DISSERTATION

ANTIOXIDANT PROPERTIES OF DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS

Submitted by

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In partial fulfillment of the requirements

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY SALEH MOBARAK AL-TURKI ENTITLED ANTIOXIDANT PROPERTIES OF DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

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ABSTRACT OF DISSERTATION

ANTIOXIDANT PROPERTIES OF DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS

The fruit and pits of date palm (*Phoenix dactylifera* L.) cultivars from the United States (US) and Saudi Arabia (SA) were analyzed for their total phenolic contents and antioxidant activity for two years. The amount of total phenolic compounds and antioxidant activity in all date fruit and pit cultivars tested in this study, at the Tamar stage, were significantly different. Total phenolic contents of fruit ranged from 507.03 (Gur SA) to 225.02 (Medjool US) mg Gallic Acid Equivalents (GAE)/100g FW and antioxidant activities ranged from 1400.14 to 228.06 μmole TEAC/100g of fresh weight (ABTS) in Deglet Noor US and Khalasa US respectively. DPPH of fruit ranged from 117.75 to 165.42 μmole TEAC/100g of fresh weight in Deglet Noor (US) and Khalasa (US) respectively. The pit, which is about 12% of date fruit weight, ranged from 66.68 (Hilali US) to 14.51 (Amir Hajj US) mg GAE/g DW total phenolics. ABTS ranged from 679.01 to 45.83 μmole TEAC/1g of dry weight in Hilali US and Hayany (US) respectively. DPPH ranged from 15.94 to 3.92 μmole TEAC/g of dry weight in Sukari (SA) and Khalasa (SA) respectively. A significant association between the total phenolic content and antioxidant activities was found in both years with ABTS and DPPH. Moreover, there was a strong relationship between measurement of antioxidant capacity by ABTS and DPPH in both years in fruit and pits. Fruit of one cultivar, Khalasa, was available both years from the (US) and (SA). Phenolic content, ABTS and DPPH radical scavenging capacity of fruit and pits differed in their antioxidant activity due to different location and other environmental factors. Fruit or pit (SA) cultivar was significantly

higher than the (US) cultivar over all treatments. Deglet Noor (US) fruit which makes up about 90% of California's date crop was found to be the best over all cultivars of those tested in this study. Hilali (US) was the best antioxidant source of date pit cultivars in (US), whereas, Sukari (SA) was the best antioxidant source of pits in (SA). Thus, this research demonstrates the potential of date fruit and pits as antioxidant functional food ingredients.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated plants on earth and its cultivation is now undertaken in many countries. Moreover, fruits of the date palm (*Phoenix dactylifera* L.) are very commonly consumed in many parts of the world and are a vital component of the diet and a staple food (Vayalil, 2002). Modern technology and scientific research have demonstrated the importance of food quality in protecting against various illnesses such as cancer and cardiac disease. Diets rich in fruits and vegetables appear to offer maximum protection against these kinds of diseases. Date fruit is a high-energy food of high sugar content, a good source of iron, potassium, and iodine, as well as low in fats and proteins (Al-Farsi et al., 2005; Saways et al., 1982) . The date fruit is listed in folk remedies for the treatment of various infectious diseases and cancer (Duke, 1992; Duke and Wain, 1981). Date fruit, one of the best foods for women after delivery, strengthen and increase the contraction rate of the uterus muscles thus facilitating delivery as well as reduce postpartum hemorrhage (Khadem et al., 2007; Nama et al., 2006). The almighty God, Allah said in the Qur'an to Mary (Peace be upon her) while she was in labor (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" (Muslim, 1990).

Food producers are interested in developing new products with an increased level of certain health-protecting compounds to satisfy consumers' interest. It is important to know which health-protecting compounds are present in raw materials, and it is important to know in what concentrations the compounds are present (Biglari et al., 2008). Therefore, detailed information about the health promoting components of dates could lead to a better understanding and an increased consumption, including their use as functional foods and ingredients in nutraceuticals, pharmaceuticals, and medicine. Based on limited studies on the phenolic profile compounds and antioxidant activity of the date fruit and pits, the relative antioxidant properties of the major cultivars grown in the United States (US) and Saudi Arabia (SA) has not been studied. The objectives of this study were to: 1) to compare antioxidant activities (free-radical-scavenging activity) and total phenolics among date fruit and pits cultivars by more than one method; 2) To characterize and compare antioxidant activities (free-radical-scavenging activity) and phenolics among ten US and five SA date fruit & pit cultivars; 3) to evaluate the contribution of total phenolics to antioxidant activity in date fruits and pits.

The format and style of this dissertation follows that of the *Journal of American Society for Horticultural Science.*

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CHAPTER 2

LITERATURE REVIEW

ORIGIN AND DISTRIBUTION

Date palm (*Phoenix dactylifera* L.) has been one of the most important fruit crops and one of the oldest plants on earth, dating back to perhaps 30–70 million years or more (Mohamed, 2004). It is believed to be indigenous in the lands around the Arabic Gulf or Mesopotamia area (currently Iraq). In ancient times, it was especially abundant between the Nile and Euphrates rivers (Morton, 1987; Pareek, 1985). From its center of origin, date palm cultivation spread through out the Arabian Peninsula, North Africa, and Middle East. The spread of date palm cultivation accompanied the expansion of Islam and peaked in southern Spain and Pakistan. Nowadays, date palm cultivation is undertaken in many countries. It is the leading fruit tree in many countries and considered an important source of wealth and food. Spanish missionaries planted date palm from pits around California in the United States of America (USA) and along the Mexican border in the late 18^{th} or early 19^{th} century (Nixon and Carpenter, 1978). Commercial date palm production in the USA is based on selected clones of cultivars introduced directly from North Africa and the Near East for research purposes. The United States Department of Agriculture (USDA) initiated these introductions near the end of the 19^{th} century. By 1910 several high quality cultivars were established in experimental gardens in the Salt River Valley of Arizona and in the Coachella Valley of California (Brown, 1982; Krueger, 1998; Sauer, 1993).

The botanical name of date palm, *Phoenix dactylifera* L, (*Palmaceae*), originates from its fruit "*Phoenix*" presumably derived from the Greek word for purple or red fruit,

and "*dactylifera*" from to the Greek word "daktulos" meaning finger like appearance of the fruit's form (Chao and Krueger, 2007; Zaid and de Wet, 2002a). The genus "*Phoenix*" comprises 12 species most of which are well known as ornamental plants. Only *P. dactylifera* has become cultivated as a fruit crop, even though there are five of these species that bear edible fruit beside date palm. *Phoenix dactylifera* L. is a woody diploid (2n = 2x = 36), dioecious species, perennial and monocotyledonous (Barrow, 1998; Sauer, 1993; Zaid and de Wet, 2002a).

DESCRIPTION OF THE DATE PALM

After: Barreveld, (1993; Eftekhari and Ramin, (2006); Zaid and de Wet, (2002a)

Roots: date palm roots originate from a ball shaped base of the trunk. It has no tap root. Four zones in the root system can be distinguished (Fig. 2.1). The first zone produces roots sprouting from the upper part of the trunk base. This zone extends deeper to about 25 cm and can play a role in respiration. The second zone and the most intense root zone has heavy root branches with rootlets spreading into the ground to collect nutritive substances and moisture. This large zone extends to about one meter below ground level. The third zone has roots from 1 to 2 m underground depending on the availability of nutritional substances in the higher zones. The fourth zone is at least 2 m underground and might extend deeper than 6 m depending on availability of water. In general, roots can be found as far as 25 m from the trunk and deeper than 6 m, nevertheless 85 % of the roots are distributed to 2 m deep and to 2 m around depending on soil characteristics, type of culture, depth of the underground water and cultivar.

Trunk: trunk girth is the same all the way up and does not increase once the canopy of fronds has completely developed without any branches (Fig. 2.1). The trunk develops from the terminal bud, called a phyllophore. It reaches 10 m to 30 m tall depending on the cultivar as well as on cultivation practices. The trunk could reach 1 m in diameter covered by the bases of the old dry fronds for several years. The cambium also disappears resulting in a constant and uniform trunk width during the palm's entire life.

Fronds: leaves are 3 m to 7 m long and have a long life of 3 to 7 years depending on cultivar, age of a palm and environmental conditions. The dead or old fronds do not drop on their own, but need to be removed under cultivation. There are over 100 green fronds on a mature date palm with one or two new fronds produced per month. Fruit bunches will be produced in a ratio of 8 fronds per bunch on a mature palm.

Flowers: inflorescences are produced from frond axils of the previous year's growth. They will be enclosed in a hard covering known as a spathe which splits longitudinal when the flowers mature exposing the inflorescence (Fig. 2.2). The spathe at the beginning is greenish then becomes brown by splitting. The annual number of spathes at each date palm is about 25 in females and more than that in males. The male inflorescence is crowded and shorter and wider than the female. These characteristics allow the recognition of the inflorescence's sex before its opening (Fig. 2.2).

Pollination: date palm is naturally pollinated by wind or insects. Traditionally commercial pollination is done by hand. Pollen is collected from male inflorescences and placed in the female spathe when it is open and ready for pollination. Otherwise,

pollen should be dried and stored until the female spathe opens. It can be stored for one year. Artificial pollination is also used to decrease thinning and thus expense because fruit set is often lower. The physical and chemical characteristics of date fruit is affected directly by pollen (Chao and Krueger, 2007; El Mardi et al., 2006; Moustafa, 2001). Metaxenia is observed in fruit size (Abdelal et al., 1983; El-Ghayaty, 1982; El-Hammady et al., 1977), fruit color and time of ripening (Al-Delaimy and Ali, 1969), and weight of fruit and pit "xenia" (Abdelal et al., 1983; El-Ghayaty, 1982; El-Hammady et al., 1977; Hussein et al., 1976; Westwood, 1993).

Fruit Growth and Development: the fruit of date palm is a drupe with a fleshy pericarp and thin and membranous endocarp (Fig. 2.3). Fruits vary in size, shape, color and quality depending on the cultivar, culture and environmental conditions (Rieger, 2005; Zaid and de Wet, 2002a). Unripe fruits are green, yellow, or red in color, and when they mature range from bright red to bright yellow, depending on cultivar. After fertilization, fruit develops from one of the three carpels within each pistillate flower. Fruit drop usually occurs 25-35 days after spathe cracking, and some cultivars have a second fruit drop after 100 days of spathe cracking (which may be referred to as "June drop") (Nay et al., 2007; Reuveni, 1986).

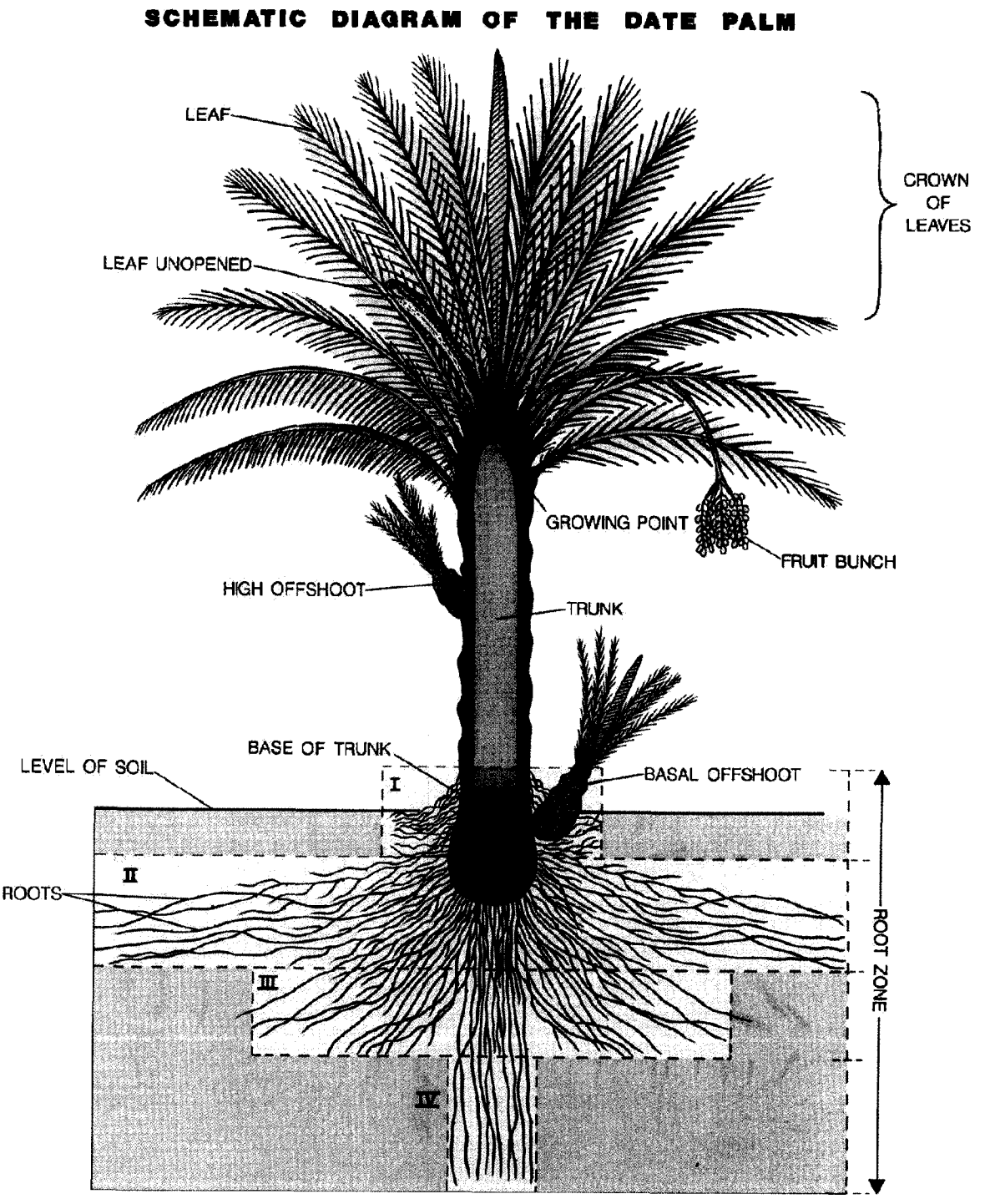

Fig. 2.1. Diagrammatic representation of date palm structure, showing attachment of offshoot to mother palm, among other morphological features. (USDA archival diagram,(Chao and Krueger, 2007)

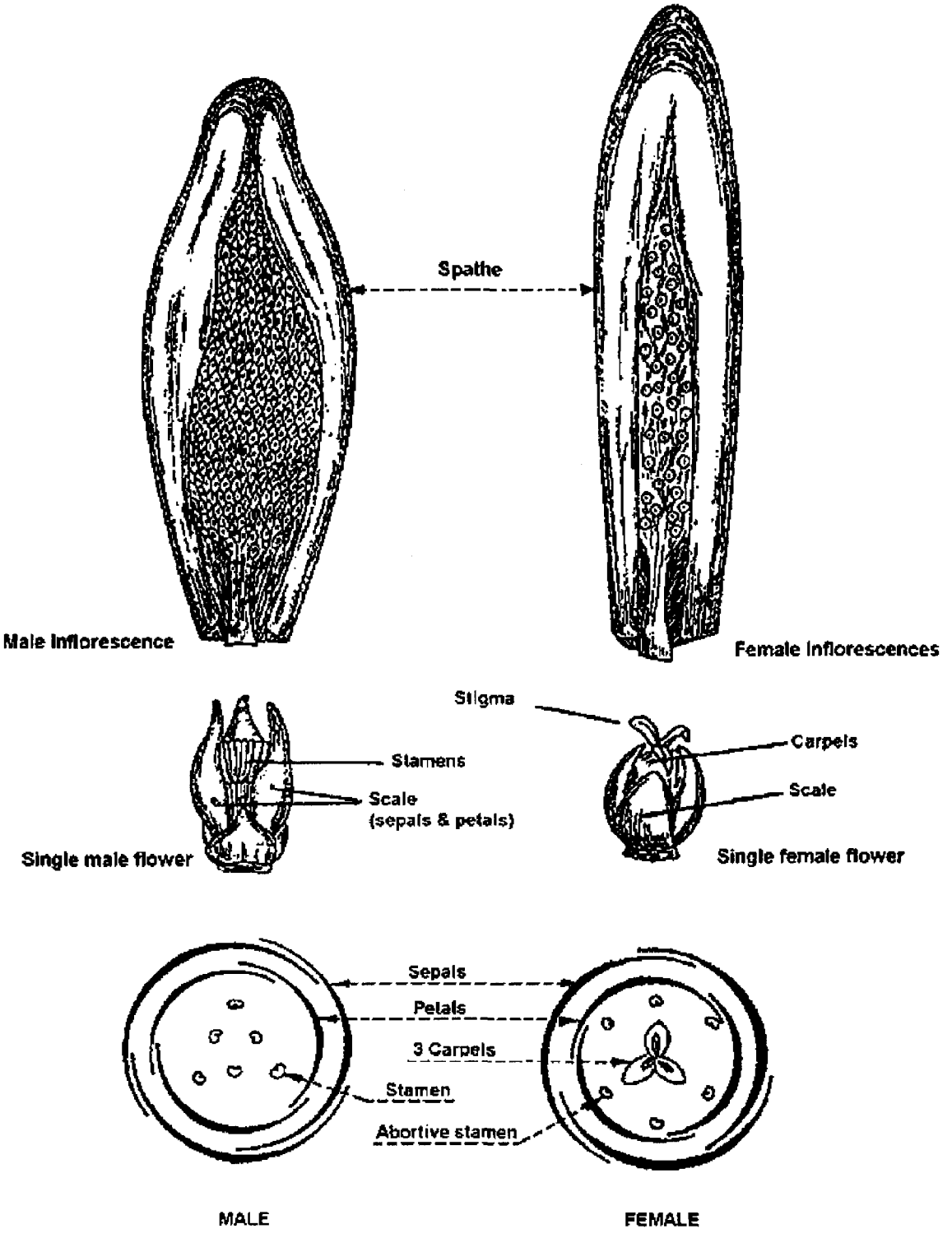

Fig. 2.2. Date palm male and female inflorescences and flowers (Zaid and de Wet, 2002a)

