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Oil characteristics and fatty acid profile of seeds from three varieties of date palm (*Phoenix dactylifera*) cultivars in Bushehr-Iran

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Studies were conducted on properties of seed oils extracted from three date palm (*Phoenix dactylifera*) varieties Shahabi, Shekar and Kabkab, grown in Bushehr-Iran. The primary objective of this study was to extract seed-oil from the three native date varieties and then analyzed their fatty acids (FAs). Also the chemical parameters of PV, AV, *p*-anisidin, and Totox values of oils, as indicators of the quality and safety, and oxidative status of edible oils, as well as refractive index as an objective method for evaluation of rancidity were also evaluated. Date seeds are approximately 10% of the fruits weight. Seeds contained about 8.5% fat that can serve as a useful source of fatty acids to replace other vegetable oils. The following mean of values from the three varieties were obtained from samples: peroxide value (1.05), acid value (1.4), *p*-anisidine value (2.54), Totox (4.64) and refractive index (1.46). Also, flame ionization based detection gas chromatography (GC-FID) revealed that oleic acid was the primary fatty acid in all varieties. It was followed by lauric, myristic, palmitic, linoleic and stearic acids and trace amounts of the other fatty acids like undecylic, capric, nonanoic (pelargonic) and caprylic acids. Depending on the regional industry, these data suggest that the potential applications of date seed oil for humans and animals are feeding, cosmetic formulations such as in body creams, shaving soap and shampoos, and pharmaceutical products.

Key words: Seed oil (*Phoenix dactylifera*), fatty acid, gas chromatography.

INTRODUCTION

Dates are fruit of the date palm (*Phoenix dactylifera* L.), that are believed to have originated in North Africa or the Middle East around the Persian Gulf, and have been cultivated and used as food since ancient times from Mesopotamia to prehistoric Egypt, possibly as early as 6000 BC (Salem and Hegazi, 1971; Naturland, 2002; Sawaya et al., 1982; Abdel-Hafez et al., 1980; Anwar, 1987). From the viewpoint of botany, *Phoenix dactylifera* L. is haply derived from a Phoenician "Phoenix," which means date palm, and "dactylifera" from a Greek word "daktulos" meaning a finger (Zaid and De Wet, 2002; Barreveld, 1993). In the Bible; because of its high

nutritional value and long life, the date palm was afore mentioned as the "tree of life" (Naturland, 2002). It is one of the most important fruit trees grown mainly in all of the southern, and with less importance, in central regions of Iran (Zaid, 2002; Ahmed et al., 1995; FAOSTAT, 2004). Iran is one of the main date-producing countries in the world; it had an annual production of 900,000 metric tons and 127,000 tons exportation in 2006 (Amer, 1994; Anonymous, 2003; Botes and Zaid, 2002; Zaid, 2002; FAOSTAT, 2004) and 14% of the world's production (6,772,068 metric tons) in 2004, that was the second major producer after Egypt (17% of world production). In 1998, Iran was the world leader in date production with a quantity of 900,000 MT. Bushehr-Iran, one of the most majorities of production, is aimed at subsistence and world and local markets. The producing percent in this area of Iran was 13.4% after Hormozgan (21.6%) and

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Khuzestan (17.6%) provinces (Biglari, 2009; Zaid, 2002; FAOSTAT, 2004). Dates play an essential role in economy and the diet of the local inhabitants (Amer, 1994; Djazayery, 2002; Botes and Zaid, 2002).

There are many varieties of date palm cultivated and some important varieties among them which are popular for their high quality are 'kabkab', 'Shekar' and 'shahabi', therefore, they were included in the study. Dates are a good source of energy, vitamins, essential nutrients, most of the macro and micro-elements (Abdel Hafiz et al., 1980; Anwar, 1987; Salem and Hegazi, 1971; Sawaya et al., 1982; Sotolu et al., 2011) and at least 15 minerals (Ahmed et al., 1995). In addition to fruit, the other parts of the date palm are used for various purposes (Eckey, 1954; Shahidi, 2005; Ahmed et al., 1995). Each fruit has one seed, consisting of a hard endocarp covered by a thin epicarp, which is approximately 10 to 46% of the fruit (Al-Hooti et al., 1998; Sotolu et al., 2011). The seeds are often used as animal feed-stuff (Ahmed et al., 1995), propagation of date palms, and other industrial applications (Shahidi, 2005). The palm pit oil contains a certain percentage of pale yellowish-green oil with pleasant odor (Shahidi, 2005; Duke and Wain, 1981; Eckey, 1954). The seed oils are important common food ingredients. Fatty acids are saturated or unsaturated carboxylic acids with a long aliphatic tail and number of carbon atoms, from 4 to 28. They are primary nutritional components found in seed oils (Shahidi, 2005; Hargrove et al., 2001; Nicolosi, 2002; Yaqoob, 2002; Sawaya et al., 1984). The palm kernel oil is used for several applications like a liniment for indolent tumors (Hartwell, 1971; Graham et al., 2000), antimicrobial effects against some microorganisms like *Escherichia coli*, alpha and beta hemolytic *Streptococci*, *Aspergillus fumigatus* and *Staphylococcus aureus* (Ekpa and Ebana, 1996) and other applications and uses in cosmetics, pharmaceuticals and food products (Ahmed et al., 1995; Al - Hooti et al., 1998; Barmak, 2011; Ekwenye and Ijeomah, 2005; Shams et al., 2010). It is a rich source of some minerals, especially iron content, total sugars and crude fats (Al-Shahib and Marshall, 2003), large quantities of fiber, and possibly resistant starch (Hamada et al., 2002; Ekpa and Ebana, 1996; Ekwenye and Ijeomah, 2005).

