DISSERTATION

Chemical composition and Antioxidant Activity of Date Palm Fruit (PHOENIX DACTYLIFERA) in Saudi Arabia

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Chemical composition and Antioxidant Activity of Date Palm Fruit (*PHOENIX DACTYLIFERA*) in Saudi Arabia

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Abstract

Dates are the fruit of the palm, which the Greeks call “Phoenix”, a word taken from the Phoenician language. Saudi Arabia is currently one of the largest dates producing countries in the world. There are several varieties of dates grown in Saudi Arabia.

Date fruits contain compounds that are potentially bioactive, with many health benefits; for example, vitamin E, carotene (precursor for vitamin A) and phenolic compounds. Dates represent an excellent source of antioxidants due to their high concentrations of phenolic compounds as well as the presence of selenoproteins. Moreover, dates are also potentially a very good source of several minerals in fact; there are at least 15 minerals found in dates.

The work presented in this thesis will determine the nutritional composition of nineteen varieties of dates sourced from four, environmentally diverse, regions in Saudi Arabia. These varieties were selected because of their popularity, economical price, as well as availability during the year. The results indicated that the range of moisture contents (10% - 30%) found within the four regions were quite similar. For levels of fat, protein and ash all varied significantly between varieties but were all very low. Carbohydrate content was variable between samples (70 - 80%). The results indicated that glucose and fructose concentrations in theses Saudi dates were generally similar and the present of sucrose being normally associated with a corresponding reduction in the level of glucose and fructose. Potassium was the major mineral found in all the varieties with concentrations as high as 1173.29 mg/100g. The mean values for phosphorus in the dates from the different regions were close to each other. The amount of selenium was generally very low in all varieties and some did not contain any selenium at all. It would appear that it is variety and not region of production that has the major impact on nutritional composition.

Moreover, this research will also determine the antioxidant capacity and phenolic content of a sub-set of these varieties at four different stages of ripening. Results showed that the levels of phenolics, anthocyanin and antioxidant capacity all decreased throughout development in all the seven selected varieties. There were strong correlations between this antioxidant capacity and the total phenolic and anthocyanin levels suggesting that these are major contributors to this nutritional property of dates.

A preliminary screen tentatively identified some phenolic compounds and indicates that there may be some compositional variation between date varieties.

Keywords: chemical composition; Antioxidant; date palm; Saudi Arabia

Declaration

I hereby declare that this thesis has been genuinely carried out by myself and has not been used in any previous application for a degree. The invaluable participation of others in this thesis has been acknowledged where appropriate.

Dedication

To my Parents, my wife and my lovely children

Acknowledgements

In the name of Allah, the Most Gracious and the Most Merciful. Alhamdulillah, all praises to Allah, the Almighty, for the strengths and His blessing in completing this thesis.

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Greatest thanks are given to my parents who have done so much for me. Though, thousands of kilometres away, they never stopped their love, prayers and support. And special thanks to my brothers and sisters for their generous support. To those who indirectly contributed in this research, your kindness means a lot to me. Thank you very much.

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Abbreviations	Symbols Caption
AA	Antioxidant activity
AAPH	[2, 2'-azobis (2-amidinopropane) hydrochloride]
ABTS	2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt
AE	antiradical efficiency
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists' methods
ATP	Adenosine triphosphate
AUC	Area under the curve
C6-C3-C6	Flavonoids carbon structure
CaCO3	Calcium carbonate
DFC	Date fibre concentrates
DNA	Deoxyribonucleic acid
DPPH	(diphenyl-2-picrylhydrazyl) radicals
DPRC	Date Palm Research Center
DW	Dry weight
FAE	Ferulic acid equivalents
FAO	Food & Agriculture Organisation
FC	Reagent Folin–Ciocalteu reagent
FL	fluorescein
FRAP	Ferric-reducing antioxidant power
FW	Fresh weight
GAE	Gallic acid equivalents
GI	glycemic index
GPx	glutathione peroxidase
H2SO4	Sulfuric acid
HCl	Hydrochloric acid
HPAEC	High performance anion exchange chromatography
HPLC	High performance liquid chromatography
ICP	inductively coupled plasma,
IU	International Unit
K4SO4	Potassium sulfate

LDL	Low-Density Lipoproteins
Mg	milligramme
min	minute
ml	millilitre
mM	millimolar
NADP	D-glucose 1-dehydrogenase
NaOH	Sodium hydroxide
NaOH	Sodium hydroxide
NH3	Ammonia
NH4OH	Ammonium hydroxide
NSP	non-starch polysaccharides
OHC	oil-holding capacity
ORAC	Oxygen radical absorbent capacity
ORACFL	lipophilic and hydrophilic
PON1	Serum paraoxonase 1
RID	refractive index detector
ROS	reactive oxygen species
RP-HPLC	reverse phase-HPLC
SD	Soft dates
SODs	superoxide dismutases
Std	Standard deviation
TE	Trolox equivalent
TEAC	Trolox equivalent capacity
TPC	Total phenolic content
TPTZ	Tripydyltriazine
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
WHC	water-holding capacity
WHO	World Health Organization
°C	degree celsius
1D	one dimensional

Chapter 1 Introduction

1.1 Background and Rationale

Fruits and vegetables contain compounds that are potentially bioactive, with many health benefits; for example, the vitamins C and E, carotene (precursor for vitamin A) and phenolic compounds. Vitamin C is a water-soluble vitamin which acts as an antioxidant to remove free radicals from the plasma of blood, before they attack the low-density lipoproteins and the cell membrane. Vitamin E is a fat-soluble vitamin which also acts as an antioxidant protecting lipids from oxidation (Brigelius, 1999). This is why vitamins C and E are important in the prevention of diseases of the heart and arteries, and of cancer (Rossig et al., 2001). Beta-carotene also acts as a fat soluble antioxidant and has also been implicated in the prevention of diseases of the heart and arteries, and of cancer (Devasagayam et al., 2004). Date fruit represent an excellent source of antioxidants due to their high concentrations of phenolic compounds, including flavonoids and anthocyanidins, as well as the presence of selenoproteins. Moreover, dates are also potentially a very good source of several minerals in fact; there are at least 15 minerals in dates. The percentage of each mineral in dates varies from 0.1 to 916 mg/100 g date, depending on the type of mineral. In addition, dates contain at least six vitamins including vitamin C, vitamin B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A (Al-Shahib et al., 2003). Also dates contain protein that includes 23 types of amino acids. Therefore, they could be an important source of carbohydrates and fibre and energy.

Dates are the fruit of the palm, which the Greeks call “Phoenix”, a word taken from the Phoenician language. Saudi Arabia is currently one of the largest dates producing countries in the world. There are several varieties of dates grown in Saudi Arabia. These in turn are grown in a number of areas with diverse environmental and soil conditions and can be consumed at a number of stages of development. It is thus very important to consider the locally available dates and to determine their nutrient composition for the purpose of increasing the production of such fruits that may be particularly beneficial.

Numerous studies have been conducted to study the benefits of the date fruit and to examine their chemical composition. The impact of environment and stage of development in particular have not been extensively researched previously.

The work presented in this thesis will therefore determine the nutritional composition of nineteen varieties of dates sourced from four, environmentally diverse, regions in Saudi Arabia. These varieties are well known because of their common preference, popularity, economic price, as well as the high availability during the year.

A potential nutritional benefit of dates may lie in their antioxidant capacity and consequent ability to quench free radicals. Free radicals are molecules that are very unstable; therefore, they look to bond with other molecules, often modifying their structure and perpetuating the detrimental process. Free radicals are responsible for aging, tissue damage, and possibly some diseases such as cancer (Halliwell, 1994). The human body can be exposed to free radicals from both external and internal sources. External sources include environmental pollution, solar ultraviolet radiation, the photo-dissociation of ozone that gives the excited oxygen atom O (1D), and poisons like industrial cleaners.

Epidemiological studies have confirmed the existence of an inverse relationship directly between eating at least five servings of fruits and vegetables a day and low incidence of chronic diseases such as heart disease (Bazzano et al., 2002; DH, 1994) and some types of cancers: especially colon, rectum and stomach cancers (Steinmetz & Potter, 1996; DH, 1998). The studies reported that rich fruits and vegetables were high in antioxidants such as vitamins E, C Ascorbic acid, Carotenoids and minerals Selenium and Zinc and Flavonoids such as Procyanidin & Catechin (Ness & Powles, 1997).

Fruit and vegetables have attracted several researchers to carry out studies on free radicals (Bagchi et al., 2000) and it was found that the extract of grape seeds, which contains a compound called Proanthocyanidin, has the ability to counter the activity of free oxygen by 84% compared to vitamin E, and by 439% compared to vitamin C. Also, (Moreno et al, 2000) concluded that the phenolic compounds found in fruit give better results as antioxidants for the protection of Low-Density Lipoproteins (LDL) than conventional vitamins such as vitamin E and C. On the other hand, this study stated that a water extract from dates had the ability to

significantly counter fat oxidation at a concentration of 2 mg/ml, but there was no assessment of phenolic compounds in the study (Vayalil, 2002). Moreover, in a study in Oman, researchers had been monitoring the Procyanidin composition in dates, but did not measure their activity as an antioxidant (Al-Farsi et al, 2005).

Based on the results of these studies, it is necessary to estimate the phenolic compounds in selected varieties of dates and assess their antioxidant activity. Moreover, this research will also determine the antioxidant capacity of a sub-set of these varieties at four different stages of ripening.

Several different methods are available and have been used to assess the total antioxidant capacity of plant extracts, such as the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). In this thesis the total antioxidant capacities of dates were evaluated using FRAP assay. In addition, the total phenolic contents of dates samples were estimated using the classical Folin–Ciocalteu reagent in this thesis. A preliminary screen to determine if there were differences in the phenolic compounds associated with varieties and stage of development was also carried out. Moreover, Anthocyanin levels were also determined to observe whether antioxidant activity of dates can be attributed to phenolic compounds.

1.2 Hypotheses and research questions

The specific hypotheses and objectives addressed by this thesis include:

1. **Nutritional composition has been shown to vary between date varieties this will be true for those grown in Saudi Arabia.**

   Objective: Data on date varieties grown in Saudi Arabia is relatively sparse. Thus this thesis will carry out a screen of nineteen of the major varieties grown in Saudi Arabia.

2. **Environmental conditions may impact on nutritional composition of dates.**

   Objective: A comparison between date varieties grown in four environmentally diverse regions of Saudi Arabia will be compared.

3. **Antioxidant activity (AA) and total phenolic content (TPC) will also vary in dates**

Objective: this has been relatively poorly investigated in the past. These two parameters will be investigated in the Saudi Arabian date to determine if there is any relationship between total phenolic content (TPC) and antioxidant activity (AA).

4. **Physiological stage of maturity of the dates may influence their nutritional composition.**
   Objective: Dates will be assessed at four stages of development for their antioxidant and phenolic composition.

1.3 Thesis Outline

In this thesis, the following outline is presented:

Chapter 1: presents a general introduction about date fruit, necessity of study on antioxidant activity in dates.

Chapter 2: includes the general introduction of the history of date fruits focuses on date production in the world and in Saudi Arabia, the stages of date ripening and the storage of dates. Also this chapter reviews some of the literature about the chemical composition of dates and presents the definitions of free radicals and antioxidants in general, followed by a review of antioxidant activity in date fruit.

Chapter 3: lists all the used materials as well as the methodology of all assays throughout the thesis. Also, this chapter illustrates statistical analysis that was used for analysis of raw data.

Chapter 4: presents date variety selection and characteristics and proximate composition analysis. Moreover, this chapter shows sugar and mineral composition of date varieties and total phenolic content (TPC) and antioxidant capacity of date varieties. The chapter illustrates comparison of same date varieties from different regions. Finally, follow up study to investigate variation in sugar, FRAP and TPC content of date varieties is presented.

Chapter 5: assesses the impact of stage of development on the nutritional composition of date fruit. This chapter consists of four subsections: first, the result of total phenolic content (TPC). Second, antioxidant activity (AA). Third, a preliminary screen to profile compounds using HPLC. Fourth, anthocyanin content.

Chapter 6: presents general discussions of results. Discussion of new assays are presented.

Chapter 7: consists of general conclusions and recommendation for future studies.

Chapter 2 Literature Review

2.1 Introduction

Fruits and vegetables contain compounds that are potentially bioactive, with many health benefits; for example, the vitamins C and E, carotene (precursor for vitamin A) and phenolic compounds. Vitamin C is a water-soluble vitamin which acts as an antioxidant to remove free radicals from the plasma of blood, before they attack the low-density lipoproteins and the cell membrane. Vitamin E is a fat-soluble vitamin which also acts as an antioxidant protecting lipids from oxidation (Jailal and Grundy, 1992). This is why vitamins C and E are important in the prevention of diseases of the heart and arteries, and of cancer (Basu et al., 1999). Beta-carotene also acts as a fat soluble antioxidant and has also been implicated in the prevention of diseases of the heart and arteries, and of cancer (Basu et al., 1999). Date fruit represent an excellent source of antioxidants due to their high concentrations of phenolic compounds, including flavonoids and anthocyanidins, as well as the presence of selenoproteins. Moreover, dates are also potentially a very good source of several minerals, vitamins, carbohydrates and fibre. Therefore, they could be an important source of these micronutrients and energy.

Dates are the fruit of the palm, which the Greeks call “Phoenix”, a word taken from the Phoenician language. Saudi Arabia is currently one of the largest dates producing countries in the world. There are several varieties of dates grown in Saudi Arabia. These in turn are grown in a number of areas with diverse environmental and soil conditions and can be consumed at a number of stages of development. It is thus very important to consider the locally available dates and to determine their nutrient composition for the purpose of increasing the production of such fruits that may be particularly beneficial.

Numerous studies have been conducted to study the benefits of the date fruit and to examine their chemical composition; this chapter aims to summarise research into the chemical composition of dates. The definitions of free radicals and antioxidants are presented in general in the second part, followed by a review of antioxidant activity in date fruit.

2.2 Description of date palm

This section represents the main parts of date palm tree:

1- *Roots*: date palm roots start from a ball shaped base of the trunk. It has no tap root. There are four zones in the root system (Figure. 2.1). In the first zone root grows from the upper part of trunk base. In the second zone or intense root zone the heavy root branches with rootlets spreading into the ground to absorb nutritive substances and moisture. In the third zone roots are from 1 to 2 m underground depending on the availability of nutritional substances in the higher zones. In the fourth zone roots are at least 2 m underground and might extend deeper than 6 m depending on availability of water. Generally, roots can be found as much as 25 m from the trunk and deeper than 6 m; however, 85 % of the roots are spread to 2 m deep and to 2 m around depending on type of culture, soil characteristics, depth of the underground water and cultivar.

2- *Trunk*: is the same all the way up and it does not have more than once canopy of leaves that has been fully developed without any branches (Figure. 2.1). It reaches 10 m to 30 m tall subject to the cultivar and it reaches 1 m in diameter. The cambium disappears causing a constant and uniform trunk width during the palm's entire life.

3- *Fronds*: leaves reaches 3 m to 7 m long and have a long life of 3 to 7 years' subject to cultivar, age of a palm and environmental conditions. There are about 100 green fronds on a mature date palm with one or two new fronds produced per month.

4- *Flowers*: From frond axils of the previous year's growth the inflorescences are produced. Theses inflorescences are enclosed in a hard covering known as a spathe which splits longitudinal when the flowers mature exposing the inflorescence (Figure 2.1). At the beginning the spathe is greenish then converts brown by splitting. The number of spathes at each date palm is around 25 in females that more than in males. The male inflorescence is overcrowded, shorter and wider than the female. From these characteristics the inflorescence's sex is recognized of before its opening (Figure. 2.1).

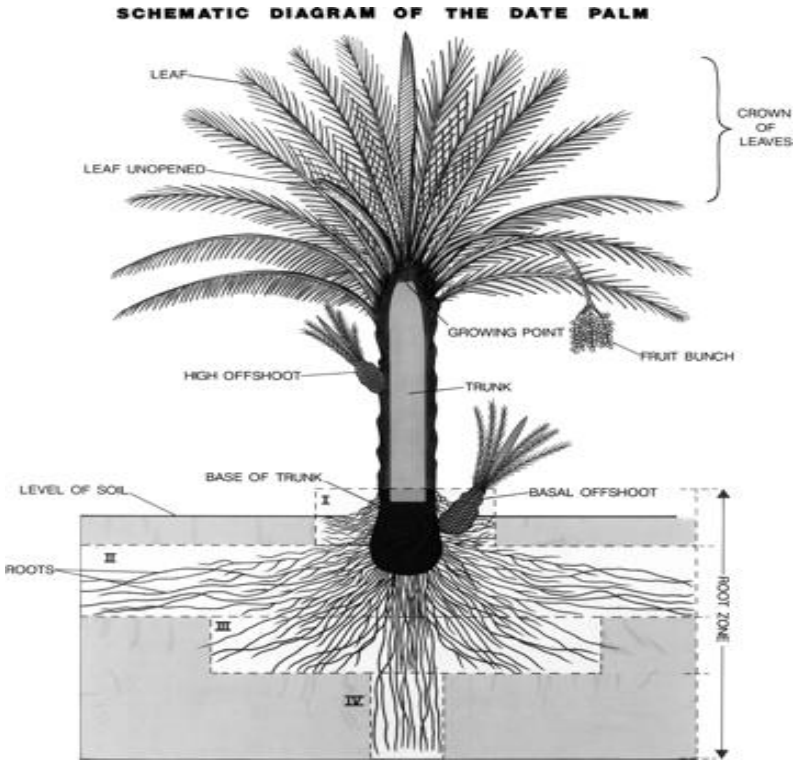


Figure 2.1: Diagrammatic representation of date palm structure, showing attachment of offshoot to mother palm, among other morphological features. (USDA archival diagram, (Chao and Krueger, 2007)

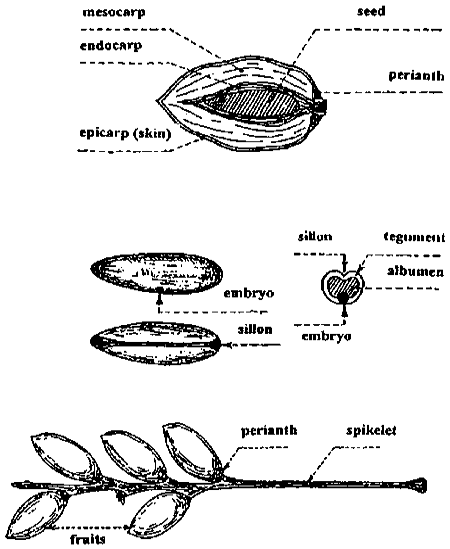


Figure 2.2: Morphology and anatomy of date palm fruit and pit (Zaid and de Wet, 2002a)

The fruit of date palm is fleshy fruit with thin skin and a central stone containing

the seed (Figure 2.2). Fruits are different in size, shape, color and quality depending on the cultivar, culture and environmental conditions (Rieger, 2005; Zaid and de Wet, 2002a). Immature fruits have green, yellow, or red color, and when they mature range from red to yellow, depending on cultivar. After pollination, fruit grows from one of the three carpels within each pistillate flower.

2.3 Environmental characteristics of Date palm

Date palm needs deep sandy loam soils to growing. Though, they can be grown in a wide range of other soil types. Date palm can grow in alkaline and saline soils but its productivity will greatly reduce (Barreveld, 1993; Pareek, 1985; Rieger, 2005). Common climate for date palm growing is normal winter temperature and long hot summer with little rain, but with rich underground water near the surface for irrigation. The best temperature during the period from pollination to fruit ripening is between 25-30°C average temperatures depending on cultivar.

2.4 History of date fruit (Phoenix dactylifera L.)

Palm trees were initially planted by the Phoenicians and later transferred to the Mediterranean. However, dates were known long before that: some historians say that the origin of the palm was in Babylon, while others have said that Harcan, an island in Bahrain, was its original home and that it was later transferred to Babylon (The Ministry of Agriculture, 2005). The palm occupied a privileged position for the ancient peoples of the earth. It was called the tree of life, and excavations carried out in the tombs of the Pharaohs indicate their great appreciation for palm trees.

2.5 Date production

2.5.1 Production worldwide

Based on the FAO report in 2014, the main date-producing countries in the world are Egypt, Iran, Saudi Arabia, United Arab Emirates, Pakistan, Algeria, Iraq, Sudan, China and Libya. Table 2.1 shows the main date-producing countries arranged according to the amount of production in 2014.

Table 2.1: Top ten date producers – 2014 (1000 short tons)

Egypt	1,227	United Arab Emirates	588
Iran	1,056	Algeria	570
Saudi Arabia	934	Sudan	482
Iraq	747	South Sudan	330
Pakistan	612	Oman	280
Source: UN Food & Agriculture Organisation (FAO)			

2.5.2 Date production in Saudi Arabia

Saudi Arabia is one of the largest date producing countries in the world. The date's ability to tolerate arid environmental conditions, combined with environmental conditions (hot and dry regions), makes Saudi Arabia quite unique for date cultivation (Ibrahim and Khalif, 1998 and Alkhateeb and Ali-Dinar, 2002). The total land area of Saudi Arabia amounts to 2.15 million square kilometers, out of which 52,684 million hectares represent the arable land that could be utilized for agriculture. Presently, only 4.19 million hectares are cultivated with various agricultural crops. Around 193,000 hectares are cultivated with perennial crops (fruits), where date palms represent 142,000 hectares of this area, i.e. 73.6% of the total cultivated area of perennial crops, equivalent to a commercial value of 1.3 billion pounds. Rangeland area is estimated to be 170 million hectares, and forest area 2.7 million hectares (The Ministry of Agriculture, 2005). When ripe, the fruit may be yellow to reddish-brown in colour (Figure 2.3). Dates are found in clusters and each bunch may weigh about 10 kg. A fully productive palm can support up to ten bunches and yield about 100 kg of fruit (Zaid, 1999).

Figure 2.3: Mature Date fruit

2.6 Stages of date ripening

Date palm fruits pass through five stages of development to reach full maturity. The whole process is lengthy and takes approximately seven months. The texture and sweetness of the date is closely related to its stage of maturity and ripeness (Zaid, 1999). Several external and internal changes are observed in colour and chemical composition during the growth and development of the dates. Based on the Arabic practice, date development can be classified into five stages: Hababouk, Kimri, Khalal, Rutab and Tamar – and the same terms have been internationally accepted (Al-Shahib and Marshall, 2003; Fadel et al., 2006; Zaid, 1999).

Hababouk stage

This is the first stage and lasts for four to five weeks post fertilization. The fruits are small and completely covered by the calyx, leaving only one sharp end of the ovary visible. The fruit in this stage is pea-sized and weighs about a gram (Ahmed et al., 1995; Al Noimi and Al-Amir, 1980; Zaid, 1999; Al-Shahib and Marshall, 2003; Fadel et al., 2006) (Figure 2.4).

Kimri stage (also called green stage)

This stage is the longest and lasts for a total of nine to fourteen weeks. In this stage, the fruit is rectangular in shape, quite hard and becomes green and has a bitter taste (Ahmed et al., 1995; Al Noimi and Al-Amir, 1980; Al-Shahib and Marshall, 2003; Fadel et al., 2006; Zaid, 1999) (Figure 2.4).

Khalal stage (full-size, crunchy)

In this stage, the color changes from green to greenish-yellow, yellow, pink, scarlet or red depending on the variety. This stage lasts for six weeks at the end of which the fruit is physiologically mature, hard and ripe. The fruit reaches its maximum weight and size at the end of this stage. A rapid increase in sucrose content accompanies this stage following a decrease in the water content (around 50-85% moisture content) (Ahmed et al., 1995; Al Noimi and Al-Amir, 1980; Fadel et al., 2006; Zaid, 1999) (Figure 2.4).

Rutab stage (Ripe, Soft)

This stage lasts between two to four weeks. The apex starts to ripen and the texture of the fruit becomes soft and translucent. The astringency from the previous stage is regularly lost and the fruit starts acquiring a brown or black colour. Due to the continuing loss of moisture the weight further decreases. There is a gain in total sugars and solids with concomitant increase in the rate of conversion of sucrose to simpler sugars (Ahmed et al., 1995; Al Noimi and Al-Amir, 1980; Fadel et al., 2006; Zaid, 1999) (Figure 2.4).

Tamar stage (ripe sun-dried or final stage in the ripening)

This is the last stage of ripening and the date appears dry. The semi-dry and dry dates will have nearly 50% each of sucrose and reducing sugars. The percentage of sugar to water is sufficient to prevent fermentation of these sugars.

Within a bunch, the fruits ripen over a month and not all at the same time. In most varieties, the skin adheres to the soft flesh and wrinkles as the inner flesh shrinks. The colour of the fruit darkens with time (Ahmed et al., 1995; Al Noimi and Al-Amir, 1980; Fadel et al., 2006; Zaid, 1999) (Figure 2.4).

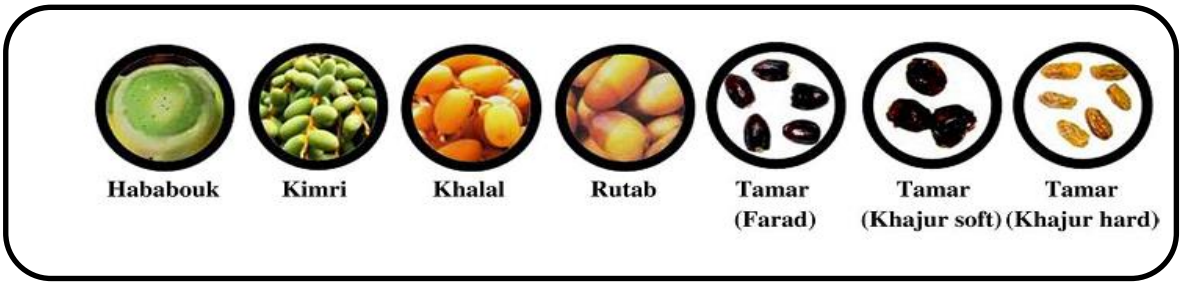


Figure 2.4: Different stages of date ripening (Baliga, M.S., et al., 2010)

Khalal (Fresh), Rutab (Semi-fresh), tamar (Semi-dry) and tamar (dry) are all suitable for human consumption.

Several studies have described the physical and chemical development of dates as they go through these various stages (Sawaya et al., 1982; 1983; Mustafa et al., 1986; Siddiqui and Gupta, 1994; El-Zoghbi, 1994; Ahmed and Ahmed, 1995; Al-Hooti et al., 1997; Myhara et al., 1999; Al-Shahib and Marshall, 2003).

Date fruits are collected and marketed at three stages of their development (Khalal, Rutab and Tamar) depending on cultivar, climatic conditions and market demand. Date collected usually starts from middle of August until October for late cultivars. Date fruits can be used for human consumption in every stage of fruit development (Sidhu, 2006) unlike other fruits.

2.7 Varieties of dates grown in Saudi Arabia

There are several varieties of dates grown in the kingdom of Saudi Arabia that are classified depending on the area of cultivation. A description of these dates and at what stage of development people prefer to consume them is given in Appendix 1.

2.8 Storage of dates

There are four main stages in the storage of dates:

1-The dates are delivered to a huge, open-air processing area where teams of men wash them to remove dust.

2- The dates are sun-dried for about 10 days.

3-After drying, each date is carefully sorted by hand and weighed into plastic bags containing about five kilograms of dates.

4-During processing, the packaged dates are stacked on pallets and then pressed with concrete-block weights for at least one month.

After that the dates are released into the market according to demand. After being released into the market, the dates are expected to have a shelf life of up to two years at room temperature (<25 °C).

Different factors can affect date quality, such as temperature and water activity. The content of antioxidant compounds (phenolics and flavonoids) of dates can increase in amount after the storage stage.

2.9 Dates' uses

Date wine was used as an alcoholic drink for pleasure, nutrition, medicine, ritual, remuneration, and funerary purposes (Cherrington, 1925). Ancient Egyptians were employed date fruit or its juice in several medicinal remedies. In some Arabic countries, dates vinegar is produced, and date juice known as `dibbis' or syrup "honey date" are made from the date fruit pulp (Pareek, 1985).

Recent innovations include chocolate covered date fruits and products such as sparkling date juice, used in some Islamic countries for special religious events such as Ramadan (Al-Hooti et al., 1997). Dry and soft date fruits may be pitted and stuffed with fillings such as almonds.

Date pits have been included in animal feed to enhance growth, an action that has been ascribed to an increase in the plasma level of oestrogens 'or testosterone' (Bahmanpour et al., 2006).

Treatment with aqueous extract of date flesh or pits significantly reduced ill effects and suggested that the induced liver damage can be improved by treatment of extracts from date flesh or pits (Al-Qarawi et al., 2004; Bahmanpour et al., 2006).

Date may soon play a larger role than just an international snack and could be potentially one of the best foods for the future (Al-Shahib and Marshall, 2003; Miller et al., 2003; Platkin, 2007).

2.10 Chemical composition of dates

The general composition of data fruit is shown in table 2.2. Date pulps generally contain easily digestible sugars (70%) – mainly glucose, sucrose and fructose – dietary fibers and low levels of proteins and fats (Al Farsi and Lee, 2008). They also contain several vitamins, such as riboflavin, thiamine, biotin, folic and ascorbic acid (vitamin C), which are essential for the body (Al Farsi and Lee, 2008). The pulp is also rich in minerals such as iron, calcium, cobalt, copper, fluorine, magnesium, manganese, potassium, phosphorus, sodium, copper, sulfur, boron, selenium and zinc (Al Farsi and Lee, 2008; Ali Mohamed and Khamis, 2004). Consumption of one hundred grams of dates can provide over 15% of the suggested daily allowance for selenium, copper, potassium and magnesium (Al Farsi and Lee, 2008). In several varieties, potassium can

be found at a concentration as high as 0.9% in the flesh, while it is as high as 0.5% in some seeds. Dates contain fundamental fluorine that is useful in protecting teeth against decay (Al Farsi et al., 2005; Al Farsi and Lee, 2008; Ali Mohamed and Khamis, 2004).

When compared to the flesh, date seeds contain higher amounts of protein and fat and are also high in dietary fiber (Al Farsi and Lee, 2008). Furthermore, the seeds also have aluminum, cadmium, chloride, lead and sulphur in various proportions. Recently, the nutritional quality (dietary fiber, proximate analysis and micronutrients) of eighteen leading varieties of date pits – Khalas, Barhe, Lulu, Shikat alkahlas, Sokkery, Bomaan, Sagay, Shishi, Maghool, Sultana, Fard, Maktoomi, Naptit Saif, Jabri, Kodary, Dabbas, Raziz and Shabebe – cultivated in the United Arab Emirates have been studied (Habib and Ibrahim, 2009). The results showed significant variability in the amounts of macronutrients and micronutrients and also that these pits are excellent sources of dietary fiber (Habib and Ibrahim, 2009).

Table 2.2: Composition of various essential nutrients and phytochemicals in dates.

Composition	Lowest reported	Highest reported
Moisture (g/100 g)	7.20	50.40
Fat (g/100 g)	0.10	1.40
Ash (g/100 g)	1.00	1.90
Protein (g/100 g)	1.10	2.60
Amino acids (mg/100 g)		
Alanine	30.00	133.00
Arginine	34.00	148.00
Aspartic acid	59.00	309.00
Cysteine	13.00	67.00
Glutamic acid	100.00	382.00
Glycine	42.00	268.00
Histidine	0.10	46.00
Isoleucine	4.00	55.00
Leucine	41.00	242.00
Lysine	42.00	154.00
Methionine	4.00	62.00
Phenylalanine	25.00	67.00
Proline	36.00	148.00
Serine	29.00	128.00
Threonine	23.00	95.00
Tryptophan	7.00	92.00
Tyrosine	15.00	156.00
Carbohydrates (g/100 g)	52.60	88.60
Fructose	13.60	36.80
Glucose	17.60	41.40
Sucrose	0.50	33.90
Fibre (g/100 g)		
Soluble	0.40	1.30
Insoluble	3.03	7.40
Total	3.57	10.90
Minerals (mg/100 g)		
Mg	31.00	150.00
Na	1.00	261.00
Ca	5.00	206.00
P	35.00	74.00
K	345.00	1287.00
Mn	0.01	0.40
Fe	0.10	1.50
Zn	0.02	0.60
Cu	0.01	0.80
Se	0.24	0.40
Vitamin (µg/100 g)		
A (Retinol)	3.00	44.70
B1 (Thiamin)	50.00	120.00
B2 (Riboflavin)	60.00	160.00
B3 (Niacin)	1274.00	1610.00
B6 (Pyridoxal)	165.00	249.00
B9 (Folate)	39.00	65.00
C (Ascorbic acid)	400.00	16000.00
α-Carotenoids (µg/100 g)	3.00	3.00
β-Carotenoids (µg/100 g)	2.50	146.00
Zeaxanthin (µg/100 g)	33.00	33.00
β-Zeaxanthin (µg/100 g)	9.00	9.00
Lutein (µg/100 g)	28.00	541.00
Neoxanthin (µg/100 g)	184.00	381.00
Phenolics (mg/100 g)	3.91	661.00
Anthocyanins (mg/100 g)	0.20	1.50

***(From Al Farsi and Lee, 2008)**

Some studies have investigated the variation in the chemical composition of specific date varieties. Hasnaoui et al., (2010) investigated the major nutrient content and the importance of the control of water activity in preventing microbiological contamination among a range of date varieties. They found that the flesh of all varieties showed high levels of total sugars (62.60-83.32%) and small amounts of protein (2.3-3.85%), ash (2.15-3.46%) and fat (0.1-0.46%) on a dry-matter basis. Although the mineral contents varied widely, all varieties could be an important source of potassium. Ahmed et al., (1995) analyzed the chemical composition of fruits from twelve varieties of date palm that are consumed in the United Arab Emirates. The study showed that glucose and fructose increase rapidly with maturation from kimri through Khalal and Rutab to Tamar. Total sugars may represent over 50% of the fresh weight at Tamar, and these values, together with low moisture contents, encourage resistance to fungal spoilage after harvest. They found a differences in moisture contents (lowest value=9.2 and maximum value =32.1). For protein, lipid and ash there were no major differences between varieties at the Tamar stage. Minerals accumulated in the fruits as well, and the researchers suggested they could be an important source of potassium for regular consumers. Sulieman et al., (2012) investigated the physical and chemical properties, as well as microbiological characteristics, of five date palm cultivars cultivated in Sudan. The study showed that the physical characteristics, like fruit weight, length, flesh thickness, and seed weight, differed significantly between the various cultivars. Although most of the cultivars had similar chemical components, there were a few differences. However, the microbiological analyses proved that no significant differences were found between the cultivars.

Despite the wide number of varieties of dates, there seems to be very little difference in their gross chemical composition. In general, the amount of carbohydrates, including reducing sugar (fructose, galactose and glucose) and non-reducing sugar (sucrose), always represents around a total of 70% of the date's dried weight. However, there have been limited studies on this aspect or indeed on the impact of development or environmental conditions on nutritional status.

2.10.1 Moisture content

There is a relationship between the stage of maturity of the date and moisture content. The moisture content of dates decreases as they ripen. In the Kimri stage the

average moisture content is 83.6%, while in the Khalal stage it is about 65.9%, and it continues to decrease through the Rutab stage (43%) and in the tamar stage (24.2%). Also, Al-Shahib & Marshall (2002) found that the moisture content of 13 varieties of dried date in this stage averaged 12.7%. With the knowledge that the percentage of humidity in relation to the occurrence of microbial corruption is 70%, the Tamar stage moisture content (24.2%) is well below this level.

