. 1 filter paper, and incubated at 30 °C for 10 days to achieve static fermentation.

The Characterization of BC

After fermentation, the BC film was washed and soaked thoroughly in distilled water for 2–3 days. Then, it was boiled in 1% (w/v) sodium hydroxide solution (NaOH) (SCHARLAU, Spain) for 1 h and washed with distilled water until the pH measured about 7.0. Next, the wet weight of the BC film was determined. After that, the sample was dried at 60 °C until it reached a constant dry weight, and the moisture content of BC was calculated to investigate water loss after drying. Finally, the structure of the dried BC film has been characterized using Fourier transform infrared (FTIR) spectroscopic scanning between 4,000 and 400 cm^{-1} to explain a structure-function group of BC (Gea et al., 2011).

Table 1

Components and concentrations of media with LWT and LWM

Components	HS	CM	LWT												
			01	02	03	04	05	06	07	08	09	10	11	12	13
Glucose (g/L)	20	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Peptone (g/L)	5	5	5	5	–	–	5	5	–	5	–	5	–	–	–
Yeast (g/L)	5	5	5	–	5	–	5	5	5	–	5	–	–	–	–
Na_2HPO_4 (g/L)	2.7	2.7	2.7	2.7	2.7	–	–	2.7	–	–	2.7	2.7	2.7	2.7	–
Citric acid (g/L)	1.5	1.5	1.5	1.5	1.5	–	1.5	–	1.5	1.5	–	–	1.5	–	1.5
LWT (%v/v)	–	–	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Coconut water (L)	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
H_2O (L)	1	–	1	1	1	1	1	1	1	1	1	1	1	1	1
	LWM														
	HS	CM	01	02	03	04	05	06	07	08	09	10	11	12	13
Glucose (g/L)	20	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Peptone (g/L)	5	5	5	5	–	–	5	5	–	5	–	5	–	–	–
Yeast (g/L)	5	5	5	–	5	–	5	5	5	–	5	–	–	–	–
Na_2HPO_4 (g/L)	2.7	2.7	2.7	2.7	2.7	–	–	2.7	–	–	2.7	2.7	2.7	2.7	–
Citric acid (g/L)	1.5	1.5	1.5	1.5	1.5	–	1.5	–	1.5	1.5	–	–	1.5	–	1.5
LWM (%v/v)	–	–	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Coconut water (L)	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
H_2O (L)	1	–	1	1	1	1	1	1	1	1	1	1	1	1	1

Note. LWT = Liquid waste from tamarind preserve; LWM = Liquid waste from mango preserve; HS = Hestrin-Schramm medium; CM = Coconut medium

RESULTS AND DISCUSSION

Physicochemical Composition of Liquid Waste from the Fruit Preservation Process

Sour tamarind and sour mango are the most preserved fruits because of their sour taste, and this is a seasonal fruit that will have a great annual seasonal impact due to overconsumption. However, the tamarind and mango preservation process use as much as 70% sugar, so this study uses liquid waste from tamarinds and mangos as the raw material to produce BC. The LWT has a pH of 2.66, which is lower than the pH of LWM is 2.72. The total soluble solids of LWT contained 26.3 °Bx of total sugar (237.50 g/L), and the total soluble solids of LWM had 24.6 °Bx of total sugar (231.90 g/L). The sugar components of the liquid waste were in the order sucrose > glucose > fructose by HPLC analysis (Table 2). The ratio of sucrose, glucose, and fructose in the LWT was 20:1:1; the ratio was similar for LWM. However, the fermentation used to preserve food uses sugar, resulting in liquid waste with a high sugar concentration (Amit et al., 2017; Zahan et al., 2017).

