ASSESSMENT OF ANTIOXIDANT POTENTIAL OF DATE (*PHOENIX DACTYLIFERA*) FRUITS FROM IRAN, EFFECT OF COLD STORAGE AND ADDITION TO MINCED CHICKEN MEAT

by

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LIST OF ABBREVIATIONS / SYMBOLS

Abbreviations / Symbols	Caption
AA	Antioxidant activity
ABTS	2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt
AE	Antiradical efficiency
AEAC	L-ascorbic acid equivalent antioxidant capacity
AGE	Advanced glycation end product
AOAC	Association of Official Analytical Chemists' methods
AOCS	American Oil Chemists' Society titration method
AUC	Area under the curve
CEQ	Catechin equivalents
CHD	Coronary heart disease
DD	Dry date
DPF	Date palm fruit
DPPH	(diphenyl-2-picrylhydrazyl) radicals
DW	Dry weight
EO	Essential oils
FAO	Food & Agriculture Organisation
FAOSTAT	Food & Agriculture Organization statistics
FC Reagent	Folin–Ciocalteu reagent
FRAP	Ferric-reducing antioxidant power
FW	Fresh weight
GADF	Grape antioxidant dietary fibre

GAE	Gallic acid equivalents
HPLC	High performance liquid chromatography
MA	Malonaldehyde
MAP	Modified-atmosphere packaging
MCM	Minced chicken meat
MFM	Minced fish muscle
ORAC	Oxygen radical absorbent capacity
PAL	Phenylalanine ammonia-lyase activity
POD	Peroxidase
PPO	Polyphenol oxidase
PUFA	Polyunsaturated fatty acids
PV	Peroxide value
ROS	Reactive oxygen species
SD	Soft dates
SDD	Semi-dry dates
SPM	Spectrophotometric peroxide method
TBA	Thiobarbituric acid
TBA-MA	Thiobarbituric acid- malonaldehyde
TBARS	Thiobarbituric acid reactive substance
TCA	Trichloroacetic acid
TEAC	Trolox equivalent antioxidant capacity
TEP	Tetraethoxypropane
TEP	1,1,3,3-tetraethoxy- propane
TFC	Total flavonoid content
TPC	Total phenolic content

TPTZ	Tripydyltriazine
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
ANOVA	Analysis of variance
MANOVA	Multivariate analysis of variance
DA	Discriminant analysis
DF	Discriminant functions
PCA	Principal component analysis
CA	Cluster analysis
HAT	Hydrogen atom transfer
ET	Electron transfer
Std	Standard deviation
LDPE/PA/LDPE	Low density polyethylene/polyamide/low density polyethylene

PENILAIAN POTENSI ANTIPENGOKSIDAAN BUAH KURMA (*PHOENIX DACTYLIFERA*) DARI IRAN TERHADAP KESAN PENYIMPANAN PADA SUHU SEJUK DAN PENAMBAHAN KE DALAM DAGING AYAM CINCANG.

ABSTRAK

Bahagian yang boleh dimakan (edible part) daripada buah kurma (*Phoenix dactylifera*) dari Iran dianalisis bagi mengetahui aktiviti antipengoksidaan (antioxidant activities, AA) dengan menggunakan kaedah TEAC (cerakin ABTS) dan penurunan ferik (ferric reducing) / kaedah kuasa antipengoksidaan (antioxidant power method) (cerakin FRAP). Kandungan fenolik total (total phenolic content, TPC) dan kandungan flavonoid total (total flavonoid content, TFC) pada DPF diukur menggunakan kaedah meter warna Folin-Ciocalteau dan kaedah meter warna aluminum klorida. Sampel yang digunakan termasuklah 4 jenis kurma lembut (soft dates, SD) yang dikenali sebagai Kurma Madu, Kurma Bam, Kurma Jiroft dan Kurma Kabkab; 3 jenis kurma separa kering (semidry dates, SDD) yang dikenali sebagai Kurma Sahroon, Kurma Piarom dan Kurma Zahedi; dan 1 jenis kurma kering (dry date, DD) iaitu Kurma Kharak. Analisis korelasi menunjukkan bahawa terdapat hubungan yang linear di antara AA dan TPC atau TFC pada DPF. Varieti kurma lembut (SD, Bam) dan kurma kering (DD, Kharak) disimpan pada suhu 4 °C selama 6 bulan, dan ditambah seminggu lagi pada suhu 18 °C. Sebatian antipengoksida (TPC dan TFC) pada buah kurma meningkat semasa penstoran. Analisis kelompok (cluster analysis) yang diaplikasikan pada data TPC dan TFC sebelum dan selepas penstoran, menunjukkan dua kelompok SD dan DD yang secara statistiknya adalah signifikan. Hal ini menandakan bahawa TPC dan TFC mempunyai perlakuan yang berbeza berdasarkan jenis kurma. Kedua-dua sistem antipengoksida yang diuji

didapati merencatkan pengoksidaan lipid. Keputusan PV dan TBARS menunjukkan bahawa 4% Kharak ekstrak adalah paling berkesan untuk merencatkan pengoksidaan lipid MCM berbanding kurma ekstrak's yang lain. Penyelidikan ini mencadangkan potensi buah kurma Iran digunakan sebagai suatu kandungan makanan yang boleh berfungsi sebagai antipengoksida.

