Deanship of Graduate Studies

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Optimization of Bio-ethanol Production from Low-cost Medjool Date Fruit

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Optimization of Bio-ethanol Production from low-cost Medjool Date Fruit

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Optimization of Bio-ethanol Production from low-cost Medjool Date Fruit

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Dedication

With the grace of Almighty Allah, I write this expression of gratitude on the occasion of obtaining my Master's degree. I dedicate this achievement to the soul of my father, who have been an inexhaustible source of support and inspiration at every stage of my journey.

I extend my heartfelt thanks to my dear mother, my loving wife, and my wonderful family for their unlimited support and constant encouragement, which played a significant role in achieving this milestone. Moments of joy and challenging times were made easier thanks to their warm presence.

On this occasion, I express special thanks to my dear friends who have been an integral part of my journey. A word of gratitude to all of you for always being there to provide support and motivation.

Declaration

I Certify that this thesis submitted for the degree of Master of Science in environmental studies is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for higher degree to any other university or institution.

Anas Ahmad Elias Alhaddad

Signature: ……………………

Date: 23/12/2023

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Abstract

In the Jordan Valley area / West Bank, tons of date palm fruit wastes of the Medjool variety (*Phoenix dactylifera L.*) are produced annually during harvesting and industrial processing. Generally, 15% of the annual Date fruit production is considered a low-cost category (1.9 USD/kg) with high nutritional value. In 2023, this category is expected to be 2,700 tonnes, thus leading to high losses for the farmers, while demand for this class is deficient at local and international markets. This study aimed to study the potential of converting these low-cost fruit classes into high-value bio-ethanol as a by-product using the yeast *Saccharomyces cerevisiae*. Design of Experiment (Stat-Ease) software was used to design and run different experiments to optimize the fermentation process under three variables (sugar contents, yeast dose, and duration of fermentation). At the same time, temperature and pH values were fixed at 30° C and 6.5, respectively. Sixteen runs were conducted in the Environmental research lab at Al Quds University.

Results revealed that the highest bio-ethanol yield was observed when the sugar content was 18.86 Brix. At the same time, the impact of yeast dose and duration of fermentation have a minimum impact on bio-ethanol production. The optimum yield was 6.48% bio-ethanol after incubation of 48 h and a sugar content of 18.86 Brix. The amount of bio-ethanol produced could help the farmers generate additional income with long shelf time, an alternative for selling their low-cost date fruit after the harvesting for a low price. One kilogram of low-cost date fruit can produce 357.95 mL of bio-ethanol, so one ton of low-cost dates could produce 357.95 L.

إنتاج الإيثانول من ثمار نخيل المجهول المنخفضة التكلفة

إعداد: أنس أحمد إلياس الحداد
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الملخص

في منطقة وادي الأردن / الضفة الغربية، يتم إنتاج أطنان من نفايات ثمار نخيل التمر من صنف المجهول (*Phoenix dactylifera L.*) سنويًا أثناء الحصاد والتجهيز الصناعي. بشكل عام، تعتبر نسبة 15% من إنتاج ثمار التمر السنوي ضمن فئة منخفضة التكلفة (1.9 دولار أمريكي لكل كيلوغرام) ذات قيمة غذائية عالية. في عام 2023، من المتوقع أن تبلغ هذه الفئة 2,700 طن، مما يؤدي إلى خسائر كبيرة للمزارعين، بينما الطلب على هذه الفئة ضعيف في الأسواق المحلية والدولية. هدفت هذه الدراسة إلى إختبار إمكانية تحويل هذه الفئات منخفضة التكلفة من الفواكه إلى إيثانول حيوي عالي القيمة كمنتج ثانوي باستخدام خميرة *Saccharomyces cerevisiae* . تم استخدام برنامج تصميم التجارب (Stat–Ease) لتصميم وتشغيل تجارب مختلفة لتحسين عملية التخمير تحت ثلاثة متغيرات (محتوى السكر، جرعة الخميرة، ومدة التخمير). في الوقت نفسه، تم تثبيت قيم درجة الحرارة والرقم الهيدروجيني على 30 درجة مئوية و6.5 على التوالي. تم إجراء ستة عشر تجربة في مختبر البحوث البيئية في جامعة القدس.

كشفت النتائج أن أعلى عائد من الإيثانول الحيوي تم ملاحظته عندما كان محتوى السكر 18.86 بريكس. في الوقت نفسه، كان لجرعة الخميرة ومدة التخمير تأثير ضئيل على إنتاج الإيثانول الحيوي. كان العائد الأمثل نسبة 6.48% من الإيثانول الحيوي بعد حضانة لمدة 48 ساعة ومحتوى سكري قدره 18.86 بريكس. يمكن أن يساعد مقدار الإيثانول الحيوي المنتج المزارعين في توليد دخل إضافي مع فترة صلاحية طويلة، بديلًا لبيع ثمار التمر منخفضة التكلفة بعد الحصاد بسعر منخفض. يمكن أن ينتج كيلوغرام واحد من ثمار التمر منخفضة التكلفة 357.95 مل من الإيثانول الحيوي، لذلك يمكن أن ينتج طن واحد من التمور منخفضة التكلفة 357.95 لترًا.

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List of Abbreviations

Brix	Unit of Sugar
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
$	United States Dollar
Kg	Kilogram
i.e.	i.e. stands for the Latin id est, or "that is"
df	Degree of freedom
VIF	Variance inflation factor
O.D.	Optical Density
%	Percent
mL	Milliliter
L	Liter
g	Gram
mg	Milligram
KH_2PO_4	Potassium dihydrogen phosphate
K_2HPO_4	Potassium hydrogen phosphate
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
$FeSO_4.7H_2O$	Ferrous sulfate heptahydrate
^{o} C	Celsius Degree
MoA	Ministry of Agriculture - Palestine
Low Cost	Dates with price lower than 1.93 $

Chapter One

Introduction & Background

- **1.1 Background**
- **1.2 Problem Statement**
- **1.3 Study Justification**
- **1.4 Study Goal**
- **1.5 Study Period**
- **1.6 Study Location**
- **1.7 Study Question**

Chapter One: Introduction & Background

1.1 Introduction

The date palm tree, also known as *Phoenix dactylifera L.*, is one of the oldest trees in the world. Palm cultivation has been around for at least 5,000 years since it was practiced by the Sumerians (Nixon, 1951). Most of Western Asia and Northern Africa are also included in its range. In addition, it may be cultivated in several areas in South America, North America, and Australia (Al-Harrasi et al., 2014). Around the year 10,000, the date palm tree most likely emerged in the ancient Hejaz region of Saudi Arabia, which is now a part of modern-day Saudi Arabia (Assirey, 2015). The date palm tree is one of the few fruit trees that can withstand harsher environmental conditions than most (El-Juhany, 2010). Dates are an essential part of the diet of those who live in dry and semiarid climates (Tavakolian et al., 2013).

Approximately forty countries across the globe are responsible for data production, as stated by the Food and Agriculture Organization of the United Nations (FAO). The production of dates is dominated by the Middle Eastern countries of Egypt, Saudi Arabia, Iran, Algeria, and Iraq, with Palestine coming in at position number 30 (FAOSTAT, 2022b).

Egypt was the world's leading country for date fruit producers in 2020, with 1,690,959 tons, followed by the Gulf Cooperation (Pariona, 2017). The Palestinian farmers in the Jordan Valley are growing two primary varieties of Date Palm trees, the Medjool and Berhy; according to the statistical survey 308,000 are Medjool and only 340 trees of Berhy. (I. Bsharat, 2023)

The physical characteristics of the date fruit, such as size, shape, color, taste, skin texture, and optical inspection of the consumers, are crucial factors for setting the price (Abdul-Hamid et al., 2020; Kamal-Eldin et al., 2018). These are also challenges for the grower during the year. Fertilization, irrigation scheduling, pest management, pollination, thinning the punches, and uncontrolled weather conditions are critical factors for improving fruit quality to the export standard (Ghazzawy et al., 2023). Managing all these key factors is difficult for most Jordan Valley farmers. According to a personal interview with Palestinian growers, the price of one kg at the farm gate ranges between 3.03 and 4.69 $ for regular and organic growers, respectively.

