CULTURE CONDITION OF BACTERIAL CELLULOSE FORMATION IN ROTTEN PINEAPPLE (*Ananas comosus*) FOR NATA DE COCO PRODUCTION

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Introduction

Coconut (*Cocos nucifera)* is a multipurpose plantation crop found in tropical and subtropical regions of the world. The liquid endosperm of the fruit is called coconut water and it is a waste product in coconut processing industries. Things have been changed with popularity of production of nata de coco by using wasted coconut water. Nata de coco is a bacterial cellulose (BC), produced by *Acetobacter xylinum* by fermenting coconut water. It is a chewy, translucent product which in high in cellulose, low in fat and calories, and no cholesterol. Therefore, it has potential to control weight, protect against colon and rectum cancer. Unlike the cellulose from wood pulp, the BC has a high purity, unique strength, an ultra-fine structure, and it is biodegradable. Isolation and purification of BC are also simple. Therefore, new trends have been evolved using bacterial cellulose, such as skin therapy, producing wound care products, artificial blood vessels and paper manufacturing. Current production of Nata de coco needs 15 to 20 days of undisturbed fermentation to produce 1.5 cm layer of Nata. It is considerably long time duration for production when it comes to demand and supply.

Rotten fruits are the main media for *Acetobacter xylinum* and pineapple (*Ananas comosus*) is most popular one. It is a major fruit crop in Sri Lanka and large quantities of its fruit component are wasted. There is a high chance of utilizing agricultural waste such as pineapple parts and coconut water for development of industries. Starter culture for nata de coco production was not readily available in Sri Lanka to produce nata de coco for the world market. Thus, this study was conducted to identify suitability of pineapple fruit component for starter culture preparation and identify the maximum production of nata de coco in coconut water medium.

Methodology

Starter culture media for *Acetobacter xylinum* were prepared by using different fruit component of pineapple to identify the most effective part for the starter culture growth. Pineapple fruit was washed with clean water. Peels and flesh were separated and cut in to small pieces. Pineapple peel (A), mix of pineapple peels and flesh (B) and pineapple flesh(C) were used to prepare three different media. Water was added into fruit component in 2:1 ratio. Brix of the solution was adjusted 14 with table sugar. Nitrogen source was supplied by adding 5% $(NH_4)_2SO_4$. The pH of the solutions was adjusted to 4 with glacial acetic acid. Sterilized jam bottles were used as the container for starter culture and they were covered by sterilized muslin cloths to create the aerobic condition. Each treatment was placed under the clean static environment. To evaluate the 14 days' culture condition, 14 samples were arranged with three replicate for three treatments to supply static condition for starter culture growth. Temperature, pH, ^{0}bx,

total sugar of each treatment was determined up to 14 days. Identification test of *Acetobacter xylinum* was done for each treatment by Hestrin and Schramm (HS) medium. Starter culture (0.1 mL) was inoculated in to 5 mL of HS broth and incubated at 30 ^{o}C for 7 days. After 7 days, pellicle containing media was selected and it was streaked on GEY agar media and incubated at 30 ^{o}C for 2 days. Isolated colony was inoculated to 5 mL of HS broth and incubated at 30 ^{o}C for 7 days. Pellicles containing media were confirmed for BC by boiling with 0.5 M NaOH for 15 min.

Mature coconut water was obtained from desiccated coconut factory at Katana. Coconut water was boiled 30 min and cooled to room temperature. The surface pellicle and pineapple pieces of starter culture of different treatment were removed and inoculated at 1:5 (starter culture: coconut water) ratios into transparent trays. Brix of the all the solutions were adjusted to 14 with table sugar. Nitrogen source was supplied by adding 5% $(NH_4)_2SO_4$. The pH of the solutions was adjusted to 4 with glacial acetic acid. Three replicates were maintained and they were covered with muslin cloth and allowed to stand for 14 days in static environment. After 14 days' heights of nata de coco layer was measured and pellicles were conformed for BC. Harvested nata de coco layers were washed four times with distilled water and it was boiled 0.5 M NaOH for 20 minutes. The samples were repeatedly washed with distilled water four times and were dried in an oven at 80 ^{o}C up to constant weight for dry weight analysis. All treatments were arranged according to the complete randomized design (CRD). SAS software package was used to analyze the data quantitatively.

