Characterization of Bacterial Cellulose (Nata de coco) from Lychee

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Abstract. Bacterial cellulose or Nata de coco is the polysaccharide produced from \textit{Acetobacter xylinum} grown in coconut water. This research was investigated the possibility to obtain bacterial cellulose from lychee. The optimum ratio of lychee juice and coconut water and sugar concentrations needed for producing bacterial cellulose were studied. The ratio of lychee juice to coconut water was set to 0:1 (control), 1:2, 1:1, 2:1 and 1:0 with 10\% \textit{A. xylinum} starter culture and 12\% sugar. The results showed that optimized ratio of lychee juice to coconut water was 2:1 which thickness and firmness were 1.2+0.2 centimeter and 284.9+15.6 N, respectively. The bacterial cellulose from lychee contained 98.44\% moisture content, 0.53\% ash and 2.62\% fiber with average overall liking scores at moderate level. When compared the addition of sucrose into the medium at 10\% and 12\% for bacterial cellulose production. Results showed no correlation between the thickness and sugar used. This research demonstrated that lychee juice mixed with coconut water at 2:1, with 10\% \textit{A. xylinum} starter culture and 10\% sugar, potentially produce the bacterial cellulose.

Keywords: Bacterial cellulose, \textit{Acetobacter xylinum}, Lychee, Coconut

1. Introduction
Nata de coco is a chewy, jelly-like food, contain high cellulose content and considered as a type of dietary fiber food stuffs. This product is a common dessert in Thailand and other Southeast Asia countries. Nata de coco, a bacterial cellulose, is produced from the fermentation of coconut water by \textit{Acetobacter xylinum} \cite{1}. Bacterial cellulose (BC) can produce from a wide range of carbon and nitrogen sources including fruit juices (coconut, pineapple, oranges, apples) and vegetal extracts (from black tea, green tea, litchi) \cite{2}. Fruits are characterized by their amount of sugars, low pH and quantities which enable to be used for alternative feedstock for production of BC. Yang et al. \cite{3} showed the lychee extract produced 2.53 g/L of the BC membrane after 2 weeks of static fermentation. In Thailand, lychee (\textit{Litchi chinensis} Sonn.) which originated in south China are commercially grown in the northern region and considered as the fourth largest lychee producer in the world \cite{4}. Lychee is a subtropical fruit with high commercial value, due to its slightly acid taste, semi-translucent white aril, excellent aroma and attractive red skin \cite{5}. However, marketing of lychee is constrained by the perishable nature of the fruit, and consequently have a short shelf life. As a result, it is necessary to added value by processing of lychee. The over production of lychee makes it suitable for the production of BC, since its inexpensive, good sources of carbon and nitrogen. Their use could also
significantly reduce the waste from postharvest loss of lychee. Therefore, lychee could be the low-cost carbon substrate for produce safe and good quality of BC which could be possible commercial uses of this biopolymer.

2. Methods

2.1 Starter culture preparation
Seeding inoculum of bacterial strain *Acetobacter xylanum* subsp. *Xylum* TISTR 975 was obtained from Institute of food research and product development (Kasetsart University, Thailand). The starter medium was prepared from 1,000 mL of filtered coconut water, 5% glucose, 0.1% Na₂HPO₄ and 1% acetic acid. After autoclaved at 121°C for 15 min, 10% ethanol was added into the medium. The cultivation was initiated by inoculating 10% of seeding inoculum into glass bottle contain 200 mL medium. The inoculated media were incubated at room temperature (28-30°C) for 5 days until the BC pellicles appear. These media were further used as a starter culture for BC production.

2.2 Production of bacterial cellulose from lychee medium

2.2.1 Effect of lychee/coconut volume ratio
Fresh lychee fruits (*Litchi chinensis* Sonn.) cv. Hong Huay at commercially mature stage were peeled and pitted. Lychee juices were obtained by homogenizer and filtered through cheesecloth. The ratio of lychee juice to coconut water was set to 0:1 (control), 1:2, 1:1, 2:1 and 1:0. The medium was adjusted total sugar to 12%, 0.1% (NH₄)₂SO₄ and pH 4.5 before autoclave at 121°C for 15 min. After cool to room temperature, 10% *A. xylinum* starter culture were added in sterile condition. The medium was left for 10 days at room temperature. The sheet of BC produced was harvested followed by cut into cubes of equal dimensions and boiled in water for 5 min. The BC cubes were immersed in water overnight with repeated changing of water to remove the sour odour.

2.2.2 Effect of sugar supplement
Experiment was carried out to investigate initial sucrose concentration for BC production. The optimum ratio of lychee juice to coconut water was supplemented with sucrose to 10% and/or 12%. BC production was conducted as same as the above method.

