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

by

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LIST OF ABBREVIATIONS

AFM	-	Atomic force microscopy
Abs	-	Absorbance
AM	-	Amylose
AMP	-	Amylopectin
AMG	-	Amyloglucosidase
ANOVA	-	Analysis of variance
BLA	-	*Bacillus licheniformis* alpha amylase
CCD	-	Central composite design
CV	-	Correlation of variance
CN	-	Cyanide
DNS	-	Dinitro salisyclic acid
DoE	-	Design of experiment
DMSO	-	Dimethyl sulfixe oxide
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_3BO_3	-	Boric acid
H_2SO_4	-	Sulfuric acid
hr	-	hour
ha	-	hectare
I_2KI	-	Iodide solution
J/g	-	joule per gram
KH_2PO_4		Monopottasium 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
$Na_2SO_3.7H_2O$	-	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 hactare

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
°C	-	degree Celsius
$°C/min^{-1}$	-	degree Celsius per minute
^{13}C-NMR	-	Carbon 13 Nuclear Magnetic Resonance

LIST OF SYMBOLS

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

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 menunjukan bahawa kandungan karbohidrat adalah sebanyak 91.17% manakala jumlah ketara amilosa masing-masing adalah 16.6% dan 17.1%. Phospohorus 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^{o}C. Pengampulan dan kebolehlarutan terjadi sebagai akibat dari gelatinisasi dari granul kanji. Semua ciri-ciri asas, memberikan kesan yang baik untuk tepung ini untuk digunakan sebagai bahan asas dalan 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^{o}C untuk 12.72 minit. Keadaan optimum untuk pemtongan adalah pada 60.75^{o}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 entensively. 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% and17.1% 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 polyhedric 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 sacharification 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 sacharification, 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