ASSESSMENT OF ANTIOXIDANT POTENTIAL OF DATE (*PHOENIX DACTYLIFERA*) FRUITS FROM IRAN, EFFECT OF COLD STORAGE AND ADDITION TO MINCED CHICKEN MEAT

ABSTRACT

Edible parts of date palm (*Phoenix dactylifera*) fruits (DPF) from Iran were analyzed for antioxidant activities (AA) using TEAC method (ABTS assay) and the ferric reducing/ antioxidant power method (FRAP assay). The total phenolic content (TPC) and total flavonoid content (TFC) of the DPF were measured using Folin-Ciocalteau and aluminum chloride colorimetric methods respectively. The samples used included four types of soft dates (SD) namely Honey date, Bam date, Jiroft date and Kabkab date; three types of semidry dates (SDD) namely Sahroon date, Piarom date and Zahedi date and one type of dry date (DD) which was Kharak date. Dry date's variety had the highest AA, TPC and TFC as compared to those of other dates. Correlation analyses indicated that there was a linear relationship between AA and the TPC or TFC of DPF. Both Bam and Kharak date varieties were stored at 4 °C for six months followed by additional one week storage at 18 °C. Antioxidant compounds (phenolics and flavonoids) of the dates increased following storage. Cluster analysis applied on TPC and TFC data before and after storage indicated that TPC and TFC had different behaviors according to the types of dates. Effect of the addition of aqueous extracts of Bam and Kharak dates were on lipid oxidation in minced chicken meat (MCM) during storage in the dark for up to 20 days at 4°C, was studied. Both date extracts were found to inhibited lipid oxidation. PV and TBARS results indicated that 4 % Kharak extracts was the most effective at inhibiting lipid oxidation of MCM than any other date extracts. This research suggests the potentials of Iranian dates to be used as antioxidative functional food ingredients.

CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

The fruit of the date palm (Phoeheonix dactylifera) is an important commercial crop in Middle East countries. Dates are a good source of energy, vitamins, and elements like phosphorus, iron, potassium, as well as a significant amount of calcium (Gamil Abdel-Hafez *et al*., 1980; Anwar, 1987). The world production estimate of dates in 2004 was 6,772,068 metric tonnes; Iran (14 % of world production) is the second major producer after Egypt (17 % of world production) (FAO, 2004).

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

In addition, there are at least 15 minerals in dates. The percentage of each mineral in dried dates varies from 0.1 to 916 mg/100 g date, depending on the type of mineral. The minerals and salts found in various proportions include boron, calcium, cobalt, copper, fluorine, iron magnesium, manganese, potassium, phosphorous, sodium and zinc. Dates contain elemental fluorine that is useful for protecting teeth against decay. Selenium, which experts believe helps to prevent cancer and boost the

immune system, is also found in dates. 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. Polysaccharide materials from dates have been used as a functional food and a source of active components in the development of drugs. Dates contain at least six vitamins including vitamin C, vitamin B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A (Ahmed *et al*., 1995).

Because of the lack of information in the literature on the phenolic compound of Iranian date cultivars, an attempt was made to characterize eight date cultivars collected from selected date-growing regions in Iran. In this study, only eight types of the commonly consumed dates in Iran were used. These types are well known because of their common preference, popularity, economic price, as well as the high availability through out the year.

Recent studies revealed that consuming high amounts of fruits and vegetables will reduce the risk of a number of chronic diseases (Nicoli *et al*., 1999). This is attributed to the presence of a group of phytochemicals: dietary fibre, phenolics, natural antioxidants, and other bioactive compounds. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu *et al*.,1998). The antioxidative effect is mainly due to phenolic components, such as flavonoids (Pietta, 1998), phenolic acids, and phenolic diterpenes (Shahidi *et al*., 1992). The antioxidant activity of phenolic compounds is a result of their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

Many of these phytochemicals possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of cancer. (Velioglu *et al*., 1998).

Vayalil (2002) and Mansouri *et al*., (2005) reported that dates have phenolic compounds (mainly cinnamic acids) and flavonoids (flavones, flavonols and flavanones) that provide antioxidant activities. Al-Farsi *et al*., (2005a, b) determined the compositional and sensory characteristics of three native sun-dried date (Phoenix dactylifera) varieties grown in Oman, comparing the antioxidant activity, anthocyanins, carotenoids, and phenolics for each different variety.

