Deanship of Graduate Studies
Al-Quds University

Antioxidant Activity and Phenolic Content of various Date Palms (*Phoenix dactylifera* L.) Fruits from Palestinian Territories

Lara Hamdan Obeyat

M.Sc. Thesis

Jerusalem – Palestine

1434/2013

Antioxidant activity and Phenolic content of various date palms (*Phoenix dactylifera* L.) fruits from Palestinian territories

Prepared by:
Lara Hamdan Obeyat

B.Sc. Chemistry. Bethlehem University. Palestine

Supervisor: Dr. Imad Odeh
Co- Supervisor: Dr. Fuad Al-Rimawi

A thesis Submitted in Partial fulfillment of requirement for the degree of the Master of Applied and Industrial Technology, Al-Quds University

1434/2013

AL-Quds University
Deanship of Graduate Studies
Applied and industrial Technology Program

Thesis approval

Antioxidant activity and Phenolic content of various date palms (*Phoenix dactylifera* L.) fruits from Palestinian territories

Prepared by: Lara Hamdan Obeyat
Registration No: 20912634

Supervisor: Dr.Imad Odeh
Co-Supervisor: Dr. Fuad Al- Rimawi

Master thesis submitted and accepted, Date:
The name and signature of examining committee member are as follows:

1-Head of Committee: Dr. I. Odeh Signature……
2-Internal Examiner: Dr. K.Sawalha Signature.....
3-External Examiner: Dr. I.Abu Skair Signature.....

Jerusalem-Palestine

1434/2013

To my beloved parents Hamdan and Nadia, to my beloved husband Anan and my son Omar to my sisters Yara, Tamara and Alaa', to my brothers Qassam and Mohammad, to my aunt Samia, to my mother in law Nae'la and to my friends for their love, patience, support, continuous encouragement, and understanding have lightened up my spirit to finish this study and this thesis

Declaration

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this thesis has not been submitted for a higher degree to any other university or institution.

Signed:

Lara H. Obeyat

Date:

Acknowledgments

Praise and thanks to Allah for granting me grace, wisdom, and the opportunity to pursue the master study program. I would like to express my grateful appreciation to Dr.Imad Odeh and Dr. Fuad Al-Rimawi my supervisors for their great leadership and valuable comments. I am also grateful to Mr. Abdel Al- Fattah Al-Hroub and to Mr. Mohammad Qabbajeh for their invaluable help during my experimental work.

Abstract

A variety of date palm fruits collected at different maturation (harvesting) stages were obtained from farmers in the Jericho area of the Jordan valley and were analyzed for their total phenolics content (TP), total flavonoids content (TF), and antioxidant activity (AA) using standard chemical methods. Thus, seven date palm samples (Madjhool, Ahmar balade, Asfar balade, Barhi balade, Barhi Iraqi, Zahedi, and Rotab) were assayed by Folin-Ciocalteau reagent for their total phenolics content, by FRAP for their antioxidant activity, and by colorimetric assay for their total flavonoids content. The results showed that the date of harvesting and the type of date palm fruit contributed much to the composition of the seven date palm varieties investigated in this study reflected by total phenolics, total flavonoids, and antioxidant activity. During the date of harvesting (from June to September 2011), total phenolics content varied between 18.72 - 38.75, 40.80 - 65.70, 42.40 - 231.40, 32.30 - 52.24, 13.75 - 31.46, 22.68 - 55.76 and 35.92 - 40.88 mg gallic acid equivalents (GAE)/100g dry weight for Madjhool, Ahmar balade, Asfar balade, Barhi balade, Rotab, Zahedi, and Barhi Iraqi, respectively. Regarding total flavonoids content, it also varied during date of harvesting, it ranged between 4.46 - 9.46, 5.01 - 5.66, 3.93 - 9.6, 1.72 - 4.26, 2.61 - 4.95, 7.85 - 8.40, and 1.98 - 2.48 mg catechin equivalents/100g dry weight sample for Madjhool, Ahmar balade, Asfar balade, Barhi balade, Rotab, Zahedi, and Barhi Iraqi, respectively. The antioxidant activity (FRAP assay) measured during different dates of harvesting was found to be in the range of 263.9 - 396.7, 181.0 - 251.0, 163.0 - 658.0, 142.0 - 229.0, 142.0 - 268.0, 300.4 - 719.3, and 228 - 268 µmol Trolox equivalents/100g dry weight for Madjhool, Ahmar balade, Asfar balade, Barhi balade, Rotab, Zahedi, and Barhi Iraqi, respectively. Maturity stage of the date palm was found to have an effect on the total phenolics and total flavonoids content as well as on the antioxidant activity of the various types of the date palms investigated in this study. Results indicated that total phenolics content, total flavonoids content, and antioxidant activity of the date palm is increasing during maturation stages for Barhi balade, Zahedi, Barhi Iraqi, Madjhool, and Rotab, while decreasing for Ahmar balade, and Asfar balade.

Correlation analyses indicated that there was a linear relationship between antioxidant activity and total phenolics content, and between antioxidant activity and total flavonoids content for all date palm varieties investigated in this study with correlation coefficients of better than

0.98 (for the relationship between antioxidant activity and total phenolics content) and 0.90 (for the relationship between antioxidant activity and total flavonoids content).

It is expected that our results will be useful to farmers particularly in their selection of date palm fruits with high content of the bioactive compounds to meet the increasing demand on such health products. These compounds can also be utilized for production using biotechnological applications through *in vitro* studies like plant cell culture by application of bioreactors.

Table of contents

List of Tables

List of Figures

List of scheme

List of Appendices

List of Acronyms

AA: Antioxidant Activity.

AAA: Arab Agronomist Association.

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid

DW: Dry Weight.

FAO: Food Agriculture Organization.

FAOSTAT: Food Agriculture Organization Statistics.

FRAP: Ferric Reducing Antioxidant Power.

GAE: Gallic Acid Equivalent.

MOA: Ministry of Agriculture.

PARC: Palestinian Agricultural Relief Committee.

PCBS: Palestinian Central Bureau of Statistics.

TEAC: Trolox Equivalent Antioxidant Capacity

TFC: Total Flavonoid Content.

TPC: Total Phenolic Content.

TPTZ: 2,4,6-tri(2-pyridyl)-1,3,5-triazine.

WBGS: West Bank Gaza Strip.

CHAPTER ONE
INTRODUCTION

1.1. Background and Rationale

Interest in phytochemical contents and antioxidant activity of fruits and vegetables is increasing highly in recent years. Recent studies have shown that the majority of antioxidant activity in fruits or vegetables may originate from the polyphenolic compounds [1]. The presence of phenolic compounds in fruits and vegetables has been studied fairly well. In addition to their important functions in plant defense mechanisms and external stresses [2], they also affect the quality, color and taste of the fruits and their products like juice and fruit slice [3]. In low concentration, phenolics may protect food from oxidative deterioration; however at high concentration, they (or their oxidation products) may participate in discoloration of foods [4].

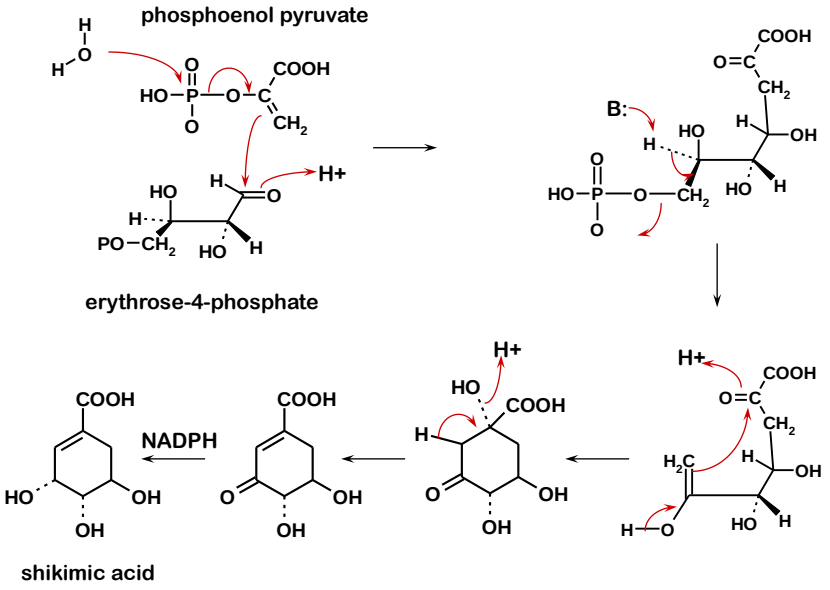
1.1.1. Bioactive compounds

Bioactive compounds in plants are compounds produced by plants having pharmacological or toxicological effects in man and animals. Although nutrients elicit pharmacological or toxicological effects when ingested at high dosages (e.g. vitamins and minerals), nutrients in plants are generally not included in the term bioactive plant compound. The typical bioactive compounds in plants are produced as secondary metabolites. Thus, a definition of bioactive compounds in plants is: secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals [5].

1.1.2. Synthesis and purpose in plants

Secondary metabolites are produced within the plants besides the primary biosynthetic and metabolic routes of compounds aimed at plant growth and development, such as carbohydrates, amino acids, proteins and lipids. They can be regarded as products of biochemical “side tracks” in the plant cells and not needed for daily functioning of the plant.

Phytogenetically, the secondary bioactive compounds in plants appear to be randomly synthesized – but they are not useless junk. One such class of secondary metabolites are phenols that are synthesized through the biochemical enzymatic shikimate cycle and these make up the bulk of the antioxidant activity observed in a variety of plants. [Scheme I]



Scheme I: Shikimic acid formation

Several of the metabolites are found to hold important functions in the living plants. For example, flavonoids can protect against free radicals generated during photosynthesis. Terpenoids may attract pollinators or seed dispersers, or inhibit competing plants. Alkaloids usually ward off herbivore animals or insect attacks (phytoalexins). Other secondary metabolites function as cellular signalling molecules or have other functions in the plants. Those plants producing bioactive compounds seem to be the rule rather than the exception. Thus, most plants even common food and feed plants are capable of producing such compounds. However, the typical poisonous or medicinal plants contain higher concentrations of more potent bioactive compounds than food and feed plants [5].

1.2. Date palm as the oldest cultivated plant in the Middle East

1.2.1. Botanical description

According to Rieger, The Arecaceae, or palm family is a large distinct family of woody monocotyledonous plants, containing up to 2600 species distributed over 200 genera. The date palm *Phoenix dactylifera L*. is one of the three economically important fruit crops in the palm family. There are estimated 3000 cultivars of date palm worldwide. It has been cultivated 6000 years ago, one of the oldest fruits planted [6].

1.2.2. World Date Production

According to FAOSTAT, world production of dates has increased from approximately 1.8 million tons in 1961-1965 up to 6.0 million tons in 2005. [Table 1.1] and [Table 1.2] show production figures throughout different years [7].

Table 1.1: Production and trade of dates worldwide

Year	Production MT	Import MT	Import in1000 $	Export MT	Export in1000 $
1961-1965	1838781	329612	48781	345398	37292
1966-1970	1916607	343763	52853	375535	41681
1971-1975	2207750	364723	78168	371512	66608
1976-1980	2549519	290835	136602	311819	108404
1981-1985	2645256	205555	162841	208477	149272
1986-1990	3168065	306503	212041	304887	206699
1989	3348675	353843	232717	354550	222235

Table 1.2: The highest twenty date producing countries in the world in 2008

Rank	Area	Production (In $1000)	Production (MT)
1	Egypt	415702	1326133
2	Iran (Islamic Republic of)	315478	1006406
3	Saudi Arabia	309081	986000
4	United Arab Emirates	217861	755000
5	Pakistan	213193	680107
6	Algeria	173275	552765
7	Iraq	121099	476318
8	Sudan	105325	336000
9	Oman	77574	255871
10	China	42318	135000
11	Tunisia	35826	127000
12	Libya Arab Jamahiriya	31347	150000
13	Yemen	17304	55204
14	Morocco	15067	72700
15	Qatar	6759	21564
16	Mauritania	6018	19200
17	Chad	5736	18300
18	Israel	5666	18078
19	United States of America	5374	17146
20	Niger	5200	16589

1.2.3. Agriculture in Palestine

The area of West Bank is 5845 square kilometer and that of Gaza Strip is 360 square kilometer. The cultivated area in West Bank is 1660 square kilometer while that of Gaza Strip is 180. Although the total geographic area is small, Palestine has an advantage of having different landscapes, climatic environment, soil, temperatures, rain quantities and altitudes of sea level. In general Palestine has the climate of the Mediterranean area, long warm summer and moderate cold winter. The average rain fall ranges from 100 to 700 millimeters. This specialty gives Palestine the flexibility of having different agricultural crops at different seasons. Jordan Ghor or Jordan Valley is a natural green house, whereby a warm temperature prevails in winter which allows unique farming different sorts of agricultural products. Also the quantity of underground water in West Bank is approximately 600-800milion cubic meter, of which 15-20% only can be used by the Palestinians and the rest is taken by the Israelis. In Gaza Strip the estimated underground water is 50-70 million cubic meters. The amount of water which goes annually to the agriculture sector is around 60% of the total consumption that is 174 million cubic meters which is used in irrigating 12.5% of total cultivated lands in WBGS [8].

1.2.4. Location of date palm cultivation in Palestine

1.2.4.1. Gaza Strip

Gaza Strip, 360 square KM, is well known for date palm agriculture, as it grows there without irrigation. Rain fall is around (300-400 mm) annually also underground water is available no deeper than (1-3 m) under the sand. One thousand dunum are planted by Hayani date palm [8].

1.2.4.2. West Bank

Jordan Valley, AlGhor, is part of West Bank, which around (400 square Km), is well known for date palm agriculture. Jericho was once called the city of date palms, for its tremendous number of date palm trees. Lately many are investing in date palm agriculture, around (5000 dunum) is being cultivated with Madjhool brand. The rest of the areas are being planted with around (35) different cultivars of date palm, around houses, streets and farms. Most of it is consumed the rest is processed into date palm paste [8].

In early 1980 PARC was interested in date palm agriculture, as it well adapted to the salty lands of Jericho. Very few farmers started working in the field, who soon were convinced that there is a positive economical opportunity as it happened in the village of Zbeidat and Marj Najeh and Jericho [8].

After several years PARC established a plan to encourage the farmers to increase date palm cultivation, Hayani product in Gaza Strip and gave them all support and guidance. Also in parallel AAA had a plan to increase the Madjhool product in the Jordan Valley Ghor. The estimated number of trees that the AAA and the MOA made available, in the past seven years was 25,000 cultivars, nearly all were Medjool type. The number of Madjhool trees is almost 100,000, 70% of which are in Jericho [8].

[Table 1.3] shows the area, yield, and production of date palm fruit trees in the PT by crop and type 2007-2008 [8].

Table (1.3): Area, yield and production of date palm fruit trees in the PT by crop and type 2007-2008

Date crop	Bearing		Unbearing		Total area	Production
	Rainfed	Irrigated	Rainfed	Irrigated		
Area	20	3953				
Yield	1200	1005		3925	7898	3997

Source: PCBS 2011
Area in dunums, yield in kg/dunum, production in tons

[Table 1.4], and [Table 1.5], show the production of Madjhool dates in tons in West Bank before 2000 till 2010 [8].

Table (1.4): Production of Madjhool in tons in Jericho governorate before 2000 till 2004

Area	Before 2000	2000	2001	2002	2003	2004
Jericho	71	182	62	115	87	195
Duyuk+Nuemeh	4	4	0	0	8.5	23.5
Ojah+ Fasayel	0	0	0	0	0	37
Jeftelek	60	37.5	42	7	45.5	128
Zbeidat	12	35.5	24.5	21	21.5	102.5
Total	147	259	128.5	143	162.5	486

Table (1.5): Production of Medjool date in Jericho governorate for 2005-2010

Area	2005	2006	2007	2008	2009	2010	Total
Jericho	167.5	121.5	306.5	743.5	295.05	224.33	2051
Duyuk+Nuemeh	3.5	6	16	32	20	20	97.5
Ojah+ Fasayel	0	3	28.5	7.5	14.545	23.89	76
Jeftelek	176	178	35	227.5	342.15	283.65	936.5
Zbeidat	9	18	37.5	108	50.5	29.450	389.5
Total	356	326.5	423.5	1118.5	1682	650	3550.5

Source: Jericho Directory of agriculture/division of plant protection and marketing

1.2.5. Consumption of date fruits In Palestine

The local average yearly consumption of date palm has increased lately to 0.9 Kg per person. The reason for the increase in consumption might be due to the normal increase in population as the consumption of date palm increased from 1994-2000 by 28.4%. The consumption is expected to reach in 2020, up to 190% of that of 1994. On the other hand the total production of date palm in 2008 was 525 Tons and this represents 21.3% of total quantity needed by local markets. The difference is imported either from Israel or the Arab countries [8].

The date palm import is around 0.17% of total food consumption, the reason behind this is the natural consumption pattern of the Palestinian consumer as well as the weak promotion for the nutritional and health benefits of the date palm, and the unavailability of adequate marketing infrastructure [9].

1.2.6. Stages of date palm ripening

Dates ripen in four stages, which are known throughout the world by their Arabic names kimri (unripe), khalal (full-size, crunchy), rutab (ripe, soft), tamr (ripe, sun-dried). At the kimri stage there is a rapid increase in size, weight, and reducing sugars; it is the period of highest acid activity and moisture content (up to 85 %). All factors level off at the end of this stage when the fruit starts to turn yellow (or red according to variety). At this point the date seed could already germinate and the fruit is botanically mature. At the khalaal stage weight gain is slow but sucrose content increases, moisture content goes down, and tannins will start to precipitate and lose their astringency. In some varieties this latter process evolves rapidly, which make them already palatable at the khalaal stage and one could speak of commercial maturity for this type of fruit at this stage [10].

With the tips of the fruit starting to turn brown, the Rutab stage sets in which is characterized by a decrease in weight due to moisture loss, a partial (the degree depending on the variety) inversion of sucrose into invert sugar and a browning of the skin and softening of the tissues. The moisture content goes down to about 35 % and the dates at this stage are sold as fresh fruit [Table 1.6] [11].

Table (1.6): Water content of a date fruit during its maturation from Khalal to Tamar stage

Stage	Water content (%)
Kimri and Early Khalal	85
Late Khalal	50
Early Rutab (tip browning)	45
50% Rutab	40
100% Rutab	30
Tamar	24 and less

(FAOSTAT, 2004)

Only when the dates are left to ripen further on the palm will they turn into tamr, climatic conditions permitting, characterized by a moisture content at which the date is self-preserving. The upper limit for the date to be self-preserving lies at around 24-25 % [11].

1.2.7. Chemical composition of date fruits

Date palm fruits are a good source of energy, vitamins, and elements like phosphorus, iron, potassium, as well as a significant amount of calcium [12], [13].

Date palm fruits have higher caloric content and more essential minerals and vitamins than most other fruits. Date palm fruits contain a high percentage of carbohydrates (total sugars, 44- 88 %), fat (0.2-0.5 %), protein (2.3-5.6 %), 15 kinds of salts and minerals, vitamins, and a high percentage of dietary fiber (6.4-11.5 %). The flesh of dates contains (0.2- 0.5 %) oil, while the seed contains (7.7-9.7 %) oil. In fact, the weight of the seed is (5.6- 14.2 %) of the date [14].

Date fruits are an excellent source of phenolics and therefore possess an extremely high antioxidant capacity. Date fruits have been reported to contain various phenolics, such as protocaechuic, P- hydroxy benzoic, vanillic, syringic, caffeic, coumaric, ferulic, hydroxy benzoic, hydroxyl cinnamic acids, which contribute significantly to total antioxidant activity [15].

1.3. Antioxidant activity

1.3.1. Antioxidant activity in fruits

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants [16].

The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [16].

1.3.2. Antioxidant Activity Assays

1.3.2.1. ABTS assay

ABTS assay (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)]) is designed to measure the overall antioxidant capacity within a given sample.

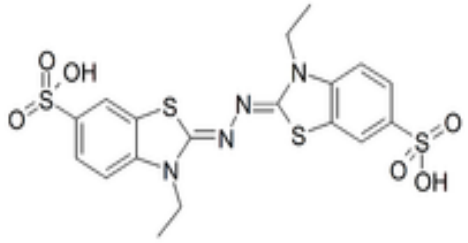


Figure 1.1:2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)

ABTS is frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods. In this assay, ABTS is converted to its radical cation by addition of sodium persulfate. This radical cation is blue in color and absorbs light at 734 nm. The ABTS radical cation is reactive towards most antioxidants including phenolics, thiols and Vitamin C. During this reaction, the blue ABTS radical cation is converted back to its colorless neutral form. The reaction may be monitored spectrophotometrically. This assay is often referred to as the Trolox equivalent antioxidant capacity (TEAC) assay. The reactivity of the various antioxidants tested is compared to that of Trolox, which is a water-soluble analog of vitamin E [17].

1.3.2.2. FRAP assay

FRAP (Ferric Reducing Antioxidant Power). In the FRAP method the yellow Fe^{3+} TPTZ complex (2,4,6-tri(2-pyridyl)-1,3,5-triazine) is reduced to the blue Fe^{2+} TPTZ complex by

electron-donating substances under acidic conditions. Any electron donating substances with a half reaction of lower redox potential than Fe^{3+}/Fe^{2+} TPTZ will drive the reaction and the formation of the blue complex forward [18].

1.3.2.3. Folin–Ciocalteu reagent

The Folin–Ciocalteu reagent which is a mixture of tungstates and molybdates works on the mechanism of oxidation–reduction reaction. The method strongly relies on the reduction of the mixture heteropolyphosphotungsates–molybdates by the phenolic compound which results in the formation of blue coloured chromogen. The phenolic compounds react with Folin–Ciocalteu reagent only under basic conditions adjusted by sodium carbonate solution. Under Basic conditions it has been observed that the phenolic compound undergoes dissociation to form a phenolate anion which reduces the Folin–Ciocalteu reagent i.e. the mixture of tungstates and molybdates rendering a blue coloured solution. The colour intensity of the formed blue chromogen can be measured by the absorbance readings using a spectrophotometer [19].

1.3.2.4. Aluminum chloride colorimetric method

The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols . In addition, aluminium chloride forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids [20].

1.4. Previous Studies

Scientific literature does not contain any reports that deal with antioxidant activity and phenolics content or flavonoids content of date palms (*Phoenix dactylifera* L.) fruits from Palestinian territories. Therefore, a detailed study of the Palestinian date palm constitutes a valuable addition to the available literature. Abundant literature dealing with antioxidant activity came out of the middle east region. Thus, In Iran, Vayalil (2002) and Mansouri et al., (2005) reported that palm dates have phenolic compounds (mainly cinnamic acids) and flavonoids (flavones, flavonols and flavanones) that provide antioxidant activities [21], [22].

Al-Farsi et al., (2005) and Al-Farsi et al., (2007) have determined the compositional and sensory characteristics of three native sun-dried date (Phoenix dactylifera) varieties grown in Oman, comparing the antioxidant activity, anthocyanins, carotenoids, and phenolics for each different variety, and found that total phenolics content ranged from 172 to 246 mg of gallic acid equivalent/100 g and antioxidant activity 146-162 µmol Trolox per gram (on a fresh weight basisi) [15][23].

Abdul Ameer A. Allaith, (2008) evaluated sixteen cultivars of date palm (*Phoenix dactylifera* L.) grown in Bahrain for their antioxidant activity, and total phenolics content at different ripening stages using the ferric reducing antioxidant power (FRAP) assay and reported that a sharp decrease in antioxidant activity was found to be associated with the fruit ripening. The highest total antioxidant activity was found at biser (unripe) stage, with a mean FRAP value of (5.71 ± 4.31 mmol/ 100 g fresh weight), followed by rutab (soft and ripped) with FRAP values of (1.2 mmol/ 100 g fresh weight) and tamer (dried fruit) (0.94 ± 0.21 mmol/ 100 g fresh weight) and the average of phenolics at biser and rutab stage were (196.8 ± 72.1 and 116.7 ± 44.1 mg GAE/ 100 g fresh weight) [24].

Biglari *et al*., (2008) analyzed edible parts of date palm (Phoenix dactylifera L.) fruits from Iran at different maturity stages for their antioxidant activities using 2,2'-azinobis (3 ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\cdot+}$) assays and the ferric reducing/antioxidant power method (FRAP assay).in this study, total phenolics content and total flavonoids content were also measured using Folin-Ciocalteu reagent and aluminum chloride method, respectively. The antioxidant activity (ABTS assay) of the date palm were 22.83–41.17, 47.6–54.61 and 500.33 µmol Trolox equivalents/100 g dry weights for soft dates, semi dry dates and dry dates, respectively. The antioxidant activity (FRAP assay) per 100 g dry weigh sample were 11.65–20, 19.12–29.34 and 387.34 µmol FRAP for soft dates, semi dry dates and dry dates, respectively. The total phenolics content ranged from 2.89 to 4.82, 4.37 to 6.64 and 141.35 mg gallic acid equivalents (GAE)/100 g dry weigh, while total flavonoids content ranged from 1.62 to 3.07, 1.65 to 4.71 and 81.79 mg catechin equivalents (CEQ)/100 g dry weigh sample for soft dates, semi dry dates and dry dates, respectively.