Table 2

Composition of LWT and LWM

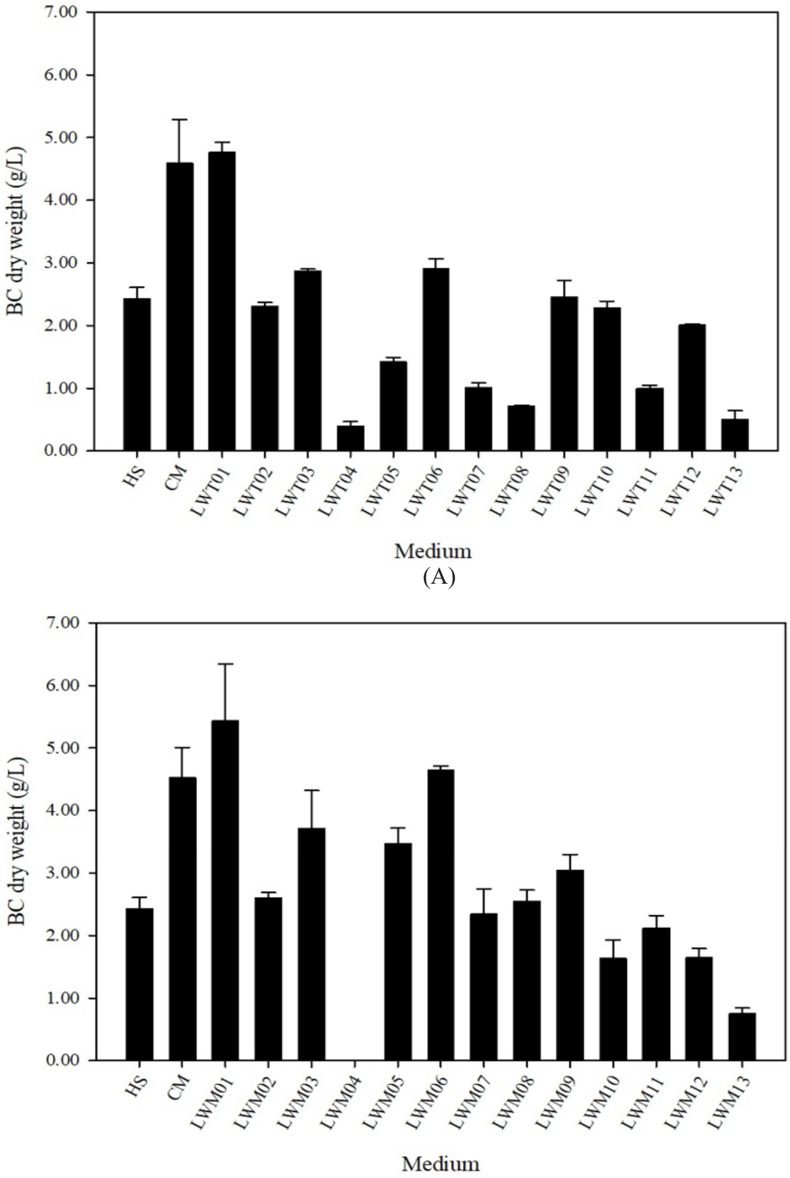
	Composition	LWT	LWM
Sugar (g/L)	Total sugar	237.50	231.90
	Fructose	10.40	11.40
	Glucose	10.50	10.50
	Sucrose	216.60	210.00
pH		2.66	2.72
Total soluble solid (°Brix [a])		26.3	24.6
Nitrogen		0	0

Note. [a]Degrees Brix (°Bx) is the sugar content of an aqueous solution; One degree Brix is 1 g of sucrose in 100 g of solution. LWT = Liquid waste from tamarind preserves; LWM = Liquid waste from mango preserves

LWT and LWM were potential carbon and energy sources for culturing *A. xylinum* for BC production. The HS medium used for BC production generally contains not less than 20 g/L of sugar. The liquid waste has high sugar: 8.5% (v/v) liquid waste was added to make a sugar concentration of 20 g/L in the medium. However, the minimum amount of glucose to support BC culturing was 5 g/L (Çakar et al., 2014; Chen et al., 2019; Nguyen et al., 2022).

Production of BC from Liquid Waste from the Fruit Preservation Process

The *A. xylinum* growth and BC production in LWT and LWM were better than in the HS and CM, and the *A. xylinum* produced a high yield of BC from LWT01 and LWM01, which produced an average dry weight of BC of 4.76±0.16 and 4.43±0.91 g/L, respectively. The organic and inorganic nitrogen and citric acid were added to the media, and the yield of BC dry weights is presented in Figure 2.



(A)

(B)

Figure 2. BC produced by *A. xylinum* in (A) LWT and (B) LWM (B), respectively

Then the LWT and LWM volume was adjusted to 8.5%, 12.5%, 17%, 25%, and 35% (v/v), with corresponding total sugar concentrations of 20, 30, 40, 60, and 80 g/L. Other components were added to the HS medium formulation before sterilization in the autoclave, inoculation with 10% (v/v) *A. xylinum*, and incubation at 30 °C for 10 days.