With the advent of the field of functional food and nutraceuticals, any information on the health-promoting components of dates will enhance the knowledge and appreciation for the uses of dates in these health-promoting products. Therefore the antioxidant activity (AA) of Iranian dates was determined and presented in this study. Several different methods are available and have been used to assess the total antioxidant capacity of plant extracts, such as the ABTS assay (Miller *et al*., 1993), the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996).

In the present study, we evaluated the total antioxidant capacities of dates using an improved ABTS method (Cai *et al*., 2004 and Re *et al*., 1999), an improved FRAP assay. We also estimated the total phenolic contents of these plants using the classical Folin–Ciocalteu reagent, and we investigated the relationship between the total antioxidant capacities and phenolic contents in the samples tested. Furthermore, we used aluminum chloride colorimetric method to identify flavonoids in the dates.

These data will be helpful for comparison of the antioxidant activities and phenolic compounds of different types of dates and also useful for understanding their chemical constituents and functionality.

Studies on other fruits indicated the possibility that storage can have an effect on the level of antioxidant compounds (Kalt, 2005; Kevers *et al*., 2007). Many fruits tend to lose stability during storage, but dates are relatively stable over a long period of time when refrigerated. This prompted us to study the influence of low temperature storage on antioxidant compounds in dates.

Chicken meat is internationally used in food recipes because of its low fat and nutritious content (Chouliara *et al*., 2007). Tichivangana and Morrissey (1985) have proved the fact that the oxidation of meat takes place in the order of fish>poultry>pork>lamb. Considering that poultry is perishable, the most important goal for industries is the retardation of oxidation for the shelf-life of the poultry products.. The hurdle technology concept (Leistner, 1995) is one of the modern trends to reach this goal, which includes the use of natural food preservatives to maintain minimal processing and to avoid lipid oxidation.

One of the most important causes of quality deterioration in chicken meat is lipid oxidation which begins almost instantly after slaughter, releasing a variety of breakdown products which produce off-odours and flavours (Gray and Pearson, 1994; Faustman and Cassens, 1990). Autoxidation of lipids is carried out by a free radical chain reaction. The initial step in this reaction is the formation of peroxide that further degrades into several reactive compounds including malonaldehyde

(MA) (Ranken, 1994; Raharjo and Sofos, 1993). These secondary products are responsible for undesirable or "rancid" flavors (Shahidi, 1994).

It is possible to use the antioxidant extract in dates to retard lipid oxidation of meat during chill storage. The main advantage of using aqueous extracts from date palm fruits is the absence of chlorophyll that has been indicated as a pro-oxidant in herb-meat systems that promote the oxidation of lipids during storage (He and Shahidi, 1997; Endo *et al*., 1985). Successful application of aqueous extract of dates in food systems may enhance the value of dates palm fruits, thus expanding the utilization of the fruits as an inhibitor against lipid oxidation.

In this research, the effect of aqueous extract of date palm fruit (Kharak and Bam) on lipid oxidation of minced chicken meat during chill storage was studied. Aqueous extract was used in order to exclude the possible interference of known lipophilic substances such as carotenoids, steroids, etc (Vayalil, 2002). Factorial design was also employed to evaluate the effect of three factors: the types of dates, the level of antioxidant application and the storage time on three responses of interest that are pH, PV and TBA in order to assess the shelf stability of minced chicken meat.

To assess the level of lipid oxidation of chicken meat during storage, both primary (PV) and secondary oxidation products (TBA) were measured. Primary lipid oxidation was evaluated by means of the PV. TBA is a widely used indicator for the assessment of degree of secondary lipid oxidation (Nishimoto *et al*., 1985). Oxidation of lipids is assessed by the TBA (thiobarbituric acid) assay which is based on the reaction between TBA and MDA (malondialdehyde).

1.2 Hypotheses and research questions

Dates considered as a fresh fruit rank number 5 in the production list of tropical and sub-tropical fruits after citrus, mangoes, bananas and pineapples. As a dried fruit, dates easily top the list over raisins, figs and prunes. There have been several studies on the antioxidant activities of various fruits, herbs, and plants. Therefore, the hypothesis of this study declares the existence of antioxidant activity in date palm fruit.

The specific questions were brought forth for discussions include:

1. Is antioxidant activity (AA), total phenolic content (TPC) and total flavonoid content (TFC) of Iranian date noticeable?
2. Whether there is a possible relationship between total phenolic content (TPC) and antioxidant activity (AA) and also total flavonoid content (TFC) of Iranian date?
3. Is there any increase in antioxidant compounds in dates after long-term cold storage?
4. What is the application of Iranian dates accessions as new potential sources of natural antioxidants and phenolic compounds in a real system like minced chicken meat?