According to the Palestinian Ministry of Agriculture (MoA) and the Palestinian Date Palm Growers Association, the Date fruit waste ranges between 10% and 25 % of the total yield. In comparison, about 50% of the yield is suitable for international export, and 15% is considered a low-cost class with a price of 1.10-1.38 $, while the average production cost per 1 kg of fruit is 1.93 $. The export company (Packinghouses) exports the goods to the international market at an average price of 7 USD, equivalent to 7.73 $. According to the MoA and Packinghouses, the low-cost class was 2,700 tonnes, so converting the low-cost date fruit class into valuable products with high economic value, such as Date Syrup, Date Vinegar, or Date Bio-ethanol (Ghnimi et al., 2017; Hossain et al., 2014; Taghizadeh-Alisaraei et al., 2019)

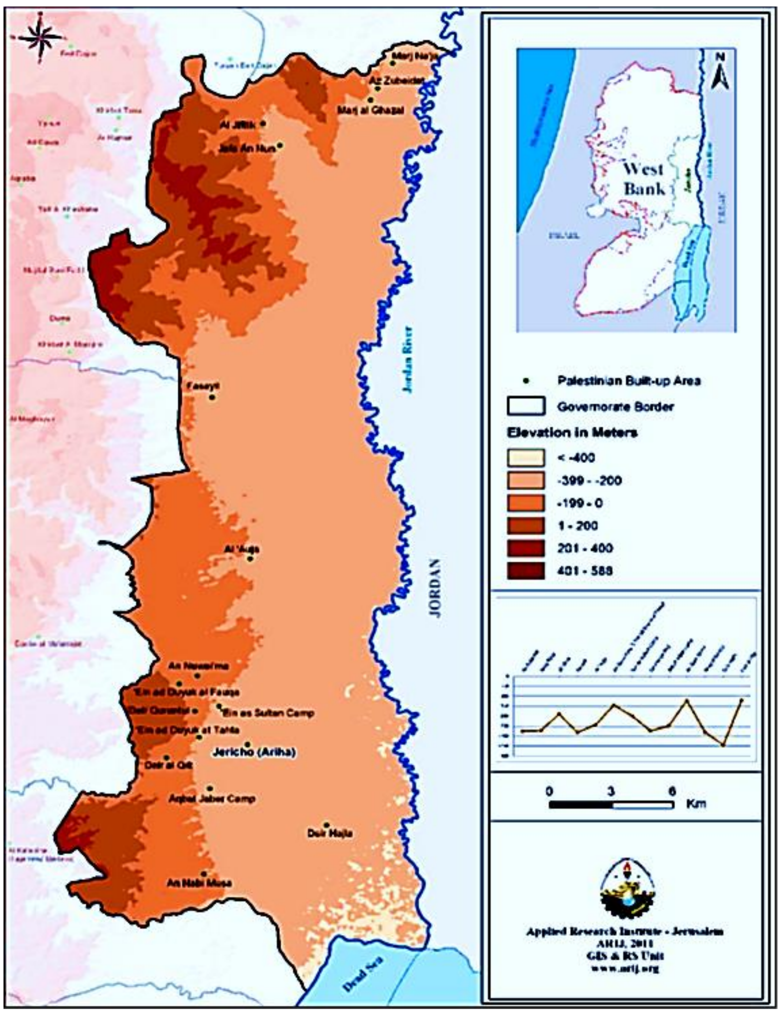

Figure 1.1: The geographical distribution of date palm stations in Jericho governorate in Jordan Valley (Khalilia et al., 2022)

It was anticipated that by 2023, the number of palm date trees in Palestine would reach 400,000 trees, most of which are located in the Jordan Valley, where figure 1.1 illustrates the distribution of dates based on the stations, with about 280,000 trees in the Jericho-Al Uja area, and the rest extend in the Fassayel, Jeftlic, lower Faria, Marj Najeh areas (Palestinian Ministry of Agriculture, 2022). Well, in 2023 it reached around 335,671 trees distributed as 332,181 as Medjool, 2,255 as Berhi, 263 as Dejlet Nour, and 972 trees as Baladi type (Daragmeh Awad et al., 2023). According to the data provided by FAOSTAT, Palestine's annual date production fluctuated from a low of 6,005 tons to a high of 12,030 tons between 2011 and 2014, with an average of 8,134 tons produced over the previous decade (FAOSTAT, 2022a).

Consumers do not accept low-date quality due to its low cost. According to Abbès et al. (2011) there is an estimation of date rejection. Hence, between 10 and 15% of Medjool dates are rejected by consumers in Palestine due to their perceived low cost. According to the new data collected for this season in 2023 from the Palestinian Ministry of Agriculture, wastes ranged between 10% and 30%. As a result, date growers suffer financial loss. This percentage falls within the normal range of 10 to 50 percent across the globe (Taghizadeh-Alisaraei et al., 2019).

1.2 Problem Statement

Palestinian farmers and packing houses have challenges in selling low-cost date fruit products, while the nutritional quality of the fruit is not less than that of the high classes. Where In 2023 the expected volume was 2,700 tons of low-quality dates. The production cost for one kg of date fruit is 2 USD, while the price for one kg of low-quality date fruit (LQDF) is 1 USD; this causes losses for many farmers.

1.3 Study Justification

Excellent and profitable use of low-cost dates to create bio-ethanol as the principal product could be an appropriate solution to the problem of low-cost fruit and producing high-quality natural products. The study aims to propose a methodology for optimizing bio-ethanol production using the response servce methodology as Box-behnken method using Stat Ease Design of Experiment software (Tenkolu et al., 2022).

1.4 Study Goal

The main goal of this study is to evaluate bio-ethanol production from low-cost dates with different sugar concentrations through the fermentation process using statistical analysis by Stat Ease Design Expert version 13 software.

1.5 Study Period

- September/October – 2022: Collection of low-cost dates from local farms
- October/November – 2022: Design and run the initial screening tests and collect data.
- September – 2023: Collection of low-cost dates from local farms.
- September / October – 2023: Design, run, and collect experiment data.

1.6 Study Location

All study experiments were done in the Research Laboratory of Water and Environment in cooperation with the biology department at Al-Quds University.

1.7 Study Question

This study answers the following research question:

1. What are the best parameters (time/yeast content/sugar percentage) to get the best bio-ethanol yield?

Chapter Two

Literature Review

- **2.1 Palm date Status and distribution in Palestine**
- **2.2 Date Fruit and Palestine Economy**
- **2.3 Date Fruit Composition**
- **2.4 Bio-ethanol Production**
- **2.5 Previous Studies**

Chapter Two: Literature Review

2.1 Palm date Status and distribution in Palestine

Date Palm is considered one of the most important fruit crops in Palestine. It has significant socio-economic importance due to its commercial, nutritional, environmental, social, health, and religious values. (Radwan, 2017) Cultivated date palms have existed in Palestine for 5,000 years. Date palm cultivation in Palestine exists in Jericho, the Jordan Valley in the West Bank, and the Gaza Strip. (Abu-Qaoud, 2015) The average economic life of a date palm tree is 40 to 50 years, but some can still be productive for a more extended period. (Chao & Krueger, 2007). The date palm tree has lower water demand, 1105 m^3 /year.dunum compared to other crops such as bananas, tomatoes, and bell b peppers, in addition to its tolerance to harsh weather and higher water salinity of up to 8 mS/cm (Abd Rabou & Radwan, 2018). According to the Ministry of Agriculture statistics, in September 2021, the number of palm farms in Palestine reached 571 farms, employing more than 5,000 workers. The predicted production of dates for the 2023 season was 18,320 tons (Sabarneh, 2022)

The date palm sector faces varied challenges, such as water scarcity, water salinity, high production costs, low prices for low-cost classes, and weak industrial processing. In 2019, according to the Palestinian Ministry of Agriculture, 10,980 tons of date were produced in the West Bank-Palestine, estimated to double within a a few years. (Sabarneh, 2022) the newly collected data from the 2023 season showed an increase in date production of 17,886 tons (Table 2.1).

Table 2.1: The increase in Medjool palm cultivation according to the Palestinian Ministry of Agriculture. (Daraghmeh & Haddad, 2023)

Year	Production/ton	Area/dunums
2005	90	1,100
2006	100	1,400
2007	110	1,650
2008	450	2,350
2009	776	3,000
2010	1,000	4,700
2011	1,400	7,000
2012	2,000	10,000
2013	2,970	12,000
2014	4,000	14,000
2015	4,500	15,000

2016	5,800	17,000
2017	7,000	18,000
2018	9,500	21,500
2019	10,980	23,000
2020	12,516	19,629
2021	11,866	23,669
2022	14,497	20,382
2023	17,886	21,872

* Production and area numbers represented each year's Fruitful trees .

