

# Quality Characteristics and Oxidative Stability of Date Seed Oil During Storage

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Studies were conducted on some quality characteristics of date seed oil from two date palm cultivars (*Phoenix dactylifera L.*): Deglet Nour and Allig. The following values were obtained for Deglet Nour and Allig cultivars respectively: unsaponifiable matter 8.92–7.76%, iodine value 45.49–44.08, total phenols 520.81–220.32 µg/g oil, chlorophylls 0.86–0.58 µg/g oil, viscosity 18.50–20.50 mPa.s, refractive index 1.457–1.462, acidity 1.06–2.10 mg KOH/g oil and peroxide index 16.00–25.00 meq O<sub>2</sub>/100 g oil. Lipid fractionation of date seed oil yielded 98.30–97.30% neutral lipids, 97.26–96.90% triglycerides, 0.25–0.18% phospholipids and 0.36–0.31% sterols. Gas-liquid chromatography revealed that the major unsaturated fatty acid was oleic acid (39.17–42.13%) while the main saturated fatty acid was lauric acid (24.34–22.56%). Date seed oil showed high medium molecular weight triglycerides (C32–C42, about 27%). Extinction coefficients at 232 nm and 270 nm increased slightly during 40 days in oven (60°C) showing that date seed oil could be easily stored. The production of oil from date seeds provides the use of a renewable resource, and at the same time adding value to agricultural products.

*Key Words:* date seeds, oil, quality, oxidation, storage

## INTRODUCTION

Date seeds (*Phoenix dactylifera L.*) may have extractable high-value-added components. However very little use has been made of these pits, rather, they are discarded or used in animal feed. Few works have been undertaken on date palm seeds, focused particularly in their chemical composition (El-Shurafa et al., 1982; Al-Showiman, 1990; Devshony et al. 1992; Al-Hooti et al., 1998; Hamada et al., 2002). Pits of the date palm are a waste product of many industries based on the technological transformation of the date fruits or on their biological transformation (Besbes et al., 2004). Then, a large quantity of date seeds could be easily collected from the date processing industries or from the

waste products (representing nearly 30% of the production in Tunisia) coming either directly from the palm grove or from the gap-conditioning stations. Date pits, a low-cost agricultural by-product, was a good precursor of the production of activated carbons which are the most popular used adsorbents (Girgis and El-Hendawy, 2002; Banat et al., 2003). Some minor molecules, such as a water-soluble galactomannan and an alkali-soluble heteroxylan were extracted from date seeds and then characterised (Ishrud et al., 2001; Ishrud et al., 2003).

It is well known that the average weight of date pits is about 10–15% of date weight (Almana and Mahmoud, 1994; Hussein et al., 1998). In Tunisia, lipid fraction of date seed could amount to over 1000 tons annually, assuming a mean proportion of 10% of seed in date fruit and 10% oil content of seeds. In a previous paper we reported that the oxidative stability of date seed oils, measured by Rancimat, was higher than that of most vegetable oils and comparable to that of olive oil; they can also protect against UV light responsible for most cellular damage (Besbes et al., 2004).

The aim of this work was to determine some quality characteristics of date seed oils and to study the effect

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in their oxidation of storage in accelerated conditions (60°C) to evaluate the potential use of date seeds as a new source of lipids for human uses.

## MATERIALS AND METHODS

### Samples

The seeds of the two cultivars: Deglet Nour and Allig were directly sampled from 50 kg of waste date fruit having the same origin (National institute of Arid Zone Degach, Tunisia) and collected at *Tamr* stage (full ripeness). The seeds were soaked in water, washed to free them of any adhering date flesh, air-dried and then dried (12 h) at about 50°C. Date pits, from each variety, were separately milled in a heavy-duty grinder to pass 1–2 mm screens and then preserved at –20°C until analyses.

### Methods

#### *Lipid Extraction and Preservation*

Lipid extraction was carried out with an SER 148 solvent extractor (Velp Scientifica, Europe) equipped with 6 Soxhlet posts. About 15 g of powdered date seeds were used for each oil extraction with petroleum ether (40–60°C, Merck). The operational conditions were (Besbes *et al.*, 2004): 30 min immersion time with thimble immersed in boiling solvent. Washing time: 60 min of reflux washing.