1.3 Objectives

The main objectives of this research was the assessment of antioxidant activity (AA), total phenolic content (TPC) and total flavonoid content (TFC) of eight different types of dates from Iran, and to study the application of date extract to retard lipid oxidation in minced chicken meat (MCM):

- To evaluate antioxidant activity (AA), total phenolic content (TPC) and total flavonoid content (TFC) of soft, semi-dry, and dry dates from Iran.
- To demonstrate a possible relationship between phenolic content and antioxidant activity (AA) and also between total phenolic content (TPC) and total flavonoid content (TFC).
- To evaluate effect of long term cold storage on antioxidant compounds, total phenolic content (TPC) and total flavonoid content (TFC) in dates.
- To determine the effect of different date varieties (Bam and Kharak) extract with different concentration (control, 2% and 4%) on pH and lipid oxidation (TBA and POV) in minced chicken meat (MCM) during storage in the dark for up to 20 days at 4°C.

1.4 Thesis Outline

In this dissertation, the following outline is presented: the evaluation of antioxidant activity (AA), total phenolic content (TPC) and total flavonoid content (TFC) of selected varieties of Iranian date fruit and the changing of phenolic content during long cold storage of two varieties, as well as the effect of aqueous extract of theses two varieties on retarding lipid oxidation in minced chicken meat (MCM) during chilled storage for up to 20 days.

Chapter *One* is a general introduction about the date fruit, necessity of study on antioxidant activity in fruit to find new sources of phenolic compound to promote health and application of redox properties of these compounds to inhibit deterioration of lipid in food.

In Chapter *Two*, the general introduction of the history of date fruits focuses on date production in the world and in Iran, the stages of date ripening, the storage of dates, and the chemical composition of the date fruit, and also briefly, the antioxidant activity in dates and in other fruits. Also this chapter reviews some of the literature about the effect of hot or cold storage on the phenolic compound and the application of natural antioxidants in food to retard lipid oxidation.

Chapter *Three* lists all the used materials as well as the methodology of all assays throughout the study. Statistical analysis that was used for analysis of raw data is illustrated in this chapter.

Chapter *Four* consists of three subsections: first, the result of antioxidant activity (AA), total phenolic content (TPC) and total flavonoid content (TFC) calculated based on fresh and dry weight. Also, the relationship between total antioxidant activity and total phenolic content, percentage of inhibition, descriptive statistics, multivariate analysis, discriminant function, and principal component analysis (PCA) were also presented. Second, the results of the effect of long-term cold storage on antioxidant compounds in dates, descriptive statistics of dates before and after storage at 4 °C for 6 months and cluster analysis (CA) is accessible. Third, the results of proximate analysis, pH, TBAR and Peroxide value and the results of experimental design and the results of ANOVA and Tukey's test for pH, PV, and TBA at different concentration and storage time, also the main and interaction plot (data means) for pH , PV, and TBA are placed in this chapter.

The fifth and final chapter consists of general conclusions. In this chapter, recommendation for future studies about other potentials for the date fruit and antioxidant compounds from dates or date seeds was presented.

CHAPTER 2

LITERATURE REVIEW

2.1 Date as the oldest cultivated plant in the Middle East

2.1.1 History of date fruits (Phoenix dactylifera)

As far back as 4000 years B.C., dates were already appreciated and commercially cultivated in what is known today as southern Iraq. In the Old World, the near East and Africa were the regions where dates were grown in large quantities. The botanical name of the date palm, Phoenix dactylifera L. is presumably derived from a Phoenician "Phoenix," which means date palm, and "dactylifera" derived from a Greek word "daktulos" meaning a finger.

Date fruits (Phoenix dactylifera) have been an important harvest in arid and semiarid regions of the world since earliest times; they have always played a significant role in the economic and social lives of the people of these regions. It is considered a vital component of their daily diet. Therefore dates are considered to be a staple food. The date is a well known fruit which has been consumed throughout the world for a long time. It is one of mankind's oldest cultivated plants and has been used as food for about 6000 years. The date palm is considered to be one of the most important fruit trees in all of the southern regions of Iran.

2.1.2 Productions

The main date-producing countries in the world are Iran, Iraq, Egypt, Saudi Arabia, Pakistan, U.A.E., Algeria, Morocco, Libya, Tunisia, USA, and in small

quantities in Spain, Mexico, Yemen, Israel. As can be seen in Table 2.1 based on FAO report in 2005 Iraq is the first producer in the world.

Table 2.1 Top ten dates producers in 2005 (1000 tonnes) based on FAO report.

Top Ten Dates Producers — 2005 (1000 tonnes)	
Iraq	7,170.00
Saudi Arabia	4,970.49
Egypt	1,170.00
Iran	880.00
United Arab Emirates	760.00
Algeria	516.29
Pakistan	496.58
Sudan	328.20
Libya	150.00
China	130.00
World Total	**16,696.56**

Source:
UN Food & Agriculture Organisation (FAO)

2.1.3 Dates in Iran

Dates are produced in 35 countries worldwide and cultivated on about 2.9 million acres of land. The world production estimate of date in 2004 was 6,772,068 Metric Tonnes, and Iran (14 % of world production) is the second major producer after Egypt (17 % of world production) (FAO, 2004).

