



**Two Stage Pretreatment of Dates Seeds for
Fermentable Sugar Production**

by

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Dedication

I would like to dedicate this work to my beloved grandmother Bakhitah, my mother Jumah and my father Salem Hasan who provided me the unconditional love and support to keep my confidence high to achieve my goal. Otherwise, I could not have reached where I am today. I would also like to dedicate this work to my supervisor Dr. Ku Syahidah Ku Ismail who worked hard to make this work successful. Last but not least, my sister Alesheg Salem and my brothers Haythem and Salem-remain the cornerstone of my life; you honor me with your loyalty. As always, I am indebted to their sacrifice and devotion.

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LIST OF ABBREVIATIONS

<i>A. niger</i>	<i>Aspergillus niger</i>
CBU/g	Cellobiase units per gram
CCRD	Central composite rotatable design
DI	Deionized water
DNS	3,5-dinitrosalicylic
EEA	European Environment Agency
EP	European petroleum
FPU/g	Filter paper unit per gram
FTIR	Fourier transform infrared
g	gram
g/g	gram/gram
g/L	gram per liter
g/Lh	gram per liter hour
h	Hour
HMF	Hydroxymethylfurfural
kg	Kilogram
L	Liter
M	Molar
mg/cm ³	milligram per cubic centimeter
mg/g	milligram per gram
mg/mL	milligram per milliliter
min	Minute
mL	Millilitre
mM	millimolar

OD	optical density
ppm	parts per million
QP	Qatar petroleum
RDA	Recommended Dietary Allowance
rpm	Revolutions per minute
RSM	Response surface methodology
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
SPORL	Sulfite pretreatment to overcome recalcitrance of lignocellulose
TGA	Thermogravimetric analysis
U/mL	Unit per milliliter
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight

Prarawatan Dua Peringkat bagi Biji Kurma Untuk Penghasilan Gula Penapaian

ABSTRAK

Pada masa kini, biji kurma merupakan bahan buangan dan menyumbang kepada masalah pelupusan bahan terbuang. Untuk menyelesaikan isu ini, adalah perlu untuk mencari nilai ekonomi bagi biji kurma supaya ia dapat digunakan sebaiknya tanpa terbuang begitu sahaja. Bahagian lignoselulosa yang terdapat di dalam biji kurma sebahagian besarnya mengandungi hemiselulosa, selulosa dan lignin. Hemiselulosa dan selulosa boleh dihidrolisis kepada gula dan kemudian boleh ditapai menjadi etanol. Maka, objektif kajian ini ialah untuk menilai kesan terhadap suhu, masa tindakbalas dan kelajuan pengadukan terhadap proses hidrolisis asid menggunakan kaedah satu faktor dalam satu masa (OFAT). Untuk hidrolisis enzim menggunakan selulase, proses pengoptimuman dilakukan menggunakan OFAT dan CCRD. Untuk kaedah OFAT, tiga faktor telah digunapakai, iaitu kepekatan selulase, suhu dan tempoh tindakbalas. CCRD dengan dua pembolehubah untuk 'response surface methodology' (RSM) telah digunakan untuk menilai secara serentak kesan terhadap kepekatan enzim dan tempoh tindakbalas terhadap jumlah kepekatan gula penurun selepas hidrolisis. Kajian mendapati biji kurma mengandungi 27.34% lignin, 20.63% selulosa dan 13.49% hemiselulosa. Keadaan optimum untuk hidrolisis asid secara OFAT diperolehi apabila kepekatan asid sulfurik adalah 1%, 70°C selama 2 jam, dan kelajuan pengadukan pada 200 rpm. Di bawah keadaan ini, jumlah kepekatan maksimum gula penurun adalah 29.81 g/L. Peringkat kedua menggunakan enzim selulase untuk menghidrolisis kandungan selulosa di dalam serbuk biji kurma kepada gula penapaian. Model statistik kuadratik telah dibentuk untuk mengaitkan di antara pembolehubah hidrolisis terhadap jumlah kepekatan gula. Keadaan optimum untuk hidrolisis enzim yang didapati daripada OFAT adalah pada 90 FPU/g selama 10 jam pada 40°C. Manakala untuk CCRD, keadaan optimumnya ialah apabila aktiviti enzim selulosa adalah 120 FPU/g selama 6 jam pada 45°C. Di bawah keadaan optimum ini, kepekatan maksimum gula penurun ialah 31 g/L. Pengesahan model menunjukkan tidak terdapat perbezaan signifikan di antara nilai yang diperhatikan dan nilai yang diramalkan. Jumlah kandungan gula yang dihasilkan daripada proses dua peringkat ialah 60.8 g/L, 80% daripada nilai teori.