Correlation analyses indicated that there was a linear relationship between antioxidant activity and the total phenolic contents or total flavonoids contents of date palm fruit [25].

Saafi *et al*., (2009) evaluated the total phenolics content and the antioxidant activity of four date palm fruit varieties grown in Tunisia. All measurements were made at the 'tamar' stage- the final stage of fruit ripeness. The date varieties were found to be rich in total phenolic ranging from 209.42 mg of equivalent gallic acid ⁄ 100 g fresh weight to 447.73 mg equivalent gallic acid ⁄ 100 g fresh weight. The date varieties studied were characterized by a high antioxidant activity ranging from 866.82 to 1148.11 µmol Equivalent Trolox ⁄ 100 g fresh weight by the ABTS method [26].

Ardekani.M *et al*., (2010) has determined the antioxidant activity and total phenolic compounds of 14 different varieties of date palm (*Phoenix dactylifera* L., Arecaceae) seed extracts from Iran using 5 solvents [water, methanol, methanol (50%), DMSO, and water: methanol: acetone: formic acid (20:40:40:0.1)] using Ferric reducing antioxidant power assay for antioxidant activity and Folin-Ciocalteu reagent for total phenolics content. DMSO extract of the "Zahedi" variety had the highest antioxidant effect (37.42 mmol/100 g dry plant) and total phenolic content (3541 mg /100 g dry plant) among these 14 varieties and 5 solvents. There was a significant correlation between the total phenolic content and antioxidant activity of the "Zahedi" variety DMSO extract, which can indicates that polyphenols are the main antioxidants [27].

Singh *et al.* (2012) have determined total flavonoids content, total phenolics content and antioxidant activity of different palm date variety from Oman, and results showed that total flavonoid contents of date fruit varied considerably from (19 to 66 mg CEQ/100g DW). Results of this study showed that higher flavonoids values were associated with Rutab stage which indicates that the drying process may have a destructive effect on these compounds [18].

CHAPTER TWO
PURPOSE OF THE PRESENT WORK

The overall objective of this study will be to evaluate the total antioxidant capacities of dates from Palestinian territories using an improved FRAP assay, determine the total phenolics content of these plants using Folin–Ciocalteu reagent, and investigate the relationship between the total antioxidant activities and phenolics content in the samples tested. Furthermore, aluminum chloride colorimetric method will be used to identify flavonoids in the date palm fruits. HPLC method will be also used to determine the concentration of different phenolics compounds (Gallic acid, P-hydroxybenzoic acid, Vanillic acid, Caffeic acid, Syringic acid, and Ferulic acid).

The data will be helpful for comparison of the total antioxidant activities, total phenolics content and total flavonoids content of different types of date palm fruits and also useful for understanding their chemical constituents and functionality.

2.1. Hypotheses and research questions

There have been several studies on the antioxidant activities of various fruits, herbs, and plants in Iran, Morocco, Algeria, Tunisia, Oman, Bahrain and others. Therefore, Hypothesis of this study declares the existence of antioxidant activity in date palm fruits in Palestinian territories.

The specific questions for discussions include:

1. Are antioxidant activity, total phenolics content and total flavonoids content of Palestinian date noticeable?
2. Whether there is a possible relationship between total phenolics content and antioxidant activity and also between total flavonoids content and antioxidant activity of Palestinian date?
3. Is there any change in total antioxidant activity, total phenolics content and total flavonoids content in palm dates as a function of harvesting time?

2.2. Objectives and aims

The main objectives of this study are:

1. To evaluate the antioxidant activity, total phenolics content and total flavonoids content of methanolic extracts from seven different types of date palm fruit at different maturity stages grown in the Palestinian territories using FRAP methods, Folin-Ciocalteau assay and aluminum chloride colorimetric methods, respectively.
2. To determine the concentration of different individual phenolic compounds by using HPLC with UV detector method.
3. To demonstrate a possible relationship between phenolics content and antioxidant activity and also between total flavonoids content and antioxidant activity.

CHAPTER THREE
EPERIMENTAL

3.1. Plant material

Seven date cultivars were used in the study; Zahedi, Barhi balade, Barhi Iraqi, Madjhool, Rutab, Ahmar balade, and Asfar balade.

Date palm samples were collected in the 18th of June, 24th of July, 4th and 20th of September 2011, at different maturity stages, and stored in the freezer at -15 ◦C for later analysis. All cultivars were grown in Aqabet jabber camp in Jericho and obtained from a local farmer.

3.2.Chemicals and Reagents

The chemicals and reagents used for analyzing the antioxidant compounds in dates were: 2,4,6-tripyridyl- S-triazine (TPTZ), Ferric Chloride trihydrate, potassium persulphate, sodium acetate, sodium carbonate. Folin–Ciocalteu reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) . Chemicals were obtained from Sigma–Aldrich.

All reagents were prepared according to standard procedures.

FRAP reagent was prepared according to Benzie and Strain (1999) by the addition of 2.5 ml of a 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 ml of 20mM FeCl3.6H2O and 25 ml of 0.3 M acetate buffer at pH3.6 [28].

0.3 M Acetate buffer at pH 3.6 was prepared according to British Pharmacopeia by dissolving 16.8g of acetic acid and 0.8g of sodium hydroxide in 1000 ml of distilled water.

10 mM TPTZ (M.wt = 312.34 g/mol) was prepared by dissolving 0.312g TPTZ in 100ml of distilled water.

40Mm HCl was prepared by dissolving 3.77ml of stock HCl solution (10.6M) to 1000ml with distilled water.

5% $NaNO_2$ was prepared by dissolving 5g of $NaNO_2$ in 100ml of distilled water.

10% $AlCl_3$ was prepared by dissolving 10g of $AlCl_3$ in 100ml of distilled water.

3.3. Methodology

3.3.1Extraction

Extraction of polyphenols was done as described by Biglari et al., 2008. Briefly, the edible part of date palm fruits (100 g) was crushed and blended for 3 min with a blender. The palm fruits were then extracted with 300 ml methanol–water (4:1, v/v), at room temperature for 5 hours using an orbital shaker. The extracts were then filtered and the supernatant were concentrated under reduced pressure at 40°C for 3 to 4 hour using a rotary evaporator to obtain the date palm fruit methanol crude extract. The crude extract was kept in dark glass bottles at -15°C until used. The storage conditions (time and temperature) were similar for all cultivars [25].

3.3.2. Measurement of Antioxidant Activity by FRAP assay

The Antioxidant Activity of date palm fruit extracts was determined using a modified method of the assay of ferric reducing/antioxidant power (FRAP) of Benzie and Strain, 1999.Freshly prepared FRAP reagent (3.0 ml) were warmed at 37°C and mixed with 40 µl of date palm fruit extract and the reaction mixtures were later incubated at 37°C. Absorbance at 593 nm was read with reference to a reagent blank containing distilled water which was also incubated at 37 °C for up to 1 hour instead of 4 min, which was the original time applied in FRAP assay [28]. Aqueous solutions of known Fe (II) concentrations in the range of (2 - 5 mM) (FeSO4.6H2O) were used for calibration [29].

3.3.3. Total phenolics content (Folin–Ciocalteau assay)

Total phenolics were determined using Folin–Ciocalteau reagents Singleton & Rossi, 1965. Date palm fruit extract 40 µl were mixed with 1.8 ml of Folin–Ciocalteu reagent (prediluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min, and then 1.2 ml of sodium bicarbonate (7.5%) was added to the mixture. After standing for 60 min at room temperature, absorbance was measured at 765 nm [30]. Aqueous solutions of known gallic acid concentrations in the range of (100 – 500 ppm) were used for calibration. Results were expressed as mg gallic acid equivalents (GAE)/100 g sample [30].

3.3.4. Total flavonoids

The determination of flavonoids was performed according to the colorimetric assay of Kim, Jeong, and Lee, 2003.Distilled water (4 ml) was added to (1 ml) of date palm fruit extract. Then, 0.3 ml of 5% sodium nitrite solution was added, followed by 0.3 ml of 10% aluminum chloride solution. Test tubes were incubated at ambient temperature 25°C for 5 min, and then 2 ml of 1 M sodium hydroxide were added to the mixture. Immediately, the volume of reaction mixture was made to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink color developed was determined at 510 nm. Aqueous solutions of known Catechin concentrations in the range of (50 – 100 ppm) were used for calibration and the results were expressed as mg catechin equivalents (CEQ)/100 g sample [31].

3.3.5 Determination of different phenolic compounds by HPLC with UV detector

3.3.5.1. HPLC conditions

C_{18} Column (250 mm x 4.6 ID)
Mobile phase: water: Methanol (82/18 volume ratio) + 2%acetic acid
Wavelength: 280 nm
Run time (min): 60.00
Injection volume: 20 µl.

3.3.5.2. Phenolic Standards

Six phenolic compounds were used as standards; Gallic acid, p-hydroxybenzoic acid, Vanillic acid, Caffeic acid, Syringic acid, and Ferulic acid [Figure 3.1].

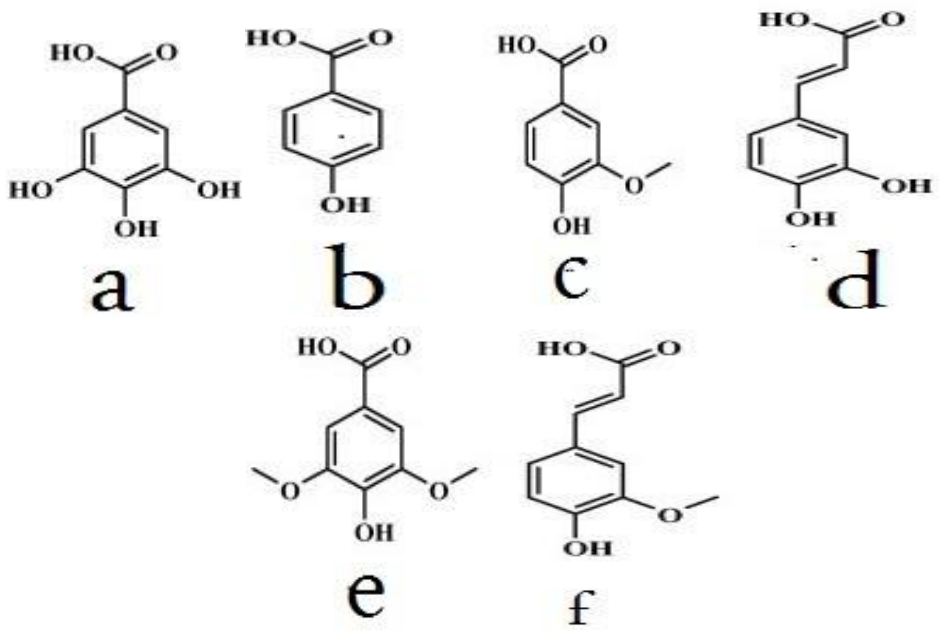


Figure 3.1: Structures of the phenolic compounds were used as standards; (a) Gallic acid, (b) p-hydroxybenzoic acid, (c)Vanillic acid, (d) Caffeic acid, (e)Syringic acid, and (f)Ferulic acid.

Three concentrations of the six phenolic compounds (10, 50, 100 ppm) were prepared and used for calibration.

CHAPTER FOUR
RESULTS & DISCUSSION

4.1. Assays Results

Seven varieties of Palestinian date palm fruits were analyzed for their total phenolics content, total flavonoids content and for their antioxidant activity. Date palm fruits were collected from one geographical region (Aqabet Jaber camp in Jericho) at different harvesting times, extending from June to late September. Table (4.1) shows the results of the total phenolics content, the total flavonoids content and the antioxidant activity of the seven palm date varieties.

Table 4.1: AA, TPC and TFC of different date varieties from Palestine (based on dry weight); results are expressed as average ± SD

Variety name	Date of harvesting	Total Phenolic Content (mgGAE /100DW)	Antioxidant activity FRAP (µmol/100 g DW)	Total Flavonoid Content (mgCEQ/100gDW)
Madjhool	18/6/2011	18.72±0.2	263.9±0.4	4.46±0.06
	24/7/2011	27.72±0.3	333.1±1.5	5.13±0.06
	04/9/2011	36.9±0.2	385.0±0.9	9.36±0.1
	20/9/2011	38.75±0.1	396.7±1.3	9.46±0.06
Ahmar balade	18/6/2011	65.70±0.02	251.0±0.14	5.66±0
	24/7/2011	55.55±0.1	236.0±0.5	5.01±0.06
	04/9/2011	48.60±0.1	220.0±0.5	5.48±0.1
	20/9/2011	40.80±0.03	181.0±1.3	5.18±0.1
Asfar balade	18/6/2011	231.4±0.1	658.0±2	9.6±0.2
	24/7/2011	86.79±0.06	179.0±1.8	6.76±0.1
	20/9/2011	42.39±0.4	163.0±0.9	3.93±0.1
Barhi balade	18/6/2011	32.30±0.04	150.0±0.6	1.72±0.7
	24/7/2011	33.20±0.05	142.0±1.6	1.84±0.1
	04/9/2011	42.40±0.2	204.0±1	3.07±0.1
	20/9/2011	52.24±0.1	229.0±0.9	4.26±0.03
Rotab	18/6/2011	13.75±0.07	142.0±1	2.61±0.2
	24/7/2011	17.22±0.05	180.0±1.2	3.82±0.1
	20/9/2011	31.46±0.08	268.0±0.56	4.95±0.04
Zahedi	18/6/2011	22.68±0.8	300.4±1.6	7.85±0.1
	24/7/2011	55.76±0.2	719.3±3	8.40±0.1
Barhi Iraqi	18/6/2011	35.92±0.2	228.0±0.6	1.98±0.1
	24/7/2011	40.88±0.06	268.0±0.7	2.48±0.1

4.1.1. Total Phenolics Content

The total phenolics content of Madjhool ranged between 18.72 and 38.75 mg GAE/100g DW as seen in [Table 4.1], between June and late September. According to [Figure 4.1] we observed an increase in total phenolics in the Madjhool date palm fruit during the indicated period which implied that TPC of this variety increases with maturity.

Results of the total phenolics content of Ahmar balade date variety showed that the maximum concentration was found in June (65.70 mg GAE/100g DW). From the results it is evident that the Ahmar balade date variety is rich in phenolics which ranged between 65.70 and 40.80 mg GAE/100g DW as shown in [Table 4.1]. Ahmar balade date variety showed a decrease in total phenolics upon changing the harvesting time from June to late September [Figure 4.2].This is consistent with previously reported results in which adecrease in antioxidant activity was associated with fruit ripening [24,25].

Similarly, the total phenolics content of Asfar balade decreased from June to late September [Figure 4.3] and ranged between 231.4 to 42.39 mg GAE/100g DW [Table 4.1]. But this date variety is richer in total phenolics than the Ahmar balade variety. In fact, the total phenolics constituent of Asfar balade date variety was the highest among other date varieties that were collected in the same month [figure 4.9].

From the assay results in [Table 4.1] it is obvious that the total phenolics content of the Barhi balade date variety ranged between 32.30 to 52.24 mg GAE/100g DW, and as seen in [Figure 4.4] there was an increase in total phenolics with date of maturation from June to late September.

Also, the total phenolics content of Rotab ranged between 13.75 and 31.46 mg GAE/100g DW as shown in [Table 4.1], and in [Figure 4.5] we can see the increase in total phenolics in this variety as a function of harvesting time [June to late September].

Zahedi and Barhi Iraqi date varieties were collected in June and July because according to the farmers these varieties are consumed at this stage of ripening.

Zahedi total phenolics content was 22.68 mg GAE/100g DW in June and 55.76 mg GAE/100g DW in July almost double the amount as shown in [Figure 4.6] and these are high amounts of phenolics compared with Madjhool, Barhi balade, and Rotab varieties that were collected in the same months as demonstrated in [Figure 4.9] and [Figure 4.10].

The total phenolics content of Barhi Iraqi was 35.92 mg GAE/100g DW in June and 40.88mg GAE/100g DW in July. There was an increase in total phenolics as the harvesting time changed between these two months [Figure 4.7].

All date palm varieties reached their highest amount of total phenolics in late September except Ahmar balade and Asfar balade where their highest amount was in June [Table 4.1]. When compared to other date palm fruits, Asfar balade exhibited the highest phenolic content in June [Figure 4.8] and in July [Figure 4.9]. On the other hand, Barhi balade had the highest phenolics content in September reaching to about 1.5 times the content of the other cultivars analyzed [Figure 4.10].

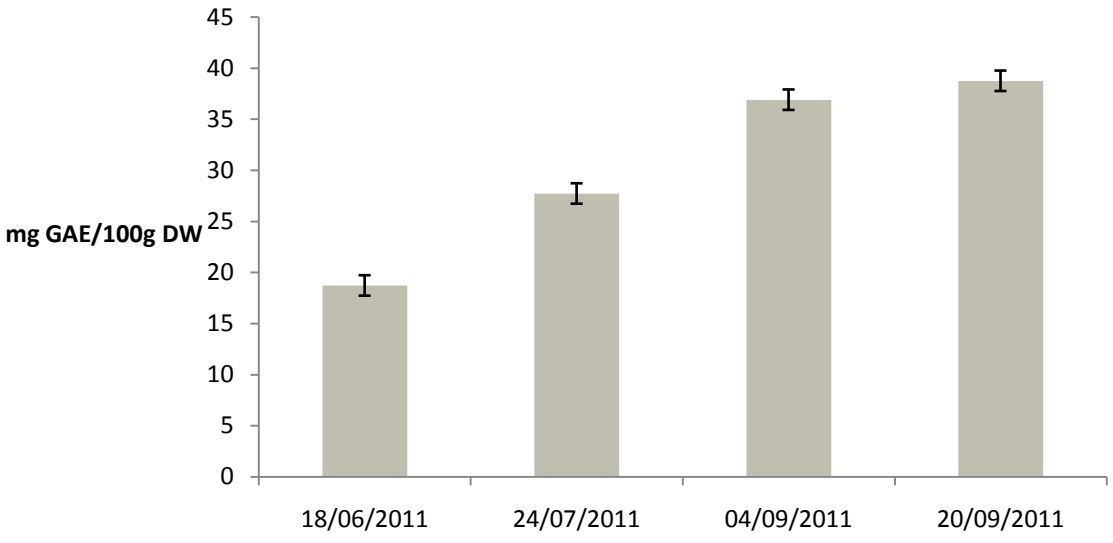


Figure 4.1: Total phenolics of Madjhool date palm harvested between June to late September 2011.

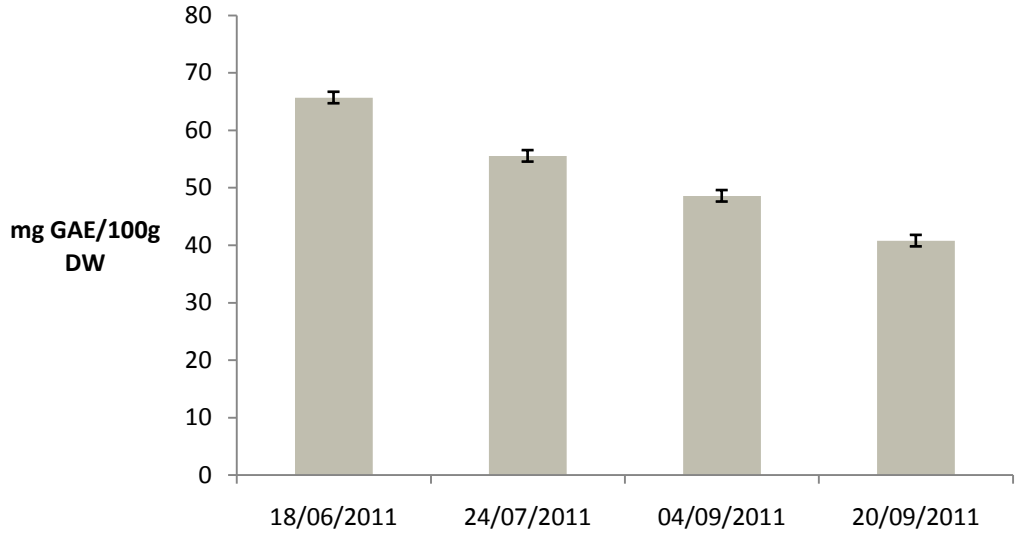


Figure 4.2: Total phenolics of Ahmar balade date palm harvested from June to late September 2011.

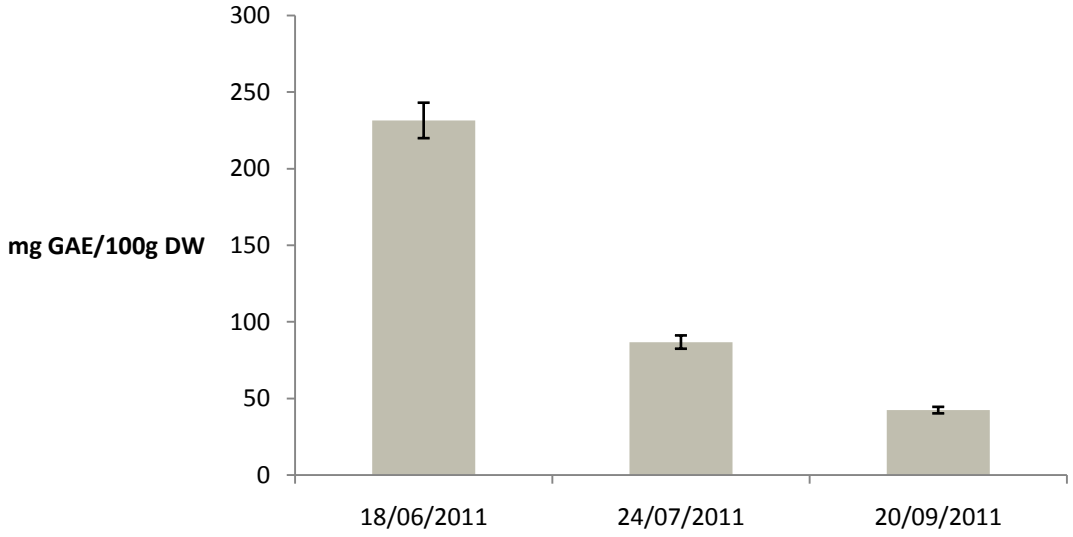


Figure 4.3: Total phenolics of Asfar balade date palm harvested between June to late September 2011.

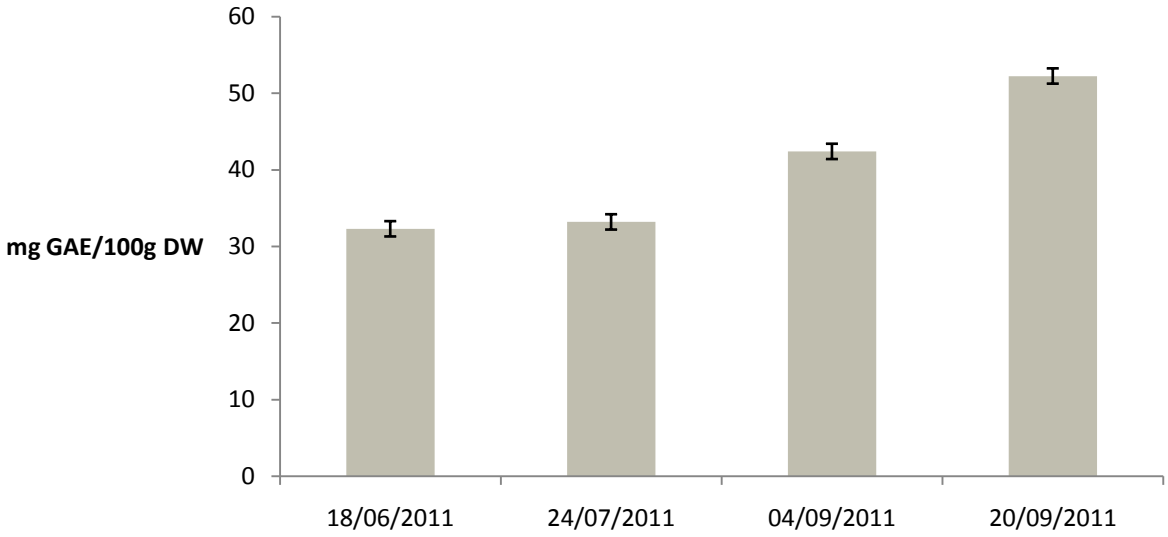


Figure 4.4: Total phenolics of Barhi balade date palm harvested between June to late September 2011.

