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Preparation the Substrate from Palmyra Palm Fruit by *Candida stellimalicola* Fermentation for Acetic Acid Production

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Abstract

The ripe palmyra palm or toddy palm (*Borassus flabellifer* Linn.) has the yellow-orange pulp with proximate pH 4.47-5.1 and total soluble solid 5.01±0.15 °Brix. In this research, the yeast *Candida stellimalicola* isolated from the ripe palm fruit pulp was used to ferment the palm fruit juice to ethanol and applied into acetic acid production. The effects of ammonium sulphate as the nitrogen source and sugars (glucose and sucrose) as the carbon source on ethanol fermentation were investigated. The ammonium sulphate concentrations at 300, 500 and 700 mg/L significantly increased the produced ethanol content ($p<0.05$). The supplementation of 500 mg/L ammonium sulfate gave the highest ethanol content at 5.75±0.09% after 7 days. The palmyra palm fruit juice was adjusted into 10 °Brix and 15 °Brix by sucrose and glucose addition for fermentation at 30°C and shaking condition within 7 days. There was a significant difference in the ethanol content between glucose and sucrose ($p<0.05$). In addition, 10 and 15 °Brix glucose showed the ethanol content with 5.43±0.13 and 5.40±0.44%, respectively ($p>0.05$). Whereas, using 10 °Brix sucrose showed the ethanol content with 1.82±0.45 and closely to 15 °Brix (1.70±0.41%). For scale up at 6 liters, the fermentation of palmyra palm fruit juice with 10 °Brix glucose and 500 mg/L ammonium sulfate supplementation was carried out at room temperature (31±2°C) and 300 rpm/min. As the results, the ethanol content was approximately 3.92±0.15% after 14 days. In the future this product will be used for acetic acid production.

Keywords : *palmyra palm fruit, ethanol, Candida stellimalicola, glucose, ammonium sulfate*

1. Introduction

Palmmyra palm or toddy palm (*Borassus flabellifer* Linn.) is a kind of palm. It is tropically found in many provinces in Thailand (Phetchaburi, Supanburi, Songkhla, Ayudthaya, Kanchanburi, NakhonPatham and Pattani). The mesocrape or sweet pulp has yellow-orange colour that can be used in several foods such as kanomtaan, ice cream and cake. Additionally, palm fruit also was a substrate for *Saccharomyces cerevisiae* to produce alcohol (Theivendirarajah and Chrystopher, 1986).

In order to produce vinegar, fermentation process includes two stages: alcoholic fermentation by yeast,followed by acetification by acetic acid bacteria. For alcoholic fermentation, different fruits such as apple, banana, grape, coconut water strawberry and cherry, can be used as the fermentable substrates. In this research, palmyra palm fruit juice was used as a potential substrate for ethanol production by the yeast *C. stellimalicola* isolated from palmyra palm fruit juice. The effects of carbon sources and nitrogen sources supplemented in the juice for the high ethanol production were investigated.

2. Materials and Methods

2.1 Palmyra palm fruit ripe preparation

Palmyra palm fruit ripe was harvested in Pattani province in southern of Thailand. The fruits were washed in tap water and peeled before extraction. The pulp was hand-extracted in water (the ratio of pulp:water= 1:2) until the pulp was dissolved completely to obtain palmyra palm fruits juice. The palmyra palm fruits juice was pasteurized at 65^{o}C for 5 min (Leuangnapa, 1990) and subsequently stored in refrigerator at –20^{o}C until use. The pH value and total soluble solid (TSS) were measured by a digital pH meter (Sartorius, Germany) and a refractometer (Atago, Tokyo, Japan), respectively. Acidity was estimated by titration with 0.1 NaOH (AOAC, 2000). The minerals of palmyra palm fruits juice were analyzed by Scientific Equipment Center, Prince of Songkla University

2.2 Yeast culture

C. stellimalicola was isolated from palmyra palm fruit ripe. The pure culture was inoculated in yeast extract peptone dextrose broth (YEPD broth; yeast extract 10 g, bacteriological peptone 20 g, D-glucose 20 g in 1000 ml distilled water) incubated at 30^{o}C for 24 h and stored at 4^{o}C.