There is also a relationship between temperature and moisture content (at a constant temperature) and this is described practically and theoretically by a moisture sorption isotherm. For instance, the chemical composition and water sorption isotherms of two Omani date varieties, Fard and Khalas, were determined by Myhara et al., 1998. Moisture sorption isotherms conducted at three temperatures displayed a crossing effect due to the dissolution of crystalline sugars at higher temperatures and moisture contents. Both varieties varied from 9.4 to 1.6 kJlmo1 as the moisture content changed from 5.0 to 40.0%. Differences in the moisture sorption behaviour of the two varieties were credited to compositional differences. However, compositional data alone could not act as a universal moisture sorption model because other complicating factors, such as crystalline sugar dissolution, were involved.

2.10.2 Fat

Dates contain small amounts of fat mainly concentrated in the skin (2.5-7.5%), and this has more physiological importance in the protection of the fruit than in contributing to the nutritional value of the date flesh (0.1-0.4%). Sawaya et al. (1983) reported that the crude fat content ranged from 0.1 to 0.46% for dates grown in Saudi Arabia and similar values were found by Ahmed et al. (1995) for dates grown in the United Arab Emirates; however, these are low compared to those of some Iranian varieties (0.4 to 0.9% of fat) (Ejlali et al. 1975).

In addition, fatty acids occur in the seed as a range of saturated and unsaturated acids, the seeds have been shown to contain 14 types of fatty acids, but only eight of these occur in the flesh and seed (see Table 2.3). Unsaturated fatty acids include palmitic, oleic, linoleic and linolenic acids. The oleic acid content of the seeds varies from 41.1 to 58.8%, which suggests that date seeds could be used as a source of oleic acid.

Table 2.3: Fatty acid content of date flesh (g/100 g)

Saturated fatty acid	Range
C12:0	0.6 _5.4
C14:0	0.3 _2.3
C16:0	1.7 _1.8
C17:0	0.01
C18:0	0.3 _0.7
C20:0	0.01
Unsaturated fatty acid	
C18:1(9)	3.2 _5.1
C18:2(6,9)	0.7 _0.8

***Data from Al-Showiman (1990).**

2.10.3 Protein

Dates are reported to contain relatively low levels of protein (from 1.10% to 2.60%) as seen in Table 2.2. Proteins are considered one of the most complicated nutrients; in addition, they are one of the most labile and difficult to purify. Proteins occur in an extensive series of forms and they have a correspondingly wide series of physical properties. The amino acid composition of the protein is nutritionally important. Thus essential amino acids such as lysine, threonine, valine, methionine, leucine, isoleucine and phenylalanine, histidine, and tryptophan must be included in the diet for maintenance of proper nitrogen stability in normal adult humans. This is because these amino acids cannot be produced in the human body (Durkin, 2012). Essential amino acids such as lysine, threonine, valine, methionine, leucine, isoleucine and phenylalanine, histidine, and tryptophan are essential in the structure and function of all living cells (Durkin, 2012).

Sadiq et al., (2013) investigated the proximate analysis, mineral composition, phytochemical constituents and amino acids of the dates' fruit flesh and seeds. The results showed that the flesh and seeds both contain essential and non-essential amino acids. Indeed, dates were found to contain all 23 types of amino acids. Some of these amino acids are not present in the most popular fruits, such as oranges, apples and bananas.

2.10.4 Dietary Fibre

The term “total dietary fiber” covers a large number of complex organic compounds, including, non-starch polysaccharides and lignin. These substances (cellulose, hemicelluloses, pectins, hydrocolloids, resistant starch and lignin) are not attacked by the human digestive enzymes. A healthy diet should include an adequate amount of dietary fiber. Soluble fiber has been shown to help control diabetes by decreasing high blood sugar as well as lowering high cholesterol, specifically low-density lipoprotein (LDL) cholesterol. Insoluble fiber increases the body’s ability to process food and the rate at which it is processed through the digestive system.

Dates are generally a good source of dietary fibre, and are also sodium-free, fat-free, and cholesterol-free. Each of these factors is important for reducing the risk of developing heart disease and cancer. The fiber found in dates comes in two forms: soluble and insoluble.

Al-Shahib and Marshall (2002), who surveyed the total dietary fibre contents of 13 date varieties from various countries, found that the percentage of total dietary fibre was in the range of 6.4-11.5%, depending on variety and degree of ripeness. They analyzed the dietary fibre content of three sun-dried date varieties (Fard, Khasab, and Khalas) and found that total dietary fibre contents ranged from 6.26 to 8.44 g/100 g fresh weight (Al-Farsi et al.,2005).

In the study by (Borchani et al., 2010), eleven date cultivars were collected at the tamar stage (full ripeness), which is characterized by dry dates allowing easy preservation. Date fibre concentrates (DFC) were extracted and analyzed for their proximate content (moisture, fibre, protein, lipid and ash) and some functional properties, such as water-holding capacity (WHC) and oil-holding capacity (OHC). DFC presented high dietary fibre content (90.71 - 93.92 g/100 g dry matter). The results showed that dates could be an important source of highly techno-functional fibers that could be used in food formulations.

According to Spiller (1993), the dietary fibre content of widely retailed dried dates (rutab and tamar stages) was estimated at 4.4%, with 3.2% insoluble and 1.2% soluble.

2.10.5 Carbohydrates

The range of carbohydrates found in the human diet illustrates the nature of the task facing the analyst who wishes to follow the recommendations published by FAO/WHO (1998) for measuring the carbohydrates in foods independently. It is inadequate to consider the carbohydrates as a single component of foods; since, different carbohydrates have distinctive metabolic and physiological properties. Carbohydrates thus basically consist of free sugars, disaccharides and polysaccharides.

Dates are particularly rich in reducing sugars, especially fructose and glucose with smaller amounts of mannose and maltose, and non-reducing sugars, such as sucrose, can represent around 80% of the dry matter. In general, in the Tamar stage of development the high percentage of sugar content renders the date extremely resistant to microbial spoilage. Several papers have investigated the total sugars in dates along with their ratio and changes during development. Ahmed et al. (1995) analyzed the main chemical composition of twelve date palms from the United Arab Emirates. The results showed that glucose and fructose increased rapidly with maturation from the Kimri stage through to the Khalal, Rutab and Tamar stages. They also found that at the Khalal stage, the water levels in the fruits are still high enough to allow fungi introduced by bird or insect damage to proliferate freely at the expense of the available sugars. There is a rapid build-up of glucose and fructose from Khalal onwards and this indicates that the date is an excellent source of readily available carbohydrates. Total sugars may represent over 50% of the fresh weight at the Tamar stage, and these values together, with low moisture content, encourage resistance to fungal spoilage after harvest. Fructose often represents around 50% of the total sugars. This high level is of dietary significance, since fructose tends to lead to a lower level of postprandial hyperglycemia than would the same intake of glucose (Al-Qblan, 1999). In addition, Marbet et al. (2008) analyzed the main chemical composition and the water content of 10 date palms from Tunisia. For all analysis, the Deglet Nour variety was taken as reference. Compositional analysis showed that the littoral varieties were very rich in reducing sugars (26 to 51 %) compared to Deglet Nour, which was rich in sucrose (54%). Elleuch et al. (2008) studied the sugar content of Deglet Noor and Allig dates and showed that sucrose was a major constituent of Deglet Noor, whereas in Allig reducing sugars were more prevalent, with equal amounts of fructose and glucose. This

difference was potentially ascribed to the presence of high invertase activity in Allig dates (Fayadh and Al-Showiman, 1990; Elleuch et al., 2008).

In comparison to the soluble sugars dates contain a smaller amount of polysaccharides such as cellulose, hemicelluloses and starch (Shinwari, 1993). Elleuch et al. (2008) analyzed the main chemical composition of two date palm fruit from the Degach region (Tunisia). The study concluded that the two varieties had similar contents of total non-starch polysaccharides (NSP)(5.71–5.85%). Thus although higher in soluble sugars dates may also represent a potential source of dietary fiber.

2.10.6 Vitamins

Dates contain a wide range of vitamins including A, A1, B, B1, B2, B3, B5, B6, and C (Table 2.2). Dates are a good source of vitamin A (containing 149 IU per 100 g), which is known to have antioxidant properties and to be essential for vision. Although the date fruit does not meet the recommended daily intake for vitamin A which is at 600 micrograms, the amount contained in the fruit is significant enough for the fruit to be included in a meal plan where the aim is to increase the vitamin A intake. The recommended daily intakes of vitamin B_1, B_2, B_3, B_5, and B_6 are 1.4 milligrams, 1.6 milligrams, 18 milligrams, 6 milligrams, and 2 milligrams respectively (Lenntech, 2017). The date palm fruit is also rich in vitamin C whose recommended daily intake is 76 milligrams (Lenntech, 2017). The consumption of the date palm fruit helps towards the recommended daily intakes.

Additionally, it is also required for maintaining healthy mucus membranes and skin. The consumption of dates could help to protect from lung and oral-cavity cancers. Although, dates meet some of the daily nutrition requirements because of its vitamins (riboflavin, thiamine, biotin, folic and ascorbic acid) that are eaten fresh or dried which avoids the damaging factors such as cooking, canning or milling associated with several other foods.

2.10.7 Minerals

Dates contain calcium, magnesium, phosphorus, potassium, iron, zinc, copper, manganese and selenium (Table 2.2). Several studies have investigated the mineral content of dates. For instance, Al-Hooti et al. (1995) reported on the mineral content of

five cultivars of dates at various ripening stages. They found that the iron content decreased in four tested cultivars from Kimri to Tamar ripening stage, whereas it increased in cv. Lulu. In contrast, the study showed that the percentages of phosphorus, potassium, calcium, sodium, magnesium and zinc decreased in all five tested cultivars of dates from the Kimri to Tamar stage. Potassium is an essential mineral that the body needs to maintain proper muscle contractions, including contractions of the heart muscle. Potassium also promotes a healthy nervous system and efficient metabolism in the body. One serving of dates contains 240 milligrams of potassium, which is more than the amount of potassium found in bananas (Al-Shahib and Marshall, 2003).

2.11 Nutritional Studies on dates

The major nutritional impacts from the consumption of date fruit is likely to arise from either their high sugar content or antioxidant capacity. Rock et al. (2009) investigated the effect of two varieties of dates (Medjool and Hallawi) on serum-oxidative status, glucose and lipid levels in healthy human subjects. The volunteers were asked to consume 100 g/day of either variety of dates for a period of four weeks, after which they were tested for variations in the serum parameters. The study showed a major decrease in the levels of triacylglycerol observed, possibly due to the amount of dietary fiber in the dates. Although an increase in the serum-glucose level concentrations on postprandial samples was observed, the fasting serum-glucose levels remained unaffected. The reason for the unaffected serum- glucose levels was attributed to the decreased serum triacylglycerol levels and oxidative stress during the trial. The presence of phenolic compounds, especially ferulic acid and coumaric acid derivatives, may have been responsible for the observed free-radical-scavenging effects so increasing of theses acids leads to free-radical effects decreasing. A decrease in basal serum-oxidative stress and the susceptibility of serum to AAPH [2, 2'-azobis (2-amidinopropane) hydrochloride]-induced lipid peroxidation was shown in the Hallawi date consumption. The existence of higher phenolic concentrations and catechins (antioxidants), as well as the different metabolism, absorption and bioactivity of the various phenolic compounds in Hallavi dates may have contributed to the observed increased antioxidant capacity, as well as serum high-density lipoprotein-associated PON1 activity, as compared to the Medjool variety. The results justify the various in vitro studies, showing that dates in general, and the Hallawi variety in particular, are

beneficial and can be included in our regular diet without having to worry about any harmful effects (Rock et al., 2009).

The impact of sugar in the diet is often measured by the use of the glycemic index. The glycemic index (GI), first proposed in 1981 by Jenkins et al, is a system of classifying food items by their glycemic response. The GI of a food depends upon the rapidity of digestion and absorption of its carbohydrates, which is determined largely by its chemical and physical properties. A specific food's GI can be determined by measuring the rise in blood glucose after ingestion of a quantity of that food containing 50 g carbohydrate equivalent compared with the same amount of carbohydrate from a reference (such as glucose or white bread) taken by the same subject (Wolever et al, 1991 and Aston et al, 2008). Using glucose as the reference, a GI of ≤55 (i.e. ≤55% of the reference) is considered low, 56-69 is considered medium, and ≥70 is considered high (Jenkins et al,1981).

The GI for dates has been measured in several studies. (Alkaabi et al, 2011) determined the composition of five common types of dates grown in the UAE (Fara'd, Lulu, Bo ma'an, Dabbas and Khalas) and calculated their glycemic indices. The study concluded that the mean glycemic indice of the dates for healthy individuals were 54.0 ± 6.1, 53.5 ± 8.6, 46.3 ± 7.1, 49.1 ± 3.6 and 55.1 ± 7.7 for Fara'd, Lulu, Bo ma'an, Dabbas and Khalas, respectively. Equivalent studies on subjects with type 2 diabetes gave very similar values (46.1 ± 6.2, 43.8 ± 7.7, 51.8 ± 6.9, 50.2 ± 3.9 and 53.0 ± 6.0). There were no statistically significant differences in the GIs between the control and the diabetic groups for the five types of dates, nor were there any statistically significant differences among the dates' GIs ($df = 4$, $F = 0.365$, $p = 0.83$). Another study (Miller et al, 2002) compared the GIs of one popular UAE date variety (Khalas) when consumed according to five different preparations: the early-ripened (Rutab) stage, the traditionally stored and commercially processed dehydrated (Tamar) stage, and both Rutab and commercial Tamar stages in mixed meals with plain full-milk yoghurt. The purpose of this study was to determine and compare the GIs of three popular varieties of dates, commercially processed and available in the UAE. The study demonstrated that the mean glycemic indexes of the dates were 35.5 for Khalas, 49.7 for Barhi and 30.5 for Bo ma'an. There was a significant difference between the results for Bo ma'an and for the other 2 varieties.

The measurement of sugar content in a food item is of importance, especially considering the negative effects that sugar has on the human body. Studies have

shown that the increased consumption of sugar is associated with an increase in the risk of cardiovascular diseases (Bray & Popkin, 2014). One such study was performed by Yang, Zhang & Gregg (2014). Yang, Zhang & Gregg (2014) based their study on the meta analyses of sugar consumption among Americans who were above two years. Yang, Zhang & Gregg (2014) also found that the association between the increased consumption of sugar and the risk for cardiovascular disease was statistically significant. This effect was manifested through increased mortality from cardiovascular diseases among the people who exhibited an increased consumption of sugar.

A study performed by Te Morenga, Mallard & Mann (2013) found that there was a tendency by people who consumed more levels of dietary sugar to gain more body weigh when compared to people whose sugar consumption was low. This was determined through the meta-analyses and systematic reviews of both cohort studies and randomized controlled trials. The increase in body weight was attributed to the increase in the calorie consumption on the account of increase sugars. In their randomized controlled trial, Ebbeling et al., (2012) found that an increased risk for obesity was associated with the increased consumption of sugars. The increase in caloric intake resulting from the increase sugar intake results in increased weight gain as found by Te Morenga, Mallard & Mann (2013). If the increase gain in body weight coincides with reduced physical activity, one may develop obesity. These finds were also reported by de Ruyter et al. (2012) who also found a statistically significant association between the increased intake of dietary sugars and the occurrence of obesity.

High sugar content has also been shown to contribute to other negative health effects. For instance, Hu & Malik (2010) found a statistically significant association between high sugar intake and type 2 diabetes mellitus in addition to obesity. These findings were also reported by Malik et al., (2010). High sugar intake was also associated with increased risk for dyslipidaemia by Welsh et al., (2010). A similar finding was reported by Welsh et al., (2011) where dyslipidaemia was more prevalent among people who reported a high sugar intake compared to their counterparts who had a low sugar intake.

The association between high sugar intake and hypertension was reported by Brown et al., (2011). The causative effects are related to the effect of high sugar intake on body weight and how the increased body weight causes an increase in the blood pressure. Other causes can also be explained by the occurrence of comorbidities such

as clogged arteries and the need for the heart to work harder to pump oxygen to the increased body mass. Chen et al. (2010) studied adults in the United States and found that a reduction in the amount of dietary sugar consumed resulted in a decrease in the blood pressure of an individual.

These effects make it important to determine the sugar content in various food items. Nutritionists and other healthcare professionals need this knowledge when planning for interventions and developing meal plans for patients with various needs and illnesses. The findings on the sugar contents of the palm data plants are important to the overall purpose of the project. The findings go towards providing a holistic knowledge of the nutritional composition of the date palm tree. The sugar composition in their various stages of maturity helps determining the stage at which the sugar content in the date fruit is appropriate for individuals with different needs.

2.12 Antioxidants

A potential nutritional benefit of dates may lie in their antioxidant capacity and consequent ability to quench free radicals. Free radicals are molecules that are very unstable; therefore, they look to bond with other molecules, often modifying their structure and perpetuating the detrimental process. Free radicals are responsible for aging, tissue damage, and possibly some diseases such as cancer as reported in a study by Pham-Huy, He & Pham-Huy (2008) and Lobo et al., (2010). The human body can be exposed to free radicals from both external and internal sources. External sources include environmental pollution, solar ultraviolet radiation, the photo-dissociation of ozone that gives the excited oxygen atom O (1D), and poisons like industrial cleaners. An example of an internal source is the superoxide-driven Fenton reaction in which O_2. Although, oxygen is a critical element for life, it can create damaging by-products during normal cellular metabolism (Al-Saikhan, 2000). The damaging effects of O_2 could be attributed to the formation of oxygen radicals as found by Sharma et al., (2012). The researchers find that the oxygen radicals are part of the reactive oxygen species that causes the continued oxidative damage which eventually results in the death of the cells. This hypothesis was generalized and changed to the "superoxide theory of O_2 toxicity" after the discovery of a class of enzymes, superoxide dismutases (SODs), that show particularly for catalytic removal of superoxide free radical, O_2.-.

The new superoxide theory developed by Indo et al., (2015) states, " *Superoxide is the origin of reactive oxygen and nitrogen species (RONS) and, as such, causes various redox related diseases and aging*." High oxidative potential can cause a range of damaging effects within the body, such as the oxidation of lipids and DNA damage through attack by reactive oxygen, especially the process of oxidative deamination.

The body is protected from the action of these free radicals by a combination of endogenous mechanisms and from dietary antioxidants. Endogenous mechanisms include the action of SOD enzymes mentioned previously. Dietary antioxidants are present in many foods, especially plants, where they can act to inhibit the oxidation which could result in a decrease in nutritional value and sensory quality (Pokorny et al., 2001). Young & Woodside (2001) defines antioxidants as any substance that when present at low concentrations compared to those of an oxidizable substrate appreciably delay or avoid the oxidation of that substrate, and argument that is reported by Vaya & Aviram (2001). Antioxidants have been shown through research to increase longevity and good health outcomes (Sykiotis et al., 2011, Maulik et al., 2013).). Therefore, interest in antioxidants has been increasing because of their high capacity in scavenging free radicals related to various diseases (Silva et al., 2006).

There are two basic types of antioxidants, primary and secondary. Primary antioxidants intercept and stabilize free radicals by donating active hydrogen atoms. Phenols represent the two main types of primary antioxidants. Secondary antioxidants prevent formation of additional free radicals by decomposing the unstable hydro-peroxides into a stable product. Antioxidants have various mechanisms of action (Table 2.4).

Table 2.4 Mechanisms of antioxidant activity (Pokorny et al., 2001)

Antioxidant class	**Mechanism of antioxidant activity**	**Examples of antioxidants**
Proper antioxidants	Inactivating lipid free radicals	Phenolic compounds
Hydroperoxide stabilisers	Preventing decomposition of hydroperoxides into free radicals	Phenolic compounds
Synergists	Promoting activity of proper antioxidants	Citric acid, ascorbic acid
Metal chelators	Binding heavy metals into inactive compounds	Phosphoric acid, Maillard compounds, citric acid
Singlet oxygen quenchers	Transforming singlet oxygen into triplet oxygen	Carotenes
Substances reducing hydroperoxides	Reducing hydroperoxides in a non-radical way	Proteins, amino acids

Antioxidants can vary extensively in chemical structure (Pokorny et al., 2001). Vegetables and fruits contain a wide range of naturally occurring antioxidant components. The antioxidant capacity of a vegetable or fruit is depended on compounds such as vitamin C, vitamin E, carotene (which is converted to vitamin A) and phenolic compounds.

"*Ascorbic acid, as a water-soluble antioxidant, is an essential nutrient and can act to "scavenge" aqueous peroxyl radicals before these destructive substances have a chance to damage the lipids, so it may be one of the first lines of defense. It works along with vitamin E, a fat-soluble antioxidant, and the enzyme glutathione peroxidase to stop free radical chain reactions*" (Naidu, 2003).

Vitamin E is the combined name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins and which exhibit antioxidant properties (Herrera and Barbas, 2001 and Packer et al., 2001). Alpha-tocopherol is the most widely used form of vitamin E in the diet of many Americans because of its bioavailability and the fact that it is the preferential form absorbed by the body (Jiang et al., 2001). "*It has been claimed that α-tocopherol is the most important lipid-soluble antioxidant, and that it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction*" (Herrera and Barbas, 2001; Traber and Atkinson, 2007). Therefore, this will remove the free radical intermediates and prevent the oxidation reaction from continuing. The oxidised α-tocopheroxyl radicals formed in

this process may be recycled back to the active reduced form by reduction by other antioxidants, such as retinol, ascorbate or ubiquinol as reported by Lobo et al., (2010).

Beta-carotene is probably the most studied of the carotenoids in terms of nutritional value. Al-Turki (2008) argued that carotenoids are, “a potent antioxidant as well as a major precursor for Vitamin A." It is one of numerous carotenoids, natural plant pigments found in deeply coloured vegetables and fruits. Beta-carotene has the ability to quench singlet oxygen and thus protect the cell membrane lipids from the harmful effects of oxidative degradation. This is an argument that has been advanced through empirical studies by Ramel et al., (2012). A physical reaction occurs in which the carotenoid receives the energy from the excited oxygen. The result is that the carotenoid molecule becomes excited, hence the quenching effect (Pandey, Krishnan & Sebastian, 2000 and Al-Turki (2008). The therapeutic effect of the beta carotene that is experienced in the erythropoietic protoporphyria results from the quenching of the singlet oxygen. Although the ability of beta-carotene and other carotenoids to quench excited oxygen, it is limited, due to the fact that the carotenoid itself can be oxidized during the process (autoxidation). It may function as a pro-oxidant and can activate proteases at higher concentrations. In addition, carotenoids are also thought to quench other oxygen free radicals to singlet oxygen. The beta carotene might react directly with the peroxyl radical at low oxygen tensions; this may give some synergism to vitamin E which reacts with peroxyl radicals at higher oxygen tensions (Brar, 2007). Carotenoids also have a number of other biological actions, including immuno-enhancement; inhibition of mutagenesis and transformation; and regression of premalignant lesions as reported in (Fouad, 2007). Consumption of carotenoid-rich foods has been correlated with prevention of cancer, cardiovascular diseases and other degenerative processes relating to oxidative stress (Stahl and Sies, 2003; 2005).

There are around 5000 known plant phenolics and model studies have confirmed that many of them have antioxidant activity (Karadeniz et al., 2005). The antioxidant activity of phenolics is primarily related to their redox properties. These redox properties enable the phenolic compounds to assume a variety of behaviors. These include the behavior of reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Sylvie et al., 2014). The antioxidant activity of the phenolic compounds is influenced by the number, and location, of hydroxyl groups present in addition to the presence or absence of 2-3 double bond and 4-oxofunction (Kumar & Padney, 2013). Flavonoids are a major family of low molecular weight

polyphenolic compounds. "*They are colored compounds, e.g. red, blue, and yellow pigments in the plant kingdom*," (Al-Saikhan, 2000). Most studies on the health benefits of phenols have focused on flavonoids as these are among the most widely distributed of the many phenols and are most abundant in food. Although flavonoids are commonly assumed to be non-nutritive agents; interest in these substances has increased due to their possible effects on human health, many of which are positive and include anti-cancer properties, anti-inflammatory, and anti-viral properties (Lee, Kang & Cho, 2007). Flavonoids present in plants possess various health benefits which have been attributed to their free radical scavenging activities that lead to a reduction in certain chronic diseases and the prevention of some cardiovascular disorders and specific kinds of cancerous processes (Tapas et al., 2008). Flavonoids not only have antioxidant properties. They also have an inhibitory effect on various enzymes that have been showed through studies to have tumorigenesis effect. These enxymes include prostaglandin synthase, lipoxygenase and cyclooxygenase (Araujo et al., 2012). The content of different types of flavonoids is influenced by a number of factor, some of which include the cultivar and the stage of maturation (Rastakhiz et al., 2015). The antioxidant activity of flavonoids, such as anthocyanins, is significantly affected by the method of measurement. The final measurement of the content of antioxidants in a fruit can be influenced by factors such as the conditions in the catalase oxidation and the substrate used (Antolovich et al., 2000).

2.13 Antioxidant content in date fruit

2.13.1 Phenolics

Several groups have measured the phenolic content of dates. Al-Farsi et al., (2005a; 2005b) showed that the total content of phenolics ranged from 134 to 280 mg of ferulic acid equivalents (FAE)/100 g, and 217-343 mg of FAE/100 g in fresh and sun-dried date varieties (Fard, Khasab, and Khalasa), respectively. The phenolic profiles of seven different cultivars of ripe date fruits grown in Algeria was studied by Mansouri et al., (2005). Their results showed that total phenolic content ranged from 2.49 to 8.36 mg gallic acid equivalents (GAE)/100 g fresh weight. In contrast (Wu et al., 2004; 2004) reported much higher contents of total phenolics in Deglet Noor and Medjool cultivars, which contained 661 and 572 mg of GAE/ 100 g fresh weight.

Sixteen cultivars commonly grown in Bahrain were evaluated for their total phenolic contents at the Tamar stage (Allaith, 2008). The results showed that the average total phenolic content was 152.10 mg of GAE/ 100 g of edible portion, which is equivalent to 85.9 mg per 100 g FW. A study of samples from Iran reported phenolics ranging from 2.89 to 141.35 mg GAE/100 g DW (Biglari et al., 2008). Recently, Chaira et al. (2009) also found that among the date cultivars of Tunisia, the Mermella variety had the lowest phenolic content (5.73 mg/100 g fresh weight), while the Korkobbi variety had the highest (54.66 mg/100 g fresh weight). The total phenolic content ranged from 2.89 to 4.82, 4.37 to 6.64 and 141.35 mg gallic acid equivalents (GAE)/100 g dw,

Various techniques have been used for the determination of natural antioxidants in food such as electrochemical HPLC. Many dates studies have used HPLC to measure flavonoid content. Eid et al. (2013) and Hammouda et al. (2013) have used high performance liquid chromatography (HPLC) and reverse phase-HPLC (RP-HPLC) to compare the flavonoid content during the developmental stages in date varieties from Saudi Arabia and Tunisia. In addition, (Odeh et al, 2014) identified individual phenolic compounds using HPLC.

Phenolic acids are divided into two general classes as either derivative of hydroxybenzoic acid or hydroxycinnamic acid (Manach et al., 2004). Examples and general structures of phenolics are shown in Figure 2.5. They contain a hydroxylated benzene ring with one or more carboxyl groups attached directly or indirectly to it. Date fruit have been shown to contain several phenolic acids. Nine bound phenolic acids (gallic, protocatechuic, p-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric, ferulic, and o-coumaric acid) were tentitatively identified and four free phenolic acids (protocatechuic acid, vanillic acid, syringic acid, and ferulic acid) have been found. Ferulic acid was the major phenolic acid found in all the date cultivars (Al-Farsi et al., 2005; 2007). The phenolic profiles of seven Algerian varieties of date were analyzed by Mansouri et al. (2005) and the results showed that they contained p-coumaric, ferulic and sinapic acids, some cinnamic acid derivatives and three different isomers of 5-o-caffeoyl shikimic acid. Studies with three varieties of Omani dates have shown the existence of both free (protocatechuic acid, vanillic acid, syringic acid, and ferulic acid) and bound phenolic acids (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, and o-coumaric acid) (Al Farsi et al., 2005).

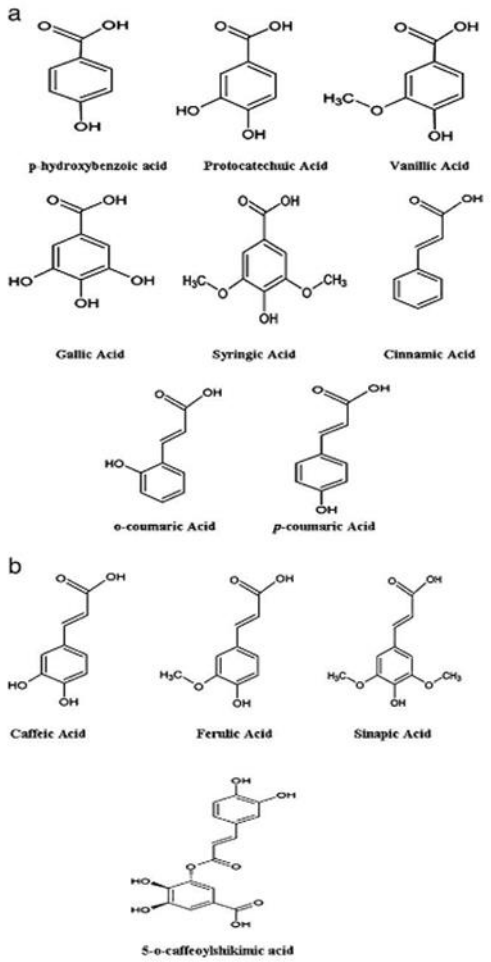


Figure 2.5: Structures of phenolic acids present in dates

In addition, dates are also considered as a good source of flavonoids, anthocyanins and procyanidins. Approximately 8000 types of flavonoids have been discovered (Duthie, 2003). The flavonoids are based on a C6-C3-C6 skeletal of carbon structure and are further sub-divided into flavones, flavonols, flavonones, isoflavones, flavanols and anthocyanins (Stewart et al., 2000). Hong et al. (2006) assessed the flavonoid content in the Deglet Noor variety during the Khalal stage of maturity. They identified thirteen flavonoid glycosides of luteolin, quercetin, and apigenin (Figure 2.6). It was also observed that both methylated and sulfated forms of luteolin and quercetin were present as mono-, di-, and triglycosylated conjugates, while apigenin was present only as the diglycoside. Quercetin and luteolin formed primarily O-glycosidic linkages, whereas apigenin was present as the C-glycoside. As of today, dates also have the unique distinction of being the only food to contain flavonoid sulphates (Hong et al., 2006).

Chaira et al. (2009) recently reported that the Tunisian date variety Korkobbi contains the highest content of flavonoids (54.46 quercetin equivalents/100 g fresh weight).

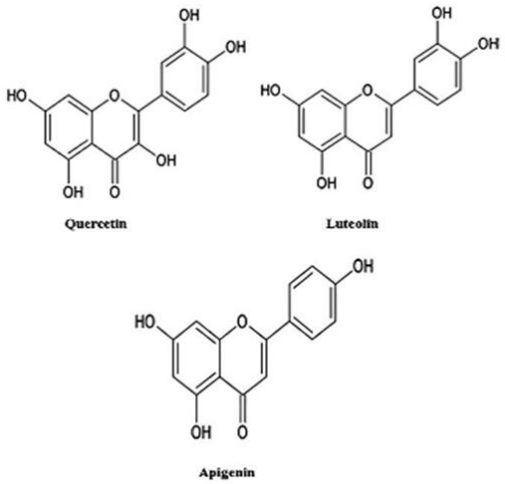


Figure 2.6: Structures of flavonoids present in dates

Anthocyanins (Figure 2.7) are water-soluble vacuolar pigments and might appear as red, purple, or blue. Anthocyanins are found in many fruits, cereals, vegetables. Anthocyanins have numerous health benefits (Mazza, 2007). Studies by Al Farsi et al. (2005b) have shown that, among the analyzed fresh date varieties, the Khasab contains the highest amount of anthocyanins (1.5 mg/100 g), followed by the Fard (0.9 mg/100 g) and Khalas (0.87 mg/100 g) variety, and that a direct relationship existed between the levels of anthocyanin and the fruit colour. Anthocyanins were detected only in fresh dates, because they may be destroyed upon sun-drying (Al Farsi et al., 2005b).

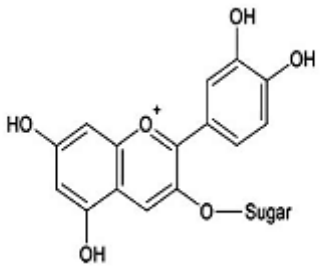
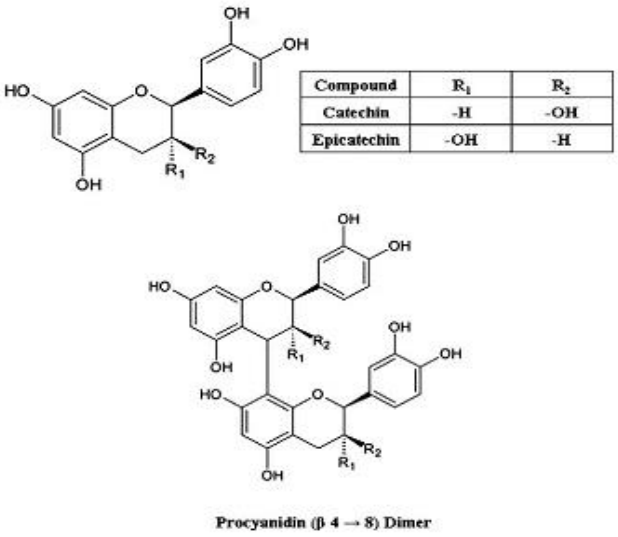


Figure 2.7: Structures of anthocyanin present in dates

Procyanidins are condensed tannins and the major precursors of blue-violet and red pigments in fruits, vegetables, nuts, seeds, barks, and flowers (Fine, 2000). Procyanidins have been extracted from the Deglet Noor variety of dates at the Khalal stage of maturity using an acetone–water–acetic acid solvent-extraction method (Hong

et al., 2006). Chemical analysis in Hong's study suggested that the procyanidin existed as higher-molecular-weight polymers, undecamers through heptadecamers, and decamers (Hong et al., 2006) (Figure 2.8).



Compound	R_1	R_2
Catechin	-H	-OH
Epicatechin	-OH	-H

Figure 2.8: Structures of procyanidins present in dates

The ratio and concentrations of these constituents depend on the category of the fruit, stage of fruit picking, location and soil conditions. These phytochemicals can all contribute to the nutritional and organoleptic properties of the fruit (Abdelhak et al., 2005; Abdul and Allaith, 2008; Al Farsi et al., 2005b;). Processing of dates can also impact their phenolic content. Comparative studies with fresh and dried Fard dates have shown a major increase in phenolic content upon drying, due to the degradation of tannins and the action of enzymes at higher temperatures (Al Farsi et al., 2005).

2.13.2 Sterols

Sterols, or steroid alcohols, are a subgroup of steroids with a hydroxyl group at the 3-position of the A-ring, and are amphipathic lipids. Sterols of plants are called phytosterols and possess many health benefits (Liolios et al., 2008). Kikuchi and Miki (1978) analyzed the sterols of the date fruit and observed that they contain cholesterol, campesterol, stigmasterol, ß-sitosterol and isofucosterol (Figure 2.9).