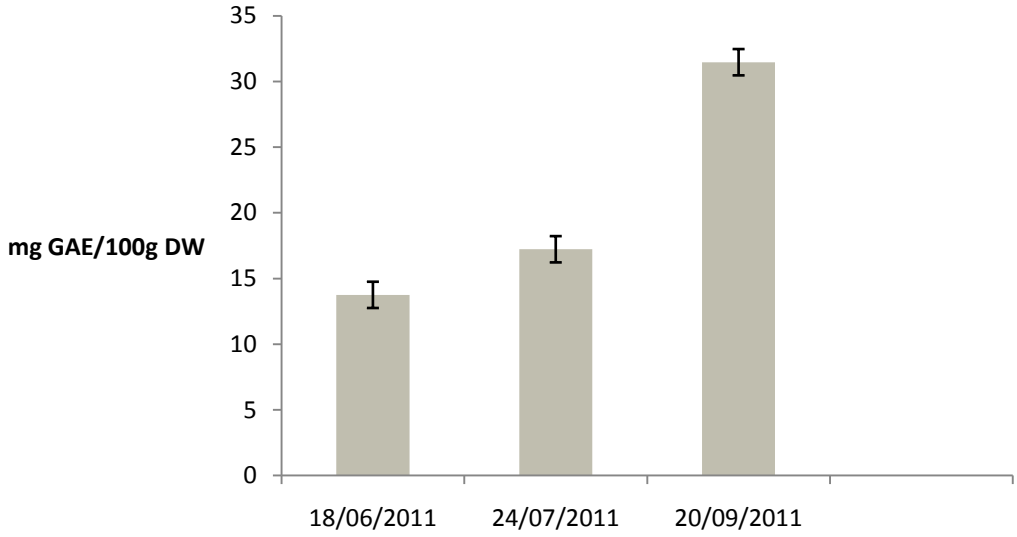


Figure 4.5: Total phenolics of Rotab date palm harvested between June to late September 2011.

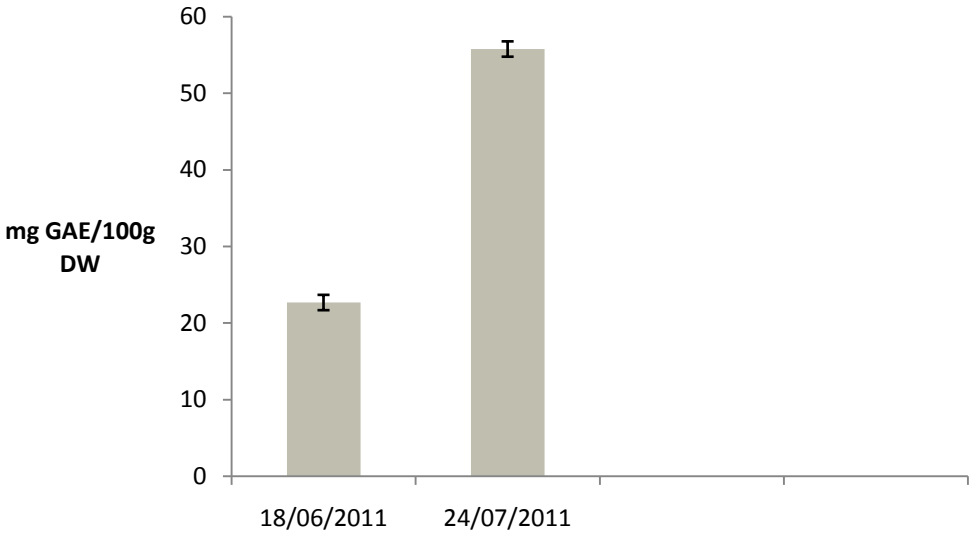


Figure 4.6: Total phenolics of Zahedi date palm harvested between June to July 2011.

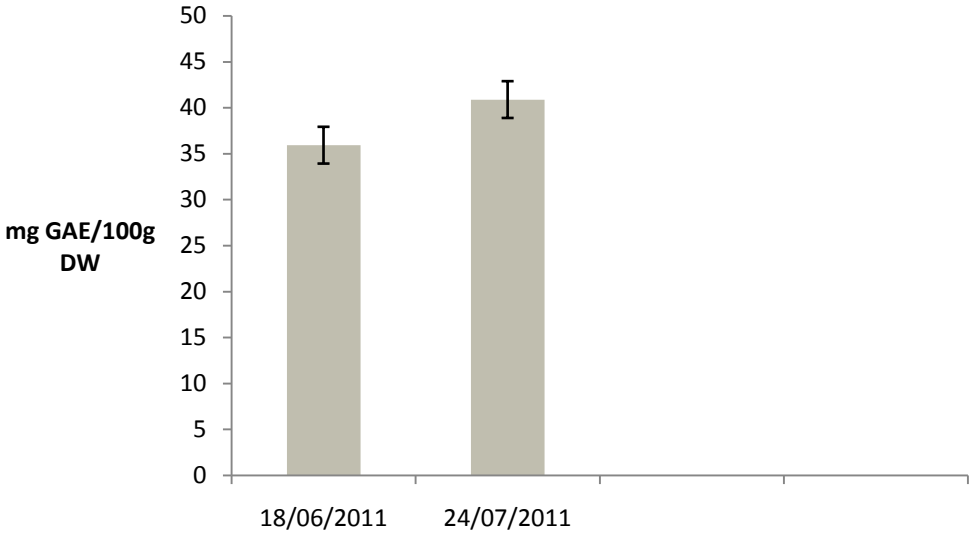


Figure 4.7: Total phenolics of Barhi Iraqi date palm harvested between June to July 2011.

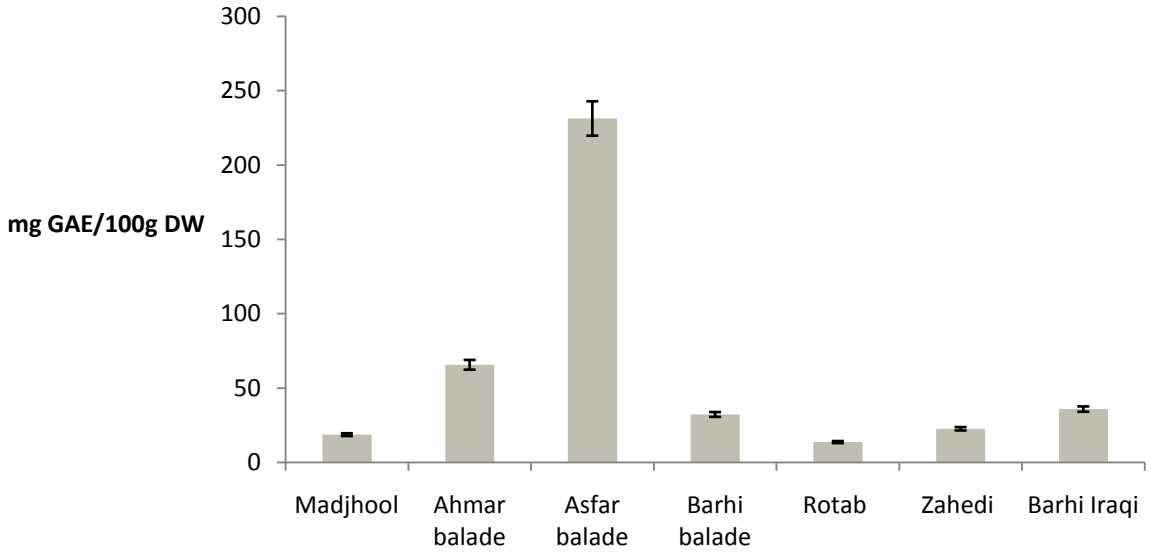


Figure 4.8: Total phenolics of all date palm varieties obtained on 18/06/2011.

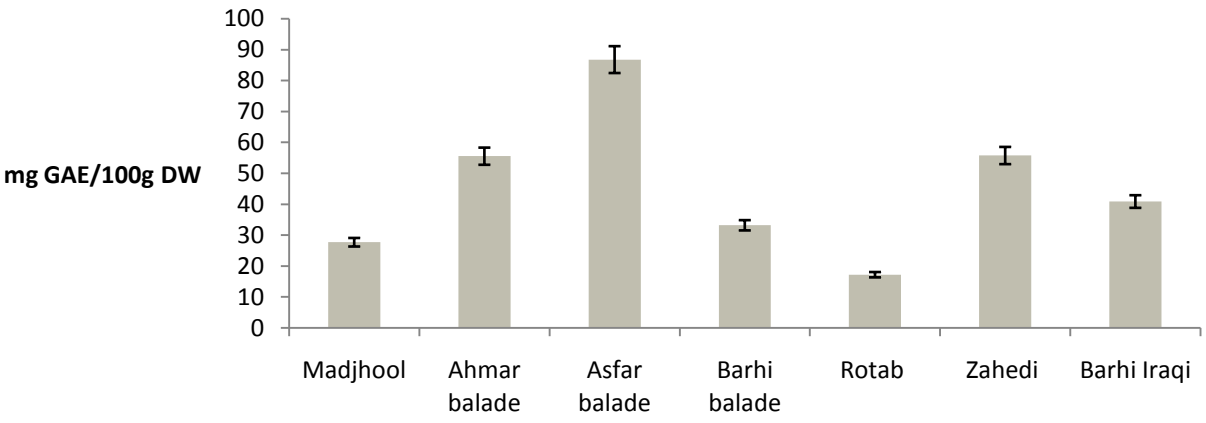


Figure 4.9: Total phenolics of all date palm varieties obtained on 24/07/2011.

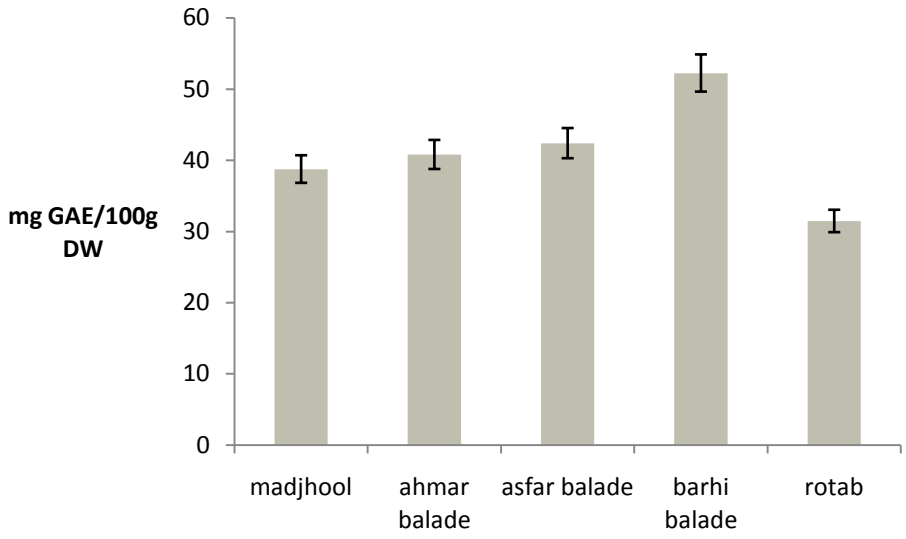


Figure 4.10: Comparison between all date varieties harvested in late September 2011.

4.1.2 Antioxidant Activity (FRAP assay) Results

The antioxidant activity of date palm fruits is attributed primarily to the presence of water soluble radical scavenging compounds particularly phenols and flavonoids. Many factors also contribute to the amount of antioxidants present among them are the date palm variety, the extent of ripening and the geographical origin. In his study, seven date palm varieties obtained from one geographical area (Jericho, Palestine) at different maturity stages were used. The antioxidant activity was measured using the FRAP assay. This aasay is based on the measurement of the ability of the substance to reduce Fe^{+3} to Fe^{+2} ion and it reveals the electron donating potential of tested compounds. The Fe^{+2} ion is measured spectrophotometrically through the determination of its colored complex with 2, 4, 6-Tris (2-pyridyl) -s-triazine, (TPTZ) at 593 nm.

The antioxidant activity of Madjhool increased successively from June to September and reached the climax in late September [Figure 4.11]. The total antioxidant activity of Madjhool date variety ranged between 263.9 and 396.7 μmol/100g DW [Table 4.1].

Similarly, [Figure 4.14] shows the reducing ability of Barhi balade date fruit extract at different collection times and it is clear that the antioxidant activity ranged between 150.0 and 229.0 μmol/100g DW [Table 4.1]. From these results we can conclude that the total antioxidant activity is directly proportional to the degree of maturation.

The antioxidant activity of Rotab reached its climax in late September [Figure 4.15] and varied between 142.0 to 268.0 μmol/100g DW as shown in [Table 4.1].

According to the FRAP assay results that are shown in [Table 4.1], the total antioxidant activity of Ahmar balade ranged between 251.0 and 181.0 μmol/100g DW and as demonstrated in figure (4.12) there was a progressive decrease in total antioxidant activity with date ripening, although this date variety was still rich in antioxidants.

The total antioxidant activity of Asfar balade ranged between 658.0 and 163.0 μmol/100g DW [Table 4.1]. It appears, from these results, that there was a decrease in total antioxidants as a function of harvesting time with the highest amount being in June [Figure 4.13].

The Zahedi total antioxidant was 300.4 μmol/100g DW in June and almost double the amount in July 719.3 μmol/100g DW as shown in [Figure 4.16] and these are the highest amounts of antioxidants when comparing this variety with the others that were collected in the same month as seen in [Figure 4.19].

The total antioxidants of Barhi Iraqi were 228.0 μmol/100g DW in June and 268.0 μmol/100g DW in July. There was an increase in total antioxidants as we went from June to July as seen in [Figure 4.17].

All date palm varieties reached their highest amount of total antioxidants in late September except Ahmar balade and Asfar balade. Their highest amount was in June and decreased through July and September successively. These results are in complete agreement with the total phenolics content determined as milligram gallic acid per 100 gram of dry date palm fruit [Table 4.1]. Comparison between all date palm varieties that were collected in 18/06/2011

shows Asfar balade leading with the highest amount of antioxidants and Rotab trailing with the lowest amount [Figure 4.18].

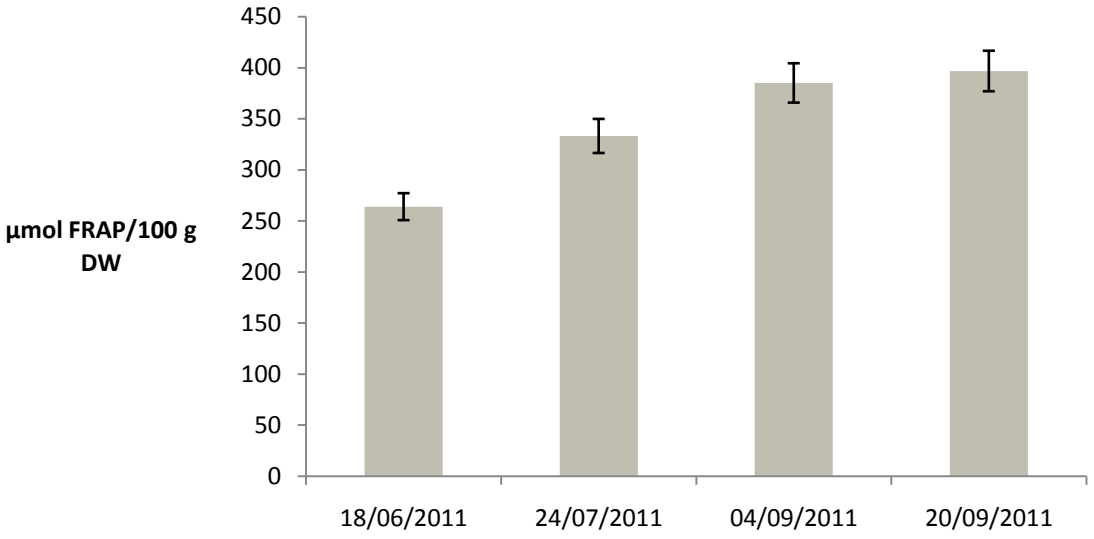


Figure 4.11: Antioxidant activity of Madjhool date palm harvested between June to late September 2011.

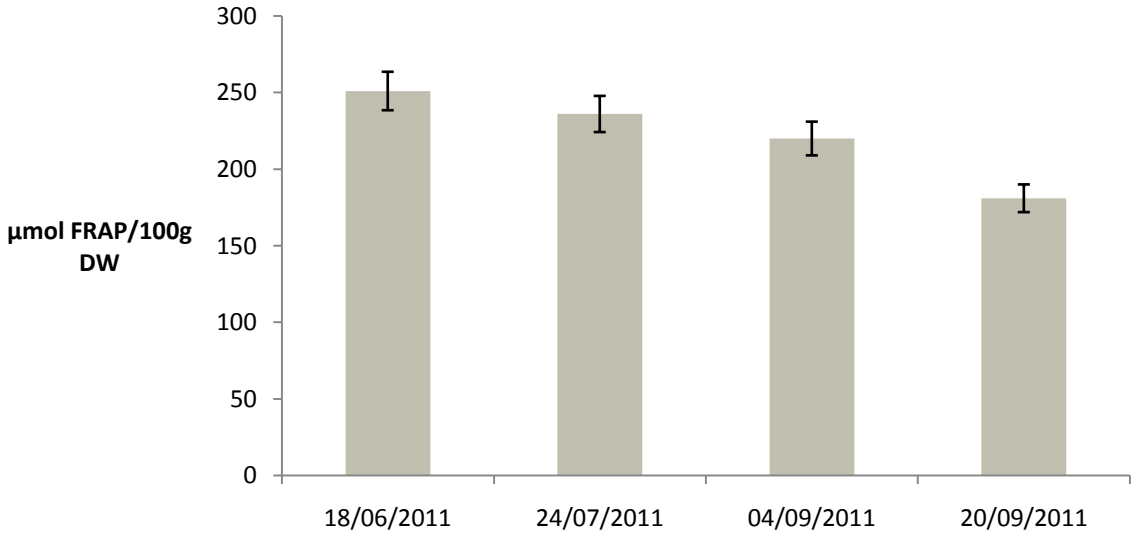


Figure 4.12: Antioxidant activity of Ahmar balade date palm harvested between June to late September 2011.

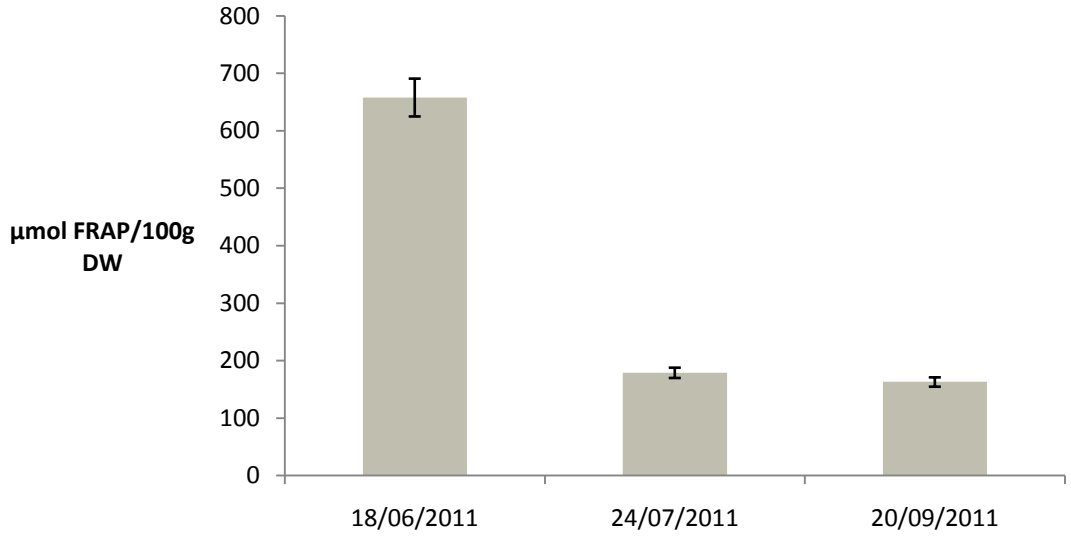


Figure 4.13: Antioxidant activity of Asfar balade date palm harvested between June to late September 2011.

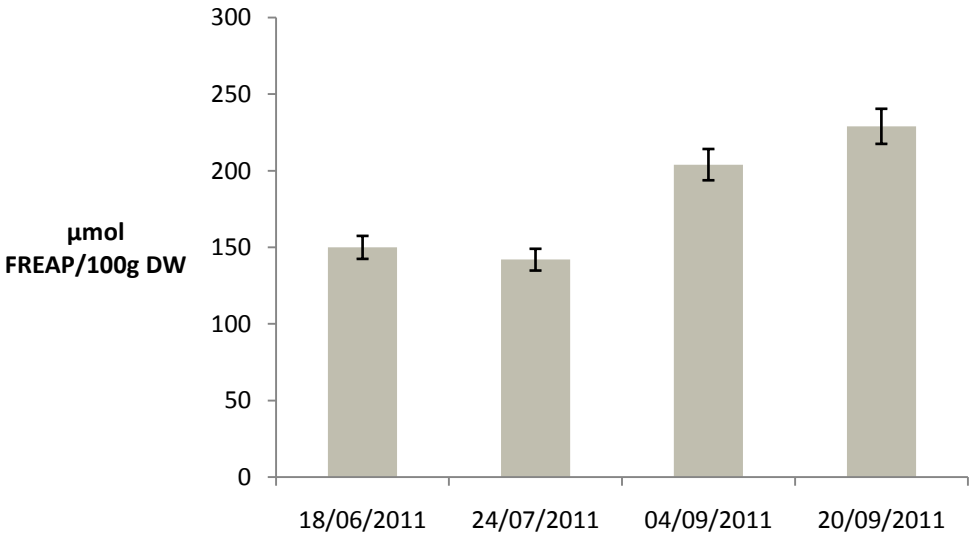


Figure 4.14: Antioxidant activity of Barhi balade date palm harvested between June to late September 2011.

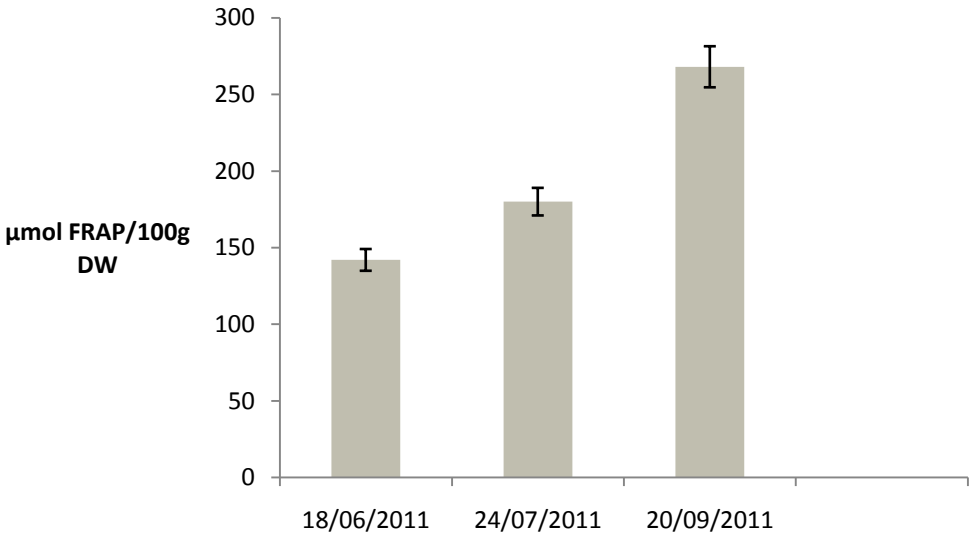


Figure 4.15: Antioxidant activity of Rotab date palm harvested between June to late September 2011.

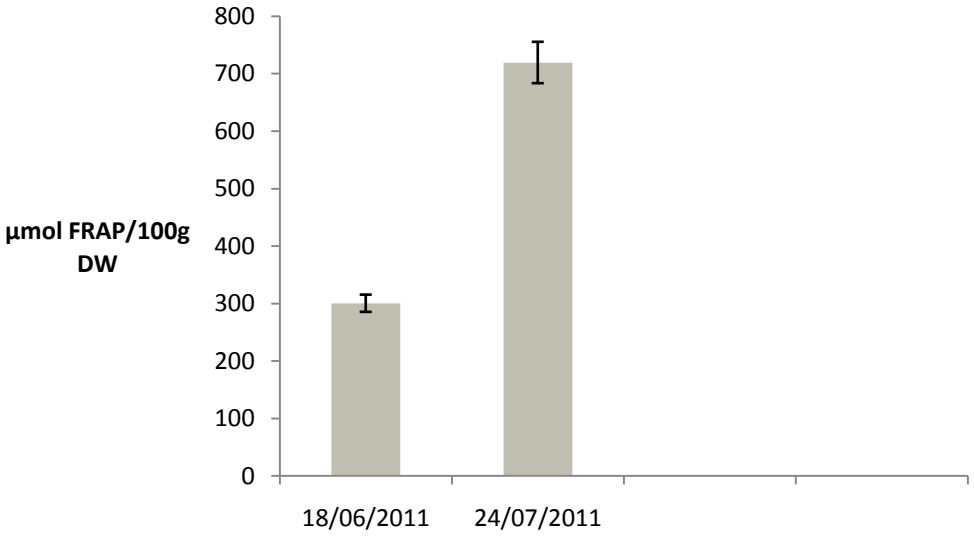


Figure 4.16: Antioxidant activity of Zahedi date palm harvested between June to July 2011.