The BC production had a high yield of 6.60±0.04 g/L of dry weight in LWM 25%, and the carbon sources from LWT had potential BC products of 5.50±0.30 g/L when 30 g/L (12.5%) sugar was used in the medium. However, in the medium, BC cannot be produced at sugar ratios of 25% and 35% or sugar concentrations of 60 g/L and 80 g/L.

Increasing the sugar concentration of the medium from 20 g/L or adding more than 8.5% liquid waste to the LWT and LWM media increased the BC. However, in LWM, the fiber of bacteria decreased when the sugar concentration was increased to 80 g/L, or 35% liquid waste was added. The BC cannot be produced in the media containing LWT with sugar concentrations of 60 g/L (25%) and 80 g/L (35%). Perhaps the elevated sugar concentration affects growth because of the inhibition of the substrate (Dikshit & Kim, 2020; Kouda et al., 1998; Väljamäe et al., 2001). The yield of BC is presented in Figure 3.

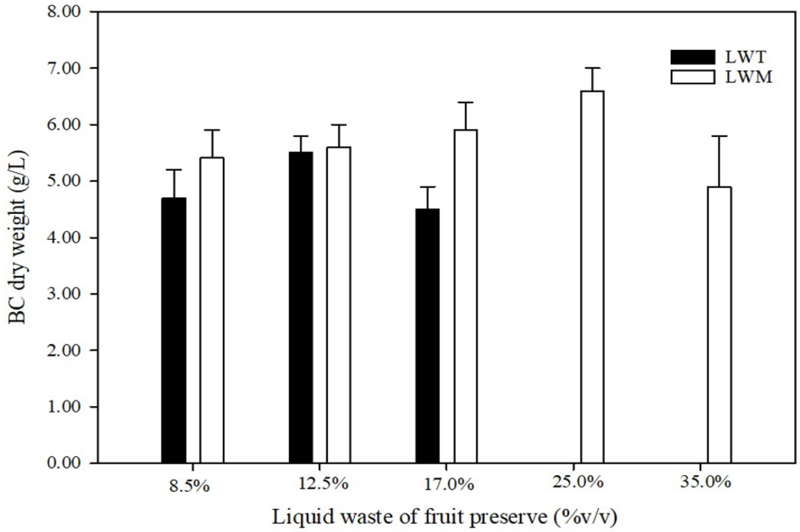


Figure 3. BC produced by *A. xylinum* and bacteria strain in HS medium liquid waste of fruit preserve between LWT and LWM

The pH of LWT and LWM decreased after the BC was harvested (Table 3). Photographs of the washed BC produced are presented in Figure 4(A), and the dry BC is presented in Figure 4(B). The moisture content of BC ranged between 96–97%; this result means that the fiber structure of BC can hold large amounts of water (Rebelo et al., 2018; Schrecker & Gostomski, 2005).

Table 3

The volume of cellulose produced and properties of fiber from LWM and LWT

Liquid waste	Volume (% v/v)	Total concentration in liquid waste (g/L)	pH		% Moisture content
			Initial	Final	
LWM	8.5	20.0	4.2	3.94	96.68
	12.5	30.0	4.2	3.97	97.24
	17.0	40.0	4.2	3.91	96.68
	25.0	60.0	4.2	3.87	97.21
	35.0	80.0	4.2	3.71	97.17
LWT	8.5	20.0	4.2	3.85	96.64
	12.5	30.0	4.2	3.97	97.23
	17.0	40.0	4.2	3.91	96.52
	25.0	60.0	4.2	3.72	0.00
	35.0	80.0	4.2	3.63	0.00

Note. LWT = Liquid waste from tamarind preserves; LWM = Liquid waste from mango preserves

