Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XIX, Issue 1- 2020, pag. 92 - 97

EFFECT OF $(NH_4)_2SO_4$ ON SOME QUALITIES OF NATA DE PINA PRODUCED BY *ACETOBACTER XYLINUM*

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Received 10th March 2020, accepted 30th March 2020

Abstract: *Pineapple juice (Ananascomosus L. cv. Pattavia) has high amounts of sugar, making it potential to be produced into Nata.The study will enable efficient utilization of pineapple juice and will also provide a new product dimension to the pineapple farmers who are not getting the right price for their product. The objectives of this research were to study the various contents of $(NH_4)_2SO_4$on total soluble solids, pH, % ethanol, thickness and yield of Nata de pina from pineapple juice (Ananascomosus L. cv. Pattavia) fermented by Acetobacter xylinum. $(NH_4)_2SO_4$ was used as a nitrogen source and its contents for Nata de pina production at 30°C for 15 days were 0.3, 0.4 and 0.5 % w/v. Increasing 0.3 - 0.5 %w/v $(NH_4)_2SO_4$ contents improved to utilize total soluble solids and % ethanol of Nata de pina and the lowest pH of Nata de pinawas obtained from using 0.5 % w/v of $(NH_4)_2SO_4$. Using 0.5 %w/v of $(NH_4)_2SO_4$led to the highest values of thickness and yield of Nata de pina after 15 days which were 12.14 mm and 100.02 g, respectively.*

Keywords: *$(NH_4)_2SO_4$, Nata, Acetobacter xylinum*

1. Introduction

Nata de coco, a kind of bacterial cellulose is an indigenous dessert well known in Thailand, which is produced from coconut water fermented by *Acetobacter xylinum*. After a period of the fermentation, a layer of gelatinous sheet is formed on the surface of the fermented coconut water. The sheet is cut into cubes. As a dessert, the washed cubes are usually served with flavoured syrup, jelly or other fruit cocktails. Presently, Nata de coco is manufactured at an industrial scale not only in Thailand but also in Indonesia and some are exported to countries like Japan [1].

A. xylinum is widely found in nature and is a common contaminant in the industrial production of vinegar by *A. aceti*. *A. xylinum* has been isolated from rotting fruits, vegetables and fermenting coconut water. Many strains of *A. xylinum* are able to produce cellulose in varying amounts and growing on a wide variety of substrates, such as glucose, sucrose, fructose, invert sugar, ethanol and glycerol [2]. Cellulose production by *A. xylinum* has been noted both in static as well as agitated cultures [3] and is known to be affected by the type and concentration of sugar, nitrogen source and pH [4]. Commonly, Nata de coco is produced by coconut water fermentation with *A. xylinum*, the substrate

is changed to pineapple juice which itis interesting since changing the substrate might affect specific characteristics of Nata such as texture, flavor, taste, color and others.

In addition, nitrogen source is an important factor for microorganism growth, which can influence on cellulose production [5].Ramana et al.[6]reported that the effect of nitrogen sources on cellulose membrane production by *Acetobacter xylinum*was evaluated. The strain was able to utilize a wide range of protein and nitrogen sources such as $(NH_4)_2SO_4$, peptone, soybean meal, glycine, casein hydrolysate, and glutamic acid for cellulose synthesis. It was found that $(NH_4)_2SO_4$ could produce higher yields of bacterial cellulose compared with other nitrogen sources. Moreover,Jagannath[7]studied the effect of $(NH_4)_2SO_4$concentrations of 0.25 - 0.50 %w/w on the production of Nata de coco, a form of bacterial cellulose, by *Acetobacter xylinum*. Maximum thickness of nata was obtained 0.5 %w/w of $(NH_4)_2SO_4$. This $(NH_4)_2SO_4$ concentration also produced the good qualities of Nata de coco with a smooth surface and soft chewy texture.