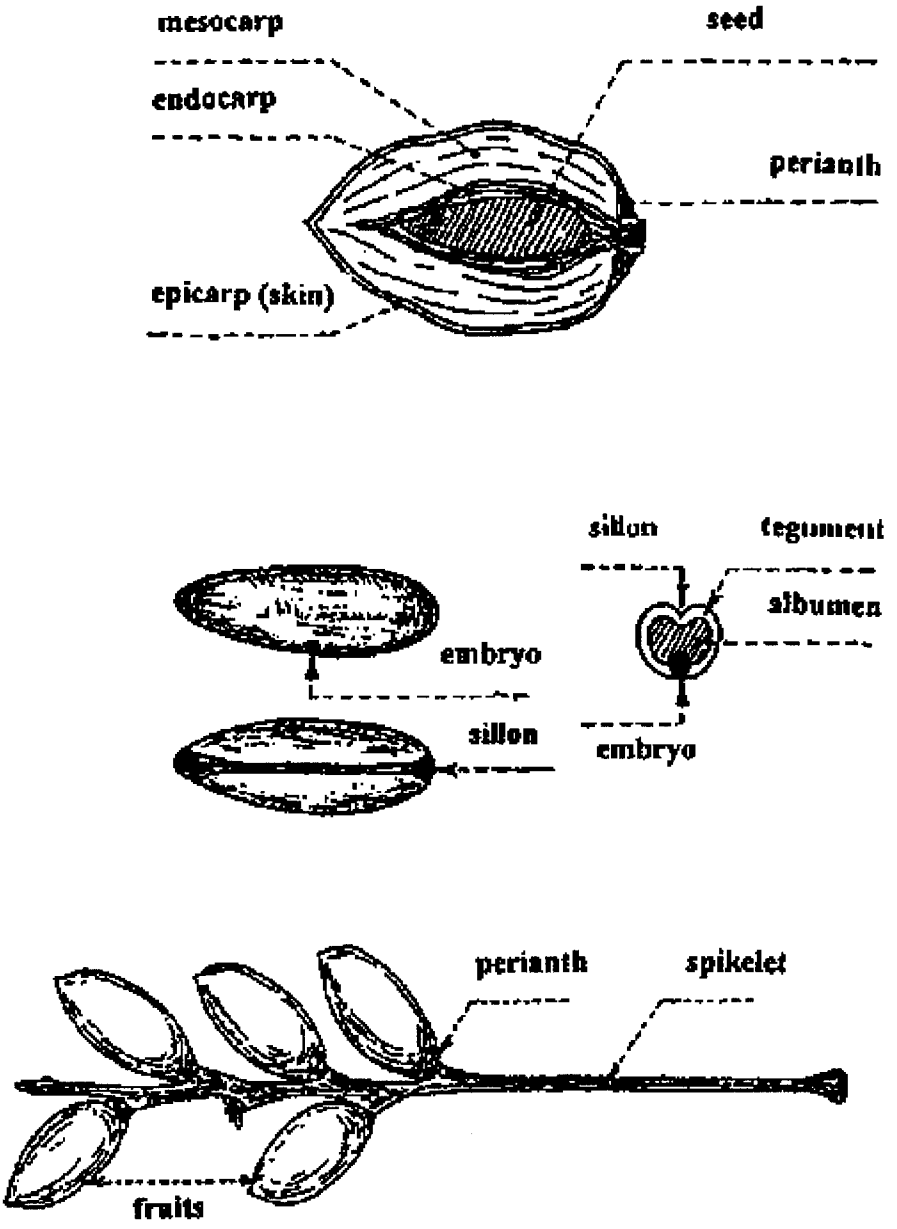

Fig.2.3. Morphology and anatomy of date palm fruit and pit (Zaid and de Wet, 2002a)

Nixon, (1950) and Roy et al., (1995) pointed out that three groups of date cultivars exist according to their fleshiness, fruit texture, and moisture under normal conditions of ripening: soft date (more than 30 % moisture,e.g. Barhee, Halawy, Hayany, Khadrawy, Medjool, Khalasa), semi-dry (20 % to 30 % moisture, e.g. Dayri, Deglet Noor, Zahidi, Ajwa , Anbara, Mabroom, Barni, Hilali), and dry (less than 20 % moisture, e.g. Thoory, Ruthana, Nabtat Ali, Sukari).

Growth and developmental stages of date palm fruit are often classified on the basis of change in color and chemical composition of the fruit, and are usually referred to in terms derived from the Arabic language. These terms have been used internationally by several authors Reuveni, (1986); Shabana and Al Sunbol, (2006); Zaid and de Wet, (2002a). They are:

1. **The *Hababouk* Stage:** it starts soon after fertilization and is characterized by loss of two unfertilized carpels, and continues until the beginning of the *Kimri* Stage.
2. **The *Kimri* Stage:** (also called green stage or depressed period) at this stage the fruit is quite hard, and increases in size and weight with the color rapidly changes from green to a color characteristic of the cultivar during *Khalal* stage. Fruit is not suitable for eating because it remains turgid and contains a substantial amount of water soluble tannins.
3. **The *Khalal* Stage:** (also called *'Bisr'*) the fruit is physiologically mature and the rate of gain in size and weight decreases slightly as the fruit reaches full size and weight. Fruits are hard ripe and the color changes completely from

green to greenish yellow, yellow, pink, red or scarlet depending on the cultivar. At this stage color of the pit changes at the end from white to brown.

4. **The *Rutab* Stage:** at this stage the tip of the apex starts ripening with changes in color to brown or black. Fruit becomes soft, and increase in soluble tannins. Fruits continue to decrease in fresh weight due to loss of moisture and increase in reducing sugar and a rapidly increasing rate of conversion of sucrose. A gain of total sugars and total solids characterizes this stage.
5. **The *Tamar* Stage:** this is the stage when the dates are fully ripe as they completely change in color to dull brown or almost black. The skin in most cultivars adheres to the flesh, and wrinkles as the flesh shrinks. At this stage, the date contains the maximum total solids and has lost most of its water. The sugar water proportion is sufficiently elevated to prevent fermentation. This is the best condition for storage.

Hence, date fruits are harvested and marketed at three stages of their development (*Khalal*, *Rutab* and *Tamar*) depending on cultivar, climatic conditions and market demand. In fact, harvest normally starts mid-August and extends through October for late cultivars. Picking fresh dates for market or as a fresh soft fruit at Rutab stage starts after the fruit turn to the ripe color and the lower half of the fruit soften and develop the characteristic brown color normally found in fully mature fruit. Unlike many other fruits, date fruits can be consumed or used for human consumption in every stage of fruit development (Sidhu, 2006).

Date pits, also called pips, stones, kernels, or seeds form part of the essential date fruit that contain a single pit about 1.2-3.6 cm long, 6-13 mm thick with a weight range from 0.5 to 4.0 g according to cultivar, environment and growing conditions (Barreveld, 1993; Zaid and de Wet, 2002a). In the Arabian Peninsula and remote parts of the desert a coffee like beverage is sometimes prepared from date pits by roasting and grinding in a similar way as for coffee beans (Ali-Mohamed and Khamis, 2004; Barreveld, 1993; Haynes and McLaughlin, 2000). According to Al-Hooti et al., (1998) date palm pits contain 9.0 % fat of which 56.1 % is oleic acid, 11.6 % linoleic acid, 8.3 % lauric acid, 6.0 % myristic, and 2.6 % stearic acid. Phytochemical and chromatographic screening showed other organic compounds, such as proteins, alkaloids, steroids, vitamins, phenols, triterpenes, and other classes of compounds, to be present (Al-Showiman, 1990) . Estrone is also present in date palm pits (Dewick, 1997) . On the other hand, a research article devoted to possible toxic effects of this adulterant, states "date pits are roasted by dry heat, then ground to a similar powder as for coffee. The color is a little lighter, the odor is fairly agreeable, and when mixed with coffee is difficult to detect" (Hussein et al., 1983). This was the only research report found that addressed the possible toxicity effect date pits adulteration of coffee. In based on a rat feeding trial, they concluded that date pits as an adulteration in coffee was of extremely low toxicity. The authors suggested the possible lethal dose for a 70 kg man was more than 1.136 liter from an interpolation of the rat trial.

There are about 5000 named cultivars of date palm known to exist all over the world, but only a few important ones have been evaluated for their performance and fruit quality (Al-Ghamdi, 1996; Al-Hooti et al., 2002). In 1950, Nixon described 160 imported

date cultivars in the United States. The study by Johnson and Hodel, (2006) found only 16 imported commercial cultivars in the United States, originating from four countries: Algeria, Egypt, Iraq and Morocco. Nixon, (1952) also described 40 American cultivars that had been selected and reproduced from the imported cultivars. Since that time, a few more American cultivars have been added. Deglet Noor is the most important commercial date cultivar, representing about 70-75 % of production; Medjool is second with 20-25 %; all other commercial cultivars account for only a few percent of total production. In the kingdom of Saudi Arabia there are 400 cultivars (Sawaya, 1986); however, only 50-60 of these are considered economically important (Al-Turki, 2002).

Most prominent and commonly grown cultivars of date palm are Amir Hajj, Barhee, Deglet Noor, Gur, Halawy, Hayany, Hilali, Khadrawy, Khalasa, Khunizi, Medjool, Shaishi, Sukari, and Zahidi which were described by Asif et al., (1986); Morton, (1987); Nixon, (1950); Zaid and de Wet, (2002a).

Amir Hajj originated in Iraq and refers to "Leader of the Pilgrimage". Fruit is yellow, ripening to amber with oblong-oval shape. The skin is thin and tender and shrinking with flesh. Flesh is soft. Pit is grayish brown, although lighter at the apex and base and somewhat darker on sides. The germ pore is variable although usually central or a little above with deep and narrow furrow.

Barhee originated in Basra, Iraq where it is regarded as one of the best dessert dates with the hot summer winds that called '*barh*'. This cultivar makes a good vigorous palm with fairly heavy trunk, but produce very few offshoots (about 3 - 5 per palm). It is considered a mid to mid-late season in fruit production. Fruit is attractive yellow in

Khalal Stage and cordate in shape. It is generally valued as a fresh date in the *Rutab* stage is amber and becomes very soft and can be easily separated from the skin. The fruit develops into a golden brown color in the early *Tamar* stage and the shape becomes broadly ovate to somewhat rounded (egg-shaped). At *Rutab* stage, the fruit is very sweet, has an excellent flavor, with little astringency, distinguishing it from all other date cultivars. In international commerce, Barhee is marketed and consumed only as fresh fruit on strands, at the *Khalal* stage. The pit fills the whole volume of the pit cavity. It is light brown to wood brown; oblong, slightly wider.

Deglet Noor refers to "date of the light" in Arabic. It is a leading date in Algeria and Tunisia. It is the main California crop. It is high yielding but not very tolerant of rain and atmospheric humidity. Fruit color at *Khalal* stage is light red while the *Rutab* stage is soft amber, and at *Tamar* stage it is slightly deeper light bloom with a oblong-ovate shape. The skin is medium thick and adheres to the flesh. It has excellent flavor but is late ripening. Pit is medium brown; narrowly elliptical with the germ pore centrally located with a furrow usually closed through the middle, continuing as a slight depression near the apex and base with the ventral surface more or less flattened.

Gur is a popular commercial cultivar in AI-Ahsa Oasis, Saudi Arabia. The palm is fairly well adapted to variable conditions. It is a fast growing palm and early ripening date of good quality. Fruit at the *Rotub* stage is yellow and elongated.

Halawy refers to "sweet" in Arabic. The fruit is extremely sweet and originated in Iraq. It is especially tolerant of humidity and early ripening. Fruit at *Khalal* stage is yellow,

while the *Rutab* is light amber and *Tamar* is golden brown. Fruit sometimes retain dry areas near the base. The shape is oblong with thin shrinking skin and flesh is thick and soft. Pit is a grayish brown and mottled at the base. They are narrowly oblong and slightly wider above the middle with a germ pore central or nearly so.

Hayany originated in Egypt. It is one of the most cold-tolerant. Fruit at *Khalal* stage is deep red color while *Rutab* and *Tamar* are colored red to almost black. The shape of the fruit is oblong-elliptical with skin of medium thickness, and flesh that is soft. It is considered early ripening. Pit is grayish brown, with the oblong, germ pore variable, and a narrow and shallow ventral furrow.

Hilali refers to "of the new moon". It is one of the very late ripening cultivars of the region. It is a fast growing, robust palm that produces offshoots in the early stages of growth. The offshoots root easily and have very good survival. Fruit is yellow and shaped broadly oval or somewhat obovate. The skin is thin, usually shrinking with the flesh and becoming finely wrinkled and flesh soft. Pit is dark brown and obovate-elliptical or oblong obovate. The germ pore is above middle with a furrow open above middle and shallow.

Khadrawy originated in Iraq and Saudi Arabia. It is early ripening. This cultivar has small edible fruit and it is quite tolerant of rain and humidity. Fruit at *Khalal* stage is yellow, while *Rutab* is amber or slightly greenish and *Tamar* is reddish brown. The fruit shape is oblong elliptical to oblong ovate. Skin is somewhat thick and tender, but flesh is soft and melting. Pit is dark brown, becoming lighter toward

the base. The germ pore is variable, but usually central or slightly below. The furrow is variable, sometimes narrow and shallow to deep.

Khalasa refers to "quintessence" or "choice". It is considered as the "Perfection of the date", and many rank it as the best date fruit of the world. It is believed to have originated in AI-Ahsa, Saudi Arabia. It is a mid-season date considered a delicacy as both fresh and dry, and retains flavor even in storage. The palm requires good air and soil drainage. In very dry conditions excessive drying of the skin occurs. Fruit at *Khalal* stage is yellow while the *Rutab* is light amber and *Tamar* is deep amber to reddish brown .Fruit shape is oblong-oval with the base oblique. Skin is thin while the flesh very tender and melting. Pit is dark to medium brown and narrowly oblong-elliptical. The germ pore is central or so while the furrow is usually open but variable.

Khunizi fruit at *Tamar* is dark red. Fruit usually withstand high humidity levels. They are relished fresh in season or dry outside the season.

Medjool the origination of this cultivar is "unknown", although plants were originally imported from Morocco. It has a medium size trunk with short to medium fronds. Fruit is yellow-orange with clear dark red strips at the *Khalal* stage. It is amber at the *Rutab* stage and transparent dark brown to black at *Tamar* stage. Mature fruit color is related to the climate and growing conditions. The fruit is a highly attractive fruit, very large and elongated - broadly oblong oval. The fruit skin is irregularly wrinkled, but at *Tamar* stage it shrinks. The thickness of the flesh and taste is excellent and sweet. Pit is dark brown and oblong or oblong-elliptical in a large percentage of the fruit. On each side of

the pit there is a protrusion forming a "wing shape" that is typical of Medjool and which can be used to distinguish it from all other cultivars.

Shaishi is a slightly robust palm with wide fronds and is well adapted to local conditions. It usually shows luxuriant growth with a thick barrel like trunk in early stages. It is a mid season cultivar with fruits that are medium to large in size and plump and firm in texture and of good quality. Some fruits often retain small areas of dry flesh at the base. Skin separation of fruits is common in this cultivar. This cultivar is generally of lower quality and produce value.

Sukari originated in Baghdad, Iraq and refers to "of candy". Fruit is a medium sized and soft with amber color date. It is a rare and highly esteemed cultivar. In humid weather it has may be severely damaged with souring and cracking, the latter in transverse apical lines. Fruit shape is oblong-elliptical and skin quite tough. It is early ripening.

Zahidi palms are stout, fast growing and heavy bearing. They are also drought resistant, but has slight tolerance of high humidity. Fruit at *Khalal* stage is yellow while the *Rutab* stage has two parts with softer portions that are light brown and drier basal portions that are slightly yellow. The *Tamar* stage also has softer portions reddish brown with drier portions little light brown. The fruit shape is obovate with thick and tough skin that adheres to the flesh. Flesh is firm with the drier flesh becoming hard. Pit is grayish brown and oblong with the germ pore central or nearly so. The furrow is variable but commonly closed in the middle.

DATE PALM GENERAL CULTURE

Soils and climate: deep sandy loam soils are considered the best for date palm growing. However, they can be grown in a wide range of soil types. Maximum water holding capacity and good drainage are desirable. Date palm can grow in alkaline and saline soils but in such soils its growth and productivity are greatly reduced (Barreveld, 1993; Pareek, 1985; Rieger, 2005). Successful date palm culture requires moderate winter temperature and an intensely long hot summer with little rain, and very low humidity during the period from pollination and to fruit maturity and harvest, but with abundant underground water near the surface for irrigation. An old folk saying describes the date palm as having "Its feet in the water and its head in the fire". Date palms are grown in a nearly rainless belt between 15° and 35° N, and can grow from 12.7 °C to 27.5 °C average temperature ,withstanding as high as 50 °C and sustaining short periods of frost at temperature as low as -5 °C. Below 7 °C shoot growth of a date palm is zero, above this level growth is active and reaches its optimum at about 32 °C; the growth will continue at a stable rate until the temperature reaches 38 °C/40 °C when it will start decreasing. The optimum temperature during the period from pollination to fruit ripening is between 25-30°C average temperature depending on cultivar. The heat requirement to ripen the fruit varies with cultivar and ranges from 4,200 to over 5,000 accumulated growing degree days (GDD), considering the growth zero value for the flowering process is18°C (Chao and Krueger, 2007; Pareek, 1985; Zaid and de Wet, 2002c).

Propagation: there are three methods for date palm propagation (Chao and Krueger, 2007; Nixon and Carpenter, 1978; Zaid and de Wet, 2002b). The most common

method is the vegetative propagation of offshoots, which ensures the genetic identity of maternal cultivars. Offshoots develop from axillary buds on the trunk near the soil surface during the date palm's juvenile stage. Offshoots, after 3 to 5 years of attachment to the parental palm, produce roots and can be removed and planted. This is about the age that the offshoots will begin to produce flowers and, in female lines, fruit. The second propagation method is to use chance seedlings from sexual crosses. Seedlings are not identical to the maternal trees and not uniform genetically, varying greatly in their production and fruit quality. About 50 % of the seedlings are male although they cannot be identified until trees began to flower after 4 to 5 years. Production and fruit quality from seedling-derived groves are greatly reduced compared with groves developed from offshoots. Moreover, seedlings have a longer juvenile phase, and flower only when 4-10 years in age (Pareek, 1985) . The third date propagation method is through tissue culture (Zaid and de Wet, 2002b). Tissue culture propagation of date palms from shoot tips through either embryogenesis or organogenesis was first developed in the 1970s to 1980s. Organogenesis can be achieved using auxiliary buds and apical meristems, whereas embryogenesis can be done through callus stage from various meristematic tissues like shoots, young fronds, stem, rachilla, and so forth. Cultivars respond to tissue culture differently, and different optimal conditions are needed for each cultivar. It takes about 6 years to reach production through the tissue culture process and 8 years to reach commercial quantities. In general, tissue culture progenies have similar characteristics as those derived from offshoot propagation. However, one of the main problems with tissue culture propagation is somaclonal variation (off types). These somaclonal variants exhibit several a typical phenotypes, including variegation in fronds, variation in leaf structure

and overall plant growth patterns, trees that do not form inflorescences or produce abnormal floral development, and trees that produce seedless parthenocarpic fruits. Most somaclonal variants can be detected in the early stages; however, some can only be detected in the field, several years after planting or after flowering, fruit set, and maturation of the trees. The frequency of somaclonal variation in tissue culture derived date palms can be sometimes very high, but mechanisms causing these variations are unclear and are under investigation (Gurevich et al., 2005).