The Medjool variety was introduced by the beginning of the nineties, which succeeded in extending from the Dead Sea in the south to the village of Marj Na'ja in the north, giving high production and quality results. (I. Bsharat, 2021). There is still an increase in the areas planted in palms due to the high salinity tolerance of the crop. The fruitful palm trees increased from 1,100 dunums in 2005 to 21,872 in 2023, a significant expansion.

2.2 Date Fruit and Palestine Economy

During the last two decades, about 300,000 Medjool-type date palm trees have been cultivated in the Jordan Valley. The export of date fruit to the international market ranks increases yearly (Table 2.2). Palestine exports date mainly to the United Arab Emirates, E.U. countries, Saudi Arabia, Turkey, Malaysia, Russia, USA, and Indonesia. (R. Bsharat, 2022)

Table 2.2: Palestine Exported Dates to International Market between 2009-2022 (R. Bsharat, 2022)

Year	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
Exports (Tons)	**70**	**200**	**500**	**700**	**800**	**1,400**	**2,000**	**3,000**	**2,286**	**2,778**	**4,385**	**5,072**	**6,555**	**6,425**

Few studies have been conducted on the feasibility of date palm cultivation in Palestine; the agricultural sector is essential to the Palestinian economy. It produces 11–20% of Gross domestic product, accounts for 25% of total exports, and is the third largest employer engaging 15% of the workforce. (Alataweneh, 2013). One of the studies published in 2007 by the owner of one of the most significant date farms in Palestine (Daiq, 2006) dealt with the high economic feasibility of date palm cultivation and its impact on the Palestinian economy. According to the study, Date fruit consumption increased by 28.4% from 1994 to 2000.

COVID-19 harms the export of Date fruit commodities to the international market; this decreases the price from 4.14 to 2.48 $/kg. The current export value of this commodity ranges between 35 and 40 million dollars USD/year (Daragmeh, 2023). The main agricultural exports from the State of Palestine are olive oil, dates, grapes, strawberries, medicinal herbs (Thym), and almonds. In contrast, the bulk expansion in agricultural product export goes on the share of Date fruit. Palestinian exports of dates have risen from US$ 324,000 in 2007 to US$ 1.2 million in 2010, reflecting an absolute growth of over 250 %. (International Trade Center, 2018)

The sector offers working places for both genders during the year. On average, every date palm tree offers 1.5 to 2 working days, in addition to the packaging houses that offer working places for women. Indirect works also to the suppliers of fertilizers and another supporting sector.

In 2021, Palestine emerged as the 9th largest global exporter of Dates, both fresh and dried, with a total export value of $55.4 million. During the same period, Dates, fresh or dried, secured its position as the 4th most exported product from Palestine. The primary destinations for Palestine's Dates, fresh or dried, were Turkey ($9.4 million), the United Kingdom ($7.52 million), the United Arab Emirates ($5.75 million), Saudi Arabia ($5.45 million), and Germany ($3.86 million). Notably, the most rapidly expanding export markets for Palestine's Dates, fresh or dried, between 2020 and 2021 were observed in Saudi Arabia (an increase of $5.36 million), the United Arab Emirates (an increase of $2.54 million), and the Netherlands (an increase of $2.31 million). (The Observatory of Economic Complexity (OEC), 2021)

2.3 Date Fruit Composition

Date fruit is an excellent source of nutritional and health benefits. The chemical composition of dates includes carbohydrates, dietary fiber, proteins, fats, minerals and vitamins, enzymes, phenolic acid, and carotenoids, all directly linked to nutritional and health benefits for consumers. (Ibrahim et al., 2021) The chemical composition of dates can vary depending on the cultivar, soil conditions, agronomic practices, the ripening stage, and farm management (Al-Kahtani & Soliman, 2012). A high percentage of sugar in the dates fruit provides a good rapid energy source. (Al-Shahib & Marshall, 2003) Dates are a rich source of nutrients and sugars (70–80%) in the form of glucose, fructose, and sucrose. (Al-Farsi et al., 2007)

Table 2.3: Part of Medjool Date Fruit Per 100g (Amadou, 2016; Devshony et al., 1992; USDA, 2015)

Component	Value
Moisture (g)	21.32
Fat (g)	0.15
Protein (g)	1.81
Fiber (g)	6.70

Sugars, total (g)	66.47
Ash (g)	1.67
Vitamin A (IU)	149
Thiamine (mg)	0.050
Riboflavin (mg)	0.060
Niacin (mg)	1.610
Vitamin B6 (mg/mL)	0.249
Vitamin C (mg)	0
Folate (µ)	15

2.4 Bio-ethanol Production

Bio-ethanol is considered one of the most important renewable energy sources that can partially replace fossil fuels. (Louhichi et al., 2013) The most crucial advantage of bioenergy is that it reduces dependence on nonrenewable fossil fuel sources. It can also provide opportunities to convert renewable organic waste materials into energy. Moreover, biomass with high sugar content allows the use of fermentation. (Djelal et al., 2017)

Fermentation technology is used to preserve food and derive valued by-products from food materials across the globe. (Chandrasekaran & Bahkali, 2013). Bio-ethanol production via yeast fermentation may provide an economically competitive source of energy. (Djelal et al., 2017). Syrup resulting from date by-products constitutes a favorable medium for yeast development. (Chniti et al., 2014) *Saccharomyces cerevisiae* is traditionally used for bio-ethanol production. *Saccharomyces cerevisiae* can produce 48.8 $g.L^{-1}$ bio-ethanol from pineapple cannery waste and 120.68 $g.L^{-1}$ bio-ethanol from sweet sorghum juice. (Nigam, 2000)

2.5 Previous Studies

Zohri and Etnan (2000) The seedless date solution was used to produce bio-ethanol after mixing, decanting, clarifying, and filtrating it. The total sugar concentrations in the prepared date juice were adjusted to 13.5, 18.0 and 22.5%. Date juice with 18% sugar content was the most suitable, and economic concentration could be fermented to bio-ethanol by using each of *Saccharomyces cerevisiae* or *S. bayamus* in submerged fermenters. The higher yield bio-ethanol production was recorded using S. bayamus grown on date juice with 18% sugar concentration at 30°C and pH 3.5. The fermentation rate under these conditions was 2.0 Brix consumed per 12 hours, and the fermentation was completed in 84 hours, resulting in an bio-ethanol concentration of 9.2%. The fermented date juice was distilled at the end of fermentation, and alcohol with nearly 93% concentration was produced.

Bassam (2001), studied the ability of two strains of *Saccharomyces cerevisiae* and *Candida* utilis to utilize the date Juice. The results showed that s. cerevisiae has a high ability to metabolize date juice for bio-ethanol production. The data on optimization of physiological conditions of fermentation, pH, temperature, and sucrose concentration showed similar effects on immobilized and free cells of *S. cerevisiae* and *C. utilis* in batch and immobilized fermentation of s. cerevisiae. A maximum yield of 12.8%, 13.4% w/v, and bio-ethanol was obtained from 22g/L sucrose when fermentation was carried out at pH 4.5 and 30 C using *S. cerevisiae*.

Louhichi et al. (2013), A comparison was made between Soxhlet and solvent extraction of a juice obtained from the dates. The alcoholic fermentation of this juice by *Saccharomyces cerevisiae* was investigated under sugar concentration near 200 $g.L^{-1}$ at 30 ^{o}C and natural pH. The results showed that all the tested varieties allowed bio-ethanol production with a concentration of around 25% (V/V). Moreover, the yeast used in the fermentation process is capable of producing alcohol even at a pH of 3.8.

