



THERMO-ENZYMATIC HYDROLYSIS OF
BITTER CASSAVA STARCH:
FUNDAMENTAL AND PROCESS
OPTIMIZATION STUDIES

by

NOORULNAJWA DIYANA BT YAACOB

(0731110215)

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LIST OF ABBREVIATIONS

| | | |
|--------|---|---|
| AFM | - | Atomic force microscopy |
| Abs | - | Absorbance |
| AM | - | Amylose |
| AMP | - | Amylopectin |
| AMG | - | Amyloglucosidase |
| ANOVA | - | Analysis of variance |
| BLA | - | <i>Bacillus licheniformis</i> alpha amylase |
| CCD | - | Central composite design |
| CV | - | Correlation of variance |
| CN | - | Cyanide |
| DNS | - | Dinitro salicylic acid |
| DoE | - | Design of experiment |
| DMSO | - | Dimethyl sulfoxide |
| DF | - | Degree of freedom |
| D.A. | - | Dextrinizing activity |
| DSC | - | Differential Scanning Calorimetry |
| DE | - | Dextrose equivalent |
| DP | - | Degree of polymerization |
| Da | - | Dalton |
| e.g. | - | example |
| e.t.c. | - | et cetera |
| EU | - | Europe Union |

| | | |
|---------------------------------|---|--|
| EM | - | Electron microscopy |
| FFA | - | Free fatty acid |
| g | - | gram |
| g/g | - | gram per gram |
| GL | - | glycolipids |
| GC | - | Gas Chromatography |
| HPLC | - | High Performance Liquid Chromatography |
| HCl | - | Hydrogen chloride |
| H ₃ BO ₃ | - | Boric acid |
| H ₂ SO ₄ | - | Sulfuric acid |
| hr | - | hour |
| ha | - | hectare |
| I ₂ KI | - | Iodide solution |
| J/g | - | joule per gram |
| KH ₂ PO ₄ | - | Monopotassium Dihydrogen phosphate |
| KI | - | Potassium Iodide |
| kg | - | kilogram |
| kV | - | kilovolt |
| L | - | liter |
| MAP | - | Monoammonium phosphate |
| mg | - | milligram |
| min | - | minute |
| max | - | maximum |
| mg/ml | - | milligram per milliliter |

| | | |
|--|---|---|
| ml | - | milliliter |
| M | - | Molar |
| mM | - | miliMolar |
| mA | - | miliampire |
| NaOH | - | Sodium Hydroxide |
| nm | - | nanometer |
| Na ₂ SO ₃ ·7H ₂ O | - | Sodium sulfite heptahydrate |
| NA | - | Not detectable |
| N | - | Normality |
| NaCl | - | Sodium Chloride |
| OH | - | Hydroxyl |
| OFAT | - | One factor at time |
| PRESS | - | Predicted residual error sum of squares |
| PHI | - | Peak High Index |
| ppm | - | part permillion |
| PL | - | Phospholipids |
| RSM | - | Response surface methodology |
| rpm | - | rotation per minute |
| sec. | - | second |
| SPSS | - | Statistical Package for the Social Sciences |
| SEM | - | Scanning electron microscopy |
| TEM | - | Transmission Electron Microscopy |
| TMA | - | Thermal mechanical analysis |
| t/ha | - | tone per hectare |

| | | |
|------------------------------------|---|--------------------------------------|
| TG | - | Triglyceride |
| USA | - | United State of America |
| vs. | - | versus |
| v/v | - | volume per volume |
| v/w | - | volume per weight |
| w/g | - | watt per gram |
| w/v | - | weight per volume |
| XRD | - | X-ray diffractometer |
| μl | - | microliter |
| μm | - | micrometer |
| $^{\circ}\text{C}$ | - | degree Celsius |
| $^{\circ}\text{C}/\text{min}^{-1}$ | - | degree Celsius per minute |
| ^{13}C -NMR | - | Carbon 13 Nuclear Magnetic Resonance |

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LIST OF SYMBOLS

| | | |
|------------|---|---------------------------------|
| T_o | - | onset temperature |
| T_p | - | peak temperature |
| T_c | - | conclusion temperature |
| ΔH | - | Enthalpy of gelatinization |
| θ | - | Theta |
| $^\circ$ | - | degree sign |
| α | - | alpha |
| D | - | Absorbance of the enzyme sample |
| D_0 | - | Absorbance of control |