Results and Discussion

Translucent gel layer was observed on top of the pineapple peel containing media and it is positive for *Acetobacter xylinum* conformation test and BC test. However, gel like layer was not observed on top of the pineapple peel with flesh and pineapple flesh containing media and starter culture of these treatments wasn't positive for *Acetobacter xylinum* conformation test.

There is a significant difference ($p<0.05$) among three treatments for total soluble solids. Total soluble solids of starter culture decreased within 14 days (figure 1). Starter culture containing pineapple flesh with peel (B) showed a rapid decrease of total soluble solids from 4th to 5th day compared to A and C. Starter culture containing pineapple peel had the highest total soluble solids level at the final stage. According to the observation bacterial cellulose forming microorganisms can be survived between 5 to 14^{0} bx level.

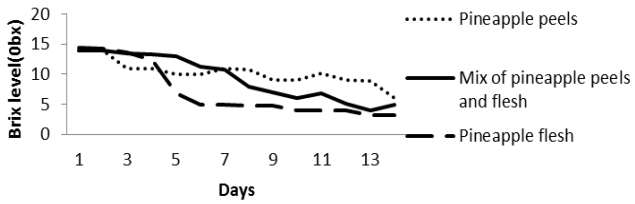

Figure 1. Changing pattern of brix level during 14 days

There is a significant difference ($p<0.05$) among three treatments for total sugar. Total sugar of starter culture decreased during 14 days (figure 2). A rapid decreasing pattern of total sugar is clearly shown by the starter culture of pineapple flesh from 1st day to 5th

day. Starter culture containing pineapple peel has significantly higher final total sugar concentration compared to other treatments. Results revealed that, bio cellulose forming microorganisms has shown the lowest sugar utilization than the other microorganisms in B and C media.

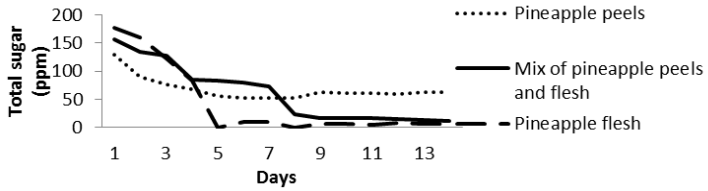

Figure 2. Changing pattern of total sugar during 14 days

There is a significant difference ($p<0.05$) among three treatments for pH values during the 14 days. pH value of starter culture media of all the treatments decreased over time (figure 3). A rapid decrease can be clearly observed from the starter culture of pineapple flesh with peel from 2nd day to 4th day. Starter culture containing pineapple peel has medium pH level compared to other treatments.

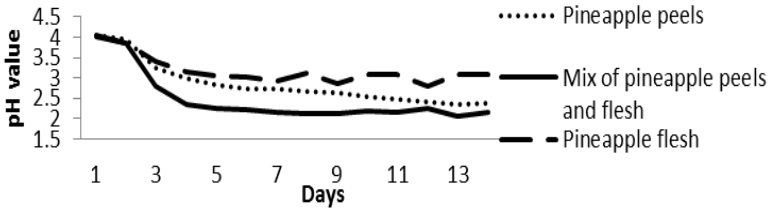

Figure 3. Changing pattern of pH during 14 days

Starter culture which was produced from pineapple peel produced a 1.45 cm thickness and 14.72% dry matter containing nata de coco layer after introducing to the coconut water medium. However, nata de coco layers were not observed on top of the media obtained from pineapple peel with flesh and pineapple flesh. The media containing pineapple flesh is not a good for existing of the microorganism. Pineapple peel which is a waste material in fruit industry is a good substrate for the growth of the bacteria forming bacterial cellulose.

Conclusions

Fruit component of the pineapple directly affected to the starter culture growth. Pineapple peel was the best fruit part that could be used for nata de coco starter culture preparation. Bacterial cellulose forming microorganisms can be survived 5 to 14 ^{0}bx level and medium pH (3-2.7) containing pineapple peel starter culture media. The pineapple peel starter culture produced a gel layer on top of the pineapple peel when it has bacterial cellulose forming microorganisms.

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