However, there are no studies of the various contents of $(NH_4)_2SO_4$ on total soluble solids, pH, % ethanol, thickness and yield of Nata obtained from pineapple juice (*Ananascomosus*L. cv. Pattavia) fermented by *Acetobacter xylinum*. Therefore, Nata de pineapple production by pineapple juice supplemented with $(NH_4)_2SO_4$ is expected to be a new product, which might be specific characteristics unlike Nata de coco product. The present work used pure cultures of *A. xylinum* to obtain a consistent product and will benefit the pineapple based farmers and communities to produce good quality Nata de pinawith superior physical properties.

2. Matherials and methods

2.1 Maintenance of *A. xylinum*

Acetobacter xylinum (TISTR 975) culture obtained from Thailand Institute of Scientific and Technological Research (TISTR), Thailand was maintained on tomato agar slants. Two hundred grams of fresh tomatoes and 500 ml of distilled water were boiled for 30 min. This tomato infusion was filtered and mixed with 100 g yeast extract, 50 g sucrose, 2.5 g peptone and 20 g agar. The volume was made up to 1,000 ml with distilled water and sterilized at 121^oC for 15 min. *A. xylinum* was streaked on these slants and incubated at 30^oC for 4 days.

2.2 Preparation of Nata de pina starter

A. xylinum grown on tomato agar slants was inoculated into sterilized media containing 20 g^{-l} glucose, 5 g^{-l} yeast extract, 5 g^{-l} peptone and 2.7 g^{-l} K_2HPO_4. The original glucose medium byWatanabe&Yamanaka[2]was modified to exclude citrate and the pH adjusted to 4.2 with glacial acetic acid. The inoculated media was incubated statically at 30^oC for 7 days.

2.3 Preparation of pineapple juice medium

Pineapple juice (*Ananascomosus*L. cv. Pattavia) was obtained from pineapple fruits purchased locally in Phitsanulok, Thailand. The juice was steamed for 30 min in a laboratory autoclave, then supplemented with varying amounts of $(NH_4)_2SO_4$(0.3, 0.4 and 0.5 % w/v) , 6 %v/v of ethanol 95 % and adjusting the pH 5 with glacial acetic acid. The surface pellicle from the agar slants were removed and inoculated (10%w/v) into 2,000 ml the medium taken in trays (L x B x H:30 x 25 x 6 cm).

The trays were covered with muslin cloth and allowed to stand for 15 days.

2.4 Harvesting and processing of Nata de pina

The sheet of Nata de pinaformed after 15 days was harvested when it was about 0.8 - 1.0 cm thick, washed repeatedly with water to remove glacial acetic acid and cut into cubes of equal dimensions. The cut cubes of Nata were immersed in water for 24 h with repeated changing of water to remove the sour odour.

2.5 Thickness and yield

The thickness of the Nata layer was measured with a micrometer. This was done in 5 different points of the surface of the Nata. The average data obtained were used. The yield of Nata de pina was measured in weight (g) [8].

2.6 pH and ethanol concentration

The pH of the samples was measured by using a pH meter (Thermo Orion model 420, USA). The measurement was carried out in triplicate [9]. Ethanol concentration was measured using an ebulliometer (Alla model 99002-ca, France)[8].

Total soluble solids (TSS)

The Nata was used for extracting juice with a commercial juice extractor. Filtered supernatant juice was used for determination of TSS. TSS was measured by a digital refractometer (ATAGO PR-101, Tokyo, Japan).

2.7 Statistical analysis

All determinations were performed in triplicate and results were expressed as the mean $\pm$ standard deviation calculated using spreadsheet software Microsoft Excel. This was carried out in a completely randomized design (CRD) which the data were analyzed by an analysis of variance ($p \leq 0.05$) and means were compared with Duncan's multiple range test. The results were processed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for Windows.