The obtained seed oils were drained under a stream of nitrogen and then stored in a freezer (–20°C) for subsequent analyses.

#### *Physico-Chemical Analyses*

AOCS official methods (AOCS, 1997) were used for the determination of the unsaponifiable matter (Method Ca 6b-53), acidity (method Cd 3d-63), iodine value (method Cd 1–25) and peroxide value (method Cd 8–53).

The refractive index was determined using an Abbe refractometer (Bellingham & Stanley Ltd, United Kingdom) at 20°C.

K232 and K270 extinction coefficients were calculated from absorption at 232 nm and 270 nm respectively, with a UV spectrophotometer (Hewlett-Packard, HP 8452 A) using a 1% solution of oil in cyclohexane and path length of 1 cm (AOAC, 1997).

Total phenols, expressed as caffeic acid ( $\mu\text{g/g}$  of oil) were determined at 725 nm using Folin-Ciocalteu reagent as described by Salvador *et al.* (2003).

Chlorophyll content was determined according to Cc 13d-55 method (AOAC, 1997).

Viscosity was followed at 25°C with stress Tech

Rheologica Rheometer (Rheologica Instruments AB, Lund, Sweden) conducted with a steel cone-plate (C 40/4) under a constant shear rate of  $100\text{s}^{-1}$ .

Lipid class separation was done according to Vaghela and Kilara (1995) with few modifications. Separation was performed using SPE cartridges, amino-propylsilica ( $\text{NH}_2$ ) and octadecylsilica (C18) (Mega Bond Elut) activated before use by rinsing with 20 mL hexane, preventing the column from drying out. The oil sample was diluted in 2 mL of chloroform and then placed onto the column. Lipid classes were recovered by sequential elution with 18 mL each of  $\text{CHCl}_3$  / Iso-propanol (2:1),  $\text{Et}_2\text{O}$  / acetic acid (98:2), and MeOH to separate neutral lipids, free fatty acids and phospholipids, respectively. Neutral lipids classes were then recovered by sequential elution with 12 mL hexane, 36 mL hexane/ $\text{MeCl}_2$  /  $\text{Et}_2\text{O}$  (89:10:1), 36 mL hexane/ethyl acetate (95:5), 36 mL hexane/ethyl acetate (85:15), 18 mL  $\text{CHCl}_3$  / MeOH (2:1) to separate steryl esters, triglycerides, sterols, diglycerides and monoglycerides, respectively. The different lipid classes were evaporated at 40 °C under reduced pressure and then classes content were expressed as percentage of total lipids. Sample were analysed in triplicate by reusing the same SPE column. Before reuse, the column was rinsed with 20 mL of acetone and then dried by passing nitrogen through it.

Fatty acids composition was determined using Hewlett-Packard 5890 Series II gas chromatograph (HP co., Amsterdam, the Netherlands) as described in a previous paper (Besbes *et al.*, 2004).

Triglycerides groups were separated by gas chromatography according to Ce 5–86 method approved by the Association of Official Analytical Chemists (AOAC, 1997). The triglycerides analyses were performed on Hewlett-Packard 5890 gas chromatograph (HP co., Amsterdam, the Netherlands) equipped with flame ionisation detector (FID) and a fused silicated column (65% phenyl, 35% methylpolysiloxane, 25 m  $\times$  0.25 mm  $\times$  0.10  $\mu\text{m}$  film thickness). The oven temperature was programmed from 220°C to 350°C at 4°C/min and the injector and the detector temperatures were set at 350°C and 380°C respectively. Nitrogen was the carrier gas (1 bar). The peak areas of duplicate injections were measured with an HP computing integrator. Values were the average of two determinations.

