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PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHANGES DURING FERMENTATION AND STORAGE OF NIPA SAP (Nypa fruticans Wurmb)

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science.

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PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHANGES DURING FERMENTATION AND STORAGE OF *NIPA* SAP (*Nypa fruticans* Wurmb)

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The purpose of this study is to evaluate the changes in the physico-chemical and microbiological profiles of *nipa* sap during natural fermentation. Natural fermentation of *nipa* sap involves the breakdown of carbohydrate materials under anaerobic condition with the activity of microorganisms and enzymes present. The process of fermentation is responsible for various changes of properties of *nipa* sap. The development of ethanol in the sap has always been the main concern related to the halal requirement and standard quality of the sap. Therefore, it is important to determine the basic properties of the sap that can be a reference to the producer.

The sap from *nipa* palm (*Nypa fruticans*) was collected from local collectors. Samples were fermented at 30^0C for 63 days. Physical, chemical, and microbiological analyses were carried out at seven-day intervals, starting from day 0 (fresh tapping) until day 63. Physical (colour and transmittance value) and chemical analyses (ash, moisture, crude protein content, pH, total titratable acidity, total soluble solid, ethanol, sugar and organic

acid contents) were carried out. The ethanol content was analysed using Headspace Gas Chromatography Mass Spectrometer (GC-MS), while the sugar and organic acid contents were analysed using High Performance Liquid Chromatography (HPLC) with Refractive Index and UV detectors, respectively.

Results showed that the physical and chemical properties of fresh *nipa* sap were significantly different ($P<0.05$) compared to the fermented sap. The pH value decreased from the initial pH of 7.25 in fresh *nipa* sap to 3.16 in the fermented sap. The decrease in pH value correlated with the increase in organic acids content, which consisted mainly of lactic, acetic, and succinic acids. Total acidity was recorded to be 1.18% (v/v) in fresh *nipa* sap, and 4.59% (v/v) in the 63 day fermented sap. Succinic acid became the main contributor to the acidic condition in fermented *nipa* sap, with 1.83% v/v during the 63 days of fermentation, followed by lactic acid (1.67% v/v), acetic acid (0.98% v/v), tartaric acid (0.10% v/v), and pyruvic acid (<0.01% v/v). The initial concentration of ethanol in fresh *nipa* sap was 0.11% (v/v). Drastic increase in the ethanol content for the sample was recorded during the first seven days of fermentation (6.66% v/v), before beginning to drop slightly from day 21 (6.43% v/v) of fermentation until day 63 (5.72% v/v). The changes in other volatile compounds were also observed during fermentation, including higher alcohols, acetoin, diacetyl, and esters. Total sugar in fresh *nipa* sap was 16.73% (w/v), with sucrose as the main sugar present (13.33% w/v), followed by fructose (1.40% w/v), glucose (1.27% w/v), and maltose (0.73% w/v). The sugar concentration declined over the fermentation process, giving a different sugar composition in each interval day. Fructose (0.80% w/v) became the main sugar produced on the 63rd day of fermentation, followed by sucrose (0.60% w/v), glucose

(0.34% w/v), and maltose (0.33% w/v). Identification of microbial species was done using the Analytical Profiling Identification system, API. The five species of yeast were identified, namely *Saccharomyces cerevisiae*, *Cryptococcus humicola*, *Candida guilliermondii*, *Kloeckera* spp., and *Stephanoascus ciferii*. Five species of lactic acid bacteria identified were *Lactobacillus plantarum*, *Pediococcus* spp., *Streptococcus thermophilus*, and *Lactobacillus brevis*. Acetic acid bacteria were isolated at the later stage of fermentation and identified as *Acetobacter* and *Gluconobacter*.

It is concluded that the microbial species were responsible for giving the different characteristics of *nipa* sap during the fermentation process. It also appeared that activities brought about by microorganisms in the early stages helped the activities in the successive stages. Three stages of fermentation were revealed, including alcoholic, lactic, and acetic acid fermentation. Each stage showed specific properties and importance in order to ensure the complete cycle of the fermentation process. Further studies with a large number of samples and from various sources should be carried out in order to increase the precision of the study.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

PERUBAHAN FIZIKO-KIMIA DAN MICROBIOLOGI DALAM FERMENTASI DAN PENYIMPANAN NIRA NIPAH (*Nypa fruticans* Wurmb)

By

NUR AIMI BINTI RADI

Jun 2013

Pengerusi : Fatimah Abu Bakar, PhD

Fakulti : Institut Penyelidikan Produk Halal

Tujuan kajian ini adalah untuk menilai perubahan fiziko-kimia dan profil mikrobiologi nira nipah semasa berlangsungnya fermentasi semulajadi. Proses fermentasi semulajadi nira nipah melibatkan penguraian bahan-bahan karbohidrat dalam keadaan anaerobik dengan kehadiran dan aktiviti pelbagai mikroorganisma dan enzim. Fermentasi yang berlaku menyebabkan perubahan pelbagai sifat air nira nipah. Penghasilan ethanol dalam nira sentiasa menjadi perhatian utama yang berkaitan dengan keperluan halal dan kualiti standard nira. Oleh itu, adalah penting untuk menentukan sifat-sifat asas nira yang boleh dijadikan sebagai rujukan kepada pengeluar.