In recent years Iran has been a leading date fruit producer in the world with an annual production of 900,000 metric tons from 218000 ha of cultivated land and 127,000 tons exportation in 2006. Even today, in spite of the dramatic socioeconomic changes in Iran, dates continue to play an essential role in the diet of the local

inhabitants (Amer, 1994; Anonymous, 2003; FAOSTAT, 2004). Date fruits are grown mainly in South, South East, and South West of the country, and with less important in central Iran.

The date palm starts to produce fruits at an average age of 5 years, and continues production with an average yield of 400–600 kg/tree/year for up to 60 years (Shinwari, 1993; Al-Shahib and Marshall, 2003). Each fruit is a one-seed berry consisting of a fleshy mesocarp covered by a thin epicarp; a hard endocarp surrounds the seed, and these stones (pits) are often used as animal feed. The fruits are arranged on spike lets bearing 20–60 individual dates, and a number of such spike lets are attached to a central stalk to form a bunch (5–30 bunches per tree)(Ahmed *et al*., 1995).

The most exquisite species of dates are produced in Iran. The fruit has a large variety and more than 400 varieties of the fruit, some of which acclaimed worldwide, are produced in the southern, southwestern, and southeastern palm groves of Iran. Due to climatic restrictions and the tropical nature of the date palm, dates are produced in 14 provinces of Iran together with Ghasre Shirin and Soumar in Kermanshah province, Tabas in the southern Khorassan province, Khor and Biabanak in Isfahan province, Taroud in Semnan province and extending from central to southern Iran. In the year 1997, about 230 thousand hectares of land were under date palm cultivation, 83.6 percent of which are productive. Hormozgan province, with a date productivity rate of 29.7 %, ranks first in the country followed by the provinces of Kerman, Khouzestan, Boushehr, and Fars, having a productivity rate of 18.8, 16.2, 11.8, and 11.6 percentages respectively. The production of dates from 192 hectares of palm groves in the country is approximately 877 tons with

Kerman province (including Jiroft area) having the highest production share of 22.7 percent.

Table 2.2 The main producing areas in Iran are:

Province	Producing percent
Hormozgan	21.6 %
Khusistan	17.6 %
Bushehr	13.4 %
Beluchistan	12.2 %
Fars	12.3 %
Kerman	21.1 %

(FAOSTAT, 2004)

The yield of dates depends on many conditions (Al-Shahib and Marshall, 2003). Some fruits ripen early in the season, whilst others are not mature until the end of the season (August/September), and the mature fruits can very widely in their sensory and chemical characteristics (Dowson, 1982). All edible varieties of date pass through four distinct stages of ripening and, the Iranian terms (Kimir, Khalal, Rutab and Tamr) are used to represent, respectively, the immature green, the mature full coloured, the soft brown and hard raisin-like stage of development (Al-Shahib and Marshall, 2003).

The dates could be consumed as fresh fruits at Khalal and Rutab stages (short shelf- life), or at Tamer stage (good storability). Besides the direct consumption of the fruit, the dates are used locally in many ways, including the production of date extract and syrup (El-Shaarawy *et al*., 1989; El-Nakhal *et al*., 1989; Sawaya *et al*., 1989; Sawaya *et al*., 1982).

About 400 different dates' cultivars grow in Iran (Anonymous, 2003), but among these only a few (58) have been investigated so far to some extent for their chemical composition (Table 2.3 & 2.4). In spite of this extensive exploitation of the dates, little information has been published about the chemical composition of the fruit. (Rouhani and Bassiri, 1976).

Table 2.3 Number of date varieties described per country

Country	Number of varieties described	Author/Reference
Egypt	26	Brown, 1924
Egypt and Sudan	22	Mason, 1925
Iran	400	FAO, 1996
Iraq	370	Dowson, 1923
Morocco	244	Saaidi, 1974
Tunisia	250	Kearney, 1906
USA	196	Nixon, 1950

(FAOSTAT, 2004)

Table 2.4 Some variety of Iranian dates.

Variety	Type	Harvest time	Color	Moisture	Growing region
Honey	Soft	August	Reddish brown	18-22 %	Semnan Province
Bam	Soft	August September	Dark brown to black	16-20 %	Kerman Province
Jiroft	Soft	August	Reddish brown	18-22 %	Kerman Province
Kabkab	Soft	Mid September	Black-brown	14-18 %	Fars Province
Sahroon	Semi-dry	October	Dark brown	10-14 %	Hormozgan Province
Piarom	Semi-dry	October	Black brown	11-15 %	Hormozgan Province
Zahedi	Semi-dry	October	Yellow	10-14 %	Fars and Khuzestan Province
Kharak	Dry	November	Golden	7-9 %	Fars Province