Two Stage Pretreatment of Date Seeds for Fermentable Sugar Production

ABSTRACT

Currently, date seeds are considered as waste and contribute to the waste disposal problem. To mitigate this issue, it is desirable to impart an economic value on date seeds so that they can be utilized instead of going to waste. The lignocellulosic part of date seeds are composed mainly of hemicellulose, cellulose and lignin. Hemicellulose and cellulose can be hydrolyzed into sugars which can then be fermented into ethanol. Thus, the objective of this research is to evaluate the effect of temperature, reaction time and agitation speed on the acid hydrolysis using the one factor at a time (OFAT) method. For the enzymatic hydrolysis using cellulase, the optimization was done using OFAT and CCRD. In the OFAT method, three factors were applied, cellulase concentration, temperature and time of reaction. A two variable Central Composite Rotatable Design (CCRD) for response surface methodology (RSM) was employed to evaluate the simultaneous effect of enzyme concentration and reaction time on the obtained total reducing sugar concentration after hydrolysis. It was found that the date seeds contain 27.34% lignin, 20.63% cellulose and 13.49% hemicellulose. The optimal condition for acid hydrolysis using OFAT was found to be at 1% sulphuric acid concentration, 70°C for 2 hours, and agitation speed of 200 rpm. Under these conditions, the maximum concentration of reducing sugars obtained was 29.81 g/L. The second stage uses cellulase enzyme to hydrolyse the cellulose content in powdered date seeds into fermentable sugars. A validated quadratic statistical model was developed to relate the hydrolysis variables to the total sugar concentration. The optimal conditions of enzymatic hydrolysis as obtained from OFAT was when the activity of cellulase enzyme was at 90 FPU/g for 10 hours at 40°C. While for CCRD, the optimum condition was obtained by 120 FPU/g for 6 hours at 45°C. Under the optimum condition, the maximum concentration of reducing sugars obtained was 31 g/L. Validation of the model indicated that there was no variance between the observed and predicted values. The total sugar content obtained from the two stage process altogether was 60.8 g/L, 80% from the theoretical value.

CHAPTER 1

INTRODUCTION

1.1 Biomass as an alternative energy source

At present, the issues of fossil fuels depletion and global warming have shifted global attention to energy production derived from renewable and sustainable sources. Energy can be derived from biomass which is renewable and sustainable in the form of biofuels such as bioethanol. This process is a promising avenue as it can simultaneously reduce agricultural waste and provide carbon neutral, renewable heat, power and transport fuels (Khan et al., 2013). There are various methods for the production of biofuels from eco-friendly biomass (Fujita et al., 2016). Biomass resources comprise those which are received from cultivation and forestry as well as from agro- and wood industries. It also includes waste sources from construction and demolition as well as municipal wastes. In agriculture, bioethanol can be produced from biomass with high starch content such as corn (Gumienna et al., 2016), potatoes, sugar cane, wood fibre, wheat, apple pomace (Evcan & Tari, 2015) and palm dates (Gaily et al., 2012). According to the European Environment Agency (EEA) (Khan et al., 2013), the use of biomass for clean energy generation in the European Union could significantly increase in the next decade without harming biodiversity, soil and water resources. The potential biomass available in Europe is sufficient to support the ambitious renewable energy targets in an environmentally responsible way.