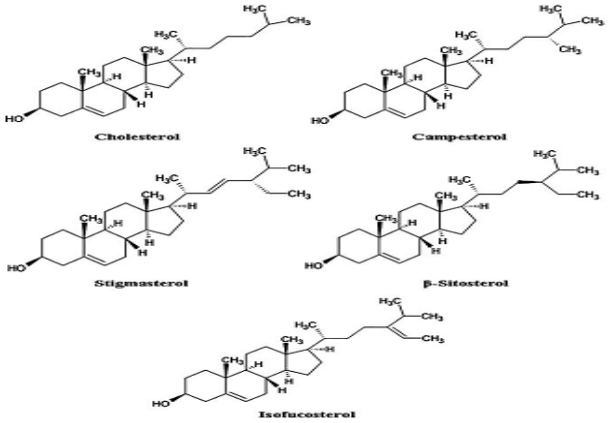


Figure 2.9: Structures of sterols present in dates

2.13.3 Carotenoids

Carotenoids are considered as a class of natural fat-soluble pigments, and influence the coloration of the plant. They contain vitamin A and protect the cell from the deleterious effects of free radicals by acting as antioxidants (Di Mascio et al., 1991). Studies have also shown that dates include the carotenoids lutein, ß-carotene and neoxanthin (Boudries et al., 2007) (Figure 2.10). In the Algerian fresh date varieties of Deglet Noor, Tantebouchte and Hamraya, the ß-carotene content is reported to be 6.4, 3.3 and 2.5 µg/100 g, while that of the lutein is 156, 28 and 33.6 µg/100 g, respectively (Boudries et al., 2007; Al Farsi and Lee, 2008). A major drop in carotenoid levels occurs during the transition from Khalal through Tamar stage; during the ripening process the levels of pro-vitamin A increase slightly in the Deglet Noor variety, while in Tantebougte and Hamraya its levels decrease (Boudries et al., 2007). Al Farsi et al. (2005b) analyzed the total carotenoid contents in both fresh and

dried varieties of Fard, Khasab and Khalas and the results showed that loss of carotenoids occurs during sun drying.

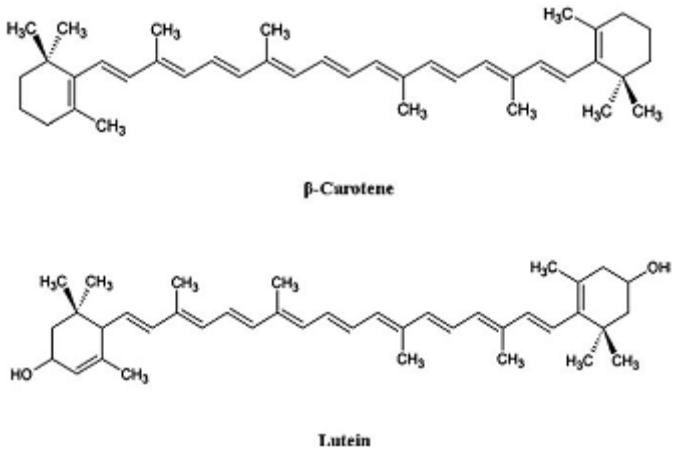


Figure 2.10: Structures of carotenoids present in dates

Several studies had investigated the antioxidant potential of dates and attempted to relate this to their composition especially in terms of phenolics. For instance, Mansouri et al. (2005) studied Algerian dates. The total phenolic compounds (TPC) and antiradical efficiency of the Algerian ripe date palm fruits were 2.49 - 8.36 mg gallic acid equivalents (GAE) per 100 g fresh weight and 0.08 - 0.22 mg GAE per 100 g, respectively. Native sun-dried date varieties from Oman have also been studied [15,16]; the TPC and antioxidant activity (AA) of Omani dates was 172 - 246 mg GAE per 100 g and 146 - 162 l/mol Trolox equivalents per gram (on a fresh-weight basis), respectively (Al-Farsi et al., 2007). (Al-Farsi et al., 2005) measured the antioxidant capacity of three date cultivars from Oman, using the Oxygen Radical Absorbance Capacity (ORAC) approach, and found them to be a good source of antioxidants (11687 to 20604 µmol of Trolox equivalents/g). Total antioxidant activity (lipophilic and hydrophilic $ORAC_{FL,}$ using fluorescein (FL) as the fluorescent probe) of two date cultivars was measured by (Wu et al., 2004) and they found values (from 23.87 to 38.95 µmol of Trolox equivalents/g). Mansouri et al., (2005) investigated the antioxidant potentials of Algerian dates using the 2-diphenyl-1-picrylhydrazyl (DPPH) approach and showed that the antioxidant activity ranged from 0.08 to 0.22 units of antiradical efficiency (AE) (Antiradical efficiency=1/EC50, Efficient concentration (EC50) = µg sample/µg DPPH: amount of antioxidant needed to decrease the initial DPPH concentration by 50%). The average antioxidant activity was recorded as 0.94 µmole Trolox equivalent/100 g FW using the Ferric Reducing Antioxidant Power (FRAP) approach in Bahraini date cultivars (Allaith, 2008). One study found that the antioxidant

activity of four types of soft dates (SD) namely Honey date, Bam date, Jiroft date and Kabkab date; three types of semi-dry dates (SDD) namely Sahroon date, Piarom date and Zahedi date and one type of dry date (DD) which was Kharak date as measured by the 2,2′-azinobis 3-ethylbenzothiazoline-6-sulphonic acid diammonium salt (ABTS assay) ranged from 22.83 to 500.33 µmole Trolox equivalent/100 g dry weights (DW) and the antioxidant activity (FRAP assay) ranged from11.65to 387.34 µmole Trolox equivalent/100 g DW (Biglari et al., 2008). These major variations between date samples could be due to various reasons such as variety, extraction techniques used and instrumental analysis (manual or automated). There is no standard method for antioxidant analysis, and this also contributes to variations in the estimation.

Guo et al., (2003), using the FRAP assay, reported that dates had an antioxidant activity of 23.67 mmol / 100 g of wet weight. They also demonstrated that this was the second highest antioxidant capacity out of 28 fruits tested and that were generally consumed in China. The consumption of date fruits at Tamar stage was shown to provide a total antioxidant value equivalent to various other common fruit and vegetables such as sweet cherry, orange fruit and Brussels sprouts (Blomhoff, 2005). As a result, dates are considered as a good source of antioxidants when compared to these foods.

In vitro studies by Vayalil (2002) showed that the aqueous extract of date fruit was an effective scavenger of superoxide and hydroxyl radicals and was able to inhibit iron-induced lipid peroxidation and protein oxidation in the rat brain homogenate in a concentration-dependent manner (Vayalil, 2002). Animal studies have also shown that oral feeding of the p-coumaric acid in dates increases the expression of antioxidant enzyme genes in rat cardiac tissue (Yeh et al., 2008).

The observed antioxidant activity of dates has been attributed to phenolic compounds, anthocyanins, flavonoid glycosides and procyanidins present in the fruits; the antioxidant activity decreases due to sun-drying and ripening (Abdul and Allaith, 2008; Al Farsi et al., 2005). There is a relation between antioxidant activity and fruit ripening as a strong decrease in antioxidant activity was found to be associated with the fruit as they ripen. A study by Allaith (2008) concluded that the phenolics were the major contributor for the antioxidant activity. Antioxidant components are not only

very significant for functional properties of date fruits or pit (oxidation resistance, taste, colour and texture) but could also have many health benefits.

Selenium found in dates could also contribute to the antioxidant benefits arising from their consumption. Several studies have shown that this critical trace element exerts its antioxidant function generally in the form of selenocysteine residues that are an integral constituent of ROS-detoxifying selenoenzymes (GPx, thioredoxin reductases and possibly selenoprotein P) (Steininbrenner and Sies, 2009). When considered as a whole, it is very clear that the presence of diverse phenolic compounds and selenium may have been responsible for the observed free-radical scavenging and antioxidant effects of dates (Ferguson et al., 2004).

2.14 Purpose of project

Although there is a significant literature on the composition of ripe date fruit in general there is relatively little in relation to those varieties as grown in Saudi Arabia. Evidence suggests that variety, environmental conditions during cultivation and stage of ripening could all impact significantly on the nutritional status of the fruit. The impact of environment and stage of development in particular have not been extensively researched previously.

The work presented in this thesis will therefore determine the nutritional composition of nineteen varieties of dates sourced from four, environmentally diverse, regions in Saudi Arabia. Moreover, this research will also determine the antioxidant capacity of a sub-set of these varieties at four different stages of ripening. To achieve this purpose, the researcher will determine the nutritional content of nineteen varieties of date in Saudi Arabia. The sampling of the dates will be varied to include dates from for environmentally diverse regions in Saudi Arabia. The researcher will then determine and compared the antioxidant activity and the total phenolic content in the dates at different stages of development.

There is no standard method for measuring antioxidant activity. Since the long term aim of this project was to focus on phenolics TPC was chosen as this assay gives an indication of the phenolic level as well as being an antioxidant assay. It is normal procedure however, to use at least two independent antioxidant assays thus FRAP was also employed since this was a technique that was already established in the laboratory.

Chapter 3 Material and methods

3.1 Plant material

In this section two different screens were carried out:

Initial screen- This was a preliminary screen designed to investigate the range of nutrient content in commercially available date fruit across Saudi Arabia. Dates were collected of different genotypes, from commercial markets from 4 different regions. Only one sample of dates was collected in each case and analysis was carried out on mixed samples in order to obtain as accurate a mean as possible. Since the exact source of each sample was not known variations due to soil, climate, harvest and transport could not be assessed. However, preparation and analysis of the dates was fully controlled. However, since the samples from each region were likely to have been subject to similar conditions impact of genotype could be partially implied. Comparisons between the 4 regions could also be used to give a general idea of any potential impact of environmental conditions.

Detailed analysis; From the preliminary screen TPC, FRAP and sugar composition were selected as the three nutritional parameters for further investigation. The preliminary screen allowed the identification of genotype/region combinations of date samples showing high and low levels of either TPC or FRAP or diverse sugar compositions. For this analysis date samples were collected from six different commercial markets and analysis carried out in triplicate on individual fruit of dates.

Thus, preparation and analysis of the dates was again tightly controlled. The exact source of the dates (actual farm), harvest procedure and transport are unknown but using dates from six different markets may indicate or reduce variation due to these parameters. The main variation tested in this instance will be genotype.

Dried dates (Barhi, Rushodia, Safawi, Segae, Ajwa, Anbara, Khodry, Daglet Noor, Rushodia, Ruthana, Sukkari, Nabtat Ali, Khodry, Thawee, Sullaj, Segae, Nabtat Ali, Khalas, Khalas, Ruzeiz, Shebebi, Shahal, Shaishee And Hilali) were obtained from local shops from Saudi Arabia; for more details see Appendix A. All dates were stored at 5ºC before analysis.

Triplicate samples of the dates were opened and the stones removed before the flesh was ground up coarsely in a domestic food processor (Kenwood Gourmet, model FP310; Kenwood Ltd, Hampshire, UK) as described by Kirk & Sawyer, 1991. Samples

were dried in a vacuum oven at 70ºC to constant weight (Kirk & Sawyer, 1991). After drying, they were ground to a powder using a pestle and mortar and stored in sealable polythene bags in a desiccator.

Fresh date samples were harvested from the Date Palm Research Center (DPRC) of King Saud University in Riyadh, Saudi Arabia during the summer seasons of 2011and 2012. Seven popular varieties of dates were selected (Barhi, Ajwa, Khalas, Nabtat Seif, Khodry, Sukkari and Segae) and fruit from three palm trees of each variety were sampled. Fruits were stored at -20°C until required. Dates were harvested at four different stages of maturity: Khalal (Fresh), Rutab (Semi-fresh), Tamar (Semi-dry) and Tamar (dry). Identification of each variety was visually verified by the experienced farmers working in the Centre.

Arabic terms are used to describe developmental and maturation stages of the date (Barreveld, 1993). Khalal (fresh) is the unripe but full-colored (yellow or red) date with a hard texture shown in figure 3.1(A). Rutab (semi-fresh) is the ripening stage, characterized by tissue softening, half colour changes and the start of the loss of astringency shown in figure 3.1(B). Tamer (semi-dry) is the last ripening stage, showing full colour changes to light or dark brown and complete loss of astringency shown in figure 3.1(C). Tamer (Dry) is the fully softened but dried dates that contain the maximum total solid with high keeping quality shown in figure 3.1(D). Note that the dates' varieties were dried by the sun. The temperature ranged between 35ºC to 40ºC and the samples were exposed to indirect sunlight for about a week. Typical examples of each stage are shown in Figure 3.1. During the grinding 200 ml of extraction solution was added for TPC and Anthocyanin.

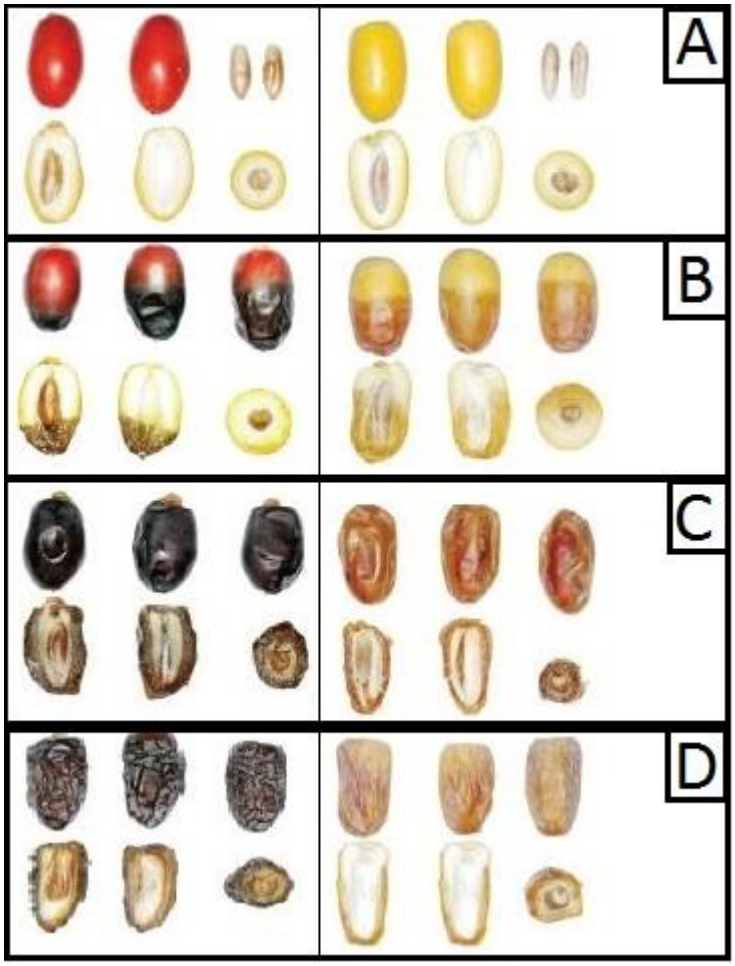


Figure 3.1: Typical ripening stages of date fruit. (A)Khalal (fresh), (B) Rutab (semi-fresh), (C) Tamar (semi dry) and (D) Tamar (dry).

Following the results from the preliminary screen sub-sets of the region/variety of dates were selected for a more in-depth study (Table 3.1). The physical characteristics of the date varieties selected are shown in Appendix 1. The varieties were selected to include those with the highest and lowest values for antioxidant activity, phenolic content and sugar composition. For each variety a sample was collected from six independent markets and then assays were carried out on individual dates from each market sample as appropriate.

Table 3.1: Date varieties used in new screen

varieties
Khondry
Rushodia
Shalahal
Sullaj
Daglet Noor
Anbra

One fruit of dates from each market were then analysed for the highest and lowest variety for each appropriate parameter. In addition, a further date from each of the six other collected samples was also analysed for all parameters.

The following nutritional parameters were chosen for this more in-depth study: (a) Antioxidants are one of the components that naturally occur in plants. The amount of antioxidants in a food item, probably a fruit or vegetable, is important and of interest to consumers and food scientists. This is because antioxidants are thought to protect the body from the effects of free radicals, in combination with other physiological mechanisms. As such, the knowledge of the antioxidant composition of different foods can help in choosing the food items to include in one's meal (Silva et al., 2006). (b) Phenolic acids are other compounds of interest to food consumers. Ozcan et al. (2014) reported that these phytochemicals have immense health benefits, some of which include antimicrobial and antioxidant properties. These properties can help protect one from the effects of degenerative diseases, allergies, infections, and inflammations (Ozcan et al., 2014). It is these properties that make the total phenolic compound in a food item a measure of interest to consumers. And (c) sugars, glucose is one form in which carbohydrates are absorbed by the human body. A food item that contains a high amount of glucose is of interest because it readily provides the energy in body upon consumption. The presence of glucose means that even before the rest of the food item is digested, the glucose can be absorbed readily to provide the body with the necessary components for energy metabolism. Fructose is another of the simple sugars in addition

to glucose that are found in many fruits. The amount of fructose in a fruit is a predictor for the total sugar content. As a result, its determination is important and of interest to consumers. The six varieties of the date palm fruit were analyzed to determine the percentage concentration of sgars.

3.2 Compositional analysis

3.2.1 Proximate composition analysis

For the purpose of this thesis the date samples were analyzed using methods as described by the Association of Analytical Chemists in Official Methods of Analysis; 16th ed;(AOAC 1995)). For moisture, the vacuum oven method 934.06 was used, for protein the Kjeldhal nitrogen method 920.152 was used, for ash method 940.26 was used and for minerals method 985.35 was used. For fat method 945.16 was used as described in the 15th ed (AOAC 1990).

Unblemished dates were selected and washed. The fruit were weighed then the calyxes and seeds were removed, and the remaining pulp cut into small pieces and 100 g homogenized in a blender (Waring Commercial Blender Single-Speed w/ Stainless Steel Jar) and stored in fridge at 5^{o}C.

3.2.1.1 Moisture

The moisture content in dates was determined using a vacuum oven, (Heraeus RVT 360) based upon the assay described by AOAC, 1995, The lower temperature was used to remove the moisture (70^{o}C for 48h) so problems associated with degradation of heat-labile substances could be reduced.

Drying containers were placed in an oven at 100°C for one hour, allowed to cool in a desiccator for 30 min and weighted (W1) 5 gm of fresh date into a clean and dried container and weighted (W2). Then the samples were dried in the vacuum oven at 70°C < 25 mm Hg for 48 hours, the container was transferred to the desiccator for 30 min to cool and reweighed (W3).

The percentage moisture was calculated by the following formula:

$$\% \text{ Moisture} = \frac{W2 - W3 \times 100}{W2 - W1}$$

After samples had dried, they were ground to a powder using a Waring Commercial Blender Single-Speed w/ Stainless Steel Jar and stored in sealable polythene bags in a desiccator until required for further analysis.

3.2.1.2 Fat

Fat content was measured using a Soxhlet extractor. The dry material is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The extraction solvent to be used is taken into a distillation tube and the Soxhlet extractor is placed onto this tube. The Soxhlet is then equipped with a condenser.

First empty extraction tube was weighted (W2). Then 200 ml of Petroleum ether (boiling range 40-60°C) and 5g of moisture free date sample (W1) was wrapped in filter paper and placed in to clean thimble and then introduced in the extraction tube and boiled for 16 hours.

Then the extraction tube is put into the Soxhlet apparatus. Turned on water and heater to start extraction and boiled for 16 hours. Transferred extract tube with ether washing and evaporated ether on water bath. Then placed the extraction tube including fat (W3) in an oven at 105^{o}C for 2 hours and cooled it in a desiccator at room temperature.

Calculation

The percentage of fat extracted is equal to the difference between the weights of the tube before extraction of the sample and after extraction of the sample divided by the original weight of the sample, ×100:

$$\%\text{Fat extract} = \frac{(W3 - W2)}{W1} \times 100$$

Where:

W1=Weight of sample

W2=Weight of dried empty tube

W3=Weight of dried extraction tube after fat extraction

3.2.1.3 Protein

Protein in the samples was determined by the Kjeldahl method using Markam Still Distillation Apparatus (Khalil and Manan, 1990).

Chemicals and reagents

Sulphuric acid, concentrated, A.R. grade,

Catalysts: Potassium sulphate and copper sulphate,

Sodium hydroxide solution, 50%. Weigh 500 g sodium hydroxide, dissolve in water and make up to 1 L with H_2O,

Ammonium sulphate, A.R

Hydrochloric acid, 0.1 N. Pipette 8.3 mL of concentrated hydrochloric acid to approximately 500 mL distilled H_2O in 1 L volumetric flask soaked in ice cold water. Allow to cool and make up to 1L with distilled H_20,

Sodium tetraborate decahydrate (borax) AR: 0.47g was weighed accurately into a 250mL conical flask, then dissolved in about 50mL water and a few drops of methyl red indicator solution added. The sample was then titrated with the hydrochloric acid from a burette until the colour changed to pink at the endpoint. [Methyl red indicator solution: dissolve about 1g of methyl red in 600mL ethanol and dilute with 400 mL water.]

Measurements

One g of dried samples was placed in a digestion flask. Then 10-15 ml of concentrated H_2SO_4 and 8 g of digestion mixture i.e. K_4SO_4: CuSO4 (8: 1w/w) was added. The flask was swirled to mix the contents completely then placed on a heater until the mixture became a clear blue green colour. Two hours was needed to complete this stage. The digest was cooled and transferred to a 100 ml volumetric flask and volume made up to the mark by adding distilled water. Ten milliliters of digest was placed in the distillation tube and 10 ml of 0.5 N NaOH was slowly added. Distillation was carried out for a minimum of 10 mins and the NH3 produced collected as NH_4OH in a conical flask containing 20 ml of 4% boric acid indicator solution. Then the distillate was titrated

against 0.1 N HCl solution until the pink colour appeared. A blank sample was routinely run. Percentage of protein of the sample was calculated using the following formula:

$$\%N = \frac{(S - B) \times N \times 0.014 \times D \times 100}{\text{Wt. of the sample } \times \text{ V}}$$

% Protein = 6.25 × %N

Where

S = Sample titration reading

B = Blank titration reading

N = Normality of HCl

D = Dilution of sample after digestion

V = Volume taken for distillation

0.014 = Milli equivalent weight of Nitrogen

6.25 = Correction factor)

3.2.1.4 Carbohydrate

Total carbohydrate composition was estimated by subtracting the sum of the contents of moisture, protein, lipid, and ash from 100% as described by Al-Farsi et al., 2008.

3.2.1.5 Sugars

Sugar content (glucose, fructose and sucrose) were measured using either HPAEC (in-depth analysis) or HPLC (preliminary screen) methods.

There are some significant differences between the new and preliminary screen. The differences are attributed to the differences in the methods and procedure used in the measurement of the parameters. For the preliminary screen, fruits from the same variety were mixed and weighed at 100 grams. The assays were then formulated and the measurements for sugar composition. For the in-depth analysis, a sample of individual fruits from the same varieties were used to formulate assays that were then

analyzed for sugar composition. An average for the six assays of the six fruits from the same variety will collected from six different markets was then determined.

3.2.1.5.1 Sugar analysis using high performance anion exchange chromatography (HPAEC)

Sugars were extracted by homogenising one g of date tissue in 10mL of deionised water. The samples were then centrifuged at 3000g for 20min. Samples were diluted 1:1000 with 50mM NaOH before being analysed by HPAEC. Glucose, fructose and sucrose were analysed using Dionex ICS-3000 Reagent-FreeTM Ion Chromatography equipped with Dionex ICS-3000 system, electrochemical detection using ED 40 and computer controller. The CarboPac^{TM} PA 20 column (3 x 150 mm/; Dionex, USA) was used and the mobile phase was 50 mM NaOH with a flow rate of 0.5 mL/min. The injection volume was 10 µl and the column temperature was 30°C. The column was flushed at 1.0 mL/min with 200 mM NaOH between runs for 10 min to remove any carbonates bound to the column. Standard sugar samples were prepared from a stock consisting of 1g of each sugar dissolved into 1L of deionised water, which was then diluted to create 2gL-1, 1gL^{-1}, 0.5gL^{-1} and 0.25gL^{-1}. Quantification of the sugars in the samples were accomplished using the standard curves prepared from relative peak areas of the standard sugars. A typical HPAEC trace is shown in figure 3.2

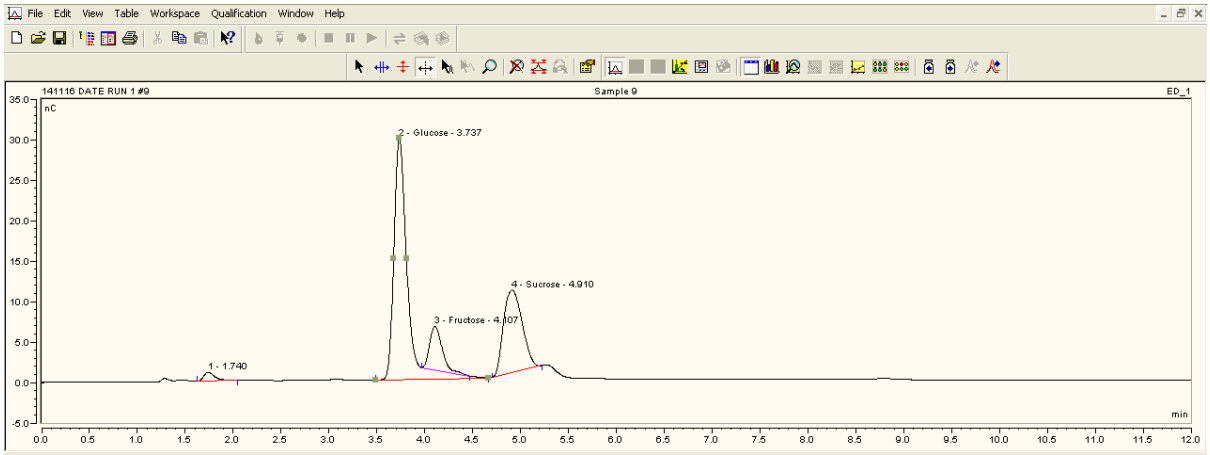


Figure 3.2 Typical HPAEC trace of sugars in date extracts

3.2.1.5.2 HPLC analysis of sugars

Chemicals and reagents

Reagent-grade water, Type I, 18 MΩ-cm resistance, filtered through a 0.2 μm filter immediately before use. Sodium acetate, anhydrous (Fluka P/N 71183). Sodium hydroxide, 50% (Fischer P/N SS254-500).Potassium hexacyanoferrate (III), ACS reagent, ≥ 99% powder (Sigma-Aldrich P/N 393517). Zinc sulfate, monohydrate (Sigma-Aldrich P/N 96495). D-glucose (Sigma-Aldrich P/N G-5250). Sucrose ; 4.8-grade, 99.998%. All chemicals and reagents used were analytical grade.

Measurements

Five gram of date was homogenized in 300 ml of distilled water using (Gowe® Digital Display Electric Lab Mixer Lab Mixing AM200S-H 30L 50-1800rpm), heated at 100^{o} C for 30 min and then 1 gram of $CaCO_3$ added, 3 ml of lead acetate and a further 500 ml of distilled water was added. The sample was then homogenized for 5 min using (Gowe® Digital Display Electric Lab Mixer Lab Mixing AM200S-H 30L 50-1800rpm) and centrifuged at 3000g for 20 min at room temperature. The clear extract (50 ml) was collected and 5g of potassium oxalate was added then it was filtered using a 0.45μm filter. Sugar standards and sample solutions (10 μL), were then analysed by HPLC.

Sugars were estimated by HPLC, according to the method described (AOAC, 1995)

The system consisted of an Injector (Model SIL-10A,), pump (LC-10 AD) and refractive index detector (RID-6A) all from Shimadzu, Kyoto, Japan. Samples were separated using a Supelcosi LC-NH2 (Supelco, Bellefonte, PA) column (4.6 ×250 mm). The mobile phase was acetonitrile/water (80/20 v/v) at an average flow rate of 2.5 ml / min. Sample was run for 15 minutes. Peak areas were calculated from calibration curves generated by dilution of a 2% standard solution and sugar contents determined using an integrator type C-R7A (Shimadzu Chromatopac Data Processor).

Calculation

Amount of each sugar (g/100g) = (ASP * CSTD / A STD) * (V/W)

Where: ASP = area/peak height of each sugar in sample solution

ASTD = area/peak height of sugar standard

CSTD = concentration of sugar standard (g/100 mL)

V = total volume of prepared sample solution (mL)

W = weight of sample (g)

Results are reported to the nearest 0.1 g/100 g

3.2.1.6 Ash

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, and provides an indication of the total amount of minerals present.

Dry ashing method was used in this thesis. In dry ashing, the dates are incinerated in a crucible made of silica, porcelain or platinum. The date matrix must be destroyed by heating gently at first to char the sample and then at 500°C in a muffle furnace (Wills, Balmer and Greenfield, 1980) to prevent foaming of lipids and sugars, until a white or light-grey residue is produced. The common procedure is described by Osborne and Voogt (1978) and in the AOAC Official Methods (sees Sullivan and Carpenter, 1993).

All the crucibles were prepared by first washing using soap and water and then soaked in 50% nitric acid for 18 hours. Crucibles were then rinsed using distilled water and soaked in 50% HCL Hydrochloric acid for 18 hours. Finally, all the crucibles were washed and rinsed using deionized water and dried in an oven at 105°C for half an hour. All the crucibles were then placed in a desiccator for 30 min to cool, after which the crucibles were weighed (W1).

Measurements

The dates were mashed and one gram of each sample placed in a crucible and weighed (W2). The dates are then gradually heated in a furnace at temperatures up to 550°C, starting from 100°C for half an hour, then 200°C for half an hour and then to 550°C for 5 hours; this total process took two to three hours, then the muffle furnace was switched off and crucibles left to cool before being weighed (W3).

Calculations

Percent of ash is calculated by following formula:

$$\%\text{Ash} = \frac{\text{Differencein Wt. of Ash} \times 100}{\text{Wt. of sample}}$$

Difference in wt. of Ash= W3 –W1

Wt of sample W2

Report test results (in g per 100 g sample) to one decimal place.

3.2.1.7 Energy

Energy was calculated by multiplying carbohydrate by 3.75, protein by 4 and fat by 9 (M. A. Al-Farsi and C. Y. Lee). Then the calculated values were converted to calories per 100 gm of the sample.

Energy calculation:

Energy (kcal/100g) = carbohydrate (g/100g) × 3.75 + protein (g/100g) ×4 + fat (g/100g) × 9

3.2.1.8 Minerals

The mineral content was determined by either Flame photometer or inductively coupled plasma, ICP following dry wet digestion as described by (Allen, 1975).
Dates were dried in an oven at 70°C, and the dried samples ground into a fine powder using a mestle and mortar. The powder samples were stored in air sealed plastic containers at room temperature until analyzed. 0.5 g of sample was placed in a 250 mL beaker. Then the sample was digested by the addition of 3.5 ml of 95%concentrated Sulphuric acid and 2 ml of 30% Hydrogen peroxide (H2O2) and heating at 250°C for 30 min. The beaker was covered by watch glass (to avoid losses by excessive reaction) during the heating. . After cooling, 1 ml of 30% H2O2 was added and the beaker again covered with watch glass, and heated slowly until the effervescence subsided. After cooling, a further 7 mL of 30% H2O2 was added. The samples were then refluxed without boiling for an additional 15 minuntil the samples went clear. Samples were transferred to a 50 mL volumetric flask, made up to volume with deionized distilled water.
Standard solutions were prepared as follows:

For elements estimated on the ICP

* Second point	*First point	Element
10.0	1.00	Ca
10.0	1.00	Mg
10.0	1.00	Fe
1.00	0.2	Cu
1.00	0.2	Mn
1.00	0.2	Se
1.00	0.2	Zn

*All concentrations in 1 mg (ppm)

For elements estimated on the Flame photometer device:

1- For K:

Concentration (mg/l)	0	10	20	40	80	100
Reading	0	17	32.5	55	87	100

2- For Na:

Concentration (mg/l)	0	10	20	40	100
Reading	0	22.5	37	57.5	100

Measurements

The samples after digest were analyzed to determine the content of Ca, Mg, P, Fe, Cu, Zn, Mn and Se by inductively coupled plasma (ICP) and K, Na by Flame photometer which carried out by technicians in the soil department at King Saud University.

3.3 Total phenolic content (TPC)

This method was adapted from Spanos and Wrolstad, (1990) and based on the original method of Singleton and Rossi, (1965) as slightly modified by Wilson, (2005).

Three fruit were cut into small pieces and mixed together, and then one gram was taken, containing both flesh and skin, and homogenized using a Polytron in 15ml of Methanol. Samples were then the centrifuge at 3000g for 30 min at room temperature. The clear extract was collected and used for the estimation of TPC values. Three replicates for each cultivar were tested.

Gallic acid was used to prepare a standard curve. A stock was made up daily by dissolving 50 mg gallic acid into 1 ml ethanol in a volumetric flask and making this

up to 50ml with distilled water. This stock solution was then used to prepare a range of standards as shown in table 3.2.

Table 3.2 Gallic acid standard solutions

Concentration gallic acid GA	200	400	600	800
Gallic Acid (ml)	0.2	0.4	0.6	0.8
water (ml)	0.8	0.6	0.4	0.2

Duplicate 0.1 ml samples of both the gallic acid standards and unknowns were placed in test tubes and 0.6 of distilled water added. 0.5 ml of Folin-Ciocalteu reagent was added into each tube. Then the tubes were covered and mixed for 30 seconds and left for 6 min at room temperature. After 6 min, 1.5 ml of Na2CO3 (7.5 g / 100 d H2O) was added to all tubes. The tubes were covered and mixed for 30 seconds. Then the tubes were placed in a water bath set to 75 °C for 2 hours. After cooling to room temperature, absorbance was read at 765 nm. Results were expressed as mg Gallic acid equivalents (GAE)/ 100 g of fresh weight (FW).

3.3.1 HPLC

Chemicals and reagents

Acetate buffer 300 mM pH 3, sodium acetate trihydrate and glacial acid.

Extraction

Three date fruit of each variety and stage of development were cut into small pieces and well mixed. One gram of the edible part (flesh and skin) was homogenized using a Polytron mixer in 50ml of 300 mM acetate buffer (pH 3). These samples were put in the centrifuge at 3000rpm for 20 minutes at room temperature. The clear extract was collected and used for the estimation by HPLC.

Measurements

An HPLC system was used that consisted of a model Perkin Elmer 200 series HPLC with diode array detector set at 280nm, equipped with an automatic injector (10µl injection volume), Supelco (Bellefonte PA, USA) Discovery C18 column 15cm long, 4.6mm internal diameter, particle size 5µm. Equilibration to starting conditions 80%

0.02M sodium phosphate buffer pH 2.4: 20% methanol for 15min at 1ml/min, then changing to 80% methanol: 20% buffer on a linear gradient over 25min at 1ml/minute operating at room temperature. Dionex Chromeleon software was used for data capture and analysis.