***Date palm harvest, postharvest handling*:** date fruits are harvested at or near maturity. However, they may be harvested prior to full maturity for special markets. Harvest is generally by hand, generally by cutting entire bunches at one time. In some cases, individual fruit may be picked, where bunches are shaken to remove only the ripe fruit. Each date palm may be picked up to 8 times over a period of 2-3 months to obtain consistent maturity (Rieger, 2005), with access to the crown of the tree being by way of climbing or mechanical lifts. Completely mechanized harvest by shakers, such as those used in some other perennial crops, is not developed enough for routine commercial use at this time (Glasner et al., 2002). In many traditional areas of date palm production, where the bulk of production is by small farms with limited resources, date fruits are usually transported directly to open-air markets. Because of their low moisture content, date fruits can be successfully stored for some time without specialized storage conditions. However, in more industrial forms of date fruit production, packing and storage of date fruits use specialized equipment and facilities. Packing houses for date fruits often use equipment that has been modified from devices used for other crops. Various processes in the packinghouse are used to maintain or improve fruit quality.

Fumigation for the elimination of insect pests is commonly practiced (Glasner et al., 2002; Rieger, 2005; Rygg, 1977) . Fumigation has mostly used methyl bromide; however, because of environmental concerns, methyl bromide is being phased out. Alternatives to methyl bromide treatment include alternative chemicals, controlled atmospheres, and physical control methods (Glasner et al., 2002). Fruit quality may be manipulated through hydration, dehydration, or curing as appropriate, and date fruits are generally stored under refrigerated conditions in industrial production for a year or more if humidity is kept below 20 %. Fresh dates (*Khalal* stage) can be stored for up to 8 weeks under the same conditions (Glasner et al., 2002; Rieger, 2005; Rygg, 1977). Sorting and grading of dates is generally performed by hand rather than by electronic devices at this time. Fruit quality of dates can be lowered by physiological and pathological factors (Rygg, 1977). Physiological defects include blacknose, associated with high humidity during the *Khalal* stage; black scald, associated with abnormally high temperatures; and puffiness of the date fruits associated with high temperature and humidity. Storage conditions may also promote fruit defects such as darkening of the skin and sugar spots.

DIET CONTRIBUTION AND USES OF DATE FRUIT

Modern technology and scientific research have demonstrated the importance of food quality in protecting against various illnesses such as cancer and cardiac disease. Diets rich in fruits and vegetables appear to offer maximum protection against these kinds of diseases. Food producers are interested in developing new products with an increased level of certain health-protecting compounds to satisfy consumers' interest. It

is important to know which health-protecting compounds are present in raw materials, and it is important to know in what concentrations the compounds are present.

Since the early 1900's, with imported date palm trees and careful selection, date fruits have been an important Californian agricultural product. The United States Department of Agriculture's (USDA) scientists maintain one of the world's largest date-palm collections safeguarding specimens of more than 50 different types of high quality. Date palm fruits are very commonly consumed in many parts of the world and are a vital component of the diet and a staple food (Vayalil, 2002) because date fruit is a high-energy food of high sugar content, a good source of iron, potassium, and iodine, as well as low in fats and proteins (Vayalil, 2002). The date fruit is listed in folk remedies for the treatment of various infectious diseases and cancer (Duke, 1992).

Date fruit or its juice was employed in Ancient Egypt in many medicinal remedies. Date wine was used as an alcoholic beverage for pleasure, nutrition, medicine, ritual, remuneration, and funerary purposes (Cherrington, 1925). The ancient Egyptians made at least seventeen types of beer and at least twenty-four kinds of wine, some of which were used as ingredients of medicines (Ghalioungui, 1979). In some Arabic countries, alcohol or liquor known as 'arak' vinegar, and date juice known as 'dibbis' or syrup "honey date" are made from the date fruit pulp(Pareek, 1985). Dry or soft date fruits may be eaten out-of-hand, or may be pitted and stuffed with fillings such as almonds and walnuts. Recent innovations include chocolate covered date fruits and products such as sparkling date juice, used in some Islamic countries for special occasions and religious times such as Ramadan (Al-Hooti et al., 1997)

Date palm fruit folk medicine covers a wide spectrum and is described as follows: "an aphrodisiac, contraceptive, demulcent, diuretic, emollient, estrogenic, expectorant, laxative, pectoral, purgative, refrigerant, the date is listed in folk remedies for ague, anemia, asthma, bronchitis, cancer, catarrh, chest, condylomata, cough, diarrhea, eyes, fatigue, fever, flu, gonorrhea, endurations, longevity, piles, pterygia, splenitis, sterility, stomachache, thirst, toothache, tuberculosis, urogenital ailments, vaginitis, virility, warts, and whitlows." (Duke and Wain, 1981). Its use is also mentioned and applied in relation to "cancers, indurations or tumors of the abdomen, gum, liver, mouth, parotids, spleen, stomach, testicle, throat, uterus, and viscera." (Hartwell, 1971).

Also reported by Duke and Wain, (1981) is a superstition that may have a scientific rational behind it: "The pollen of a male date palm mixed with water is a charm against childlessness," as the pollen, contains estrogenic hormone 'estrone' and has exhibited gonadotrophic activity on immature rats. "Egyptians also believe that to swallow one, two, or three date stones will prevent child-bearing for many years." Therefore, while less convincing the date may be seen as both a potential fertility aid and contraceptive.

It has been proven experimentally that date fruit extracts have been shown to increase sperm count in guinea pigs and enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats (Al-Qarawi et al., 2004). Also, the pollen grains of date palm have been used by Egyptians to improve fertility in women (Bajpayee, 1997). 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).

Middle Easterners believe that consumption of dates, particularly in the morning on an empty stomach, can reverse the actions of any toxic material that the subject may have been exposed to. Saudi researchers thought to assess the ability of date flesh and pits to prevent some of the toxic actions of carbon tetrachloride on animals' liver. Treatment with aqueous extract of date flesh or pits significantly reduced ill effects and suggested that the induced liver damage can be ameliorated by treatment of extracts from date flesh or pits (Al-Qarawi et al., 2004; Bahmanpour et al., 2006).

Earlier, because date fruits are commonly consumed in many parts of the world and are a vital component of the diet in most of the Arabian countries, Kuwaiti researchers carried out a preliminary study for the first time on date fruits' antioxidant and anti-mutagenic properties '*in vitro*'. Results indicated that antioxidant and anti-mutagenic activity in date fruit is quite potent and implicates the presence of compounds with potent free-radical-scavenging activity (Vayalil, 2002).

Furthermore, Saudi researchers tested the local folk medicine claim that date fruits are beneficial in gastric ulcers in humans on animals. The results indicated that the aqueous and ethanolic extracts of the date fruit and, to a lesser extent, date pits, were indeed effective in ameliorating the severity of gastric ulceration. It was postulated that the basis of the gastro-protective action of date fruit extracts may be multi-factorial, and may include an antioxidant action (Al-Qarawi et al., 2005).

Recent research has been conducted upon the modern cultivars of *Phoenix dactylifera* growing all around the world, so it is not surprising that there is such interest and renewed hope for what the ancient cultivars of the Date Palm may have in store. Date

palms produce many products that are useful to humans. In these days of concerns over obesity, it is well to remember that sweet, sun-ripened dates make a tasty, fat- and cholesterol-free snack, supplement for bakery confections, furthermore, dates 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). Date fruits provide carbohydrates and protein, fiber, magnesium, iron, potassium, phosphorous, and a minute amount of sodium. Some date palms e.g. "Californian" are sodium-free and contain several vitamins; A, B-complex (B_1 B_2 B_6 niacin, pantothenic acid), and C (ascorbic acid). For those adherents to the Glycemic Index 'GI' diet, research proves that date fruits (Khalasa – the best cultivar of Arabian dates) when eaten alone or in mixed meals with plain yoghurt have a low glycemic index (Miller et al., 2002; Miller et al., 2003). Consumption of dates may also be of benefit in glycemic and lipid control of diabetic patients (Miller et al., 2002). Date fruits could be useful in the attenuation of H_2O_2-induced oxidative stress-mediated skin diseases in human skin, possibly due to antioxidant properties (Dammak et al., 2007). In addition, date fruits are an especially good food source for the elderly, the frail and convalescent. Puri et al., (2000) observed that there is a scientific basis for feeding certain plants to mothers after child birth and to invalids with relatively poor immune status, among which is the date palm (*Phoenix dactylifera*). Date fruit was found to enhance haemagglutinating antibody titers, plaque-forming cell counts in the spleen and macrophage migration index as an index of cell-mediated immunity (Vayalil, 2002). Studies have also shown the antibacterial (Sallal and Ashkenani, 1989) and antifungal (Sallal et al., 1996; Shraideh et al., 1998) properties of date fruits. In addition, previous *in vivo* studies indicated the

recorded content of vitamin C in the date flesh as 0.179 % and 0.137 % in pits (Al-Qarawi et al., 2004).

ANTIOXIDANTS

Antioxidants (oxidation inhibitors) represent a class of substances that vary widely in chemical structure that reduce oxidative damage (Pokorny et al., 2001). They can be defined as any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delay or prevent the oxidation of substrate (Gutteridge, 1993; Vaya and Aviram, 2001). Since Harman, (1956) and Tappel, (1968) reported in their classic papers the provocative contention that antioxidants might provide longevity, their thoughts have been echoed repeatedly and research in the area of these substance has grown logarithmically (Zoecklein, 1999). Antioxidants have been found to act as defensive and protective agents against oxidative species in the human body, food, and plants, inhibiting the decomposition of oxidation products which result in decreased nutritional value and sensory quality (Pokorny et al., 2001). Even though, oxygen is an essential element for life, it can create damaging by-products during normal cellular metabolism (Al-Saikhan, 2000). Gerschman et al., (1954) proposed that the damaging effects of O_2 could be attributed to the formation of oxygen radicals. This hypothesis was popularized and converted into the "superoxide theory of O_2 toxicity" after the discovery of a class of enzymes, superoxide dismutases (SOD_S), that appear specific for catalytic removal of superoxide free radical, $O_2^{\bullet -}$. In its simplest form, the superoxide theory states that O_2 toxicity is due to excess formation of $O_2^{\bullet -}$ and that the SOD enzymes are major antioxidant defenses (Halliwell, 1996).

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 diverse mechanisms of action (Table 2.1). The most important mechanism is their reaction with lipid free radicals, forming inactive products (Pokorny et al., 2001).

Table 2.1. 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

In other words, antioxidants can be classified into two groups according to their solubility; hydrophilic antioxidants (water-soluble), such as the majority of phenolic compounds and ascorbic acid, and lipophilic antioxidants (fat-soluble) such as carotenoids and vitamin E (Namki, 1990).

Free radicals: free radicals are chemicals that have one or more unpaired electrons and can react with a range of biological molecules such as nucleic acids and proteins, resulting in cell damage. Free radicals are constantly generated in our body as a by-product of breathing oxygen, exercising and breaking down food for energy. A free

radical is any species capable of independent existence that contains one or more unpaired electrons (Haliwell and Gutteridge, 1985). An unpaired electron is one that occupies an atomic or molecular orbital by itself. The simplest free radical is atomic hydrogen. Since a hydrogen atom has only one electron, it must be unpaired. Radicals can be formed by several mechanisms, such as adding a single electron to a non-radical. They can form when a covalent bond is broken if one electron from the bonding pair remains on each atom (homolytic fission). Some bonds are hard to break, e.g. temperatures of 450 °C to 600 °C are often required to rupture C–C, C–H, or C–O bonds. Indeed, combustion of organic compounds proceeds by free radical mechanisms. Other covalent bonds fragment more easily; just trimming your fingernails can cleave disulphide bonds in keratin to generate sulfur radicals (Symons, 1996). Radicals can react by more than one way:

a) Radical Plus Radical: If two free radicals meet, they can join their unpaired electrons and make a covalent bond (a shared pair of electrons). The product is a non-radical. A biologically relevant example is the fast reaction of nitric oxide ($NO^{\bullet}$) and superoxide ($O_2^{\bullet-}$):

   Nitric Oxide ($NO^{\bullet}$) + Superoxide ($O_2^{\bullet-}$) = Peroxynitrite ($ONOO^-$)

b) Radical Plus Non-radical: Most molecules found in the human body are not radicals. Hence, any reactive free radical generated is most likely to react with a non-radical. When a free radical reacts with a non-radical species, a free radical chain reaction results and new radicals are formed, which may be more or less reactive than the original radical. Attack of reactive radicals upon membranes or lipoproteins starts the free radical chain reaction called lipid peroxidation. There

is growing evidence that lipid peroxidation takes place in human blood vessel walls and contributes to the development of atherosclerosis, raising the risk of stroke and myocardial infarction. If OH radicals are generated close to DNA, they can attack the purine and pyrimidines and cause mutations. For example, the purine guanine is converted into 8-hydroxyguanine and other products, which can cause errors during DNA replication (Halliwell, 1994; 1996).

Free radicals play an important role in a number of biological processes, some of which are necessary for life, such as the intracellular killing of bacteria by neutrophil granulocytes. Free radicals have also been implicated in certain cell signaling processes (Pacher et al., 2007). Moreover, the transfer of electrons is basic to energy production and many metabolic processes. However, if the chain reaction goes on in an uncontrolled manner, cell membrane damage can occur resulting in altered cell function, mutation and even cell death. The two most important oxygen-centered free radicals are superoxide and hydroxyl radical. They are derived from molecular oxygen under reducing conditions. However, because of their reactivity, these same free radicals can participate in unwanted side reactions resulting in cell damage. Many forms of cancer are thought to be the result of reactions between free radicals and DNA, resulting in mutations that can adversely affect the cell cycle and potentially lead to malignancy. Some of the symptoms of aging such as atherosclerosis are also attributed to free-radical induced oxidation of many of the chemicals making up the body (Albano, 2000). In addition free radicals contribute to alcohol-induced liver damage, perhaps more than alcohol itself (Llesuy et al., 2001; Pacher et al., 2007).

Antioxidants neutralize free radicals by donating one of their electrons, ending the electron-stealing reaction. The antioxidants do not themselves become free radicals by donating an electron because they are stable in either form. They act as scavengers, helping to prevent cell and tissue damage that could lead to cellular damage and disease. Fruits and vegetables can provide an abundant supply of different types of antioxidants, along with other, less well-understood, components that could be important factors in achieving optimum health benefits. A great variety of the antioxidants found in foods are also available in nutritional supplement form. It is a matter of some debate whether the higher amounts of antioxidants that can be taken in supplement form, offset the theoretical advantage of the combined benefit of all components of the food source. Much research still needs to be done on this question, and on the role and mechanism of action of specific antioxidants in different disease states (Landolph, 2000).

Antioxidant Compounds: fruits and vegetables contain abundant different naturally occurring antioxidant components. The majority of the antioxidant capacity of a fruit or vegetable is from compounds such as vitamin C, vitamin E, β–carotene (which is converted to vitamin A) and phenolic compounds that are capable of counteracting the damaging effects of oxidation.

Vitamin C, ascorbic acid, as a water-soluble antioxidant, is an essential nutrient and unique position to "scavenge" aqueous peroxyl radicals before these destructive substances have a chance to damage the lipids, so it may be one of the first line 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 collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties (Herrera and Barbas, 2001; Packer, 1991; Packer et al., 2001). Of these, α-tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and using this form (Brigelius-Flohe and Traber, 1999). 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). This would remove the free radical intermediates and prevent the oxidation reaction from continuing. The oxidised α-tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol (Wang and Quinn, 1999).

Beta-carotene is probably the most familiar and well-studied of the carotenoids. It is a potent antioxidant as well as a major precursor for Vitamin A (Albanes, 1999). It is one of several carotenoids, natural plant pigments found in deeply colored fruits and vegetables. The antioxidant function of beta-carotene is due to its ability to quench singlet oxygen, scavenge free radicals and protect the cell membrane lipids from the harmful effects of oxidative degradation (Krinsky and Deneke, 1982; Santamaria et al., 1989). The quenching involves a physical reaction in which the energy of the excited oxygen is transferred to the carotenoid, forming an excited state molecule (Krinsky, 1993). Quenching of singlet oxygen is the basis for beta-carotene's well known therapeutic efficacy in erythropoietic protoporphyria (a photosensitivity disorder) (Mathews-Roth, 1993). The ability of beta-carotene and other carotenoids to quench

excited oxygen, however, is limited, because the carotenoid itself can be oxidized during the process (autoxidation). Burton and Ingold (1984) have shown that beta-carotene autoxidation *in vitro* is dose-dependent and dependent upon oxygen concentrations. At higher concentrations, it may function as a pro-oxidant and can activate proteases. In addition to singlet oxygen, carotenoids are also thought to quench other oxygen free radicals. It is also suggested that beta carotene might react directly with the peroxyl radical at low oxygen tensions; this may provide some synergism to vitamin E which reacts with peroxyl radicals at higher oxygen tensions (Brar, 2007; Cotgreave, 1997). Carotenoids also have been reported to have a number of other biologic actions, including immuno-enhancement; inhibition of mutagenesis and transformation; and regression of premalignant lesions (Fouad, 2007). Consumption of carotenoid-rich foods has been related to prevention of cancer, cardiovascular diseases and other degenerative processes involving oxidative stress (Stahl and Sies, 2003; 2005).

Phenolic compounds, there are approximately 5000 known plant phenolics and model studies have demonstrated that many of them have antioxidant activity (Karadeniz et al., 2005; Robards et al., 1999). The antioxidant activity of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evans et al., 1995). Their antioxidant activity is generally based on the number and location of hydroxyl groups present as well as the presence of a 2-3 double bond and 4-oxofunction (Rice-Evans and Miller, 1998). The flavonoids, a large family of low molecular weight polyphenolic compounds, include the flavones, flavonols, flavonones, isoflavones, flavan-3-ols and anthocyanins (Stewart et al., 2000). Although flavonoids are generally considered non-nutritive agents, interest

in these substances has risen because of their possible effects on human health (Hertog et al., 1992b). Flavonoids embrace a wide variety of compounds, which are the product of photosynthesis in plants. They are colored compounds, e.g. red, blue, and yellow pigments in the plant kingdom (Al-Saikhan, 2000). In addition to their antioxidant activities, flavonoids inhibit enzymes such as prostaglandin synthase, lipoxygenase and cycloxygenase, closely related to tumorigenesis, and may induce detoxifying enzymes such as glutathione S-transferase (Lee et al., 1995). Many kinds of flavonoid have been reported in fruits and vegetables and their types and contents vary with cultivar and maturation (Hertog et al., 1992a; 1992b).

ANTIOXIDANT CONTENT OF DATE PALM

Fresh dates were found to contain 2,546 mg/100 g phenolics, and are much higher in total phenolics than cranberries which have the highest total phenolics among the fresh fruits, 678 mg/100 g (Vinson et al., 2005). The protein in dates contains 23 types of amino acids, some of which are not present in the most 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 (Al-Shahib and Marshall, 2003).