#### *Oven Test*

Twenty samples (20 g) of each date seed oil variety were poured into separate 50 mL open beakers (4 cm diameter, 6 cm height) without stirring, and then kept in the dark in an oven at  $60 \pm 1^\circ\text{C}$ . The temperature of 60°C is used as a rapid method to simulate the storage in real conditions (Nissiotis and Tasioula-Margari, 2002). One sample from each variety was randomly

removed from the oven at a predetermined time, cooled at room temperature (15 min) and then analysed for specific extinctions at 232 nm and 270 nm and chlorophylls content.

## RESULTS AND DISCUSSION

### Date Seed Oil's Quality Indices

The relative percentage weight of lipids compared with weight of dried pits was about 10.19% for Deglet Nour cultivar and about 12.67% for Allig cultivar (Besbes et al., 2004). The physico-chemical quality parameters (Table 1) were generally similar to those cited by Hamada et al. (2002) and El-Shurafa et al. (1982), but higher than those reported by Devshony et al. (1992).

Deglet Nour seed oil contained slightly more unsaponifiable matter and chlorophylls than Allig seed oil (0.89% against 0.77% and 0.86 µg/100g against 0.58 µg/100g, respectively). The oil obtained from Deglet Nour seeds contained near 60% more total phenols than that obtained from Allig seeds (520.8 mg/kg against 220.3 mg/kg). This seemed to be the principal cause of the higher oxidative stability reported in a recent study (44 h against 33 h determined by Rancimat) of Deglet Nour seed oil (Besbes et al., 2004). A high correlation was observed between total polyphenol content and oxidative stability of olive oil (Papadopoulus and Boskow, 1991; Montedoro et al., 1993; Caponio et al., 1999; Salvador *et al.*, 2001). It is worth noting that date seed oil has a higher phenol content compared to most edible oil except olive oil which was particularly considered as a source of phenolic compounds in Mediterranean diet. It has been reported that the phenol compounds content in olive

oil varies from 100 mg/kg to 1000 mg/kg (Tuck and Hayball, 2002). This may explain that date seed oil presented comparable oxidation induction times (~33–44 h) to virgin olive oil (Besbes et al., 2004).

Date seed oils had a low refractive index indicating that they may contain fatty acids with medium–short hydrocarbon chains. Viscosity of date seed oil is one of the lowest among most vegetable oils but similar to that of oleic acid and raspberry seed oil (Oomah et al., 2000). This also suggested that date seed oil had a high medium–short chain fatty acids and triglycerides contents. In fact, a trend in the relationship between fatty acids chain length and viscosity was observed (Geller and Goodrum, 2000; Gustone et al., 1986).

The acidity and free fatty acids (FFA) were similar to those reported by Devshony et al. (1992) and El-Shurafa et al. (1982). FFA contents of Deglet Nour seed oil were similar to that of extra-virgin olive oil for which the upper threshold limit is 1% free acidity. The low free-FFA content of date seed oil indicated that it could be edible. Allig seed oil had higher acidity, peroxide value and  $K_{232}$ ,  $K_{270}$  extinctions coefficients compared to Deglet Nour seed oil. This may be due to the higher stability of Deglet Nour seed oil during the extraction procedure.

Iodine value ranged from ~44.1 for Deglet Nour to ~45.5 for Allig. These values were within ranges with those for date palm pits (Devshony et al., 1992; El-Shurafa et al., 1982), but lower than those for olive oil, almond oil, sesame oil, soya oil and sunflower oil (Pearson, 1972). This may be due to the fact that date seed oil contained less polyunsaturated fatty acids (PUFA).

### Date Seed Oil's Lipid Classes

Edible fats and oils are a complex mixture of triglycerides (TG: 90–95%), diglycerides (DG: 1–2%), free fatty acids (FFA: ~0.5%), phospholipids (PL) and other minor components (~2.5% in the case of oil and 10% in the case of animal fats; Ruiz-Gutiérrez and Pérez-Camino, 2000).

Like all vegetable oils, triglycerides fraction of date seed oil represented the major part of the lipid fraction (97.26% for Deglet Nour and 96.90% for Allig, Table 2). These proportions were slightly lower to those of olive oil (98.5%–99%).