Chniti et al. (2014), studied the ability of *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii*, and *Candida pelliculosa* for bio-ethanol production on dates syrup. In batch fermentation, the bio-ethanol concentration was depended on the initial sugar concentration and the yeast strain. For an initial sugar concentration of 174.0 ± 0.2 $kg.m^{-3}$, maximum bio-ethanol concentration was 63.0 ± 0.1 $kg.m^{-3}$ during *S. cerevisiae* growth, namely higher than the amounts achieved during *Z. rouxii* and *C. pelliculosa* growth, 33.0 ± 2.0 $kg.m^{-3}$ and 41.0 ± 0.3 $kg.m^{-3}$ respectively. Contrarily, only *Z. rouxii* could grow on 358.0 ± 1.0 $kg.m^{-3}$ initial sugar amount, resulting in 55.0 ± 1.0 $kg.m^{-3}$ bio-ethanol produced.

Chniti et al. (2017) was tested three yeasts, *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii* and *Candida pelliculosa*, for bio-ethanol production on dates' syrup. In batch fermentation, the bio-ethanol concentration depended on the initial sugar concentration and the yeast strain. For an initial sugar concentration of 17.4°Brix, the maximum bio-ethanol concentration was 63 g/L during S. cerevisiae growth, higher than the amounts achieved during *Z. rouxii* and *C. pelliculosa* growth, 33 g/L and 41 g/L, respectively. On 35.8 Brix initial sugar amount, only *Z. rouxii* was able to grow, resulting in 50 g/L bio-ethanol production, showing an inhibitory effect on S.cerevisae and *C. Pelliculosa* due to the osmotic stress resulting from the high sugar concentration.

Chapter Three

Materials & Methods

- **3.1 Materials**
- **3.2 Methodology**
    - 3.2.1 Experimental Design
    - 3.2.2 Date samples collection
    - 3.2.3 Palm dates Juice Preparation
    - 3.2.4 Yeast Culture Preparation
    - 3.2.5 Preparation of Fermentation medium for bio-ethanol production
    - 3.2.6 Responses Measurements

Chapter Three: Materials & Methods

3.1 Materials

The following contents were used in this study:

- U.V./Vis Spectrophotometer (Hach), Gas Chromatography, brix meter, sugar brix kit 10mL test tubes, rack-tubes, incubator, Wireloop, beakers, flasks, Autoclave, pH meter, and Petri dishes.
- The chemicals are glucose, urea, K_2HPO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, malt extract agar base with mycological peptone, and yeast extract were bought from Sigma-Aldrich.
- *Saccharomyces cerevisiae* is purchased from Sigma company as live culture.

Table 3.1: Instrument details

Instrument	Methodology	Reference
pH-mater	After calibration, the electrode immersed in the solution and when the reading stabilized, the result was recorded	(Skoog et al., 2019)
Brix meter	After calibration, the cover of the brix meter was opened and the sample was added, then the result was obtained	(NIELSEN, 2010)
UV/Vis Spectophotometer	The measurements were obtained according to the general instructions of spectrophotomer manuals	(Skoog et al., 2019)
Gas Chromatography	The measurements were obtained according to the general instructions of Gas Chromatography manuals	(Snyder et al., 2011)

Table 3.2: Chemicals and biological material

Chemicals / Biological Material	Reference/Lot Number	Company
Yeast from *Saccharomyces cerevisiae*	BCCD3501	Sigma Aldrich
Glucose (Dextrose: Corn Sugar)	21K0010	Sigma Aldrich
K_2HPO_4	22K0085	Sigma Aldrich
$MgSO_4.7H_2O$	90K0850	Sigma Aldrich
$FeSO_4.7H_2O$	98517	Human Chemicals
Malt extract agar base	0000507172	HiMedia

3.2 Methodology

3.2.1 Experimental Design

The experiment design was used to identify the number of experiments used to optimize bio-ethanol production. We applied the response surface methodology randomized Box-Behnken design using Stat Ease Software version 13. As described in Table 3.3, this study has three factors: Time (h), Yeast (*Saccharomyces cerevisiae)* concentration (O.D.), and sugar concentration (Brix), while outcomes will be exported as bio-ethanol (%). In this experiment, the tested yeast O.D. was between 0.2 and 2.0; the period was between 48 and 240 hours, whereas sugar concentration was between 5 and 35 brix. The software suggested the table 3.3 with 16 runs, where each run has 3 replicates. The pH-value was fixed at 6.5, and the temperature was fixed at 30 ^{o}C during all experiment runs. *As many articles stated, Saccharomyces cerevisiae was used because it is the most common used in bioethanol production during the fermentation process.*

Table 3.3: Experiment design for the factors and responses in this study. Data arranged according to Factor 1.

Run	**Factor 1**	**Factor 2**	**Factor 3**	**Response 1**
	A: Time (Hour)	**B: Yeast (OD)**	**C: Sugar (Brix)**	**Bio-ethanol (%)**
1	48	1.1	35 ± 0.50	
2	144	2	5 ± 0.25	
3	240	1.1	5 ± 0.26	
4	144	0.2	5 ± 0.25	
5	144	1.1	20 ± 0.30	
6	144	0.2	35 ± 0.50	
7	144	1.1	20 ± 0.40	
8	240	0.2	20 ± 0.39	
9	240	2	20 ± 0.36	
10	144	1.1	20 ± 0.38	
11	144	2	35 ± 0.49	
12	240	1.1	35 ± 0.50	
13	48	1.1	5 ± 0.24	
14	48	0.2	20 ± 0.50	
15	144	1.1	20 ± 0.48	
16	48	2	20 ± 0.43	

3.2.2 Samples collection

Date fruit samples around 10 kg were collected from Jericho farms, which were soft and low in cost from the Medjool type dates (Figure 3.1).

Figure 3.1: Collection of Low-cost dates

3.2.3 Dates Juice Preparation

The seeds of the fruits were removed, and the dates were washed and cut into small pieces, then cooked at 80 °C for 30 minutes (figure 3.2) to minimize the impact of other microorganisms during the fermentation process and to enable any bio-ethanol residules in the dates to volatile because it needs around 78 °C to be volatile. Two kg of date flesh was mixed with 6.0 L of water (1:3 ratio) (to obtain higher brix). The mixture was primarily filtered with white sheets of clothes and the formed liquid, then stored at 4 °C in a refrigerator (to supress any microbial growth in that time). Sugar brix was measured, and it was 35. The concentrated date juice was diluted to prepare the optimum solutions used in this study based on the following formula: $C_1V_1 = C_2V_2$

Figure 3.2: Date Juice preparation steps

3.2.4 Yeast Culture Preparation

The culture medium consisted of 8.0 g/L malt extract agar base with mycological peptone, 10.0 g/L glucose, 3.0 g/L yeast extract, and 20 g/L agar, where the value was 6.5. Yeast medium was autoclaved at 121^{o} C for 15 min and poured into Petri dishes (Figure 3.3). The same solution was prepared without adding agar to form yeast culture broth and autoclaved and poured into 10 mL test tubes. Both were stored at 4 °C for yeast culture after that.

Figure 3.3: Preparation of yeast medium agar for *Saccharomyces cerevisiae* growth.

Saccharomyces cerevisiae was grown on yeast medium (with agar) at 30 °C for 48 hours (Figure 3.4). The yeast was added to yeast culture broth to form the proper yeast concentration, as mentioned in Table (3.3), for bio-ethanol production to use in the fermentation process. The following procedures describe the method for yeast preparation.

1. The yeast culture broth was prepared and separated into a 10 mL test tube.
2. The solution was a blank in the spectrophotometer with an optical density of 600 nm.
3. At least one yeast colony was added using the Wireloop to yeast broth test tube, mixed well, and measured on a spectrophotometer.

4. The last step was repeated by adding more colonies to the solution to get the required concentration per 10 mL.

Figure 3.4: Saccharomyces cerevisiae after growing on yeast medium agar.