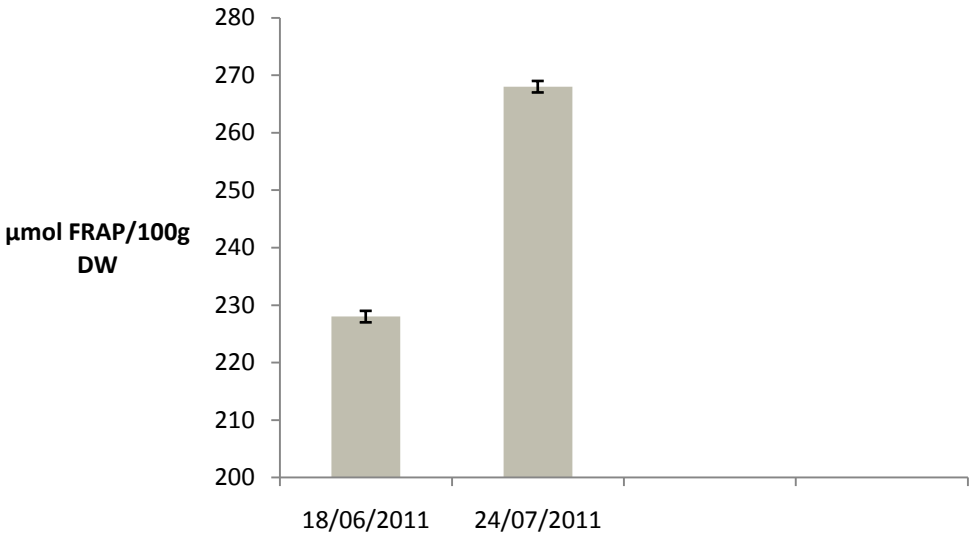


Figure 4.17: Antioxidant activity of Barhi Iraqi date palm harvested between June to July 2011.

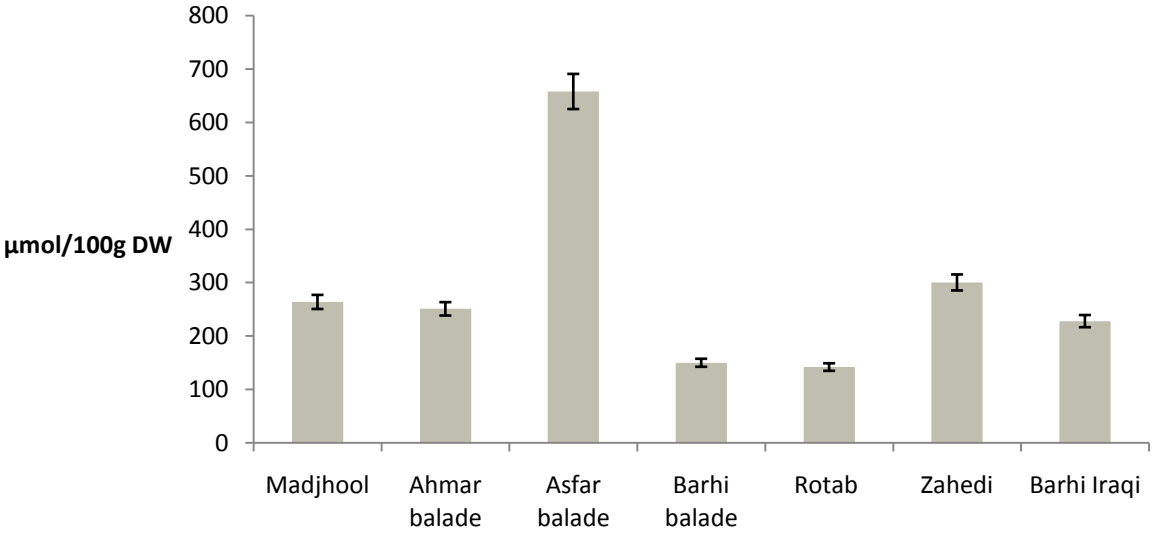


Figure 4.18: Antioxidant activity of all date palm varieties obtained on 18/06/2011.

While comparison between all date varieties that were collected in 24/07/2011 from Aqbet jabber camp shows Zahedi with the highest amount of antioxidants followed by Madjhool, Barhi Iraqi, Ahmar balade, Rotab, Asfar balade and Barhi balade [Figure 4.19].

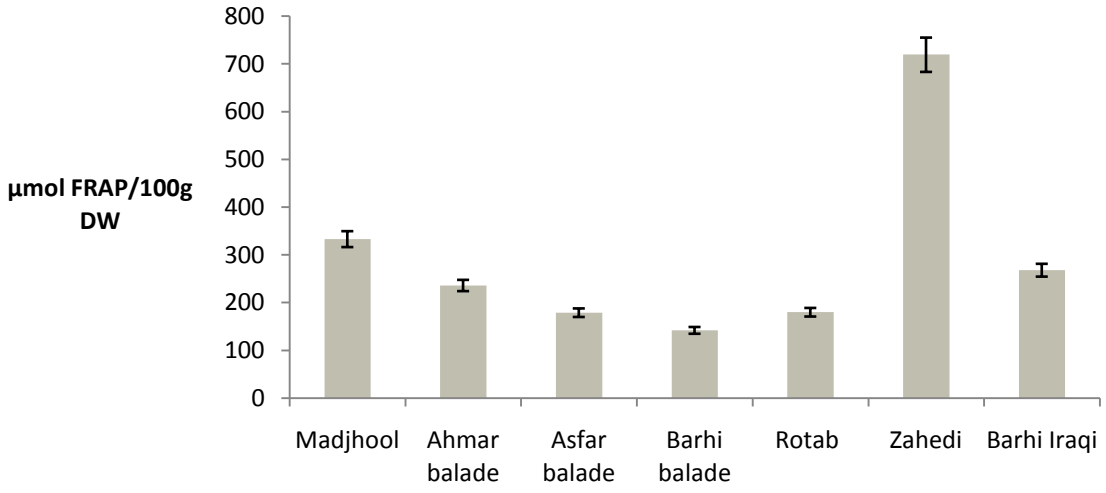


Figure 4.19: Antioxidant activity of all date palm varieties obtained on 24/07/2011.

On the other hand, comparison between all date varieties that were collected in 04/09/2011 from Aqbet jabber camp shows Madjhool with the highest amount of antioxidants followed by Ahmar balade and Barhi balade [Figure 4.20].

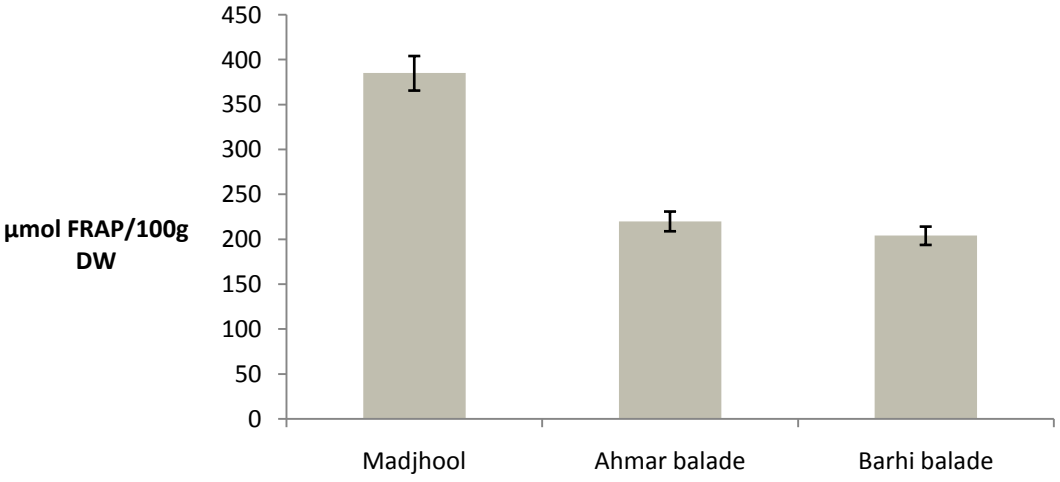


Figure 4.20: Antioxidant activity of all date palm varieties obtained on 04/09/2011.

In late September, Madjhool had the highest amount of antioxidants when comparing it with other varieties followed by Rotab, Barhi balade, Ahmar balade and Asfar balade [Figure 4.21]. Overall, the antioxidant activity of the date palm fruits obtained from Jericho, Palestine is superior to others reported earlier in the literature e.g. date palm from Iran where their antioxidant activity ranged between 11.65 and 387.34µmol FRAP [24].

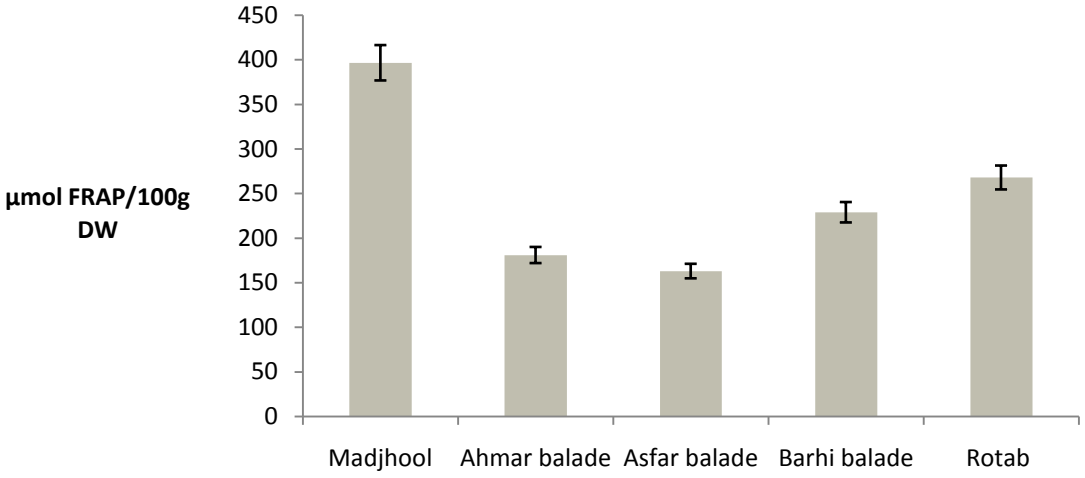


Figure 4.21: Antioxidant activity of all date palm varieties obtained on 20/09/2011.

4.1.3 Total Flavonoids Content Results

The total flavonoids content was determined using the aluminium chloride colorimetric method. The results showed [Table 4.1] that total flavonoids content in Madjhool date palm fruit ranged between 4.46 and 9.46 mg CEQ/100g DW and as demonstrated in [Figure 4.22] there was an increase in their concentration as we went from June to late September.

The total flavonoids content in Ahmar balade varied with harvesting time. Early harvest (June) produced the highest amount of flavonoids (5.66 CEQ/100g DW) while late harvest decreased the amount by about 8% (5.18 CEQ/100g DW) [Figure 4.23]. Also, the total flavonoids content of Asfar balade changed following the same pattern as Ahmar balade. Thus, in June it was 9.60mg CEQ/100g DW while in September it decreased to 3.93 mg CEQ/100g DW [Figure 4.24].

In summary, for both Ahmar balade and Asfar balade date palm fruits the highest flavonoid values were in June as shown in [Table 4.1]. This indicated that the drying process might have a destructive effect on these compounds [17]. Also, it is worthy to note that the Asfar balade variety has about 70% more flavonoids than the Ahmar balade one.

In contrast, the maximum flavonoids content in Barhi balade , and Rotab was attained in late September in total agreement with that of Madjhool. Thus the total flavonoids content of Barhi balade date fruit ranged between 1.72 and 4.26 mg CEQ/100g DW [Figure 4.25]. While the total flavonoid contents of Rotab date fruit reached its climax in late September as seen in figure (4.26) ranging between 2.61 and 4.95 mg CEQ/100g DW [Table 4.1].

Zahedi total flavonoids content was 7.85 mg CEQ/100g DW in June and 8.40 mg CEQ/100g DW in July as shown in [Figure 4.27]. While the total flavonoids content of Barhi Iraqi was 1.98 mg CEQ/100g DW in June and 2.48 mg CEQ/100g DW in July [Figure 4.28]. As these two date palm fruits were harvested only in June and July, only two samples were available for analysis.

As demonstrated earlier, all date palm varieties exhibited their highest amounts of flavonoids in late September with the exception of Ahmar balade and Asfar balade which had the highest concentration in June.

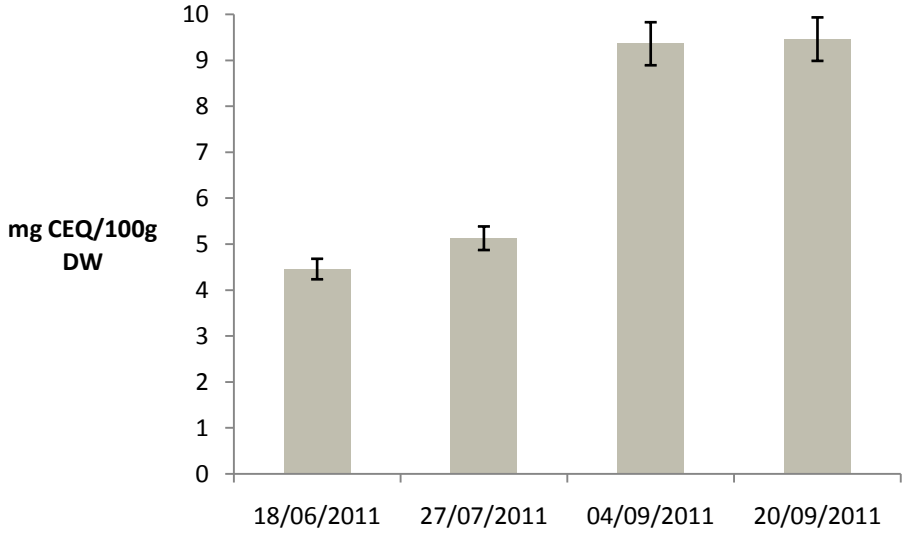


Figure 4.22: Total flavonoids of Madjhool date palm harvested between June to late September 2011.

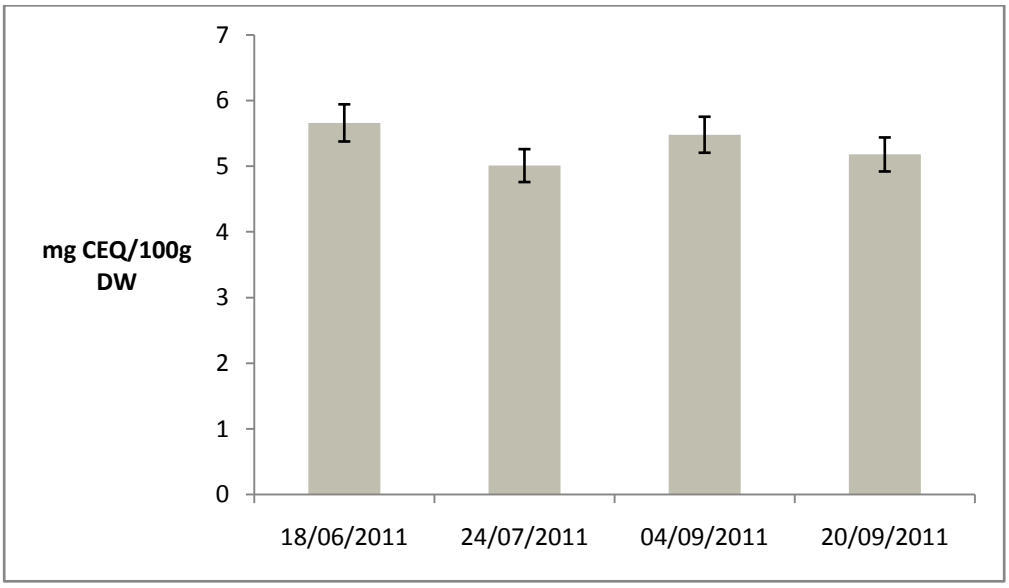


Figure 4.23: Total flavonoids of Ahmar balade date palm harvested between June to late September 2011.

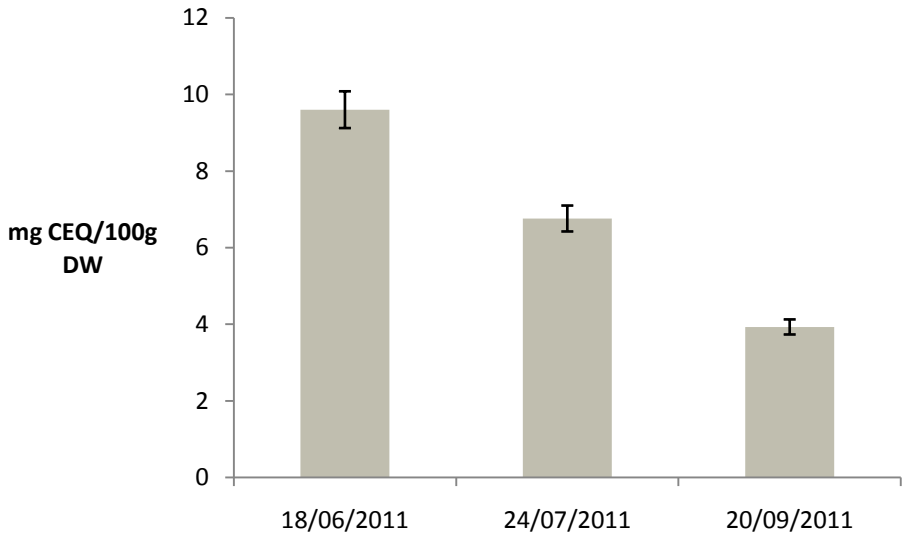


Figure 4.24: Total flavonoids of Asfar balade date palm harvested between June to late September 2011.

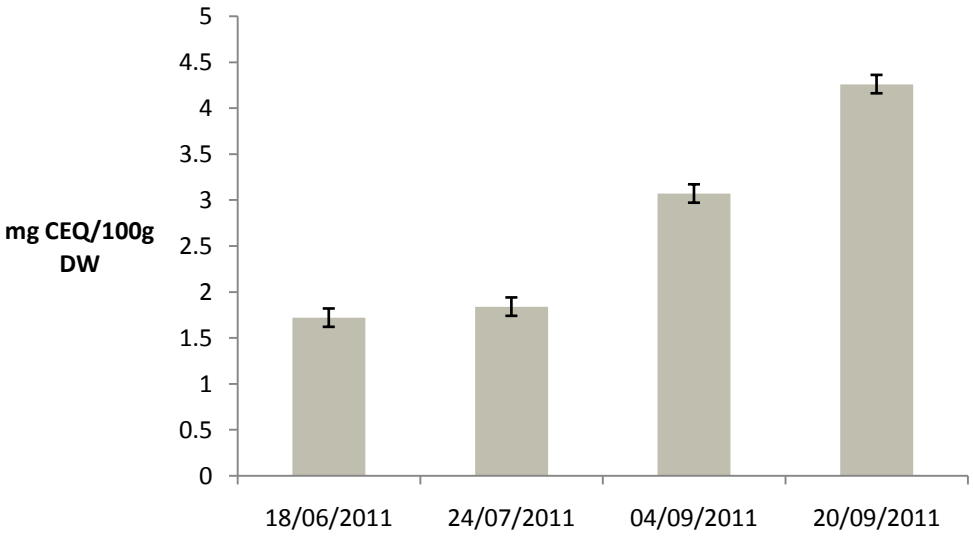


Figure 4.25: Total flavonoids of Barhi balade date palm harvested between June to late September 2011.

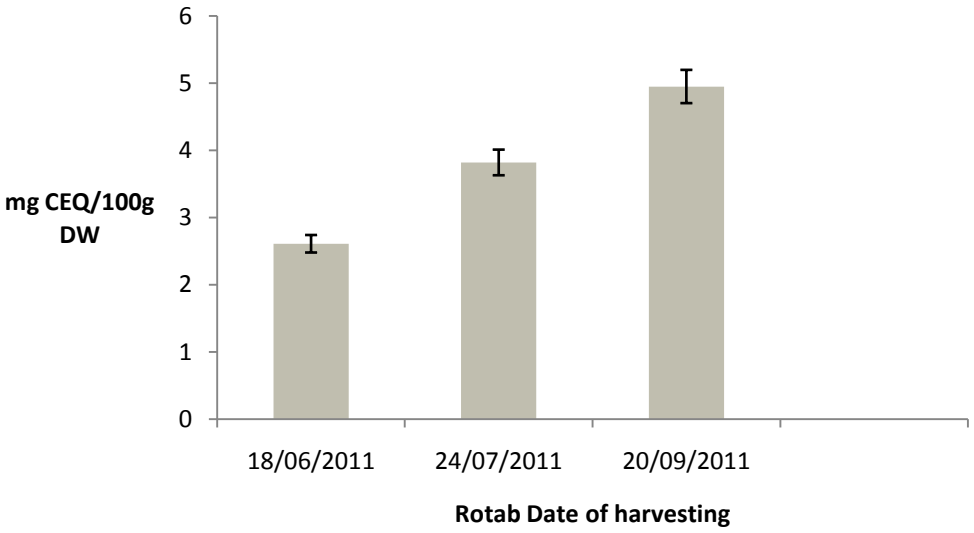


Figure 4.26: Total flavonoids of Rotab date palm harvested between June to late September 2011.

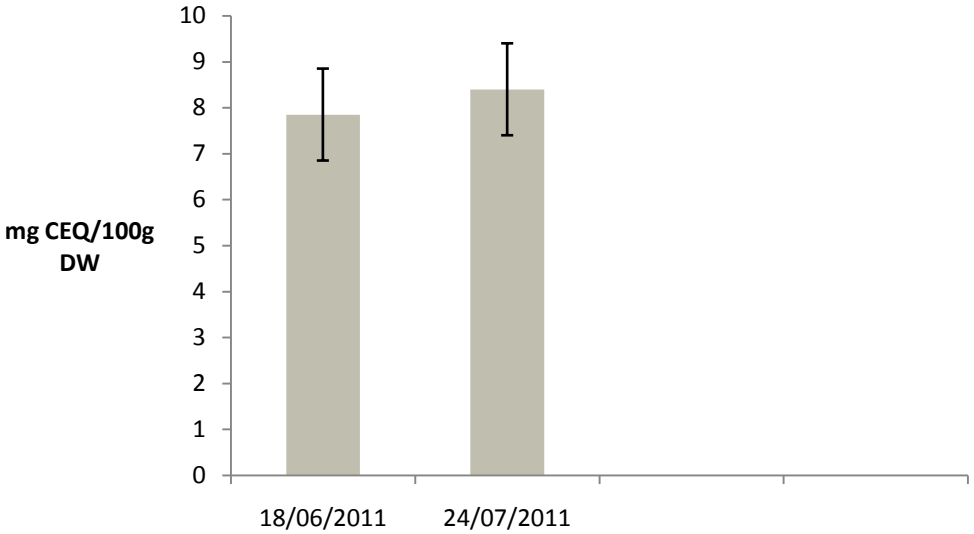


Figure 4.27: Total flavonoids of Zahedi date palm harvested between June to July 2011.

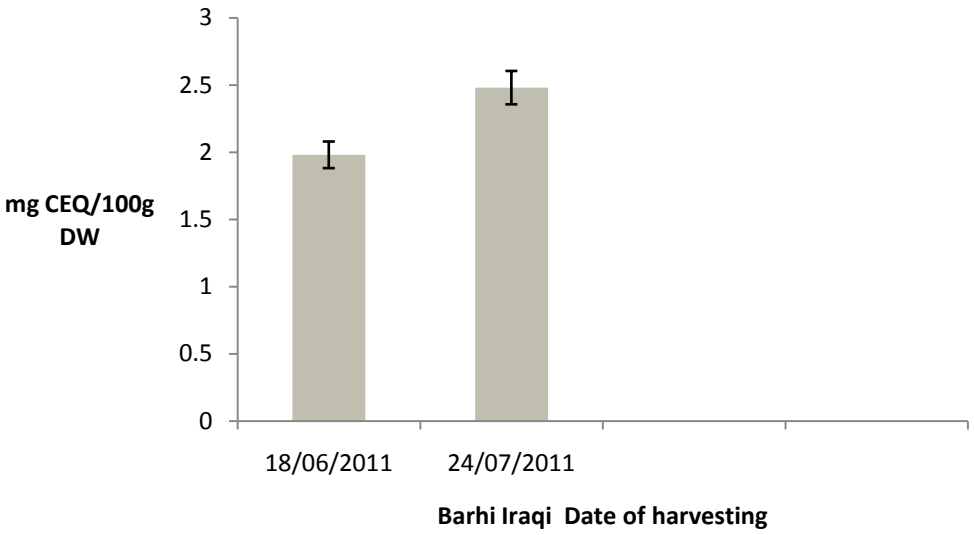


Figure 4.28: Total flavonoids of Barhi Iraqi date palm harvested between June to July 2011.

Figure (4.29) shows a comparison between all date varieties that were collected in 18/06/2011 from Aqbet jabber camp. Asfar balade has the highest amount of total flavonoid contents comparing it with other varieties followed by Zahedi, Ahmar balade, Madjhool, Rotab, Barhi Iraqi and Barhi balade.