The range of saturated and unsaturated fatty acid profiles of date seeds may vary among individual varieties (Al-Hooti et al., 1998; Walid, 2003). Fatty acids are determined as fatty acid methyl esters (FAMES) mainly by gas-liquid chromatography (GLC) with flame ionization detectors (FID) (Rahul et al., 2009). There are some date-packaging factories in Bushehr that discard their date seeds as waste or utilize them on a small scale as animal feed. Also there is no information in the literature on fatty acid profiles of date seeds in the cultivar chosen for this study. Therefore, the primary objective of this study was to extract seed-oil from the three native date varieties grown in Bushehr -Iran namely, Kabkab, Shekar and Shahabi, and analyzes their fatty acids (FAs);

because of the presence of oils in date seeds, it is suitable for rancidity and their reaction with oxygen produces toxic compounds. Therefore, the chemical parameters of PV, AV, *p*-anisidine and Totox values of oils as indicators and safety of the quality and oxidative status of edible oils were also evaluated. The peroxide value (PV) is a parameter specifying the content of oxygen as peroxide, especially hydro peroxides in a substance. The primary oxidation products are normally measured with peroxide value test (PV), and the secondary products with *p*-anisidine test. AnV and (2 × PV) can be combined into the Totox number. The acid value (AV), a common parameter in the specification of fats and oils, is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1 g of fat, and it is a measure of the free fatty acids (FFA) present in the fat or oils. In addition to the above, refractive index as an objective method for evaluation of rancidity in date seed oil was measured.

MATERIALS AND METHODS

All solvents, chemicals and standards for the determination of fatty acids were analytical grade and purchased from Merck (Germany) and Sigma (USA).

Sample preparation

The area covered for this study with possibility of date productions because of their geographical and climatic conditions, and the concentration of palm plantations were Dashtestan farms around Bushehr province in South of Iran. Approximately 10 kg of mature date were collected randomly and separately for each of the three varieties of Kabkab, Shahabi and Shekar from the mentioned local farm in Bushehr, at the beginning of the 2011 harvest season in September. There were three cultivars in each of the farms. The samples were transported to the toxicology laboratory, where a minimum quantity of 1 kg of seeds was extracted from each variety of mature date. Damaged seeds were discarded and air-dried after washing with distilled water to remove any adhering flesh. The cleaned seeds were crushed by mortar and then were ground to powder in a domestic grinder to pass a 2 mm screen. Samples were kept at 4°C in PE (Polyethylene) bags until analysis.

Extraction of seed oils

The oil content of date seed samples were prepared with Soxhlet apparatus according to American Oil and Chemical Society Official Method (1998) and the AOCS Ba 3-38 (1998) method. The oil was extracted from date seed samples using an n-hexane solvent extractor for both percolation and immersion under conditions of the test.