3.3.2 Anthocyanin determination

This method was based on that of Guisti and Wrolstad (2001) using pH-differential method. One gram of date was homogenized in 80 ml of distilled water using (Gowe® Digital Display Electric Lab Mixer Lab Mixing AM200S-H 30L 50-1800rpm) for one min then sonicated for fifteen min. Particulates were removed by centrifugation at 1500g for 10 min. Clear extract (1 mL) was placed into a 25 ml volumetric flask, made up to a final volume 25ml with pH 1.0 buffer solution (1.49 g of KCl/100 mL of distilled water) and mixed. Another 1 mL of extract was similarly placed into a 25 mL volumetric flask, made up to a final volume 25ml with pH 4.5 buffer (1.64 g of sodium acetate/100 mL of distilled water), and mixed. Then the absorbance was measured in a UV-1601 Shimadzu spectrophotometer (Shimadzu) at 510 nm and at 700 nm. Then it was calculated as Ab = (A510nm - A700nm) pH1.0 - (A510nm - A700nm) pH4.5 with a molar extinction coefficient for cyanidin 3-glucoside of 26900. The results were used in the following equation and made as milligrams of cyanidin 3-glucoside equivalents per 100 g of fresh weight.

$$anthocyanins\ (mg\ c\text{-}3\text{-}gE/100\ g) = \frac{Ab}{eL} \times MW \times D \times \frac{V}{G} \times 100$$

Where *Ab* indicates absorbance, *e* is cyanidin 3-glucoside molar absorbance (26900), *L* is cell path length (1 cm), *MW* abbreviates of molecular weight of anthocyanins (449.2), *D* is a dilution factor, *V* is the final volume (mL), and *G* is the sample weight (mg).

3.3.3 Depth Analysis

There were some differences between the new and preliminary screen in sample preparation for TPC and FRAP assays. In preliminary screen 100g of fruit from

the same variety were mixed and then replicate samples taken. However, in the new screen replications were made using individual fruit from each of the six market samples. An average for the six assays of the six fruits from the same variety collected from six different markets was then determined.

Sample preparation

The six individual samples will then be analysed using the same protocols as for the preliminary screen to give an n=6.

Assays

We will determine Total Phenolic (TPC) and Ferric Reducing Antioxidant Power (FRAP) on all 36 samples collected.
The extractions and assays will follow the same experimental protocols as utilized in the preliminary screen.

3.4. Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay used in this thesis was as described by Benzie and Strain, (1996).

Chemicals and reagents

300mM Acetate buffer (pH 3.0) was prepared by mixing 0.94g sodium acetate anhydrous, 8ml glacial acetic acid, and 492ml water.
10mM [2, 4, 6-Tripyridyl-s-Triazine (TPTZ)] solution was prepared by dissolving 0.156g TPTZ in 5ml of 40mM HCl.
20mM Iron III Chloride Hexahydrate was prepared by dissolving 0.0271g in 5ml distilled water.
FRAP reagent was prepared fresh by adding 25ml of Acetate buffer (300 mM) to 2.5ml TPTZ(10mM) and 2.5ml Iron III Chloride hexahydrate (20mM).
Trolox (2mM) solution was prepared by dissolving 5mg of Trolox in 20ml ethanol and used to prepare 0.1mM-1mM trolox standards as shown in table 3.3.

Table 3.3 Trolox standards for the FRAP assay

Concentration of trolox (mM)	0.2	0.4	0.6	0.8	1.0
Volume of 2mM trolox (ml)	0.2	0.4	0.6	0.8	1.0
Volume of ethanol (ml)	0.8	0.6	0.4	0.2	0.0

One gram of the edible part of the date was cut into small pieces and homogenized using a Polytron in 50ml of 300 mM acetate buffer (pH 3). Samples were then centrifuged at 3000xg for 20 min at room temperature. The clear extract was collected and used for the estimation of FRAP values.

First 100µl of FRAP reagent was added to each well of a 96 well microtitre plate. Then 10µl of either standard or extracted sample were added in duplicate. The plate was leaved for 5 minutes to incubate at room temperature and then it was read at 630 nm in a plate reader.

Results are expressed as mmol trolox equivalence (TE) /100g fresh weight.

3.5 Statistical analysis

Statistical analysis was carried out by one-way ANOVA using SPSS software. ANOVA is a statistical technique that assesses potential differences in a scale-level dependent variable by a nominal-level variable having 2 or more categories. A one-way ANOVA has just one independent variable which in this thesis was (nutrient level. In conducting an ANOVA, there is an attempt to determine if there is a statistically significant difference among the groups (varieties, regions and ripening). Where a significant difference is indicated further analysis is required to determine exactly where the group differences lay.

At this point post-hoc t tests, examining the mean differences between the groups, are carried out. There are several multiple comparison tests that can be conducted that will control for Type I error rate, including the Bonferroni, Scheffe, Dunnet, and Tukey tests. In this thesis Tukey tests was used.

The analysis was carried out using SPSS. The groups for analysis by creating a grouping variable called either region, nutrient variable and ripening (the independent variable), giving the samples -Barhi_Madina a value of "1", Rushodia_Madina a value of "2" and Safawi_Madina a value of "3" and so on for the 21 other samples until Hilali_Eastern with a value of 24. To complete the test, the

dependent variable was selected such as (sugar, FRAP, etc.). After this one-way ANOVA selected, Post Hoc multiple comparisons were selected then Tukey was selected with significance level of 0.05 to compare one selected variety with other in all regions or between the same regions. After the tables were created, mean difference and significance and standard deviation were presented. Descriptive option was selected to show number of replications, means and standard deviation.

There were different stages of the statistical analysis in this thesis. firstly, it is important to identify the problem of scientific research and the objective of the scientific research and its purpose as a comparison between two or more phenomena, the interconnection between phenomena, and studying the differences between two phenomena and their effect on the human or the environment. In this thesis it is important to consider the locally available dates and to determine their nutrient composition for increasing the production of such fruits that may be particularly beneficial. Then, determination of statistical aspect of the relevant problem such as case study in order to collect the largest amount of information about the phenomenon. After that data and variables are categorized into groups. As in this thesis it is important to determine the nutritional composition of nineteen varieties of dates sourced from four, environmentally diverse, regions in Saudi Arabia. These varieties are well known because of their common preference, popularity, economic price, as well as the high availability during the year. As the impact of environment and stage of development have not been extensively researched previously.

Finally, descriptive results are presented with a clear and understandable explanation. In this thesis standard deviation is used because we want to assess how widely distributed some measurements are not indicate the uncertainty around the estimate of the mean measurement, so we do not need the standard error of the mean. Standard deviation is a measure of variability. We calculated the standard deviation of a sample as an estimate of the variability of the population from which the sample was described. Note that the standard errors were very small.

The results were prepared as averages of three replicates ± standard deviation; also a one-way ANOVA test and Tukey test were used to show the differences among varieties of each stage and also between varieties of each region. The difference was considered significant at $P \leq 0.05$. Duncan's test ($p < 0.05$) was used to determine significant differences between means and Pearson's test was employed to perform the correlation analysis.

Chapter 4 Composition of Saudi Arabian Date Fruit

4.1 Nutritional value of dates compared to other fruit

Dates represent a significant staple in some regions of the world and some data banks have directly compared the nutritional composition of dates with similar dried food and nuts (USDA 2008). However, similar comparisons of dates to other fruit do not seem to be available on the databases. Thus in the first instance a comparative data base was prepared using data from McCance and Widdowsons The composition of foods (2014). Tables 4.1 and 4.2 show this comparison between the nutritional value of dates and other fruits either fresh or dried.

A comparison using these tables shows that dates have good nutritional value when compared to some other fruits such as apple, apricots, figs, graps, pears, peaches and pineapple.

Table 4.1: Nutritional value of fresh dates and other fresh fruits

Fresh								
Proximates	Apples	Apricots	Dates	Figs	Grapes	Pears	Peaches	Pineapple
Water (g)	84.5	87.2	60.7	84.6	81.8	83.8	88.9	86.5
Total nitrogen (g)	0.06	0.14	0.24	0.21	0.06	0.05	0.16	0.06
Protein (g)	0.4	0.9	1.5	1.3	0.4	0.3	1.0	0.4
Fat (g)	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.2
Carbohydrate (g)	11.8	7.2	31.3	9.5	15.4	10.0	7.6	10.1
Energy (kcal) (kcal)	47	31	124	43	60	40	33	41
Total sugars (g)	11.8	7.2	31.3	9.5	15.4	10.0	7.6	10.1
Glucose (g)	1.7	1.6	16.2	5.2	7.6	2.3	1.1	2.0
Fructose (g)	6.2	0.9	15.1	4.1	7.8	7.1	1.1	2.5
Sucrose (g)	3.9	4.6	Tr	0.3	0.1	0.7	5.2	5.5
NSP (g)	1.8	1.7	1.8	1.5	0.7	2.2	1.5	1.2
Inorganics								
Sodium (mg)	3	2	7	3	2	3	1	2
Potassium (mg)	120	270	410	200	210	150	160	160
Calcium (mg)	4	15	24	38	13	11	7	18
Magnesium (mg)	5	11	24	15	7	7	9	16
Phosphorus (mg)	11	20	28	15	18	13	22	10
Iron (mg)	0.10	0.50	0.30	0.30	0.30	0.20	0.40	0.20
Copper (mg)	0.02	0.06	0.12	0.06	0.12	0.06	0.06	0.11
Zinc (mg)	0.1	0.1	0.2	0.3	0.1	0.1	0.1	0.1
Chloride (mg)	Tr	3	210	18	Tr	1	Tr	29
Manganese (mg)	0.10	0.10	0.20	0.10	0.10	Tr	0.10	0.50
Selenium (µg)	Tr	(1)	(1)	Tr	(1)	Tr	(1)	Tr
Iodine (µg)	Tr	N	N	N	1	1	3	Tr
Vitamins								
Carotene (µg)	18	405	(18)	(150)	17	18	114	18
Retinol Equivalent (µg)	3	67	(3)	(25)	3	3	19	3
Vitamin D (µg)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vitamin E (mg)	0.59	N	N	N	Tr	0.50	N	0.10
Vitamin K1 (µg)	5.60		5.60		8.60	3.60	5.80	0.21
Thiamin (mg)	0.03	0.04	0.06	0.03	0.05	0.02	0.02	0.08
Riboflavin (mg)	0.02	0.05	0.07	0.03	0.01	0.03	0.04	0.03
Niacin (mg)	0.1	0.5	0.7	0.4	0.2	0.2	0.6	0.3
Tryptophan/60 (mg)	0.1	0.1	0.7	0.2	Tr	Tr	0.2	0.1
Vitamin B6 (mg)	0.06	0.08	0.12	0.08	0.10	0.02	0.02	0.09
Vitamin B12 (µg)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Folate (µg)	1	5	25	N	2	2	3	5
Pantothenate (mg)	Tr	0.24	0.21	0.22	0.05	0.07	0.17	0.16
Biotin (µg)	1.2	N	N	N	0.3	0.2	(0.2)	0.3
Vitamin C (mg)	6	6	14	2	3	6	31	12

1. Nutrient values within round brackets, ( ), are estimated.
2. A trace value for a nutrient is represented by Tr.
3. Where a nutrient is present in significant quantities, but there is no reliable information on the amount, the value is represented by N.
4. Footnote information is appended to the field to which it applies and is enclosed in square braces, [ ], and is separated from the corresponding value by three space characters.
Source: McCance and Widdowsons The composition of foods (2014)

Table4.2: Nutritional value of dried dates and other dried fruit

Dried Proximates	Apples	Apricots	Dates	Figs	Pears	Peaches	Pineapple	Raisins
Water (g)	21.6	14.7	14.6	16.8	18.4	15.5	9.3	13.2
Total nitrogen (g)	0.32	0.77	0.53	0.57	0.25	0.55	0.41	0.34
Protein (g)	2.0	4.8	3.3	3.6	1.6	3.4	2.5	2.1
Fat (g)	0.5	0.7	0.2	1.6	0.5	0.8	1.3	0.4
Carbohydrate (g)	60.1	43.4	68.0	52.9	52.4	53.0	67.9	69.3
Energy (kcal) (kcal)	238	188	270	227	207	219	276	272
Total sugars (g)	60.1	43.4	68.0	52.9	52.4	53.0	67.9	69.3
Glucose (g)	8.6	20.8	(35.4)	28.6	11.9	(7.9)	13.6	34.5
Fructose (g)	31.7	10.0	(32.6)	22.7	36.8	(7.9)	17.0	34.8
Sucrose (g)	19.9	12.6	Tr	1.6	3.6	(37.1)	37.3	Tr
NSP (g)	9.7	7.7	4.0	7.5	8.3	7.3	8.1	2.0
Inorganics								
Sodium (mg)	16	56	10	62	15	6	13	60
Potassium (mg)	540	1880	700	970	750	1100	1080	1020
Calcium (mg)	16	92	45	250	55	36	120	46
Magnesium (mg)	16	65	41	80	35	54	110	35
Phosphorus (mg)	43	120	60	89	65	120	67	76
Iron (mg)	0.50	4.10	1.30	4.20	1.00	6.80	1.30	3.80
Copper (mg)	0.11	0.40	0.26	0.30	0.30	0.63	0.74	0.39
Zinc (mg)	0.5	0.7	0.4	0.7	0.5	0.8	0.7	0.7
Chloride (mg)	1	35	370	170	5	11	200	9
Manganese (mg)	0.50	0.40	0.30	0.50	0.20	0.80	3.40	0.30
Selenium (µg)	Tr	(7)	(3)	Tr	(2)	(8)	Tr	(8)
Iodine (µg)	Tr	N	N	N	5	23	Tr	N
Vitamins								
Carotene (µg)	91	645	(40)	(64)	91	445	(120)	12
Retinol Equivalent (µg)	15	105	(7)	(11)	15	74	(20)	2
Vitamin D (µg)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vitamin E (mg)	1.45	N	N	N	Tr	N	0.68	N
Vitamin K1 (µg)								3.70
Thiamin (mg)	N	Tr	0.07	0.08	N	Tr	N	0.12
Riboflavin (mg)	N	0.20	0.09	0.10	N	0.19	N	0.05
Niacin (mg)	N	3.0	1.8	0.8	N	5.3	N	0.6
Tryptophan/60 (mg)	0.3	0.6	1.5	0.5	Tr	0.7	0.5	0.2
Vitamin B6 (mg)	N	0.17	0.19	0.26	N	0.10	N	0.25
Vitamin B12 (µg)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Folate (µg)	Tr	14	13	9	Tr	(14)	Tr	10
Pantothenate (mg)	Tr	0.70	0.78	0.51	N	(0.30)	N	0.15
Biotin (µg)	N	N	N	N	N	N	N	2.0
Vitamin C (mg)	Tr	Tr	Tr	1	Tr	Tr	Tr	1

1. Nutrient values within round brackets, ( ), are estimated.
2. A trace value for a nutrient is represented by Tr.
3. Where a nutrient is present in significant quantities, but there is no reliable information on the amount, the value is represented by N.
4. Footnote information is appended to the field to which it applies and is enclosed in square braces, [ ], and is separated from the corresponding value by three space characters.

Source: McCance and Widdowsons The composition of foods(2014)

4.2. Date variety selection and characteristics

The first objective was to assess variability in nutritional composition between the major date varieties as grown in Saudi Arabia. Since a second objective was to explore the potential impact of environmental conditions these were collected from across the country from regions which are characterized by the diversity of climate. The date producing regions of Saudi Arabia can be effectively divided into four environmentally disparate areas that have massive underground water reserves and excellent climatic conditions. The climates in these areas are all hot and dry in the summer and cold in winter.

1 - Al Madina region (M): located in the west of the kingdom and the agricultural area is around 10,019 Hectare. Temperatures are ranging from 28-42 ° C in summer and between 8-22 ° C in winter.

2 - Al Qaseem region (Q): located nearly in the middle of the kingdom and the agricultural area is around 14,597 Hectare. Temperatures are ranging throughout the year between 12 to 41 ° C.

3 - Riyadh region (R): located in the middle of the kingdom and the agricultural area is around 36,861 Hectare. Temperatures are ranging throughout the year between 20 to 50 ° C.

4 - Eastern region (E): (Al-Ahsa) is the largest Oasis in the world, in the Eastern Province of Saudi Arabia. Agricultural area is around 11,697 Hectare. The annual average temperature is 25 ° C. The climate in these areas is hot and dry in the summer and cold in winter.

Six varieties of dates were collected from each of the four regions. Morphologic characteristics of the fruit were taken into consideration for the cultivar collection. The varieties selected from each region are shown in table 4.3. In total 19 different varieties were chosen due to their popularity, commercial importance, and preference in taste and consumption. Where possible samples of the same variety were collected from each region but this was not always possible as each region grows those varieties best suited

to the prevailing conditions. Thus only 5 of the selected varieties were represented in more than one region. Note that these varieties differ in size and colour.

Table4.3: Date varieties, and their regional origin, used in this study.

Al Madina	Al Qaseem	Riyadh	Eastern
Barhi	Khodry	Khodry	Khalas
Rushodia	Daglet Noor	Thawee	Ruzeiz
Safawi	Rushodia	Sullaj	Shebebi
Segae	Ruthana	Segae	Shahal
Ajwa	Sukkari	Nabtat	Shaishee
Anbara	Nabtat	Khalas	Hilali

The physical characteristics of the date varieties selected are shown in Appendix 1.

4.2 Proximate composition analysis

The twenty-four samples of date were collected from four different regions of Saudi Arabia. The dates were collected at harvest maturity and subjected to a proximate compositional analysis to determine moisture, protein, fat, carbohydrate, ash and energy content. One set of dates was collected in each case and the analysis carried out on three repetitions from a sample (500g) pooled from several individual fruit. This represents a preliminary screen of the date varieties and whilst providing an accurate measure of the mean values from each sample cannot, at this stage, be subjected to statistical analysis. A more in-depth follow up study was then undertaken to assess the variability across samples for individual dates and markets for those varieties found to exhibit the highest variation from the preliminary screen.

The results for the preliminary screen are shown in table 4.4. Moisture content varied between the 24 date varieties with a range of between 10 and 30%. The range of moisture contents found within the four regions were quite similar; 10.249-20.271 g/100g with an overall mean of 15.6 in Qaseem dates, 11.685-23.44 g/100g with an overall mean of 16.2 in Riyadh dates, 11.464-29.833 g/100g with an overall mean of 19.0 in Eastern region dates and 11.648-27.935 g/100g with an overall mean of 20.3 in Madina region dates.

Levels of fat, protein and ash all varied between varieties but were all very low. Mean fat content from each of the regions ranged from the lowest value of 0.171g/100g in Madina dates through 0.18g/100g in Qassem dates and 0.23g/100g in Eastern region dates to the highest level of 0.29g/100g in Riyadh dates. Thus, there were no noticeable differences in fat content of dates from the different regions.

Mean protein content of dates from the four regions were 2.31 g/100g, 2.52 g/100g, 2.94 g/100g and 2.97 g/100g in Eastern region dates, Riyadh dates, Madina dates and Qassem dates, respectively. Again, these are all very similar and there were no noticeable variations between regions. Mean ash contents of dates from each of the four regions were again similar at 1.66, 1.77, 1.95 and 2.13g/100g in Qassem dates, Eastern dates, Riyadh dates, and Madina dates, respectively.

Table 4.4: Proximate chemical composition (g/100g fresh weight) of date flesh from twenty-four varieties and four regions

		Moisture	Fat	Protein	Carbohydrate	Ash	Energy (Kcal)
M1	Barhi-Madina	22.35±0.170	0.14±0.049	3.10±0.039	72.97±0.291	1.44±0.116	287.30±1.023
M2	Rushodia-Madina	11.65±0.342	0.22±0.003	2.81±0.064	83.65±0.225	1.68±0.062	326.87±1.076
M3	Safawi-Madina	25.74±0.097	0.11±0.002	2.38±0.082	70.03±0.108	1.73±0.102	273.18±0.092
M4	Segae-Madina	14.01±0.310	0.38±0.038	3.02±0.107	80.27±0.555	2.33±0.145	316.46±1.522
M5	Ajwa-Madina	20.07±0.093	0.50±0.025	2.87±0.038	73.45±0.152	3.11±0.173	291.38±0.382
M6	Anbara-Madina	27.94±0.172	0.49±0.058	3.44±0.087	65.64±0.151	2.50±0.098	264.32±0.562
Q1	Khodry-Qaseem	20.27±0.189	0.16±0.007	3.30±0.085	73.99±0.345	2.27±0.250	292.14±1.546
Q2	Daglet Noor-Qaseem	18.12±0.074	0.48±0.057	2.45±0.101	77.42±0.171	1.53±0.006	304.45±0.497
Q3	Rushodia-Qaseem	10.25±0.195	0.12±0.002	2.92±0.128	85.43±0.371	1.28±0.115	333.11±0.974
Q4	Ruthana-Qaseem	13.72±0.237	0.33±0.005	3.00±0.053	81.75±0.319	1.21±0.086	321.49±1.057
Q5	Sukkari-Qaseem	19.56±0.185	0.66±0.058	2.45±0.108	74.84±0.215	2.50±0.115	296.37±1.256
Q6	Nabtat Ali-Qaseem	11.51±0.237	0.12±0.003	3.66±0.079	83.52±0.338	1.18±0.114	328.95±1.238
R1	Khodry-Riyadh	21.45±0.417	0.17±0.025	3.76±0.056	72.47±0.627	2.15±0.153	288.33±1.933
R2	Thawee-Riyadh	11.69±0.401	0.16±0.004	2.52±0.060	83.97±0.662	1.66±0.286	326.44±2.349
R3	Sullaj-Riyadh	23.44±0.184	0.24±0.009	2.19±0.117	72.47±0.174	1.67±0.231	282.66±1.066
R4	Segae-Riyadh	16.37±0.209	0.44±0.020	2.25±0.185	78.59±0.116	2.36±0.065	307.62±1.000
R5	Nabtat saif-Riyadh	11.97±0.355	0.18±0.003	2.36±0.088	83.36±0.300	2.14±0.035	323.63±1.263
R6	Khalas-Riyadh	12.57±0.050	0.53±0.010	2.07±0.139	83.10±0.267	1.73±0.173	324.69±0.428
E1	Khalas-Eastern Region	15.35±0.186	0.50±0.042	1.62±0.131	80.75±0.304	1.79±0.115	313.77±1.271
E2	Ruzeiz-Eastern Region	21.99±0.109	0.26±0.030	2.71±0.102	73.15±0.170	1.90±0.088	287.48±0.825
E3	Shebebi-Eastern Region	11.46±0.129	0.31±0.003	2.43±0.016	84.00±0.230	1.79±0.153	327.50±0.897
E4	Shahal-Eastern Region	18.87±0.142	0.22±0.004	2.15±0.144	76.90±0.213	1.86±0.114	298.98±0.944
E5	Shaishee-Eastern Region	16.31±0.080	0.16±0.003	2.49±0.087	79.49±0.105	1.55±0.058	309.50±0.082
E6	Hilali-Eastern Region	29.83±0.115	0.11±0.002	2.47±0.077	65.88±0.290	1.71±0.172	257.87±1.037

*Results are expressed as mean values of three determinations ± SD.

Carbohydrate content of the 24 date varieties was calculated as described in the methods as an assumption that this was the remaining mass after correction for moisture, protein, fat and ash. The analysis of the data showed that as expected, the value of carbohydrates was very high in all dates tested but again showed variation between varieties. As such, the averages, and ranges in brackets, for the 6 varieties from each of the four regions were 74.33(65.637-83.645), 76.7 (65.883-80.752), 79 (72.467-83.973), and 81.09 (73.97-85.60)g/100g in Madina dates, Eastern region dates, Riyadh dates and Qassem dates, respectively.

These values, along with those for protein and fat were used to calculate the expected energy content of these dates. The mean energy values, with ranges in brackets, for the four regions were 293.2 (264.316-326.869) in Madina region dates, 299.2 (257.871-327.498) in Eastern region dates, 308.9 (288.331-324.691) in Riyadh dates and 312.8 (292.138-333.109) calorie/ 100 g in Qaseem dates. These mean values appeared to be very close for all regions.

Dates are a good source of rapid energy due to their high carbohydrate content (70 - 80%). Most of the carbohydrates in dates are in the form of fructose and glucose, which are easily absorbed by the human body (Al-Farsi et al., 2007). Thus further analysis of these date samples was carried out to measure the levels of these sugars, along with sucrose.

4.3 Sugar Composition of date varieties

In addition to the proximate composition described above the sugar composition was further investigated by determining the levels of glucose, fructose and sucrose in the 24 varieties of dates. The results are shown in table 4.5.

Table 4.5: Sugar composition of the 24 date varieties from Saudi Arabia

		% Glucose	% Fructose	% Sucrose	% Total Sugar
M1	Barhi-Madina	36.38±0.123	33.46±0.174	00.00±0.000	69.64±0.256
M2	Rushodia-Madina	19.62±0.055	17.47±0.181	33.42±0.169	70.68±0.157
M3	Safawi-Madina	31.32±0.109	30.13±0.167	00.00±0.000	61.45±0.075
M4	Segae-Madina	33.33±0.552	31.28±0.825	00.00±0.000	63.61±1.592
M5	Ajwa-Madina	36.02±0.093	33.21±0.182	00.00±0.000	69.23±0.271
M6	Anbara-Madina	28.98±0.198	25.85±0.144	00.00±0.000	55.50±1.049
Q1	Khodry-Qaseem	34.45±0.069	32.90±0.429	00.00±0.000	66.69±0.423
Q2	Daglet Noor-Qaseem	11.22±0.100	10.37±0.567	49.81±0.671	70.27±1.184
Q3	Rushodia-Qaseem	26.85±0.094	24.89±0.024	19.25±0.045	70.90±0.135
Q4	Ruthana-Qaseem	27.21±0.256	27.69±0.401	04.10±0.084	61.35±1.403
Q5	Sukkari-Qaseem	05.94±0.089	04.47±0.166	52.50±0.038	62.95±0.128
Q6	Nabtat Ali-Qaseem	23.13±0.364	21.40±0.990	19.47±0.693	65.72±1.678
R1	Khodry-Riyadh	36.71±0.235	34.24±0.252	00.00±0.000	71.12±0.072
R2	Thawee-Riyadh	34.71±0.086	32.73±0.063	00.00±0.000	67.44±0.090
R3	Sullaj-Riyadh	40.60±0.237	37.40±0.305	00.00±0.000	78.67±0.670
R4	Segae-Riyadh	36.53±0.337	33.75±0.659	03.27±0.055	73.48±0.164
R5	Nabtat saif-Riyadh	09.43±0.157	07.80±0.007	47.85±0.209	65.29±0.191
R6	Khalas-Riyadh	39.10±0.143	35.19±0.150	00.00±0.000	74.64±0.088
E1	Khalas-Eastern Region	32.57±0.695	29.69±0.141	11.24±0.001	73.83±0.204
E2	Ruzeiz-Eastern Region	34.44±0.080	32.78±0.163	00.00±0.000	67.22±0.095
E3	Shebebi-Eastern Region	19.61±0.071	18.77±0.137	28.43±0.105	66.90±0.150
E4	Shahal-Eastern Region	34.93±0.203	32.40±0.164	00.00±0.000	67.33±0.096
E5	Shaishee-Eastern Region	25.45±0.094	23.31±0.099	20.03±0.297	69.13±0.174
E6	Hilali-Eastern Region	35.32±0.262	33.44±0.158	00.00±0.000	69.33±0.158

*Results are expressed as mean values of three determinations ± SD.

This analysis identified glucose, fructose, and sucrose in the date varieties. However, sucrose was not detectable in all the varieties of date fruits analyzed. Out of the 24 varieties analyzed, 13 of these did not have any sucrose in them. This translates to 54.1% of the varieties that did not have sucrose. This is in keeping with the findings by Mortazavi, Arzani & Barzegar (2010) who reported that the predominant sugars in the date fruit were fructose and glucose.

The percentage of total sugar again varied and ranged from the lowest value of 55.5 g/100g as found in the Anbara-Madina variety to the highest value of 78.7 g/100g in Sullaj-Riyadh. However, we can say that total sugar levels in general of these Saudi dates from the four regions do not differ very much with mean values of 65.02 g/100g

in Madina dates, 66.30 g/100g in Qassem dates, 68.96 g/100g in Eastern region dates and 71.77 g/100g in Riyadh dates.

Table 4.5 indicated that glucose and fructose concentrations in individual varieties were generally similar but varied considerably between varieties ranging from about 5% of each sugar in the Sukkari variety from Qaseem to around 35-40% in many other varieties. There was less variation between regions. The highest mean value for glucose was found in Riyadh dates (32.85 g/100g) followed by Madina dates (30.94 g/100g), Eastern region dates (30.39 g/100g) and Qassem dates (21.47 g/100g). For fructose the corresponding mean values were 30.18 g/100g in Qassem dates, 28.40 g/100g in Eastern region dates 28.57 g/100g in Madina dates 20.2 g/100g in Riyadh dates. It is interesting to note that the ranking for fructose is the inverse that for glucose. As seen from Table 4.5, sucrose was absent in many of the date varieties. The present of sucrose being normally associated with a corresponding reduction in the level of glucose and fructose. The level of sucrose again varied significantly between those varieties that contained this sugar. The highest level (52.5 g/100g) was found in the Sukkari variety from Qaseem, which not surprisingly was the variety that also contained the lowest levels of glucose and fructose. The lowest level (3.27 g/100g) was found in the Segae variety from Riyadh. This variety had levels of glucose and fructose similar to those varieties with no sucrose.

4.4 Mineral composition of date varieties

In this preliminary study, both macro and micro minerals (trace elements) were measured. The content of the major mineral components of the 24 date varieties namely Ca, K, Mg, Na and P are presented in Table 4.6. Values for the trace elements Cu, Fe, Mn, Se and Zn are shown in in Table 4.7.

Table 4.6: Major mineral content of 24 date varieties (results are expressed as mg/100g)

regions	Calcium (Ca)	Potassium (K)	Phosphorus (P)	Magnesium (Mg)	Sodium (Na)
Barhi-Madina	67.49±0.356	904.66±18.945	81.28±0.587	71.57±0.054	3.30±0.103
Rushodia-Madina	83.96±0.157	846.13±14.931	59.04±1.476	65.60±0.237	2.40±0.128
Safawi-Madina	37.47±0.129	739.90±7.502	55.75±1.288	53.18±0.091	2.16±0.181
Segae-Madina	49.69±0.548	819.31±4.047	71.32±0.740	58.62±1.041	1.34±0.023
Ajwa-Madina	43.08±0.326	963.58±22.735	62.47±1.253	65.84±0.105	3.06±0.227
Anbara-Madina	88.87±0.115	1173.29±11.925	66.31±0.957	75.38±0.696	4.34±0.160
Khodry-Qaseem	60.31±0.891	906.73±6.175	53.51±0.408	52.30±0.722	1.33±0.090
Daglet Noor-Qaseem	49.98±0.274	714.90±10.174	67.29±1.398	52.01±1.789	2.43±0.064
Rushodia-Qaseem	64.11±0.314	749.42±7.412	58.26±0.961	53.26±1.593	1.52±0.292
Ruthana-Qaseem	78.39±0.308	768.04±15.716	65.63±1.287	85.62±0.168	1.25±0.079
Sukkari-Qaseem	67.34±0.159	664.94±17.209	77.94±1.428	64.98±0.356	5.13±0.177
Nabtat Ali-Qaseem	47.86±0.102	700.86±8.968	50.13±1.005	44.58±0.318	1.28±0.069
Khodry-Riyadh	54.82±0.999	810.46±11.017	48.07±0.405	47.23±1.207	2.92±0.010
Thawee-Riyadh	73.09±0.496	889.13±6.889	68.36±1.285	55.74±0.317	2.77±0.306
Sullaj-Riyadh	48.61±0.111	831.93±6.537	70.48±0.933	54.49±1.185	1.20±0.213
Segae-Riyadh	49.66±0.049	756.58±8.834	61.51±0.728	51.43±0.918	3.91±0.066
Nabtat saif-Riyadh	50.64±0.310	815.70±21.388	47.66±0.893	35.58±0.798	0.81±0.095
Khalas-Riyadh	45.15±0.384	750.07±6.350	53.56±0.808	54.35±0.824	1.34±0.403
Khalas-Eastern Region	43.85±0.164	749.41±12.885	53.61±1.142	52.46±1.097	0.97±0.056
Ruzeiz-Eastern Region	88.20±0.047	843.73±23.390	50.72±0.755	50.50±1.035	2.92±0.074
Shebebi-Eastern Region	78.36±0.280	860.32±3.092	53.94±0.647	50.35±0.103	3.93±0.031
Shahal-Eastern Region	43.62±0.208	725.77±9.706	37.90±1.307	44.86±1.435	2.90±0.029
Shaishee-Eastern Region	79.65±0.107	730.11±16.892	50.02±1.644	43.64±1.082	1.14±0.133
Hilali-Eastern Region	66.21±0.099	855.93±5.763	60.31±0.932	55.63±1.069	2.91±0.025

*Results are expressed as mean values of three determinations ± SD.

Table 4.7: Trace element content of 24 date varieties (results are expressed as mg/100g fresh weight)

regions	Copper (Cu)	Iron (Fe)	Manganese (Mn)	Selenium (Se)	Zinc (Zn)
Barhi-Madina	0.84±0.062	1.33±0.071	0.60±0.021	0.20±0.015	0.51±0.014
Rushodia-Madina	0.52±0.036	1.49±0.041	0.41±0.012	0.36±0.010	0.49±0.030
Safawi-Madina	0.58±0.056	1.72±0.058	0.58±0.018	0.33±0.026	0.53±0.054
Segae-Madina	0.16±0.023	1.52±0.080	0.40±0.010	0.08±0.015	0.30±0.037
Ajwa-Madina	0.38±0.026	0.99±0.061	0.51±0.017	0.40±0.015	0.31±0.038
Anbara-Madina	0.26±0.066	1.77±0.189	0.43±0.010	0.16±0.026	0.29±0.038
Khodry-Qaseem	0.30±0.062	1.47±0.110	0.55±0.034	0.25±0.020	0.55±0.056
Daglet Noor-Qaseem	0.31±0.051	1.26±0.023	0.41±0.023	0.10±0.030	0.38±0.015
Rushodia-Qaseem	0.58±0.075	1.50±0.105	0.36±0.006	0.00±0.000	0.39±0.017
Ruthana-Qaseem	0.30±0.046	1.44±0.223	0.60±0.021	0.00±0.000	0.51±0.049
Sukkari-Qaseem	0.80±0.089	1.60±0.114	0.52±0.011	0.11±0.036	0.70±0.024
Nabtat Ali-Qaseem	0.27±0.075	1.47±0.058	0.40±0.010	0.00±0.000	0.38±0.019
Khodry-Riyadh	0.32±0.068	1.51±0.158	0.43±0.023	0.21±0.062	0.51±0.040
Thawee-Riyadh	0.95±0.044	1.16±0.042	0.39±0.020	0.00±0.000	0.32±0.015
Sullaj-Riyadh	0.38±0.055	1.09±0.011	0.35±0.020	0.00±0.000	0.37±0.045
Segae-Riyadh	0.11±0.076	1.56±0.094	0.35±0.020	0.04±0.015	0.28±0.022
Nabtat saif-Riyadh	0.75±0.097	1.05±0.029	0.30±0.016	0.19±0.037	0.21±0.030
Khalas-Riyadh	0.78±0.024	1.40±0.207	0.30±0.029	0.07±0.005	0.30±0.034
Khalas-Eastern Region	0.74±0.032	1.30±0.167	0.31±0.013	0.07±0.025	0.28±0.020
Ruzeiz-Eastern Region	0.09±0.026	1.25±0.180	0.44±0.029	0.01±0.010	0.24±0.036
Shebebi-Eastern Region	0.93±0.065	1.38±0.213	0.39±0.035	0.00±0.000	0.35±0.041
Shahal-Eastern Region	0.52±0.076	1.04±0.012	0.33±0.024	0.16±0.035	0.56±0.016
Shaishee-Eastern Region	0.87±0.095	1.06±0.033	0.24±0.036	0.00±0.000	0.34±0.030
Hilali-Eastern Region	0.28±0.030	1.60±0.098	0.38±0.025	0.05±0.006	0.52±0.054

*Results are expressed as mean values of three determinations ± SD.