2.3 Analysis of BC

2.3.1 Physiological quality
Color of BC cubes were recorded as L* (brightness), a* (red-green) and b* (yellow-blue) using a ColorQuest XE (Hunter Associates Laboratory Inc., USA). The hardness of BC cubes was determined by texture analyzer (TA-XT plus). Thickness of BC sheet was measured using vernier caliper.

2.3.2 Chemical quality
The moisture content (%w/w) was determined based on the weight loss of the samples when dried under hot air oven. Crude ash and fiber were determined by AOAC method [6]. Reducing sugar was determined by dinitrosalicylic acid (DNS) method.

2.3.3 Sensory evaluation
Hedonic evaluation was carried out by 30 untrained panels. Color, odor, flavor, texture and overall acceptability of BC cubes were evaluated by the 7- and 9-point Hedonic scale from 0 (extremely disliked) to 7 (very like) and 9 (extremely liked) for the 1st and 2nd experiment, respectively.

2.4 Statistical analysis
The expressed values are means ± standard deviation of triplicate replication. The differences between treatment means were determined by Duncan’s multiple range test at p<0.05 by the SPSS statistics version 18.
3. Results and Discussion

3.1 Effect of lychee/coconut volume ratio

The effect of lychee juice/coconut water ratio on BC production was carried out at 0:1, 1:2, 1:1, 2:1 and 1:0 as shown in Table 1. After 10 days cultivation, the maximum thickness of BC production was 1.63 cm when coconut water (control) was employed as a media followed by the mixture of lychee juice and coconut water with 1.23 cm thickness. In contrast, the BC produced in lychee juice media was only 0.5 cm in thickness. This indicates that lychee juice alone as a fermentation media was not appropriate for BC production.

Maximum and minimum hardness was seen for the BC cubes from the coconut water (control) and lychee juice as a fermenting medium, respectively. The hardness of BC cubes from 1:2 and 2:1 lychee/coconut ratio were slightly lower than that in control.

The moisture content of BC cubes from lychee and coconut water media were range from 97.68-98.44%.

The ash of the BC cubes from medium containing high ratio of lychee juice (1:1, 2:1 and 1:0) was much higher than that obtained in coconut water (0:1 and 1:2).

Table 1. Effect of lychee/coconut volume ratio on physicochemical properties of BC cubes.

| Lychee/coconut | Thickness (cm) | Hardness (N) | Moisture (%) | Crude ash (%) |
|----------------|----------------|--------------|--------------|--------------|
| 0:1 (control)  | 1.63 ± 0.06a   | 512.53 ± 42.63a | 97.68 ± 0.05 | 0.05 ± 0.01a |
| 1:2            | 1.23 ± 0.12b   | 269.27 ± 27.92c | 98.39 ± 0.09 | 0.06 ± 0.01a |
| 1:1            | 1.23 ± 0.15b   | 117.13 ± 11.27b | 98.35 ± 0.39 | 0.11 ± 0.05b |
| 2:1            | 1.23 ± 0.06b   | 284.87 ± 15.62c | 98.44 ± 0.22 | 0.18 ± 0.02c |
| 1:0            | 0.50 ± 0.01a   | 33.93 ± 3.21a   | 98.21 ± 0.82 | 0.12 ± 0.01b |

Values are the mean ± SD (n=3); different letters indicate significant differences by Duncan test at P<0.05.

The lightness (L*) of BC cubes trended to decrease with the ratio of lychee medium (Table 2). However, there were no significant difference of BC cubes color (L* and a*). The b* value (yellow-blue) were significantly different among the lychee/coconut volume ratio.

Table 2. Effect of lychee/coconut volume ratio on color (L*a*b*) of BC cubes.

| Lychee/coconut | L* (lightness) | a* (red-green) | b* (yellow-blue) |
|----------------|----------------|----------------|------------------|
| 0:1 (control)  | 53.51 ± 0.90a  | -2.50 ± 0.16    | -8.87 ± 0.43a    |
| 1:2            | 51.70 ± 1.09a  | -2.27 ± 0.17    | -7.78 ± 0.46ab   |
| 1:1            | 48.53 ± 7.38a  | -2.29 ± 0.38    | -7.46 ± 0.25b    |
| 2:1            | 51.30 ± 2.02a  | -2.06 ± 0.27    | -7.59 ± 0.69b    |
| 1:0            | 49.38 ± 1.64a  | -2.06 ± 0.10    | -7.91 ± 0.93ab   |

Values are the mean ± SD (n=3); different letters indicate significant differences by Duncan test at P<0.05.