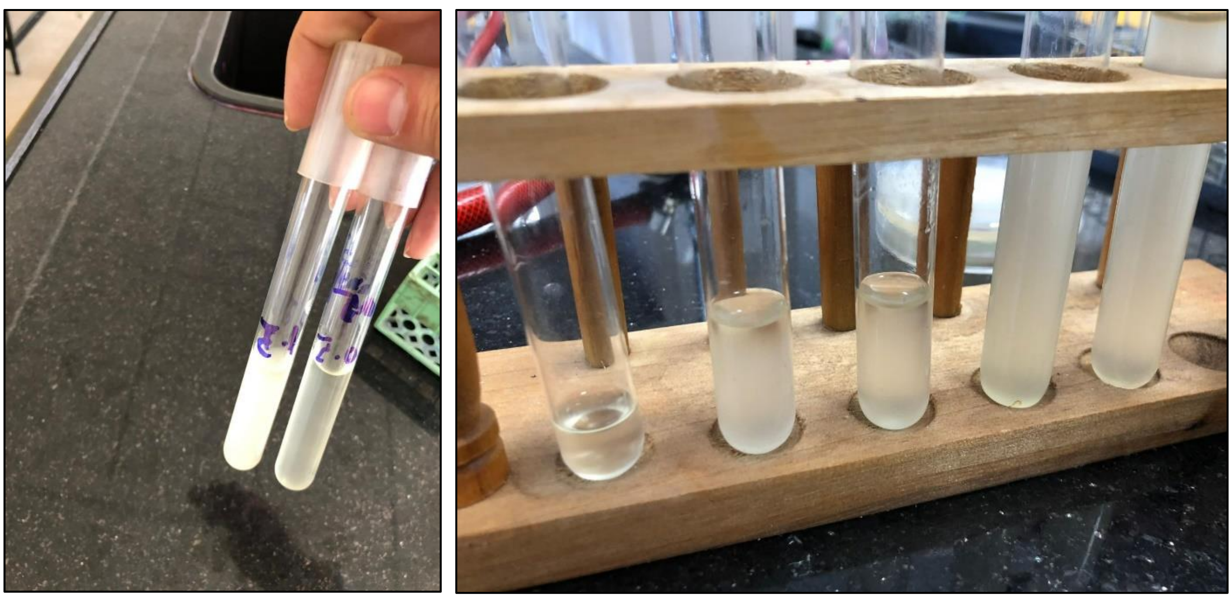

Figure 3.5: Preparation of Yeast culture with different concentrations.

3.2.5 Preparation of fermentation medium for bio-ethanol production

The following components were added (10 g/L of glucose, 3 g/L of urea, 0.5 g/L of KH_2PO_4, 0.5 g/L of K_2HPO_4, 0.5 g/L of $MgSO_4.7H_2O$, and 0.01 g/L of $FeSO_4.7H_2O$) to one liter of date juice, then the solution was autoclaved at 121 ^{o}C for 15 min (based on laboratory procedures to autoclave microbials chemicala). The fermentation process to produce bio-ethanol was performed in a 250 mL flask; 100 mL of fermentation media were inoculated with 1 mL yeast culture. Flasks were made airtight with rubber stoppers to maintain anaerobic conditions. Each flask had an air-piper connected to the rubber and water bottle to remove excess gas and bubbles. All samples were fermented at 30^{o} C according to the previous experiment design.

Figure 3.6: Autoclaving process for all samples.

Figure 3.7: Experiment preparation and setup.

3.2.6 Responses measurements

Bio-ethanol percentage was tested in Al-Quds Center for Measurement and Analysis at Al-Quds University using gas chromatography. Sugar brix was tested using a brix meter, and the results were analyzed using Stat Ease experiment design software.

Figure 3.8: Samples preparation for analysis.

Chapter Four

Results & Discussion

- **4.1 Results & Discussion**
  - 4.1.1 Data Analysis for Bio-ethanol Production
  - 4.1.2 Bio-ethanol Production Equation
  - 4.1.3 Bio-ethanol Production Optimization

Chapter Four: Results and Discussion

4.1 Results & Discussion

In this experiment, 16 runs were designed to study the effect of growing time, yeast, and sugar concentrations on the productivity of bio-ethanol from date juice and after the fermentation process. Results are illustrated in Table 4.1. The table shows each run and the assigned level of each factor in addition to the response. The run includes either a low, middle, or high level of each factor based on previous screening tests in 2022 (Data not shown).

Table 4.1: The effect of growing time, yeast, and sugar concentrations on bio-ethanol production.

Run	Factor 1	Factor 2	Factor 3	Response 1
	A: Time (Hour)	B: Yeast (OD)	C: Sugar (Brix)	Bio-ethanol (%)
1	48	1.1	35 ± 0.50	4.7 ± 0.21
2	144	2	5 ± 0.25	2.3 ± 0.10
3	240	1.1	5 ± 0.26	2.6 ± 0.10
4	144	0.2	5 ± 0.25	2.0 ± 0.12
5	144	1.1	20 ± 0.30	4.9 ± 0.22
6	144	0.2	35 ± 0.50	4.0 ± 0.15
7	144	1.1	20 ± 0.40	6.1 ± 0.06
8	240	0.2	20 ± 0.39	6.7 ± 0.32
9	240	2	20 ± 0.36	7.8 ± 0.38
10	144	1.1	20 ± 0.38	4.4 ± 0.26
11	144	2	35 ± 0.49	6.9 ± 0.20
12	240	1.1	35 ± 0.50	5.9 ± 0.21
13	48	1.1	5 ± 0.24	1.2 ± 0.15
14	48	0.2	20 ± 0.50	5.9 ± 0.10
15	144	1.1	20 ± 0.48	7.4 ± 0.21
16	48	2	20 ± 0.43	6.5 ± 0.15

4.1.1.1 ANOVA for Quadratic Model

The analysis of results using design expert software (Analysis of Variance ANOVA) showed that the Model F-value of 6.72 implies the model is significant with a P-value of 0.0155. There is only a 1.55% chance that an F-value this large could occur due to noise. Only sugar concentrations were significant, with a P-value of 0.0028 (table 4.2). In this case, C, C^2 are significant model terms. Growing time and yeast concentration had no significant influence.

Table 4.2: ANOVA for Quadratic model for Response 1: Bio-ethanol

Source	Sum of Squares	df*	Mean Square	F-value	p-value	Status
Model	57.40	9	6.38	6.72	0.0155	Significant
A-Time	2.76	1	2.76	2.91	0.1390	
B-Yeast	3.00	1	3.00	3.16	0.1258	
C-Sugar	22.45	1	22.45	23.64	0.0028	Significant

AB	0.0625	1	0.0625	0.0658	0.8061	
AC	0.0100	1	0.0100	0.0105	0.9216	
BC	1.69	1	1.69	1.78	0.2306	
A²	0.6806	1	0.6806	0.7168	0.4297	
B²	1.50	1	1.50	1.58	0.2554	
C²	25.25	1	25.25	26.59	0.0021	Significant

* df: Degree of Freedom

4.1.1.2 Coefficients in Terms of Coded Factors

The coefficients in Table 4.3 represent the expected change in response for each unit change in the corresponding factor, assuming all other factors remain constant. The intercept (5.70) is the average response when all factors are set to zero in an orthogonal design. In an orthogonal design, factors are set up to be independent of each other. This means that changing one factor does not influence the others, leading to a clearer understanding of the individual factor effects.

Variance Inflation Factor (VIF) measures how much the variance of an estimated regression coefficient increases if your predictors are correlated. In an orthogonal design, VIFs are 1, indicating no multicollinearity (correlation between predictors). If VIF > 1, there's multicollinearity, and higher VIF values suggest more severe correlation. The rule of thumb is that VIFs less than 10 are tolerable.

Table 4.3: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df*	Standard Error	95% CI Low	95% CI High	VIF
Intercept	5.70	1	0.4872	4.51	6.89	
A-Time	0.5875	1	0.3445	-0.2555	1.43	1.0000
B-Yeast	0.6125	1	0.3445	-0.2305	1.46	1.0000
C-Sugar	1.68	1	0.3445	0.8320	2.52	1.0000
AB	0.1250	1	0.4872	-1.07	1.32	1.0000
AC	-0.0500	1	0.4872	-1.24	1.14	1.0000
BC	0.6500	1	0.4872	-0.5422	1.84	1.0000
A²	0.4125	1	0.4872	-0.7797	1.60	1.0000
B²	0.6125	1	0.4872	-0.5797	1.80	1.0000
C²	-2.51	1	0.4872	-3.70	-1.32	1.0000

* df: Degree of Freedom

4.1.1.3 Fit Statistics

Coefficient of Determination (R^2) measures the proportion of the variance in the dependent variable (response variable) that is predictable from the independent variable (predictors). An R^2 of 0.9097 suggests that about 90.97% of the variability in the dependent variable is explained by the independent variable. Adjusted R^2 is similar to R^2 but adjusts for the number of predictors in the model. In this case, it is 0.7743, which accounts for the model complexity. (See table 4.4).