1.2 Ethanol

Ethanol is a renewable fuel source that is produced by the fermentation and distillation process. The most common source of ethanol in the United States in 2008 is corn (Wallander et al., 2011). However, it can be produced from other products such as sugar cane, saw grass, and other natural products that is conducive to the fermentation/distillation process.

1.2.1 Chemical formula of ethanol

According to the International Union of Pure and Applied Chemistry (IUPAC), the molecular formula of ethanol is C_2H_5OH . One molecule of ethanol contains 2 atoms of carbon, 6 atoms of hydrogen and 1 atoms of oxygen (Fig. 1.1). Ethanol is an alcohol because of the functional group $-OH$.

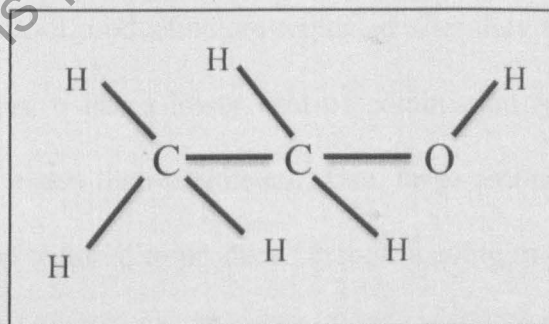


Figure 1.1 Molecular structure of ethanol

1.2.2 Properties of ethanol

Pure ethanol is a polar solvent that is water soluble and has a flash point of 12.78°C. Ethanol has a vapour density of 1.59, making it heavier than air. Consequently, ethanol vapours do not rise in the atmosphere and will constantly seek lower altitudes. The specific gravity of ethanol is 0.79. Ethanol has an auto-ignition temperature of 422°C and a boiling point of 78.33°C. Ethanol is also not as toxic as gasoline or methanol, and carcinogenic compounds are not presented in pure ethanol.

1.2.3 Advantages and disadvantages of ethanol as a fuel

There are both advantages and disadvantages associated with the use of ethanol as an alternative fuel to petroleum. Unlike petroleum, ethanol is a renewable resource, burns more cleanly in air than petroleum, producing less carbon (soot) and carbon monoxide. The use of ethanol as opposed to petroleum is carbon neutral, provided that the crops required for ethanol production are replanted after they are harvested. Ethanol also has its disadvantages; it has a lower heat of combustion (per mole, per unit of volume, and per unit of mass) than petroleum. Also, large amounts of arable land are required to produce crops required to produce ethanol, leading to problems such as soil erosion, deforestation, fertiliser run-off, and salinity issues. The disposal of waste fermentation liquors will also cause major environmental problems. Another issue is that typical current engines would require modification to utilize high concentrations of ethanol.

1.2.4 Ethanol applications

Ethanol has many useful properties which can be utilized in a wide-range of products such as beverages, green chemicals, pharmaceuticals, plastics, cosmetics, industrial products (solvents, paint) and increasingly, as a renewable transport fuel.

1.3 Problem statement

Worldwide, date palm (*Phoenix dactylifera*) agro-industry and processing are continuously increasing due to great importance in human nutrition. Tonnes of date seeds were wasted after consumption which lead to environmental problems. It is also a waste of raw materials since the date seeds contains high amount of carbohydrate which has the potential to be converted into something valuable. Thus, there is a need to find a suitable application to utilize the date seeds. Unlike date fruits, date seeds don't compete with food supply and pose no harm to the world's food security. In this research, date seeds were assessed as a raw material for fermentable sugar production, which can be converted to ethanol. Moreover, studies on the use of date seeds for the production of ethanol are still scarce.

1.4 General objective

The objective of this research project is to convert the date seeds to simple reducing sugars for ethanol production using a two-stage pretreatment process; acid hydrolysis followed by enzymatic hydrolysis.