(Al-Farsi et al., 2005a; 2005b) found that the mean 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. Moreover, Fresh date cultivars were found to be a good source of anthocyanins (0.24-1.52 mg of cyanidin 3-glucoside equivalent/100 g), carotenoids (1.31-3.03 mg/100 g), free phenolic acids (2.61-12.27 mg/100 g), bound phenolic acids (6.84-

30.25 mg/100 g) and selenium (0.36 to 0.53 mg/100 g FW). In another study of three different Omani' cultivars (namely Mabseeli, Um-sellah, and Shahal), Al-Farsi et al., (2007a) found that date pits contained 3102-4430 mg of GAE/100 g fresh weight of total phenolics. Date fruit ranged in total phenolics from 172 mg of GAE/100 g FW to 246 mg of GAE/100 g FW. Mansouri et al., (2005) studied the phenolic profiles of seven different cultivars of ripe date fruits grown in Algeria. They found that total phenolic content ranged from 2.49 to 8.36 mg gallic acid equivalents (GAE)/100 g fresh weight. (Wu et al., 2004a; 2004b) reported much higher contents of total phenolics in Deglet Noor and Medjool cultivars than those cultivars studied by Mansouri et al., (2005), which were contain 661 and 572 mg of GAE/ 100 g fresh weight. A study of sixteen fruit cultivars commonly grown in Bahrain were evaluated for their total phenolic contents at Tamar stage. The average of total phenolic contents was 152.10 mg per 100 g of edible portion, which is equivalent to 85.9 mg per 100 g FW (Allaith, 2008). Samples from Iran ranged from 2.89 to 141.35 mg gallic acid equivalents (GAE)/100 g DW (Biglari et al., 2008). Date cultivars have different levels and patterns of phenolic acids. Nine phenolic acids (gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, and *o*-coumaric acid) were tentatively identified and four free phenolic acids (protocatechuic acid, vanillic acid, syringic acid, and ferulic acid). Ferulic acid was the major phenolic acid for all date cultivars (Al-Farsi et al., 2005a; 2007b).

The three date fruit cultivars from Oman, studied by Al-Farsi et al., (2005a); (2005b) were found to be a good source of antioxidants (11,687 to 20,604 μmol of Trolox equivalents/g) using Oxygen Radical Absorbance Capacity (ORAC). Antioxidant activity of petroleum ether pit extracts was 8.16%, while methanolic and water extracts of pits

were devoid of any activity (Mohamed and Al-Okbi, 2005). Al-Farsi et al., (2007a) found that the antioxidant activity was 146 to162 μmole Trolox equivalents per g with ORAC method (on a fresh weight basis). Wu et al., (2004a) measured total antioxidant activity (lipophilic and hydrophilic $ORAC_{FL}$) of two date cultivars and found values lower than those reported by Al-Farsi et al., (2007a). Mansouri et al., (2005) studied the antioxidant potentials of Algerian dates and found that the antioxidant activity ranged from 0.08 to 0.22 values of antiradical efficiency (AE) using DPPH. Allaith, (2008) reported that the average of antioxidant activity was 0.94 μmole Trolox equivalent/100 g FW using the Ferric Reducing Antioxidant Power (FRAP) in Bahraini date cultivars. The Iranian study found that the antioxidant activity (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 significant variations among date samples could be due to varietal, extraction techniques used and instrumental analysis (manual or automated). Unless there is standard method for antioxidant analysis, such variations could exist. The high antioxidant content of dates is supported by Vayalil, (2002) and Guo et al., (2003). Although these researchers used different methods and different extraction solvents, which make quantification difficult. Vayalil, (2002) stated that potent antioxidant and antimutagenic activities of dates implicate free radical scavenging activity. In addition, Guo et al., (2003) reported that dates had the second highest antioxidant value of 28 fruits commonly consumed in China. The antioxidant activity of figs, prunes, and raisins when determined by the $ORAC_{FL}$ assay were found to be 34, 86, and 30 μmole of TE/g fresh weight, respectively (Halvorsen et al., 2002; Wu et al., 2004a). It is worth mentioning

that consumption of date fruits at Tamar stage also provides a total antioxidant value equivalent to several common fruits such as sweet cherry, orange fruit and Brussels sprouts (Blomhoff, 2005). Thus, compared to these fruits, dates are considered as a good source of antioxidants. A sharp decrease in antioxidant activity was found to be associated with the fruit ripening. It was concluded that the phenolics were the major contributor for the antioxidant activity (Allaith, 2008). Antioxidant components are not only very important for functional properties of date fruits or pits (oxidation resistance, taste, color and texture) but could also have many health benefits. In many ways, dates may be considered as an almost ideal food.

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CHAPTER 3

ANTIOXIDANT PROPERTIES OF DATE FRUIT (*PHOENIX DACTYLIFERA* L.) CULTIVARS

INTRODUCTION

The fruit of the date palm (*Phoenix dactylifera* L.) is historically regarded as one of the most important fruit crops worldwide. It has been particularly important in the Arabian Peninsula of the Middle East; as well as in southern Africa, Australia, Mexico, and the United States in southern California, Arizona, and Texas, over the past three centuries (Chao and Krueger, 2007). Date fruit is considered a vital component and staple food in countries where it is cultivated. Date fruits provide very nutritious food supplying large amounts of vitamins and daily requirements of carbohydrates and proteins for a healthy diet, including important minerals like potassium, iron, calcium, and phosphorous. Growing evidence indicates that the consumption of fruits and vegetables is considered as essential to good health and protection against many chronic diseases such as cardiovascular diseases (Nicoli et al., 1999). Javanmardi et al., (2003) pointed out that phytochemicals from fruits have been shown to possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of degenerative diseases in humans. The interest in antioxidants has been increasing because of their high capacity in scavenging free radicals related to various diseases (Silva et al., 2007).

Date fruits were found to be a good source of antioxidant components (Al-Farsi et al., 2005a; 2007b) where they may contain a high level of antioxidants along with other fresh and dried fruits (Halvorsen et al., 2002; Wu et al., 2004b). The antioxidant

properties of date fruits - as other fruit - vary depending on their content of phenolic components and vitamins C and E, carotenoids and flavonoids (Al-Farsi et al., 2005b; Gu et al., 2003; Mansouri et al., 2005; Saura-Calixto and Goñi, 2006).

Because there are only a few research publications on phenolic compounds and antioxidant activity of date fruits information on the relative antioxidant status of the major cultivars grown in the United States (US) and Saudi Arabia (SA) is limited. The objectives of the present research were to characterize and compare antioxidant activities (free-radical-scavenging activity) and phenolic contents among ten US and five SA date fruit cultivars, to measure antioxidant properties using more than one method and to evaluate the contribution of total phenolic content to antioxidant activity in date fruits.

MATERIALS AND METHODS

Plant materials:

Mature date palm fruit of 15 cultivars (*Phoenix dactylifera* L.) were provided by air freight courtesy of the USDA-ARS National Clonal Germplasm Repository for Citrus & Dates - Riverside, California, USA and from authenticated date palms of Saudi Arabian farmers (Table 3.1). Fruits were used at tamar stage (full ripeness) from 2006 and 2007 harvests. Upon arrival at the laboratory, the samples were stored at -20 °C until extraction and analysis.

Table 3.1. Date palm fruit cultivars (*Phoenix dactylifera* L.) used in this research.

No.	Source	Genus	Species	Cultivars
1	United States (US)	*Phoenix*	*dactylifera*	Amir Hajj
2	United States (US)	*Phoenix*	*dactylifera*	Barhee
3	United States (US)	*Phoenix*	*dactylifera*	Deglet Noor
4	United States (US)	*Phoenix*	*dactylifera*	Halawy
5	United States (US)	*Phoenix*	*dactylifera*	Hayany
6	United States (US)	*Phoenix*	*dactylifera*	Hilali
7	United States (US)	*Phoenix*	*dactylifera*	Khadrawy
8	United States (US)	*Phoenix*	*dactylifera*	Khalasa
9	United States (US)	*Phoenix*	*dactylifera*	Medjool
10	United States (US)	*Phoenix*	*dactylifera*	Zahidi
11	Saudi Arabia (SA)	*Phoenix*	*dactylifera*	Khalasa
12	Saudi Arabia (SA)	*Phoenix*	*dactylifera*	Shaishi
13	Saudi Arabia (SA)	*Phoenix*	*dactylifera*	Sukari
14	Saudi Arabia (SA)	*Phoenix*	*dactylifera*	Gur
15	Saudi Arabia (SA)	*Phoenix*	*dactylifera*	Khunizi

Dry matter of date fruits:

Samples were analyzed for dry matter (DM %) using about five hundreds milligrams of the edible portion (flesh and skin) of two fruits of each cultivar. Tissue was weighed and cut into small pieces then dried at 70°C under vacuum (100 mmHg) until a constant weight was obtained (after 14 days). Three replicates for each cultivar were applied.

Extraction method:

There is no one satisfactory solvent extraction method for antioxidants and phenolics or even for a specific class of these components. This is because the chemical nature of antioxidants and phenolics differ from simple to very highly polymerized (Shahidi and Naczk, 2003). Therefore, the extraction of antioxidant compounds and total phenolics of the two fruit cultivars (Khalasa and Sukari) was tested using durations of 15 min., 30 min., or 60 min. with five hundred milligrams of edible portion (flesh and skin).Two fruits of each cultivar were ground using liquid nitrogen (LN_2) with a mortar and pestle. Tissue was extracted with 10 ml of cold acetone 80% (-20 °C), followed by centrifugation of the sample at 6000 rpm for 15 min. at 4 °C. One milliliter of supernatant of each sample was centrifuged to dryness at 45 °C for two hours using a VacufugeTM, "Eppendorff" and stored at - 80 °C for further analysis. Three replicates for each cultivar were tested. The best extraction duration for measurement of phenolic content and antioxidant capacity was used thereafter for all samples.

Measurement of date fruit total phenolic content:

Total phenolic content was determined using Folin-Ciocalteu reagent adapted from Spanos and Wrolstad, (1990) and based on the original method of Singleton and Rossi, (1965) as slightly modified by Wilson, (2003). This assay is based on the color reaction of phenolics with a phosphomolybdic-phosphotungstic acid reagent (Folin-Ciocalteu reagent, Fluka). Gallic acid solution was used to prepare a standard curve for estimation of total phenolic content as gallic acid equivalents. Gallic acid solution was made up daily by dissolving 25 mg into 25 ml 80 % acetone in a volumetric flask for best accuracy. A standard curve was prepared from 7.0 ml of stock gallic acid with 3.0 ml 80 % acetone. Then gallic acid standard curve dilutions were prepared using volumes as shown in (Table 3.2).

Table 3.2. Gallic acid proportions used for total phenolics standard curve dilutions.

µg / ml in assay	µl Stock Standard	ml de-ionized water (µl)
0	0	3.5 (3500)
40	200	3.3 (3300)
80	400	3.1 (3100)
120	600	2.9 (2900)
160	800	2.7 (2700)
200	1000	2.5 (2500)

In triplicate microplate wells, 35 µl of standard curve dilutions of gallic acid were pipetted per cell to constitute the standard curve. Vacufuged stored samples were reconstituted with 1.0 ml 80 % acetone and vortexed. Then 100 µl of extract sample was diluted with 900 µl de-ionized water and vortexed. In triplicate microplate wells, 35 µl of diluted samples were pipetted per cell. With multichannel pipettors 150 µl of Folin-Ciocalteu reagent (fresh dilute full strength SIGMA reagent diluted1/10 with dH_2O) was added into each well. The microplate was covered with adhesive film and mixed on a platform shaker at 400 rpm for 30 seconds, then held for 5 minutes at room temperature. After 5 min. 115 µl of 7.5 % Na_2CO_3 (7.5 g / 100 dH_2O) was added to all wells with a multichannel pipettor. The microplate was covered with adhesive film and mixed on a platform shaker at 400 rpm for 30 seconds. After that, the microplate was placed on a heating pad that was set to 45 °C for 30 minutes and covered with an insulating styrofoam cover. After standing to cool for 60 min. at room temperature, absorbance was read at 765 nm wavelength, at 45 °C using a spectrophotometer/ microplate reader (SPECTRA *max*-Plus^{384}, Molecular devices, Sunnyvale, CA) with uv-vis spectral scanning, computer-controlled SOFT *max* PRO data analysis and reporting software. Results were expressed as µg GAE/ml. A Microsoft Excel® regression spreadsheet was used to calculate the concentration of Gallic acid equivalents of liquid sample in GAE/100 g of fresh weight (FW).

Measurement of date fruit antioxidant capacity:

There has not been agreement as to one preferred method over others to measure the antioxidant capacities of food (Wu et al., 2004a). Both methods ABTS & DPPH are among the most popular spectrophotometric methods widely and commonly used in vitro

for determining the antioxidant capacity of foods and chemical compounds (Awika et al., 2003; Kim et al., 2002). These methods are usually expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

Evaluation of antioxidant activity using the ABTS method:

Antioxidant capacity was measured by -2,2'Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium Salt ~ 98% (Sigma Aldrich Co.) ABTS assay using the modified method of Miller and Rice-Evans, (1997), based on the original by Miller and Rice-Evans, (1996) as slightly modified by Wilson, (2003). This procedure measures the relative ability of antioxidant substances to scavenge the $ABTS^{*}$ free radical. Oxidation of ABTS by manganese dioxide (MnO2) (Sigma Aldrich Co.) to the activated $ABTS^{*+}$ Radical, is compared with standard amounts of the synthetic antioxidant Trolox (6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid ~ 97%, (Sigma Aldrich Chemical Co.), the water-soluble vitamin E analogue. This technique is based on the reduction of the blue-green $ABTS^{*+}$ radical by electron- or hydrogen-donating antioxidants, which was estimated by spectrophotometric/microplate analysis (SPECTRA *max*-Plus^{384}, Molecular devices, Sunnyvale, CA) and computer-controlled SOFT *max* PRO data analysis and reporting software. Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

Briefly, the basic principle for this measurement was as follows: a stock solution of ABTS was prepared by weighting 40 mg ABTS and adding 15 ml de-ionized water in a small beaker with 1-3 gram of MnO_2 (manganese dioxide, SIGMA # M-1656) mixed and reacted for 20 min. at room temperature. The solution was filtered through Whatman® (No.1) filter paper in a Buchner-Funnel by Vacuum Pump (DUO-Seal).

Excess MnO_2 was filtered by passing the solution through a 0.2 µM syringe filter into a flask. This solution was diluted with about 10-15 ml of 5.0 mM Phosphate Buffered Saline (PBS) pH 7.4 and mixed well to read an absorbance of exactly 0.70 ($\pm$ 0.02) at 734 nm. on temperature 30 °C by adding 25 µl dH_2O to 250 µl $ABTS^{*+}$ in a microplate well. It was then placed in a controlled heating block at ~ 30 °C during use. Fresh ABTS*+ radical cation solution was prepared each working day.

Trolox stock standard solution (0.5 mM) was prepared by diluting 6.26 mg Trolox into a 50.0 ml volumetric flask, in 5.0 mM PBS (pH 7.4). Fresh standards were prepared every 4 weeks. This solution was vortexed and sonicated for ~ 10 mins. to thoroughly dissolve the Trolox. A range of dilutions (Table 3.3) was prepared daily from frozen aliquots of the standard curve.

Table 3.3. Trolox standard curve dilutions used in Eppendorf tubes for ABTS.

Standard #	µM in assay	µl Trolox stock	Ml PBS
1	0	-	1000
2	5	110	890
3	10	220	780
4	15	330	670
5	20	440	560
6	25	550	450

Vacufuged stored samples were reconstituted with 1.0ml 80 % acetone by vortexing. In Eppendorf tubes, a set of three dilutions was prepared with 80 % acetone ranging 1/1 to 1/100 of 20 mg/ml extract. The assay was carried out on 96-well microplates (12 wells/row) and run one row at a time. A Trolox standard curve was run first (2x each standard conc.), and three different dilutions of each extract were run in triplicate as three wells in the same row plus blanks for each dilution.

25 µl Trolox standard or samples were pipetted into wells across the row, and 25 µl of 5 mM PBS was added to blank wells. 250 µl ABTS solution was added by multichannel pipettor to all wells across each row of the microplate, except extract blanks. The microplate was placed on a platform shaker at 300 rpm for 10 seconds and absorbance read at 734 nm, at 30 °C, exactly one minute after addition of ABTS. A Microsoft Excel® spreadsheet was used to calculate the antioxidant capacity as Trolox Equivalent Antioxidant Capacity (TEAC) in µmole TEAC/100g sample fresh weight (FW) compared to the standard. A coefficient of variation (<10 %) was used to control treatment, reading and sample preparation variability.

Evaluation of antioxidant activity using the DPPH method:

Antioxidant radical scavenging activity was also determined using the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH*) microplate assay with methanol according to Lu and Yeap Foo, (2000), based on the original by Brand-Williams et al., (1995). Briefly, A 0.1 mM of DPPH (1,1-diphenyl-2-picrylhydrazyl) was prepared by dissolving 7.889 mg DPPH in 100 ml volumetric with 100 % CH_3OH with sonication for three hours followed by store in the refrigerator. The DPPH stock was adjusted to read 0.90 AU in one microplate test cell by using 100 % CH_3OH (started with 18.0 ml DPPH

stock and added 12.0 ml of 100 % CH_3OH then fined tuned to attain 0.90AU). Stock Trolox (1.0 mM) was prepared by dissolving 12.52 mg Trolox in a 50 ml volumetric with 5.0 mM PBS, then stored as1.0 ml aliquots at – 20 °C. A standard curve was made by preparing Trolox dilutions from 0 to 90 µM concentrations using 1.0 mM Trolox and 5.0 mM PBS in 1.5 mL Eppendorf tubes as noted in Table 3.4.

Table 3.4. Trolox standard curve dilutions used in Eppendorf tubes for DPPH analysis.

µM Trolox	Stock Trolox (µL)	5 mM PBS (µL)
0	0	1000
20	220	780
30	330	670
40	440	560
50	550	450
60	660	340
70	770	230
80	880	120
90	990	10

15 µl Trolox standard curve dilutions of each concentration or well-mixed sample solutions were added in triplicate cells. Then 285 µl DPPH stock at 0.90 AU was added to all wells and mixed using a multichannel pipettor. The microplate was covered after standing for 3 min. and the decrease in the absorbance was read at 515 nm. at exactly 25 °C using a spectrophotometer/ microplate reader (SPECTRA *max*-Plus^{384}, Molecular

devices, Sunnyvale, CA) and computer-controlled SOFT *max* PRO data analysis and reporting software. Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC). A Microsoft Excel® spreadsheet was used to calculate the antioxidant capacity as μmole TEAC/100g sample fresh weight (FW) as compared to the standard.

Data statistical analysis:

Two years (2006 & 2007) data were subjected to random analysis of variance using the GLM procedure based on the general linear model procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC, 2005). Tukey's procedure, Honestly Significant Difference, (HSD) at $\alpha = 0.05$ probability level was used to detect the differences between treatment means. Relationship between total phenolic content, ABTS and DPPH variable were analyzed based on the Spearman's rank correlation coefficient (r) procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC, 2005).

RESULTS

Extraction methods:

Table 3.5 compares the effect of extraction methods on total phenolic contents and antioxidant capacity, using three different durations. Two cultivars were used as representative of date fruits to evaluate the extraction duration process. Significant ($p < 0.05$) differences existed among different durations used, with some minor exceptions. Extraction for one hour gave the highest total phenolic content and antioxidant capacity in both cultivars. Thus, one hour extraction duration was selected to evaluate the total phenolic contents and antioxidant capacity for all cultivars in this study.