Nira dari pokok nipah (*Nypa fruticans*) telah dikumpulkan daripada pembekal tempatan. Sampel telah difermentasi pada 30ºC selama 63 hari. Analisis fizikal, kimia dan mikrobiologi telah dijalankan pada selang setiap tujuh hari, bermula dari hari 0 (sejurus selepas pengumpulan nira segar) sehingga 63 hari. Analisis fizikal (warna dan nilai transmitans) dan analisis kimia (kandungan abu, kelembapan, dan kandungan protein

mentah, pH, jumlah keasidan tertitrat, jumlah pepejal larut, kandungan etanol, kandungan gula, dan kandungan asid organik) telah dijalankan. Kandungan etanol dianalisis menggunakan "Headspace Gas Chromatography Mass Spectrometer" (GC-MS), manakala kandungan gula dan asid organik dianalisis menggunakan "High Performance Liquid Chromatography" (HPLC) dengan masing-masing menggunakan pengesan Indeks Biasan dan UV.

Hasil analisis menunjukkan sifat fizikal dan kimia nira nipah segar adalah berbeza secara signifikan ($P < 0.05$) dengan nira fermentasi. Nilai pH menurun dari pH awal 7.25 nira segar kepada 3.16 nira fermentasi. Penurunan dalam nilai pH boleh dikaitkan dengan peningkatan kandungan asid organik, yang utamanya terdiri daripada asid laktik, asetik, dan succinic. Keasidan jumlah adalah 1.18% (v/v) bagi nira segar, manakala 4.59% (v/v) bagi nira terfermentasi hingga hari ke-63. Asid suksinik merupakan penyumbang utama kepada keadaan asid nira nipah terfermentasi (1.83% v/v) selama 63 hari, diikuti oleh asid laktik (01.67% v/v), asid asetik (0.98% v/v), asid tartarik (0.10% v/v), dan asid piruvik (<0.01% (v/v). Kepekatan awal kandungan etanol nira nipah segar adalah 0.11% (v/v). Peningkatan drastik kandungan etanol untuk sampel dicatatkan dalam tempoh tujuh hari pertama fermentasi (6.66% v/v), sebelum mula jatuh sedikit selepas 21 hari fermentasi sehingga 63 hari (5.72% v/v). Perubahan sebatian lain yang tidak menentu turut diperhatikan semasa penapaian, termasuk alkohol yang lebih tinggi, acetoin, diacetyl, dan ester. Jumlah gula nira nipah segar dianalisis adalah sebanyak 16.73%(w/v), dengan sukrosa sebagai gula utama (13.33% w/v), diikuti oleh fruktosa (1.40% w/v), glukosa (1.27% w/v), dan maltosa (0.73% w/v). Kepekatan gula penurun terhasil akibat fermentasi, memberikan komposisi gula yang berbeza pada setiap

peringkat penilaian. Fruktosa (0.80% w/v) menjadi gula utama wujud pada hari ke-63 penapaian, diikuti oleh sukrosa (0.60% w/v), glukosa (0.34% w/v), dan maltosa (0.33% w/v). Pengenalpastian spesies mikrob telah dilakukan menggunakan system Analytical Profiling Identification, API. Lima spesies yis telah dikenalpasti, iaitu *Saccharomyces cerevisiae, Cryptococcus humicola, Candida guilliermondii, Kloeckera* sp, dan *Stephanoascus ciferii*. Lima spesis bakteria asid laktik dikenalpasti iaitu *Lactobacillus Plantarum, Pediococcus* spp., *Streptococcus thermophilus,* dan *Lactobacillus brevis.* Bakteria asid asetik telah diperincikan pada peringkat akhir fermentasi dan dikenalpasti sebagai *Acetobacter* dan *Gluconobacter.*

Kesimpulannya, bahawa spesis mikrob bertanggungjawab menyebabkan perubahan ciri-ciri nira nipah semasa fermentasi. Aktiviti mikroorganisma pada peringkat awal membantu aktiviti-aktiviti mikroorganisma di peringkat seterusnya. Tiga peringkat fermentasi telah dinamakan, iaitu fermentasi asid alkohol, laktik, dan asetik. Setiap peringkat memberikan ciri-ciri tertentu dan penting untuk menghasil kitaran lengkap proses fermentasi. Kajian lanjut dengan sebilangan besar sampel dan dari pelbagai sumber perlu dijalankan dalam usaha untuk meningkatkan ketepatan kajian.

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I certify that an Examination Committee has met on **date of viva** to conduct the final examination of **name of student** on his **degree** thesis entitled "**title of thesis**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at other institution.

NUR AIMI BINTI RADI

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