(www.sahravi.com)

2.1.4 Stages of date ripening

Dates ripen in four stages, which are known throughout the world by their Arabic names *kimri* (unripe), *khalal* (full-size, crunchy), *rutab* (ripe, soft), *tamr* (ripe, sun-dried). At the kimri stage there is a rapid increase in size, weight, and reducing sugars; it is the period of highest acid activity and moisture content (up to 85 %). All factors level off at the end of this stage when the fruit starts to turn yellow (or red according to variety). At this point the date seed could already germinate and the fruit is botanically mature. At the khalaal stage weight gain is slow but sucrose content increases, moisture content goes down, and tannins will start to precipitate and lose their astringency. In some varieties this latter process evolves rapidly, which make them already palatable at the khalaal stage and one could speak of commercial maturity for this type of fruit at this stage (Al-Shahib and Marshall, 2003). With the tips of the fruit starting to turn brown, the rutab stage sets in which is characterized by a decrease in weight due to moisture loss, a partial (the degree depending on the variety) inversion of sucrose into invert sugar and a browning of the skin and softening of the tissues. The moisture content goes down to about 35 % and the dates at this stage are sold as fresh fruit (Table 2.5).

Table 2.5 Water content of a date fruit during its maturation from Khalal to Tamar stage.

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

(FAOSTAT, 2004)

Only when the dates are left to ripen further on the palm will they turn into tamr, climatic conditions permitting, characterized by a moisture content at which the date is self-preserving. The upper limit for the date to be self-preserving lies at around 24-25 % (Al-Hooti *et al*., 1997)

2.1.5 Storage of date

In the season of tamr harvest, some industries receive the fruits in amounts that far exceed the markets' immediate capacity. Thus, most tamr dates are stored and then released into the market according to demand. Date industries usually store tamr at −3 °C up to a year. After packing and releasing to the market, the fruits are expected to have a shelf life of up to 2 years at room temperature (~25 °C). Since quality parameters are affected by storage, it is very important to understand the effect of such storage conditions on the different characteristics and consumers' acceptability of the date fruit.

The literature contains plenty of work on the effect of storage on different fruit and vegetable attributes (Kevers *et al*., 2007; Tavarini et al., 2008; Leja *et al*., 2003). Unfortunately, few if any of those studies and reports dealt with the effect of storage conditions on date quality parameters. Thus, research is needed to investigate the effect of current methods of storage on different date quality and physical attributes, and ultimately devise ideal conditions of storage suitable for the fruit. The date fruit has received disproportionately little attention, despite its importance in Iran and many other countries and other regions.

2.1.6 Chemical composition of date fruit

Dates are considered a complete diet and a very important item of food. With plenty of vitamins and minerals, date have 25 % more potassium than bananas while being free of fats, sodium, and cholesterol. Dates play an important role in the diet and treatment of obesity and are the most important source of energy and food in date producing areas. Dates distinguish themselves therefore from most other fruit in that they have a botanical maturity and at least 3 distinct commercial maturation levels, the sweet khalaal, the rutab, and the tamr stage. According to variety and growth conditions date fruits (tamr) vary in shape, size and weight. Usually they are oblong though certain varieties may reach a near round shape. Length and width may vary from respectively 18 and 8 mm to 60 and 32 mm but averages at 40 and 20 mm. Average weights per fruit is about 7 to 10 grams.

Knowledge of the qualitative and quantitative chemical composition of date fruit is of high importance:

2.1.6.1 Moisture content

The date goes from one extreme of moisture content (85 % at the early kimri stage) to another (5-10 % in dry desert dates). In between there are several levels of importance, i.e. about 50-60 % for sweet khalaal, about 35-40 % for rutab, around 24 % for entering the zone of self-preservation, and about 20 % at which a large amount of dates are marketed because they are safe to store but have still retained a pliable and attractive texture. With the knowledge that moulds are unable to grow in an atmosphere below 70 % relative humidity (for yeasts and bacteria even higher),

while dates are in equilibrium with surrounding air, in the surrounding air with 70 % relative humidity, moisture content of date will be 24 %. Rutab at 35 % is well above this level and must be considered perishable. Moisture content can be artificially manipulated by drying (either in the sun or by dehydration) to remove, or by (vacuum) hydration and steaming, to add water. Apart from microbial attack increasing moisture content also tends to increase biochemical processes in the dates such as darkening and softening. Increasing moisture content tends to decrease relative sweetness and bring out more strongly the specific date flavours, hence the preference, generally speaking, for softer and rutab dates.