Sterol fraction was considered as the major unsaponifiable fraction in many oils. A high level of sterols were determined in both date seed oils, which made up about 0.35 g /100g Deglet Nour seed oil and about 0.30 g/100g in Allig seed oil. Sterols are related to the quality of oils, and the quantification of individual sterol components is fundamental to check authenticity (Salvador et al., 2001; Lercker and Rodriguez-Estrada, 2000). Authenticity of oil mean sterols content in date seed oils was higher compared

**Table 1.** Quality indices of date seed oils.

Quality Indices <sup>a</sup>	Cultivars	
	Deglet Nour	Allig
(Mean ± SD, n = 3)		
Unsaponifiable matter (g/kg)	8.92 ± 0.39	7.76 ± 0.34
Chlorophylls (µg/g)	0.86 ± 0.05	0.58 ± 0.07
Total phenols (µg/g)	520.8 ± 10.2	220.3 ± 6.3
Refractive index (at 20 °C)	1.457 ± 0.001	1.462 ± 0.001
Viscosity (mPa.s)	18.50 ± 0.50	20.50 ± 0.30
Acid value (mg KOH/g oil)	1.06 ± 0.06	2.10 ± 0.14
Free fatty acids (% oleic acid)	0.53 ± 0.03	1.05 ± 0.07
Peroxyde value (meq O <sub>2</sub> /100g)	16.0 ± 0.5	25.0 ± 0.4
$K_{232}^a$	0.54 ± 0.01	0.90 ± 0.01
$K_{270}^a$	0.66 ± 0.01	0.82 ± 0.02
Iodine value (g/100g)	45.5 ± 0.2	44.1 ± 0.3

<sup>a</sup> $K_{232}$  and  $K_{270}$ : specific extinction coefficients at 232 nm and 270 nm respectively.

**Table 2.** Lipid classes of date seed oils.

Lipid Classes	Cultivars	
	Deglet Nour	Allig
	(g/100 g Oil, Mean $\pm$ SD, $n = 3$ )	
Neutral lipids	98.30 $\pm$ 0.54	97.40 $\pm$ 0.88
Triglycerides	97.26 $\pm$ 0.49	96.90 $\pm$ 0.50
Diglycerides	0.23 $\pm$ 0.07	0.20 $\pm$ 0.05
Monoglycerides	0.09 $\pm$ 0.01	0.13 $\pm$ 0.03
Free fatty acids	0.33 $\pm$ 0.07	0.55 $\pm$ 0.05
Phospholipids	0.25 $\pm$ 0.05	0.18 $\pm$ 0.02
Sterols	0.36 $\pm$ 0.04	0.31 $\pm$ 0.08

to extra virgin olive oil ( $\sim 0.15$ g/100g, Salvador et al., 2001). Both date seed oils studied have a good freshness state regarding their low content in free fatty acids, monoglycerids and diglycerids. Deglet Nour seed oil showed a slightly lower FFA (0.33g/100g oil against 0.55g/100g oil, respectively). This confirmed the results obtained previously by official methods. Fractionation of lipid classes by solid fat extraction (SPE) could be very important to study functional properties of each date seed oil fraction such as emulsifying properties of phospholipids and monoglycerids.

#### Fatty Acid Composition

The major fatty acid found in both cultivars was oleic acid, ranging from 39.17% for Deglet Nour seed oil to 42.13% for Allig seed oil (Table 3). This was in agreement with what was reported in literature (Al-Showiman, 1990; Devshony et al., 1992; Al-Hooti et al., 1998). However, Al-Hooti et al. (1998) found a higher content of oleic acid (53.3–58.8%) in date seed oil from other origins. Fatty acid composition of date seed oil seemed to vary slightly with cultivars.

Deglet Nour and Allig seeds oil may be regarded as an oleic-lauric oil because oleic acid was the most abundant followed by lauric acid. Al-Hooti et al. (1998) also reported that date seed oils were oleic and linoleic, or oleic and palmitic. In a previous study Deglet Nour seed oil was regarded as an oleic-lauric oil, while Allig seed oil was an oleic-linoleic oil. The few differences between our previous results and the present ones, for Allig seed oil, may be explained by differences in climatic conditions from year to year. In general, fatty acid composition of date seed oils may be affected by differences between varieties, cultural conditions and climatic conditions (Al-Shahib and Marshall, 2003).