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ABSTRAK

Ciri-ciri asas kanji ubi kayu yang akan digunakan di dalam penghasilan bioetanol dikaji dengan terperinci. Di dalam kajian ini, ubi kayu yang tidak boleh dimakan (*Manihot esculenta*) telah digunakan sebagai bahan mentah bagi kanji di mana ia akan melalui hidrolisis enzimatik untuk menghasilkan glukosa. Kemudiannya melalui proses penapaian bagi mendapatkan bioetanol. Analisis hampiran terhadap kanji ini menunjukkan bahawa kandungan karbohidrat adalah sebanyak 91.17% manakala jumlah ketara amilosa masing-masing adalah 16.6% dan 17.1%. Phosphorus dan abu menunjukkan nilai terendah dan kadar kelembapan air adalah 10.5%. Nitrogen dan jumlah lemak boleh diabaikan. Dengan menggunakan pelbagai peralatan analisis, ciri-ciri dapat dikenalpasti. Didapati bahawa asas kanji ini memiliki bentuk polihedrik dengan memvisualisasikannya di bawah SEM dan permukaannya halus tanpa kehadiran liang-liang. Di bawah XRD, pola menunjukkan bahawa ubi kayu dikelaskan sebagai kanji jenis A dan suhu gelatinisasi mereka tinggi, 89.4°C. Pengampulan dan kebolehlarian terjadi sebagai akibat dari gelatinisasi dari granul kanji. Semua ciri-ciri asas, memberikan kesan yang baik untuk tepung ini untuk digunakan sebagai bahan asas dalam industri bioetanol. Hidrolisis enzimatik kanji dari sumber-sumber alam dikenalpasti sebagai aplikasi berpotensi dalam penghasilan komersial bioetanol. Kesan dari pelbagai pembolehubah proses dipelajari untuk penukaran optimum pati ubi kayu menjadi glukosa menggunakan α -amilase dan amyloglucosidase. Kanji adalah polisakarida tersimpan yang berasal daripada tumbuhan, yang tidak dapat ditukarkan ke gula dengan mudah. Pemotongan kanji memerlukan gelatinisasi terlebih dahulu dengan rawatan pemanasan, pencairan oleh amylase dan penukaran untuk gula oleh amyloglucosidase. Untuk mendapatkan kepekatan glukosa yang lebih tinggi; pencairan dan proses sakarifikasi harus dioptimumkan. Rekabentuk eksperimen komposit factorial penuh dan rekabentuk komposit berpusat (CCD) digunakan dalam perancangan eksperimen dan analisis keputusan. Kajian awal telah dilakukan untuk mengetahui pembolehubah yang berpotensi untuk kedua-dua proses. Keberkesanan α -amilase dalam pencairan adalah ditentukan oleh dextrinizing aktiviti (DA) sedangkan prestasi amyloglucosidase berdasarkan pada kepekatan glukosa. Keadaan optimum untuk pencairan bagi 35% buburan ubi kayu adalah dengan menggunakan 0.33% BAN480L di dalam penimbal natrium acetate (pH7) pada 85°C untuk 12.72 minit. Keadaan optimum untuk pemotongan adalah pada 60.75°C, pH4.53, dengan menggunakan AMG300L 0,2% dalam masa 40min. Sebuah model kecukupannya sangat memuaskan apabila kadar pekali untuk pencairan dan sakarifikasi masing-masing adalah 0.9977 dan 0.9795.

ABSTRACT

Fundamental characterization of cassava starch that will be used in bioethanol production was studied extensively. In the present study, non edible cassava (*Manihot esculenta*) is used as the raw material for starch, which undergoes enzymatic hydrolysis to produce glucose then precede the fermentation to obtain bioethanol. Proximate analysis of this starch showed that the carbohydrate content is 91.17% while apparent and total amylose are 16.6% and 17.1% respectively. Phosphorus and ash showed the lowest value and the moisture content is 10.5%. Nitrogen and Total fat are negligible. By using various analytical equipments, its characteristics were identified. It was found that the root starch has a polyhedral shape by visualizing under SEM and the surface was smooth with no evidence of pores. Under XRD, the pattern shows that the cassava was classified as A-type starch and their gelatinization temperature was high, 89.4°C. Swelling and solubility take place as a result of gelatinization of starch granule. All the fundamental characteristics, gave a good impact for this starch to be used as a raw material in bioethanol industry. Enzymatic hydrolysis of starch from natural sources finds potential application in commercial production of bioethanol. The effects of various process variables were studied for optimum conversion of cassava starch to glucose using α -amylase and amyloglucosidase. Starch is a reserved polysaccharide of plant origin, which cannot be converted to sugar easily. Starch saccharification requires prior gelatinization by heat treatment, liquefaction by α - amylase and conversion to sugars by amyloglucosidase. In order to get higher glucose concentration; liquefaction and saccharification processes must be optimized. Full factorial composite experimental design and central composite design (CCD) were used in the design of experiments and analysis of results. Preliminary study was done to investigate the potential variable for these two processes. The performance of α - amylase in liquefaction was determined by dextrinizing activity (D.A.) while the performance of amyloglucosidase was based on glucose concentration. The optimal condition for liquefaction for 35% cassava starch slurry was obtained by using 0.33% BAN480L in sodium acetate buffer (pH 7) at 85°C for 12.72 min. The optimal conditions for saccharification were found to be at 60.75°C, pH 4.53, using 0.2% AMG300L in 40 min. A model adequacy was very satisfactory, as coefficient of determination were 0.9977 and 0.9795 for liquefaction and saccharification, respectively.

CHAPTER 1

INTRODUCTION

1.1 Overview

Demand for energy is increasing day by day. This phenomenon occurred due to the growing of population and worldwide societies are becoming more industrialized. Energy demand was related to the fossil fuel reserved. While the demand of the energy is increased, fossil fuel reserved is depleted. Since most of the energy that produces today is generated by fossil fuel, a new alternative source of energy should be find out to overcome the situation.

Bachar (2007) stated that *“Only 1.08 trillion barrels of petroleum reserves are left on Earth, and only one new barrel is found for every four us”*. With over 31.03 billion barrels consumed annually worldwide (2006) and rapidly increasing, there is less than 25.4 years left”. Based on the statistical analysis that have done by Bachar (2007), the depleted of fossil fuel reserved will become a serious problem for the world if there is no action to be taken. Statistics that have done by International Energy Statistics for Europe petroleum stock shows the decreasing from 134 ‘000 barrels in 2005 to 812’000 barrels in 2009. Petroleum stock in Europe, Asian & Oceania, and OECD declined every year. The same phenomenon occurred in Asia & Oceania and OECD country.

PETRONAS was incorporated on 17 August 1974 as the national oil company of Malaysia, vested with the entire ownership and control of the petroleum resources in the country. It has since grown from just being the manager and regulator of Malaysia’s upstream sector into a fully integrated oil and gas corporation, ranked among the