1.4.1 Specific objectives

1. To evaluate the effects of sulfuric acid concentration, temperature, agitation speed and reaction time on acid pretreatment of dates seeds to produce fermentable sugar via OFAT method.
2. To analyze the effects of enzyme concentration, reaction temperature and time on enzymatic pretreatment of dates seeds to produce sugar via OFAT method.
3. To optimize the sugar production for the enzymatic pretreatment of date seeds after applying the acid pretreatment via CCRD.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Today, global reserves of liquid fuels are estimated to meet just over half of the global demand by 2023, which indicates that the remaining 50% of the required fuel will have to be met with other sources (Sun & Cheng, 2002). Environmental concerns have increased over the damaging tailpipe; emission of carbon monoxide, oxides of nitrogen and other ozone-forming pollutants by a large volume combustion of petroleum-based fuel. To prepare for the uncertainty of future oil stock and reduce air contamination, it is necessary to invest in alternative energy carriers, which improves energy safety and decreases emission (Sun & Cheng, 2002). This chapter will discuss the work done on bioethanol production from biomass including date fruits, as well as the pretreatment methods used for cellulosic biomass.

2.2 Date palm trees

Globally, there exist more than 20 kinds of dates (Abdul Afiq et al., 2013). Date palm trees (*Phoenix dactylifera* L.) can tolerate highly saline conditions, drought, and high temperatures, earning its status as an important symbol of life in the desert (El-Juhany, 2010). It is one of the oldest trees that have been cultivated since ancient times, where the date fruits, branches, roots, and seeds all have useful properties (El-Juhany et al. 2010). Palm fronds are an important source for ethanol production as they contain

about 58% cellulose and 22% hemicelluloses (Sulieman et al., 2013). Date palm trees are grown in more than 40 countries with the Arab countries being the major manufacturer and exporter of dates in the world. Based on the Food and Agriculture Organization of the United Nations (2009), the global annual production of dates was about 7.4 million tons where 5.4 million tons were contributed by the Arab countries (Al-Khalifah et al., 2013). In 2010, the total global production of dates exceeded 7 million tons, producing around 1 million tons of seeds per year (Abdul Afiq et al., 2013).

Dates are a suitable source for bioethanol production. They contain considerable amounts of inverted sugars, glucose and fructose (Chniti et al., 2014). The flesh of dates is made out of 73% to 83% sugars. Even dates of a lower grade showed the same sugar content as dates of high quality. Fermentation of sugars is an anaerobic biological process in which sugars are converted to alcohol by the action of microorganisms, usually yeast (Sulieman et al., 2013).

2.2.1 Size of date palm trees

The date palm trees are evergreen palm trees that can reach 15 to 40 m in height. Its fasciculate root system can grow up to a depth of 6 m (Zaid et al., 2002). The stem is cylindrical, straight, up to 1 to 1.1 m in diameter. Female date palm trees start fruiting within 4 to 6 years and reach complete production after 15-20 years. Forty kg of date fruits can be produced annually, and more than 100 kg is possible with intensive management. When farmed with low levels of inputs and management, date palm trees may produce 20 kg fruits or less annually (Chao & Krueger, 2007).

2.2.2 Date fruits

The date fruit is a rectangle, one-seed berry, with a fat and sweet pericarp (Fig. 2.1). Dates differ very much in size ranging from 18 to 110 mm long, weighing from 2 to 60 g per date, and the hues ranging from yellow to black. The date seed (also called hollow, stone or seed) is hard coated, oblong and weighs 0.5 to 4g (Zaid et al., 2002).