It is worth noting that the degree of unsaturation of these date seed oils was lower than that of common seed oils, since date seed fat had much lower linoleic acid content. In spite of this low level of unsaturation, date seed oil may present an interesting potential for different uses. In fact, Al-Shahib and Marshall (2003)

**Table 3.** Fatty acid composition of date seed oils.

Fatty Acid <sup>a</sup>	Cultivars	
	Deglet Nour	Allig
	(g / 100g Total Fatty Acids, Mean $\pm$ SD, $n = 3$ )	
Capric C10	0.68 $\pm$ 0.04	0.44 $\pm$ 0.01
Lauric C12	24.34 $\pm$ 1.06	22.56 $\pm$ 0.36
Myristic C14	11.17 $\pm$ 0.70	10.33 $\pm$ 0.04
Myristoleic C14	–	0.33 $\pm$ 0.09
Palmitic C16	8.47 $\pm$ 0.29	8.30 $\pm$ 0.08
Palmitoleic C16	0.18 $\pm$ 0.08	0.12 $\pm$ 0.02
Stearic C18	2.57 $\pm$ 0.25	2.65 $\pm$ 0.06
Oleic C18	39.17 $\pm$ 0.62	42.13 $\pm$ 0.91
Linoleic C18	11.99 $\pm$ 0.44	11.66 $\pm$ 0.07
Linolenic C18	1.39 $\pm$ 0.88	1.01 $\pm$ 0.30
SAFA	47.23 $\pm$ 0.96	44.29 $\pm$ 0.66
MUFA	39.34 $\pm$ 1.10	42.58 $\pm$ 1.15
PUFA	13.39 $\pm$ 1.62	12.67 $\pm$ 0.68

<sup>a</sup> SAFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acid.

suggested that date seeds would be a useful source of edible oils for human consumption or for medicinal use.

#### Triglyceride Composition

The most abundant triglycerides fraction was C48 followed in descending order by C42, C44, C46, C50, C52, C54, C38 and C40 (Table 4). Both date seed oils presented a high content of medium molecular weight triglycerides (C32–C42) which ranged from 26.46% to 27.24% for Deglet Nour and Allig, respectively. Palm oil and soya oil were rather constituted by high molecular weight triglycerides (100% C46–C54 for palm oil and 100% C50–C56 for soya oil) (Collomb et al., 1998).

The presence of medium molecular weight triglycerides in date seeds oils reflect the presence of medium and short chain fatty acids (especially lauric acid)

**Table 4.** Triglycerides composition of date seed oils.

Triglycerides	Cultivars	
	Deglet Nour	Allig
	(g/100g total fatty acids, mean $\pm$ SD, $n = 2$ )	
Total C38	4.87	4.94
Total C40	2.60	2.80
Total C42	18.99	19.50
Total C44	15.83	16.48
Total C46	14.88	15.59
Total C48	22.68	21.13
Total C50	8.94	8.87
Total C52	5.46	5.35
Total C54	4.87	4.01

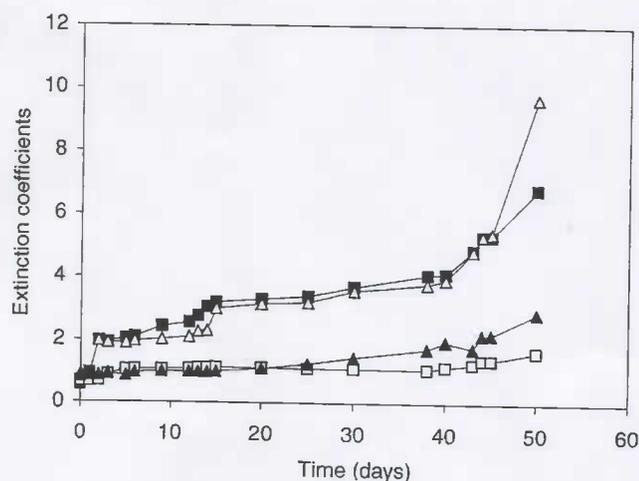
which were responsible for the low viscosity of date seed oil.