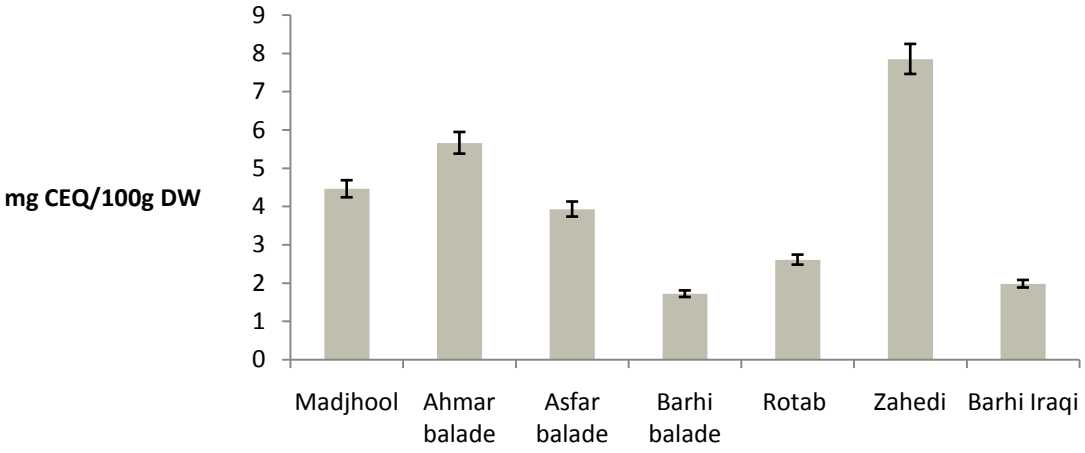


Figure 4.29: Total flavonoids of all date palm varieties obtained on 18/06/2011.

Figure (4.30) shows a comparison between all date varieties that were collected in 24/07/2011 from Aqbet jabber camp. Zahedi has the highest amount of total flavonoid contents comparing it with other varieties followed by Asfar balade, Medjool, Ahmar balade, Rotab, Barhi Iraqi and Barhi balade.

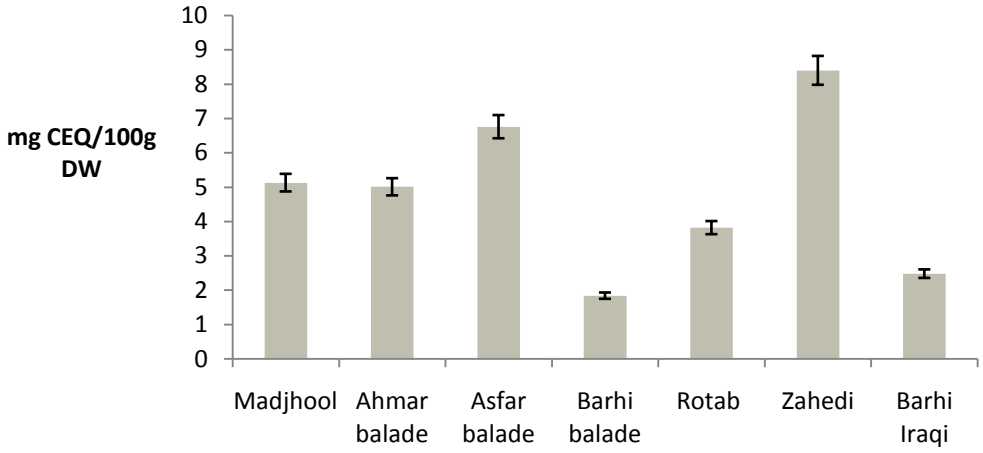


Figure 4.30: Total flavonoids of all date palm varieties obtained on 24/07/2011.

Figure (4.31) shows a comparison between all date varieties that were collected in 04/09/2011 from Aqbet jabber camp. Medjool has the highest amount of total flavonoid contents comparing it with other varieties followed by Ahmar balade and Barhi balade.

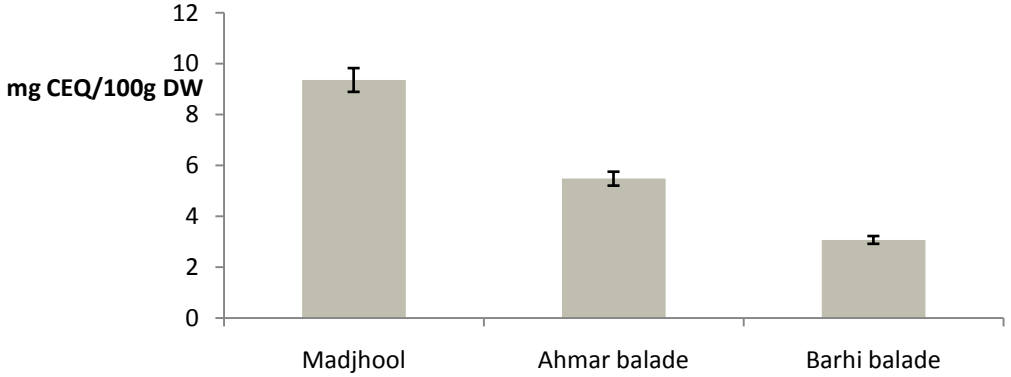


Figure 4.31: Total flavonoids of all date palm varieties obtained on 04/09/2011.

Figure (4.32) shows a comparison between all date varieties that were collected in 20/09/2011 from Aqbet jabber camp. Madjhool has the highest amount of total flavonoid contents comparing it with other varieties followed by, Ahmar balade , Rotab, Barhi balade and Asfar balade.

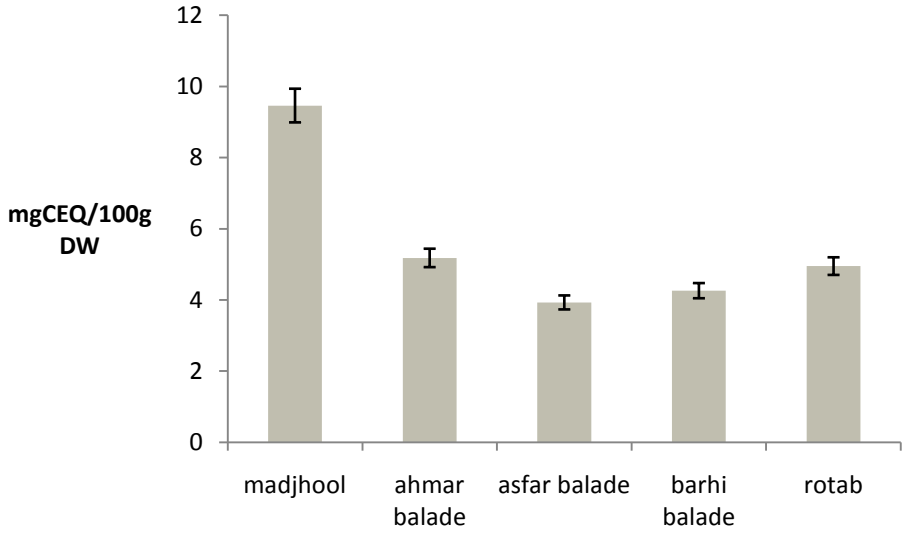


Figure 4.32: Total flavonoids of all date palm varieties obtained on 20/09/2011.

4.2. Correlation analyses

A correlation between antioxidant activity and total phenolics content as well as between antioxidant activity and total flavonoids content was performed for all date palm varieties used in this study at different harvesting dates. Results have confirmed the correlation between antioxidant activity and total phenolics content for all date palm varieties where a linear relationship was obtained with a correlation coefficients r^2 of better than 0.90, see [Figures 4.33(a) to 4.39(a)]. Furthermore, a positive correlation between antioxidant activity and total flavonoids content with a correlation coefficient r^2 of better than 0.80, see [Figures 4.33(b) to 4.39(b)]. These results confirmed that antioxidant activity of palm dates arises from mainly phenolic compounds and flavonoids, and demonstrates the potential of Palestinian dates as antioxidant functional food ingredients. Similar results were obtained by Biglari et. al. (2008)

where they got a linear relationship between antioxidant activity and total phenolics content or total flavonoids content. Similar results were obtained also by Neo Y. et. al. (2010) where a high positive correlation was obtained between total phenolics content and FRAP antioxidant activity assay with a correlation coefficient of 0.999.

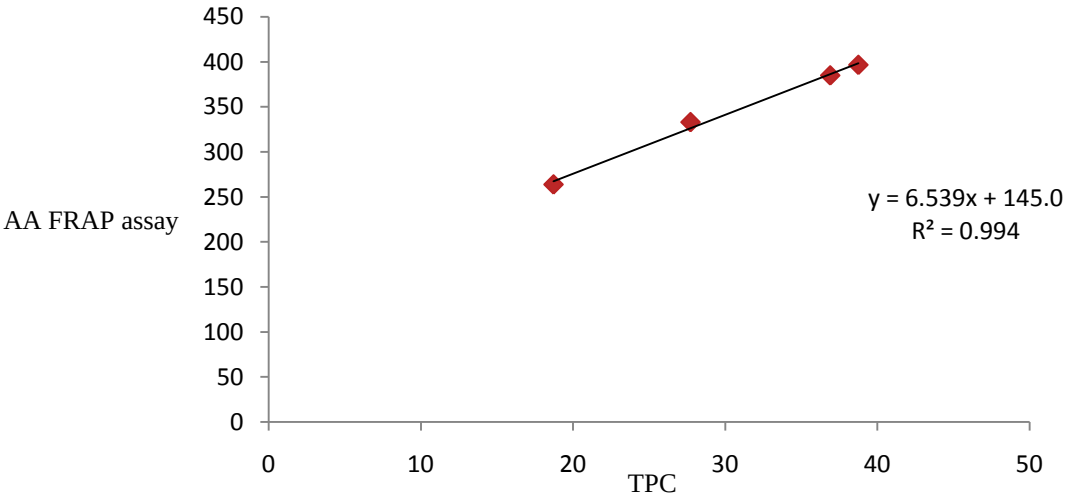


Figure 4.33: (a) antioxidant activity vs. total phenolics content for Madjhool palm date harvested from June to late September 2011.

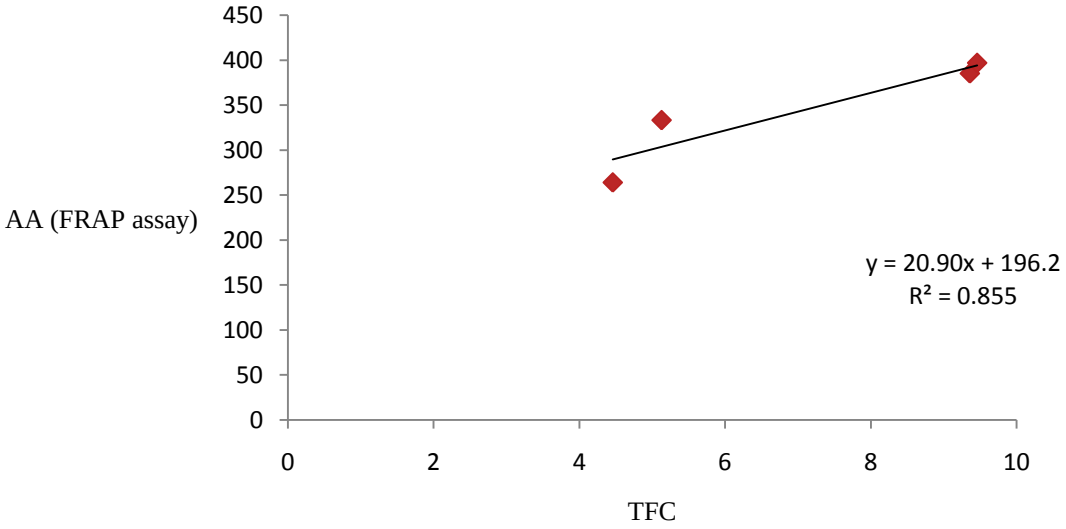


Figure 4.33: (b) antioxidant activity vs. total flavonoids content for Medjool palm date harvested from June to late September 2011.

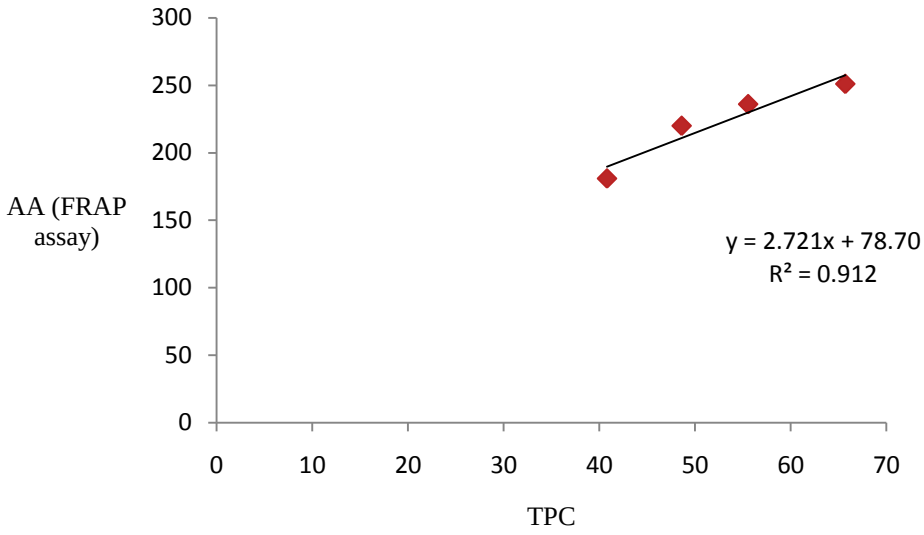
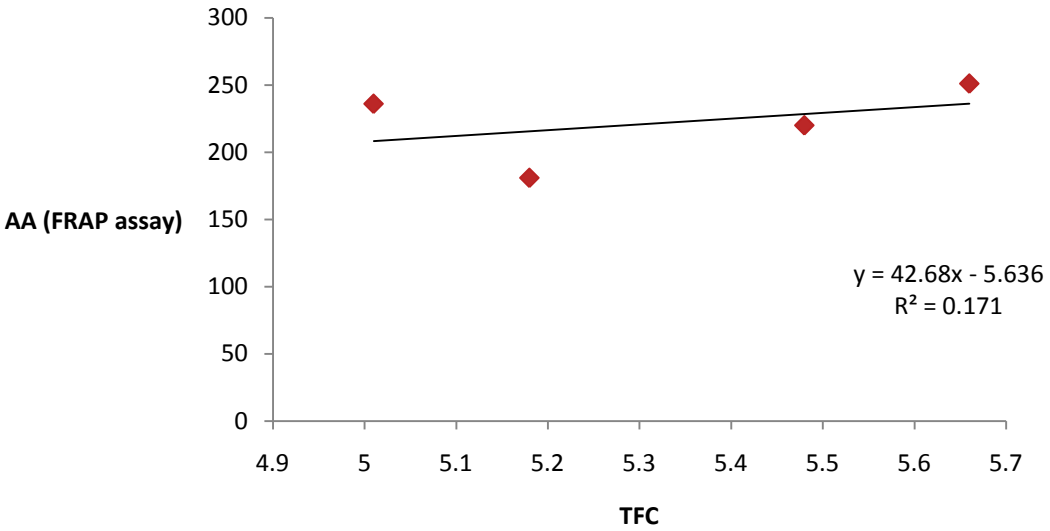


Figure 4.34: (a) antioxidant activity vs. total phenolics content for Ahmar balade palm date harvested from June to late September 2011.



Fifure 4.34: (b) antioxidant activity vs. total flavonoids content for Ahmar balade palm date harvested from June to late September 2011.

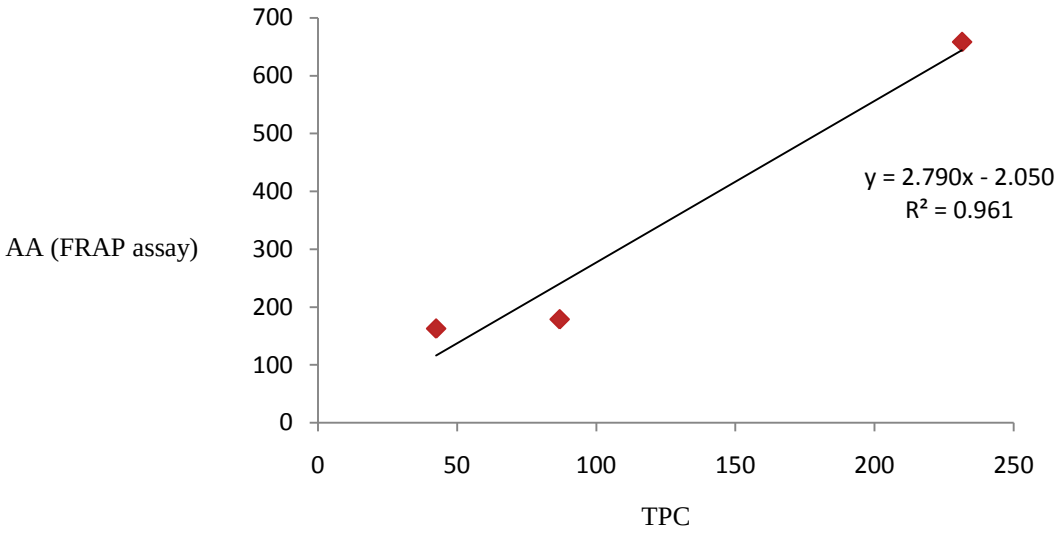


Figure 4.35: (a) antioxidant activity vs. total phenolic content for Asfar balade palm date harvested from June to late September 2011.

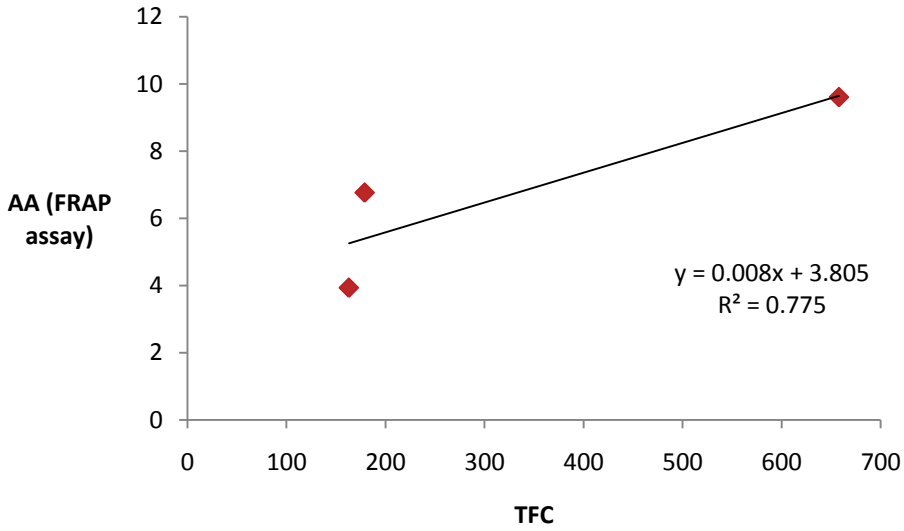


Figure 4.35: (b) antioxidant activity vs. total flavonoid content for Asfar balade palm date harvested from June to late September 2011.

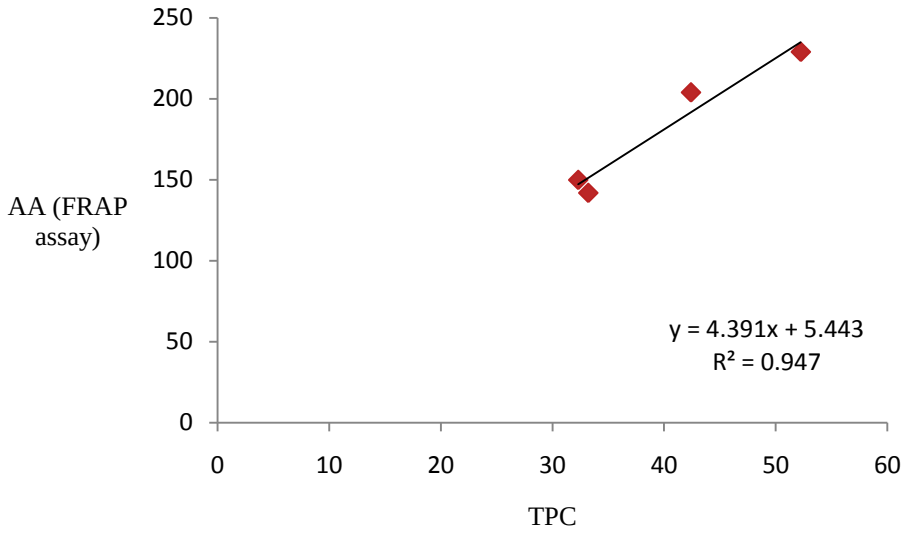


Figure 4.36: (a) antioxidant activity vs. total phenolic content for Barhi balade palm date harvested from June to late September 2011.

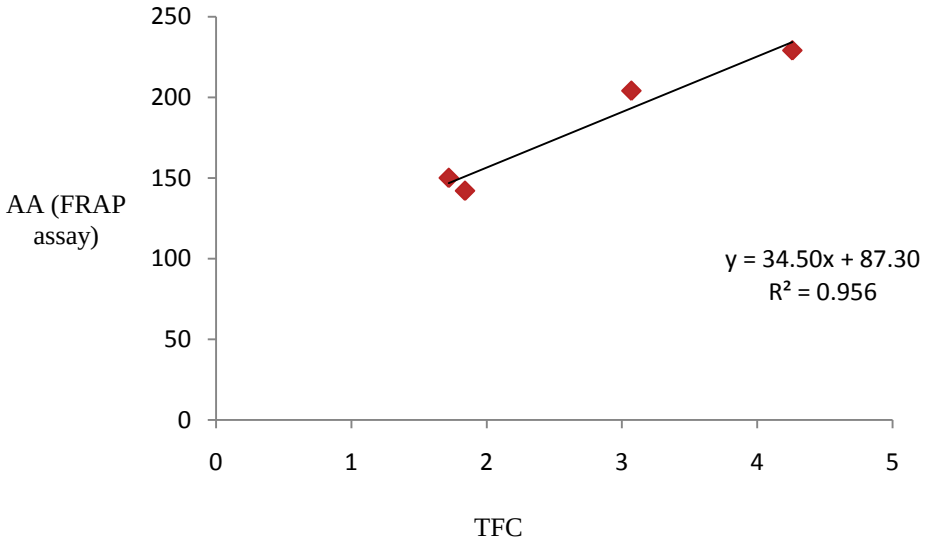


Figure 4.36: (b) antioxidant activity vs. total flavonoid content for Barhi balade palm date harvested from June to late September 2011.

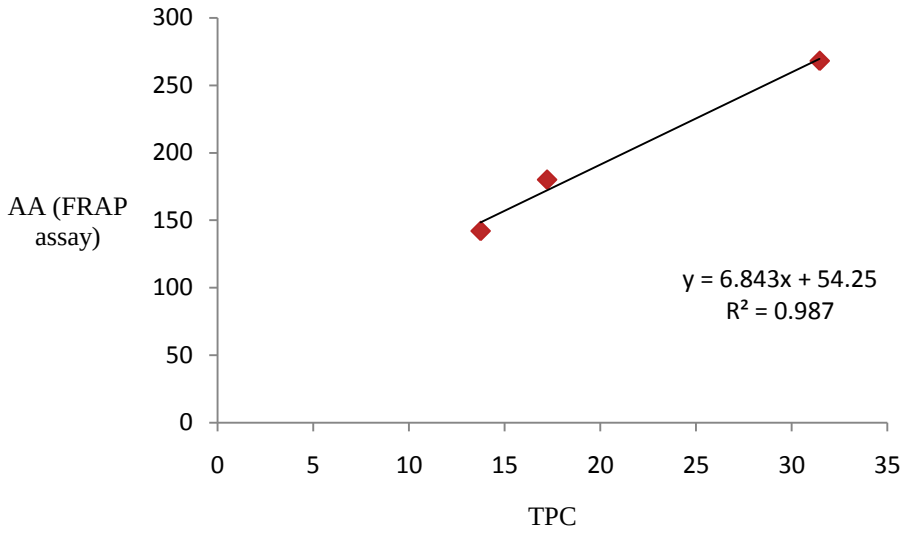


Figure 4.37: (a) antioxidant activity vs. total phenolic content for Rotab palm date harvested from June to late September 2011.

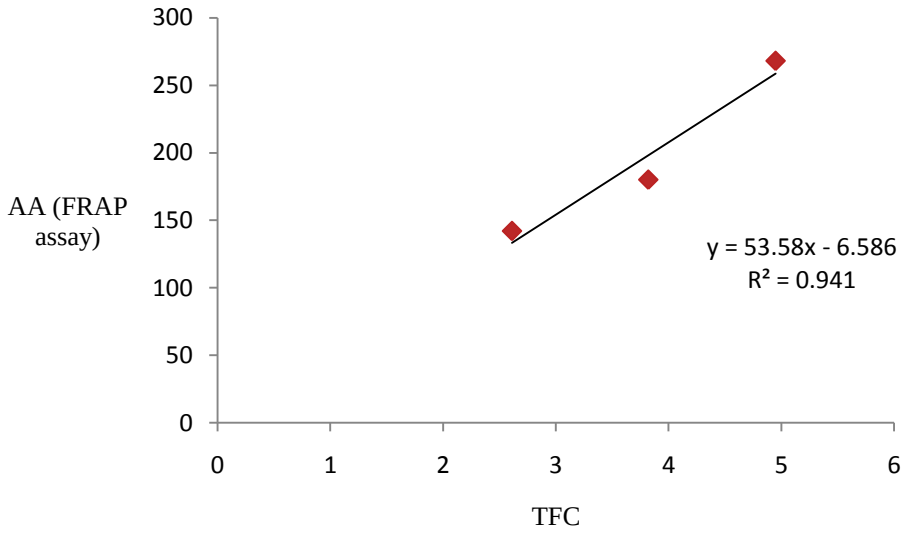


Figure 4.37: (b) antioxidant activity vs. total flavonoid content for Rotab palm date harvested from June to late September 2011.