Table 3.5. Comparison of extraction duration tested for contents of total phenolics and antioxidant capacity in Khalasa (SA) and Sukari (SA) cultivars[z]

Extraction duration	Total phenolics[y] (mg GAE/100g FW)	Antioxidant capacity[w] (μmole TEAC/100g FW)
Khalasa Fruit 15 min.	445.84a	2274.45d
Khalasa Fruit 30 min.	402.07b	3851.21b
Khalasa Fruit 1 hour	440.75a	4541.56a
Sukari Fruit 15 min.	330.82d	3210.49c
Sukari Fruit 30 min.	361.36c	3969.03b
Sukari Fruit 1 hour	349.14cd	4160.10b

[z] Data are expressed as mean (n=3) on a fresh weight basis. Means followed by the same letter, within a column are not significantly different by least significance difference (LSD) at $\alpha = 0.05$. [y] Total phenolics, expressed as milligrams of gallic acid equivalents (GAE) per 100g of fresh weight. [w] Antioxidant capacity is expressed as micromoles of trolox equivalent antioxidant capacity (TEAC) per 100g of fresh weight.

Dry matter of date fruit:

Dry matter percentages (DM %) of 15 date fruit cultivars for both years are presented in Tables 3.6 and 3.7. DM% ranged from 71.04 to 88.04 % in Medjool US and Gur SA cultivars respectively. Moreover, there was a weak correlation ($p = 0.0861$) in dry matter of cultivars for the two years, $r = 0.26$.

Measurement of date fruit total phenolic content:

The amount of total phenolic contents varied in different accessions and ranged from 507.03 (Gur SA) to 225.02 (Medjool US) mg GAE/100g FW. The highest total phenolic levels were detected in Gur SA, Khunizi SA, and Deglet Noor US, and the lowest in Medjool US, Khalasa US, and Amir Hajj US (Tables 3.6 and 3.7). Furthermore, a significant ($p < 0.0001$) variation due to years (Y), cultivars (C), and their interactions (Y x C) was found (Table 3.8). Total phenolic was greater in the second year than the first year over all cultivars except Khalasa SA (Fig. 3.1.a.). Significant ($p < 0.0001$) association between total phenolic content and antioxidant activities were found in both the first and the second year: ($r = 0.82$), ($r = 0.74$) with ABTS ($r = 0.84$), and ($r = 0.89$) with DPPH, respectively (Table 3.9). As a result, cultivars with high total phenolics tended to have higher antioxidant capacity. On the other hand, weak correlations ($p = 0.0007$ & $p = 0.0127$) between total phenolic content and dry matter was found in the first and second year: ($r = 0.49$) and ($r = 0.37$), respectively (Table 3.9).

Measurement of date fruit antioxidant capacity:

Means for antioxidant activity of the 15 date fruit cultivars for both methods ABTS and DPPH methods were highly significantly among cultivars (Table 3.6 &3.7) according to Tukey's procedure, Honestly Significant Difference, (HSD) at $\alpha = 0.05$ probability level. ABTS ranged from 1400.14 to 228.06 µmole TEAC/100g of fresh weight in Deglet Noor US and Khalasa US respectively. Deglet Noor US, Khunizi SA, and Gur SA were the highest over all cultivars while Medjool US, Khalasa US, Barhee US were the lowest of all cultivars.

Halawy US, Khalasa SA, Shaishi SA, Khunizi SA demonstrated greater ABTS activity in the first year (Fig. 3.1.b). Significant ($p < 0.0001$) differences between years (Y), among cultivars (C), and their interactions (Y x C) were found (Table 3.8).

DPPH ranged from 117.75 to 165.42 μmole TEAC/100g of fresh weight in Deglet Noor US and Khalass US respectively. Also, Deglet Noor US, Khunizi SA, and Gur SA were the highest over all cultivars and Medjool US, Khalasa US, Barhee US were the lowest over all cultivars. The second year DPPH activity was greater than the first year except Khalasa US, Khalasa SA, and Khunizi SA, which were higher in the first year. Significant difference ($p < 0.0001$) between two years was also detected (Table 3.8 & Fig.3.1.c).

From Table 3.9 Spearman's rank correlation coefficient shows there is a strong relationship between measurement of antioxidant capacity by ABTS and DPPH in both years where the first year was ($r = 0.93$) and the second year was ($r = 0.78$) at ($p < 0.0001$). Weak correlations ($p = 0.004$ & $p = 0.0471$) between ABTS and dry matter were found for both the first and the second year: ($r = 0.42$) and ($r = 0.30$), respectively. Weak correlations ($p = 0.0112$ & $p = 0.00994$) between DPPH and dry matter were also found for the first and the second year: ($r = 0.38$) and ($r = 0.25$), respectively (Table 3.9).

In general, Deglet Noor US, Khunizi SA and Gur SA were the best over all fruit cultivars, while, Khalasa US, Barhee US and Amir Hajj US were the poorest over all fruit cultivars (Fig. 3.2) based upon on index that is the sum of total phenolics plus ABTS and DPPH for years one and two.

Fruit of one cultivar, Khalasa, was available both years from the US and SA. Phenolic content, ABTS and DPPH radical scavenging capacity and percentage of dry matter of fruit were all significantly higher from the SA source (Table 3.10).

Table 3.6. Antioxidant properties measured as total phenolics, ABTS and DPPH for 15 date fruit cultivars obtained from Riverside CA (US) and Saudi Arabia (SA) for first year.

		mg GAE/100g FW		μmole TEAC/100g FW				Dry Matter %	
No.	**Cultivars**	**Total Phenolics**		**ABTS**		**DPPH**		**DM**	
1	Amir Hajj US	258.42	efgh[z]	392.18	g	281.44	def	77.63	ef
2	Barhee US	313.54	de	324.53	hi	253.15	efg	79.94	cde
3	Deglet Noor US	342.33	cd	852.58	b	733.17	a	78.35	def
4	Halawy US	292.71	defg	460.23	ef	275.86	def	84.85	a
5	Hayany US	303.40	def	500.49	de	361.76	cd	80.53	cde
6	Hilali US	254.64	fgh	403.23	fg	231.71	fg	75.14	f
7	Khadrawy US	272.56	efgh	355.64	gh	271.68	defg	84.59	a
8	Khalasa US	241.90	gh	271.65	ij	215.09	fg	77.95	def
9	Medjool US	225.02	h	232.04	j	176.38	g	71.04	g
10	Zahidi US	294.88	defg	417.82	fg	332.16	de	82.86	abc
11	Khalasa SA	389.95	abc	563.12	d	478.99	b	84.26	ab
12	Shaishi SA	379.12	bc	685.35	c	441.43	bc	84.69	a
13	Sukari SA	313.91	de	517.62	de	480.51	b	81.06	bcd
14	Gur SA	430.20	ab	653.94	c	515.02	b	83.21	abc
15	Khunizi SA	435.95	a	1360.99	a	738.69	a	83.12	abc
		HSD = 56.706 at α= 0.05		HSD = 66.446 at α= 0.05		HSD = 95.458 at α= 0.05		HSD = 3.3595 at α= 0.05	

[z] With each column means followed by the same letter are not significantly different by Tukey-Kramer mean separation test at ($P \leq 0.05$)

Table 3.7. Antioxidant properties measured as total phenolics, ABTS and DPPH for 15 date fruit cultivars obtained from Riverside CA (US) and Saudi Arabia (SA) for second year.

		mg GAE/100g FW		μmole TEAC/100g FW				Dry Matter %	
No.	**Cultivars**	**Total Phenolics**		**ABTS**		**DPPH**		**DM**	
1	Amir Hajj US	323.48	g[z]	477.49	ef	329.81	fgh	83.94	a
2	Barhee US	323.65	g	437.00	fgh	220.64	hi	82.53	ab
3	Deglet Noor US	488.00	ab	1400.14	a	1117.75	a	72.95	c
4	Halawy US	345.34	fg	407.29	h	284.73	gh	85.73	a
5	Hayany US	388.15	def	628.16	cd	375.32	efg	86.85	a
6	Hilali US	357.78	fg	505.60	e	381.96	efg	87.34	a
7	Khadrawy US	363.46	fg	500.71	e	361.05	fg	86.67	a
8	Khalasa US	260.54	h	228.06	i	165.42	i	74.71	bc
9	Medjool US	365.24	fg	588.76	d	483.09	de	84.54	a
10	Zahidi US	374.18	efg	500.61	e	431.44	def	87.53	a
11	Khalasa SA	357.31	fg	463.24	efg	394.47	efg	82.83	ab
12	Shaishi SA	429.59	cde	418.62	gh	523.64	cd	84.27	a
13	Sukari SA	441.12	bcd	641.44	cd	621.75	bc	85.82	a
14	Gur SA	507.03	a	714.57	b	648.49	b	88.04	a
15	Khunizi SA	485.49	abc	663.35	bc	659.34	b	87.70	a
		HSD = 57.303 at α= 0.05		HSD = 54.247 at α= 0.05		HSD = 110.98 at α= 0.05		HSD = 8.5628 at α= 0.05	

[z] With each column means followed by the same letter are not significantly different by Tukey-Kramer mean separation test at ($P \leq 0.05$)

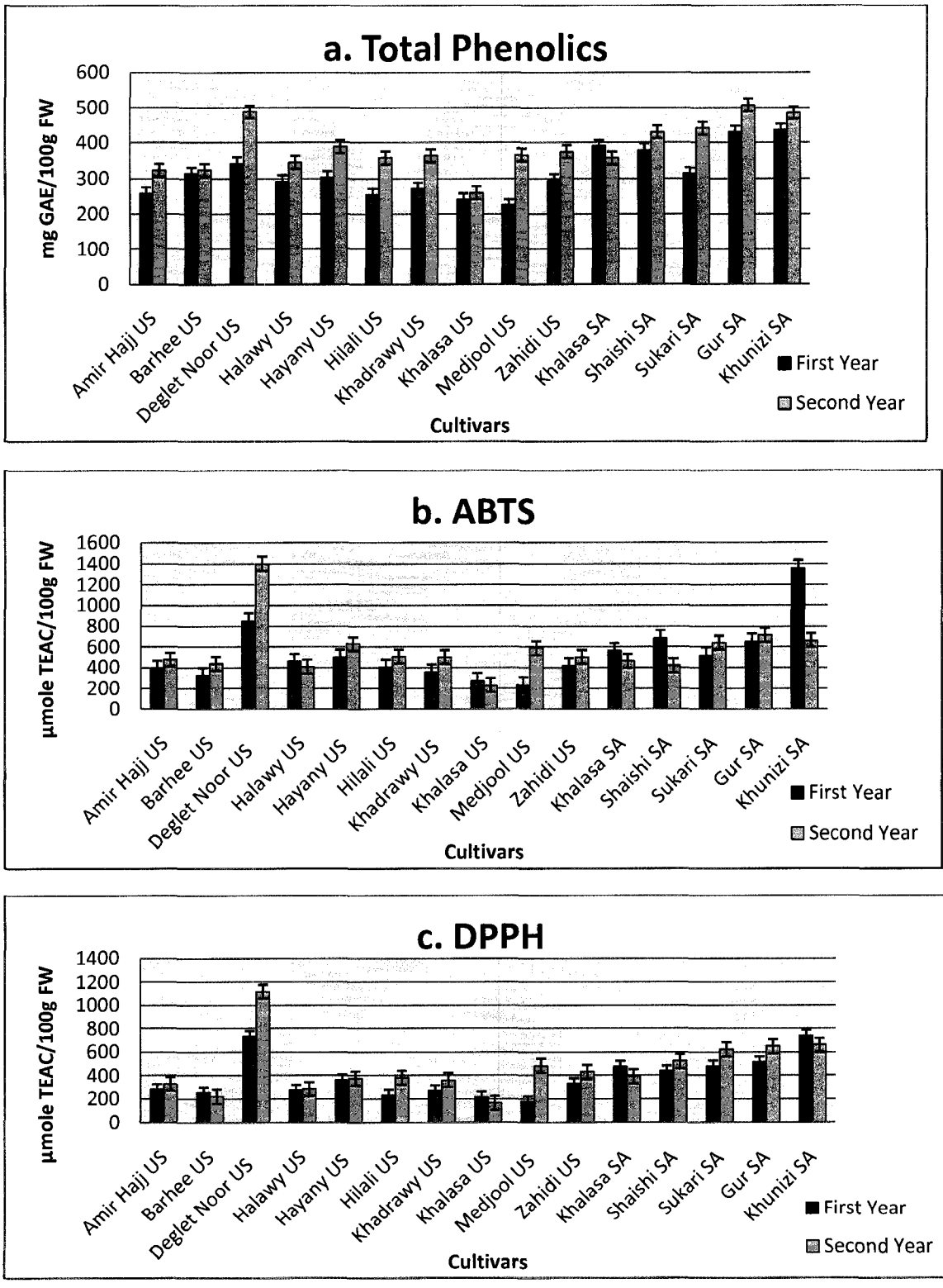

Fig.3.1. Years x cultivars interactions for total phenolics, ABTS and DPPH.

Table 3.8. Analysis of variance tested for years, cultivars and interactions.

Source	**Phenolics**	**ABTS**	**DPPH**	**Dry matter**
Year (Y)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cultivar (C)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Rep.	ns	< 0.0001	ns	ns
Y x C	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Expressed as P-values statistical significance; significant at $P \leq 0.05$

Table3.9. Spearman's rank correlation coefficient matrix (r) displayed for total phenolics, total antioxidants (ABTS & DPPH) and dry matter.

	First Year			Second Year		
	ABTS	DPPH	Dry Matter	ABTS	DPPH	Dry Matter
Total phenolics	r = 0.81858 P < 0.0001	r = 0.83623 P < 0.0001	r = 0.48445 P = 0.0007	r = 0.73845 P < 0.0001	r = 0.88524 P < 0.0001	r = 0.36880 P = 0.0127
ABTS		r = 0.92767 P < 0.0001	r = 0.42082 P = 0.004		r = 0.78090 P < 0.0001	r = 0.29763 P = 0.0471
DPPH			r = 0.3747 P = 0.0112			r = 0.24875 P = 0.0994

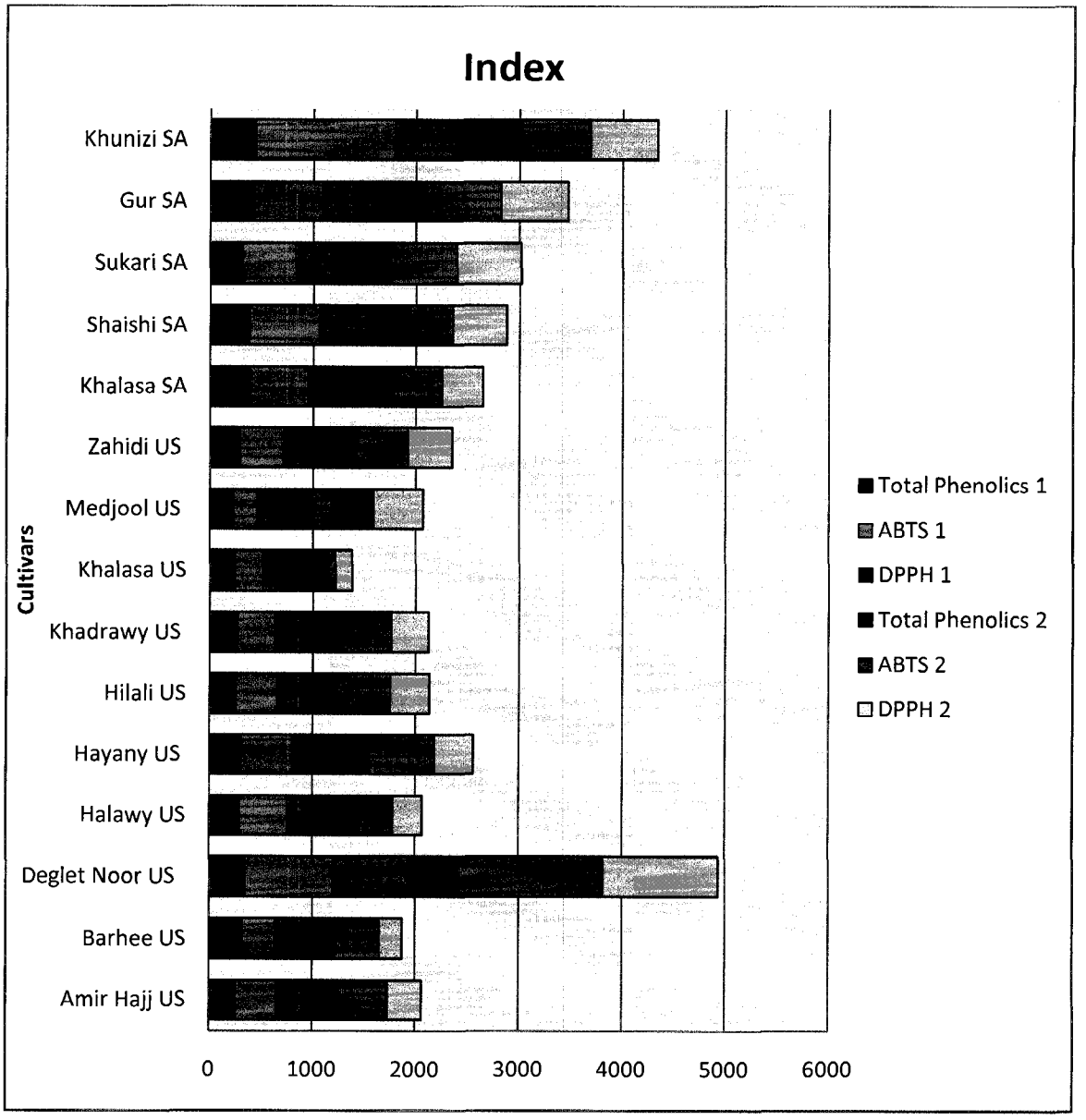

Fig. 3.2. Index ($\sum$ (Total Phenolics + ABTS + DPPH) first year + second year

Table 3.10. Comparison of antioxidant properties and dry matter between Khalasa fruits of US and SA sources.

a.		First Year							
No.	**Location**	**Phenolics**		**ABTS**		**DPPH**		**DM**	
8	**US**	241.90	b[z]	271.65	b	215.09	b	77.95	b
11	**SA**	389.95	a	563.12	a	478.99	a	84.26	a
		HSD= 23.061; α= 0.05		HSD = 24.342; α= 0.05		HSD = 41.519; α= 0.05		HSD = 3.6548; α= 0.05	
b.		Second Year							
No.	**Location**	**Phenolics**		**ABTS**		**DPPH**		**DM**	
8	**US**	260.54	b	228.06	b	165.42	b	74.71	b
11	**SA**	357.31	a	463.24	a	394.47	a	82.83	a
		HSD = 12.649; α= 0.05		HSD = 31.26; α= 0.05		HSD D= 33.563; α= 0.05		HSD = 6.1492; α= 0.05	

[z] With in each column means followed by the same letter are not significantly different by Tukey-Kramer mean separation test at ($P \leq 0.05$)

DISCUSSION

The amount of total phenolic compounds and antioxidant activity in the date fruit cultivars tested in this study, at the Tamar stage (most common edible forms of date fruit), were significantly different. Due to limitations of the samples available, it was not possible to identify the specific causes of this variation. Factors such as cultivar, growing condition, maturity, season, geographic origin, climate, location, temperature, fertilizer, soil type, disease and pest exposure, processing, storage conditions and amount of sunlight received, among others, might be responsible for the observed differences of date fruits (Al-Farsi et al., 2007b; Biglari et al., 2008; Gil et al., 2002) and may significantly influence the content of plant secondary metabolites (Wu et al., 2006).