Regarding differences between triglyceride composition of oils, triglyceride profile of date seed oil could also be used to check authenticity.

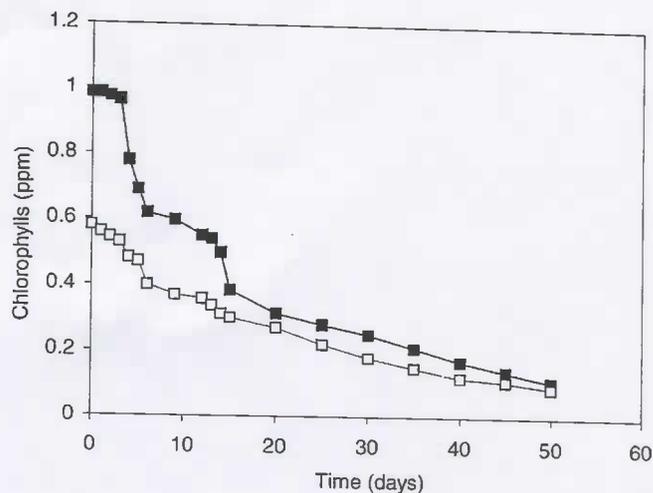
### Effect of Storage at 60°C

Quality indexes such as specific extinctions at 232 nm and 270 nm, and chlorophylls content were followed up in order to study the effect of storage in accelerated conditions (oven test at 60°C) on date seed oil. Figures 1 and 2 display the relationship between heating time at 60°C and specific extinctions and chlorophylls content.

The formation of primary compounds of oxidation such as hydroperoxides coincided with the increase of absorptivity at 232 nm. Formation of primary compounds of oxidation occurred initially at a lower rate. In fact, the extinction coefficient at 232 nm passed only from ~0.6 to ~3.8. This little increase suggested that date seed oils were resistant to oxidation. After only 6 days in the same conditions, absorptivity at 232 nm of canola oil reached about 10.26 (Vieira and Regitano-d'Arce, 2001). After 50 days in an oven, specific extinction at 232 nm became much higher for Allig seed oil. This may be explained by the fact that Deglet Nour seed oil was more resistant to oxidation. In fact, in a previous work on Allig seed oil presented a lower induction time (33 h against 44 h, Besbes et al., 2004). The primary products of oxidation are not stable under heating and then their degradation could promote the formation of secondary product of oxidation that absorb at about 270 nm (Vieira and Regitano-d'Arce,



**Figure 1.** Relationship between specific extinctions and heating time during thermal oxidation of date oil at 60°C. Deglet Nour: (■) 232 nm. (□) 270 nm. Allig: (△) 232 nm. (▲) 270 nm.



**Figure 2.** Relationship between chlorophylls content and heating time during thermal oxidation at 60°C. (■): Deglet Nour. (□) Allig.

2001). Specific extinction at 270 nm did not considerably change during 50 days in an oven (Figure 1). This also confirmed the resistance of date seed oil against the oxidative phenomenon. This resistance against oxidation of date seed oil may be explained firstly because date seed oil presented a low polyunsaturated fatty acid content, which are more sensitive to oxidation, and secondly by the presence of natural antioxidant such as phenolic compounds, sterols, tocopherols and chlorophylls (Besbes et al., 2004).

Despite differences in their initial chlorophyll content, Deglet Nour and Allig seed oil showed similar chlorophyll deterioration. Chlorophylls content of both date seed oils decreased considerably during storage at 60°C. This decrease could be due to thermal oxidation or to the destruction of these pigments. The study of the pro-oxidant or antioxidant activity of date seeds oil's chlorophylls would require the analysis of different type of pigments.

The results indicate the potential functional and economic utility of *Phoenix dactylifera L.* date seeds as a new source of oils. All these characteristics consolidate the possibility of date seed oil incorporating into food and cosmetics, and its pharmaceutical applications. Nevertheless, it is very important to support this study by integrating date seeds oils in some food and cosmetic formulations.

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