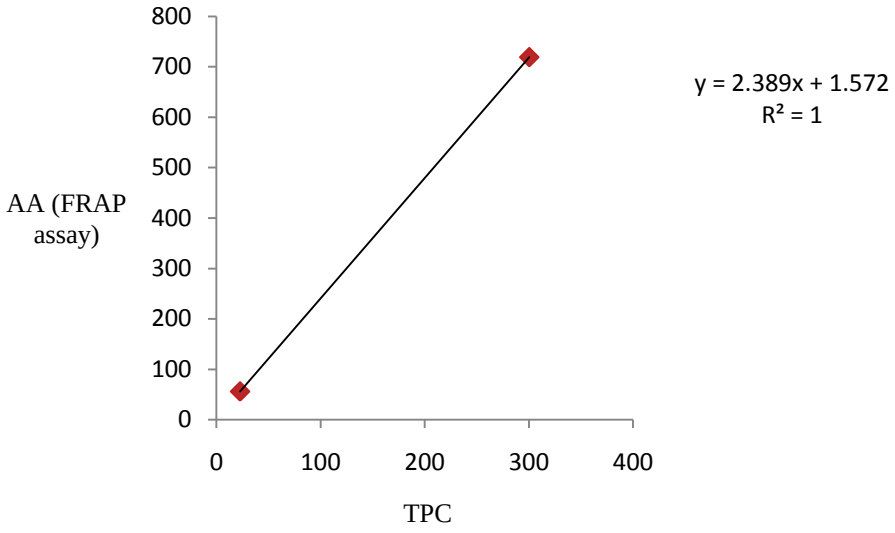


Figure 4.38: (a) antioxidant activity vs. total phenolic content for Zahedi palm date harvested from June to July 2011.

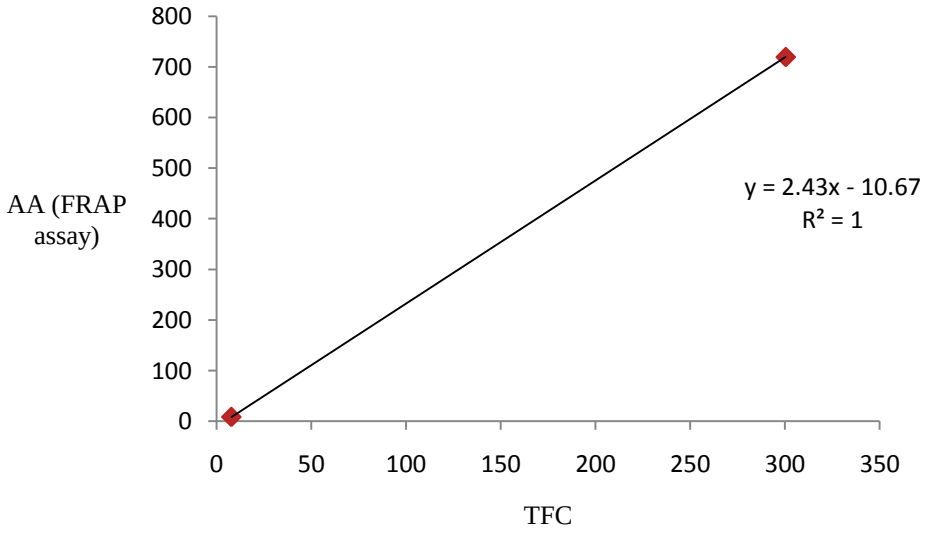


Figure 4.38: (b) antioxidant activity vs. total flavonoid content for Zahedi palm date harvested from June to July 2011.

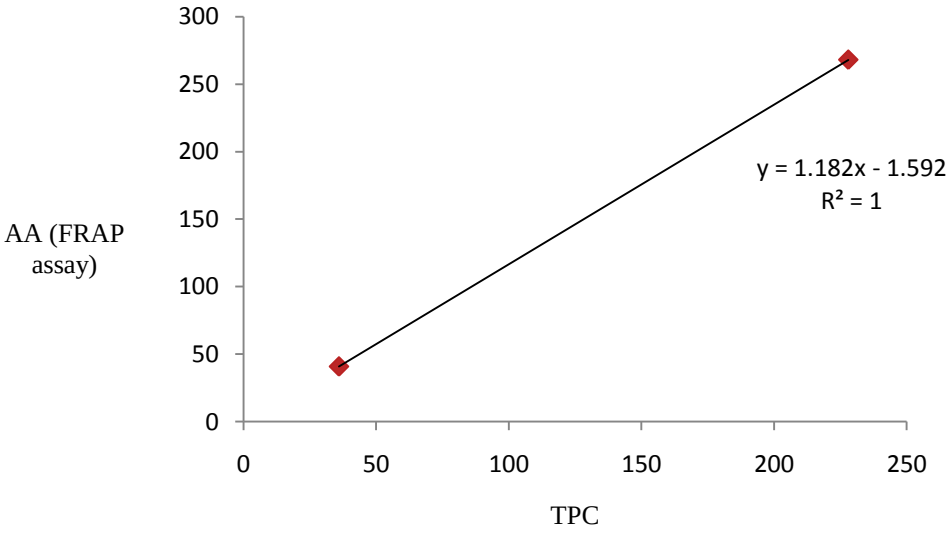


Figure 4.39: (a) antioxidant activity vs. total phenolic content for Barhi iraqi date harvested from June to July 2011.

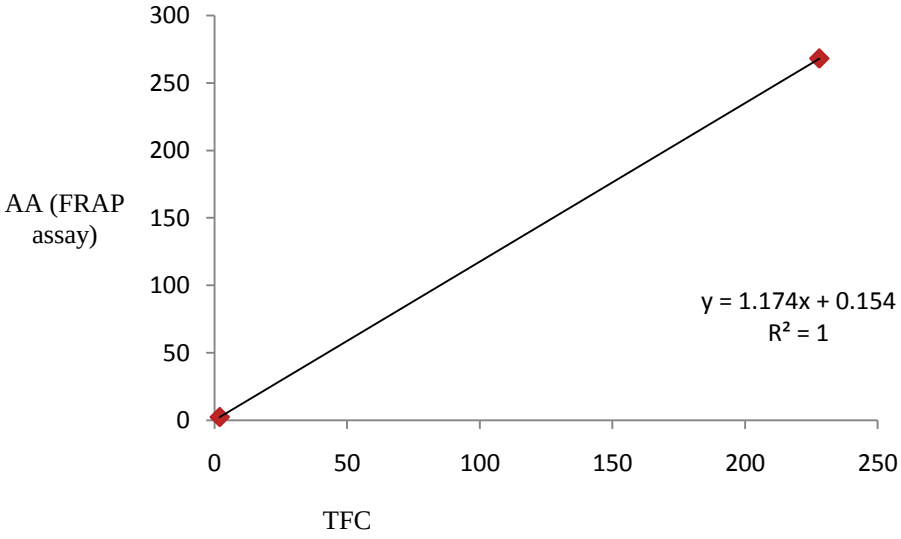


Figure 4.39: (b) antioxidant activity vs. total flavonoid content for Barhi iraqi palm date harvested from June to July 2011.

4.3. Comparison of antioxidant activity, total phenolics and total flavonoids content of Palestinian palm dates investigated in this study with those of other studies

It is interesting to compare total phenolics, total flavonoids content, and antioxidant activities of Palestinian palm dates investigated in this study with those of other countries. Various factors such as palm date variety, growing condition, maturity, season, geographic origin, fertilizers, soil type, and amount of sunlight received, experimental conditions (storage, extraction) might be responsible for any observed differences.

4.3.1. Total phenolics content

The range of total phenolics content of the seven Palestinian palm dates investigated in this study is 13.75-231.4, 17.22-86.79, 36.9-42.4, 31.46-52.24 mgGAE/100 g DW, harvested in June, July, early September, and late September, respectively. These values compared to total phenolics content obtained by Mansouri et al. (2005) for the Algerian ripe date palm fruit (2.49-8.36 mgGAE/100 g fresh weight), indicates that the Palestinian palm date varieties investigated in this study are more rich in phenolic compounds.

Comparing total phenolics content of Palestinian palm dates obtained in this study with those obtained by Biglari *et al*., (2008) for Iranian palm dates (2.89 to 6.64 mg GAE⁄ 100 g of dry weight and 141.35 mg GAE⁄ 100 g dry weight for Kharak variety), indicates that Palestinian palm dates is more rich in phenolic compounds compared to Iranian palm dates, except for Kharak date which has higher phenolic content compared to dry Palestinian palm dates.

Al-Farsi et al. (2007) reported that total phenolics content of different Omani palm date varieties ranged between 172 and 246 mg gallic acid equivalents/100 g fresh weight, which are higher than total phenolics content of Palestinian palm dates investigated in this study and comparable to those of Asfar balade palm date variety harvested in June where its total phenolics content is 231.4 mg gallic acid equivalents/100 g dry weight.

Comparing the total phenolics content of Palestinian palm dates investigated in this study with those obtained by Abdul Ameer A. Allaith, (2008) for Bahrain date palms (116.7 – 196.8 mg GAE/ 100 g fresh weight), indicates that the Palestinian palm date varieties in this study have lower phenolics content compared to Bahraini date palms, while Asfar balade palm date variety harvested in June have higher total phenolics content (231.4 mg gallic acid equivalents/100 g dry weight) compared to those of Bahraini date palms.

4.3.2. Total flavonoids content

The range of total flavonoids content of the seven Palestinian palm dates investigated in this study is 1.72-9.6, 1.84-8.40, 3.07-9.36, 3.9-9.46 mgCEQ/100 g DW, harvested in June, July, early September, and late September, respectively. Comparing these values with those obtained by Biglari *et al*. (2008) for Iranian palm dates (1.62 to 3.07, 1.65 to 4.71 and 81.79 mg catechin equivalents (CEQ)/100 g dry weight sample for soft dates, semi dry dates and dry dates, respectively), indicates that Palestinian palm date varieties are comparable to Iranian palm dates in their total flavonoids content except for dry Iranian dates which have larger total flavonoids content.

Comparing total flavonoids content for Palestinian palm dates varieties in this study with those obtained by Singh *et al.* (2012) for palm dates from Oman (19-66 and 25-34 mg catechin equivalents (CEQ)/100 gm at Rutab and Tamr stages respectively) indicates that Palestinian palm date varieties are lower compared to Omani palm dates in their total flavonoids content. Singh et al. (2012) have reported that higher flavonoids values were associated with Rutab stage which indicates that the drying process may have a destructive effect on these compounds. Our results, however, show increasing in total flavonoids content during maturation except for Ahmar balade and Asfar balade date palm varieties.

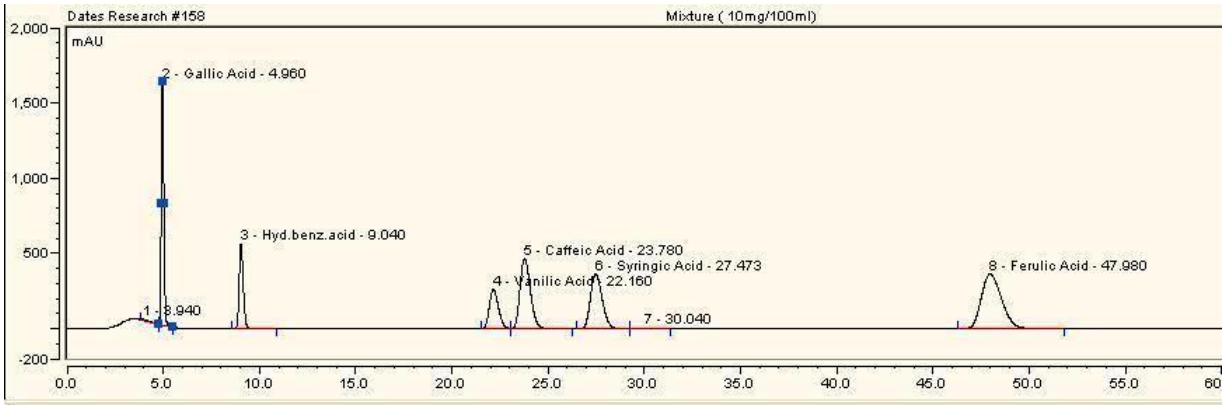
4.3.3 Antioxidant activity

The range of antioxidant activity of the seven Palestinian palm dates investigated in this study is 142.0-658.0, 142.0-719.3, 204.0-385.0, 163.0-396.7 µmol/100 g DW, harvested in June, July, early September, and late September, respectively. Comparing these values with those obtained by Bilgari et al (2008) for Iranian palm dates at different maturity stages (11.65–20, 19.12–29.34 and 387.34 µmol FRAP/100 g dry weight sample for soft dates, semi dry dates and dry dates, respectively), indicates that Palestinian palm date varieties in this study are higher than Iranian palm dates in their antioxidant activity except for dry Iranian dates which have comparable antioxidant activity to Palestinian palm date varieties investigated in this study.

Comparing antioxidant activity of Palestinian palm dates varieties in this study with those obtained by Abdul Ameer A. Allaith (2008) for Bahraini palm dates (5710, 1200, and 940 µmol⁄ 100 g fresh weight for biser (unripe) stage, rutab (soft and ripped), and tamer (dried fruit) stage, respectively) indicates that Palestinian palm date varieties are lower compared to Bahraini palm dates in their antioxidant activity.

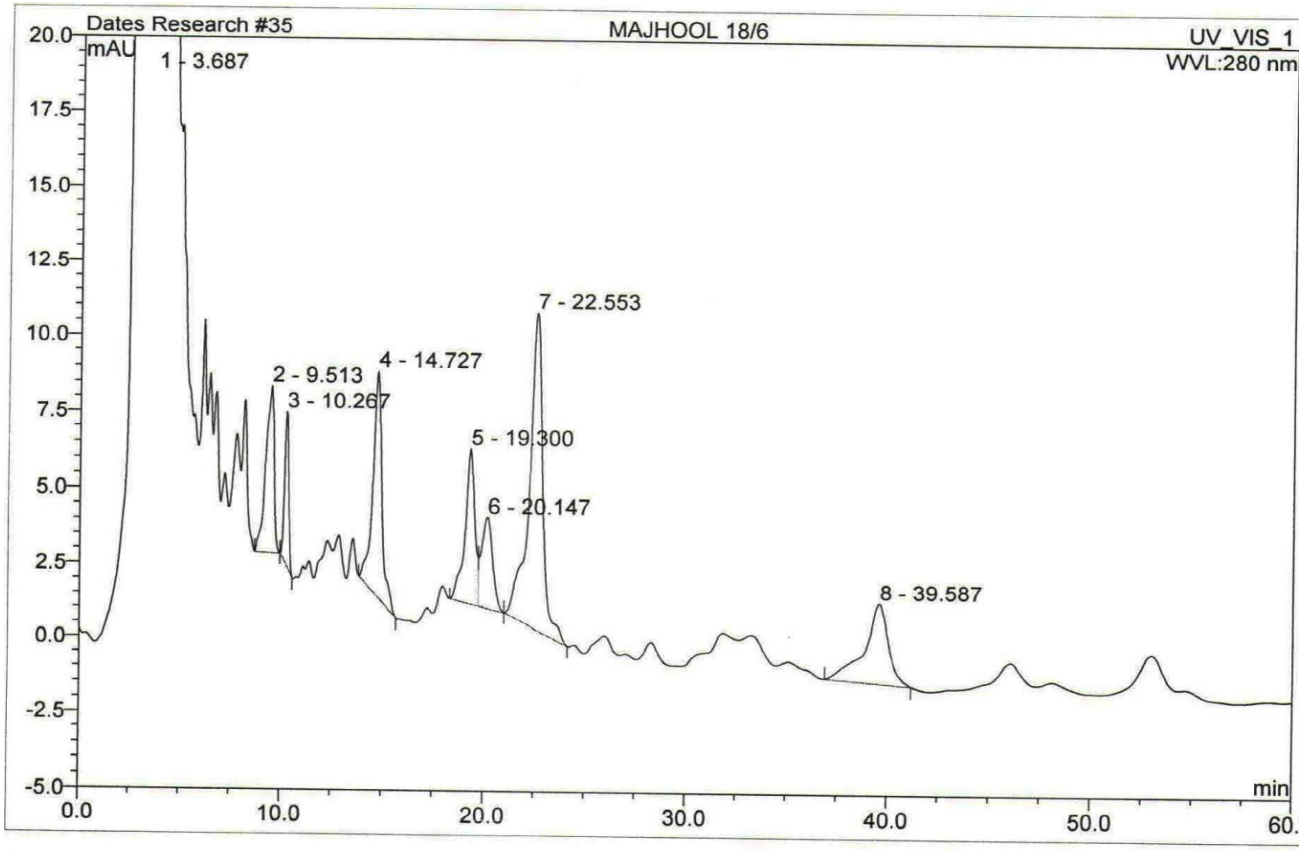
4.4. Amounts of phenolic compounds present in the palm date varieties determined by HPLC

There were six phenolic compounds in the date palm extracts identified and quantified in this study. These compounds are: Gallic acid, p-hydroxybenzoic acid, Vanillic acid, Caffeic acid, Syringic acid, and Ferulic acid. [Figure 4.40] shows a chromatogram of different phenolic compounds separated by the current HPLC method, (Figure 4.40 (a) for a standard of the six phenolic compounds while figure 4.40 (b-h) shows a chromatogram of different phenolic compounds detected in different palm date varieties). Isocractic separation was used in this study as it is a simpler method for the detection of phenolic compounds compared to gradient separation. The linearity of the HPLC method for determination of these six phenolic compounds was confirmed in the range from 10-200 ppm with high coefficient of determination r^2 larger than 0.99.



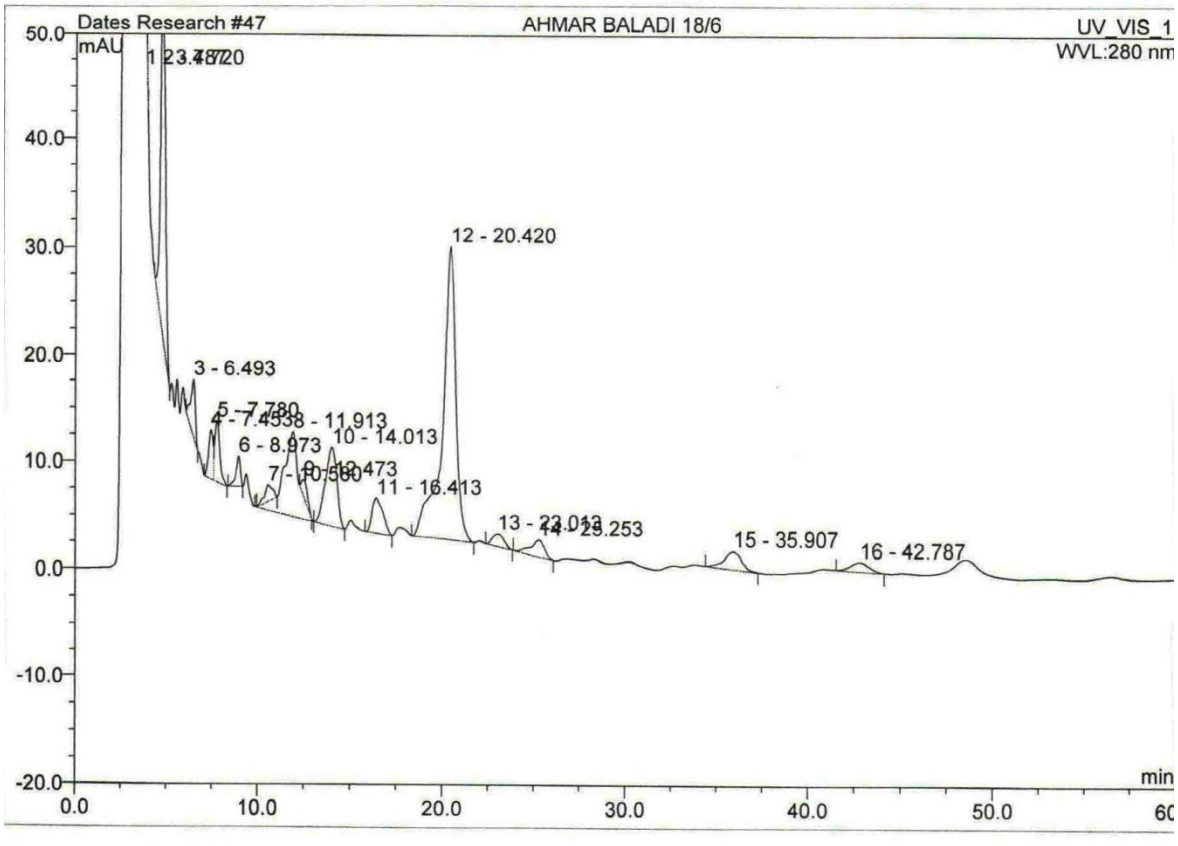
Time/min

Figure 4.40: (a) chromatogram of the six phenolic compounds. 1)gallic acid, 2)p-hydroxybenzoic acid, 3)vanillic acid, 4)caffeic acid, 5)syringic acid, 6)ferulic acid.



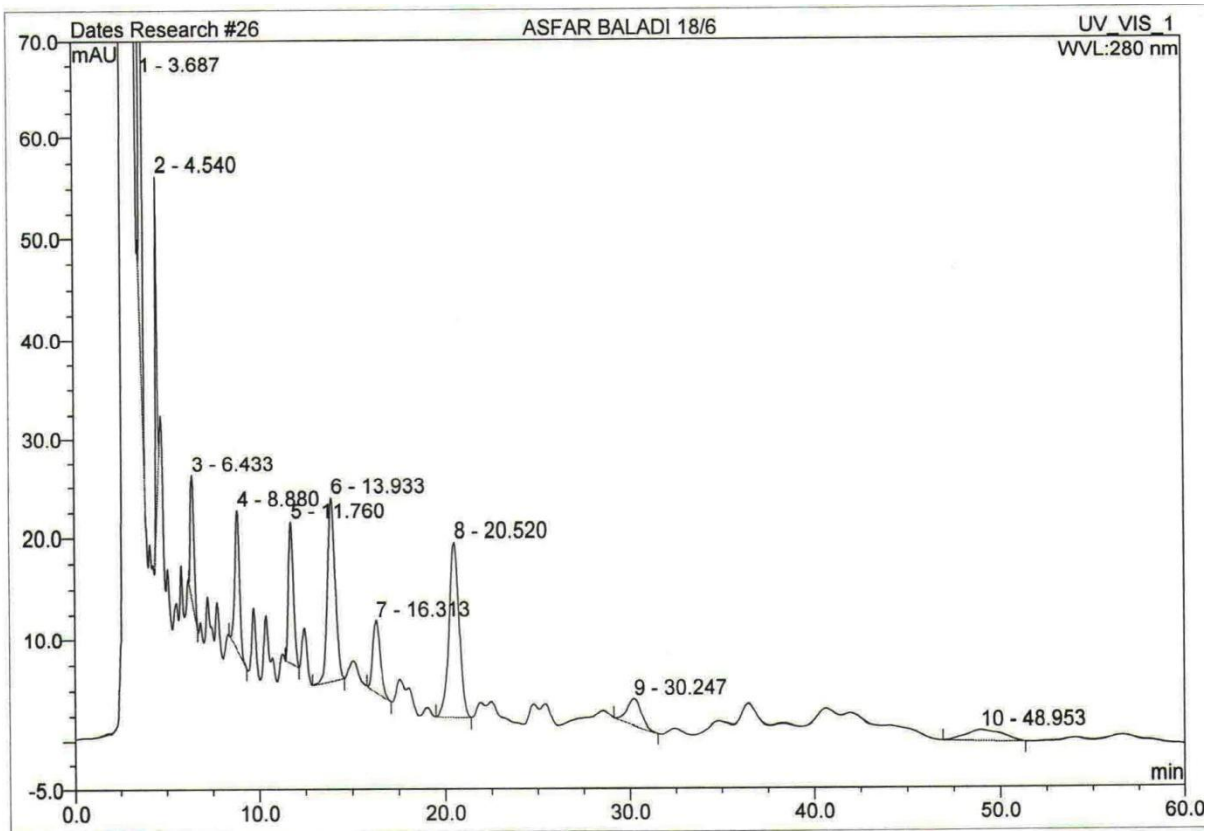
Time/min

Figure 4.40: (b) a chromatogram of different phenolic compounds detected in Madjhool palm date varieties obtained on 18/6/2011.7) vanillic acid.