Potassium was the major mineral found in all the varieties with concentrations as high as 1173.29 mg/100g. The highest regional mean value (907.81mg/100g) and the highest level of potassium (1173.29 mg/100g) were found in Madina dates and the Anbara_variety from the Madina region, respectively. There was variation in potassium content between varieties and this may be reflected by the mean contents of this macro mineral of Riyadh dates (808.97 mg/100g), Eastern region dates (794.21 mg/100g) and Qassem dates (750.81 mg/100g).

Eastern region dates contained the highest mean content (66.65 mg/100g) of calcium followed in descending order by Madina dates (61.76 mg/100g), Qassem dates (61.33 mg/100g) and Riyadh dates (53.66 mg/100g). The lowest value of calcium was found in the Safwai variety from the Madina region (37.47 mg/100g), whereas the highest (88.87 mg/100g) was encountered in Anbra variety from the Madina region. However, in contrast Qassem region dates contained the lowest mean amount of copper (0.42 mg/100g), while the highest value (0.57 mg/100g) was found in Eastern dates; thus may be indicating a reverse relationship between calcium and copper contents of dates resulting from different soils.

The mean values for phosphorus in the dates from the different regions were close to each other ranging from (51.08, 58.27 to 62.12 mg/100g) in Eastern region dates, Riyadh dates and Qassem dates respectively. The highest mean phosphorus content was shown in Madina dates (66.02 mg/100g). As seen in the table 4.7 the amount of selenium was generally very low in all varieties and some did not contain any selenium at all.

A general observation derived from this analysis was that Madina dates appeared to be the richest sources of both macro and micro minerals. Six out of ten measured minerals in this study had their highest levels in Medina dates and two others were second highest in dates from this region. It has been shown that Madina dates are favoured and liked by all Muslims. This is from a religious point and may also possibly be due to the high fertility of the Madina soil.

4.5 Total phenolic content (TPC) and antioxidant capacity of date varieties

The total phenolic content (TPC) and antioxidant capacity (FRAP) of the 24 varieties was also measured. The results are shown in Figures 4.1 and 4.2.

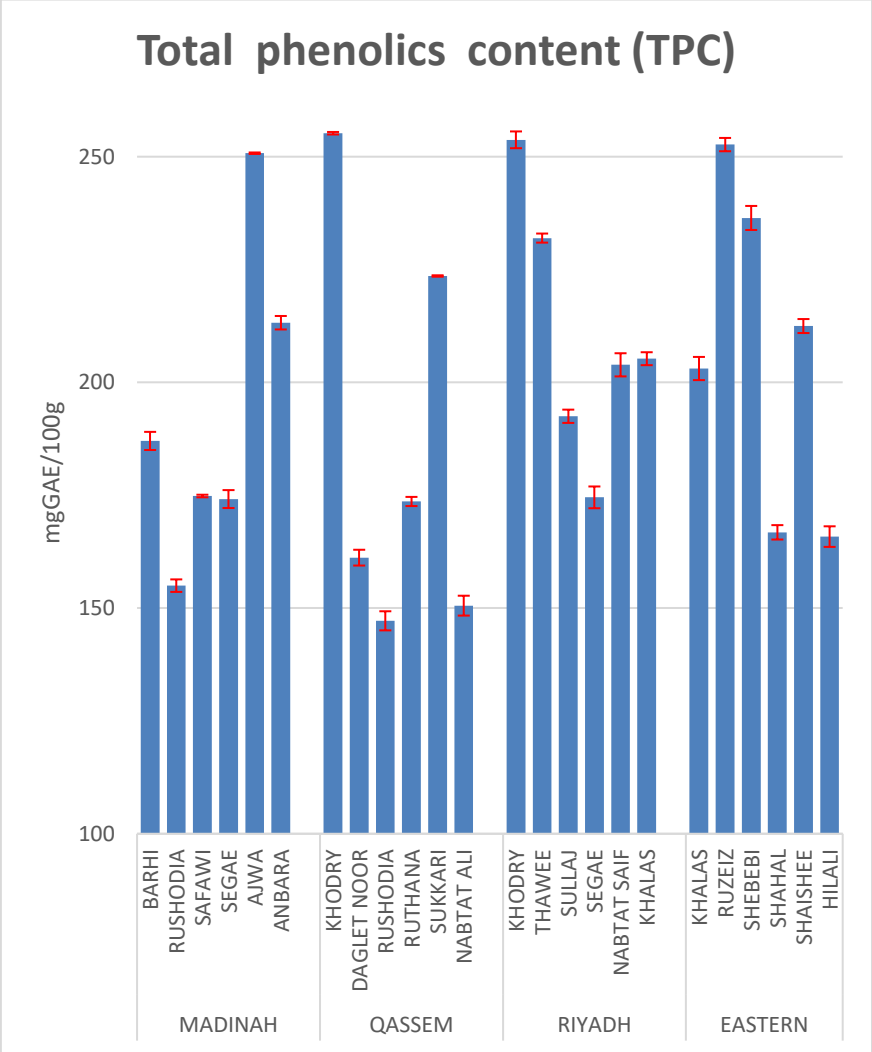


Figure 4.1 : Total phenolic content (TPC) of 24 Date varieties

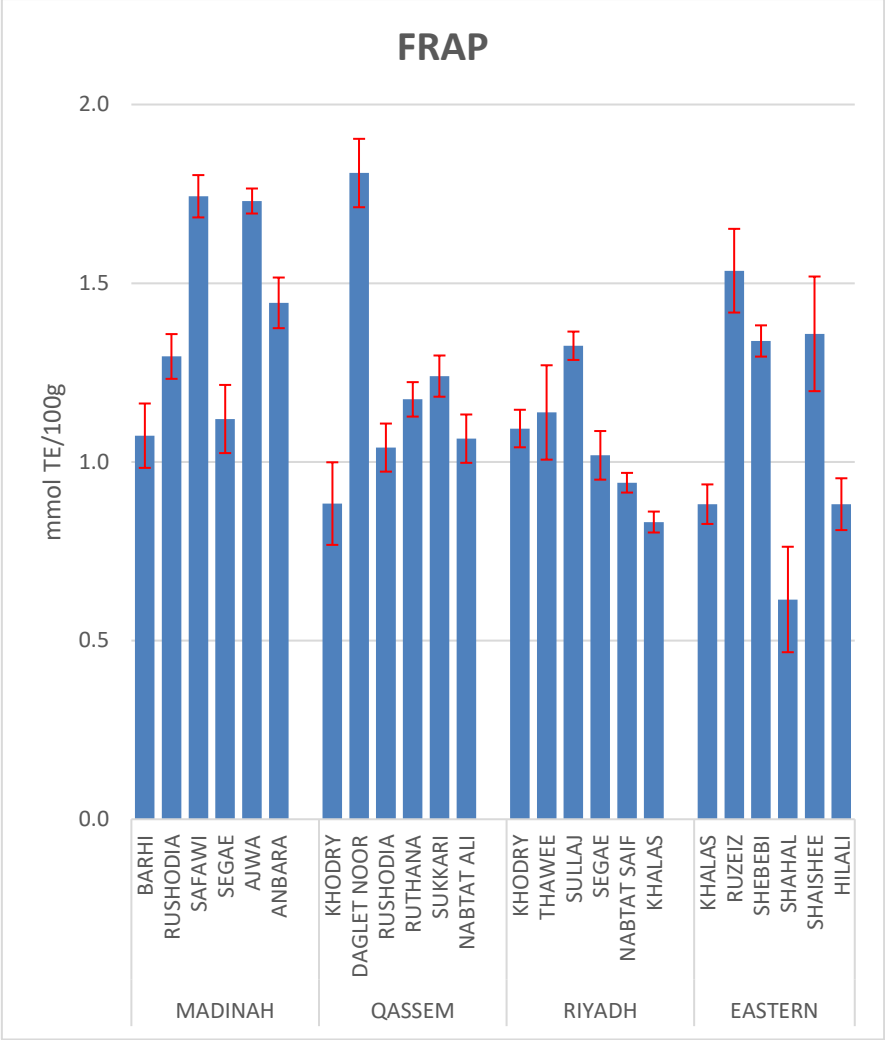


Figure 4.2: antioxidant capacity (FRAP) of 24 Date varieties

Overall Total phenolic content of the date varieties ranged between the lowest values of 147.17 ±2.12 mg GAE/100g for Rushodia-Qaseem to 255.196 ±0.29 mg GAE/100g for Khodry–Qaseem. One-way analysis of variance (ANOVA) demonstrated significant differences at $P < 0.05$ between individual varieties.

In terms of regional analysis, the Riyadh region showed the highest level of TPC with mean (210.31mg GAE/100g) followed by (206.21, 192.49 mg GAE/100g) in Eastern and Madina regions respectively. The lowest mean of TPC was found in Qassem Region dates (185.2131mg GAE/100g).

Ajwa showed the highest level of TPC (255.196 ± 0.28 mg GAE/100g) in Medina region whilst Rushodia (154.969 ± 1.39 mg GAE/100g) gave the lowest level., In the Qassem region Khodry showed the highest level of TPC (255.196 ± 0.28 mg GAE/100g) whereas, Rushodia was again the lowest (147.173 ± 2.12 mg GAE/100g).

In the Riyadh region Khodry again had the highest level of TPC (253.734 ±1.86 mg GAE/100g) whereas, Segae was the lowest (174.526 ± 2.4 mg GAE/100g). Ruzeiz showed the highest level of TPC in the Eastern Region (252.679 ± 1.48 mg GAE/100g) whilst Hilali had the lowest level (165.835 ±2.28 mg GAE/100g).

Figure 4.2 shows the antioxidant capacity of the twenty-four date varieties as measured by FRAP. The values ranged from 0.615 ± 0.147 mmoLTE/100 g in Shahal-Eastern Region variety to the highest content of 1.64 ± 0.31 mmoL/100 g in Daglet Noor-Qaseem, analysis of variance indicated significant differences at ($P < 0.05$).

Unlike the multiple comparisons of total phenolic contents determined by TPC method which mostly, were significantly different at ($P<0.05$), the multiple comparisons of antioxidant capacity of most varieties measured by FRAP were not significantly different from each other. However, there are exceptions which are listed below:

Safawi-Madina which is significantly different at P=0.031 from Khalas-Riyadh and at P=0.05 from Shahal-Eastern Region. Anbara-Madina which is significantly different at P=0.014 with Shahal-Eastern Region. Daglet Noor-Qaseem which is significantly different at P=0.004, at P=0.023 and at P=0.001 from Khalas-Riyadh, Khalas-Eastern Region and Shahal-Eastern Region, respectively.

Ruzeiz-Eastern Region TP content is showing significant difference at P=0.026 from Shahal-Eastern Region content. Shahal-Eastern Region is significantly different at P=0.036 from Rushodia-Madina and significantly different of Shaishee-Eastern Region at P=0.048 from TP content. The correlation between FRAP and TPC values was examined and the result shown in figure 4.3.

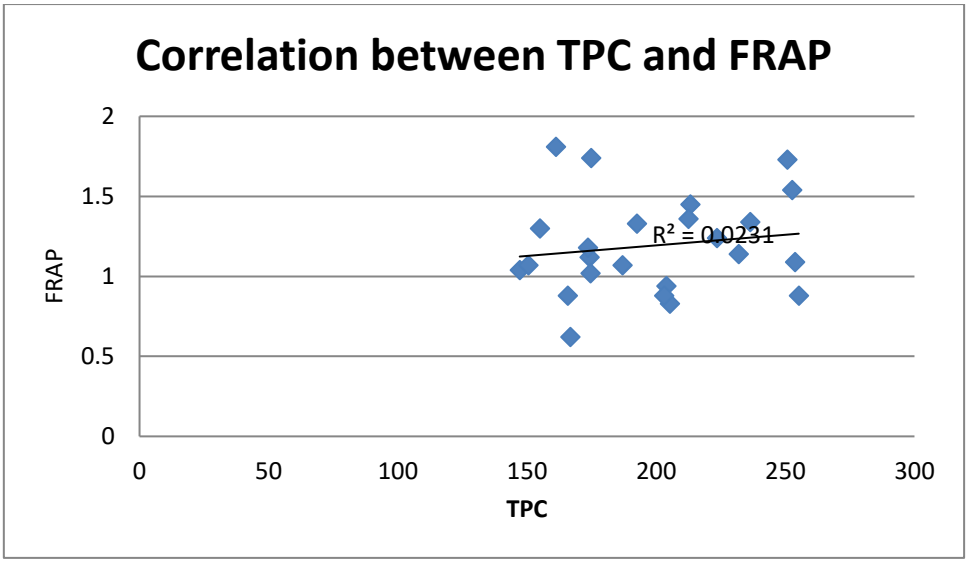


Figure 4.3: Correlation between TPC and FRAP for the 24 varieties

Figure 4.3 indicated that there was a significant correlation (R2=0.02) between TPC and FRAP. This may have been expected since they are both based on oxidative capacity but may also indicate that phenolics play a very significant role in determining the total antioxidant capacity of the dates.

4.6 Comparison of same date varieties from different regions

There were only a limited number of opportunities to select dates from the same variety but grown across different regions. A direct comparison was made for the results in these cases as a further means of identifying potential environmental influences on date composition. This comparison is summarized in table 4.8.

Table 4.8: Comparison summary

	Varieties/Region	% Glucose	% Fructose	% Sucrose	% Total Sugar	Calcium (Ca)	Potassium (K)	Phosphorus (P)	Magnesium (Mg)	Selenium (Se)	TPC	FRAP
M2	Rushodia-Madina	19.62±0.055	17.47±0.181	33.42±0.169	70.68±0.157	83.96±0.157	846.13±14.931	59.04±1.476	65.60±0.237	0.36±0.010	154.97±1.386	1.30±0.063
Q3	Rushodia-Qaseem	26.85±0.094	24.89±0.024	19.25±0.045	70.90±0.135	64.11±0.314	749.42±7.412	58.26±0.961	53.26±1.593	0.00±0.000	147.17±2.116	1.04±0.067
M4	Segae-Madina	33.33±0.552	31.28±0.825	00.00±0.000	63.61±1.592	49.69±0.548	819.31±4.047	71.32±0.740	58.62±1.041	0.08±0.015	174.15±1.986	1.12±0.095
R4	Segae-Riyadh	36.53±0.337	33.75±0.659	03.27±0.055	73.48±0.164	49.66±0.049	756.58±8.834	61.51±0.728	51.43±0.918	0.04±0.015	174.53±2.411	1.02±0.068
Q1	Khodry-Qaseem	34.45±0.069	32.90±0.429	00.00±0.000	66.69±0.423	60.31±0.891	906.73±6.175	53.51±0.408	52.30±0.722	0.25±0.020	255.20±0.283	0.88±0.116
R1	Khodry-Riyadh	36.71±0.235	34.24±0.252	00.00±0.000	71.12±0.072	54.82±0.999	810.46±11.017	48.07±0.405	47.23±1.207	0.21±0.062	253.73±1.859	1.09±0.053
R6	Khalas-Riyadh	39.10±0.143	35.19±0.150	00.00±0.000	74.64±0.088	45.15±0.384	750.07±6.350	53.56±0.808	54.35±0.824	0.07±0.005	205.25±1.433	0.83±0.029
E1	Khalas-Eastern Region	32.57±0.695	29.69±0.141	11.24±0.001	73.83±0.204	43.85±0.164	749.41±12.885	53.61±1.142	52.46±1.097	0.07±0.025	203.07±2.568	0.88±0.055

A comparison of the four varieties grown in more than one region shows that in general differences due to variety seem to be most important in each case. Notable exceptions seem to be firstly in the level of sucrose (and corresponding changes in glucose and fructose) where three out of the four varieties demonstrate a difference between the two regions. The second may be in the accumulation of selenium where two of the varieties show a difference. In the case of selenium, the two varieties: Rushodia and Segae showing a difference both have higher levels when grown in the Medina region and as such may be an environmental impact. This may reflect a higher level of this essential mineral in the richer soil in this region. There is no such obvious correlation with sucrose levels. However, this may be related to the physiological stages of maturity of the respective dates which may be influenced by environment. In the next chapter the impact of maturity on some of these parameters will be examined.

4.7 Follow up study to investigate variation in sugar, FRAP and TPC content of date varieties.

Following the results from the preliminary screen sub-sets of the region/variety of dates were selected for a more in-depth study. The physical characteristics of the date varieties selected are shown in Appendix 1. The varieties were selected to include those with the highest and lowest values for antioxidant activity, phenolic content and sugar composition. For each variety a sample was collected from six independent markets and then assays were carried out on individual dates from each market sample as appropriate.

One fruit of dates from each market were then analysed for the highest and lowest variety for each appropriate parameter. In addition, a further date from each of the six other collected samples was also analysed for all parameters.

The following nutritional parameters were chosen for this more in-depth study: (a) Antioxidants are one of the components that naturally occur in plants. The amount of antioxidants in a food item, probably a fruit or vegetable, is important and of interest to consumers and food scientists. This is because antioxidants are thought protect the body from the effects of free radicals, in combination with other physiological mechanisms. As such, the knowledge of the antioxidant composition of different foods can help in choosing the food items to include in one's meal (Silva et al., 2006). (b) Phenolic acids other compounds of interest to food consumers. Ozcan

et al. (2014) reported that these phytochemicals have immense health benefits, some of which include antimicrobial and antioxidant properties. These properties can help prevent one from the effects of degenerative diseases, allergies, infections, and inflammations (Ozcan et al., 2014). It is these properties that make the total phenolic compound in a food item a measure of interest to consumers. And (c) sugars, glucose is one form in which carbohydrates are absorbed by the human body. A food item that contains a high amount of glucose is of interest because it readily provides the energy in body upon consumption. The presence of glucose means that even before the rest of the food item is digested, the glucose can be absorbed readily to provide the body with the necessary components for energy metabolism. Fructose is another of the simple sugars in addition to glucose that are found in many fruits. The amount of fructose in a fruit is a predictor for the total sugar content. As a result, its determination is important and of interest to consumers. The six varieties of the date palm fruit were analyzed to determine the percentage concentration of fructose.

4.7.1 Ferric Reducing Antioxidant Power

The ferric reducing antioxidant power as measured for the six different varieties of the date palm selected are shown in Figure 4.4 along with the values from the preliminary screen for comparison.

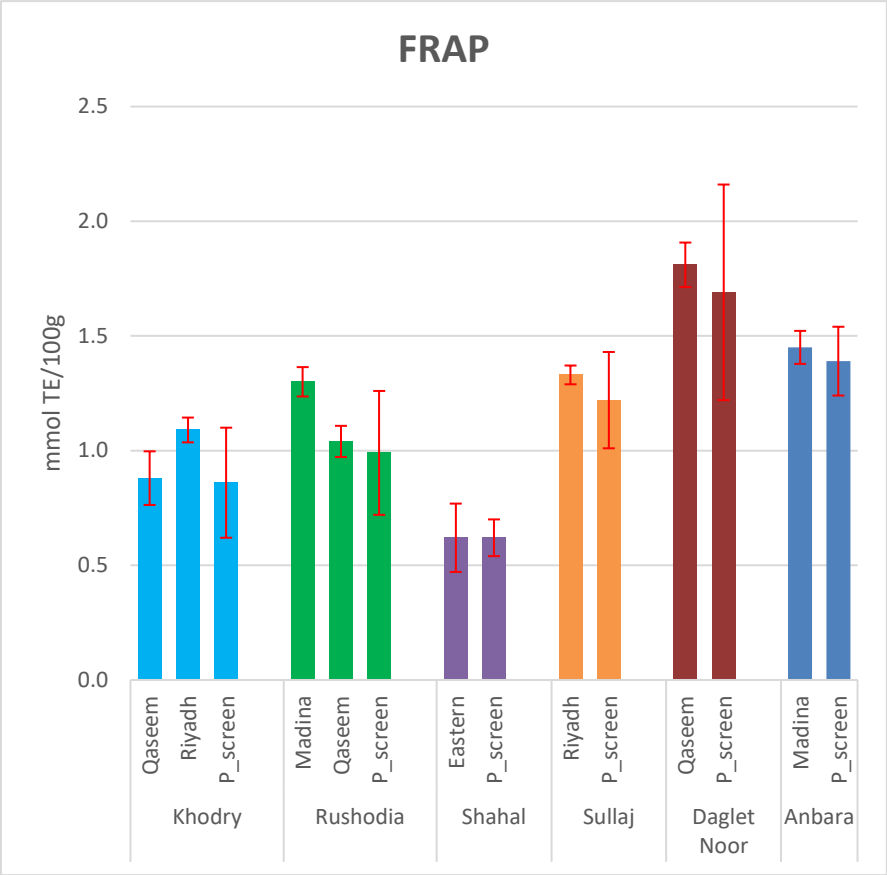


Figure 4.4: Antioxidant activity of selected date varieties.

The results showed that the different varieties of dates had different amounts of the ferric reducing antioxidant power. When the ferric reducing antioxidant power of the six varieties of the date palm fruit are compared, it is evident that the Daglet_Noor variety of the fruit has the highest amount both in terms of range and average and the ranking was Daglet-Noor.>Anbara>Sullaj>Rushodia>Khodry>Shalal. Analysis of variance demonstrated significant differences at ($P<0.05$) between varieties. Khodry was significantly different ($P<0.05$) from Daglet_Noor and Anbara. Rushodia was significantly different ($P<0.05$) from Daglet_Noor. Shahal was significantly different ($P<0.05$) from Daglet_Noor, Sullaj and Anbara. Sullaj was significantly different ($P<0.05$) from Shahal. Daglet_Noor was significantly different ($P<0.05$)

from Shahal, Khodry and Rushodia. Anbara was significantly different (P<0.05) from Shahal and Khodry.

If these values and rankings are compared with the preliminary screen (Figure 4.4) then there are some differences. The ranking in the preliminary screen was the same being Daglet-Noor.>Anbara>Sullaj>Rushodia>Khodry>Shalal. Although the absolute values for many of the varieties was greater than the preliminary screen which may reflect the fact that these were collected in a different year and different environments.

4.7.2 Total Phenolic Content

The total phenolic content of the six selected date varieties is shown in Figure 4.5 along with the values obtained from the preliminary screen for comparison.

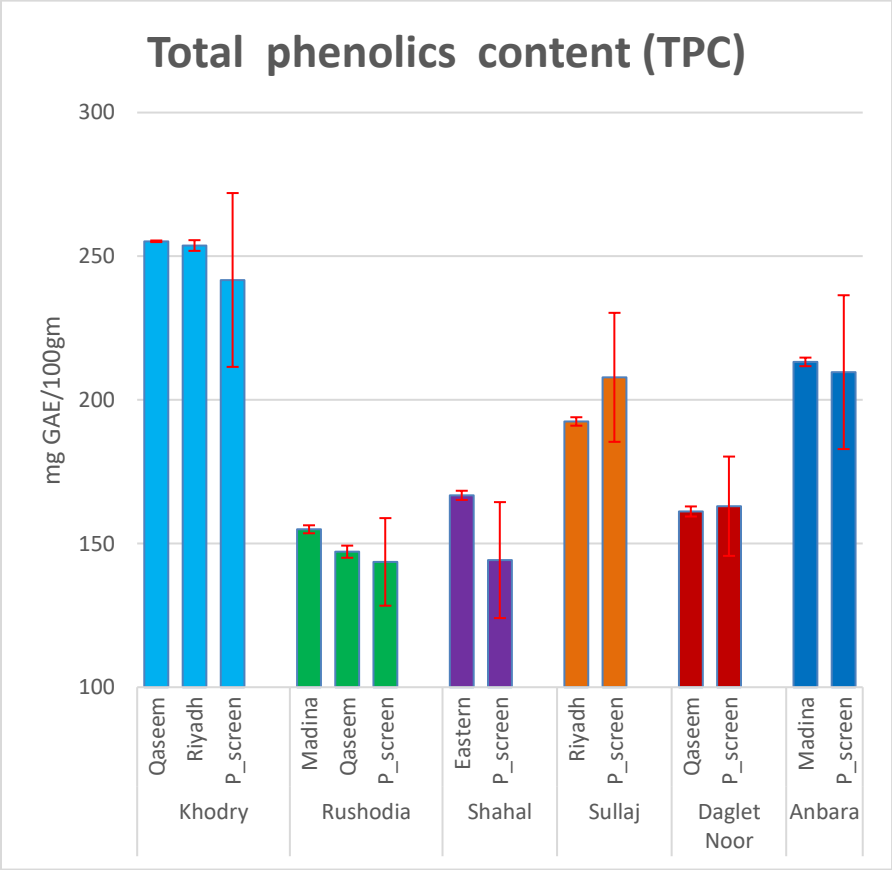


Figure 4.5: Total phenolic content of selected date varieties

The results showed that the Khodry variety had the highest total phenolic content and the ranking was Khodry>Anbara>Sullaj>Daglet-Noor>Shalal>Rushodia. Analysis of variance demonstrated significant differences at ($P<0.05$) between varieties. Khodry was significantly different ($P<0.05$) from Daglet_Noor, Shahal and Rushodia. Rushodia, Daglet_Noor and Shahal were significantly different ($P<0.05$) from Khodry, Sullaj and Anbra . Shahal was significantly different ($P<0.05$) from Daglet_Noor and Rushodia. Sullaj and Anbara were significantly different ($P<0.05$) from Daglet_Noor, Shahal and Rushodia.

These values were compared to those obtained in the preliminary screen (Figure 4.5). The ranking in this case was Khondry>Anbara>Sullaj>Shalal>Daglet-Noor>Rushodia. It can be seen that the rankings are very similar in each case apart from Shalal and Daglet Noor which have switched rank. In this case the absolute values obtained for the varieties in each screen are very similar, perhaps showing that this aspect of the date composition is not that dependent on harvest year. It was noted that in this year the temperature was higher than the previous years and may have led to faster ripening of the fruit than in previous years.

4.7.3 Glucose

Dates were collected from six separate markets and analysed for Glucose content (a). The results are compared to the values obtained from dates in the preliminary screen (b).

Table 4.9: Glucose percentage of selected date varieties

	Glucose % [(a)]	Glucose % preliminary screen [(b)]
Khodry	33.84±8.20^{AB}	34.45±0.07 ; 36.71±0.24
Rushodia	16.57±8.04^{C}	19.62±0.05 ; 26.85±0.09
Shahal	35.20±8.80^{AB}	34.93±0.20
Sullaj	39.13±8.54^{A}	40.60±0.24
Daglet_Noor	10.79±8.97^{C}	11.22±0.10
Anbara	28.44±8.02^{B}	28.98±0.20

*Means within the same column followed by the same letter are not significantly different at ($p<0.05$) according to the Tukey HSD test.

The results showed that the Sullaj variety had the highest glucose content and the ranking was Sullaj > Shalal >Khodry>Anbara>Rushodia> Daglet-Noor. Analysis of variance demonstrated significant differences at ($P<0.05$) between varieties. Khodry was significantly

different (P<0.05) from Daglet_Noor and Rushodia. Rushodia was significantly different (P<0.05) from all other varieties except Daglet_Noor. In the same way Daglet_Noor was significantly different (P<0.05) from all other varieties except Rushodia. Shahal was significantly different (P<0.05) from Daglet_Noor and Rushodia. Sullaj was significantly different (P<0.05) from Daglet_Noor, Anbra and Rushodia. Anbara was significantly different (P<0.05) from Daglet_Noor, Sullaj and Rushodia.

These values were compared to those obtained in the preliminary screen (Table 4.10). The ranking in this case was identical being Sullaj > Shalal >Khodry>Anbara>Rushodia> Daglet-Noor. It can also be seen that the absolute values were very similar in each case. The new data is a good reflection of the preliminary data for example the percentage of the Shahal-Eastern Region was measured in the preliminary screen at 34.93±0.203%. This amount was very similar to the percentage glucose for the same variety measured using the new screen at 35.20±5.80%.

4.7.4 Fructose

Dates were collected from six separate markets and analysed for Fructose content (a). The results are compared to the values obtained from dates in the preliminary screen (b).

Table 4.10: Fructose percentage of selected date varieties

Variety	% Fructose[(a)]	% Fructose preliminary screen[(b)]
Khodry	31.10±8.79^{AB}	32.90±0.43 ; 34.24±0.25
Rushodia	15.65±3.03^{C}	17.47±0.18 ; 24.89±0.02
Shahal	33.43±6.68^{AB}	32.39±0.16
Sullaj	39.76±3.62^{A}	37.40±0.30
Daglet_Noor	9.90±1.67^{C}	10.37±0.57
Anbara	28.26±3.68^{B}	25.85±0.14

*Means within the same column followed by the same letter are not significantly different at (p<0.05) according to the Tukey HSD test.

The results showed that the Sullaj variety had the highest fructose content and the ranking was Sullaj > Shalal >Khodry>Anbara>Rushodia> Daglet-Noor. Analysis of variance demonstrated significant differences at (P<0.05) between varieties. Khodry was significantly different (P<0.05) from Daglet_Noor and Rushodia. Rushodia was significantly different (P<0.05)

from all other varieties except Daglet_Noor. In the same way Daglet_Noor was significantly different (P<0.05) from all other varieties except Rushodia. Shahal was significantly different (P<0.05) from Daglet_Noor and Rushodia. Sullaj was significantly different (P<0.05) from Daglet_Noor, Anbra and Rushodia. Anbara was significantly different (P<0.05) from Daglet_Noor, Sullaj and Rushodia.

These values were compared to those obtained in the preliminary screen (Table 4.11). The ranking in this case was Sullaj > Shalal >Khodry>Anbara>Rushodia> Daglet-Noor. It can be seen that the rankings were identical in each case. The new values are almost identical to the preliminary data. Certainly, the ranking is identical, and the absolute values are only a few % different at most. That is unlikely to be statistically significant and certainly not nutritionally significant.

4.7.5 Sucrose

The amount of sucrose in the six varieties of the date palm fruit were also measured (a). The results are compared to the values obtained from dates in the preliminary screen (b).

Table 4.11: Sucrose percentage of selected date varieties

Variety	% Sucrose [(a)]	% Sucrose preliminary screen[(b)]
Khodry	0.00±0.00^{D}	0.00±0.00 ; 0.00±0.00
Rushodia	28.09±7.96^{B}	33.42±0.17 ; 19.25±0.4
Shahal	0.00±0.00^{D}	0.00±0.00
Sullaj	0.00±0.00^{D}	0.00±0.00
Daglet_Noor	49.16±6.26^{A}	49.81±0.67
Anbara	0.00±0.00^{D}	0.00±0.00

*Means within the same column followed by the same letter are not significantly different at (p<0.05) according to the Tukey HSD test.

There was no sucrose in the Khodry, Anbara, Sullaj and Shalah varieties of the dates. The results showed that the Daglet-Noor variety had the highest sucrose content and the ranking was Daglet-Noor > Rushodia. Analysis of variance demonstrated significant differences at (P<0.05) between varieties. Khodry was significantly different (P<0.05) from Daglet_Noor and Rushodia.

Rushodia and Daglet_Noor were significantly different (P<0.05) from all other varieties. Shahal was significantly different (P<0.05) from Daglet_Noor and Rushodia. Sullaj was significantly different (P<0.05) from Daglet_Noor and Rushodia. Anbara was significantly different (P<0.05) from Daglet_Noor and Rushodia.

These values were compared to those obtained in the preliminary screen (Table 4.12). The ranking was identical and the absolute values very similar in each case.

4.7.6 Total Sugar

The total sugar composition in the data palm fruit is comprised of the amount of fructose, glucose, and sucrose (a). The results are compared to the values obtained from dates in the preliminary screen (b).

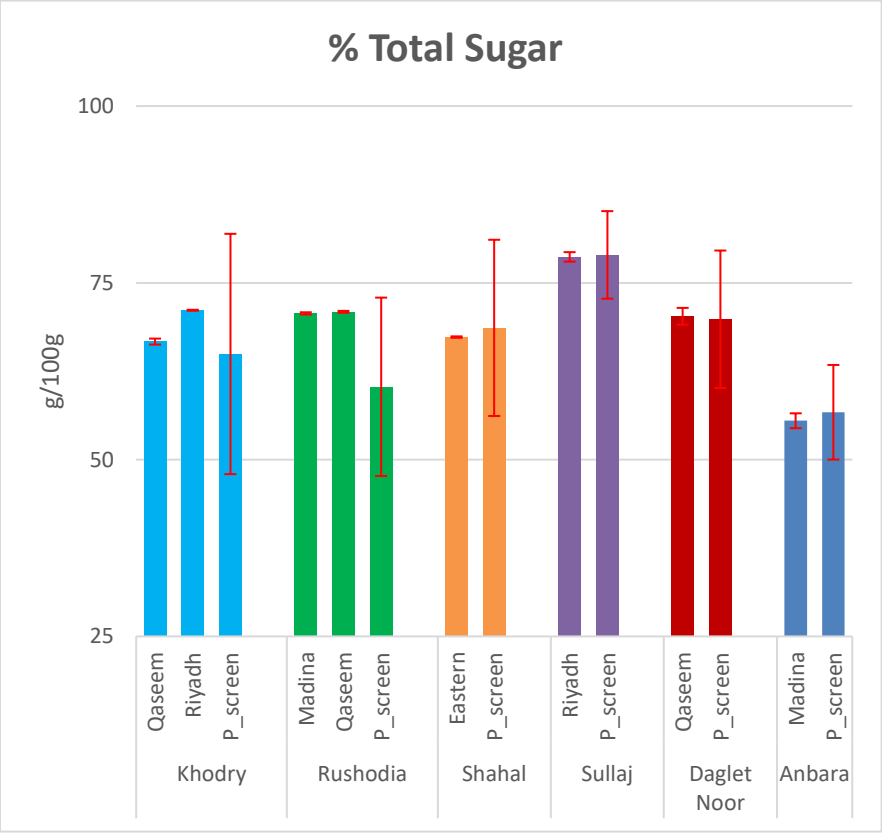


Figure 4.6: Total sugar percentage of selected date varieties

The results showed that the Sullaj variety had the highest total sugar content and the ranking was Sullaj > Daglet-Noor> Shalal >Khodry> Rushodia>Anbara. Analysis of variance

demonstrated insignificant differences at ($P>0.05$) between most varieties in the in-depth experiment. Except that Sullaj and Anbra were significantly different ($P<0.05$) from each other.

These values were compared to those obtained in the preliminary screen (Figure 4.6). The ranking in this case was Sullaj >Rushodia > Daglet-Noor> Shalal >Khodry>Anbara.

It can be seen that the rankings in this case are not identical apart from the highest and lowest varieties in each case. However, this is probably just a reflection of the fact that there are very few significant differences between varieties for this parameter. The absolute values obtained in the in-depth study are again very similar to those from the preliminary screen.

The present study suggests that there are significant variations in phenolic content and antioxidant potential between genotypes of dates. However, the use of commercially available fruit did not allow the potential impact of environmental factors- soil, climate, transport and storage- or harvest parameters- age of tree, time of harvest position on bunch – to be taken fully into consideration. Some of these may also be causal. Stage of maturity at harvest has been shown in this study to have a significant impact however, since fruit are routinely harvested at the same stage the impact on commercially available fruit is likely to be small. The aim of this research was to identify variations in nutritional value of dates across Saudi Arabia in particular with respect to phenolic content and antioxidant activity. A secondary aim was to explore potential causes of this variation in particular impact of soil/climate variations across the country. The study has identified genotype/region combinations of dates that exhibit extremes for the nutrient parameters investigated. A future study could identify, or seek to generate, several "field stations" where these genotypes are being grown all together under diverse conditions (soil type, climate etc.). The harvest protocols (stage of maturity, age of tree, position on bunch) in each case could then be harmonised at each station in order to eliminate any potential variation due to these factors. Harvesting and individually analysing at least 6 fruits from each of 3 independent trees for each genotype from each "field station" would then allow detailed assessment of the impact of genotype and environmental factors. This could be carried out over several successive harvest years to further investigate the impact of climatic parameters.