Fatty acid methyl ester (FAME) preparation and analysis

The evaluation of fatty acids in date seeds requires the preparation of fatty acid methyl esters (FAME) in order to improve volatility and to reduce peak tailing, and subsequent analysis by GC with good precision and reproducibility (Cert et al., 2000). There are several methods for preparing the FAME from fats and oils (ISO, 1998;

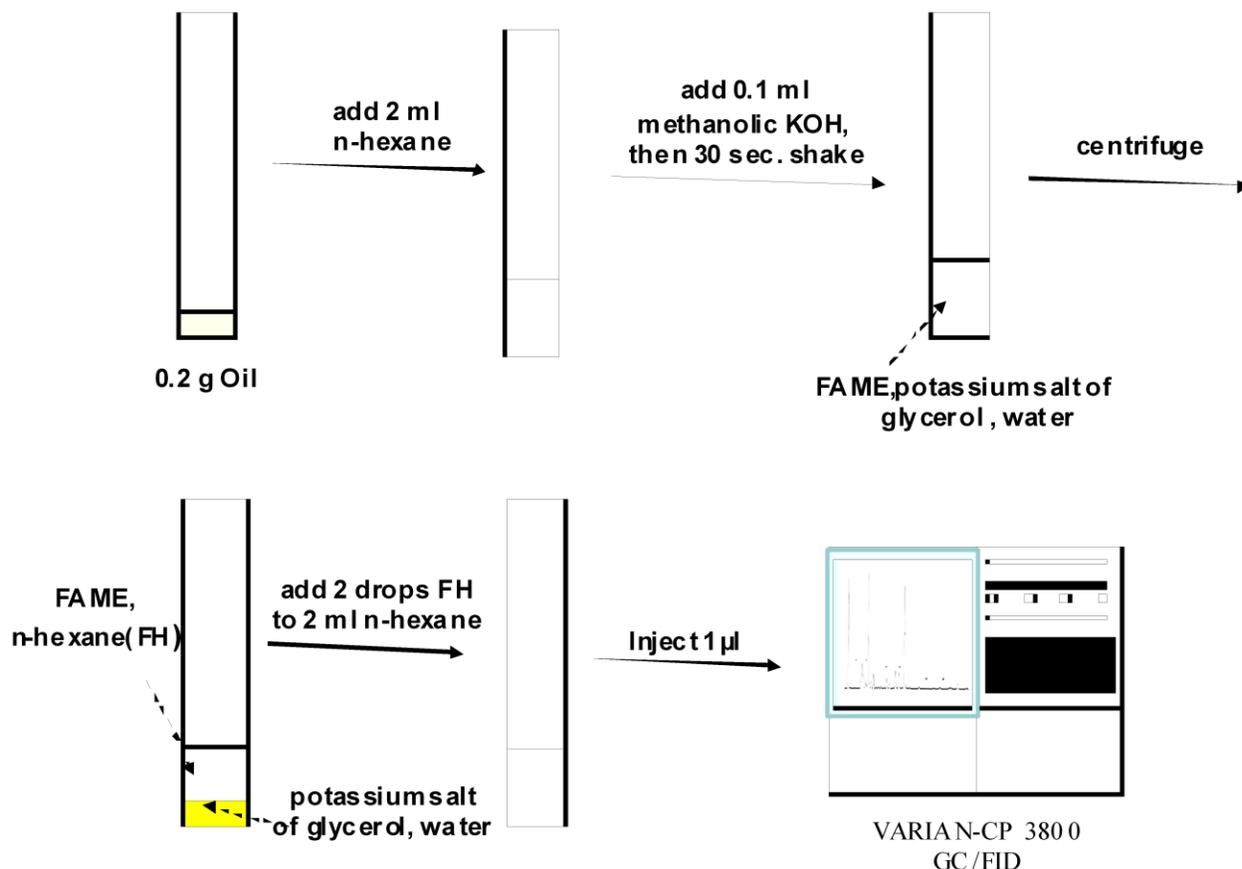


Figure 1. A schematic figure for the preparation of fatty acid methyl ester (FAME) according to AOCS method Ce 2-66.

Ayorinde et al., 1988; IUPAC, 1987; Christie, 1982; Morrison and Smith, 1964). In this study, the AOCS method Ce 2-66 (1997) was used for the preparation of FAME, which is shown as schematic in Figure 1. The FAME preparations were analyzed by GC-FID (Varian, CP- 3800) with a capillary column (FFAP, 30 m, 0.22 mm film thickness, 0.25 mm i.d.) with method of AOCS Ce 1e-91 (2001); helium was used as carrier gas. The injector and detector (FID) temperatures were maintained at 255 and 270°C, respectively and the temperature programming for the column was: 125°C (0.5 min), then to 150°C at 25°C/min (2 min), then to 200°C at 25°C/min (90 min). The Makeup, H₂, and air flows of FID detector were 25, 30 and 300 ml/min, respectively. The peaks were recognized, based on their retention times (RT) using standard FAMES. All samples were run in triplicate.