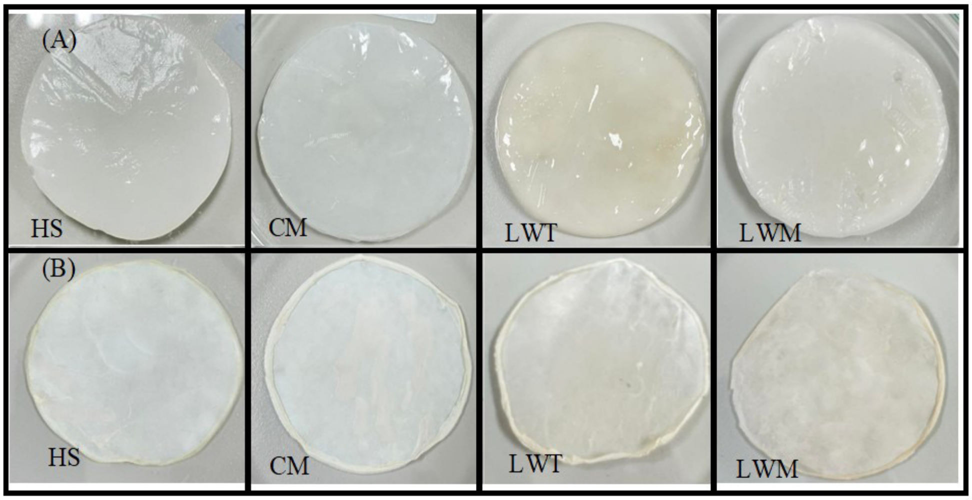


Figure 4. (A) Wet and (B) dry BC produced from *A. xylinum* in different media

The FTIR spectra of BC produced from LWT and LWM were similar to those of BC from HS and CM, presented in Figure 5. The peak between 665–670 cm^{-1} is assigned to the out-of-plane with a carbon-hydrogen bond (C-H), bending the carbon bond of carbon to the hydroxyl group (δ(C-OH)). The peak at about 1,111 cm^{-1} is assigned to the alkanes stretch (Ʋ(C-C)) ring group of polysaccharides and cellulose (Kacuráková et al., 2002; Movasaghi et al., 2008), and the peaks at 900 cm^{-1} and about 1249 cm^{-1}

are assigned to the carbonyl group (C=O) and ether (C−O−C) stretching of glucose (Carrillo et al., 2004; Wong et al., 2009). The peaks at 3,278 and 3,345 cm^{-1} are assigned to stretching oxygen-hydrogen bond (Ʊ(O-H)) of cellulose I (Moharram & Mahmoud, 2007).

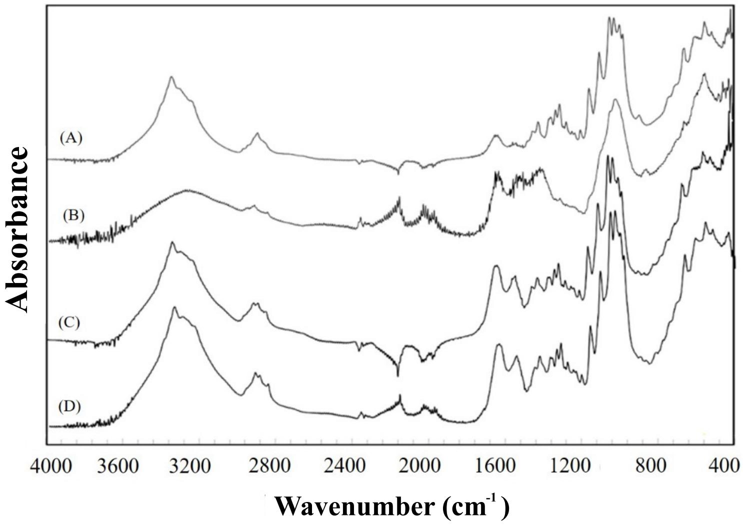


Figure 5. FTIR spectra of BC films produced from *A. xylinum* in (A) HS medium, (B) CM, (C) LWT, and (D) LWM

CONCLUSION

Liquid waste from the fruit preservation process can be used as the raw material for *A. xylinum* growth and the production of BC because it contains mainly sugar compounds. Moreover, the BC produced from this liquid waste has a structure similar to that of the BC produced from coconut water. Therefore, liquid waste has the potential as an alternative carbon source in BC production to reduce costs. The sugar ratio of LWT and LWM is similar, sucrose > glucose > fructose (20:1:1), and the sugar concentration in the liquid waste can be mixed in the medium for BC production. The results of this study show that *A. xylinum* grew and produced BC in LWT and LWM higher than in HS and CM. The BC production had the highest yield in LWM, 25%. The BC cannot be produced in a medium containing LWT with a sugar concentration over 60 g/L (25%), possibly because the high sugar concentration affects the growth due to substrate inhibition. However, the moisture content of the resulting BC ranges between 96–97%; the fiber structure of this BC can hold a large amount of water. This study is initial research for using waste from agricultural production to add value and decrease waste. In the future, it is important to continue developing uses for BC in the industrial sector.

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