Chapter 5 Total Phenolic Content and Antioxidant capacity of dates during development

In the previous chapter there was a significant variation found between date varieties in a number of nutritional factors. There could also be an impact of developmental stage of the fruit on nutritional quality and this aspect was explored in this chapter. Total phenolic content and antioxidant capacity were chosen for study since both these factors demonstrated significant variation and may reflect nutritionally important characteristics of the fruit that have been relatively poorly investigated in the past.

The determination of the antioxidant power in the dates is very important from a nutritional perspective. Benzie & Choi (2014) argue that the fact that the consumption of foods that have a high content of antioxidants are associated with increased functional longevity as well as improved health makes it important to determine the antioxidant activity in a food item It is not feasible to measure the actual amounts of individual antioxidants in a food item. It is for this reason that methods that measure the total antioxidant activity in a food item was designed (Benzie & Choi, 2014).

5.1 Description of varieties used in the study

Seven varieties of dates were selected, from among those used in the previous chapter, (Barhi, Ajwa, Khalas, Nabtat Seif, Khodry, Sukkari and Segae). Theses varieties were selected due to their traditional high consumption at all ripening stages unlike the other varieties which are consumed only at the Tamar stage. Dates were harvested at four different stages of maturity: Khalal (Fresh), Rutab (Semi-fresh), Tamar (Semi-dry) and Tamar (dry). Samples were then analysed for nutritional composition as described in materials and methods. Figure 5.1 shows the ripening stages for each of the selected varieties.

varieties/stages	Fresh	Semi-fresh	Semi-dry	Dry
Ajwa				
Barhi				
Khalas				
Khodry				
Nabtat Seif				
Segae				
Sukkari				

Figure 5.1: The ripening stages of the seven selected date varieties

5.2 Total Phenolic Content (TPC)

The TPC at the four stages of development and for the seven varieties (Barhi, Ajwa, Khalas, Nabtat Seif, Khodry, Sukkari and Segae) are shown in Figure 5.2, respectively. Analysis of variance of this TPC date demonstrated significant differences at ($P<0.05$) between varieties at all stages of development.

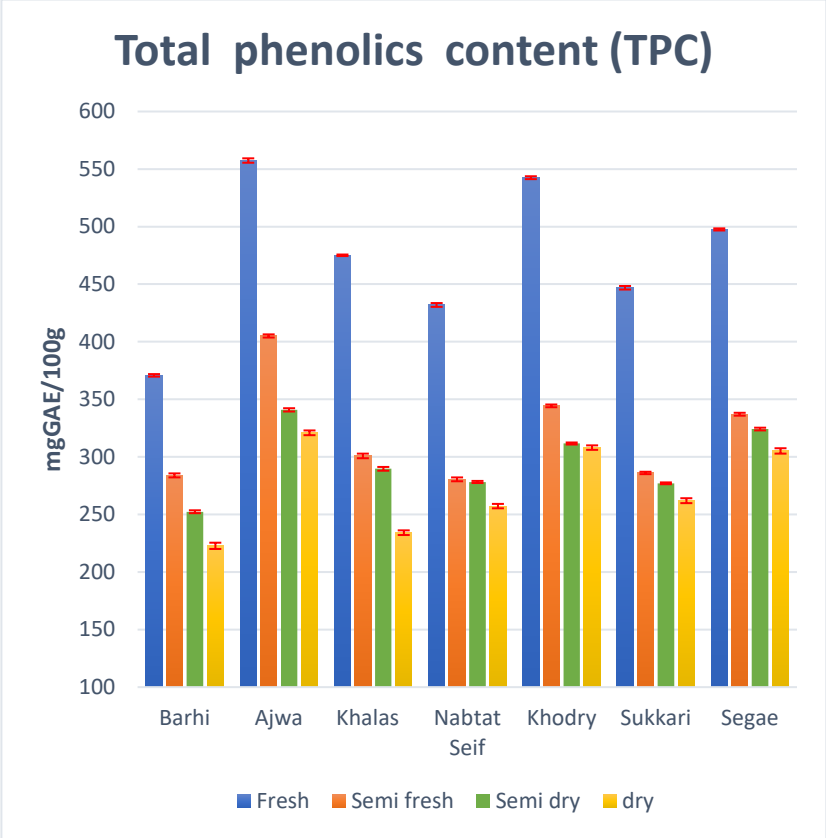


Figure 5.2: TPC of seven varieties of dates during development

From the results in Figure 5.2 can be seen that at each of the four stages of development the Barhi variety displayed the lowest TPC. In contrast Khodry, Ajwa and Segae are the three varieties with the greatest TPC at each stage of development.

It is clear from the data in Figure 5.2 that all seven varieties exhibited a steady decrease in TPC during development. However, in all cases the largest loss appears to occur between the fresh and semi-fresh stages of development. In summary, results show that the level of TPC is decreasing throughout development for all seven varieties.

5.3 Impact of development on antioxidant capacity of dates

The FRAP activity of the seven selected cultivars was determined at the four different stages of maturity (Fresh stage, Semi-fresh stage, Semi-dry stage and dry stage) and results are shown in Figure 5.3. Analysis of variance of the FRAP values demonstrated significant differences at ($P<0.05$) between varieties at all different stages of development.

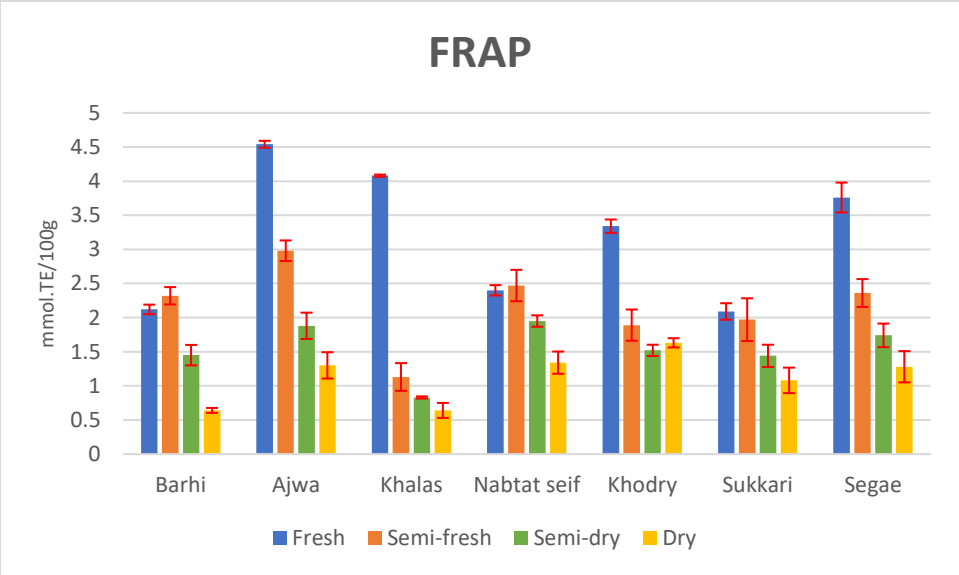


Figure 5.3: FRAP of the seven selected varieties of dates during development

From the data in Figure 5.3 it can be clearly seen that the FRAP value in all seven varieties declined during development. The highest level of FRAP shown in Figure 5.3 was in Ajwa Fresh (4.54±0.051 mmol/100g). Followed by Khalas fresh which contained (4.08±0.016 mmol/100g). Also, the lowest level of FRAP was found in Sukkari Fresh (2.09±0.121 mmol/100g) and Barhi Fresh (2.12±0.070 mmol/100g).

As for the semi-fresh varieties, FRAP values appeared to be comparatively similar however, the highest value is found (2.98±0.151 mmol TE/100g) in Ajwa semi-fresh. In addition as seen all varieties exhibited reduction in FRAP in the fresh stage. The least content (1.13±0.203 mmol TE/100g) is found in Khalas semi fresh.

As for the semi-dry varieties, FRAP values appeared to be comparatively similar however, the highest value was found (1.95±0.084 mmol TE/100g) in Nabtat seif semi-dry. Up

to now stage does not seen to affect FRAP of this variety to a great extent as there is no substantial difference (only 0.52 mmol) from content of Nabtat seif semi-fresh reported (2.47±0.230 mmol TE/100g). The least content (0.83±0.016 mmol TE/100g) is found in Khalas semi fresh. As for the dry varieties, FRAP values appeared to decrease from semi-dry stage. As seen in Figure 5.3 the highest value is found (1.63±0.068 mmol TE/100g) in Khodry dry. However, the least content (0.64±0.036 mmol TE/100g) is found in Barhi dry.

The correlation between FRAP and TPC values was examined and the result shown in figure 5.4.

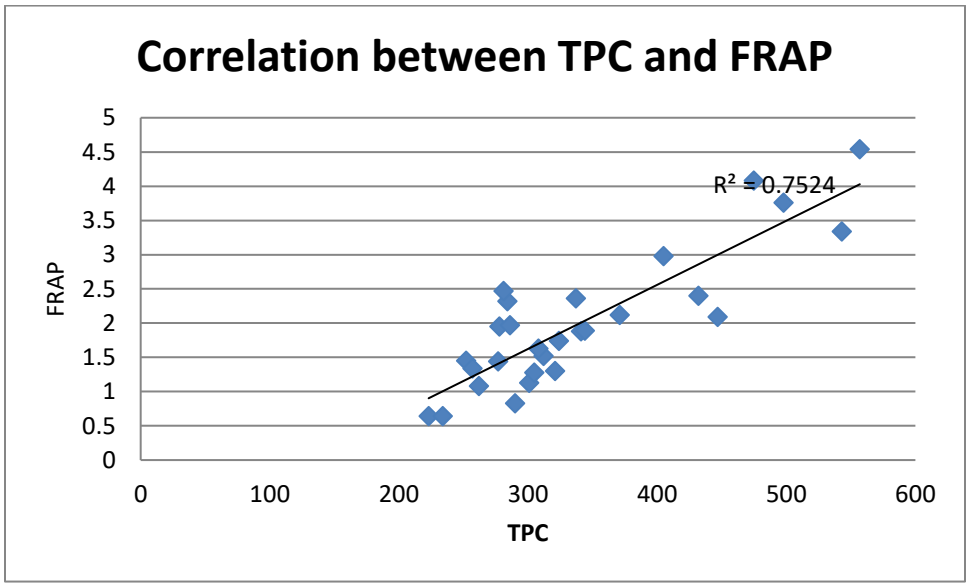


Figure 5.4: Correlation between TPC and FRAP for the seven varieties and four stages of development

Figure 5.4 indicated that there was a strong and positive significant correlation (R2=0.75) between TPC and FRAP. This may have been expected since they are both based on oxidative capacity but may also indicate that phenolics play a very significant role in determining the total antioxidant capacity of the dates, a finding that was also reported by Allaith (2007). Similar findings were also reported by Sadeghi, Valizadeh & Shermeh (2015) who reported that a statistically significant relationship existed between the total phenolic contnets and the antioxidant power in date fruits from Iran. This was based on a r^2 value of 0.974 and 0.975 and a p value of 0.01. Based on these findings, Sadeghi, Valizadeh & Shermeh (2015) concluded that the date fruits that had high total phenolic content also has a high ferric reducing antioxidant power values.

5.4 Profiling by HPLC

The two previous sections demonstrated a general decline in both TPC and FRAP during date development. This could arise from either a general decline in all the compounds responsibe or a loss of more specific compounds during development. The next aim of this project was thus to start to investigate the biochemical basis for this decline. The first step was to carry out a general metabolic profiling using HPLC.

A preliminary experiment carried out by Alicia Tan Poh (another PhD student in the laboratory) had shown that extraction solvent appeared to have minimal impact on the TPC of the extracts. In her experiment date fruit (unspecified variety) were extracted, as described in this thesis, with either methanol/water or FRAP buffer. The TPC value of the two extracts was then determined. The TPC values obtained were 2.37+ 0.039 and 2.61+ 0.21 mg GAE/g for the methanol and FRAP buffer extracts, respectively. Thus, for this initial profiling only the FRAP assay extracts were analysed.

All the FRAP extracts from the fresh, semi-fresh, semi-dry and dry dates of the seven native varieties from Saudi Arabia, namely, Barhi, Ajwa, Khalas, Nabtat seif, Khodry, Sukkari and Segae were analysed by HPLC using a procedure designed to detect phenolics. The resultant HPLC traces are shown in figure 5.3. Peaks with similar retention times between samples were assumed to represent the same compound and relative amount was estimated directly as peak area. The resultant analysis is shown in table 5.3. Peaks with the same letter (under peak identity) are assumed to be the same based on RT. Peak areas are given for each peak as the mean of three independent HPLC runs.

Table 5.1: Analysis of the peaks observed on the HPLC traces seven varieties of dates during development

Variety	Peak RT	Fresh	Semi-Fresh	Semi-Dry	Dry	Peak identity
Ajwa	2.08	16.3	7.2	0	0	A
	2.21	25	16.8	8.9	3.5	B
	2.6	0.1	0.07	0	0.13	C
	2.9	1.8	0	0.5	0.41	D
	3.2	1.2	0	1.2	0.47	E
Barhi	2.2	8.48	9.49	4.38	0.76	B
	3.2	0.98	1.31	0.44	0	E
	13.52	0.44	0.38	0	0	F
	13.87	0.71	0.64	0.63	0	G
Khalas	2.08	42.55	32.93	11.48	5.08	A
	2.24	0.99	0.7	0.74	0.31	B
	3.12	0.62	0	0	0	H
	3.39	0.39	0	0	0	E
	8.12	0	0	0	0.28	I
	12.79	0	0.6	0	0	J
Khodry	1.92	7.99	6.3	3.85	2.53	A
	2.12	35.56	33.13	17.48	13.29	B
	2.97	2.67	2.89	2.33	0.87	D
	9.97	0	0	0.4	0.27	K
	10.9	0	0	0.58	0.21	L
	12.52	0	0.76	0	0	M
	13.12	1.06	0	0.4	0	N
	13.37	1.51	1.19	0	0	F
Nabtat seif	1.95	9.47	4.72	0.31	0	A
	2.1	36.19	20.95	9.64	7.48	B
	2.37	1.5	0.63	1.19	0	P
	2.68	5.62	2.25	0.61	0	C
	2.98	4.83	1.47	2.49	1.41	D
	3.25	2.37	0	1.65	0.42	E
Segae	1.95	10.96	4.59	7.84	1.13	A
	2.1	20.98	25.03	19.47	9.78	B
	2.37	4.39	0.49	0.46	0	P
	2.69	0.92	1.15	0.52	0	C
	2.95	0	1.19	1.16	0.36	D
	7.76	0	0.86	0.75	0.46	Q
	9.85	0	0.53	0.59	0.46	K
	10.32	0	0.29	0	0.23	L
	12.8	0.77	0.7	0.58	0	J
Sukkari	1.95	10.22	7.32	5.17	2.4	A
	2.12	38.4	27.94	20.2	13.97	B
	2.59	0.63	0.81	0.39	0.25	C
	2.97	1.41	0.44	3.02	0.8	D
	3.25	0.71	0.44	0.73	0.43	E
	12.97	0.84	0	0.52	0	J
	13.18	0	0.9	0.41	0.31	N

As shown in Table 5.1 peaks with the same letter are likely to be the same in each sample. Thus, peak A occurs in all varieties except Bahri and declines very significantly during development. Furthermore, peak B occurs in all seven varieties and it drops during development. Levels are similar in all varieties except in Khodry where it appears very low. Peak D is present in five varieties out of seven; it is a very small peak in all cases except in Barhi and Khalas because it was below level of detection. It shows a drop during development.

Peak E appears low in four out of seven varieties and it drops during development. Remaining peaks are associated with only one or two varieties but in general all seem to fall during development.

It is not possible from these results to determine which, if any, of these peaks actually represent phenolics. However, the reduction in peak areas during development is consistent with the overall reduction in TPC that occurs at the same time. Similarly, it is also not possible to assign identities to these peaks. However, a number of phenolic standards were also run on the column and their retention times are shown in table 5.1.

A comparison of these standards with the data obtained for the graphs could suggest that peak E, as identified in table 5.1, could represent gallic acid which was found in Ajwa, Barhi, Khalas, Nabtat-seif and Sukkari dates. Peak F could represent Sinapic acid or Ferulic Acid which are found in Barhi and Khodry. Peak K could represent vanillic acid was found in Khodry and Segae. Moreover, peak L could represent (-) Epicatechin and is detected only in Segae. Whilst peak M could represent Epicatechin gallate (ECG) and is detected only in Khodry. Finally, peak J could represent P-coumaric acid which is found in Khalas and Segae. It must be realized however, that these are very tentative potential identifications. Similarly, several of the peaks detected in only a limited number of varieties had very low peak areas associated with them. It is thus possible that these are present in other samples but at a level below the limit of detection.

Potential identification of the other peaks is not possible. They may represent conjugated soluble phenolics or indeed other unrelated compounds. It is interesting to note however, that in most cases these also show a decline during development.

Table5.2: Retention times for standard phenolics on the HPLC

Standard	Retention Time (min)
Gallic acid	3.25
Protocatechuic acid	4.8
Epigallocatechin	6.56
Catechin	6.6
Chlorogenic acid	8.63
Epigallocatechin gallate	8.9
Vanilic acid	9.89
Caffeic acid	10.16
Syringic acid	10.16
(-) Epicatechin	10.29
Epicatechin gallate (ECG)	12.5
p-coumaric acid	12.71
Ferullic Acid	13.38
Sinapic acid	13.52
Naringin	15.85
Resveratrol	16.71
Trans-cinamic acid	20.53
Naringenin	20.9

Generally, from the potential phenolic compounds identified, gallic acid is the most common and occurs in most varieties.

If these are indeed phenolics then the results show that phenolic compounds were dependent on both the stage of maturity and variety. Generally, they decreased as maturity proceeded. The order of phenolic compounds in date fruit being full maturation is: fresh > semi-fresh> semi-dry >dry.

From the results obtained, it appears that the varieties contain more or less similar types of compounds with perhaps some small differences.

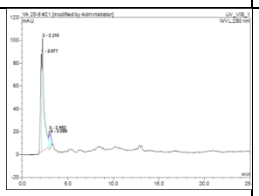
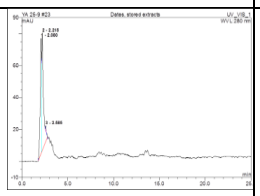
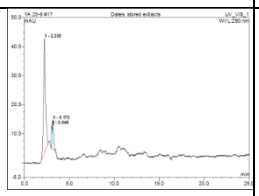
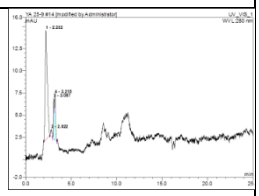
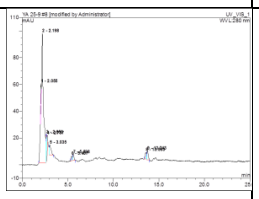
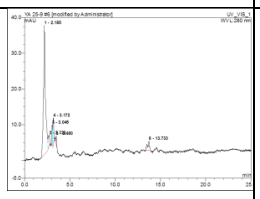
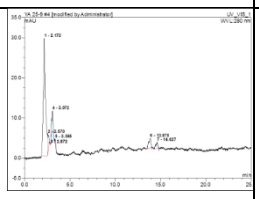
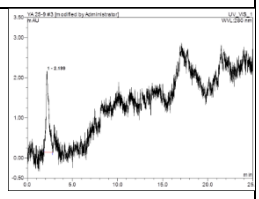
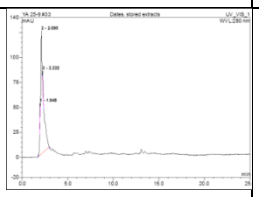
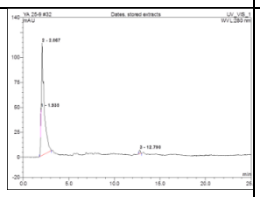
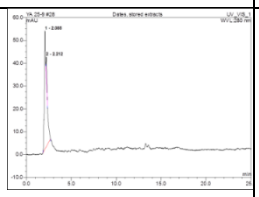
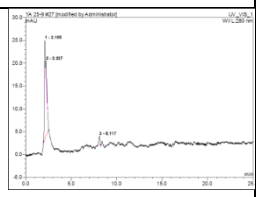
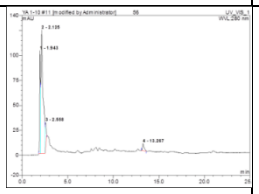
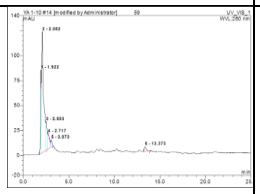
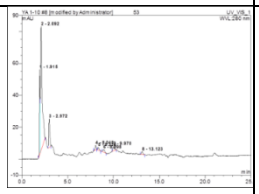
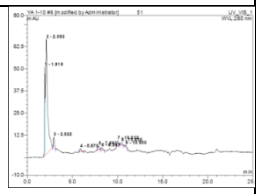
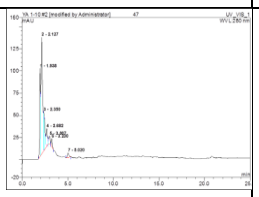
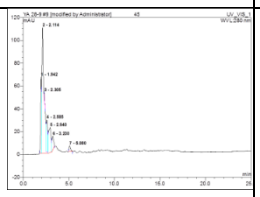
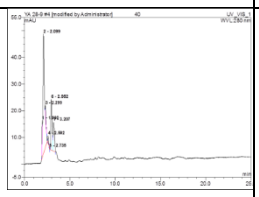
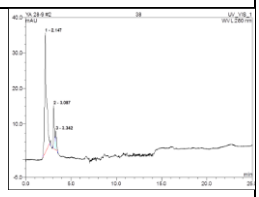
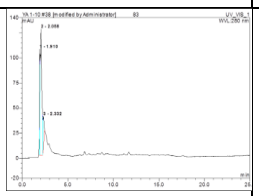
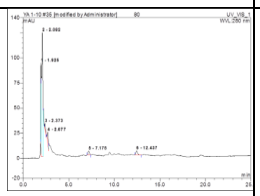
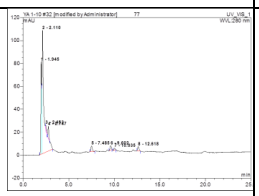
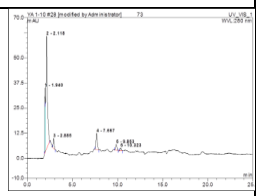
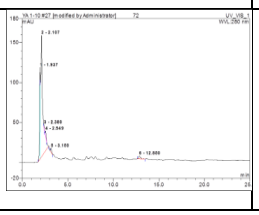
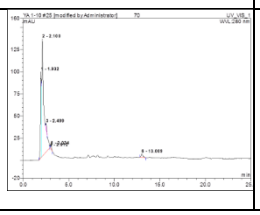
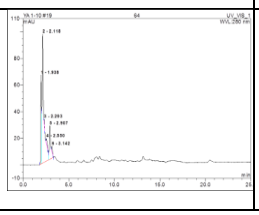
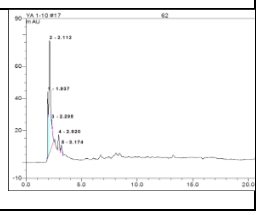
Variety	fresh	Semi-fresh	Semi-dry	dry
Ajwa				
Barhi				
Khalas				
Khodry				
Nabtat-Saif				
Segae				
Sukkari				

Figure 5.5: HPLC traces (absorbance at 280nm) of the extracts from seven varieties of dates made at four stages of development

5.5 Anthocyanin

The testing of the anthocyanin content in the dates has merit from a nutritional sense. Anthocyanin belong to the bioflavonoid phytochemical group. These are compounds that are of significance to human health. Lila (2004) reports that anthocyanins have been shown through *in vivo* and *in vitro* studies to reduce the proliferation of cancer cells in the body. Anthocyanin have also been shown to inhibit the formation of tumors. These actions are through to be achieved by the effect of anthocyanin on inhibiting the formation of cyclooxygenase enzymes as well as its potential as an antioxidant (Lila, 2004). Through the effect of anthocyanin in the human body, the protein kinase pathway that is activated by mitogen is inhibited thereby preventing the tumorigenesis (Lila, 2004). Citing these benefits, it is important to determine the anthocyanin content in various varieties of dates.

The seven selected varieties, (Barhi, Ajwa, Khalas, Nabtat seif, Khodry, Sukkari and Segae) were thus examined for their total content of anthocyanins and the results are shown in table 5.5. There was significant variation (p=0.5) between varieties at all four stages of development.

Table 5.3: Anthocyanin content of the seven selected varieties of dates during development

Anthocyanin mg/100g

varieties	Fresh	Semi fresh	Semi dry	Dry
Barhi	0.29±0.006 ^{C}	0.25±0.008 ^{D}	0.08±0.009 ^{AB}	0.04±0.015 ^{BC}
Ajwa	0.48±0.017 ^{B}	0.44±0.020 ^{AB}	0.11±0.010 ^{A}	0.06±0.006 ^{AB}
Khalas	0.33±0.005 ^{C}	0.30±0.025 ^{C}	0.05±0.009 ^{BC}	0.02±0.006 ^{CD}
Nabtat seif	0.14±0.011 ^{D}	0.13±0.016 ^{E}	0.05±0.011 ^{BC}	0.02±0.006 ^{CD}
Khodry	0.59±0.019 ^{A}	0.47±0.005 ^{A}	0.11±0.009 ^{A}	0.08±0.018 ^{A}
Sukkari	0.12±0.010 ^{D}	0.12±0.007 ^{E}	0.03±0.012 ^{C}	0.01±0.006 ^{D}
Segae	0.45±0.022 ^{B}	0.41±0.020 ^{B}	0.07±0.030 ^{ABC}	0.04±0.006 ^{BC}

*Results are expressed as mean values of three determinations ± SD.(n=?)

*Means within the same column followed by the same letter are not significantly different at (p<0.05) according to the Tukey HSD test.

Anthocyanin showed significant differences ($p < 0.05$) among the fresh varieties examined Table 5.3. As seen the highest content of Anthocyanin was present in Khodry (0.587mg / 100g), followed by Ajwa (0.479mg / 100g) and Segae (0.453 mg / 100g). However, the lowest amount of Anthocyanin was present in Sukkari (0.122 mg / 100g), followed by Nabtat seif (0.138mg/100g).

Anthocyanins, which were detected in semi-fresh dates, showed significant differences ($p <0.05$) among the varieties examined. The highest content of anthocyanins was present in Khodry (0.468 mg/ 100 g), followed by Ajwa (0.442 mg/100 g) and Segae (0.411 mg/100g). However, the lowest content of anthocyanins was present in Sukkari (0.116 mg/100 g) followed by Nabtat seif (0.129 mg/100g). As seen the content of Anthocyanins in semi-fresh stage is slightly decrease from fresh stage due to the slight sun drying for seven days from fresh to semi-fresh and they are in the same colour approximately.

In addition, Anthocyanin content was found to decrease sharply from semi-fresh stage to semi-dry stage almost One-third of the semi-fresh value. In addition, Anthocyanin showed significant differences ($p < 0.05$) among the semi-dry varieties examined. The highest content of Anthocyanin was present in Ajwa (0.108 mg / 100g), followed by Khodry (0.105 mg / 100g) and Barhi (0.083 mg / 100g). However, the lowest amount of Anthocyanin was shown in Sukkari (0.028 mg / 100g), followed by Khalas (0.046 mg/100g). Note that the varieties by the semi-dry stage had relatively small differences in their Anthocyanin contents.

Table 5.3 showed a dramatic decrease in Anthocyanin content at the dry stage for all seven varieties probably due to their degradation upon drying.

Anthocyanins, which were found in dry dates, showed significant differences ($p <0.05$) among the varieties examined. The highest content of anthocyanins was present in Khodry (0.081mg/ 100 g), followed by Ajwa (0.060 mg/100 g) and Segae and Barhi (0.041mg/100g) each. However, the lowest content of anthocyanins was present in Sukkari (0.011 mg/100 g) followed by Nabtat seif (0.019mg/100g) the same order as at the semi-dry stage.

Anthocyanins, which were found in Barhi, showed significant differences ($p <0.05$) among the stages examined. The highest content of anthocyanins was present in Fresh (0.295 mg/ 100 g), followed by semi-fresh (0.251 mg/100 g) then in semi-dry (0.083 mg/ 100 g) and the lowest amount is found in dry (0.041 mg/ 100 g).

Ajwa cultivar showed significant differences ($p < 0.05$) among the stages examined. The highest content of anthocyanins was present in Fresh (0.479 mg/ 100 g), followed by semi-fresh (0.442 mg/100 g) then in semi-dry (0.108 mg/ 100 g) and the least amount is found in dry (0.060 mg/ 100 g).

Khalas cultivar is showed significant differences ($p < 0.05$) between the stages examined. The highest content of anthocyanins was present in Fresh (0.331 mg/ 100 g), followed by semi-fresh (0.297 mg/100 g) then in semi-dry (0.046 mg/ 100 g) and the least amount is found in dry (0.021 mg/ 100 g).

Nabtat Saif cultivar is showed significant differences ($p < 0.05$) between the stages examined. The highest content of anthocyanins was present in Fresh (0.138 mg/ 100 g), followed by semi-fresh (0.129 mg/100 g) then in semi-dry (0.053 mg/ 100 g) and the least amount is found in dry (0.019 mg/ 100 g).

Khodry cultivar is showed significant differences ($p < 0.05$) between the stages in Anthocyanin content. The highest content of anthocyanins was present in Fresh (0.587 mg/ 100 g), followed by semi-fresh (0.468 mg/100 g) then in semi-dry (0.105 mg/ 100 g) and the least amount is found in dry (0.081 mg/ 100 g).

Sukkari cultivar is significantly different ($p < 0.05$) between the stages in Anthocyanin content. The highest content of anthocyanins was present in Fresh (0.122 mg/ 100 g), followed by semi-fresh (0.116 mg/100 g) then in semi-dry (0.028 mg/ 100 g) and the least amount is found in dry (0.011 mg/ 100 g).

Segae cultivar showed significant differences ($p < 0.05$) between different stages in Anthocyanin content. The highest content of anthocyanins was present in Fresh (0.453 mg/ 100 g), followed by semi-fresh (0.411 mg/100 g) then in semi-dry (0.068 mg/ 100 g) and the least amount is found in dry (0.041 mg/ 100 g). As seen, anthocyanins content in fresh and semi-fresh are close together.

In general the anthocyanin content is low at all the various stages of date development except for the Khodry cuitivar where its content of anthocyanin was clearly higher than the other varieties at the Fresh stage. This is because the colour of the skin characterizing the Fresh stage

of this cultivar, Khodry is red. These results are in agreement with those reported by Dowson and Aten (1962) and EI-Sabrout (1979) on different date palm cultivars. These differences between stages are related to the color of these cultivars Fresh is red colour at full mature stage and the red color decreases as the fruits proceed towards ripening stage while Semi-fresh is dark yellow with dark brown colour and semi-dry is brown colour less than dry. The highest total content of Anthocyanin in Fresh stage for Khodry and Ajwa were expected due to their red colour, whereas for other varities due to their yellow colour.

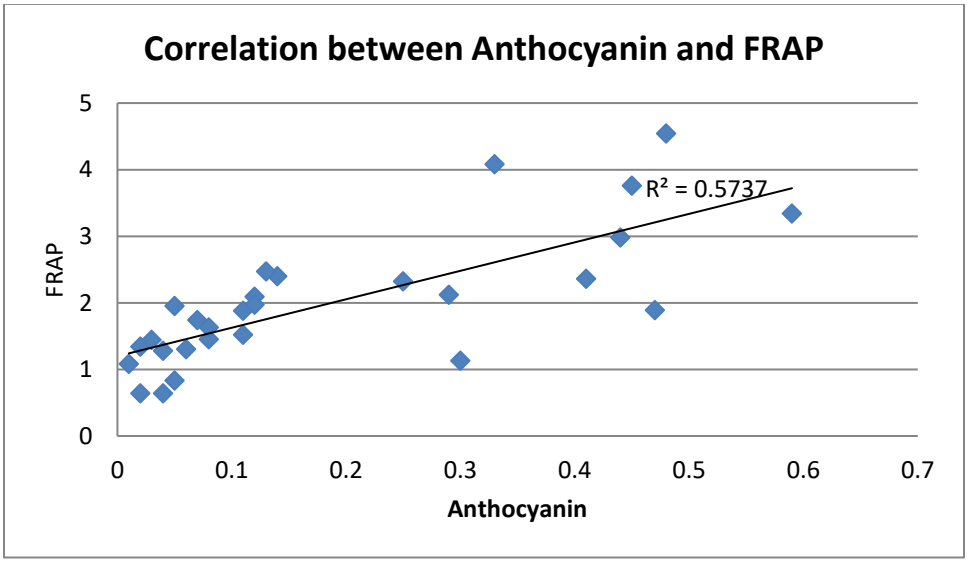


Figure 5.6: Pearson correlation graph of Anthocyanin and FRAP

Figure 5.6 indicated that there was a strong significant correlation (R^2=0.57) between Anthocyanin and FRAP. This again suggests that phenolics may represent a significant contribution to the antioxidant capacity of the date. These finding have also been reported by Nile, Kim & Keum (2015) in their study on the grape fruit. The researcher found that a statistically significant relationship existed between the free radical scavenging activity and the total anthocyanin in the fruit.

5.6 Comparison of market and field samples

There were seven opportunities to compare dates from the same variety from the first experiment (market sourced) with those obtained from the field experiments described above. A direct comparison was made for the results for TPC and FRAP as a further means of identifying potential cultivar impacts on date composition. These comparisons are summarized in figures 5.7 and 5.8.

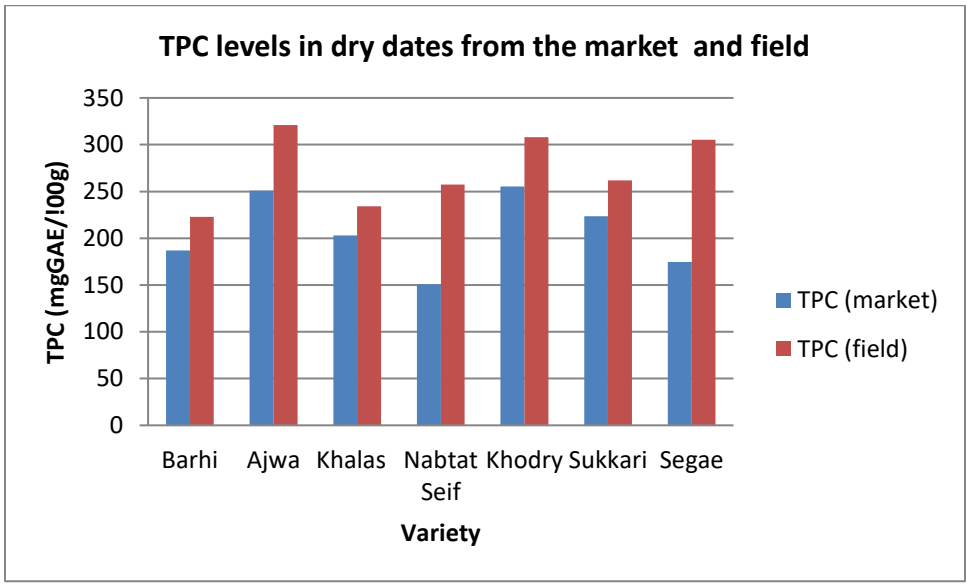


Figure: 5.7 Comparison of TPC levels in dry dates from the market and field

The levels of TPC in the market bought samples were routinely lower than those in the dry samples generated from field harvested material suggesting that there may be some losses occurring during marketing or that there has been a seasonal effect. However, the levels are nonetheless reasonably similar between the two years. Apart from Segae the field harvested varieties showed a similar trend in the market bought samples confirming the varietal differences.