Some chemical and physical analysis of date oil seeds

The chemical parameters of acidity (AV), peroxide (PV), and *p*-anisidine (*p*-AnV) were determined according to the American Oil Chemist's Society method Cd3d-63 (1999), AOCS method Cd 8-53 (1996) and the AOCS method Cd 18-90 (1996), respectively in all mentioned varieties of date seed oils. To determine *p*-anisidine value, the absorbance value was measured at 350 nm using a spectrophotometer (CECIL CE 7250–7000 Series-Bio Aquarius, England). Totox number was calculated as follows: $Totox = 2 PV + p\text{-An V}$. Also, refractive indexes (RI or *n*) of date seeds oil were recorded with AOCS Cc 7-25 (2002) method, using a digital refractometer (Krüss AR₄, Series Abbe, Germany).

Statistical analysis

The mean percentages for each fatty acid in three varieties were compared using the Chi-square test. The experiments were conducted in triplicate. To determine of AV, PVs and *p*-anisidine of the different varieties of date seed oils, analysis of variance was carried out. The descriptive statistics (mean ± standard deviation) and one-way analysis of variance (ANOVA) were conducted using MINITAB statistical package (Minitab Inc., PA, USA).

RESULTS

A range of saturated and unsaturated fatty acids were presented in all varieties of date seeds. Oleic acid was the primary fatty acid in date seeds of all three varieties with concentrations of 37.6, 31.5, and 31.8% of total fatty acids, respectively. It was followed by lauric (25.6823, 30.5096 and 30.8162%), myristic (13.2767, 16.7650 and 16.9334%), palmitic (11.9011, 13.0804 and 13.1082%), linoleic (6.9320, 4.4148 and 4.4592%), stearic acid (2.3432, 1.8534 and 1.8720%) and trace amounts of the \ other fatty acids like undecylic, capric, nonanoic (pelargonic) and caprylic acids. Despite the difference in the amount of each fatty acid in all three varieties, results indicated that there is no significant relationship between

Table 1. Total fat (% w/w) and fatty acid composition of date seed oil (g/100 g of total fatty acid) of seeds from three varieties of date palm.

Variety	Total fat (%)	Fatty acid (%)									
		Caprylic	Pelargonic	Capric	Undecylic	Lauric	Myristic	Palmitic	Linoleic	Oleic	Stearic
Kabkab	8.5±0.11	0.375±0.001	0.7789±0.12	0.5008±0.007	0.464±0.1	25.6823±0.6	13.2767±0.12	11.9011±0.12	6.932±64	37.6095±0.09	2.3432±0.98
Shekar	8.1±0.05	0.3759±0.1	0.201±0.008	0.4953±0.094	0.1724±0.1	30.5096±1.04	16.765±0.7	13.0804±0.31	4.4148±0.08	31.4783±0.434	1.8534±0.05
Shahabi	9.1±0.16	0.3627±0.11	-	0.4795±0.023	0.1741±0.011	30.8162±0.75	16.9334±0.45	13.1082±0.103	4.4592±0.65	31.7946±1.21	1.872±0.006

All values given are means ± SD of three determinations. Data show that the oleic acid and lauric acid have the highest amount among unsaturated and saturated fatty acids in three varieties, respectively.

levels of fatty acids in three varieties. Mean% ± SD of the fatty acid values and oil contents are shown in Table 1. The mean of AV, PV, *p*-An V and Totox number values were 1.4, 1.053, 2.54, 3.64 mequiv/kg respectively and RI was 1.462 for all varieties. Table 2 presents the mean of AV, PV, *p*-An V, Totox number and RI value of different samples.

DISCUSSION

In our study, the mean of saturated fatty acids of lauric, myristic, palmitic, caprylic, capric and stearic, for all varieties were 29.003, 15.658, 12.6965, 0.3712, 0.492 and 2.023% respectively of total fatty acids. Therefore, reminders were related to unsaturated fatty acids of undecylic, linoleic and oleic in varieties of Kabkab, Shekar and Shahabi, respectively. The three varieties were similar in the type of fatty acids present in their seed oil but pelargonic acid was not found in Shahabi. Despite the difference in the amounts of fatty acids, there is no significant relationship between levels of every fatty acid in all groups. The PV and refractive indexes were also not significant, while, in Shahabi variety, Totox index was slightly higher than the two others, due to its greater acidity than the other varieties. Several similar studies have examined the fatty acids as

our study. Besbes et al. (2004) in the same study on some quality characteristics of date seed oil from two Deglet Nour and Allig varieties of date palm reported that oleic acid (41.3 to 47.7%) was the main unsaturated fatty acids. The main saturated fatty acids were lauric acid (17.8%) for the Deglet Nour cultivar and palmitic acid for the Allig cultivar (15%). The oil contents were 10.19 and 12.67%. Capric (0.8 to 0.07%), myristic (9.84 to 3.12%), myristoleic (0.09 to 0.04%), palmitoleic (0.11 to 1.52%), linoleic (12.2 to 21%), linolenic (1.68 to 0.81%) and stearic acids (5.67 to 3 %) were also found.