According to Sahari *et al*., (2007), because of the lack of information in the literature on the composition of the majority of Iranian date cultivars, an attempt was made to characterise 34 major date cultivars collected from all date-growing regions in Iran. The date varieties were Mazafati (1), Kabkab (2), Zahedi (3), Estamaran (4), Shahani (5), Kaluteh (6), Zark (7), Khanizi (8), Khasooi (9), Halilei (10), Gasab (11), Ale-Mehtari (12), Holuo (13), Shahabi (14), Gantar (15), Piarom (16), Croot (17), Barhi (18), Khazravi (19), Lasht (20), Abdollahi (21), Khorst (22), Bezmani (23), Haftad-Gazi (24), Halavi (25), Maktoom (26), Deiri (27), Shah-Mohammadi (28), Khalass (29), Moslehi (30), Kharouzard (31), Gach- Khah (32), Tourz (33) and Kang-Gard (34).

Moisture was the main value in the chemical composition and its range of variation was 19.5–39.2 g/100 g, when taking all samples into account. Differences in variety, period of maturity stage and environmental conditions are exerting significant influence on chemical composition of date (Sahari *et al.*, 2007).

2.1.6.2 Sugars

Sugars contribute the most prevalent single component and in the ancient date production countries the date has been used more as a sugar source than as a fruit. All sugars in dates consist of a mixture of sucrose (C_{12} H_{22} 0_{11}), glucose (C_6 H_{12} 0_6) and fructose (C_6 H_{12} 0_6) of which the latter two are the derivations of sucrose after inversion. The relative amounts of sucrose, glucose and fructose are determined mainly by varietal characteristics, but it can be said that most dates belong to the invert sugar type, i.e. at the stage at which they are consumed, most if not all sucrose has been inverted into glucose and fructose by the enzyme invertase.

The practical and commercial sub-division of dates in "soft", "semi-dry", and "dry", based on their external qualities of texture and pliability at the tamr stage, is seemingly correlated to increasing sucrose content, but not always, with the Deglet Noor and to a certain extent the Zahdi as exceptions, the latter one also sometimes being classified as a dry date. Moisture and fibre content are expected also to play a role in determining whether a date is "soft", "semi-dry" or "dry".

Sahari *et al*., (2007) showed glucose was the main sugar in dates and its content ranged from 16.41 to 54.23 g/100 g (fresh weight basis). Fructose content range of variation was 12.62–43.31 g/ 100 g (fresh weight basis). Sucrose content in all varieties, excepting of Zark variety, were not significant; but in all varieties, statistical differences were obtained in fructose, glucose and Glu/Fru ratio. The decrease observed in the sucrose content in the Tamr stage with a simultaneous increase in the reducing sugars is attributed to the rising activity of the splitting enzyme invertase resulting from a loss of the integrity of membrane system during softening (Sahari *et al*., 2007).

This enzyme hydrolyses sucrose into glucose and fructose to completion or near completion in soft date cultivars, but the hydrolysis is only partially achieved in the semi-dry/dry date cultivars (Sawaya *et al*., 1983).

Though the ratio glucose and fructose originates from a one-to-one basis, this ratio may change during ripening, usually glucose remaining the prominent one. For practical purposes the relative percentages of sucrose, glucose and fructose are of no great importance to the consumer, though availability of glucose and fructose is sometimes propagandized as a more direct, accessible energy source to the human body.

The ratio of Glu/Fru range of variation was 1.03–1.88. Glucose and fructose were the predominant monomers of the reducing sugars as no evidence of any other sugar monomer even in trace amounts other at all varieties was found. The ratio of Glu/Fru in dates can be of considerable interest because fructose is about twice as sweet as glucose and is much less diabetogenic than glucose. It seems that differences in variety, environmental conditions and fructose content are exerting significant influence on sugar content and this ratio of date (Sawaya *et al*., 1983; Chatha *et al*., 1989; Youssif *et al*., 1989; Ahmed *et al*., 1995; Aidoo *et al*., 1996; Besbes *et al*., 2004).

2.1.6.3 Proteins and Fats

Proteins and Fats occur in small amounts in the date flesh. Fat is mainly concentrated in the skin (2.5-7.5 %) and has a more physiological importance in the

protection of the fruit than contributing to the nutritional value of the date flesh (0.1-0.4 %). Palmitic, capric and caprylic acid were identified as the major free fatty acids in the date flesh followed by linoleic, lauric, pelargonic, myristic acid and a number of others (Kikuchi and MikiI, 1974). Date pits contain a certain percentage of oil. Proteins occur in date fruit in the range of 1-3 % and though their amino acid pattern is favourable to human needs, the amounts are too small to be considered an important nutritional source. When extracting dates for sugar, proteins may create turbidity in the juice and have to be removed. Proteins also play a role in non-oxidative browning (Maillard reaction) and in the precipitation of tannins during ripening. Based on report of Sahari *et al*. (2007) from 34 Iranian date fruits, protein content ranged from 1.6 to 5.0 g/100 g (fresh weight basis) and lipids varied from 0.22 to 0.62 g/100 g (fresh weight basis). Those differences might be attributed to the variability of the studied cultivars and seasonal variation and also storage differences.