2.3 Ethanol *production experiments*

2.3.1 Starter culture preparation

For starter culture, a full loop of *C. stellimalicola* was inoculated into 200 ml palmyra palm fruits juice adjusted to 10 ^{o}Brix with glucose. This culture was incubated on rotary shaker with 110 rpm at 30^{o}C for 18-24 h.

2.3.2 Effect of nitrogen sources on ethanol production

Palmyra palm fruit juice was standardized at 15 ^{o}Brix with glucose. Ammonium sulphate [$(NH_4)_2SO_4$] was supplemented at different concentrations of 0, 300, 500 and 700 mg/l. The starter culture 10% starter culture (v/v) was inoculated into 600 ml substrate that subsequently incubated at 110 rpm, 30^{o}C for 7 days. The sample was taken out every day to measured ethanol content by ebulliometer.

2.3.3 The effect of glucose and sucrose as carbon sources on ethanol production

Palmyra palm fruit juice was standardized at 10 and 15 °Brix by glucose and sucrose. The starter culture 10 % (v/v) was inoculated into 600 ml palmyra palm fruit juice containing ammonium sulphate 500 mg/L. The fermentation was carried out for 7 days at 30°C and 110 rpm. The ethanol content was daily measured by ebulliometer.

2.3.4 Scaling up

The starter culture 10% (v/v) was inoculated into 6 liters palmyra palm fruit juice with the TSS 10 °Brix by glucose and ammonium sulphate (500 mg/L) supplementation. The fermentation was carried out for 14 days at room temperature and 300 rpm/min. The ethanol content was measured every 2 days by ebulliometer.

2.3.5 Statistical analysis

The experimental results were expressed as mean±standard deviation (SD) of triplicate. Completely randomized design (CRD) was applied in this experiment and data was presented by average means±standard error. Duncan's multiple range test used for comparison the means between samples with the statistical level of significant was set at $p \leq 0.05$.

3. Results and Discussion

3.1 Chemical property of palmyra palm fruits juice

The mature palm fruit pulp or mesocrape are shown in Figure 1. The palmyra palm fruit juice has the TSS 5.1±0.15 °Brix, acidity 0.53±0.02, pH 4.47-5.1, while mineral compositions are shown in Table 1. The TSS and minerals depend on the ripeness of the fruits. The TSS and mineral content in the palmyra palm fruit juice could be considered as the carbon source and mineral for yeast consumption in ethanol production.

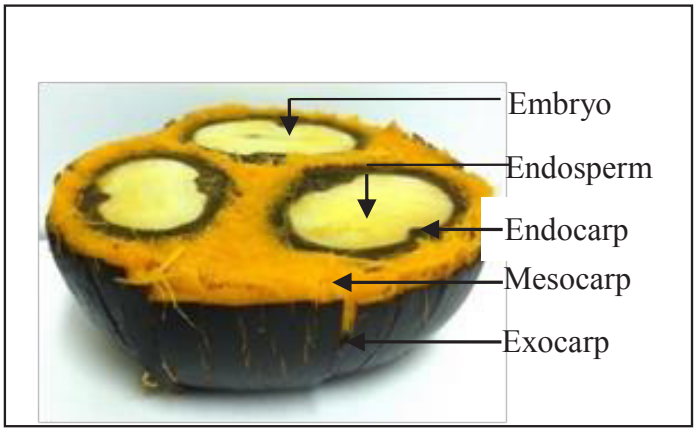

Figure 1. The palmyra palm fruit ripe.

Table 1. Chemistry property from palmyra palmfruit pulp.

Chemistry property	Results
Total soluble solids (TSS)	5.10±0.15
pH	4.47-5.1
Acidity (10%)	0.53±0.02
Trace element (mg/kg)	
Copper	< LOQ
Calcium	21.79±0.37
Iron	< LOQ
Magnesium	40.16±0.67
Potassium	845.24±5.34
Phosphorus	22.00±0.27
Sodium	51.05±3.92
Zinc	< LOQ