3. Results and discussion

3.1 TSS, pH and % ethanol of Nata de pina

TSS and ethanol valuesof Nata de pina produced by all percentages of $(NH_4)_2SO_4$decreased throughout the fermentation, affecting pH decreased as well (Fig. 1) . This might be a cause of using reducing sugars or carbon sources, appeared in pineapple juice, by *A. xylinum* in order to be substrates for the production of gluconic and ketogluconic acid[10]. Increasing in the range of 0.3 - 0.5 %w/v of $(NH_4)_2SO_4$ promoted carbon and nitrogen sources utilization by *A. xylinum*. Furthermore, among different $(NH_4)_2SO_4$percentages, the organism preferred 0.5 %w/v of $(NH_4)_2SO_4$in the presence of pineapple juice during the fermentation. On day 15, TSS value of Nata de pinaproduced by 0.5 %w/v of $(NH_4)_2SO_4$ was used 2.52 times; however, TSS value of the control (no addition of $(NH_4)_2SO_4$)was used 1.40 times.Ramana et al.[6]confirmed that among various nitrogen sources evaluated, $(NH_4)_2SO_4$was found to be a suitable substrate of *A. xylinum* along with one of the carbon sources such as sucrose/glucose or mannitol.

3.2 Thickness and yield of Nata de pina

Increasing 0.3 – 0.5 %w/v of $(NH_4)_2SO_4$ affected higher values of thickness and yield of Nata de pina, but thickness and yield of Nata de pina decreased when 0.7 %w/v of $(NH_4)_2SO_4$ added to pineapple juice (Fig. 2) . In the present study, 0.5 %w/v of $(NH_4)_2SO_4$ gave the highest

values of thickness and yield of Nata de pina produced in the end of experiment which were 12.14 mm and 100.02 g, respectively, indicating higher than those of Nata de coco (8.6 mm of thicknessand 61.3 g of yield)[7]. This was in agreement with Yamanaka et al. [11] who studied 0.5 %w/v of $(NH_4)_2SO_4$affecting to maximize the thickness of Nata produced. This result indicated thatpineapple juice as a medium was adjusted to pH 5 with acetic acidwhich was suitable for Nata de pina. This isin disagreement withJagannath et al.[7]whomodified to exclude citrate and adjusted to pH 4.2 with acetic acid was suitable for Nata de coco produced by *A. xylinum*. The use of acetic acid to bring down the pH of the juice has a better effect as compared to other acids on the growth of *A. xylinum* and Nata de pina formation [11].Vandamme et al. [12] opined that the role of acetic acid was that of an in situ control of pH. Acetic acid breaks down to CO_2 and water generating extra ATP and thereby leading to more efficient utilization of sugars for cellulose synthesis.

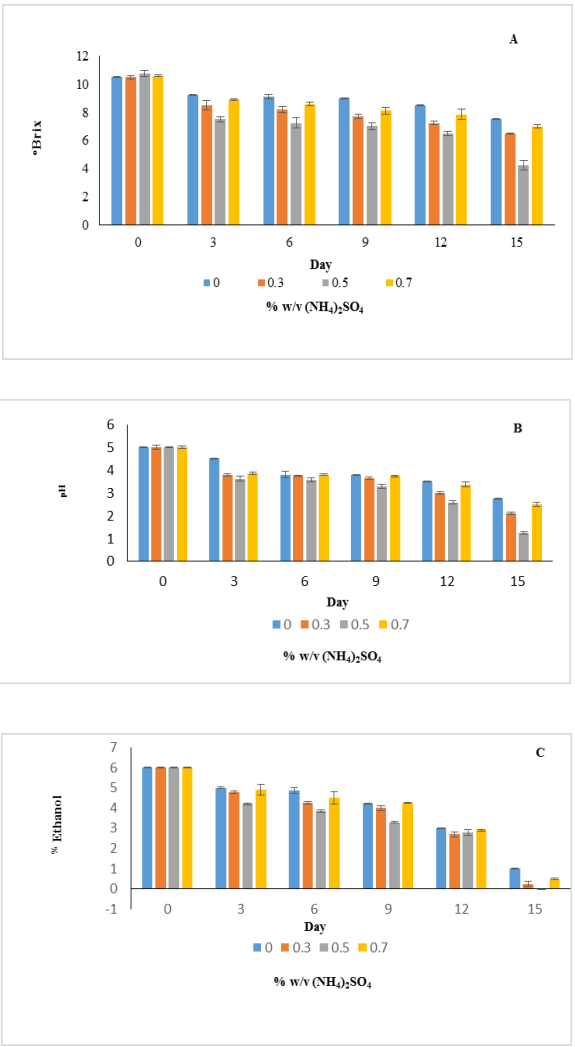

Fig. 1 TSS (A), pH (B)and % ethanol (C) of Nata de pina supplemented with various amounts of $(NH_4)_2SO_4$, α = 0.05.