The results showed that the date fruit cultivars from two locations, US and SA, had a similar level of phenolic content to those of the two date fruit cultivars studied by Wu et al., (2004a). Total phenolics of 572 to 661 mg gallic acid equivalents /100 g fresh weight in this study were higher than values reported by Al-Farsi et al., (2007a) who reported total phenolic content values between 172 and 246 mg gallic acid equivalents / 100 g fresh weight for date fruit from Omani. Al-Farsi et al., (2005a) earlier reported that Omani date fruit ranged between 217 and 343 mg/100g. Date fruit may contain a higher level of total phenolic content compared to other fresh and dried fruits (Wu et al., 2004a)

Results, also confirmed a previous report by Mansouri et al., (2005) that indicated phenolic compounds as the dominant antioxidant phytochemicals in date fruits. Moreover, a strong correlation was found between the total phenolic content value and antioxidant activity (ABTS & DPPH) values, suggesting that total phenolics assay may

be sufficient to evaluate antioxidant properties in date fruit instead of doing both measurements. The total phenolics assay is quicker and less costly than more complicated radical scavenging assays. It is reassuring, however, that all these assays are in agreement.

The averages of antioxidant activities of date fruit cultivars based on ABTS and DPPH assays as given in Table 3.6 &3.7 showed that Deglet Noor, (a US cultivar) which makes up about 90% of California's date crop (Hong et al., 2006), was found to be the over all best cultivar of those tested in this study. This cultivar was rich in total phenolics and high in radical scavenging capacity values reaching as high as 1400.14 (ABTS) and 1117.75 (DPPH) μmole TEAC/100g FW. Such high antioxidant activities have not been reported by other studies which were confined to simple extraction methods. The antioxidant activity of other date fruit cultivars has been assessed and reported by other research using different assays. Algerian date fruit cultivars have been estimated by DPPH assay and ranged from 0.08 to 0.22 of antiradical efficiency (AE = 1/EC50) (Mansouri et al., 2005). Whereas Omani date fruit cultivars showed an antioxidant activity ranging from 8.2 to 12.5 mM Trolox g^{-1} using Oxygen Radical Absorbance Capacity (ORAC) (Al-Farsi et al., 2005a). Using the Ferric Reducing Antioxidant Power (FRAP), Halvorsen et al., (2002) reported date fruit value was 1.01 mmol per 100 g FW. In the most current study reported on Iranian date fruit, Biglari et al., (2008) using an ABTS assay found the antioxidant activity ranged from 22.83 to 500.33 μmole TEAC/100g of dry weight. The differences in the antioxidant measurements among the assays may be explained, as each assay has a different mechanism of action, or used different reaction conditions.

Analysis of variance (ANOVA) detected a significant difference among all cultivars (C) of date fruit since $p < 0.0001$ (Table 3.8). This indicates that date fruits cultivars are very different relative to antioxidant activities. For instance, date fruit cultivars could be ranked in order of the index (Fig. 3.2) as Deglet Noor US > Khunizi SA > Gur SA > Sukari SA> Shaishi SA > Khalasa SA > Hayany US > Zahidi US > Hilali US > Khadrawy US > Medjool US > Halawy US > Amir Hajj US > Barhee US > Khalasa US. Khalasa US possessed both low phenolic contents and antioxidant activity.

Due to weak correlations between dry matter and total phenolic contents, ABTS and DPPH, dry matter was not considered a related variable in this study.

Antioxidant capacity and phenolic content in date fruit as affected by growing locations:

No information has been reported concerning growing location effects on antioxidant capacity and total phenolic contents of date fruit. Environmental effects on date fruit crop characteristics have not been reported in the literature. Many crops are significantly affected by location (Emmons and Peterson, 2001; Howard, et al., 2003; Peterson and Qureshi, 1993). The importance of growing locations and their interaction on phenolic contents and antioxidant activity was evaluated by testing one cultivar grown at two locations in the United States (US) and in Saudi Arabia (SA). Significant differences at ($\alpha = 0.05$) of total phenolic content and antioxidant activity ABTS and DPPH in both locations in Khalasa cultivar (Table 3.10) were found. Khalasa cultivar growing in the US (PI # 8753, Accession # 78-27) was originally obtained from Al-Hofuf city (Krueger, 1998; Nixon, 1950) which is the major urban center in the huge Al-

Ahsa Oasis in the Eastern Province of Saudi Arabia. This plant introduction record would indicate that the same cultivar was compared for the two locations in this study.

The latitude of Al-Hofuf is 25° 22' N and longitude is 49° 34' E. 32 feet (10 meters) above sea level and less than 100 mm (3.937008 inchs) rain per year. Summer is hot with temperature average exceeding 45 °C. Winter is cooler (and often cold) with average temperature ranging from 14 to 23 °C. Khalasa cultivar growing in the US was provided by the USDA-ARS National Clonal Germplasm Repository for Citrus & Dates - Riverside, California, USA where the latitude is 33° 953' N and the longitude is 117° 395' W. Riverside elevation is 847 feet (258.166 meters) above sea level with an average annual rainfall of 254 mm (10 inches) per year. Riverside, CA climate is warm during summer when temperatures tend to be in the 30's °C and cool during winter when temperatures tend to be in the 10's °C. The warmest month of the year is August with an average maximum temperature of 35 °C, while the coldest month of the year is December with an average minimum temperature of 5 °C (weather.com, 2008) . These variations in temperature between regions could change some physical characteristics and chemical composition of date fruits. Chatty and Tissaoui, (1997) reported that different temperature demands of palm species are related to their sub tropical origin and their geographic distribution. Temperature would likely influence fruit chemical content depending on the region in which they are grown. Morton, (1987) pointed out that heating during ripening of date varies between 25-30 °C depending on cultivar and location. Moreover, they refer to humidity and sun light as factors that affect the fruit. Thus the variation among the regions in the US and SA should affect Khalasa cultivar characteristics and may be related to temperature–humidity as well.

CONCLUSION

The results of this study, clearly show that different cultivars of date palm fruit have different levels of antioxidant capacity and total phenolic properties. In addition, date fruit cultivars grown in the US and SA differ in their antioxidant activity due to different location and other environmental factors. Finally, the phenolic content profile, which is strongly correlated to antioxidant activity in date fruits, may serve as a tool to support the geographic origin of these date fruit cultivars. Nevertheless, further studies are needed to characterize antioxidant properties among the cultivars for human health benefits.

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CHAPTER 4

ANTIOXIDANT PROPERTIES OF PITS FROM DATE CULTIVARS (*PHOENIX DACTYLIFERA* L.)

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) pit averages about 10 % to 15 % of date fruit weight (Almana and Mahmoud, 1994; Hussein et al., 1998). Date pits contain about 9.0 % fat, of which 56.1 % is oleic acid, 11.6 % linoeic acid, 8.3 % lauric acid, 6.0 % myristic, and 2.6 % stearic acid (Al-Hooti et al., 1998). Phytochemical and chromatographic screening have documented other compounds in date pits including carbohydrates and minerals, as well as an important source of proteins, steroids, vitamins, phenols, crude fiber (Al-Showiman, 1990; Besbes et al., 2004a; Besbes et al., 2004b; Ghazanfari et al., 2008; Hamada et al., 2002). Even though pits may have these extractable high value-added components, they generally have no specific use and are commonly discarded, other than sometimes being used as a soil organic additive or as feed for various livestock (Aldhaheri et al., 2004; Banat et al., 2004; Hamada et al., 2002). There is increasing interest to use date pits in a similar way as coffee beans as a hot beverage without the disadvantage of caffeine (Ali-Mohamed and Khamis, 2004; Banat et al., 2004; Barreveld, 1993; Hamada et al., 2002; Haynes and McLaughlin, 2000). Moreover, date pits, an inexpensive agricultural product have also been used for production of activated carbon, a popular adsorbent (Banat et al., 2003; Girgis and El-Hendawy, 2002).

In the United States and some other date palm producing countries, pits of date palm have been a waste problem to the date fruit industry (Besbes et al., 2004b; Hamada et al., 2002). The aim of this work was to study phenolic profiles which have not yet been investigated for date pits. To our knowledge, in vivo studies on the antioxidant potential of date pits are lacking. Results of in vitro antioxidant assays may not only explain the high oxidative stability of date pits, but could also help to improve the economic utility of date pits as a new source of potential beneficial health products, as well as provide data for more extensive investigations into their ability to inhibit in vivo free-radical-mediated damage. The objectives of the present research were to characterize and compare antioxidant properties (free-radical-scavenging activity) and phenolic contents in the pits of ten US and five SA date cultivars; further, to measure antioxidant properties using more than one method and to evaluate the contribution of total phenolic contents to antioxidant activity in date pits.

MATERIALS AND METHODS

Plant materials:

Mature date fruit with pits of 15 cultivars (*Phoenix dactylifera* L.) were obtained by air freight courtesy of the USDA-ARS National Clonal Germplasm Repository for Citrus & Dates - Riverside, California, USA and from authenticated date palms of Saudi Arabian farmers (Table 4.1). Date fruits were hand pitted at the Tamar stage (full ripeness) to separate the fruit from the pits from 2006 and 2007 harvests. Upon arrival at the laboratory, the samples were stored at -20 °C until extraction and analysis.

Table 4.1. Date palm cultivars (*Phoenix dactylifera* L.) used for research on antioxidant properties of pits.

No.	Source	Genus	Species	Cultivars
1	United States (US)	*Phoenix*	*dactylifera*	Amir Hajj
2	United States (US)	*Phoenix*	*dactylifera*	Barhee
3	United States (US)	*Phoenix*	*dactylifera*	Deglet Noor
4	United States (US)	*Phoenix*	*dactylifera*	Halawy
5	United States (US)	*Phoenix*	*dactylifera*	Hayany
6	United States (US)	*Phoenix*	*dactylifera*	Hilali
7	United States (US)	*Phoenix*	*dactylifera*	Khadrawy
8	United States (US)	*Phoenix*	*dactylifera*	Khalasa
9	United States (US)	*Phoenix*	*dactylifera*	Medjool
10	United States (US)	*Phoenix*	*dactylifera*	Zahidi
11	Saudi Arabia (SA)	*Phoenix*	*dactylifera*	Khalasaaa
12	Saudi Arabia (SA)	*Phoenix*	*dactylifera*	Shaishi
13	Saudi Arabia (SA)	*Phoenix*	*dactylifera*	Sukari
14	Saudi Arabia (SA)	*Phoenix*	*dactylifera*	Gur
15	Saudi Arabia (SA)	*Phoenix*	*dactylifera*	Khunizi

Dry matter of date pits:

Dry matter (DM %) was determined for pits from three replicates for each cultivar. Two pits of each replicate cultivar were cleaned by hand to free them of any adhering date flesh, and every pit was then split into four fractions. The fresh weights of pit fractions were recorded and dried at 70°C under vacuum (100 mmHg) for one week and the dry weight was obtained to calculate % dry matter (DM %).

Extraction method:

Because the chemical nature of antioxidants and phenolics differ from simple to very highly polymerized, and because they vary among plant sources due to a matrix effect, there is no one satisfactory universal solvent extraction method for antioxidants and phenolics or even for a specific class of these components, (Shahidi and Naczk, 2003). Therefore, development of an extraction method for antioxidant compounds and total phenolic of date pits was tested for two cultivars (Khalasa and Sukari) and commercial date pit powder as a standard (produced in 2005 by Emirates Dates Factory - Al-Saad). Pits of Khalasa and Sukari cultivars were cooked in an oven at 260 °C, for 20 or 30 minutes. Date pits were also roasted for 5, 10 and 15 minutes at 200 °C. Roasted pits were ground with a coffee grinder. Raw unroasted pits are much harder and were ground in a heavy-duty grinder and passed through a 1-2 mm screen. To test the best cooking/roasting times extracts were prepared using 80 % Acetone + 1 % Formic acid solution. Hydrolysis of samples was performed to remove lipids from the extract, and to liberate bound phenolic acids (Naczk and Shahidi, 2004; 2006; Shahidi and Naczk, 2003). Pits were extracted in methanol containing 1.2 N HCl (40 mL of methanol + 10

mL of 6 N HCl) according to Yi et al., (2005). Samples were dissolved in the acidified methanol solution in glass flasks and placed in a water bath at 80 °C while shaking at 200 rpm for 2 h. The hydrolyzed samples were cooled in an ice bath in the dark and filtered through a 0.2 μm syringe nylon filter.

The samples were also tested to determine the best extraction duration using 1, 3, 6, and 24 hours at 4°C. One hundred milligrams of each sample with three replicates were put into 15ml tubes, then 10 ml of the different solutions were added to each tube. The acidic hydrolysis extraction solutions used were: (80 % acetone:19 % DI water:01 % HCl); (80 % acetone:19 % DI water:01 % HNO_3); (70 % acetone:29.5 % DI water:0.5 % acetic acid); and (80% acetone:20 % DI water). After the extraction durations indicated, all samples were centrifuged at 6000 rpm for 15 min. at 4 °C. One milliliter of supernatant of each sample was vacufuged to dryness at 45 °C for two hours using a VacufugeTM, "Eppendorff" and stored at - 80 °C for further analysis. Three replicates for each cultivar were tested. The best roasting time, extraction duration, and solutions for measurement of phenolic content and measurement of antioxidant capacity were used thereafter for all samples.

While free soluble antioxidants are readily available for testing, insoluble or bound polyphenols are usually ignored in the literature. In vitro alkaline digestion extracts of date pits was obtained according to (Perez-Jimenez and Saura-Calixto, 2005). Alkaline extraction was carried out on the residuals (in the 15ml tube which was centrifuged) and left in the hood for 24 hours with 10 mL of 2M NaOH at room temperature for 1 hour in the rotator. The pH was adjusted with 10 mL of 3M acetic acid and extracted in 10 ml ethyl alcohol for one more hour.

Measurement of date pits total phenolic content:

Total phenolic content was determined using Folin-Ciocalteu reagent adapted from (Spanos and Wrolstad, 1990), and based on the original method of (Singleton and Rossi, 1965) as slightly modified by (Wilson, 2003). This assay is based on the color reaction of phenolics with a phosphomolybdic-phosphotungstic acid reagent (Folin-Ciocalteu reagent, Fluka). Gallic acid solution was used to prepare a standard curve for estimation of total phenolic content as gallic acid equivalents. Gallic acid solution was made up daily by dissolving 25 mg into 25 ml 80 % acetone in a volumetric flask for the best accuracy. A standard curve was prepared from 7.0 ml of stock gallic acid with 3.0 ml 80 % acetone. Then gallic acid standard curve dilutions were prepared using volumes as shown in (Table4.2).

Table 4.2. Gallic acid proportions used for total phenolics standard curve dilutions.

µg / ml in assay	µl Stock Standard	ml de-ionized water (µl)
0	0	3.5 (3500)
40	200	3.3 (3300)
80	400	3.1 (3100)
120	600	2.9 (2900)
160	800	2.7 (2700)
200	1000	2.5 (2500)

In triplicate microplate wells, 35 µl of standard curve dilutions of gallic acid were pipetted per cell to constitute the standard curve. Vacufuged stored samples were reconstituted with 1.0ml 80 % acetone and vortexed. Then 100 µl of extract sample was diluted with 900 µl de-ionized water and vortexed. In triplicate microplate wells, 35 µl of diluted samples were pipetted per cell. With multichannel pipettors 150 µl of Folin-Ciocalteu reagent (fresh dilute full strength SIGMA reagent 1/10 with dH_2O) was added into each well. The microplate was covered with adhesive film and mixed on a platform shaker at 400 rpm for 30 seconds, then held for 5 minutes at room temperature. After 5 min. 115 µl 7.5 % Na_2CO_3 (7.5 g / 100 dH_2O) was added to all wells with a multichannel pipettor. The microplate was covered with adhesive film and mixed on a platform shaker at 400 rpm for 30 seconds. The microplate was then placed on a heating pad that was set to 45 °C for 30 minutes and covered with an insulating styrofoam cover. After standing to cool for 60 min. at room temperature, absorbance was read at 765 nm wavelength, at 45 °C using a spectrophotometer/ microplate reader (SPECTRA *max*-Plus^{384}, Molecular devices, Sunnyvale, CA) with uv-vis spectral scanning, computer-controlled SOFT *max* PRO data analysis and reporting software. Results were expressed as µg GAE/ml. A Microsoft Excel® regression spreadsheet was used to calculate the concentration of Gallic acid equivalents of liquid sample in GAE/g of dry weight (DW).

Measurement of date pits antioxidant capacity:

There has not been agreement as to one preferred method over others to measure the antioxidant capacities of food (Wu et al., 2004). Both ABTS & DPPH are among the most popular spectrophotometric methods widely and commonly used in vitro for

determining the antioxidant capacity of foods and chemical compounds (Awika et al., 2003; Kim et al., 2002). These methods are usually expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

Evaluation of antioxidant activity using the ABTS method:

Antioxidant capacity was measured by -2,2'Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt ~ 98 % (Sigma Aldrich Co.) ABTS assay using the modified method of (Miller and Rice-Evans, 1997), based on the original (Miller and Rice-Evans, 1996) as slightly modified by (Wilson, 2003). This procedure measures the relative ability of antioxidant substances to scavenge the $ABTS^{*}$ free radical. Oxidation of ABTS by manganese dioxide (MnO2) (Sigma Aldrich Co.) to the activated $ABTS^{*+}$ Radical, compared with standard amounts of the synthetic antioxidant Trolox (6-Hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid ~ 97 %, (Sigma Aldrich Chemical Co.), the water-soluble vitamin E analogue. This technique is based on the reduction of the blue-green $ABTS^{*+}$ radical by electron- or hydrogen-donating antioxidants, which was estimated by spectrophotometric/microplate analysis (SPECTRA *max*-Plus^{384}, Molecular devices, Sunnyvale, CA) and computer-controlled SOFT *max* PRO data analysis and reporting software. Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

Briefly, the basic principle for this measurement was as follows: a stock solution of ABTS was prepared by weighting 40 mg ABTS and adding 15 ml de-ionized water in a small beaker with 1-3 gram of MnO_2 (Manganese dioxide, SIGMA # M-1656) mixed and reacted for 20 min. at room temperature. The solution was filtered through

Whatman® (No.1) filter paper in a Buchner-Funnel by Vacuum Pump (DUO-Seal). Excess MnO_2 was filtered by passing the solution through a 0.2 µM syringe filter into a flask. This solution was diluted with about 10-15 ml of 5.0 mM Phosphate Buffered Saline (PBS) pH 7.4 and mixed well to read an absorbance of exactly 0.70 ($\pm$ 0.02) at 734 nm. on temperature 30 °C by adding 25 µl dH_2O to 250 µl $ABTS^{*+}$ in microplate well. It was then placed in a controlled heating block at ~ 30 °C during use. Fresh $ABTS^{*+}$ radical cation solution was prepared each working day.