2.1.6.4 Crude fibres (non-soluble solids)

Crude fibres are usually connoted with the insoluble, non-nutritive portion of the date flesh, and mainly composed of cellulose, hemicellulose, lignins and ligno-cellulose, and insoluble proteins. During the ripening process these substances are gradually broken down by enzymes to more soluble compounds to render the fruit more tender and soft. In commercially ripe dates crude fibre amounts to 2-6 % of the date flesh, but in low quality dates for industrial purposes this percentage will be up to 10 %. In one particular determination, date flesh was found to contain (on fresh

weight basis) 1.55 % cellulose, 1.28 % hemicellulose and 2.01 % lignin (relatively high and perhaps caused by the lignin content in the skin) (Lund *et al*., 1983).

Elleuch *et al*. (2008) chose two date palm cultivars: Deglet-Nour and Allig from Tunisia and characterized chemical composition and the dietary fibre (DF). These values (on a dry matter basis: DM) were obtained respectively: sucrose 52.7 % and 13.9 %, glucose 13.7 % and 29.9 %, fructose 12.6 % and 29.0 %, total dietary fibre 14.4 % and 18.4 %, protein 2.1 % and 3 %, ash 2.5 % and 2.52 %. Insoluble DF, the major fraction of total dietary fiber (DF), constituted 9.19–11.7 % DM for Deglet-Nour and Allig, respectively. The chemical composition of these DF concentrates showed high total DF contents (between 88 % and 92.4 % DM) and low protein and ash contents (8.98–9.12 % and 2.0–2.1 % DM, respectively). The DF concentrates showed a high water-holding capacity (15.5 g water/g sample) and oil-holding capacity (9.7 g oil/g sample) (Elleuch *et al*., 2008).

2.1.6.5 Vitamins and Minerals

Of the many data found in literature related to vitamins and minerals, mainly of importance for nutritional purposes, a main conclusion emerges that dates at the stage of maturation in which they are normally and mostly consumed. Date fruit contains vitamins A, C, B_1, B_2 and niacin in reasonable amounts (Table 2.6). Date is also a good source of sodium, potassium, magnesium, calcium and iron. A fair amount of chlorine, copper, sulphur and phosphorus exist in date fruit.

Table 2.6 Vitamin Content of Date:

Vitamin	% 100 / gm
Vitamin A	4.8 - 6
Vitimain C	0.77 - 2.7
Vitamin B1	0.07 - 0.1
Vitamin B2	0.03 - 0.05
Vitamin B3	0.33 - 2.2

(FAOSTAT, 2004)

Sahari *et al*., (2007) measured mineral elements such as Na, Mg, K and Ca by ICP atomic spectroscopy in 34 major date cultivars collected from all date-growing regions in Iran and their amounts were in the range of 4.46–47.74, 18.44–79.35, 203.61–982.97 and 23.24–73.85 mg/100 g (dry weight basis), respectively. Sodium content was lower than other results reported in Pakistan, United Arab Emirates, USA, China, Oman and Tunisia (Chatha *et al*., 1989; Youssif *et al*., 1989; Ahmed *et al*., 1995; Aidoo *et al*., 1996; Myhara *et al*., 1998; Besbes *et al*., 2004). Variations could originate from the date variety, and agro-climatic as well as environmental condition.

2.1.6.6 Enzymes

Enzymes play an important role in the conversion processes that takes place during formation and maturation of the date fruit, and the activities of four of them are of particular interest to final product quality:

i. Invertase: responsible for the inversion of sucrose into glucose and fructose and related to texture and pliability.

ii. Polygalacturonase and pectinesterase: both convert insoluble pectic substances into more soluble pectins, contributing to softness of the fruit.

iii. Cellulase: breaks down cellulose into shorter chain substances with increasing solubility and eventually leading to glucose, thus decreasing fibre content.

iv. Polyphenol oxidase is responsible for biochemical changes of polyphenols to which the tannins belong; they are important in non-oxidative browning reactions of the date.

Knowledge of the functions and activity of these enzymes is important because by proper manipulation of heat and humidity the enzyme activity can be stimulated or depressed according to the desired result. Enzyme activity normally takes place in solution or moist atmosphere; the optimum temperature range usually falls between 30 and 40°C, over and below which the activity will decrease (for instance invertase at 50°C, loses 50 % activity and 90 % at 65°C after 10 minutes). Prolonged storage of dates under refrigeration or freezing is based mainly on the slowing down of enzyme activity.

Khali and Selselet-Attou (2007) studied on the effect of heat treatment (55°C/20 min) on polyphenol oxidase (PPO) and peroxidase (POD) activities and total phenolic compounds in Algerian dates (Deglet Nour variety) at Tamar (fully ripe) stage and in dates stored for 5 months at ambient temperature and in cold storage (10°C). Deglet Nour dates showed that there was a decrease in both POD and PPO activities during storage for either heat treated and non-treated dates samples.