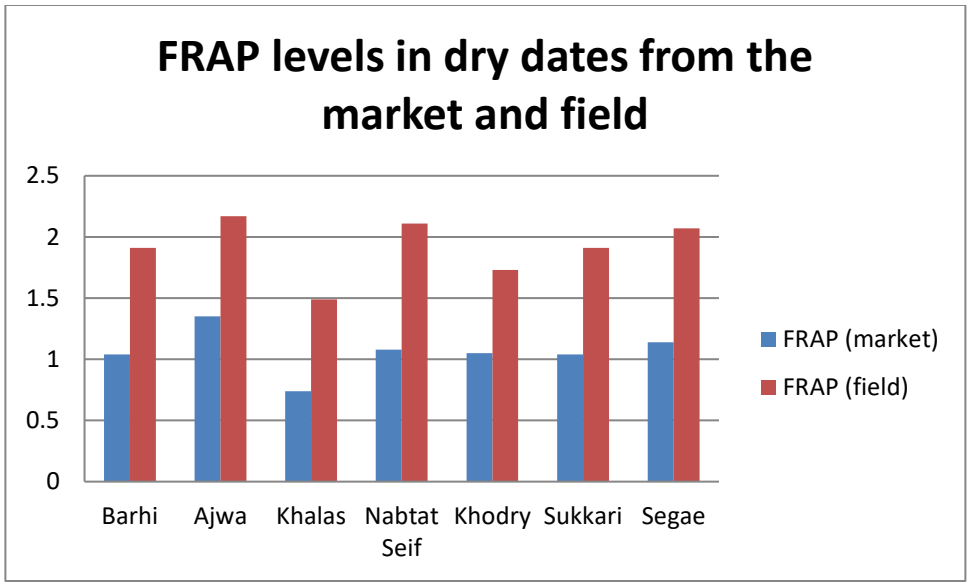


Figure: 5.8 Comparison of FRAP levels in dry dates from the market and field

As with TPC the FRAP values were generally lower in the market bought samples than in the field harvested material (figure 5.8). Again this may indicate an effect of marketing or a seasonal influence. The trend between varieities was however, similar in both cases again confirming the varietal differences.

Chapter 6 Discussion

The date tree is one of the oldest cultivated plants on earth and it has been introduced into many parts of the word. The top ten date producing countries in the world in descending order according to an FAO report in 2005, are Iraq, Saudi Arabia. Egypt , Iran , United Arab Emirates, Algeria , Pakistan , Sudan , Libya andChina, and the total World production was 16,696.56 tonnes. Other producing coutries include Morocco, Tunisia, USA, and in smaller quantities Spain, Mexico, Yemen and Israel (FAO, 2005).

Deserving mention is the fact that fruits of the date palm (*Phoenix dactylifera* L.) are common food items; consumed in many parts of the world and represent a valuable favored and indispensable component of the diet, as such they might be considered as a staple food in some areas (for example in Gulf States and some other Arab countries). To Muslims, the palm tree is a blessed tree whereas in the Gulf States date fruits are a sign of prosperity, hospitality and sympathy. The almighty God, Allah said in the Qur'an to Mary (Peace be upon her) while she was in labour (which is translated as follows) “Shake also to thee the palm-trunk, and there shall come tumbling upon thee date fruit fresh and ripe. Eat therefore, and drink, and be comforted” (Quran/Chapter 19/Verses 25 &26)**.** Furthermore, the Prophet Mohammad (peace and blessings of Allah be upon him) said in authentic narration: “Whoever eats seven date fruits in the morning; no poison will harm him until it is evening” (Sahih Muslim).

There is no doubt, that good healthy food is pivotal in protecting against todays long standing life threatening diseases and illnesses such as diabetes, cancers, cardiovascular diseases and others. And no doubt that diets rich in fruits and vegetables foster maximum protection against those diseases. Dates have medicinal uses listed in folk remedies, and were claimed to treat various infectious diseases and cancer. Dates are recommended for women as one of the best foods for them during pregnancy, strengthening and increasing the contraction rate of the uterus muscles thus facilitating delivery as well as reducing postpartum hemorrhages. Due to their antihyperlipidemic, hepatoprotective activities, they can be used as functional and essential healthy foods in the human diet (Biglari et al., 2009). Because of their antibacterial ability, immuneomodulatory activity and antifungal property, dates can be used in folk medicine to treat different infectious diseases (Baliga et al., 2011). Dates are nutriceuticals and possess

pharmacological properties that may be due to their high concentrations of minerals and a variety of other bioactive phytochemicals of varied chemical structure (Baliga et al., 2011). Dates have other potential nutritional benefits. Thus the protein contains 23 types of amino acids, some of which are not present at high levles in other popular fruits such as oranges, apples and bananas, moreover, dates contain at least six vitamins including small amount of vitamin C, and vitamins B1 thiamine, B2 riboflavin, nicotinic acid (niacin) and vitamin A (Vinson, et al.,2005). It is also clear that various extracts of dates contained potent antioxidant activity, because they inhibit *in vitro* lipid and protein oxidation and possess free radical scavenging capacity (Vayalil, 2002). Date pits can be used to improve the nutritional value of incorporated food products. Also, extracts show hepatoprotective and antimicrobial activity in rat (Biglari et al., 2009, Jassim and Naji, 2010 and Baliga et al., 2011).

The chemical composition, properties and sensory characteristics of dates has received significant research interest over the years. A few to mention are the study by Ahmed et al, (1995) who examined the chemical composition of date varieties as influenced by the stage of ripening, Al-Farsi, et al., (2005) who investigated the composition and sensory characteristics of three native sun-dried date (Phoenix dactylifera L.) varieties grown in Oman; Mohamed Elleuch, et al., (2008) who investigated the chemical composition and characteristics of dietary fiber; Chema Borchani, et al.,(2010) investigating the chemical properties of 11 date varieities in Tunisia and their corresponding fiber extracts and Abdel Moneim Sulieman, et al., (2012) who carried out a comparative study on five Sudanese date (Phoenix dactylifera L.) fruit cultivars. In addition a comprehensive review of the nutritional properties and health benefits of date fruits (Phoenix dactylifera L.) was produced during the course of this project by Saada M Al-Orf1 et al. (2012) in the Bulletin of the National Nutrition Institute of the Arab Republic of Egypt. The literature review also identified the fact that data on dates from Saudi Arabia was relatively lacking.

These papers demonstrate the importance of knowing the composition of the date and the various factors that may impact on this composition. In this study the proximate composition of nineteen varieties of date commomly consumed in Saudi Arabis, all at the Tamer stage of development was determined. This included measurements of moisture, energy, carbohydrate, fat and protein content.

The percentage moisture found in the samples tested in this thesis was quite large and ranged between 4.99%±0.49% and 21.55%±0.18%. A study performed by Parvin et al., (2015) to determine the nutritional content of date fruits (*Phoenix dactylifera L.*) in Bangladesh found that the moisture content of the date fruits that were sourced from Tunisia was responsible for between 13.2% and 14.1% of the total weight of the date fruit while the solids comprised of between 85.9% and 86.8% of the total weight of the date fruit. These values fall within the range observed in this thesis. In contrast, both the lowest and highest levels of moisture found in our study were considerably lower than the results reported by Ahmed et al. (1995). This may reflect the fact that our samples had been sourced from commercial markets and as such may have been sublected to conditions during transport and storage that had resulted in the dehydration of some of the fruit.

Crude fiber and ash also contribute to the weight of the date fruit. The lower values in the range of ash content (1.18±0.114 g/100g to 3.11 ± 0.173 g/100g) in the present study were similar to those reported by Ahmed et al., (1995) 1.3 g/100g to 1.9 g/100g. Parvin et al., (2015) found that ash contributed 2.13% to 2.18% of the total weight while crude fibers accounted for 6.05% to 6.9% of the total weight of the date fruit. The ash values in this case being within the range found in this thesis. The value for ash has been reported by several other workers (0.097±0.0015% to 0.53±0.01% versus 0.1 to 0.5%, 1.987±0.097% to 3.66±0.078% versus 1.7 to 3.0% and 0.066 to 0.106% versus 1.3 to 1.9%, respectively). Yousif et al., 1982; Sawaya et al., 1983; Booij et al., 1992; Yousif et al., 1992; Al-Hooti et al., 1997; Al-Farsi et al., 2005, 2007; Elluech et al., 2008; Hasnaoui et al., 2010; Borchani et al., 2010(Tunisian study) and Sulieman et al., 2012(Sudanese dates, Fat and protein) levels for these measurements were generally in agreements with concentrations found by us and Ahmed et al., 1995.

Our results in fat,total sugar, potassium and protein were similar to those reported by Hasnaoui et al., 2010 who found that fat ranged from 0.11g/100g to 0.57 g/100g and total sugar from 77.31 g/100g to 88.02 g/100g. And for protien 0.46 g/100g to 3.85 g/100g. Our results for fat, total sugar and protien were also similar to those reported by Borchani et al., 2010 who found that fat ranged from 0.1g/100g to 0.64 g/100g and total sugar from 61.64 g/100g to 83.32 g/100g, potassium was 792.11 mg/100g to 1626.5 mg/100g and protien 2.32 g/100g to 2.85 g/100g.

Carbohydrates represent a major component of the data fruit and are significant as both an energy sourse and as a component of the sensory quality of the fruit. The relative levels of glucose,

fructose and sucrose are impostant in this respect and as such were investigated further in this study. Individual, free sugars levels determined in our present study for sucrose ranged from as low as 0.00 to 52.56 g/100g , fructose ranged from 4.46 to 35.8 g/100g and glucose from 5.93-40.60 g/100g. The total combined sugars ranged from 55.49-78.66 g/100g. Other studies have found that the amount of carbohydrate is significantly higher in date fruits. In the study performed by Parvin et al., (2015) it was found that the amount of carbohydrates in the date fruit amounted to 50.8% to 55.0% of the total dry weight of the date fruit. Generally, glucose and fructose concentrations in our Saudi dates were similar but varied considerably between varieties and the present of sucrose is being normally associated with a corresponding reduction in the level of glucose and fructose. The lowest free sugar levels for sucrose, fructose and glucose encountered in different varities in the present study were comparatively lower than those reported by Borchani et al. (2010) who reported the lowest levels of sucrose as 1.07 g/100g, fructose as 0.63 g/100 g and glucose as 19.85 g/100g. In addition, they were comparatively lower than the lowest values reported by Sulieman et al.,(2012) which were sucrose 17.83 g/100g, fructose 0.63 g/100 g and glucose 12.88 g/100g. In contrast the highest reported levels for the sugars in this study were similar to those reported in this thesis, these being (33.42 g/100g in Rushodia , 37.40 and 40.60 g/100g in Sullaj, for sucrose, fructose and glucose, respectively). The sucrose concentrations observed in in our study were in agreement those reported by Borchani et al. (2010) while our highest value was significantly higher (more than two folds) than that reported by Sulieman et al., 2012 (52.56 versus 22.48g/100g, respectively). Additionally, the level of sugars reported by Al Farsi et al, (2005), were generally in agreement with those reported by Ahmed et al. (1995)(44.3-64.1 g/100g), Al-Hooti et al., (1997) (about 88 g/100g) and Al- Shahib W. and Marsall R.J.,(2003) (44-88 g/100g). The levels of sugars in each case were in general agreement with the range found in our study.

Hamad et al., (2015) carried out a study where they aimed to determine the nutritional quality of various varieties of date fruit in Saudi Arabia. The analysis of the type and amounts of various sugars in the date fruit was one of the objectives of the study. The findings of the current study can be compared with the study by Hamad et al., (2015) because both studies were carried out in Saudi Arabia. Comparison of the amount of sugars between the two studies showed differences. For instance, the amount of glucose and fructose in the Rashodia cultivar as reported by Hamad et al., (2015) was 42.5 ± 0.6 and 53.0 ± 0.0 g/100g, respectively. Although there was

variation in the amount of glucose and fructose between the various regions, there were still significant variations between cultivars in the amount of glucose and fructose reported in the two studies. For instance, the amount of glucose and fructose in the Rashodia cultivar in Madina was 19.62±0.055 and 17.47±0.181~~g/100g,~~ respectively. This is smaller compared to the glucose and fructose concentrations of 42.5 ± 0.6 and 53.0 ± 0.0g/100g for the Rashodia cultivar as reported by Hamad et al., (2015).

The same trend was observed in the Saffawy cultivar where Hamad et al., (2015) reported a glucose and fructose concentration of 47.3 ± 0.07 and 54.26 ± 2.4g/100g, respectively compared to the glucose and fructose concentrations of 31.32±0.109 and 30.13±0.167g/100g, respectively for the Saffawy cultivar from the Madina region seen in this study. For the same cultivar, Hamad et al., (2015) reported a sucrose concentration of 28.7 ± 1.04g/100g while the current study did not find any sucrose in the Saffawy cultivar from the Madina region.

A study performed by Mortazavi, Arzani & Barzegar (2010) offers various findings that may help explain the variations in the amount of glucose and fructose seen in the various regions as well as the variation between the current study and that performed by Hamad et al., (2015). Mortazavi, Arzani & Barzegar (2010) determined the amount of organic acids and sugars in date fruit during its growth and development process. Mortazavi, Arzani & Barzegar (2010) found that the type and amounts of organic acids in the date fruit were different at the various stages of development. For instance, the most predominant organic acid during the first stage of growth was malic acid. Succinic acid was the most predominant organic acid during the Khalal stage of development while acetic acid was the predominant organic acid during the Tamar stage of development. The concentration of the organic acids also increased towards the latter stages of growth (Mortazavi, Arzani & Barzegar, 2010). The same trend was witnessed for the amount of glucose and fructose in the date fruits during development.

During the early stages of development, the concentration of glucose and fructose in the date fruits was low. As the fruit matured, the concentration of these two predominant sugars increased significantly. The maximum level was achieved 160 days after full bloom (Mortazavi, Arzani & Barzegar, 2010). The variations in the glucose and fructose concentration of the date fruits in the current study from the various regions could be explained by the possibility that the

date fruits were harvested at different days after full bloom. This would result in a variation in the glucose and fructose concentration in the various samples.

Nutritionally, dates are high-energy foods rich in sugar content, but equally they represent a good source of minerals such as iron, potassium, and iodine (Vayalil, 2002). This thesis indicated that potassium was the major mineral found in all the varieties tested with concentrations as high as 1173.29 mg/100g. The highest mean regional value (907.81mg/100g) and the highest level of potassium (1173.29 mg/100g) were found in dates from the Madina region and the Anbara_variety from the Madina region, respectively. Our results for potassium were similar to those reported by Baliga et al.(2010) were the highest value was 1287 mg/100g, and it is also within the range reported by Ahmed et al. (1995) from 565 mg/100g to 916 mg/100g.

Eastern region dates contained the highest mean content (66.65 mg/100g) of calcium followed in descending order by Madina dates (56.76 mg/100g), Qassem dates (56.33 mg/100g) and Riyadh dates (53.66 mg/100g). The lowest level of calcium was found in the Safwai variety from the Madina region (32.47 mg/100g), whereas the highest (88.20 mg/100g) was encountered in the Ruzeiz variety from the Eastern region. Theses results were higher than those reported by Ahmed et al. (1995) which was 19 mg/100g and lower than those reported by Assirey (2014) which was 187 mg/100g.

However, in contrast Eastern region dates contained the lowest mean amount of copper (0.28 mg/100g), with the highest value (0.55 mg/100g) being found in Riyadh dates; thus this may indicate a reverse relationship between the calcium and copper contents of dates resulting from different soils. Theses results were similar to those reported by Baliga et al.(2010) from 0.01 to 0.8 mg/100g. Moreover, our results for iron were (0.99 to 1.77 mg/100g) which are simliar to those reported by Baliga et al.(2010) for iron from 0.10 to 1.5 mg/100g. Maganese levels encountered in the present study were in line with those reported by Ahmed et al. (1995) and Baliga et al.(2010) 0.5 mg/100 g and 0.4 mg/100g, respectively. Similarly magnesium levels encountered in the present study were in line with those reported by Ahmed et al. (1995) and Baliga et al.(2010) 47 mg/100 g to 82 mg/100 g and 31 mg/100 g to 150 mg/100g, respectively. For phosphorus the results in this thesis (37.90 to 81.28 mg/100g) were higher than those reported by Assirey.(2014) whose highest value was 27 mg/100g. However, they were in in line with those reported by Baliga et al.(2010) (35 to 74 mg/100g).

For Sodium our results (0.81 to 5.13 mg/100g) were lower than those reported by Assirey.(2014) , Ahmed et al. (1995) and Baliga et al.(2010) where highest soduim level were (8.9 mg/100g, 287 mg/100g and 261 mg/100g, respectively). However, our Sodium results are in agreement with those reported by Al-Shahib et al., 2003, who found that amount of Sodium at the tamar stage was 5.4 mg/100g.

The Selenium and Zinc content (0.1mg/100g to 0.40mg/100g and 0.21mg/100g to 0.55mg/100gm, respectively) in the present study were similar to those reported by Baliga et al.(2010) (0.24 mg/100g to 0.4 mg/100g and 0.02 mg/100g to 0.6 mg/100g, respectively).

In general, our minerals results were similar to those presented by Al-Farsi.(2008). Hamad et al., (2015) analyzed the mineral content of various cultivars of date palm fruits in Saudi Arabia. Their findings offer a basis for comparison. The mineral content of the various cultivars of the data palm fruit in the current study are different from those reported by Hamad et al., (2015). For instance, the amount of copper in the Rashodia cultivar from the region of Madina was 0.52±0.036 and 0.58±0.075mg/100g compared to 2.62 ± 0.212mg/100g reported by Hamad et al., (2015). The amount of zinc reported by Hamad et al., (2015) for the Saddawy cultivar was 0.923 ± 0.06mg/100g. The zinc content for the same cultivar in the current study at 0.53±0.054mg/100g was not very different. The trends show that where there were differences in the mineral content between cultivars when the two previous mentioned studies are compared, there is almost no difference in some cultivars and minerals.

Differences in mineral content can be explained by various factors that influence the concentration of minerals in fruits and vegetables. Florkowski et al., (2009) argues that the ash values of various fruits are significant factors that influence the mineral content in the fruit. Table 4.4 showed that there were differences in the ash content of the various cultivars of the date palm fruit. The differences were also observed in the ash content of the same cultivars in different regions in Saudi Arabia. The difference in the ash content is cited as a predictor for the differences in the mineral content of the date palm fruit (Florkowski et al., 2009). Another factor that might explain differences in the mineral content of the different cultivars is the genetic makeup of the various varieties of the date palm fruit (Florkowski et al., 2009). The genetics of the plant affect the sink characteristics, the manner in which the vascular tissues are distributed, and the metabolic

rates. Florkowski et al., (2009) found that various fruit varieties might have different mineral concentrations even if they are grown in the same conditions.

Pre-harvest practices and factors might also affect the mineral content of the date palm fruit. Florkowski et al., (2009) argued that the location in which the fruits were grown influences the mineral concentration. Some of the most significant pre-harvest factors include nutrient content of the soil in which the plant is grown. If there were variances in the soil nutrient content in the various regions, the differences are bound to manifest in the mineral content in the fruit. Other abiotic factors in the environment that might influence the mineral content include the bioavailability of the minerals in the soil, the pH, moisture content and the aeration of the soil. Human factors such as cultural practices in the growth of the date plant in the various regions might also affect the mineral content of the fruit. For instance, the use of plant growth regulators, thinning practices, the application of inorganic fertilizers, and pruning are highlighted by Florkowski et al., (2009) as some of the factors that might influence the mineral content of the same cultivar from one region to another.

The antioxidant potential, and related to this the phenolic content of dates, is perhaps one nutritional aspect that has received relatively less attention in the past. Safaa Y. Qusti, et al.(2012) mentioned 16 antioxidant containing food items that were cited in Holly Quran in their review "Screening of Antioxidant Activity and Phenolic Content of Selected Food Items Cited in the Holly Quran. They organised these items into three groups according to their phenolic content: (1) high (> 70 mg GAE/g (fw) and > 400 mg GAE/g (dw)), (2) moderate (10-70 mg GAE/g (fw) and 150-400 mg GAE/g (dw)) and (3) low (< 10 mg GAE/g (fw), < 150 mg GAE/g (dw)). Their results showed that in general, a good correlation could be found between antioxidant activity and phenolic acid content.

The total phenolic content (TPC) of the nineteen date vatieties was thus assessed. We have shown that the TPC in the 24 samples that we studied, ranged between 147.17±2.12 mg GAE/100g in Rushodia-Qassem to 255.20±0.28 mgGAE /100g in Khodry- Qassem. According to Vinson, et al.,(2005), dates have high levels of both free and total phenols (2,546±29 mg/100g and 1,959±244 mg/100g) in both fresh and dry versions, respectively. Amounts as high as 572- 661 mg GAE/100g phenolics in dates have been reported by Wu et al., (2004).

A significant difference in TPC between cultivars of Tunisian dates was reported by Emna Behija Saaf, et al. (2009) in their study they compared the phenolic content and antioxidant activity of four date palm (Phoenix dactylifera L.) fruit varieties grown in Tunisia. The date varieties were found to be rich in total phenolics ranging from 209.42 mg GAE/100g fresh weight to 447.73 mg GAE/100 g fresh weight. These were shown by the authors to have similar levels of TPC to those of Oman dates (values between 172-246 mgGAE/100g as reported by Al-Farsi et al., 2005, 2007, and also with those of Bahrain (Allaith, 2008) but appeared to be considerably higher than ours containing approximately double the highest and lowest levels of phenolics. The TPC of Omani dates ranged between 134 and 280 mg/100g, and between 217 and 343mg/100g, in fresh and dried dates (Al-Farsi et al., 2005). Values for fresh dates in this study were comparable to ours which ranged between 147.17 and 255.20 mg/ 100g. Khanavi et al., 2009, assessed and compared TPC and antioxidant activity in 10 varieties of Iranian dates from the Busheher region. In this evaluation four different methods of extraction were used. The extracts exhibited different amounts of total phenolics which ranged from 102.72 mg GAE/100 g to 276.85 mg/100 g. These values seem to fall within a range approximately similar to our study. In contrast, Foroogh Biglari, et al.,(2008) determined TPC and antioxidant activity in eight Iranian date cultivars, and found their TPC to be considerably lower ranging between 2.89 and 141.35 mg GAE /100 g. A multi country study (Pakistan, Egypt and Saudi Arabia) by Faqir Muhammad Anjum , et al.,(2012) found that the highest TPC was 276.85 mg GAE/100 g of the dry plant. Significant differences existed among extracts with different solvents with some exceptions. This was slightly greater than the levels encountered in our present study.

Antioxidant activity (AA) of peels, pulp and seeds of common fruits has been determined by Guo, et al., (2003). Their results for AA of fruit pulps, as measured by the FRAP assay, have shown that dates came 2nd after hawthorn, surpassing guava, kiwifruit, purple mulberry, strawberry and another 22 fruit. It should be be noted that there was more than a 90-fold difference between the FRAP values in the fruits pulps tested. As for peel AA content, dates were ranked 3rd, and for seed content they were ranked 7th. In this study the AA for date pulp, peel and seeds was 6.98±0.29, 16.69±0.55 and 1.77±0.13 mmol/100g, respectively. In comparison with results from our study, we can concluded that the AA in our 24 dates cultivars were lower, ranging from 0.62±0.147 to 1.64±0.314 mmolTE/ 100g. These comparatively higher levels of AA of dates have been reported by other researchers as well (Vayalil, 2002; Al-Farsi, 2005, 2007; E. Behigia Saafi,

2009). Wolfe et al., (2008) in their study on the cellular AA of common fruits have shown that in general berries had the highest AA values as assessed by ORAC, AA, TEAC and FRAP assays. They also demonstrated that this correlated with the TPC of the fruit. This result was not in accordance with that of Guo, et al., (2003), where two berries came next to dates while guava and kiwi had the 4th and 5th ranks. Behigia Saafi, (2009) used the Trolox equivalent capacity (TEAC) assay and Brand-Williams et al.,(1995) the DPPH method to measure AA of four Tunisian date cultivars and found it to range between 866.82 l (µmol Trolox equivalents/100 g fresh weight) and 1148.11 (µmol Trolox equivalents/100 g fresh weight). According to this Tunisian study and claim by its authors, the Tunisian date palm fruits had higher levels of AA compared to Iranian dates (by TEAC assay) and Algerian dates (by the DPPH procedure). The AA in the Iranian date varieties was measured by the FRAP method and averaged between 11.65±0.88 and 387.34±1.94 µmol/100g (Biglari et al. 2008), which were significantly lower than ours (1641.5±314 µmol/100g). As for the AA of sixteen Bahraini date palm cultivars assayed from a pooled sample of dates at various stages of development; (Biser, Rutab and Tamer) these gave a FRAP value of 4000.16±4150 µmol/100g ranging from 1000.05±360 to 9000.18±7110 µmol/100g (Allaith., 2008). These levels were considerably greater than the levels encountered in our present study. Ten varieties of Pakistani dates, extracted by four different solvents, and with AA determined by the FRAP method gave values ranging from 575.77 to 3279.48 µmol/ 100g $FeSO_4$ (Khanavi et al. 2009). These authors reported that all extracts of dates showed various levels of inhibitory effects (DPPH) ranging from 5.45% to 56.61%. Power of DPPH scavenging radicals was high and comparable to 10mg/l of α-tochopherol according to their report. Hasani., et al.,(2007) found the AA of dates tested by a β-carotene bleaching procedure to range from 9.28 to 75.96%. Also Chinese date pulp was shown to contain about 6.9 mmol/100g (6900 µmol/ 100g $FeSO_4$)(Halliwell B.,1999), which considerably surpassed the Pakistani date values indicated above. The FRAP values in these Pakistani dates were significantly higher than our values. Total AA of three premium Saudi date varieties as determined by DPPH were significantly different ranging between 2.9±0.30 to 6.6±0 µmol TE/g dry basis, (water extracts) and from 3.80±0.29 to 9.10±0.11 µmol TE/g dry basis, (alcohol extracts) (Ebtesam Abdullah Saleh1, et al. 2011).

Due to the chemical nature of antioxidants, and phenolics in particular, they can vary from simple to very highly polymerized molecules, thus there is no one suitable solvent extraction method for antioxidants and phenolics or even for a specific class of these components (Shahidi

and Naczk, 2003). Thus the range of extraction and assay methods employed in our and previous literature may be a significant factor in the large variaition in the values of these two parameters being reported.

Ebtesam Abdullah Saleh1, et al., (2011) in their study on the phenolic and antioxidant content of various Date Palm (Phoenix dactylifera L.) fruits from Saudi Arabia concluded that generally, water extracts of three premium quality date varieties (Khalas, Sukkari and Ajwa) showned significantly higher contents of total phenols than alcoholic extracts, especially in the case of the Ajwa varieity (455.88 and 245.66 mg/100g, respectively). They showed that the TPC depended on date variety and extraction solvent. Ajaw water extract contained the highest TPC (455.88 mg/100g) compared to sukkari 377.66 mg/100g and khalas 238.54 mg/100g extracts. These researchers measured TPC in alcohol and water extracts of the three date varieties at the tamer stage, and their results follow the same trend, as in our study where TPC was determined in alcohol extracts of 24 different varieties of dates from four regions of the kingdom of Saudi Arabia. The wide range of difference in TPC in two varieties; Khodry-Qassem and Rusodia –Qassem (same region) suggests that there may be little influence of locality as regards climate, soil and other elements, on nutritional composition and supporting Ebtesam Abdullah Saleh1, et al. (2011) claim, that, it may be the variety that is the main determining factor of TPC level.

Saleh Al-Turki, et al., (2010) in their study on the diversity of antioxidant properties and phenolic content of date palm (Phoenix dactylifera L.) fruits as affected by cultivar and location characterized and compared TPC and AA of fruits of ten cultivars from the USA and fruits of five cultivars from Saudi Arabia collected in 2006 and 2007. They found that fruits of cultivars collected from Saudi Arabia had higher dry matter content than those collected from the USA. In most of their tested cultivars, TPC was significantly higher during the second year of the study. The TPC in the tested varieties ranged between the highest value of (507.0 mg GAE/100 g FW to the lowest content of 225.0 mg GAE/100 g FW. Again these levels of TPC were substantially greater than the levels encountered in our present study. The wide difference seen in the study by Saleh Al-Turki, et al. (2010) could be attributed to the different methods used for measuring antioxidant activity in addition to the effect of location on antioxidant activity and phenolics content, as well; these same factors may have contributed to the variability of their results and ours.

In a study of cellular antioxidant activity of common fruits (Kelly et al., 2008), found that the highest TPC was found in blueberry and blackberry (429±10 and 412 ±6 mg GAE/100 g), respectively. Examples of other fruits tested included pomegranate (338±14 mg GAE/100 g); cranberry (287±5 mg GAE/100 g); raspberry (239±10 mg GAE/100 g); plum (239±7 mg GAE/100 g) and strawberry (235±6 mg GAE/100 g). In comparison, some Saudi dates in our present study would be ranked forth in this list for instance Khodry-Qassem at 255.20±0.28 mgGAE/100g, Khodry-Riyadh at 253.73±1.8 mgGAE/ 100g, Ruzeiz-Eastern Region at 252.68±1.5 mgGAE/ 100g, and Ajwa-Madina atb250.77±0.16 mgGAE/ 100g. Also, we can conclude that the Saudi date variety Rushodia-Qassem having the least amount of TPC (147.17±2.12 mgGAE/100 g), has a TPC level in excess of many other fruits, namely pear (94.8±0.7mgGAE/100g);pineapple (78.1±0.8mg/100g): peach (73.1±2.4mg/100g);grapefruit (71.0±1.3mg/100g);nectarine (66.3±2.1mg/100g);mango (62.6±4.2mg/100g); kiwifruit (60.4±3.3 mg/100g); orange (56.9±0.8 mg/100g); banana (54.8±1.3 mg/100g); lemon (50.8±0.9mg/100g);avocado (23.9±0.7mg/100g);cantaloupe (16.0±0.4mg/100g); honeydew (15.5±0.9 mg/100g) and watermelon (14.1±0.3 mg/100g). Vinson, et al.,(2005) compared fresh and dried fruits as regards their in vitro and in vivo antioxidants activities and stated that the best nutrient score out of six fresh fruits (apricot, cranberry, dates, figs, grapes, and plums), belonged to dates. However, the nutrient score was significantly better for fresh dates, and as for the corresponding six dried fruits, dates were only ranked fifth with apricots and figs ranked first in this instance. These nutrient scores were based on serving size which was 40 grams for all dried fruits tested, and serving sizes for the fresh were 165g for apricot, 95g for cranberry, 138g for dates, 100g for figs, 154g for green grapes and 66g for plums. Fresh dates were found to surpass cranberries the richest fresh fruits in total phenolics, as some of them contained 2,546±29 mg GAE/100g phenolics (Vinson, et al.,2005).

The observation that fresh dates seemed to have a higher nutritional composition than dried was partly the impetus to examine the impact of stage of development on date fruit composition. This also provided an opportunity to compare market varieities with fruit harvested directly from the tree. All seven Saudi date varieties in this study exhibited the same pattern of decrease and increase of TPC with stage of maturity. In the results we have shown that TPC decreased from the fresh stage to the semi-fresh stage and then retained a pattern of slight increase from this semi-fresh stage to the next 3rd and 4th stages (semi-dry and dry stages) in ascending order. This

observation may be of significance for the nutritional value of dates as their TPC was not adversely affected by stage of maturity at the later stages of development, but rather it was slightly improved in terms of phenolic content. It was also found that the ranking between varieities identified in the market bought fruit was mirrored quite closely in the field harvested material adding further strength to the varietal differences identified in the first study.

In date fruits, some early studies showed that TPC could reach 3000mg AGE/ 100 gFW at early stages of maturity, at which stage the phenols are responsible for the fruit astringency. At later stages (tamer stage), the fruit maturity is completed and it loses its astringency by the precipitation of phenols in an insoluble form (Mutlak, 1986). A decrease in total phenolic through the various stages of ripening in all the seven varieties studied was also reported in a study performed by Haider et al., (2014). This group found that there was a statistically significant difference in the total phenolic content of date fruits through their various developmental stages and that the total phenolic content decreased from the khalal to the tamar stage of development of the dates. The khalal is the earlier stage of the development while the tamar stage is the very latest stage of development of the date fruits.

The decrease in the total phenolic content as the date fruit ripens could be explained by the decrease in the content of tannins in the date fruit as it develops further. This finding was reported by Tafti & Fooladi, (2006) in a study where one of the objectives was to compare the tannin content in the date fruit at different stages of their development. The tanning content at the kimri stage was measure at 1.7% of the total solid content whilst at the khalal stage it was 0.46% a significant decrease from that reported at the earlier stage. At the tamar stage, Tafti & Fooladi, (2006) measure the tannin content in the date fruits at 0.24%. The findings show that the unripe date fruits have more tannins compared to their ripe counterparts.

Haider et al., (2014) also attributed the decrease in the phenolic content in the dates, as they progressed through the various developmental stages, to the oxidation of the total phenolic content by polyphenol oxidase. Polyphenol oxidase has a significant effect on the total phenolic content in plant-based food items. In studying its effect on cocoa beans, de Brito, Garcia & Amancio (2002) found that there was a statistically significant reduction in the total phenolic content in the cocoa beans when they were treated with polyphenol oxidase.

The differences in the TPC of the different varieties of the dates studied could also be explained by factors such as the region in which the dates were grown, the stage of ripeness, the environmental conditions during the growth of the date plant, and the postharvest handling practices (Haider et al., 2014). The storage conditions are particularly important when studying the total phenolic content in the fruit because the duration for which the dates are stored has an effect on phenylalanine ammonia lyase. This is an enzyme that is involved in the biosynthesis of the phenolic compounds in the date fruits. The longer the dates are stored, the more the effect of the phenylalanine ammonia lyase. Increased activity of the enzyme will lead to increased biosynthesis of the phenolic compounds, a factor that results in an increasing of the total phenolic compounds in the date fruit.