Further the bacteria were able to grow equally well in media containing an appropriate concentration of $(NH_4)_2SO_4$as the nitrogen source. The bacteria were able to make this transition effectively as evidenced by the good yield of Nata de pina obtained. However, the precursor in cellulose synthesis is uridine diphosphoglucose and hence bacterial cellulose production involves use of sucrose or glucose as a carbon source for growth and polysaccharide formation [12]. These conditions also positively influenced on the quality of Nata de pina in terms of water holding capacity and hardness. Thickness has a direct effect on the amount of water that Nata can hold which in turn affects the softness of the final product. Hence, Nata has high hydrophilicity which shows significant water holding capacity as well [7].

Moreover, in this experiment, ethanol was added in pineapple juice, which is a significant factor in the production of cellulose by *A. xylinum* [13]. As well, pineapple juice adjusted to pH 5 played an important role in the production of Nata cellulose (Fig. 1,2) .Budhiono et al.[14] reported that pH 5 of the pineapple juice medium had a significant effect on the thickness of Nata produced.

Verschuren et al. [10] confirmed that oxygen concentration also influenced on Nata formation.
Therefore, the growth of *A. xylinum* in trays should be adequate aeration even under static conditions. The work ofVerschuren et al. [10] has established that the first stage of cellulose fermentation isentirely oxygen controlled. When the dissolved oxygen in the medium is used up, bacteria existing only in the vicinity of the surface can maintain their activity and produce cellulose in the form of gel.

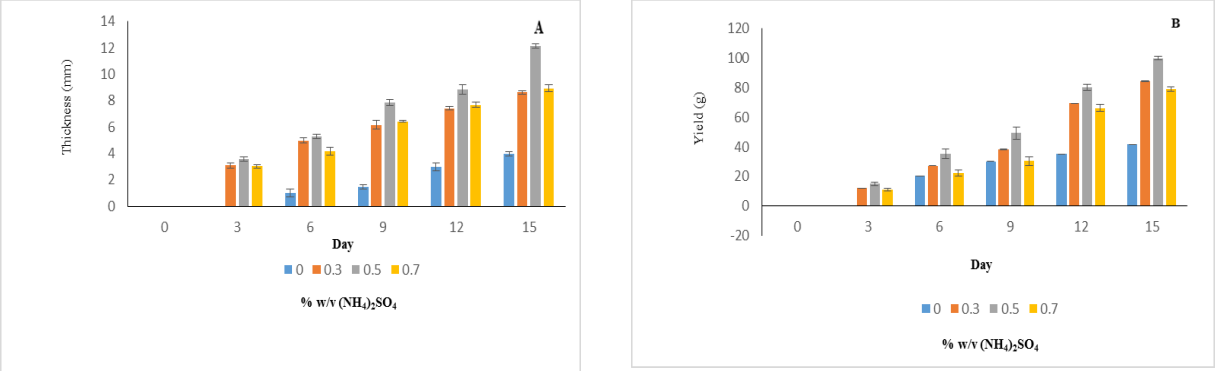

Fig. 2 Thickness (A)and yield (B) of Nata de pina supplemented with various amounts of $(NH_4)_2SO_4$, $\alpha = 0.05$.

4. Conclusion

Addition of 0.5% w/v of $(NH_4)_2SO_4$ led to benefits in production of Nata from pineapple juice by giving better yield, thickness since *A. xylinum* could use more ethanol and TSS. Bacterial cellulose in the form of Nata de pina is virtually indigestible because of its high dietary fiber content. Many health benefits like prevention of colon cancer, heart attack, and increase in blood pressure or hypertension have been attributed to Nata [15]. This work will be database for Nata production from other kinds of fruits to give a new product dimension to the general farmers who are not getting proper price for their produce.

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