Table 4.4: Fit Statistics

Std. Dev.	**0.9745**	**R^2**	**0.9097**
Mean	4.96	**Adjusted R^2**	0.7743
C.V. %	19.66	**Predicted R^2**	0.7679
		Adequate Precision	8.7781

For the comparison of Predicted R^2 and Adjusted R^2. The Predicted R^2 is given as 0.7679, and the passage states that this is in reasonable agreement with the Adjusted R^2 of 0.7743. This indicates that the model's performance is consistent with its performance on the training data. The small difference (less than 0.2) suggests that the model generalizes well.

Adequate Precision measures the signal-to-noise ratio. A higher ratio is generally desirable. In this case, a ratio of 8.7781 is mentioned, which is well above 4 (the desirable threshold). This suggests that the signal (meaningful information) in the model is strong compared to the noise (random variability). The table provides additional statistical measures for the model, such as the standard deviation, mean, and coefficient of variation (C.V. %). These metrics give insights into the distribution and variability of the data.

The passage concludes by stating that the model can navigate the design space. This means that the model is effective in understanding and representing the relationships within the data. The combination of high R^2, Adjusted R^2, and Adequate Precision suggests that the model is robust and performs well. In summary, the provided statistics and information convey that the model has good explanatory power, generalizes well to new data, and exhibits a strong signal-to-noise ratio, making it a reliable tool for navigating the design space.

4.1.1.4 Response Service Model Graphs

Figure 4.1 shows a set of three graphs labeled A, B, and C. These are response surface methodology (RSM) plots, which are used to model and analyze the effects of several independent variables on a response variable. Graph A describes the Effect of Time on Ethanol Production, this graph shows the relationship between time (in hours) and the percentage of ethanol produced. The x-axis represents time in hours, and the y-axis represents ethanol concentration as a percentage.

The solid line represents the predicted response, while the dotted lines represent the 95% confidence interval (CI) bands. The design points (actual experimental observations) are marked with red dots.

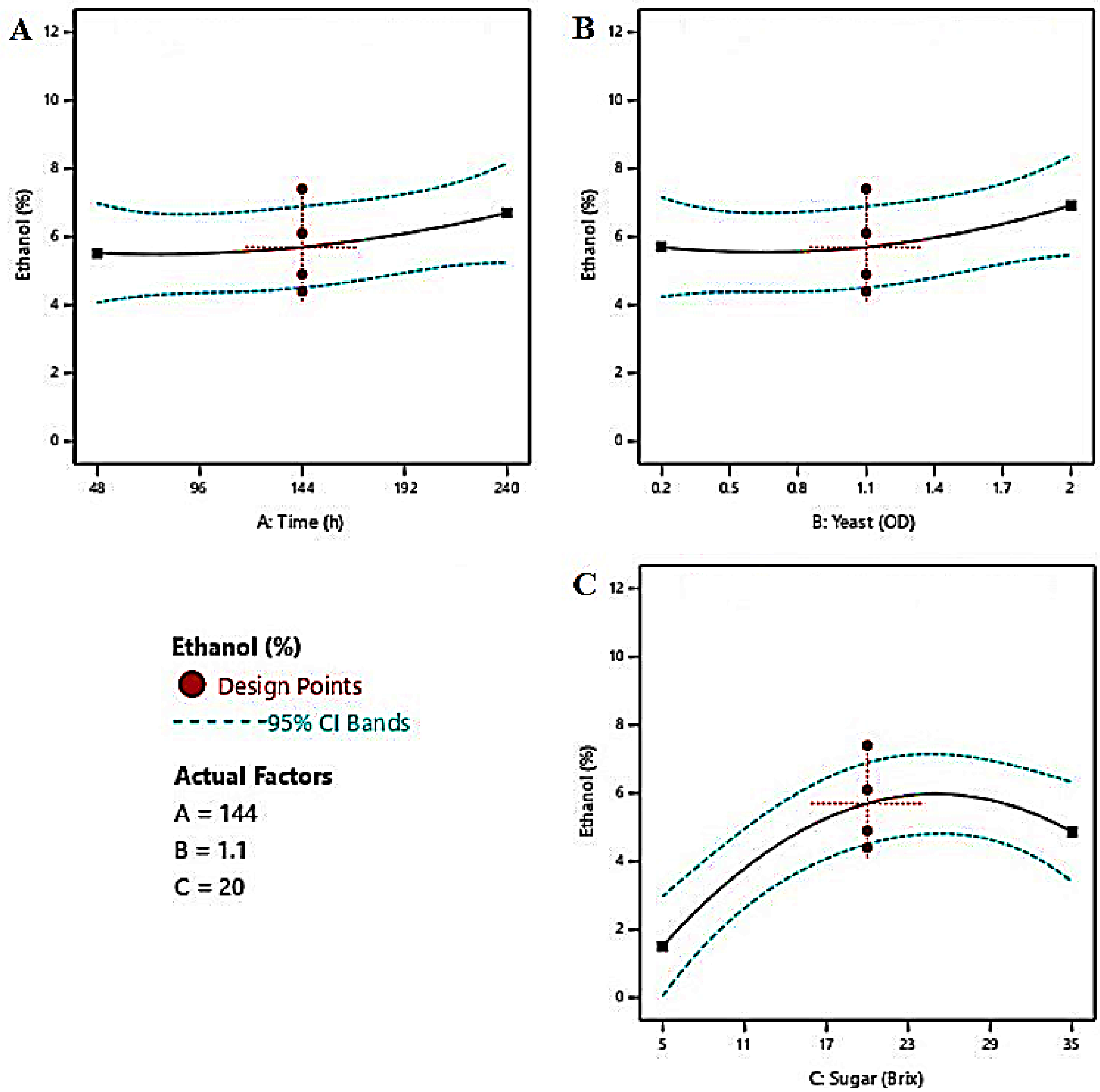

Figure 4.1: Relation between time and bio-ethanol, yeast and bio-ethanol, and sugar content with bio-ethanol.

Graph B describes the Effect of Yeast Concentration on Ethanol Production, this graph illustrates how the concentration of yeast (measured in optical density, OD) affects ethanol production. The x-axis represents yeast concentration, and the y-axis represents ethanol concentration as a percentage. As in graph A, the solid line is the predicted response with the dotted lines showing the 95% CI bands, and red dots indicate design points.

Graph C describes the Effect of Sugar Concentration on Ethanol Production, this graph displays the effect of sugar concentration (measured in Brix, a unit for sugar content) on ethanol production. The x-axis represents sugar concentration, and the y-axis represents ethanol concentration as a percentage. Similar to the other graphs, there's a predicted response (solid line) and 95% CI bands (dotted lines), with red dots for design points.

In Figure 4.1, Red dots represent the design points, and the blue dotted lines represent the 95% CI bands. Actual factors used in the experiment are listed as follows: A (time) = 144 hours, B (yeast OD) = 1.1, and C (sugar Brix) = 20.

These plots are typically used in experiments to optimize the production process, in this case, ethanol production. The graphs suggest that there is an optimal range for each factor where ethanol production is maximized. The confidence intervals provide an understanding of the precision of the predicted response. The actual factors listed may represent the optimal conditions determined by the experiment for ethanol production.

4.1.2 Bio-ethanol Production Equation

Regarding the factors used in this study, the final Equation in Terms of Coded Factors was formulated as an output for the results of varied parameters.

$$Ethanol\ (\%) = 5.7 + ((0.6 * \mathrm{A}) + (0.6 * \mathrm{B}) + (1.7 * \mathrm{C}) + (0.1 * \mathrm{AB}) - (0.0 * \mathrm{AC}) + (0.7 * \mathrm{BC}) + (0.4 * \mathrm{A}^2) + (0.6 * \mathrm{B}^2)) - (2.5 * \mathrm{C}^2)$$

Where A is Growing Time (hour), B is Yeast optical density as concentration, and C is Sugar concentration (Brix).

The coded factors equation can be used to predict the response for given levels of each factor. By default, the high levels of the factors are coded as +1, and the low levels are coded as -1. The coded equation helps identify the relative impact of the factors by comparing the factor coefficients. Due to the non-significant effects of growing time and yeast concentration and their interactions with each other and with sugar concentration, the following formula was created:

$$Ethanol\ (\%) = 6.2 + ((0.6 * \mathrm{A}) + (0.6 * \mathrm{B}) + (1.7 * \mathrm{C})) - (2.5 * \mathrm{C}^2)$$

Where A is Growing Time (hour), B is Yeast optical density as concentration, and C is Sugar concentration (Brix).