Figure 2.1: Date fruits

Dates are high in sugar content, containing 66.5 g sugar per 100 g serving of the fruit (Barrett, 2013). The following nutritional data outlines some of the key nutrients found in dates, and is based on a 100 g serving of the fruit (Barrett, 2013); fiber 6.7 g (27% RDA), potassium 696 mg (20% RDA), copper 0.4 mg (18% RDA), manganese 0.3 mg (15% RDA), magnesium 54 mg (14% RDA) and vitamin B6 0.2 mg (12% RDA). Table 2.1 shows the chemical composition of different Sudanese dates based on varieties for a 100 g serving. The moisture content of dates ranges from 8.78% for Gondaila variety to 10.68% for Jaw variety and the fat content ranges from 1.71% for Madini dates to 2.06% for Mishrig dates. The protein content ranges from 3.69% for Jaw dates to 4.09% for the Gondaila dates, while the ash content of various dates ranges

Table 2.1: Chemical composition of different Sudanese dates based on variety (g/ 100 g date fruits)

Variety	Moisture	Fat	Protein	Ash	Fiber	Carbohydrate
Gondaila	8.78	2.00	4.09	2.20	2.53	80.41
Barakawi	9.38	1.87	4.03	2.50	2.67	79.55
Jaw	10.68	1.79	3.69	2.38	2.74	78.73
Mishrig	8.81	2.06	3.92	2.19	2.78	80.27
Bittamoda	10.03	1.81	3.72	1.96	2.37	80.11
Madini	9.90	1.71	3.94	2.27	3.14	79.04

Source: Mohamed et al., (2014)

from 1.96% for Bittamoda to 2.5% for Barakawi dates. The fiber content of various dates is from 2.37% for Bittamoda dates to 3.14% for the Madini dates. The carbohydrates content varies from 78.73% for Jaw dates to 80.4% for the Gondalia dates (Mohamed et al., 2014). The data indicates that date fruits are a rich source of carbohydrates.

2.3 Date seeds

Date seeds have a solid body as shown in Fig. 2.2. It has a rectangular shape, tapered at both ends and occupies the center of the fruit. The weight ranges from 0.5 to 4 g, length from 12 to 20 mm, and width from 6 to 15 mm (Ibrahim, 2012). The seed occupies about 10 to 20 % of the full weight of the fruit and its length is usually equal to three times the width. Dates are composed of four parts as shown in Fig. 2.3 and explained in Table 2.2.



Figure 2.2: Date seeds

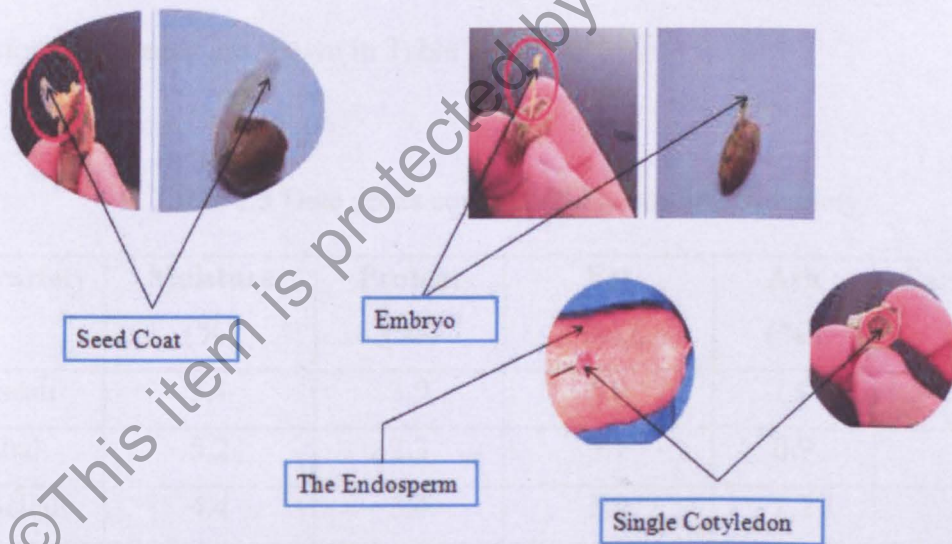


Figure 2.3: Parts of a date seed (Ibrahim, 2012)