3.2 Ethanol production

3.2.1 Effect of ammonium sulfate

C. stellimalicola, an anamorphic yeast, was isolated from palmyrah palm pulp to apply in alcoholic fermentation of the palm juice. The effects of ammonium sulphate concentrations at 0, 300, 500 and 700 mg/L as the nitrogen source on ethanol fermentation are shown in Figure 2. The increase in ammonium sulphate concentrations from 0, 300, 500 and 700 mg/L (w/v) significantly increased the produced ethanol content (p<0.05). The maximum ethanol contents were 1.90±0.15, 3.00±0.00, 5.75±0.09 and 5.84±0.05%, respectively within 5 to 7 days. In addition, the ethanol concentration in the juice supplemented with 500 and 700 mg/L of ammonium sulphate were not significantly difference (p>0.05). (Figure 2) Thus, the supplementation of 500 mg/L ammonium sulfate gave the highest ethanol content at 5.75±0.09% within 7 days.

The nitrogen source and mineral are essential for the cell growth (Bafrncova *et al*., 1999; Pramanik and Rao, 2005). The increase in nitrogen source enhanced the efficiency of wine fermentation. Ethanol fermentation at low nitrogen source showed low ethanol content (Coleman *et al*., 2007). Ammonium sulphate and ammonium dihydrogen phosphate are good nitrogen source for wine fermentation. (Nantitanon, 2006)

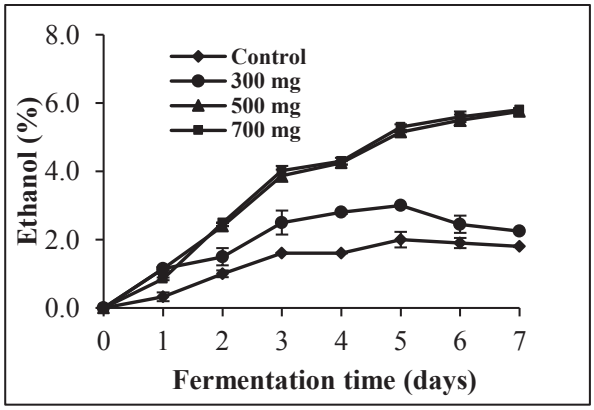

Figure 2. The effect of $(NH_4)_2SO_4$ on ethanol production in palmyra palm fruits juice with 15% (w/v) glucose for 7 days by *C. stellimalicola*.

3.2.2 Effect of carbon sources

The effect of glucose and sucrose was investigated. In ethanol fermentation, glucose and sucrose had significant difference in ethanol concentration (p<0.05). Indeed, 10 and 15^o Brix of glucose showed the ethanol content at 5.43±0.13 and 5.40±0.44%, respectively (p>0.05). Whereas, using 10 and 15 oBrix of sucrose showed the ethanol content at 1.82±0.45 and 1.70±0.41%, respectively (p>0.05). (Figure 3). C. *stellimalicola* produced the highest ethanol (5.43±0.13%) at 10^o Brix palmyra palm fruit within 7 days.

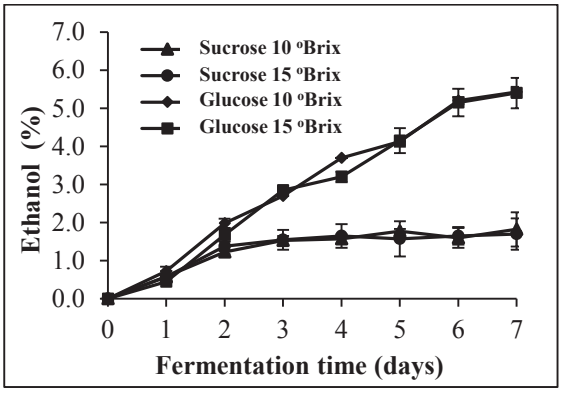

Figure 3. The ethanol production of *C. stellimalicola* in palmyra palm fruits juice with 10 and 15% (w/v) glucose and sucrose for 7 days cultivation.

Figure 4. Ethanol production in 6 L palmyra palm fruits juice with 10 % (w/v) glucose for 14 days by *C. stellimalicola*.