Trolox stock standard solution (0.5 mM) was prepared by diluting 6.26 mg Trolox into a 50.0 ml volumetric flask, in 5.0 mM PBS (pH 7.4). Fresh standards were prepared every 4 weeks. This solution was vortexed and sonicated for ~ 10 mins. to thoroughly dissolve the Trolox. A range of dilutions (Table 4.3) was prepared daily from frozen aliquots of the standard curve.

Vacufuged stored samples were reconstituted with 1.0ml 80 % acetone by vortexing. In Eppendorf tubes, a set of three dilutions was prepared with 80 % acetone ranging 1/1.1 to 1/100 of 20 mg/ml extract. The assay was carried out on 96-well microplates (12 wells/row) and run one row at a time. A Trolox standard curve was run first (2x each standard conc.), and three different dilution of each extract were run in triplicate as three wells in the same row plus blanks for each dilution.

Table 4.3. Trolox standard curve dilutions used in Eppendorf tubes for ABTS.

Standard #	µM in assay	µl Trolox stock	Ml PBS
1	0	-	1000
2	5	110	890
3	10	220	780
4	15	330	670
5	20	440	560
6	25	550	450

25 µl Trolox standard or samples were pipetted into wells across the row, and 25 µl 5 mM PBS was added to blank wells. 250 µl ABTS solution was added by multichannel pipettor to all wells across each row of the microplate, except extract blanks. The microplate was placed on a platform shaker at 300 rpm for 10 seconds and absorbance read at 734 nm, at 30 °C, exactly one minute after addition of ABTS. A Microsoft Excel® spreadsheet was used to calculate the antioxidant capacity as Trolox Equivalent Antioxidant Capacity (TEAC) in µmole TEAC/g sample dry weight (DW) compared to the standard. A coefficient of variation (<10 %) was used to control treatment, reading and sample preparation variability.

Evaluation of antioxidant activity using the DPPH method:

Antioxidant radical scavenging activity was also determined using the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH*) microplate assay with methanol according to Lu and Yeap Foo, (2000), based on the original (Brand-Williams et al., 1995). Briefly, A 0.1 mM of DPPH (1,1-diphenyl-2-picrylhydrazyl) was prepared by

dissolving 7.889 mg DPPH in 100 ml volumetric with 100 % CH_3OH and sonicated for three hours then stored in the refrigerator. The DPPH stock was adjusted to read 0.90 AU in one microplate test cell by using 100 % CH_3OH (started with 18.0 ml DPPH stock and added 12.0 ml of 100 % CH_3OH then fine tuned to attain 0.90AU). Stock Trolox (1.0 mM) was prepared by dissolving 12.52 mg Trolox in a 50 ml volumetric with 5.0 mM PBS, then stored as1.0 ml aliquots at – 20 °C. A standard curve was made up by preparing Trolox dilutions from 0 to 90 μM concentrations using 1.0 mM Trolox and 5.0 mM PBS in 1.5 mL Eppendorf tubes using (Table 4.4).

Table 4.4. Trolox standard curve dilutions used in Eppendorf tubes for DPPH analysis.

μM Trolox	Stock Trolox (μL)	5 mM PBS (μL)
0	0	1000
20	220	780
30	330	670
40	440	560
50	550	450
60	660	340
70	770	230
80	880	120
90	990	10

15 µl Trolox of standard curve dilutions of each concentration or well-mixed sample solutions were added in triplicate cells. Then 285 µl DPPH stock at 0.90 AU was added to all wells and mixed using a multichannel pipettor. The microplate was covered after standing for 3 min. and the decrease in the absorbance was read at 515 nm. at exactly 25 °C using a spectrophotometer/ microplate reader (SPECTRA *max*-Plus^{384}, Molecular devices, Sunnyvale, CA) and computer-controlled SOFT *max* PRO data analysis and reporting software. Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC). A Microsoft Excel® spreadsheet was used to calculate the antioxidant capacity as µmole TEAC/g sample dry weight (DW) compared to the standard.

Data statistical analysis:

Two years (2006 & 2007) data were subjected to analysis of variance using the GLM procedure based on the general linear model procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC, 2005). Tukey's procedure, Honestly Significant Difference, (HSD) at $\alpha = 0.05$ probability level was used to detect the differences between treatment means. Relationship between total phenolic content, ABTS and DPPH variable were analyzed based on the Spearman's rank correlation coefficient (r) procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC, 2005).

RESULTS

Dry matter of date pits:

Dry matter percentages (DM %) of 15 date pit cultivars for both years are presented in Table 4.9 &4.10. DM % ranged from 92.79 % to 84.07 in Hayany US and Amir Hajj US cultivars respectively. There is a significant ($p = 0.0076$) but weak correlation ($r = 0.39$) in dry matter of all cultivars between the two years.

Extraction methods:

Measurements of total phenolic content were used to evaluate the effect of varying cooking/roasting time for date pits extracted with 80 % Acetone + 1 % Formic (Table 4.5). Two cultivars were used as representative of date pits to evaluate the cooking/roasting period. Significant ($p \leq 0.05$) differences existed among different times used, with some minor exceptions. Roasting for 10 min. gave the highest total phenolic content in both cultivars. Thus, 10 min. roasting was selected to evaluate the total phenolic contents and antioxidant capacity for all cultivars in this study.

Total phenolics content following hydrolysis extraction by methanol containing 1.2 N HCl in a water bath for 2 hours was also highest after 10 min. roasting time for both cultivars (Table 4.6). Moreover, the values following acid hydrolysis were lower or in the same range between 5,046.30 to 1752.22 and 13,393.73 to 5,941.93 mg GAE/100g FW for Khalasa and Sukari cultivars, respectively, compared to those using extraction with 80% acetone + 1% formic acid (Table 4.5) without using the acid hydrolysis (Table 4.5, 4.6).

For extraction using different durations 1, 3, 6, and 24 hours and different solutions (Table 4.7), 1 hour extraction with the 80 % acetone produced the highest

values with all samples: 6,149.94, 4,711.73 and 3,658.49 mg GAE/100g FW for Sukari, Khalasa cultivars and commercial powder respectively.

Table 4.8 compares the total phenolic contents with free soluble antioxidant activity (ABTS) for extraction of one hour in different solutions. The 80% acetone solution (highlighted in Table 4.8) provided the best extraction with both cultivars and the commercial sample. Extraction of bound phenolics in the residues of digested extracts was not improved by any of the acid hydrolysis treatments (Table 4.8).

Measurement of date pits total phenolic content:

The content of total phenolics varied in different accessions and ranged from 66.68 (Hilali US) to 14.51 (Amir Hajj US) mg GAE/g dry matter. The highest total phenolic levels were detected in Hilali US, Sukari SA and Shaishi SA, and the lowest in Amir Hajj US, Hayany US and Gur SA (Table 4.9,4.10). Furthermore, significant ($p < 0.0001$) differences between years (Y), cultivars (C), and interactions (Y x C) was found (Table 4.11) and the first year was higher than the second year over all cultivars (Fig. 1.a.). A significant ($p < 0.0001$) association between the total phenolic content & antioxidant activity was found for the first and the second year: $r = 0.94501$, $r = 0.84374$ with ABTS $r = 0.74$ and $r = 0.74$ with DPPH, respectively (Table 4.12). As a result, cultivars with high total phenolics tended to have higher antioxidant capacity. On the contrary, non-significant correlations ($p = 0.2442$ & $p = 0.9112$) between total phenolic content and dry matter was found for the first and the second year: $r = 0.18$ and $r = -0.02$, respectively (Table 4.12).

Measurement of date pits antioxidant capacity:

Antioxidant activity means of the 15 date pit cultivars in both ABTS and DPPH assays are significantly different among cultivars (Table 4.9,4.10) according to Tukey's Honestly Significant Difference, (HSD) at $\alpha = 0.05$ probability level analysis of means. ABTS ranged from 679.01 to 45.83 μmole TEAC/1g of dry weight in Hilali US and Hayany US respectively. Khunizi SA, Deglet Noor US, Zahidi US and Sukari SA were the highest over all cultivars, on the other hand, Hayany US, Amir Hajj US and Barhee US were the lowest over all cultivars.

However; Amir Hajj US, Khadrawy US , Medjool US , Khalasa SA, had higher ABTS activity in the second year (Fig. 4.1.b). Significant ($p < 0.0001$) differences between two years (Y), cultivars (C), and interactions (Y x C) were found (Table 4.11).

DPPH ranged from 15.94 to 3.92 μmole TEAC/g of dry weight in Sukari SA and Khalasa SA respectively. Also, Hilali US, Deglet Noor US and Sukari SA were the highest over all cultivars and Amir Hajj US, Halawy US, Hayany were the lowest over all cultivars. The first year DPPH activity was higher than the second year and a significant difference ($p < 0.0001$) between two years was also detected (Table 4.11 & Fig.4.1.c).

From Table 12 Spearman's rank correlation coefficient shows there is a strong relationship between measurement of antioxidant capacity by ABTS and DPPH in both years where the first year was $r = 0.74$ and second year was $r = 0.74$ at ($p < 0.0001$). Non-significant correlations ($p = 0.6164$ & $p = 0.2546$) between ABTS and dry matter were found for both the first and the second year: $r = 0.08$ and $r = 0.17$, respectively. Correlations between DPPH and dry matter were also found to be not significant ($p =$

0.20 & p = 0.78) for the first and the second year: r = -0.19 and r = 0.04, respectively (Table 4.12).

In general, Hilali US, Sukari SA and Zahidi US were the best over all fruit cultivars, while, Hayany US, Amir Hajj US and Halawy US were the poorest over all fruit cultivars (Fig. 4.2) based upon on index made by summing total phenolics plus ABTS and DPPH for years one and two.

Pits of one cultivar, Khalasa, were available both years from both the US and SA. Phenolic content, ABTS and DPPH radical scavenging capacity and percentage of dry matter of fruit were all significantly higher from the SA source except DPPH in the first year (Table 13).

Table 4.5. Total phenolics content for cooking/roasting time for date pits using 80 % Acetone + 1 % Formic acid

	mg GAE/100g FW			
Cooking/Roasting time	Khalasa		Sukari	
05 min	3103.25	c[z]	8704.16	b
10 min	4102.97	b	7587.34	b
15 min	2901.57	c	6557.26	c
20 min	2042.81	d	6066.62	e
30 min	1752.22	e	5941.93	d
Raw	5046.30	a	13393.73	a
	LSD= 166.75		LSD= 375.71	

[z] Within each column means followed by the same letter are not significantly different by Least Significant Difference (LSD) mean separation test at ($P \leq 0.05$)

Table 4.6. Total phenolics content for hydrolysis extraction by methanol containing 1.2 N HCl in water bath at 80 C for 2 hrs.

Sample	mg GAE/100g FW	
Raw Sukari	6886.93	a[z]
10 min Sukari	4839.08	b
15 min Sukari	4277.95	c
5 min Sukari	4038.16	d
Raw Khalasa	3807.95	e
10 min Khalasa	3520.20	f
5 min Khalasa	2719.28	g
15 min Khalasa	2661.73	g
Commercial	2244.49	h
	LSD = 166.16	

[z] Means followed by the same letter are not significantly different by Least Significant Difference (LSD) mean separation test at ($P \leq 0.05$)

Table 4.7. Total phenolics content for extraction durations and different acid hydrolysis solutions.

Samples	mg GAE/100g FW		Samples	mg GAE/100g FW	
Sukari pits 1 h extract (80%acetone)	6149.94	a	Khalasa pits 24 h extract (80%acetone+ 1%HCl)	2782.00	p
Sukari pits 3 h extract (80%acetone+ 0.5% acetic acid)	6132.99	a	Khalasa pits 6 h extract (80%acetone+ 1%HCl)	2668.20	pq
Sukari pits 1 h extract (80%acetone+ 0.5% acetic acid)	5883.61	b	Commercial powder 3 h extract (80%acetone)	2631.89	pq
Sukari pits 24 h extract (80%acetone+ 0.5% acetic acid)	5530.11	c	Khalasa pits 3 h extract (80%acetone+ 1%HCl)	2585.88	q
Sukari pits 6 h extract (80%acetone+ 0.5% acetic acid)	5275.88	d	Khalasa pits 1 h extract (80%acetone+ 1%HCl)	2520.51	qr
Sukari pits 6 h extract (80%acetone)	4944.17	e	Sukari pits 24 h extract (80%acetone+ 1%HNO3)	2360.71	rs
Sukari pits 24 h extract (80%acetone)	4881.21	e	Sukari pits 3 h extract (80%acetone+ 1%HNO3)	2334.07	s
Khalasa pits 1 h extract (80%acetone)	4711.73	f	Sukari pits 6 h extract (80%acetone+ 1%HNO3)	2285.65	s
Sukari pits 3 h extract (80%acetone+ 1%HCl)	4280.75	g	Commercial powder 6 h extract (80%acetone+ 0.5% acetic acid)	2198.48	st
Sukari pits 24 h extract (80%acetone+ 1%HCl)	4130.63	gh	Commercial powder 6 h extract (80%acetone)	2075.00	t
Sukari pits 6 h extract (80%acetone+ 1%HCl)	4106.42	h	Commercial powder 24 h extract (80%acetone+ 1%HCl)	2053.21	t
Khalasa pits1 h extract (80%acetone+ 0.5% acetic acid)	3997.46	hi	Commercial powder 1 h extract (80%acetone+ 1%HCl)	1847.40	u
Khalasa pits 24 h extract (80%acetone+ 0.5% acetic acid)	3900.61	ij	Sukari pits 1 h extract (80%acetone+ 1%HNO3)	1762.66	uv
Sukari pits 1 h extract (80%acetone+ 1%HCl)	3854.61	ijk	Commercial powder 3 h extract (80%acetone+ 1%HCl)	1704.55	uv
Sukari pits 3 h extract (80%acetone)	3769.87	jkl	Commercial powder 6 h extracted (80%acetone+ 1%HCl)	1636.76	vw
Khalasa pits 3 h extract (80%acetone+ 0.5% acetic acid)	3723.86	kl	Khalasa pits 3 h extract (80%acetone+ 1% HNO3)	1474.53	w
Commercial powder 1 h extract (80%acetone)	3658.49	l	Khalasa pits 24 h extract (80%acetone+ 1% HNO3)	1142.82	x
Khalasa pits 6 h extract (80%acetone+ 0.5% acetic acid)	3455.11	m	Khalasa pits 1 h extract (80%acetone+ 1% HNO3)	1133.14	x
Khalasa pits 3 h extract (80%acetone)	3329.20	mn	Commercial powder 24 h extract (80%acetone+ 1%HNO3)	1094.40	xy
Khalasa pits 24 h extract (80%acetone)	3186.35	no	Khalasa pits 6 h extract (80%acetone+ 1% HNO3)	1082.29	xy
Commercial pits 1 h extract (80%acetone+ 0.5% acetic acid)	3181.51	no	Commercial powder 24 h extract (80%acetone)	1060.50	xy
Khalasa pits 6 h extract (80%acetone)	3079.81	o	Commercial powder 6 h extract (80%acetone+ 1%HNO3)	1021.76	xy
Commercial powder 24 h extract (80%acetone+ 0.5% acetic acid)	3072.55	o	Commercial powder 3 h extract (80%acetone+ 1%HNO3)	946.70	y
Commercial powder 3 h extract (80%acetone+ 0.5% acetic acid)	2791.69	p	Commercial powder 1 h extract (80%acetone+ 1%HNO3)	937.02	y

[z] Within each column means followed by the same letter are not significantly different by Least Significant Difference (LSD) mean separation test at ($P \leq 0.05$) (LSD = 167.19)

Table 4.8. Total phenolics content and antioxidant activity (ABTS) for free soluble antioxidants and bound polyphenols following acid hydrolysis and alkaline digestion.

Treatment	mg GAE/100g FW		µmole TEAC/100g FW	
Sukari pits 1 h extract (80%Acetone+ 1%HNO3)	6918.193	a	4510.08	d
Sukari pits 1 h extract (80%Acetone)	6583.367	b	15754.51	a
Sukari pits 1 h extract (80%Acetone+ 1%HCl)	6137.703	c	15884.56	a
Sukari pits 1 h extract (80%Acetone+ 0.5acetic acid)	5712.820	d	16122.86	a
Khalasa pits 1 h extract (80%Acetone+ 1% HNO3)	4615.977	e	2957.26	e
Khalasa pits 1 h extract (80%Acetone)	3967.108	f	14154.98	b
Khalasa pits 1 h extract (80%Acetone+ 1%HCl)	3847.033	fg	15800.15	a
Commercial powder 1 h extract (80%Acetone+ 1%HNO3)	3826.250	g	1725.42	f
Khalasa pits1 h extract (80%Acetone+ 0.5acetic acid)	3627.664	h	15448.22	a
Commercial powder 1 h extract (80%Acetone+ 1%HCl)	3350.567	i	13732.12	b
Commercial powder 1 h extract (80%Acetone)	3334.403	i	11212.83	c
Commercial powder 1 h extract (80%Acetone+ 0.5acetic acid)	2858.719	j	11546.15	c
Sukari pits 1 h digest (80%Acetone)	1161.499	k		
Sukari pits 1 h digest (80%Acetone+ 0.5acetic acid)	1122.244	kl		
Sukari pits 1 h digest (80%Acetone+ 1%HCl)	1018.332	lm		
Khalasa pits1 h digest(80%Acetone+ 0.5acetic acid)	1016.023	lm		
Sukari pits 1 h digest (80%Acetone+ 1%HNO3)	992.932	lm		
Khalasa pits 1 h digest (80%Acetone)	925.966	mn		
Khalasa pits 1 h digest (80%Acetone+ 1% HNO3)	912.112	mn		
Khalasa pits 1 h digest (80%Acetone+ 1%HCl)	909.802	mn		
Commercial pits 1 h digest (80%Acetone)	835.910	no		
Commercial pits 1 h digest (80%Acetone+ 0.5acetic acid)	815.128	no		
Commercial pits 1 h digest (80%Acetone+ 1%HNO3)	796.654	no		
Commercial powder 1 h digest (80%Acetone+ 1%HCl)	759.708	o		

[z] Within each column means followed by the same letter are not significantly different by Least Significant Difference (LSD) mean separation test at ($P \leq 0.05$)

Table 4.9. Antioxidant properties measured as total phenolics, ABTS and DPPH for 15 date pit cultivars obtained from Riverside CA (US) and Saudi Arabia (SA) for the first year.