The results obtained on sensory evaluation in terms of color, odor, flavor, texture and overall liking is presented in Table 3. From the results, color and flavor scores of BC cubes prepared from higher ratio of coconut water was more appreciated by consumers. This could be attributed to the result of color measurement (L*a*b*) from Table 2. On the other hand, BC cubes from higher ratio of lychee juice exhibited more appreciated odor. Average scores of overall linking ranged from 4.55 to 5.60 in the 7-point Hedonic scale. BC cubes prepared from lychee/coconut volume ratio at 2:1 was the preferred sample, followed by the ratio at 1:1 and 1:2. Thus, the 2:1 lychee/coconut volume was selected from the sensory and physicochemical properties for further study.

Table 3. Effect of lychee/coconut volume ratio on sensory properties of BC cubes.

| Lychee/coconut | Color | Odor | Flavor | Texture | Overall linking |
|----------------|-------|------|--------|---------|----------------|
| 0:1 (control)  | 6.15 ± 1.76b | 4.05 ± 1.19a | 5.65 ± 1.52b | 5.65 ± 1.63 | 5.40 ± 1.43ab |
| 1:2            | 5.45 ± 1.85ab | 4.00 ± 1.75a | 4.75 ± 2.10b | 5.10 ± 1.74 | 5.15 ± 1.95ab |
| 1:1            | 4.50 ± 1.73a | 3.50 ± 1.54a | 3.55 ± 1.50a | 5.20 ± 1.64 | 4.55 ± 1.43a |
2:1  5.45 ± 1.47<sup>ab</sup>  5.10 ± 1.21<sup>b</sup>  4.85 ± 0.99<sup>b</sup>  5.60 ± 1.33  5.60 ± 0.82<sup>b</sup>
1:0   4.55 ± 1.79<sup>a</sup>  5.15 ± 1.76<sup>b</sup>  4.40 ± 1.88<sup>ab</sup>  5.00 ± 2.60  4.55 ± 1.54<sup>a</sup>

Values are the mean ± SD (n=3); different letters indicate significant differences by Duncan test at P<0.05.

3.2 Effect of sugar supplement

The effect of sugar supplemented to the culture media was investigated by culturing A. xylinum in the 2:1 lychee/coconut medium with adjusted to 10 and 12ºBrix by adding sucrose. Sucrose concentration of 12ºBrix was found to maximize the thickness, fiber and reducing sugar of BC (Table 4). However, there were no significant difference of thickness, fiber and sensory evaluation (Figure 1) of BC produced from 10 and 12ºBrix medium.

Former study of Phong et al. [7] on sugar level have been reported to have no advantage on cellulose production when the level greater than 5%. The decrease in cellulose yield at high initial glucose concentration could due to some glucose metabolized to gluconic acid which inhibited cellulose production [7]. On the contrary, if initial glucose concentration supplemented was low, there were not enough carbon sources for production of cellulose by bacteria. In the present study, the sucrose concentration of 10% proved to produce appropriate BC. This agree with the studied of Jagannath et al. [8] that maximum thickness of nata was obtained at pH 4.0 with 10% sucrose concentration. They also noted that different sugars produced optimal cellulose yields at different pH values.

Table 4. Effect of sugar supplement on physicochemical properties of BC cubes.

| Initial brix | Thickness (cm) | Hardness (N) | Moisture (%) | Fiber (%) | Reducing sugar (mg/100 mL) |
|--------------|----------------|--------------|--------------|-----------|----------------------------|
| 10º          | 1.77 ± 0.06    | 221.70 ± 10.73 | 97.62 ± 0.33 | 2.18 ± 0.01 | 0.57 ± 0.01<sup>b</sup> |
| 12º          | 1.90 ± 0.01    | 207.73 ± 42.96 | 97.08 ± 0.08 | 2.62 ± 0.08 | 0.86 ± 0.01<sup>a</sup> |

Values are the mean ± SD (n=3); different letters indicate significant differences by Duncan test at P<0.05.

From our studies, lychee juice mixed with coconut water potentially use as a substrate for BC production. This support by the studied of Yang et al. [3] who produced BC from lychee extract. They found that lychee extract produced an ultrafine network nanostructure BC and had higher crystallinity of 94.0% than that from HS medium. The trace elements including magnesium (Mg) and sodium (Na) in the lychee extract were partly absorbed in the BC membrane [3].

4. Conclusion

The results demonstrate the promising advantages of using lychee as a low-cost carbon source for BC production. The present study shows that lychee juice mixed with coconut water at 2:1, with 10% A.
xylinum starter culture and 10% sugar, potentially produce the bacterial cellulose. Nevertheless, further studies are needed to study the supplement of nitrogen and phosphate sources to improve the productivity of BC from lychee.

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