The other study performed by Nehdia et al. (2010) showed that the main fatty acid of oil is oleic (50.10%). It was followed by linoleic (19.23%), lauric (10.24%), palmitic (9.83%), stearic (7.51%) and a trace amounts of the other fatty acids. The physicochemical properties include: the *p*-anisidine value 3.67; the peroxide value 3.62 meq/kg and the refractive index 1.45. Results of the study by Devshony et al. (1992) on the seeds of four date palm (*Phoenix dactylifera* L.) cultivars, Dekel Noor, Zahidi, Medjool and Haiawy, also revealed that the major unsaturated and saturated fatty acids were oleic (42.3%) and lauric (21.8 %) acids. The values of myristic, palmitic and linoleic acid were 10.9, 9.6 and 13.7%, respectively. The average content of oil and AV were obtained as 8.15% and 1.04 meq/kg.

Walid et al. (2003) obtained the findings from their study on 14 varieties of date palm *Phoenix dactylifera* L. and showed that the highest percentage of oil among the 14 cultivars was 9.3% in Shalaby date seeds. The mean of oil content in all of varieties was 6.7%. Oleic acid made up nearly half of the fatty acid in most of the seeds but the lowest detectable saturated fatty acids were caprylic and capric acids with averages of 0.4%. The highest percentages of unsaturated and saturated fatty acids among the 14 cultivars were related to the oleic and lauric acids with amounts of 49.8 to 24.1% in Suqaei and Rabeaah varieties, respectively. Also, different amounts of various fatty acids between these upper and lower range were obtained. Al-Hooti et al. (1998) analyzed five date cultivars (Bushibal, Gash Gaafar, Gash Habash, Luhu and Shahla) grown in the United Arab Emirates and found that the fatty acid content of the seeds varies from 6.3 to 10.9% for lauric acid, 5.2 to 7.0% for myristic acid, 10.6 to 13.8% for palmitic acid, 1.4 to 3.7% for stearic acid, 53.2 to 58.8% for oleic acid, 10.7 to 12.8% for linoleic acid, 0.1 to 0.2% for linolenic acid and 0.5 to 0.8% for arachidic acid.

In our study, like the previous studies, oleic acid had the highest amount among unsaturated fatty acids, but lower than their values. Our findings indicate that lauric acid was the highest amount

Table 2. Physico-chemical properties from seeds and seed oil from the three varieties of date palm (*Phoenix dactylifera*).

Physico-chemical property	Kabkab	Shekar	Shahabi
PV (mequiv/kg)	1.04±0.023	1.06±0.031	1.06±0.055
AV(mequiv/kg)	1.79±0.31	1.33±0.09	1.07±0.07
p-An V (mequiv/kg)	2.14±0.7	2.35±0.93	3.12±0.13
Totox (mequiv/kg)	4.22	4.47	5.24
Refractive index (n)	1.462±0.098	1.462±0.138	1.461±0.137

All values given are means ± SD of three determinations.

among saturated fatty acids in all varieties (Table 2), agreeing with the findings of Walid et al. (2003), Basbes et al. (2004), Al-Hooti et al. (1998) and Devshony (1992); but in results performed by Nehdia et al. (2010), lauric acid is located after the linoleic acid. The myristic, palmitic, stearic and linoleic acids in our study as previous reports were significant, although there were difference in their values. Our results for type of fatty acids were similar to those of Al-Hooti et al. (1998) but we found no linolenic acids. The mean of oil contents in previous studies ranged from 6.7 to 12.67%, whereas our results in Shekar variety corresponds with the results of Devshony et al. (1992) with amount of 8.1, although the mean of all groups in our study were slightly higher than the mentioned study. Although the results of the physico-chemical properties were close, they were some slight differences. However, result of AV in Kabkab variety was similar to Devshony et al. (1992) findings with an amount of 1.04.

Conclusion

It could be inferred from the present study generally, that date seed oil is rich in oleic and linoleic acids. We conclude that they would serve as useful source of these fatty acids and replace the other vegetable oils. However, their safety should be evaluated before use for human feed. They are renewable resources. Depending on the regional industry, these data suggest the potential application of date seed oil for cosmetics formulations such as in body creams, shaving soap and shampoos and pharmaceutical products and maybe the other new applications. Therefore, it is very important to have more research on date seed oil in the future to explore its potential useful effects.

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