4.1.3 Bio-ethanol Production Optimization

Through the analysis of Stat Ease design of experiment design, this study showed around 43 solutions as for optimization of the results with a desirability average of 68% to 74% for using this solution based on the recommendation of the software itself. According to the results of the experiments, bio-ethanol could be produced from low-cost date palm fruit (Medjool) within a range of growing time, yeast, and sugar concentrations, wherein the best solution was number 21 as shown in Figure 4.2. Its present time (48.0 hours), yeast (1.99 OD), sugar (18.86 brix), and bio-ethanol production was 6.43%. To get the maximum bio-ethanol percentage in the shortest time; yeast and moderate sugar concentrations must be increased.

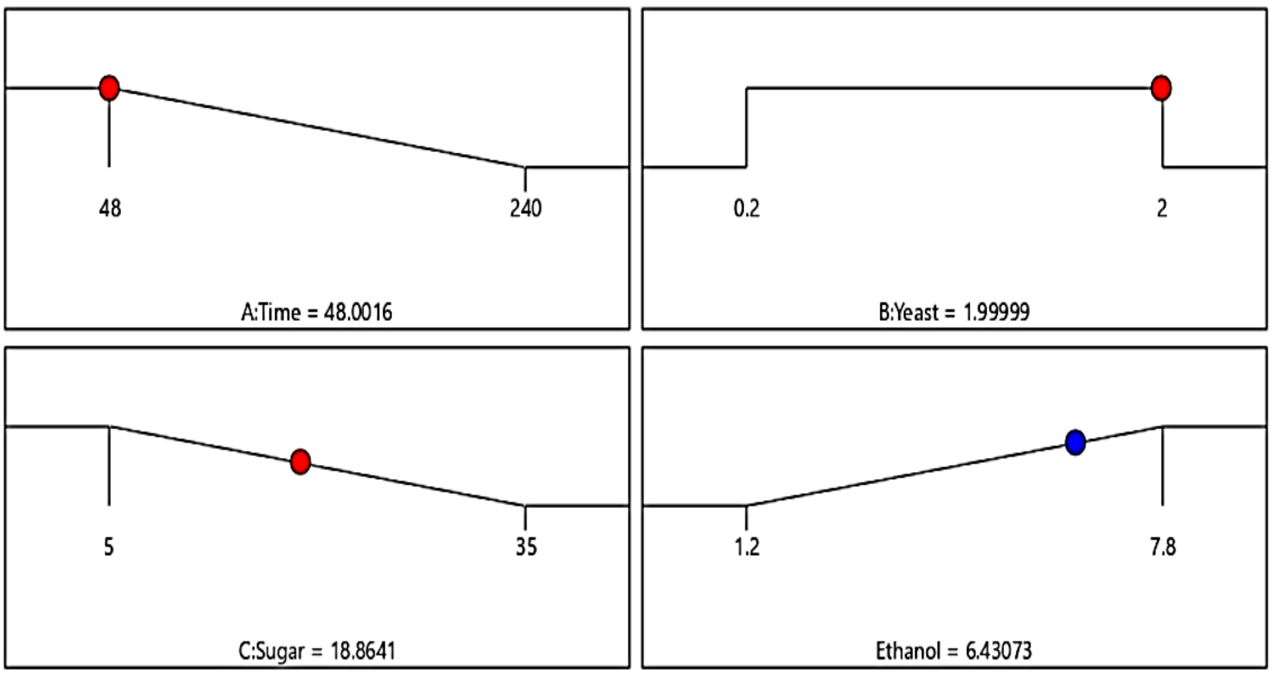

Figure 4.2: Best parameters for bio-ethanol production according to optimization conditions. The figure shown the lowest value and the highest value.

The typical plot of residuals confirms that residuals are normally distributed. On their plot graph, residuals approximately fit a straight line and do not form a shape such as an S curve that might indicate no linearity (Figure 4.3).

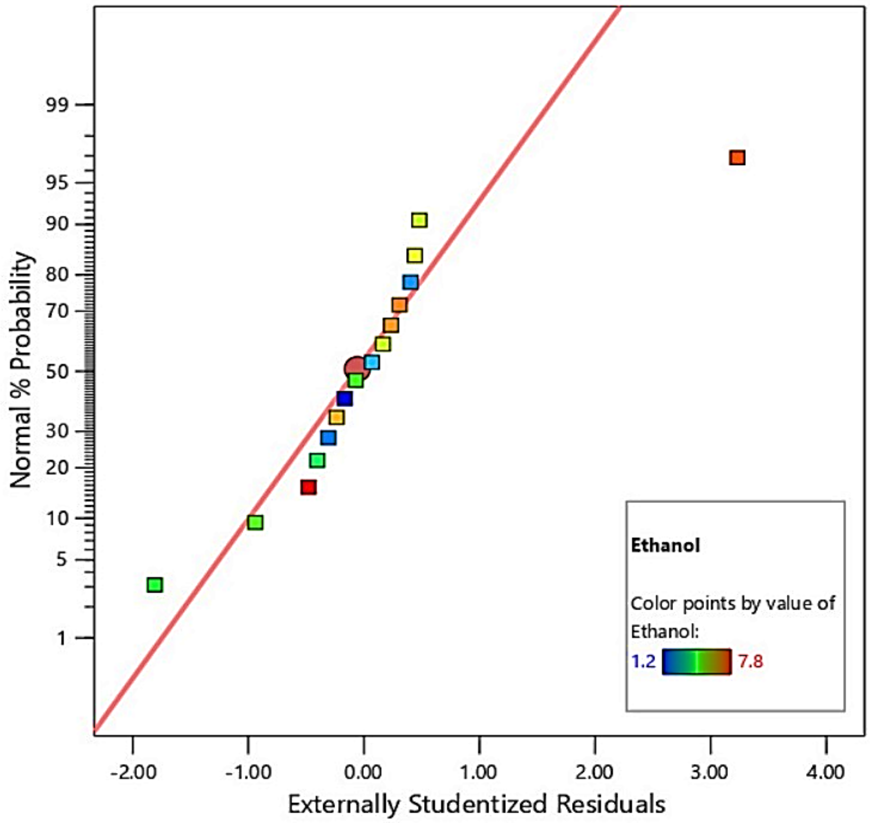

Figure 4.3: Normal Plot of Residuals

In any experiment, researchers tend to identify whether outliers heavily impact their model; the design expert provides the Residual vs. Predicted plot to find outliers. If none of the results appear below or above reference redlines, this indicates an absence of outliers (Fig. 4.4). The figure shows the model-predicted values against residuals. The random scatter around the zero line (highlighted black) can be well noticed. This indicates a good distribution of results. The two redlines at the top and bottom of the graph are set to address if there are outliers in the results. On the graph, no results fall over or below redlines, indicating the absence of outliers in this experiment.

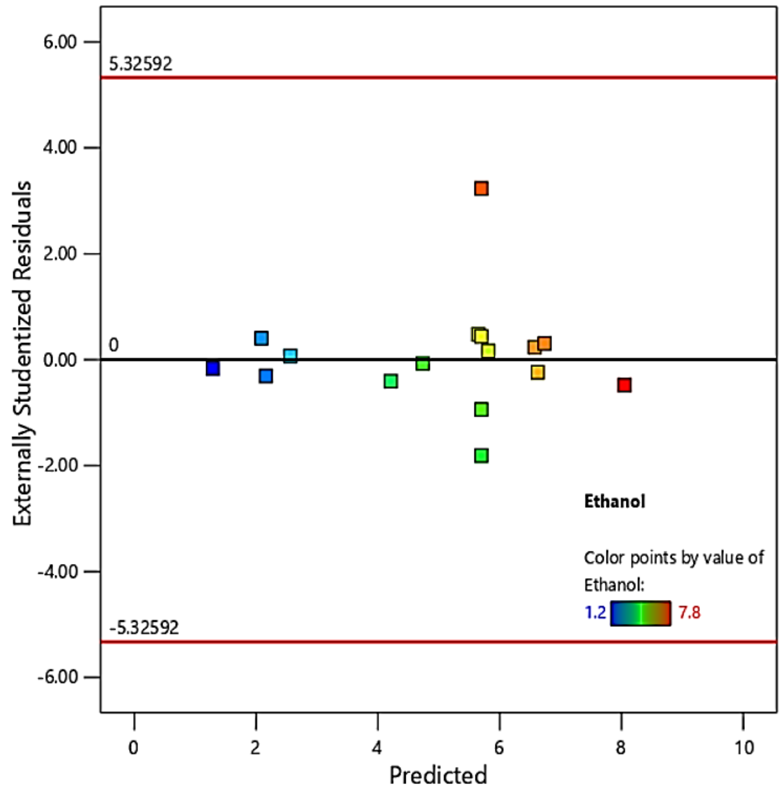

Figure 4.4: Residuals vs. Predicted

The residual vs. Run plot is helpful to detect timeline-based trends in the residuals during the experiment. Residuals should look random on the plot; if any pattern is observed, something went wrong in the experiment. The plot also looks for residuals outside limits. It addresses outliers that indicate a problem with the model, a required transformation, or a cause to verify or repeat a specific run.