		First Year							
		mg GAE/g DW		μmole TEAC/g DW				Dry Matter %	
No.	Cultivar	Total Phenolics		ABTS		DPPH		DM	
1	Amir Hajj US	14.51	j	74.71	g	5.27	gh	84.07	j
2	Barhee US	24.61	i	141.83	f	7.74	cd	85.03	h
3	Deglet Noor US	47.17	de	404.05	c	11.45	b	84.14	j
4	Halawy US	25.85	i	131.51	f	4.47	hi	86.32	f
5	Hayany US	16.35	j	53.06	g	4.48	hi	89.01	a
6	Hilali US	66.68	a	679.01	a	14.90	a	84.75	i
7	Khadrawy US	48.96	cd	339.42	d	6.01	fg	89.12	a
8	Khalasa US	31.93	gh	142.91	f	6.04	efg	85.50	g
9	Medjool US	44.53	ef	282.88	e	5.16	ghi	87.24	d
10	Zahidi US	56.32	b	527.14	b	8.43	c	87.75	c
11	Khalasa SA	34.35	g	278.46	e	3.92	i	88.52	b
12	Shaishi SA	50.48	c	417.33	c	7.32	cde	86.82	e
13	Sukari SA	66.42	a	543.26	b	15.94	a	88.62	b
14	Gur SA	31.55	h	297.57	e	6.10	efg	86.29	f
15	Khunizi SA	43.06	f	424.88	c	6.96	def	86.80	e
		HSD = 2.7049 at α= 0.05		HSD = 30.477 at α= 0.05		HSD = 1.2966 at α= 0.05		HSD = 0.1707 α= 0.05	

[z] Within each column means followed by the same letter are not significantly different by Tukey-Kramer mean separation test at ($P \leq 0.05$)

Table 4.10. Antioxidant properties measured as total phenolics, ABTS and DPPH for 15 date pit cultivars obtained from Riverside CA (US) and Saudi Arabia (SA) for the second year.

		Second Year							
		mg GAE/g DW		μmole TEAC/g DW				Dry Matter %	
No.	Cultivar	Total Phenolics		ABTS		DPPH		DM	
1	Amir Hajj US	17.12	f	100.30	g	4.54	h	90.01	a
2	Barhee US	28.60	e	117.40	fg	4.91	h	88.15	b
3	Deglet Noor US	43.75	c	322.64	cd	7.36	c	86.24	c
4	Halawy US	28.78	e	124.35	f	4.61	h	89.94	ab
5	Hayany US	19.47	f	45.83	h	4.73	h	92.79	a
6	Hilali US	63.68	a	498.94	a	12.17	a	86.05	c
7	Khadrawy US	41.48	c	383.69	b	10.22	b	90.37	a
8	Khalasa US	38.29	d	138.74	f	6.17	ef	87.24	bc
9	Medjool US	35.89	d	318.87	d	6.03	ef	88.74	a
10	Zahidi US	41.35	c	400.97	b	5.62	fg	88.99	a
11	Khalasa SA	43.80	c	340.95	c	7.24	c	89.59	ab
12	Shaishi SA	53.67	b	321.38	cd	6.91	cd	90.04	a
13	Sukari SA	52.26	b	392.93	b	6.13	ef	88.11	b
14	Gur SA	27.19	e	247.25	e	5.09	gh	91.34	a
15	Khunizi SA	43.94	c	253.63	e	6.34	de	90.46	a
		HSD = 2.7652 at α= 0.05		HSD = 21.911 at α= 0.05		HSD = 0.6548 at α= 0.05		HSD = 3.0654 α= 0.05	

[z] Within each column means followed by the same letter are not significantly different by Tukey-Kramer mean separation test at ($P \leq 0.05$)

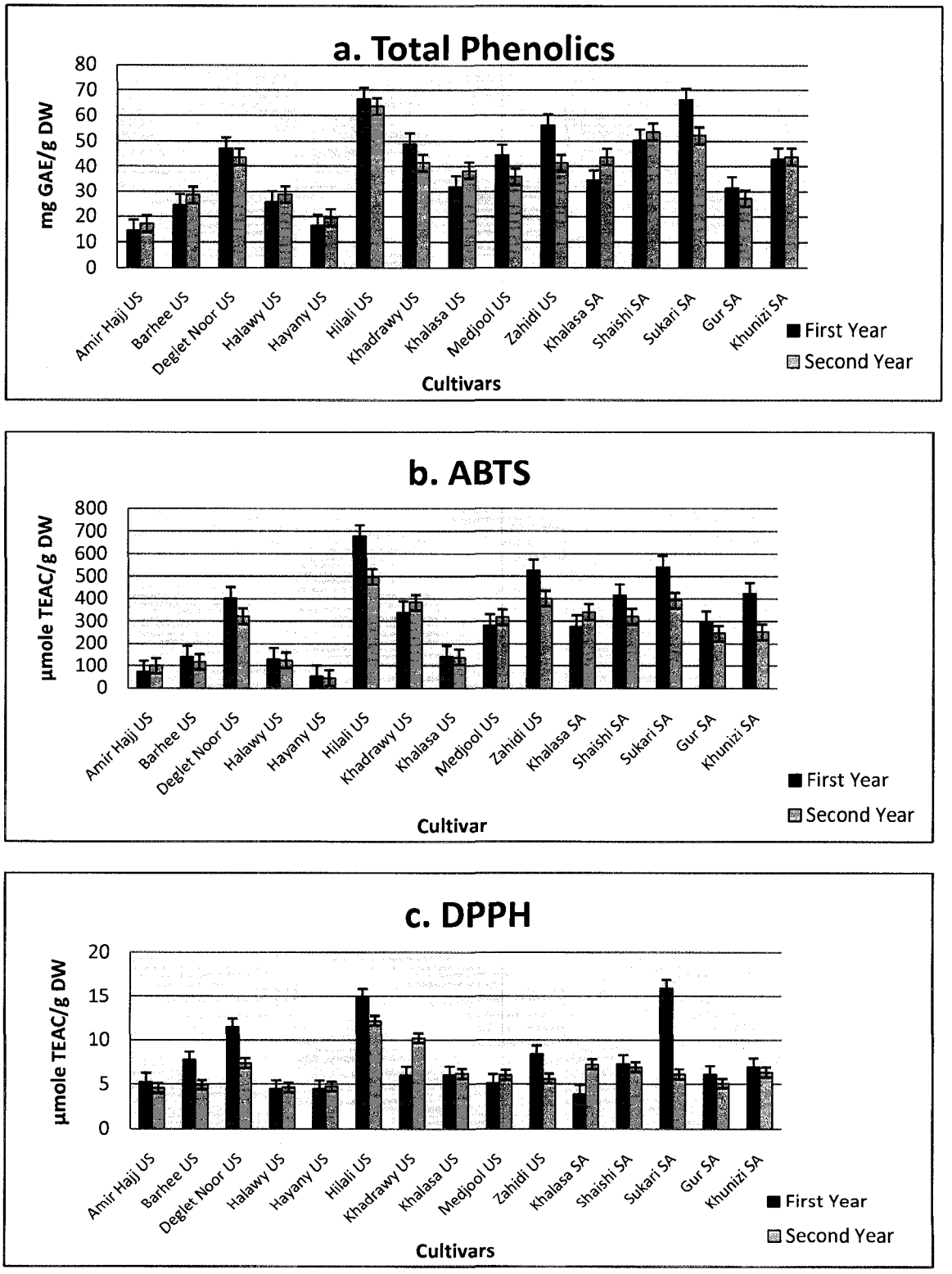

Fig.4.1. Years x cultivars interactions for total phenolics, ABTS and DPPH.

Table 4.11. Analysis of variance for years, cultivars and interactions.

Source	Phenolics	ABTS	DPPH	Dry matter
Year (Y)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cultivar (C)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Rep.	ns	< 0.0001	< 0.0001	ns
Y x C	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Expressed as P-values statistical significance; significant at $P \leq 0.05$

Table 4.12. Spearman's rank correlation coefficient matrix (r) displayed for total phenolics, total antioxidants (ABTS & DPPH) and dry matter.

	First Year			Second Year		
	ABTS	DPPH	Dry Matter	ABTS	DPPH	Dry Matter
Total phenolics	r = 0.94501 P < 0.0001	r = 0.74174 P < 0.0001	r = 0.17721 P = 0.2442	r = 0.84374 P < 0.0001	r = 0.74380 P < 0.0001	r = -0.0171 P = 0.9112
ABTS		r = 0.73737 P < 0.0001	r = 0.07672 P = 0.6164		r = 0.73618 P < 0.0001	r = 0.17341 P = 0.2546
DPPH			r = -0.19412 P = 0.2013			r = 0.04342 P = 0.7770

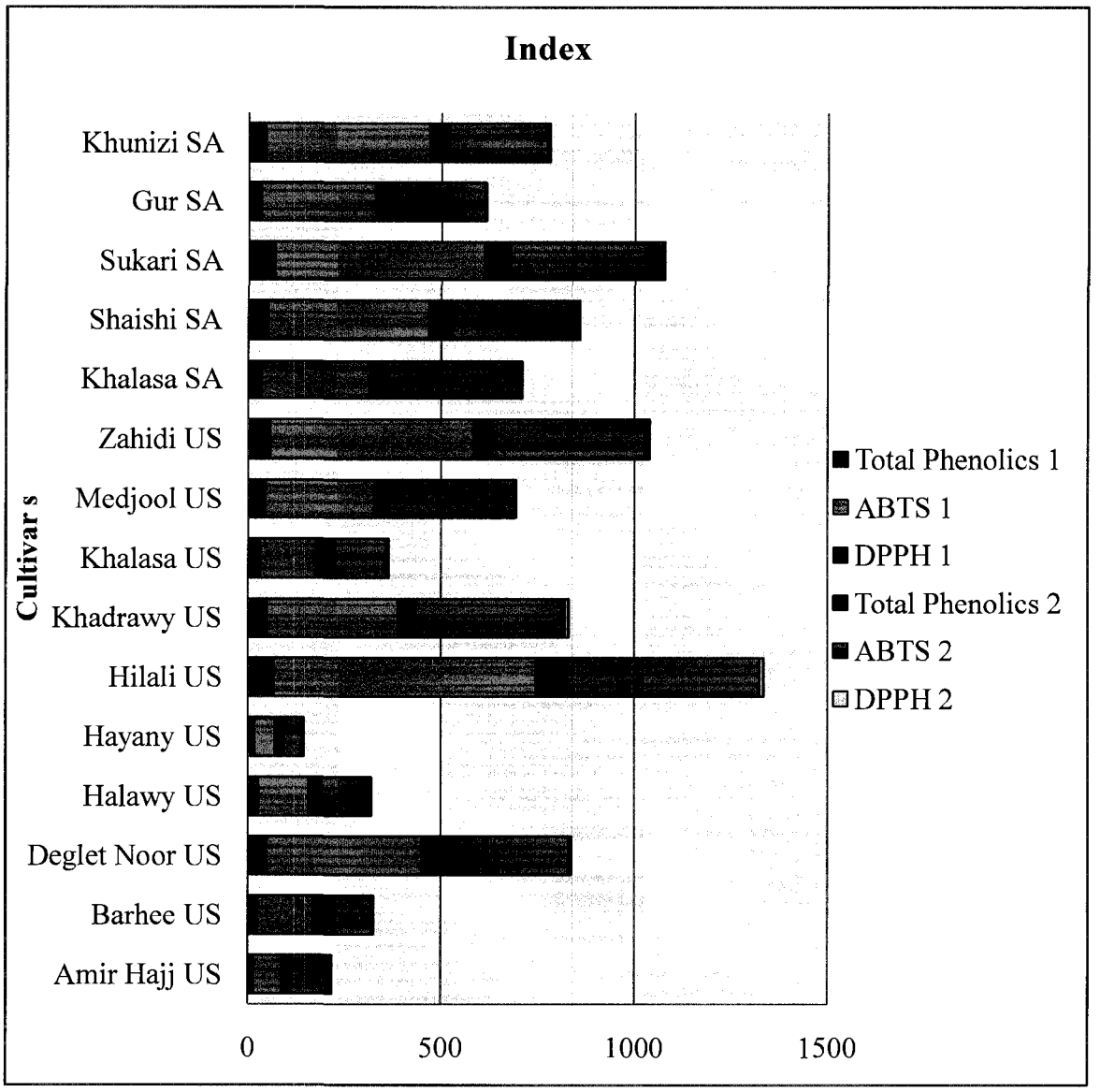

Fig.4.2. Index (∑ (Total Phenolics + ABTS + DPPH) first year + second year

Table 4.13. Comparison of antioxidant properties and dry matter between Khalasa pits of US and SA sources.

a.		First Year							
No.	**Location**	**Phenolics**		**ABTS**		**DPPH**		**DM**	
8	**US**	31.93	b[z]	142.91	b	6.04	a	85.50	b
11	**SA**	34.35	a	278.46	a	3.92	b	88.52	a
		HSD= 1.883; α= 0.05		HSD = 11.93; α= 0.05		HSD = 0.5115; α= 0.05		HSD = 0.0625; α= 0.05	
b.		Second Year							
No.	**Location**	**Phenolics**		**ABTS**		**DPPH**		**DM**	
8	**US**	38.29	b	138.75	b	6.17	b	87.24	b
11	**SA**	43.80	a	340.95	a	7.24	a	89.59	a
		HSD = 0.870; α= 0.05		HSD = 11.96; α= 0.05		HSD D= 0.537; α= 0.05		HSD = 8.371; α= 0.05	

[z] Within each column means followed by the same letter are not significantly different by Tukey-Kramer mean separation test at ($P \leq 0.05$)

DISCUSSION

The content of total phenolic compounds and antioxidant activity among date pit cultivars tested in this study were significantly different. Due to limitations of the samples available, it was not possible to identify with certainty other environmental factors that may have contributed to variation among culivars. Factors such as cultivar, growing condition, maturity, season, geographic origin, climate, location, temperature, fertilizer, soil type, disease and pest exposure, processing, storage conditions and amount of sunlight received, among others, might be responsible for the observed differences of date pits (Al-Farsi et al., 2007; Biglari et al., 2008; Gil et al., 2002) and may significantly influence the content of plant secondary metabolites (Wu et al., 2006).

The results from developing the extraction method including the best time for cooking-roasting, extraction duration and solution, revealed that 10 min. was the best roasting time, and one hour duration and 80 % acetone solution were most effective without doing hydrolysis nor alkaline digestion. These optimum processes were followed in this study. The results found in this study agree with Shahidi and Naczk, (2003) who also found that a long roasting time decreased the measurable phenolic contents in coffee beans. Ideally, about 10 minutes total roast time was adequate to produce 66.68 mg GAE/g DW of total phenolics from Hilali pits.

Results from this study showed the same strong relationships between the total phenolic content value and antioxidant activity (ABTS & DPPH) as detected in fruit samples, except ABTS values were higher. The strong and significant correlations suggest that a total phenolics assay may be sufficient to evaluate antioxidant properties in date pits instead of doing both measurements. The total phenolics assay is quicker and

less costly than more complicated radical scavenging assays. It is reassuring, however, that all these assays are in agreement.

The averages of antioxidant activities of date pit cultivars based on ABTS and DPPH assays given in Table 4.9 &4.10 showed that Hilali, (a US cultivar) was found to be the over all best cultivar of those tested in this study. This cultivar was rich in total phenolics and high in radical scavenging capacity values reaching as high as 679.01 (ABTS) and 14.90 (DPPH) μmole TEAC/g DW which could be recommended as an ideal cultivar for a source of antioxidants in US cultivars. At the same time, Sukari SA was considered as the best antioxidant source from pits of SA cultivars. Based on this study the values for Sukari SA were 643.26 (ABTS) and 15.94 (DPPH) μmole TEAC/g DW.

Analysis of variance (ANOVA) detected a significant variation among cultivars (C) of date pits, $p < 0.0001$ (Table 4.11), indicating that the cultivars of date pits are very different based on the antioxidant activity. For instance, date pit cultivars could be ranked in order of the index (Fig. 2) as Hilali US > Sukari SA > Zahidi US > Shaishi SA > Khadrawy US > Deglet Noor US > Khunizi SA > Khalasa SA > Medjool US > Gur SA > Khalasa US > Barhee US > Halawy US > Amir Hajj US > Hayany US. Hayany US possessed both low phenolic contents and antioxidant activity.

Due to weak correlations between dry matter and total phenolic contents, ABTS and DPPH, dry matter was not considered a reliable indicator of antioxidant properties in this study.

Antioxidant capacity and phenolic content in date pit as affected by growing locations:

No information has been reported concerning growing location effects on antioxidant capacity and phenolic contents of date pits. Environmental effects on date pit crop characteristics have not been reported in the literature. Many crops are significantly affected by location (Emmons and Peterson, 2001; Luke R Howard, 2003; Peterson and Qureshi, 1993). The importance of growing locations and their interaction on phenolic contents and antioxidant activity was evaluated by testing one pit cultivar grown at two locations in the United States (US) and in Saudi Arabia (SA). Significant differences at (α = 0.05) of total phenolic content and antioxidant activity ABTS and DPPH in both locations in Khalasa cultivar (Table 4.13) were found. Khalasa cultivar growing in the US (PI # 8753, Accession # 78-27) was originally obtained from Al-Hofuf city (Krueger, 1998; Nixon, 1950) which is the major urban center in the huge Al-Ahsa Oasis in the Eastern Province of Saudi Arabia. This plant introduction record confirms that the same cultivar was compared for the two locations in this study.

The latitude of Al-Hofuf is 25° 22' N and longitude is 49° 34' E. 32 feet (10 meters) above sea level and less than 100 mm (3.937008 inches) rain per year. Summer is hot with temperature average exceeding 45 °C. Winter is cooler (and often cold) with average temperature ranging from 14 to 23 °C. Khalasa cultivar growing in the US was provided by the USDA-ARS National Clonal Germplasm Repository for Citrus & Dates - Riverside, California, USA where the latitude of Riverside is 33° 953' N and the longitude is 117° 395' W. Riverside elevation is 847 feet (258.166 meters) above sea level with an average annual rainfall of 254 mm (10 inches) per year. Riverside, CA climate is warm during summer when temperatures tend to be in the 30's °C and cool

during winter when temperatures tend to be in the 10's °C. The warmest month of the year is August with an average maximum temperature of 35 °C, while the coldest month of the year is December with an average minimum temperature of 5 °C (weather.com, 2008) . These variations in temperature between regions could change some physical characteristics and chemical composition of the date fruits including pits.(Chatty and Tissaoui, 1997) reported that different temperature demands of palm species are related to their sub tropical origin and their geographic distribution. Temperature should influence fruit and their pit chemical content depending on the region in which they are grown. (Morton, 1987) pointed out that during ripening of dates temperatures vary between 25-30 °C depending on cultivar and location. Moreover, they refer to humidity and sun light as factors that affect the fruit phytochemical content. Thus the variation among the regions in the US and SA where dates are grown should affect Khalasa cultivar characteristics and may be related to temperature–humidity as well.

CONCLUSIONS

The results of this study clearly show that different cultivars of date palm pits have different levels of antioxidant capacity and total phenolic properties. In addition, date pit cultivars grown in the US and SA differ in their antioxidant activity due to different location and other environmental factors. Finally, the phenolic content profile, which is strongly correlated to antioxidant activity in date pits, may serve as a tool to support the geographic origin of these date pit cultivars. Nevertheless, further studies are needed to characterize antioxidant properties among the cultivars for human health benefits and potential value-added by products such as a hot caffeine-free beverage similar to coffee.

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