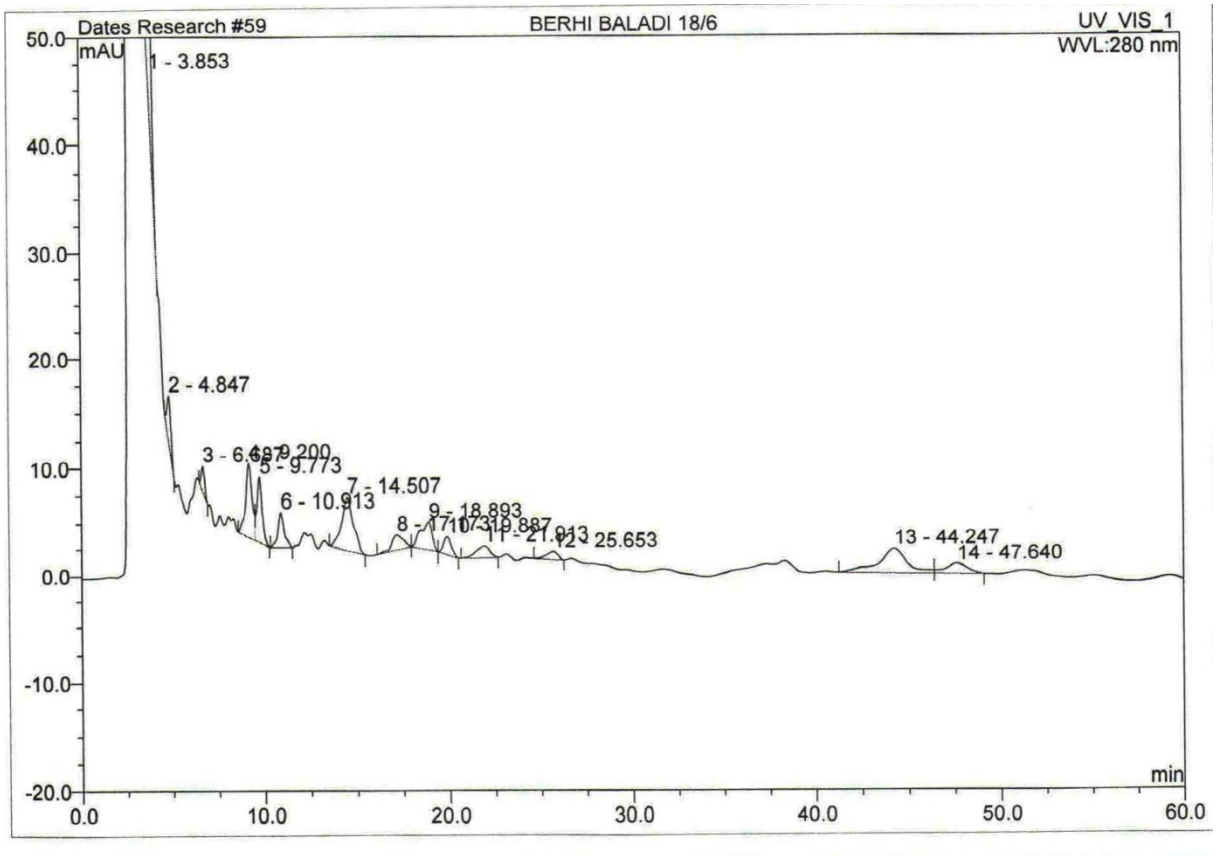
Time/min

Figure 4.40: (c) a chromatogram of different phenolic compounds detected in Ahmar balade palm date varieties obtained on 18/6/2011. 2)gallic acid, 6)p-hydroxybenzoic acid, and 13)caffeic acid.



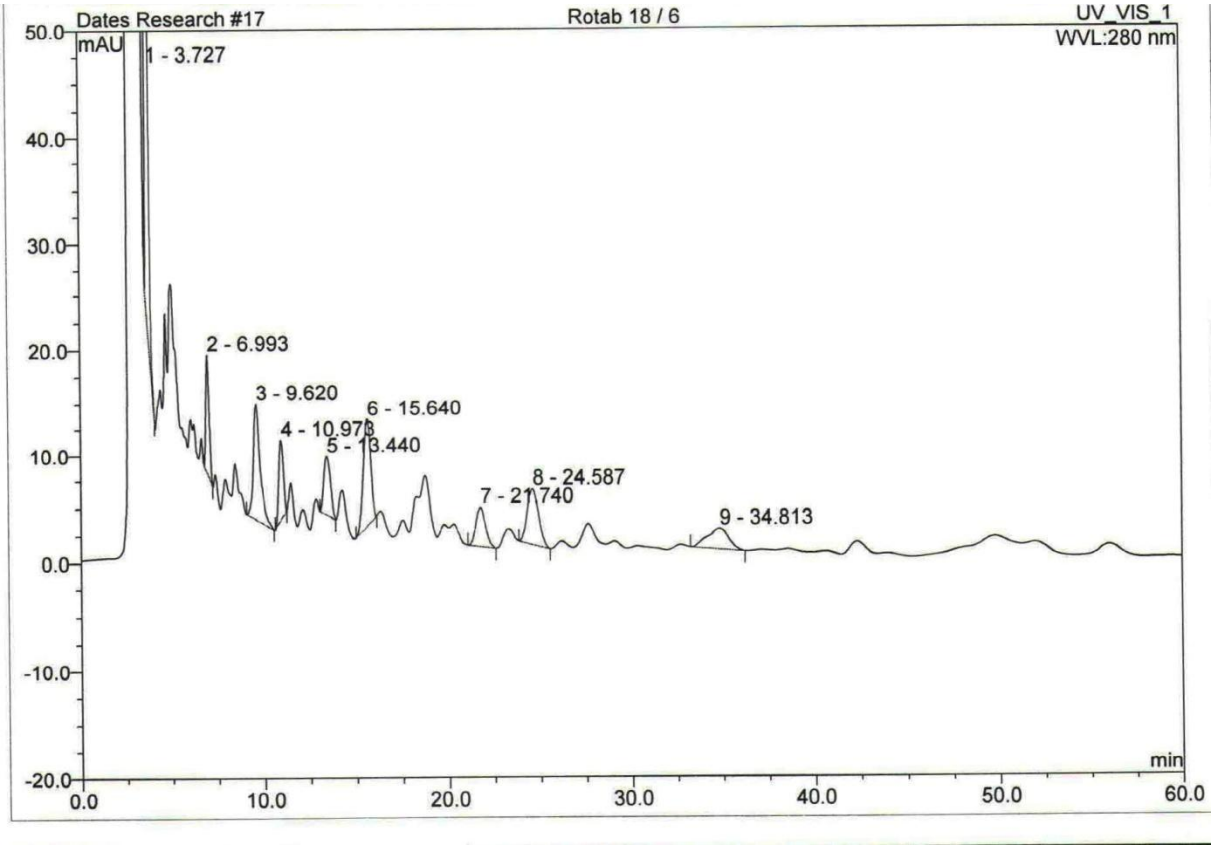
Time/min

Figure 4.40: (d) a chromatogram of different phenolic compounds detected in Asfar balade palm date varieties obtained on 18/6/2011.2)gallic acid, 4)p-hydroxybenzoic acid.



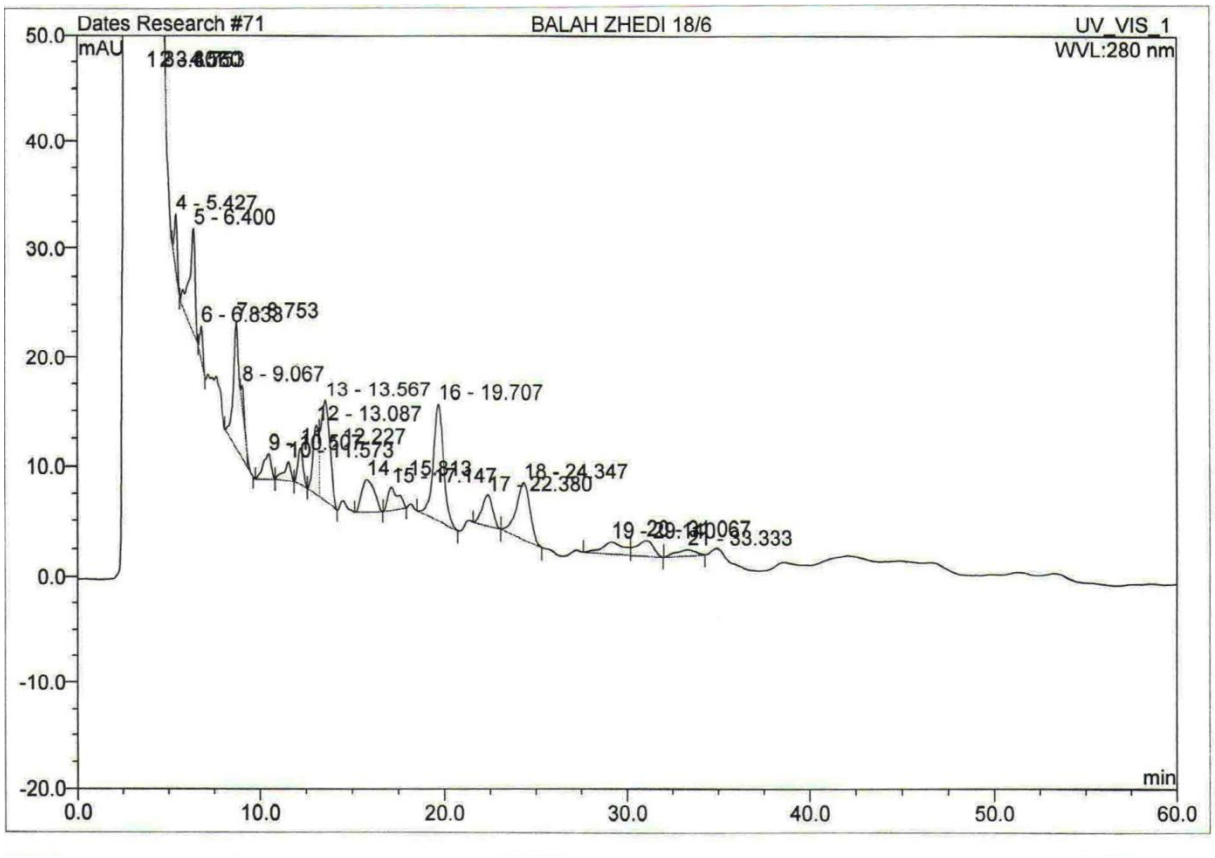
Time /min

Figure 4.40: (e) a chromatogram of different phenolic compounds detected in Berhi balade palm date varieties obtained on 18/6/2011.2)gallic acid, 4)p-hydroxybenzoic acid, and 14)ferulic acid.



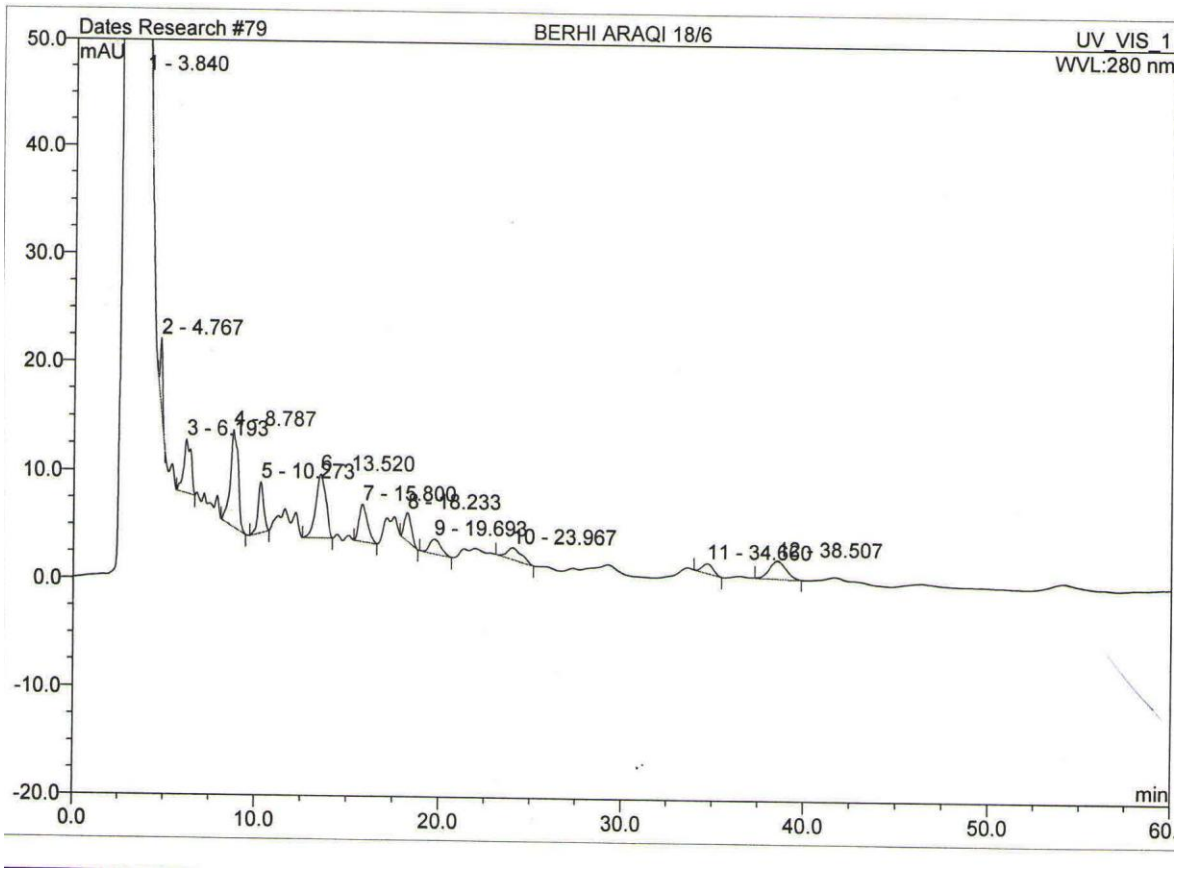
Time/min

Figure 4.40: (f) a chromatogram of different phenolic compounds detected in Rotab palm date varieties obtained on 18/6/2011.7) vanillic acid.



Time/min

Figure 4.40: (g) a chromatogram of different phenolic compounds detected in Zahedi palm date varieties obtained on 18/6/2011.3)gallic acid, 8)p-hydroxybenzoic acid.



Time/min

Figure 4.40: (h) a chromatogram of different phenolic compounds detected in Berhi Iraqi palm date varieties obtained on 18/6/2011.2)gallic acid, 3)p-hydroxybenzoic acid.

Gallic acid, appears in most of the date varieties during maturity stages and the highest value was in Asafar balade date variety (1.02 mg/100g DW), followed by Ahmar balade (0.49 mg/100g DW), Barhi balade (0.27 mg/100g DW), Rotab (0.24 mg/100g DW), Medjool (0.082 mg/100g DW), Zahedi (0.074 mg/100g DW) and Barhi iraqi (0.067 mg/100g DW).

p-Hydroxybenzoic acid was the most dominant in all date varieties and recorded the highest concentration in dry date in Asfar balade (2.8 mg/100g DW) followed by 1.85 mg/ 100g DW in Barhi balade variety, Barhi iraqi 1.23 mg/100g DW, Rotab (1.20 mg/100g DW), and Medjool (0.69 mg/100g DW).

Vanillic acid appears in the date fruits where the highest value was in Madjhool (0.46 mg/100g DW), followed by Rotab (0.43 mg/100g DW), Barhi Iraqi (0.26 mg/100g DW), Barhi balade (0.2 mg/100g DW), Zahedi (0.14 mg/100g DW), and Ahmar balade (0.12 mg /100g DW).

Caffeic acid appears in some palm date varieties and the highest value was in Ahmar balade (1.45 mg/100g DW) followed by Barhi Iraqi (0.056 mg/100g DW) and Medjool (0.021 mg/100g DW).

Syringic acid appears in Barhi Iraqi as the highest value (0.20 mg/100g DW) followed by Ahmar balade (0.19 mg/100g DW), Barhi balade (0.080 mg/100g DW) and (0.035 mg/100g DW) in Zahedi. Ferulic acid appears only in Barhi balade date variety and ranged from 0.041 to 0.52 mg/100g DW.

As seen in [Table 4.2], gallic acid, p-hydroxybenzoic acid, vanillic acid and caffeic acid appeared in Madjhool date fruit. For gallic acid it appeared in July and the value was 0.081 mg/100 g DW and then vanished, but for p-hydroxybenzoic acid it ranged between 0.38 to 0.69 mg/100 g DW from July to late september. Vanillic acid appeared in both June and July with values of 0.46 and 0.27 mg /100 g DW, respectively. While caffeic acid appeared in September and varies between 0.017 to 0.021 mg /100 g DW.

For Ahmar balade date fruit it's obvious from [Table 4.3] that the contents of phenolic compound gallic acid showed irregularity as it went from June to September and ranged between (0.44 to 0.097 mg/100 g DW) same as p-hydroxybenzoic acid which ranged between (0.058 to 0.070 mg/100g DW). While vanillic acid and syringic acid were appeared in July with 0.11 mg/100g DW) and (0.19 mg/100g DW) respectively, cafeeic acid values increased and went from (0.026 mg/100g DW) in June to (1.4 mg/100g DW) in late September.

Barhi balade HPLC chromatograph and according to our standards showed that Barhi balade contained gallic acid in the range between (0.064 mg/100g DW) in June and (0.27 mg/100g DW) in July, p-hydroxybenzoic acid in the range between (0.34 to 1.9 mg/100g DW) from June to late September, vanillic acid ranged between (0.20 to 0.063 mg/100g DW), ferulic acid ranged between (0.061 to 0.52 mg/100g DW) and syringic acid value was (0.080 mg/100g DW) in late September as seen in [Table 4.4].

Gallic acid and p-hydroxybenzoic acid appeared in Asfar balade date fruit and ranged between 0.40 to 1.02 mg/100g DW for gallic acid, and 0.90 to 2.8 mg/100g DW for p-hydroxybenzoic acid as seen in [Table 4.5]. Apparently in Rotab date fruit, gallic acid, p-hydroxybenzoic acid and vanillic acid were appeared in the HPLC chromatogram in the range shown in [Table 4.6]. Gallic acid, p-hydroxybenzoic acid, vanilllic acid, and syringic acid phenolic compounds were appeared in Zahedi date fruit as shown in [Table 4.7]. While in Barhi Iraqi date fruit gallic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid and syringic acid appeared as shown in [Table 4.8].

Table 4.2: Amounts of phenolic compounds (in mg/100 g DW) found in Madjhool date palm harvested from June to late September 2011 determined by HPLC.

	Madjhool mg/100 g DW			
Standard	18/06/2011	24/07/2011	04/09/2011	20/09/2011
Gallic Acid	n.d	0.081	n.d	n.d
p-Hydroxybenzoic Acid	n.d	0.38	0.56	0.69
Vanilic Acid	0.46	0.27	n.d	n.d
Caffeic Acid	n.d	n.d	0.021	0.017
Syringic Acid	n.d	n.d	n.d	n.d
Ferulic Acid	n.d	n.d	n.d	n.d

Table 4.3: Amounts of phenolic compounds (in mg/100 g DW) found in Ahmar balade date palm harvested from June to late September 2011 determined by HPLC.

	Ahmar balade mg/100 g DW			
Standard	18/06/2011	24/07/2011	04/09/2011	20/09/2011
Gallic Acid	0.44	0.49	0.097	n.d
p-Hydrobenzoic Acid	0.058	0.12	0.070	n.d
Vanilic Acid	n.d	0.11	n.d	n.d
Caffeic Acid	0.026	n.d	n.d	1.4
Syringic Acid	n.d	0.19	n.d	n.d
Ferulic Acid	n.d	n.d	n.d	n.d

Table 4.4: Amounts of phenolic compounds (in mg/100 g DW) found in Barhi balade date palm harvested from June to late September 2011 determined by HPLC.

	Barhi balade mg/100 g DW			
Standard	18/06/2011	24/07/2011	04/09/2011	20/09/2011
Gallic Acid	0.064	0.27	n.d	n.d
p-Hydrobenzoic Acid	0.34	0.37	0.6	1.9
Vanilic Acid	0.11	0.20	n.d	0.063
Caffeic Acid	n.d	n.d	n.d	n.d
Syringic Acid	n.d	n.d	n.d	0.080
Ferulic Acid	0.061	0.041	0.49	0.52

Table 4.5: Amounts of phenolic compounds (in mg/100 g DW) found in Asfar balade date palm harvested from June to late September 2011 determined by HPLC.

	Asfar balade mg/100 g DW		
Standard	18/06/2011	24/07/2011	20/09/2011
Gallic Acid	0.40	n.d	1.0
p-Hydrobenzoic Acid	0.90	1.2	2.8
Vanilic Acid	n.d	n.d	n.d
Caffeic Acid	n.d	n.d	n.d
Syringic Acid	n.d	n.d	n.d
Ferulic Acid	n.d	n.d	n.d

Table 4.6: Amounts of phenolic compounds (in mg/100 g DW) found in Rotab date palm harvested from June to late September 2011 determined by HPLC.

	Rotab mg/100 g DW		
Standard	18/06/2011	24/07/2011	20/09/2011
Gallic Acid	n.d	0.24	n.d
p-Hydrobenzoic Acid	n.d	0.75	1.1
Vanilic Acid	0.25	0.37	0.43
Caffeic Acid	n.d	n.d	n.d
Syringic Acid	n.d	n.d	n.d
Ferulic Acid	n.d	n.d	n.d

Table 4.7: Amounts of phenolic compounds (in mg/100 g DW) found in Zahedi date palm harvested from June to late September 2011 determined by HPLC.

	Zahedi mg/100 g DW	
Standard	18/06/2011	24/07/2011
Gallic Acid	0.035	0.074
p-Hydrobenzoic Acid	0.026	n.d
Vanilic Acid	n.d	0.14
Caffeic Acid	n.d	n.d
Syringic Acid	n.d	0.035
Ferulic Acid	n.d	n.d

Table 4.8: Amounts of phenolic compounds (in mg/100 g DW) found in Barhi iraqi date palm harvested from June to late September 2011 determined by HPLC.

	Barhi Iraqi mg/100 g DW	
Standard	18/06/2011	24/07/2011
Gallic Acid	0.067	0.048
p-Hydrobenzoic Acid	0.51	1.2
Vanilic Acid	n.d	0.26
Caffeic Acid	0.056	n.d
Syringic Acid	n.d	0.20
Ferulic Acid	n.d	n.d

CHAPTER FIVE
GENERAL CONCLUSION AND RECOMMENDATIONS

The antioxidant activities, total phenolic contents and total flavonoid contents of seven Palestinian date palm fruits were determined and presented in this study. The antioxidant activity was measured using FRAP assay while total phenolic content and total flavonoid content of the date palm fruit were measured using Folin Ciocalteau and aluminum chloride colorimetric methods, respectively.

Our results showed that the date palm fruit in Jericho is rich in antioxidants, phenolics and flavonoids when compared to results in other countries such as Tunisia.

On the basis of our finding, we conclude that date palm fruit constitutes a natural source of potent antioxidants that may prevent many diseases and could potentially be used in food and pharmaceutical formulations. However, it is very interesting to accomplish this study by other interventions to know more about the different compounds (phenolic acids, flavonoids) responsible for the antioxidant activity and also to investigate the mechanism of their action in vitro and in vivo.

We recommend carrying more in depth study of the effect of processing time and methods used on the antioxidant constitution of date palm fruits particularly the most popular date palm Madjhool. We also recommend studying the contents of phenolics and flavonoids with respect to maturation stages of the fruit, which could be achieved through close cooperation with expert farmers in the Jericho area and with biologists working in this field. This follow up will be for the benefit of the Palestinian economy taking into consideration the huge investment that is being put in the area to concentrate on plantation of wide agricultural lands with date palms.

References

1. Eberhardt, M.V., Lee, C.Y. and Liu, R.H. (2000) Antioxidant activity of fresh apples, Nature, 405: 903-904.
2. Mayr, U., Treutter, D., Santo-Buelga, C., Bauer, H. and Feucht, W. (1996) Developmental changes in the phenol concentrations of Golden Delicious apple fruits and leaves, Phytochem., 38 (5): 1151-1155.
3. Van der Sluis, A.A., Dekker, M., Skrede, G. and Jongen, W.M.F. (2002) Activity and concentration of polyphenolic antioxidants in apple juice, 1. Effect of existing production methods, J. Agric. Food Chem., 50: 7211-7219.
4. Bushway, A.A., Hu, W. and Shupp, J.M. (2002) Quality characteristics of five disease resistant apple cultivars, J. Amer. Pom. Soc., 56(2): 94-105.
5. Bioactive compounds in plants- benefits and risk for man and animals (2008). [Online].[Accessed 27th May 2013]. Available from World Wide Web: <http://www.dnva.no/geomed>
6. Rieger, M. 2006. Introduction to Fruit Crops. NY: Haworth Press, Inc.
7. FAOSTAT. (2010). [online]. [Accessed 7th January 2011]. Available from World Wide Web:<http://faostat.fao.org/default.aspx>
8. George M. Naber. (2011). Effects of investment in "Medjool" dates on developing the Palestinian economy. MA, thesis, MICAD Bethlehem University, Palestine.
9. DAIQ, I. (2007). Date Palm Economics and Cultivation Circumstances in Palestine. In: A.Zaid et al, ISHS III International Date Palm Conference, 19-24 February, Abu Dhabi, UAE.
10. Al-Shahib,W., &Marshall,R. J. (2003).Fatty acid content of the seeds from 14 varieties of date palm Phoenix dactylifera L. International Journal of Food Science and Technology , 38, 709–712.
11. Al-Hooti, S., Sidhu, J.S. and Qabazard, H. (1998). Chemical composition of seeds of date fruit cultivars of United Arab Emirates. International Journal of Food Science and Technology. 35, 44–46.