The analysis of the phenolic compounds in the date palm fruits is justifiable from a nutritional perspective owing to the significance of phenolic compounds in the human body. Phenolic compounds have been reported to be of significance in the treatment of various illnesses as well as the prevention of cancer (Hunag, Cai & Zhang, 2010). Natural phenolic compounds can be obtained from dietary plants as well as medicinal herbs. They include coumarins, stilbenes, flavonoids, quinones, lignans, tannins, curcuminoids, and phenolic acids. These phenolic compounds have different bioactivities that may confer various benefits to the human body through the chemo-preventive properties of the phenolic compounds. Some of these effects include anti-inflammatory properties, anti-mutagenic properties, antioxidant, and anti-carcinogenic properties (Hunag, Cai & Zhang, 2010). Phenolic compounds may also have other benefits to the human body that justify their analysis in food products. By controlling the cell cycle, phenolic compounds help to regulate apoptosis. They also regulate the metabolism of carcinogens and the expression of ontogenesis (Hunag, Cai & Zhang, 2010). Phenolic compounds also help inhibit, cell proliferation, migration, adhesion, and differentiation (Hunag, Cai & Zhang, 2010). The nutritional significance of phenolic compounds has also been highlighted by Cheynier (2012) who argues that the phenolic compounds influence the quality of the foods that are based on plants. It should also be remembered that phenolics are important in food processing where they are used to produce the red color in wines and fruit juices and also as a substrate to enable enzymatic browning. Phenolic compounds are also significant for their influence on the flavor of food (Cheynier, 2012, p.153). These factors make it important to analyze the phenolic compounds in a food item. The

analysis gives information on the concentration of specific compounds in a food item, and hence, its appropriateness against various health or processing needs.

There was a good correlation between TPC and AA both within varieties and at the different stages of development. There was thus a need to determine whether there was an association between TPC and AA in the dates. Other studies performed with the aim of determining the nutritional value of dates have performed these tests. For instance, Allaith (2007) determined that there was a statistically significant correlation between the total phenolic content in the date fruits and their antioxidant power. With a R2 value of 0.595, the strength of the correlation was moderate but positive. This relationship was determined at the biser stage of fruit development. This led to the conclusion that the total phenolic content in the date fruits was a significant factor that influenced their ferric reducing antioxidant power.

A study performed by Allaith (2007) found that the most antioxidant activity in the date fruit was found when the date fruit was at the biser stage of development. The FRAP value at this stage was 5.71±4.31mmol/100g FW. This stage of development coincides with the fresh fruit stage of ripening considered in this study. The finding that the highest FRAP value was in the fresh date fruits was consistent with the findings by Allaith (2007). The difference between the actual numbers is not very significant. Any differences could be explained by factors such as the different varieties of dates, the environmental conditions in which they were growth, and the pre and post handling practices as discussed earlier. These findings show that the FRAP value was lower in the semi-fresh date fruits when compared to the fresh fruit. This stage of development coincides with the rutab stage of development in the study performed by Allaith (2007). This researcher found that during this stage of date development, the FRAP value was 1.2 mmol/100g. This was a significant drop from the 5.71±4.31mmol/100g that was reported in the earlier stage of development. A similar reduction in FRAP was reported in the current study from the fresh stage of ripening to the semi-fresh stage of ripening. The nutritional value of the dates at this stage of ripening with reference to the FRAP value was significantly lower compared to the dates at the fresh stage of ripening. The finding of the lowest FRAP value in the dry stage of fruit ripening was also reported by Allaith (2007) who reported the lowest FRAP (0.94±0.21 mmol/100g) at the tamar stage of development. The tamar stage is the dry fruit stage of date development. This was not significantly different from the FRAP value reported in this study. These findings may be

important from a nutritional perspective because they show that the nutritional value of the date plants with reference to FRAP is highest when the fruits are fresh compared to when they are dried. The findings also show that the further the date fruits progressed on the stages of development, the lesser the antioxidant power.

This study did not examine the impact of maturity on other components of the fruit. However, in a study of chemical composition of date varieties as influenced by stage of development and ripening, the percentages of moisture of twelve date cultivars were found to range from 80.1% lowest value to the highest level of 85.5% at Kimri stage, from 54.5% to 76.5% at Khalal stage, from 35.9% to 50.4% at Rutab stage and from comparatively very low level of 9.2% to 29.5% at the last stage of ripening the Tamer stage (Ahmed I. et al. 1995). Similarly, sugar content (g/100g FW.) of those twelve commercial varieties of date at the different stages of ripening indicated above, respectively, ranged from 5.1 g to 7.7 g, 18.8 g to 31.9 g, from 40.8 g to 50.1 g and from 44.3 g to 64.1/100 g.

Date colour is significant and to this effect anthocyanins may make a significant contribution to both the TPC and AA. Levels of anthocyanin during the development of the seven varieties of date were thus assessed. Our results indecated that the anthocyanin content was low at all stages of date development except for the Khodry cuitivar where its content of anthocyanin was clearly higher than the other varieties at the fresh stage. Moreover, the content of anthocyanins at the semi-fresh stage was slightly decrease from the fresh stage due to the slight sun drying for seven days from fresh to semi-fresh and they are approximately the same colour. In addition, anthocyanin content was found to decrease sharply from semi-fresh stage to semi-dry stage to almost one-third of the semi-fresh value. There was a strong significant correlation between anthocyanin and AA.

Several groups have studied the anthocyanin content of different fruits and their cultivars and found them to range widely between 0 to 515mg/100g (fw) (Kalt., et al., 20000; Cantos. et al., 2002; Slimestad., and Solheim., 2002; and Moyer., et al., 2002). Anthocyanins levels as detected in three Omani fresh date varieties were significantly different (Al-Farsi, 2005). They ranged between 0.25 and 1.52mg/100g. These authors concluded that the dates they studied were poor sources of anthocyanins. Al-Farsi, (2005), reported that sun dried dates do not contain any anthocyanins, possibly to their destruction upon drying.

The study by Al-Farsi et al., (2005) found that anthocyanin was only present in the fresh dates. However, this study detected anthocyanin in the semi-fresh, semi-dry and dry dates. There was a statistically significant difference in the amount of anthocyanin as the dates progressed down the various stages of ripeness. A study by Rumainum et al., (2016) found that the amount of anthocyanin in fruits was affected by various factors among which was light. Light treatment of the fruits resulted in a decrease in the amount of anthocyanin in the flesh of the fruits. There are several studies that have addressed the changes in pigment content of dates at different stages of development. Bacha et.al, (1987) studied the physical and chemical characteristics of the fruits of Seleg, Sakhi, Khudari and Nebut Seif date palm cultivars during three stages of fruit development (Kimri, Khalal and Tamar). They concluded that pigment content was high in the Kimri stage and was then greatly reduced in the other two stages these results are similar to those in our study except that we included an extra stage of development. Studies by Al Farsi et al, (2005) have shown that among the analyzed fresh date varieties, the highest amount of anthocyanin was detected in Khasab (1.52 mg/ 100 g), followed by Fard (0.92 mg/100 g) and Khalas (0.24 mg/100 g), these were expressed as cyanidin 3-glucoside equivalents. The amount of anthocyanins in Khalas is similar to that found in our study where the amount in fresh fruit was (0.331 mg/ 100 g), followed by semi-fresh (0.297 mg/100 g) then in semi-dry (0.046 mg/ 100 g) with the least amount found in dry fruit (0.021 mg/ 100 g) and this due to a direct correlation that existed between the levels of anthocyanin and the stage of maturity.

In this study, commercial sun-drying of dates (at 30-50 °C for 7-14 days) lead to the almost complete destruction of anthocyanins. According to (Wrolstad, 2004) the degradation of anthocyanins during drying and storage is due to enzymatic and nonenzymatic browning reactions. Many enzymes have been found to be involved in the degradation of anthocyanins; these include glycosidase and polyphenol oxidase as stated by (Shahidi et al, 2004).

Our comparison of fresh and market sourced fruit also suggested a decrease in nutritional value in the latter. Although it should be noted that these samples in our study were not direcly matched. Studies have been performed that could explained this trend between dates from the market compared with dates from the field. As highlighted earlier, the postharvest handling process are very important to the nutritional quality of fruits and vegetables. Mirzaei & Garmakhany (2015) found that the drying practices and conditions used have an effect on the TPC

as well as the FRAP of dates. The researchers found that oven drying and sun drying affected the antioxidant activity and the phenolic compounds in the Iranian dates, especially when the temperatures ranged between 50oC and 80oC (Mirzaei & Garmakhany, 2015). An increase in the temperature at which the date fruits were dried resulted in a decrease in the antioxidant activity and the total phenolic content. The decrease in these values was more significant in the antioxidant activity where the value reduced from 314.2 to 210.4 GAE/100g when the drying temperature was set at 80oC compared to the reduction of gallic acid equivalents (the phenolic compound) from 667.3 to 610.5 (GAE/100g dry weight) at the same drying temperatures (Mirzaei & Garmakhany, 2015). It is possible that the dates in the market had been dried at a higher temperatures than those from the field, hence contributing to the lower FRAP and TPC values.

Our results, and those of other researchers, have demonstrated that there were significant changes in the TPC and AA levels througout date development. Phenolics represent a diverse range of chemical compounds and antioxidant activity could similarly be attributed to a number of compounds. A preliminary analysis was thus carried out to examine the extent to which the metabolic profile, in particular the phenolic profile, was conserved between varieties and stages of development. It was also hoped that this analysis would also allow a tenetative identification of potential phenolic compounds. Since our results, and those of some other researchers, had identified greater TPC and AA activities in water extracts these were used for this preliminary investigation. There were some differences observed between the HPLC profiles obtained from the seven different varieties, although this might to some extent have been confounded by the low level of signal associated with some of the peaks. It was found that the majority of the detectable peaks declined during development and although this corresponds to a general decline in TPC, especially between the fresh and semi fresh stages of development, does not imply that these peaks are associated with phenolic compounds. That said comparison of peak retention times with those of standards temtatively identified nine phenolic acids (gallic, protocatechuic, p-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric, ferulic, and o-coumaric acid). Ferulic acid being the major phenolic acid found in all the date cultivars as stated in (Al-Farsi,2005). This was only a preliinary study and was not analysed further.

6.1 Discussion new assays

The comparison of the assays for the six cultivars of the date palm fruit showed very strong correlations with that obtained in the preliminary screen during the preliminary screen. Indeed, the only really major difference between the two sets of data were the absolute values for the antioxidant activity which, whilst retaining the same ranking, tended to be higher in the in-depth screen than in the preliminary study.

These differences might have been due to the fact that the samples were not harvested during the same season. The data from the old samples preparation was collected at an earlier date when compared to the data from the new samples preparation that was collected at a later date. The difference in the seasons is a possible factor explaining the difference in the values of the same cultivar of date palm fruit for each of the three variables that were studied.

The effect of seasons on the nutritional components of the date palm fruit has been highlighted by Hamad et al., (2015,). They found that the nutritional component of the date palm fruit is affected by a variety of factors such as the season during which the date palm was growth, the conditions during growth, and extent to which the date palm fruit was exposed to sunlight. This finding makes it plausible that there might have been a difference in the values of the various variables for date palm fruits of the same cultivar harvested during different seasons.

6.1.1 Sugar Composition in the Different Cultivars of the Date Palm Fruit

In addition to the proximate composition described above the sugar composition was further investigated by determining the levels of glucose, fructose and sucrose in the 24 varieties of dates. The results reveal that the total sugar content is high and based on variety and locality. These sugars are mainly reducing sugars in the form of glucose, fructose, and non-reducing sugars (mainly sucrose). The variance in sugar composition indicates potential difference in invertase activity in these cultivars, an enzyme which is responsible for the conversion of sucrose into glucose and fructose (Hamad, et al., 2015). The existence of pronounced invertase activity in dates,

which significantly reduces its sucrose content has also been shown by Elleuch et al., (2008) and Besbes et al., (2009) in their studies on the chemical composition of dates.

Table 4.5 disclosed that sugar content ranged from the lowest value of 55.5 g/100g as found in the Anbara-Madina variety to the highest value of 78.7 g/100g in Sullaj-Riyadh. However, we can say that sugar levels in general of these Saudi dates from these four regions do not differ very much with mean values of 65.02 g/100g in Madina dates, 66.30 g/100g in Qassem dates, 68.96 g/100g in Eastern region dates and 71.77 g/100g in Riyadh dates. Generally, this is not unexpected, as it is known that dates are important source of sugar and hence, are used in many food products to sweeten them and increase flavour in these products. This result was also in agreement with that of Assirey (2015) and Hasnaoui, et al., (2010) in a previous study for other date varieties. The result was also similar to that of Besbes et al., (2009) in a study of three Tunisian dates cultivars, were these authors indicated that the sugar content was predominance in all these cultivars.

The results also indicated that glucose and fructose concentrations in these Saudi dates were generally similar but varied considerably between varieties ranging from about 5% each in the Sukkari variety from Qaseem to around 35-40% in many other varieties. There was less variation between regions. The highest mean value for glucose was found in Riyadh dates (32.85 g/100g) followed by Madina dates (30.94 g/100g), Eastern region dates (30.39 g/100g) and Qassem dates (21.47 g/100g). For fructose, the corresponding mean values were 30.18 g/100g in Qassem dates, 28.40 g/100g in Eastern region dates 28.57 g/100g in Madina dates 20.2 g/100g in Riyadh dates. It is interesting to note that the ranking for fructose is the inverse to that for glucose.

Results revealed that sucrose was absent in most of the date varieties. The present of sucrose being normally associated with a corresponding reduction in the level of glucose and fructose. The level of sucrose varied significantly between those varieties that contained this sugar. The highest level (52.5 g/100g) was found in the Sukkari variety from Qaseem, which not surprisingly was the variety that also contained the lowest levels of glucose and fructose. The lowest level (3.27 g/100g) was found in the Segae variety from Riyadh. This variety had levels of glucose and fructose similar to those varieties with no sucrose.

However, the lack and absence of sucrose in most varieties can be linked to the genetic and environmental issues that may have impact the quality and quantity structure of the sugar fraction

by changing the activity of the enzymes involved in synthesis and breakdown processes. These results are in agreement with Rastegar et al., (2012) where they indicated that sucrose experienced a complete hydrolysis into reducing sugar at different maturation stages.

6.1.2 Discussion of the Total Phenolic Content

The data collected from the preliminary screen suggested that the total phenolic content was different in the different cultivars of the date palm. This was confirmed in more in-depth screen of a limited number of varieties. The empirical data showed that total phenolic content for the Khodry variety of the date palm fruit was 241.74±30.25 mg GAE/100g, 143.61±15.24 mg GAE/100g for the Rushodia variety, 144.24±20.18 mg GAE/100g and 207.85±22.45 mg GAE/100g for the Shahal and Sullaj varieties respectively. These values show a difference from one variety of the date palm to another. Even though the different varieties have different amounts of the total phenolic content, the impact of factors such as the ripeness of the samples used, the region where the cultivars were cultivated, the time for which the dates were stored, the conditions during storage, and the environmental factors cannot be ignored (Haider et al., 2014). In both cases dates the dates for these screens were obtained commercially and as such may not have been very specific in terms of the stage of maturation or ripening of the date fruit used,

Results in this thesis from dates at various stages of development have shown a significant decline during maturation. Haider et al., (2014) found that the total phenolic contents in a date palm was different during the various maturation states. The total phenolic content was highest in the date palm fruit during the Khalal stage of ripening. This is the earliest stage when the date palm is mature. As the date palm tends towards the tamar stage, the amount of the total phenolic content reduced significantly.

Different factors can be cited to explain the decline in the total phenolic content as the date palm fruit tends from the Khalal stage to the tamar stage. Haider et al., (2014) found that due to the oxidation by polyphenol oxidase, the amount of total phenolic content also reduces. Additionally, the reduction in the amount of tannins that occurs as the date palm fruit progresses through the different ripening periods results in the reduction of the total phenolic content.

6.1.3 Discussion of Ferric Reducing Antioxidant Power

The in-depth screen confirmed the suggested conclusion from the preliminary screen that there were differences in the ferric reducing antioxidant power of the different cultivars of the date palm fruit. For instance, the analysis of the Shahal and Sullaj varieties showed 0.62±0.08 mmol TE/100g for the Shahal variety while the amount for the Sullaj variety was 1.22±0.21 mmol TE/100g. There was also a significant difference between the Khodry variety with a ferric reducing antioxidant power of 0.86±0.24 mmol TE/100g while the Rushodia has a ferric reducing antioxidant power of 0.99±0.27 mmol TE/100g.

The difference in the ferric reducing antioxidant power of different cultivars has also been highlighted by Shahdadi, Mirzaei & Garmakhany (2015). These researchers attributed the differences in the ferric reducing antioxidant power to different factor, which included the preparation of the assays. One of the aspects of the assays that was highlighted was the stage of ripening at which the sampled fruits were. The difference was explained by the capacity to donate electrons by the bioactive compounds in the date palm fruit. Shahdadi, Mirzaei & Garmakhany (2015) found that this potential, and hence the reducing power was highest during the khalal ripening stage and the lowest during the tamar ripening stage. The temperature at which the samples were stored was also a significant factor because it affects the drying of the dates. Shahdadi, Mirzaei & Garmakhany (2015) reported that as the temperatures increased, the ferric reducing antioxidant power of the date palm fruit reduced. The effects of the elements above was also highlighted by (Al-Jasass, Siddiq & Sogi, 2015). These researchers found that factors such as the maturity of the date palm fruits, the preparation of the assays, relative humidity, and the temperature also affect the ferric reducing antioxidant power (Al-Jasass, Siddiq & Sogi, 2015).

Chapter 7 Conclusion and Future work

7.1 Conclusion

Food is not consumed just for the nourishment of the human body. Some food items have therapeutic effects. For instance, one can consume carrots if they are deficient of Vitamin A in order to replenish their sources. Dates are important for their free radical scavenging properties among the other nutrients that have been highlighted in this study. The present study explored the different varieties of dates in Saudi Arabia. Firstly, performing an analysis of the food items to determine whether and by how much their nutrient components differ is important for the consumer seeking to make informed decisions about what food items to consume.

This study generated a lot of information that is beneficial to both the consumer and the scholarly world. Firstly, the study showed that different varieties of dates have different amounts of individual nutritional components. Even more importantly, the study showed that the same variety of date might have different amounts of the same nutritional component if it is grown in different regions of Saudi Arabia. This finding brought into perspective the potential reasons for these regional differences in the amount of the same nutritional component. The review of secondary sources showed that the differences could be attributed to factors such as the mineral content in the soils, the relative humidity in the different regions in Saudi Arabia, exposure to sunlight, storage, and other environmental conditions.

These findings have an impact with regard to the research questions that guided this inquiry. The study has shown that environmental conditions can have a significant impact on the nutritional components of the dates. This is evidenced by the fact that the date fruits grown in different regions with different weather and soil conditions in Saudi Arabia had different amounts of the nutritional components. The study has also shown variance in the nutritional composition in the various varieties of dates grown Saudi Arabia. This was similar to the variation shown in dates from other regions as reported in the literature.

Through the third research question, the study sought to determine whether the total phenolic content and the antioxidant power of the dates would differ from one variety to another. Both the preliminary screen and more in-depth analyses used in this study provided empirical

evidence to show variance in these two nutritional components for the different varieties. Finally, the study sought to explore whether the stage of maturity was a significant factor in the nutritional components of the dates. While the use of the descriptive data did not allow for the testing of the statistical significance of the difference, the data showed that the amount of the different nutritional components varied from one stage of ripening or maturation to another.

The results indicated that the range of moisture contents (10% - 30%) found within the four regions were quite similar. For levels of fat, protein and ash all varied significantly between varieties but were all very low. Carbohydrate content of the 24 date samples was calculated as described in the methods as an assumption that this was the remaining mass after correction for moisture, protein, fat and ash. As expected this value was very high in all dates tested and again showed significant variation between varieties. Dates are a good source of rapid energy due to their high carbohydrate content (70 - 80%). Most of the carbohydrates in dates are in the form of fructose and glucose, which are easily absorbed by the human body (Al-Farsi et al., 2007). The results indicated that glucose and fructose concentrations in theses Saudi dates were generally similar and the present of sucrose being normally associated with a corresponding reduction in the level of glucose and fructose. Again the major influence on the variation found seems to arise from varietal rather than regional factors.

Moreover, in this thesis both macro and micro minerals (trace elements) were measured. The content of the major mineral components of the 24 date samples namely Ca, K, Mg, Na and P and values for the trace elements Cu, Fe, Mn, Se and Zn were obtained. Potassium was the major mineral found in all the varieties with concentrations as high as 1173.29 mg/100g. The mean values for phosphorus in the dates from the different regions were close to each other. The amount of selenium was generally very low in all varieties and some did not contain any selenium at all. Results showed a reverse relationship between calcium and copper contents of dates potentially due to different soils.

A comparison of the four varieties grown in more than one region showed that in general differences due to variety seem to be most important in each case. Notable exceptions seem to be firstly in the level of sucrose (and corresponding changes in glucose and fructose) where three out of the four varieties demonstrated a difference between the two regions. The second may be in the

accumulation of selenium where two of the varieties showed a difference and this may well be related to differential selenium content in the soils although this was not able to be measured.

The total phenolic content (TPC) and antioxidant capacity (FRAP) of the 24 samples was also measured. Results showed that overall total phenolic content of the date varieties ranged between the lowest values of 147.17 ±2.12 mg GAE/100g to 255.196 ±0.29 mg GAE/100g. Moreover, the antioxidant capacity of the twenty-four date samples measured by FRAP showed the values ranged from 0.615 ± 0.147 mmoLTE/100 g to the highest content of 1.64 ± 0.31 mmoLTE/100 g. These values were within the ranges reported for dates from other countries other than Saudi Arabia. Although the literature range of values was very large and this may be a consequence of the different extraction and assay techniques that have been employed. In general though the variability in the Saudi varieties was found to be similar to that reported from other countries and would not probably have a major nutritional impact on the consumer.

In this thesis the impact of maturity on some of selected varieties was also examined. Seven varieties of dates were selected, from among those used in the first screen, (Barhi, Ajwa, Khalas, Nabtat Seif, Khodry, Sukkari and Segae). They were harvested at four different stages of maturity: Khalal (Fresh), Rutab (Semi-fresh), Tamar (Semi-dry) and Tamar (dry). Results showed that total phenolics decreased throughout development for all seven varieties. This was associated with a corresponding change in the antioxidant capacity of the fruit.

Total contents of anthocyanins were examined for these selected varieties. Results indicated that anthocyanin contents decrease sharply from semi-fresh stage to semi-dry stage to almost one-third of the semi-fresh value. It was noted that the seven varieties at the semi-dry stage had only small differences in their anthocyanin contents. There was a dramatic decrease in the anthocyanin content in the dry stage for all seven varieties due to destruction upon drying.

Finally, a direct comparison was made for the results in TPC and FRAP as a further means of identifying potential cultivar characteristics on date composition between the seven of selected dates and the same variety from 24 varities. The higher level of TPC and FRAP in seven cultivars because of the seven cultivars were exposure to the sun for only two weeks, compared with the 24 samples that collected from the market in the first experiment.

This thesis clearly demonstrates that the amount of phenolic compounds and the antioxidant capacities of date fruits were affected by maturation stage. Date cultivars have different levels of TPC, FRAP and anthocyanins during ripening most of which were present at the fresh stage. Moreover, it concluded that most cultivars have a significant antioxidant activity and are found to be good sources of antioxidant. These findings confirm the antioxidant potential of selected date cultivars and increase focus on the impact on health promoting antioxidative compounds in those cultivars during the four maturation stages.

7.2 Future work

There are a lot of potentially beneficial chemical compounds to be found in dates, such as fibre and vitamins. The literature has identified vitamins A, B1, B2 and niacin as being found in reasonable amounts in dates, but that other vitamins such as vitamin C are very low.

A low intake of dietary fibre is associated with a high incidence of colon cancer, heart disease, diabetes and other diseases/disorders (Anonymous, 1987). There are several methods for measuring fibre in dates. Hot water can be used to extract milled flesh (100 °C for 5 min). The insoluble and soluble dietary fibre in the dates can then be determined according to the AOAC enzymatic-gravimetric method of Prosky, Asp, Schweizer, De Vries, and Furda (1988). Similarly there are literature methods for the major vitamins. Further work should compare the varieties of dates in each region based on the content of their fibre and vitamin compounds. This current screen involved only a small number of samples, although the ranking was shown to be repeatable for antioxidant and total phenolic content between the market and field sourced samples. Future work should increase the sampling sixe and if possible combine this with more precise geographical details regarding the region of production. One key aspect might be to try to link mineral content of the dates with soil composition. This may be particularly important in the case of selenium.

Antioxidant activity has been reported in many fruits; however, dates have not received similar attention and some studies indicate that temperature affects antioxidant compounds such as: Hasegawa and Mayer, (1980); Hamdan, (1975); Benjamin et al., (1979); and Mutalk and Mann, (1984). Similarly, studies on other fruits have indicated the possibility that storage can have an effect on the level of antioxidant compounds (Kalt, 2005; Kevers et al., 2007). Many fruits tend

to lose antioxidant capacity during storage, but dates are comparatively stable and are often kept for long periods of time and refrigerated. There were significant differences in this respect found between the field harvested and market sourced material. Thus a study to examine the influence of low temperature storage on antioxidant compounds in dates could be undertaken.

This thesis has highlighted the difference in the various components in the different cultivars of the date palm fruit grown in Saudi Arabia. However, confounding factors such as the stage of ripening, relative humidity, temperature, the preparation of the assay, region, and conditions during growth has been shown to affect the nutrient composition. Using an experimental approach where all the confounding variables are controlled will help determined accurately whether the nutritional content differs significantly.

The control of the confounding variables is important for future studies because it will help create a common basis upon which the comparison can be performed. In a study where the researcher is able to control for any effect from the confounding variables, the data will clearly show whether there is a difference in the amount of the different nutrients in the same cultivars from different regions and in the same cultivars from the same regions. This is important because it gives an accurate account.

One other element needed in future studies on this matter, and one that was evidence in the work of Al-Jasass, Siddiq & Sogi (2015) among other researchers cited in this discussion is the use of inferential statistics in addition to descriptive statistics. When the new samples preparations were used in the testing of the six cultivars, only descriptive data was used in the analysis of the data. More precisely, only the maximum, minimum, and average values were produced in the analysis. The study performed by Al-Jasass, Siddiq & Sogi (2015) shows the use of inferential statistics such as ANOVA and t-tests to determine whether a set of data or the values for various nutrients in one cultivar was different in a manner that was statistically significant from another. Such findings will help the researcher determine whether the difference is as a result of the sample preparations used in the analysis or whether the difference is because of the varieties in the regions in which the dates are grown. The fact that scholars such as Hamad et al., (2015) have determined that the confounding variables are significant means that this proposal offers a different approach, which will potentially yield different perspectives.

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Appendix 1: Date cultivar details

1 Safawi*

Cultivated in Almadina Almonawara area, heavy bearer, changes to 'Khalal' or 'Bisr' at the end of June and to 'Tamar' during second half of August and consumed as Tamar.

2 Sefri

It is mainly cultivated in most areas of the kingdom; it is considered as early to midseason maturing and consumed mainly as Tamar.

3 Ajwa*

Cultivated in Almadina Almonawara area, midseason maturing, commercially important during 'Hap' season, changes to 'Khalal' or 'Bisr' stage during second half of June and to 'Tamar' during August, and consumed as 'Tamar'

4 Anbara*

It is considered as one of the best and distinguished date palm varieties at Almadina Almonawara, cultivated at a limited scale, moderate fruit bearer, matures late and consumed as Tamar.

5 Ghur

Cultivated in Al Qateef and Alhassa governorates, early maturing, changing to 'Khalal' or 'Bisr' stage during the first half of June and Rutab during the second half of June. It is consumed as Rutab.

6 Qatarah

It is mainly cultivated in Riyadh and Al Qassim areas, consumed as Rutab and matures at midseason.

7 Mabroom (Barni Al Ola)*

It is cultivated in Almadina Almonawara area which has good quality dates, consumed as Rutab and Tamar, and mature early in season.

8 Majhool

It is one of the important commercial varieties in the world which was recently cultivated in the Kingdom, particularly in Almadina Almonawara area. It is considered as the most important export variety in the international market, and its fruits contain few fibres, consumed as Tamar and mature early in season.

9 Miskani

It is a good commercial variety in the Riyadh area, bearing heavily; its fruit resemble Nabut Seif, consumed as Tamar and mature midseason.

10 Maktomi

One of the commercial varieties in the central region of the Kingdom, the fruit has few fibres and phenolic compounds, and can be consumed at most stages of maturity 'Bisr', 'Rutab' and 'Tamar', fruits mature in midseason.

11 Meneifi

Its cultivation dominates in the central region, matures late season and consumed as Rutab and Tamar.

12 Nabtat Sultan

It is an excellent variety cultivated in the central region, matures late in the season and its fruits are consumed as Rutab and Tamar.

13 Nabtat Seif

Despite its limited cultivation, it is considered one of the important varieties in the central region; it is consumed as Rutab and Tamar and matures in midseason.

14 Nabtat Ali*

An important variety date in the Qassim area; the fruit has a few fibres, consumed as Rutab and Tamar and matures in midseason.

15 Hilali

It is cultivated in Al Hassa governorate, late maturing and consumed as Rutab.

16 Wannana

It is cultivated in Al Qassim region, its fruits mature in midseason and consumed as Rutab and Tamar.

17 Barhi*

An important variety cultivated in most areas of the kingdom due to its high quality and economical returns to farmers, fruits mature in mid-season during August/September and consumed as Bisr, Rutab or Tamar, the flesh is delicious and tasty.

18 Barni Al Madina*

It is mainly cultivated in Almadina Almonawara, the flesh is semidry, late maturing, used and consumed as Rutab and Tamar.

19 Beid

It is cultivated in Almadina Almonawara, a heavy bearing variety, late maturing and consumed as Rutab and Tamar.

20 Hulwa

It is mainly cultivated in Aljouf and Hail areas and has been recently widely cultivated in Almadina Almonawara; its fruits mature in midseason and consumed at all stages of maturity Bisr, Rutab and Tamar.

21 Khesah

It is cultivated in Al Hassa area, late maturing and consumed as Rutab.

22 Khodry*

It is an important variety in several areas of the Kingdom; its fruit has a few fibres, consumed as Tamar and matures late in the season.

23 Khalas

It is considered as one of the best commercial varieties in Alhassa governorate, and spreads in most areas of the kingdom, its fruit has a few fibres, it has excellent eating quality, and can be consumed at all stages of maturity. However, its maturity season is in August and is considered as a mid-season maturing variety.

24 Khenaizy

One of the main varieties in Qateef and Al Hassa governorates, it is fast growing in areas with a high water table and bears heavily, the flesh at 'Bisr' stage is tasty and reaches full maturity in midseason (August), it is consumed as 'Tamar' and the 'Big' is normally boiled and dried before consumption and locally known as 'Salog'.

25 Deglet Noor*

This variety was recently introduced to many areas in the Kingdom, particularly Almadina Almonawara and Riyadh areas, it is considered as one of the important export varieties in the international market, and its dates are consumed as Rutab and Tamar, and mature late in the season.

26 Thawee

It is cultivated in the Riyadh region, its fruits have a medium quality and consumed as Rutab and Tamar and mature late in the season.

27 Rabeaa

It is cultivated in Almadina Almonawara area and its dates have a good quality, it is consumed as Rutab and Tamar and mature early in season.

28 Ruzeiz

It is one of the most dominant date palm varieties in Al Hassa governorate, heavy bearer cultivar with fruits that mature by midseason and consumed as 'Rutab and 'Tamar'.

29 Rushodia*

It is cultivated in AI Qassim area, its fruits mature in midseason and consumed as Rutab.

30 Ruthana*

It is cultivated in Almadina Almonawara and the Central regions, its fruits mature early in season and consumed as Rutab.

31 Sabaka

It is considered as one of Al Qassim area varieties where it is mainly cultivated, its fruits mature midseason and consumed as Rutab and Tamar.

32 Sari

It is cultivated in Al-Aflaj and Wadi-Al Dawasir governorates, early maturing and consumed as Rutab.

33 Sukkari*

It is an important cutting in the Al Qassim area and spreads to several areas of the kingdom, its fruits as Rutab and Tamar have a good to excellent taste, respectively. Fruits mature in midseason during August and consumed as Rutab and Tamar,

34 Sullaj

It is cultivated in Riyadh area; the date has a good quality, consumed as Rutab and matures in midseason.

35 Shebebi

It is cultivated at Al Hassa and Al Qateef governorates. Its fruits have a medium quality. Matured in midseason during August and consumed as Rutab and Tamar.

36 Shahal

It is cultivated in Al Hassa governorate. Fruits mature in late season starting early September and mainly consumed as Rutab. The Rutab is better quality compared to the Tamar.

37 Shaishee

It is mainly grown in Al Hassa governorate and considered as one of the good varieties. It matures in mid-season during August and consumed as Rutab and Tamar.

*Date cultivar used in project.

Appendix 2: New and Old Data

Table 1 showing the new and old data

	NEW DATA																	
	FRAP			TPC			% Glucose			% Fructose			% Sucrose			% Total Sugar		
	mmol TE/100g	Min	Max	mg GAE/100gm	Min	Max		Min	Max		Min	Max		Min	Max		Min	Max
Khodry	0.86±0.24	0.64	1.25	241.74±30.25	199.58	278.75	33.84±8.20	23.49	44.43	31.10±8.79	20.70	42.37	0.00±0.00	0.00	0.00	64.94±16.99	44.20	86.81
Rushodia	0.99±0.27	0.73	1.49	143.61±15.24	125.42	168.33	16.57±3.04	13.70	22.04	15.65±3.03	12.45	21.00	28.09±7.96	14.31	35.41	60.30±12.61	40.46	75.81
Shahal	0.62±0.08	0.52	0.77	144.24±20.18	112.50	171.25	35.20±5.80	27.07	42.62	33.43±6.68	24.25	42.64	0.00±0.00	0.00	0.00	68.63±12.46	51.32	85.26
Sullaj	1.22±0.21	1.05	1.59	207.85±22.45	184.58	236.25	39.13±2.54	35.63	42.37	39.76±3.62	35.11	44.53	0.06±0.14	0.00	0.00	78.95±6.19	70.74	86.40
Daglet_Noor	1.69±0.47	1.19	2.34	162.99±17.28	145.42	181.25	10.79±1.97	8.55	13.41	9.90±1.67	7.84	11.87	49.16±6.26	40.02	56.73	69.85±9.72	56.41	81.19
Anbara	1.39±0.15	1.14	1.54	209.65±26.77	167.92	234.58	28.44±3.02	24.47	32.00	28.26±3.68	23.61	32.77	0.00±0.00	0.00	0.00	56.70±6.69	48.09	64.78
	OLD DATA																	
Khodry-Qaseem	0.88±0.116			255.20±0.283			34.45±0.069			32.90±0.429			00.00±0.000			66.69±0.423		
Khodry-Riyadh	1.09±0.053			253.73±1.859			36.71±0.235			34.24±0.252			00.00±0.000			71.12±0.072		
Khodry	1.63±0.068			308.06±1.948			NO TEST			NO TEST			NO TEST			NO TEST		
Rushodia-Madina	1.30±0.063			154.97±1.386			19.62±0.055			17.47±0.181			33.42±0.169			70.68±0.157		
Rushodia-Qaseem	1.04±0.067			147.17±2.116			26.85±0.094			24.89±0.024			19.25±0.045			70.90±0.135		
Shahal-Eastern Region	0.62±0.148			166.78±1.596			34.93±0.203			32.40±0.164			00.00±0.000			67.33±0.096		
Sullaj-Riyadh	1.33±0.040			192.49±1.470			40.60±0.237			37.40±0.305			00.00±0.000			78.67±0.670		
Daglet Noor-Qaseem	1.81±0.096			161.17±1.748			11.22±0.100			10.37±0.567			49.81±0.671			70.27±1.184		
Anbara-Madina	1.45±0.071			213.22±1.493			28.98±0.198			25.85±0.144			00.00±0.000			55.50±1.049		