Fig. 4.5 shows residual vs. Run distribution; there were no runs outside the limits of redlines, and results are randomly scattered below and above the zero-black line. There was also no source of errors during the experiment, as no patterns were observed. Patterns are usually caused by a specific error in the experiment, for instance, if a cluster of results is above the reference zero line while the rest is below.

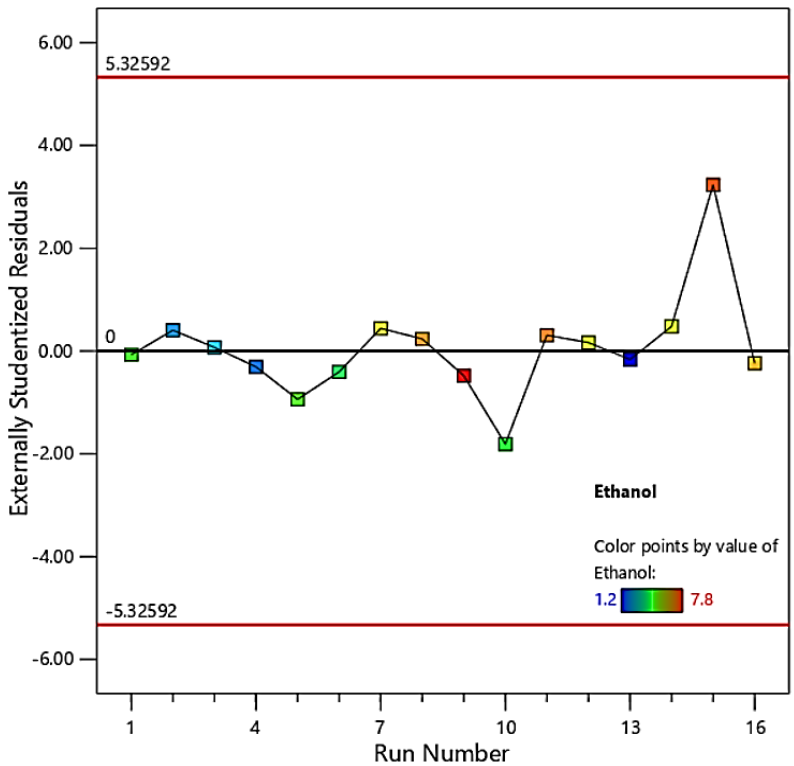

Figure 4.5: Residuals vs. Run

The Box-Cox is a transformation of non-normal independent variables to normality. Lambda (λ) is a power function that describes appropriate transformation. In this experiment, λ= 1.0, which suggests no transformation. Box-Cox plot does not recommend a better transformation than the default. In Fig 4.6 the green line indicates the best λ value (1.0 in this case).

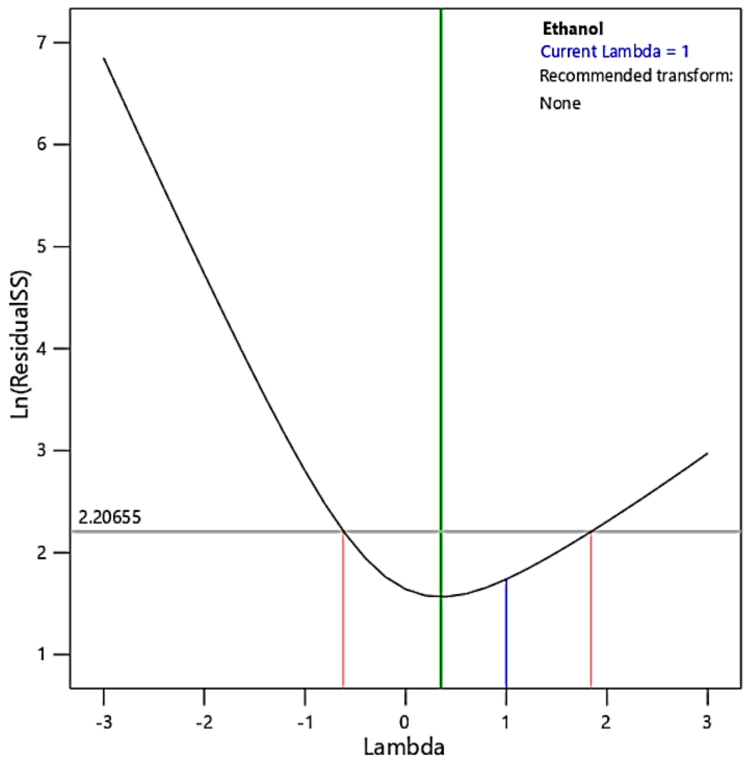

Figure 4.6: Box-Cox: Lambda

This study is considered one of the first publications that used Medjool dates to produce bio-ethanol and the first study of this type in Palestine. Meanwhile, it proves that bio-ethanol production after optimization could be 6.43% after 48 hours when beginning with yeast of 1.99 OD and sugar of 18.86 brix. This study showed higher results when using *Saccharomyces cerevisiae* than El-Shawan and Zohri (2001), who produced 4.91%, and Zohri and Etnan (2000), who could not detect bio-ethanol for the same period (48 hours), and both studies used varied Saudi dates types (Khalas, Raziz, Shabibi, and Shashi). Also, it was higher than Chniti et al. (2014), who used the Deglet-Nour date type and produced 63 $kg.m^{-3}$ or 6.3% after 72 hours; so considering the time, it is better to produce 6.43% after 48 hours than 6.3% after 72 hours. According to our results, 100 ml of the date fruit solution (1:3, W/V) could produce 6.43 mL of bioethanol, then 1 kg able to produce 3 L of 35 brix; so this volume could be diluted to 5.567 L of 18.86 brix. Well if every 100mL produce 6.43 mL then 5.567 L could produce 357.95 mL of ethanol

Chapter Five

Conclusions & Recommendations

- **5.1 Conclusions**
- **5.2 Recommendations**

Chapter Five: Conclusions & Recommendations

5.1 Conclusions

The thesis has presented a comprehensive analysis of the date palm cultivation and utilization in Palestine, with a particular focus on the potential for bio-ethanol production from low-cost date fruits. The study reveals that the date palm, particularly the Medjool variety, plays a crucial role in Palestine's agricultural sector, both economically and socially. Despite the challenges, such as water scarcity, high production costs, and low prices for low-cost dates, the cultivation of date palms remains significant in the region. The research has effectively demonstrated the feasibility of converting low-cost dates into valuable bio-ethanol, thus presenting an innovative solution to manage date fruit waste and boost the economic value of the crop.

5.2 Recommendations

1. Considering the high yield and potential of bio-ethanol production from low-cost dates, it is recommended to scale up this initiative. This could not only provide a sustainable solution for date fruit waste management but also contribute to the renewable energy sector in Palestine.
2. Developing additional products, as a natural product from bioethanol fermentation process, such as vinegar from low-cost dates to increase the economic benefits of growing date palms.
3. Establishing partnerships between farmers, government agencies, and research institutes can foster innovation in date palm cultivation and product development, including bio-ethanol.
4. Continued research in optimizing bio-ethanol production parameters and exploring new technologies in date processing can lead to more efficient and cost-effective production methods.
5. Making awareness for the farmers and local community about the benefits of date-derived products, including bio-ethanol, can increase demand and support for local produce.

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