12. Gamil-Abdel-Hafez, M., Fouad-Shalaby, A., & Akhal, I. (1980). Chemical composition of 15 varieties of dates grown in Saudi Arabia. Fourth Symposium on biological aspects of Saudi Arabia (pp. 89–91).
13. Anwar-Shinwari, M. (1987). Iron content of date fruits. Journal of the College of Science-King-Saud-University, 18(1), 5–13.
14. Ahmed, I.A., Ahmed, A.W.K. and Robinson, R.K.(1995). Chemical composition of date varieties as influenced by the stage repining. Food Chemistry. 54, 305–309.
15. Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., & Shahidi, F. (2005a). Compositional and sensory characteristics of three native sun-dried date (Phoenix dactylifera L.) varieties grown in Oman. Journal of Agricultural and Food Chemistry, 53, 7586–7591.
16. Antioxidant Activity By Aruna Prakash, PhD, Fred Rigelhof and Eugene MIller, PhD [online] (Accessed 5th March 2011). Available from World Wide Web: http://www.medlabs.com/downloads/antiox_acti_.pdf
17. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., et al.(1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26, 1231–1237.
18. Singh, V., Guizani, N., Essa, M.M., Hakkim, F.L., and 1Rahman, M.S. (2012). Comparative analysis of total phenolics, flavonoid content and antioxidant profile of different date varieties (Phoenix dactylifera L.) from Sultanate of Oman. International Food Research Journal,19, 1063-1070.
19. Reaction Mechanism of Folin–Ciocalteu reagent. [online](Accessed 16th September2011).Available from World Wide Web: http://pelagiaresearchlibrary.com/der-pharmacia-sinica/vol3-iss6/DPS-2012-3-6-680-684.pdf
20. Shahidi, F., P.K. Janitha and P.D. Wanasundara, 1992. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr., 32: 67-103.
21. Vayalil, P.K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (Phoenix dactylifera. L. Arecaceae).Journal of Agricultural and Food Chemistry. 50, 610–617.

22. Mansouri, A., Embarek, G., Kokkalou, E., & Kefalas, P. (2005). Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (Phoenix dactylifera). Food Chemistry, 89, 411–420.
23. Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M., &Al-Rawahy, F. (2007). Compositional and functional characteristics of dates, syrups, and their by-products. Food Chemistry, 104, 943–947.
24. Abdul Ameer A. Allaith.(2008). Antioxidant activity of Bahraini date palm (Phoenix dactylifera L.)Fruit of various cultivars. International Journal of Food Science and Technology. 43, 1033–1040.
25. Biglari, F., Al Karkhi, A.F.M. and Easa, A.M. (2008). Antioxidant activity and phenolic content of various date palm (Phoenix dactylifera) fruits from Iran. Food Chemistry 107: 1636–1641.
26. Saafi et al. (2009). Phenolic content and antioxidant activity of four date palm (Phoenix dactylifera L.) fruit varieties grown in Tunisia. International Journal of Food Science and Technolog, 44, 2314–2319
27. Ardekani.M. et al.,(2010) Comparison of Antioxidant Activity and Total Phenol Contents of some Date Seed Varieties from Iran. Iranian Journal of Pharmaceutical Research, 9 (2): 141-146.
28. Benzie, I.F.F. and Strain, J.J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods In Enzymology 299: 15–27.
29. Shui, G.,and Leong, L. P. (2006). Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. Food Chemistry. 97, 277–284.
30. Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16:144-158.
31. Kim, D.-O., Jeong, S. W., & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry, 81, 321–326.

Appendix A

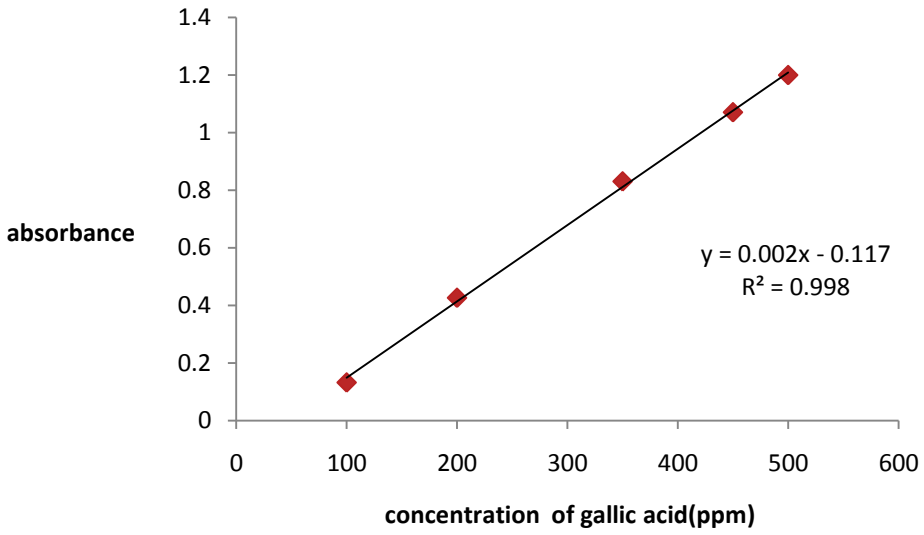
Absorbance results of AA, TPC and TFC assays

Variety Name	Date of harvesting	crude extract in ml	FRAP absorbance results	TPC absorbance results	TFC absorbance results
Medjool	18/6/2011	80	0.351	0.487	0.201
	24/7/2011	90	0.499	0.568	0.231
	4/9/2011	90	0.703	0.684	0.419
	20/9/2011	90	0.744	0.710	0.476
Ahmar balade	18/6/2011	50	1.197	0.835	0.456
	24/7/2011	50	0.994	0.772	0.404
	4/9/2011	60	0.693	0.562	0.368
	20/9/2011	60	0.563	0.429	0.348
Asfar balade	18/6/2011	130	1.663	0.841	0.429
	24/7/2011	85	0.904	0.248	0.321
	20/9/2011	90	0.825	0.189	0.124
Barhi balade	18/6/2011	85	0.263	0.179	0.084
	24/7/2011	80	0.298	0.181	0.095
	4/9/2011	55	0.413	0.568	0.226
	20/9/2011	50	0.536	0.745	0.344
Rotab	18/6/2011	55	0.133	0.342	0.193
	24/7/2011	55	0.196	0.481	0.281
	20/9/2011	55	0.455	0.803	0.363
Zahedi	18/6/2011	90	0.387	0.495	0.352
	24/7/2011	135	0.709	0.895	0.252
Barhi Iraqi	18/6/2011	80	0.332	0.396	0.102
	24/7/2011	75	0.428	0.539	0.135

Appendix B

Total Phenolic absorbance and calibration curve

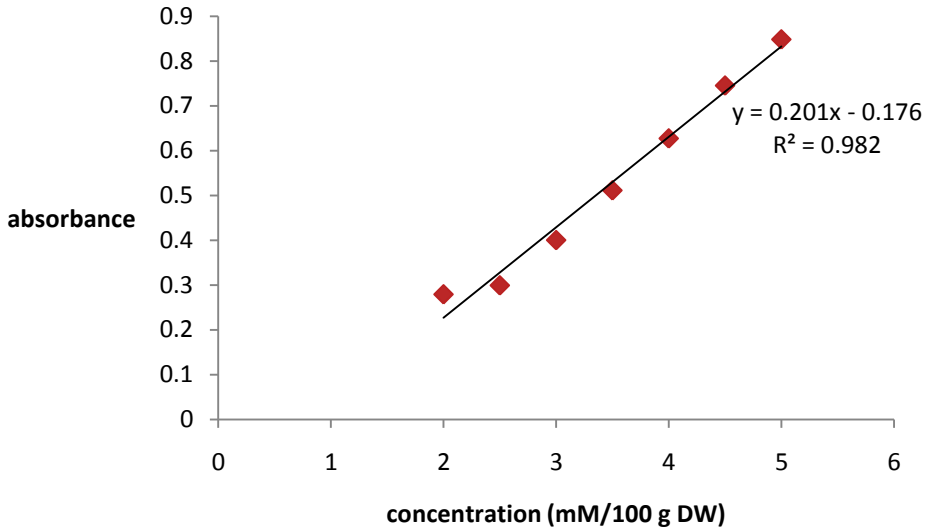
Concentration of gallic acid (ppm)	Absorbance (765nm)
100	0.132
200	0.426
350	0.830
450	1.070
500	1.199



Appendix C

FRAP absorbance (2 – 5 mM) FeSO4.7H2O) Fe (II) and calibration curve.

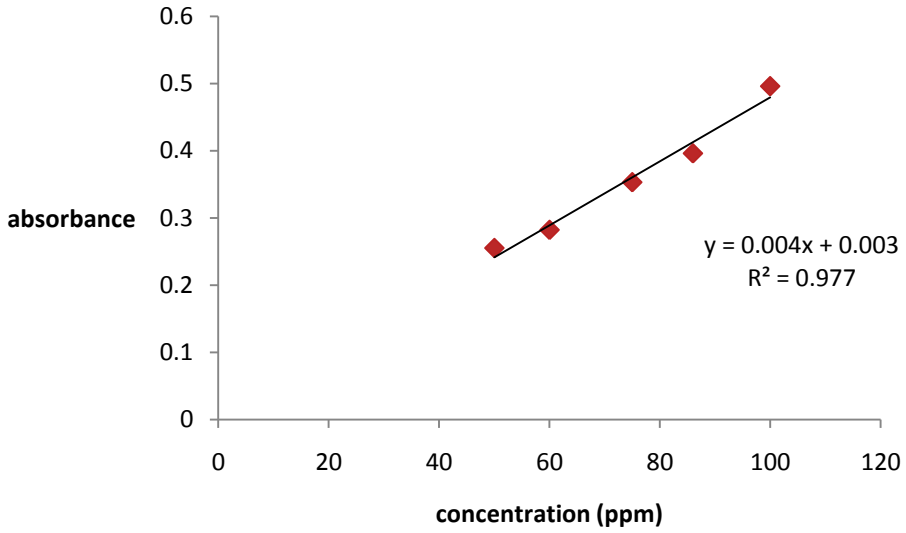
Concentration of Fe +3(mM)	Absorbance (593 nm)
2	0.279
2.5	0.299
3	0.400
3.5	0.511
4	0.627
4.5	0.745
5	0.848



Appendix D

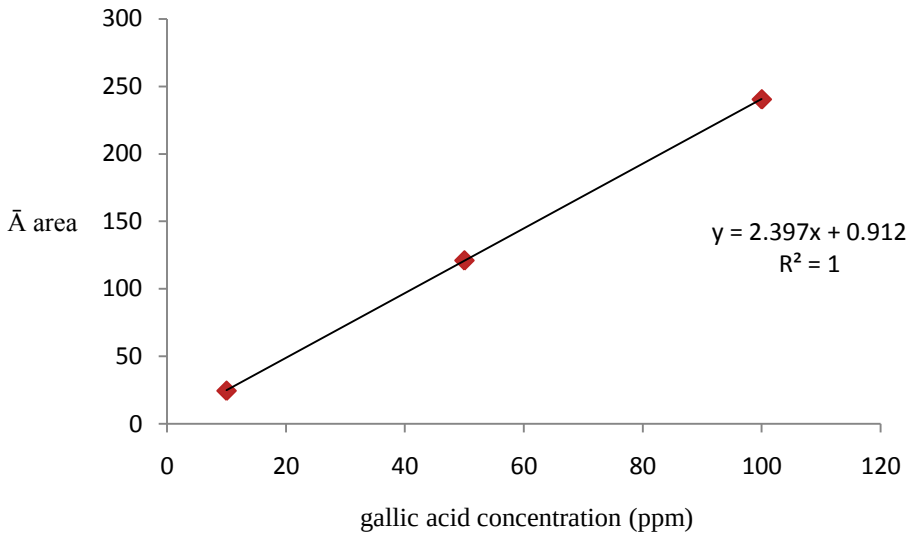
Total Flavonoid Content absorbance and calibration curve

Concentration of catechin (ppm)	Absorbance (510 nm)
50	0.255
60	0.282
75	0.353
86	0.396
100	0.496



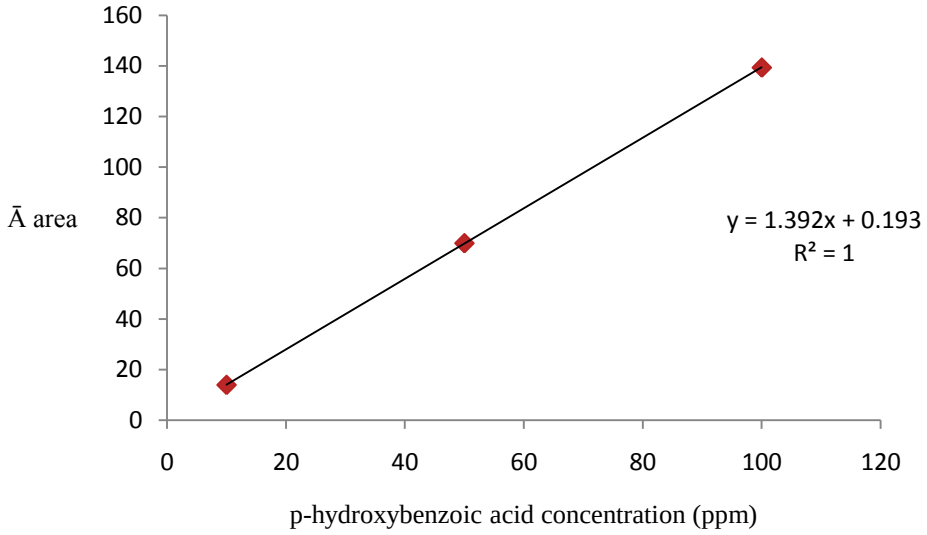
Appendix E:

Calibration curve for gallic acid (Area of gallic acid peak vs. Concentration in ppm).



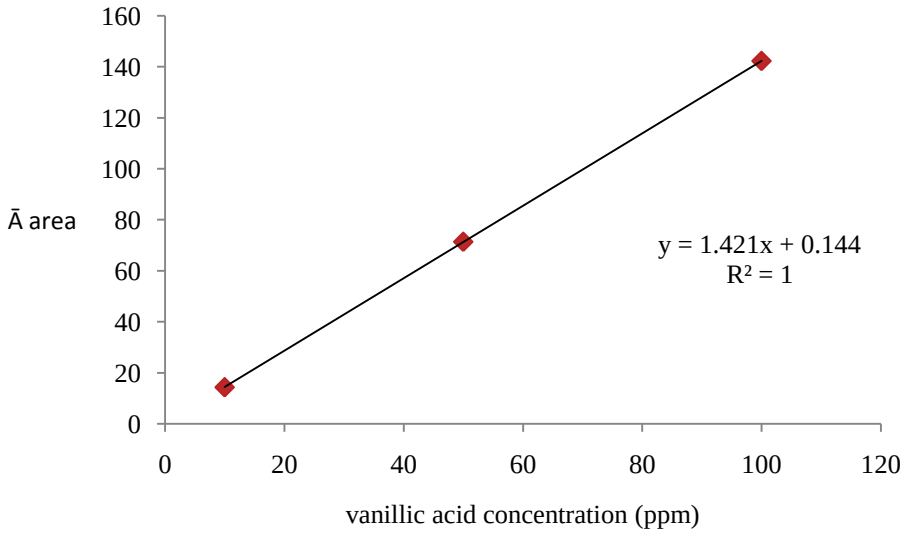
Appendix F:

Calibration curve for P- hydroxybenzoic acid (Area of P- hydroxybenzoic acid peak vs. Concentration in ppm).



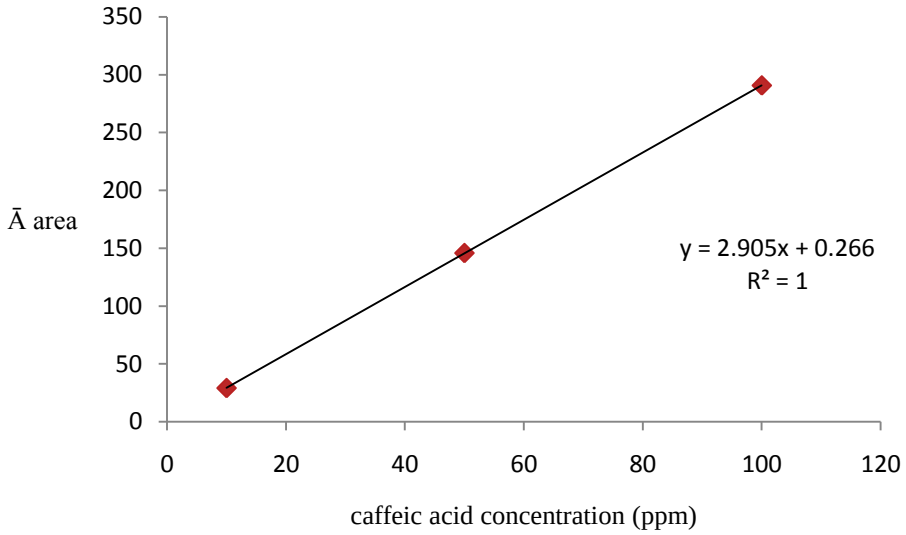
Appendix G:

Calibration curve for vanillic acid (Area of vanillic acid peak vs. Concentration in ppm).



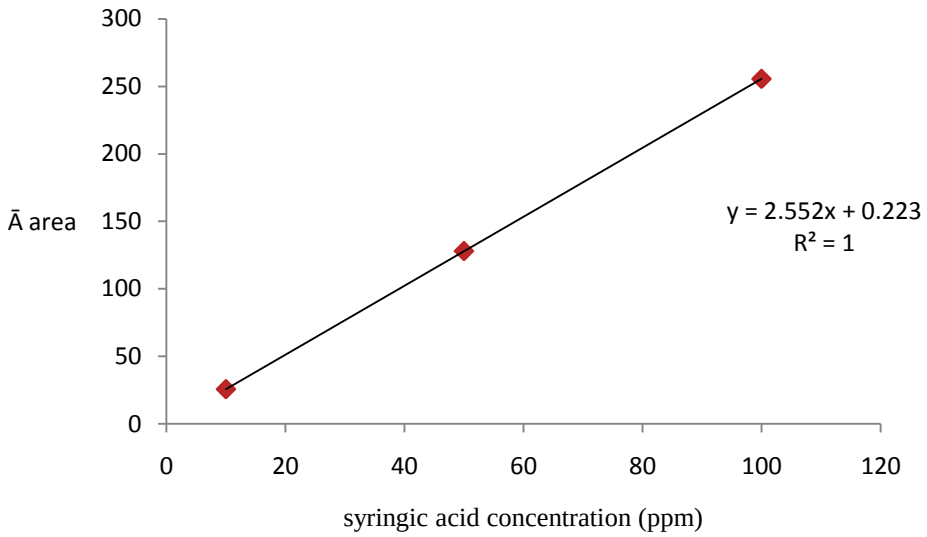
Appendix H:

Calibration curve for caffeic acid (Area of caffeic acid peak vs. Concentration in ppm).



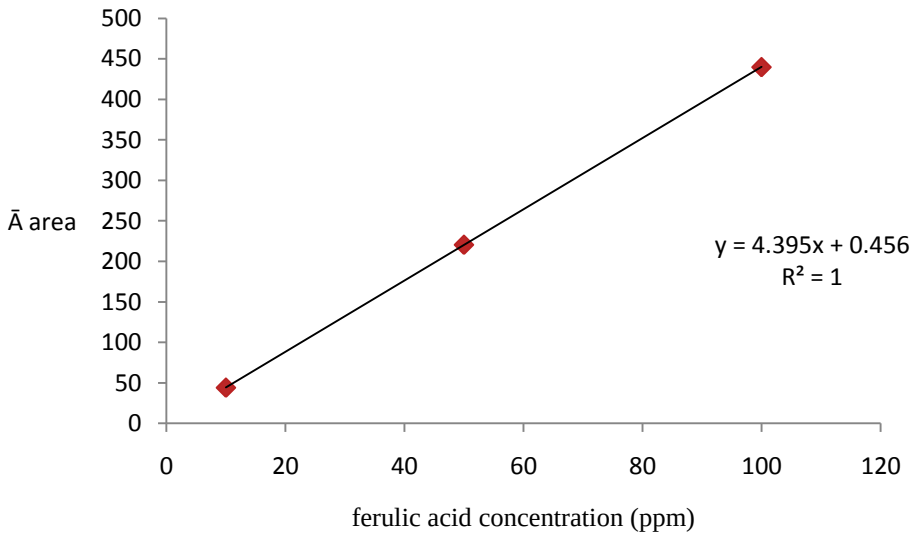
Appendix I:

Calibration curve for syringic acid (Area of syringic acid peak vs. Concentration in ppm).



Appendix J:

Calibration curve for ferulic acid (Area of ferulic acid peak vs. Concentration in ppm).



الملخص بالعربية

الهدف العام من هذه الدراسة هو تقييم محتوى مضادات الأكسدة الكلي في بعض أنواع فاكهة النخيل في الأراضي الفلسطينية باستخدام فحص FRAP وكذلك تحديد إجمالي محتوى الفينول وإجمالي محتوى الفلافونويد باستخدام فحصي (Folin –Ciocalteu and Aluminum Chloride colorimetric method) على التوالي. وقدم تم استخدام جهاز (HPLC) لتحديد تركيز بعض من مركبات الفينول.

تضمنت الدراسة تحليل سبعة أنواع مختلفة من فاكهة النخيل وهي مجهول، بلح احمر، بلح اصفر، بلح بلدي، رطب، زهيدي وبلح عراقي من منطقة عقبة جبر في مدينة أريحا وتم قطف العينات في شهر حزيران، شهر تموز، بداية شهر أيلول ونهاية شهر أيلول.

أظهرت النتائج أن تاريخ الحصاد ونوع فاكهة النخيل قد ساهمت كثيرا في إجمالي محتوى الفينول ، وإجمالي محتوى الفلافونويد، ومضادات الأكسدة. خلال تاريخ الحصاد (من حزيران إلى أيلول 2011)، إجمالي محتوى الفينول تراوح بين 18.72 حتي 38.75، 40.80 حتي 65.70، 42.40–231.40، 32.30 حتي 52.24، 13.75 حتي 31.46، 22.68 حتي 55.76 و 35.92 حتي 40.88 (mg GAE/100g DW) للمجهول، البلح الأحمر ، البلح الأصفر ، البلح البلدي ، الرطب، الزهيدي والبلح العراقي، على التوالي.

وفيما يتعلق بإجمالي محتوى الفلافونويد، وتراوحت النتائج بين 4.46–9.46، 5.01–5.66، 3.93 حتي 9.6، 1.72–4.26، 2.61–4.95، 7.85–8.40، و1.98–2.48 (mg CEQ/100g DW) للمجهول، البلح الأحمر ، البلح الأصفر ، البلح البلدي ، الرطب، الزهيدي والبلح العراقي، على التوالي.

وكانت نتائج مضادات الأكسدة تتراوح بين 263.9 حتي 396.7، 181.0 حتي 251.0، 163.0 حتي 658.0، 142.0 حتي 229.0، 142.0 حتي 268.0، 300.4 حتي 719.3، و228– 268 (µmol/100g DW) للمجهول، البلح الأحمر، البلح الأصفر ، البلح البلدي ، الرطب، الزهيدي والبلح العراقي، على التوالي.

وجد أن مرحلة النضج لها تأثير على إجمالي محتوى الفينول وإجمالي محتوى الفلافونويد وكذلك على نشاط مضادات الأكسدة. أشارت النتائج إلى أن إجمالي محتوى الفينول ، و الفلافونويد، ونشاط مضادات الأكسدة لفاكهة النخيل في تزايد خلال مراحل النضج في البلح البلدي، الزهيدي، البلح العراقي، المجهول والرطب ، بينما تتناقص ف البلح الحمر والبلح الأصفر

كما أشارت التحليلات بوجود علاقة خطية بين مضادات الأكسدة ومحتوى الفينول الكلي ، وبين مضادات الأكسدة ومحتوى الفلافانويد الكلي لجميع أصناف النخيل التي استخدمت في هذه الدراسة مع معامل ارتباط 0.98 (بالنسبة للعلاقة بين مضادات الأكسدة و محتوى الفينول الكلي) و0.90 (بالنسبة للعلاقة بين مضادات الأكسدة ومحتوى االفلافانويد الكلي).

ونتوقع أن يستفيد المزارعين من النتائج خاصة في اختيار ثمار النخيل ذات المحتوى العالي من المركبات النشطة بيولوجيا كما يمكن أيضا أن تستخدم هذه المركبات في تطبيقات التكنولوجيا الحيوية.