For scaling up, the ethanol content was approximately 3.92±0.15% after 14 days of the fermentation with 6 L palmyra palm fruit juice (Figure 4). This result was considered as low content when compared to other substrates. *Ariyasenaet al*. (2000) reported that palmyra palm fruit ripe contained flabelliferin (FB) that was the steroidal saponin or tetraglycoside (flabilliferin II). The flabilliferin II composed the bitter taste in palmyra palm fruit pulp (Jansz *et al*., 1994); and had antimicrobial effect that could decrease the ethanol fermentation. (Nikawelaet al., 1998a: Ariyasena *et al*., 2001). At 250 mg/L of FB, Saccharomyces cerevisiae growth was decreased about 50-70 %. (Nikawela *et al*., 1998b)

The produced ethanol substrate could be used in vinegar fermentation. The vinegar has yellow orange colour and distinct flavor (Figure 5). Additionally, it also contains mineral; (as 1.50±0.02 mg/kg calcium, 0.02±0.00 mg/kg iron, 7.22±0.05 mg/kg magnesium, 15.00±0.00 mg/kg phosphorus, 30.40±0.72 mg/kg potassium and 65.10±1.82 mg/kg sodium) the residual alcohol at 0.24±0.00% and acetic acid content contain 4.14±0.10% suitable for vinegar standard.

4. Conclusion

The supplementation of 500 mg/L ammonium sulfate gave the highest ethanol content at 5.75±0.09% after 7 days. *C. stellimalicola* could produce high ethanol 5.43±0.13% at 10^{o} Brix palmyra palm fruit within 7 days. For scale up at 6 liters, the fermentation of palmyra palm fruit juice with 10^{o} Brix glucose and 500 mg/L ammonium sulfate supplementation gave the ethanol content at approximately 3.92±0.15% after 14 days. The ethanol was the substrate for acetification by acetic acid bacteria. In the future, this product will be useful for acetic acid production.

5. Acknowledgements

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6. References

(1) AOAC. Official Method of Analysis. 16th ed. Verginia: The Associate Analysis Chemists. 2000.

(2) Ariyasena, D.D., Jansz, E.R. and Abeysekera, A.M. Some studies directed at increasing the potential use of palmyrah (*Borassus flabellifer* L.) fruit pulp. Journal of the Science of Food and Agriculture. 2001; 81: 1347-1352.

(3) Bafrncova, P., Smogrovicova, D.,Slavikova, I., Patkova, J. and Domeny, Z. Improvement of very high gravity ethanol fermentation by media supplementation using *Saccharomyces cerevisiae*. Biotechnology Letters. 1999; 21: 337-341.

(4) Coleman, M.C., Fish, R. and Block, E.D. Temperture - dependent kinetic model for nitrogen limited wine fermentation. Applied and Environmental Microbiology. 2007; 73(18): 5878-5884.

(5) Jansz, E.R., Nikawela, J.K., Gooneratne, J. and Theivendirarajah, K. Studies on the bitter Principle and debittering of palmyrah fruit pulp. Journal of Science of Food and Agriculture. 1994; 65: 185-189.

(6) Leuangnapa, N. Production of palmyra palm (*Borassus flabillifer* Linn.) powder and its uses in some Thai desserts. Bangkok, Thailand: Kasetsart University, MSc. Thesis.1990.

(7) Nantitanon, W. Effects of ammoniacal nitrogen addition in wine fermentation. Chiang Mai, Thailand: Chiang Mai University. MSc. Thesis. 2006.

(8) Nikawela, J.K., Abeysekara, A.M. and Jansz, E.R. Flabelliferins-steroidal saponins from palmyrah (*Borassus flabellifer* L.) Isolation by flash chromatography quantifiifation and saponin related activity. Journal of the National Science Council Sri Lanka. 1998; 26: 9-18.

(9) Nikawela, J.K., Wijeyaratne. S.C., Jansz, E.R. and Abeysekara, A.M. Flabelliferins- steroidal saponins from palmyrah (*Borassus flabellifer* L.) II. Preliminary investigations of effect on yeast and selected bacteria. Journal of the National Science Council Sri Lanka. 1998; 26: 141-150.

(10) Pramanik, K., and D.E. Rao. Kinetic study on ethanol fermentation of grape waste using *Saccharomyces cerevisiae* yeast isolated from toddy. The Institution of Engineers (India) Publications.2005; 85: 53-58.

(11) Theivendirarajah, K. and Chrystopher, R. K. Alcohol from palmyrah palm (*BORASSUS FLABELLIFER* L.) fruit pulp. Vingnanam Journal of Science. 1986; 1: 44-46.