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Evaluation of the fatty acid profiles, antioxidant activities, and total phenol and vitamin E contents of three types of Saudi date seed oils

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Abstract

Date seed oil is well known for its high content of monounsaturated fatty acids, especially oleic acid, and antioxidant compounds, such as phenols, tocopherols, phytosterols and vitamin A, which play an important role in reducing the risk of many diseases. Therefore, date seed oil extraction and processing into different pharmaceutical and cosmetic products may be economically beneficial and could be considered an important opportunity for future v industrial utilization. The main goal of this study is to investigate the fatty acid composition, antioxidant activities, and total polyphenol and vitamin E contents of oils extracted from the seeds of three types of Saudi date cultivars: Al-Raziz, Al-Khalas, and Al-Ajwa. Hexane and petroleum ether organic solvents were used for oil extraction due to the unavailability of cold extraction devices. Date seed oils extracted from the specified Saudi date cultivars were further subjected to chemical and statistical analyses using standard Methods. Total lipid extracted from the Al-Raziz, Al-Khalas, and Al-Ajwa date seeds cultivars was 10.38-12.65 g/100 g with hexane and 10.21-12.69 g /100 g with petroleum ether, with no significant differences between them. Oleic, lauric, myristic, palmitic, linoleic, and stearic acids were the most abundant fatty acids in all three types of date seed oils. Particularly, oleic acid accounted for nearly half of the total fatty acid content (48.2-51.12%) in all three types of date seed oils. Total saturated fatty acid contents were 42.2, 40.1, and 43.4% and the total unsaturated fatty acid contents were 57.8, 59.0, and 56.6% in the date seed oils of Al-Khalas, Al-Raziz, and Al-Ajwa, respectively. All date seed oils had high contents of antioxidants (41.1-63.2%), phenols (274.0-302.53%), and vitamin E (16.68-19.2%). Therefore, Al-Raziz, Al-Khalas, and Al-Ajwa date seed oils can be used for industrial food and caffeine-free coffee production and the development of cosmetic and pharmaceutics products for skin microbial disease and wound treatment. Here, date seed oils were very rich in antioxidants, phenols, and vitamin E, with some fatty acids, such as lauric acid, exhibiting antimicrobial activities. Future studies should investigate the degree of fat oxidation and changes in the total phenol, vitamin E, phytosterol, and phytochemical compound contents during date storage.

1. Introduction

The Kingdom of Saudi Arabia is among the most important date palm (*Phoenix dactylifera* L.) producers worldwide, producing 1.54 million tons of date palm annually (Alqahtani *et al.*, 2022). Date palm trees and their products are not only an important part of the social culture but also affect the national income and nutritional status of the population in Saudi Arabia. Therefore, effective industrial application of date palm trees, fruits, and waste was the main objective of the National Transformation Program in 2020 and remains a major goal of the Kingdom until 2030.

In Saudi Arabia, dates are generally used for direct consumption or production of different value-added products, such as date drinks, syrups, jams, candies, confectionaries, and sweets. However, considerable date seed (stone or kernel) waste is generated annually in date fruit processing factories and observed within the city of Mecca, especially during the Hajj season, posing a serious threat to the environment. In fact, date seeds

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contain high concentrations of bioactive compounds, such as crude fiber, dietary fiber, vitamins (A, E, and B), minerals (calcium, potassium, iron, zinc, boron, and selenium), essential fatty acids (omega-3 and 6), and sterols. They are cholesterol-free, exhibit anti-inflammatory and antioxidant effects, and have higher stability than olive oil. Date seed oil is rich in antioxidants, phenols, and vitamin E, with some fatty acids, such as lauric acid, exhibiting anti-microbial activities (Besbes, Blecker, Deroanne, Bahloul *et al.*, 2004; Bouhlali *et al.*, 2017; Karizak, 2017).

Al-Shahib and Marshall (2009) reported that date seeds contain 7.7-9.7% oil with 14 saturated and unsaturated fatty acids. Unsaturated fatty acids in date oil include palmitoleic, oleic, linoleic, and linolenic acids. The oleic acid content in date oil varies between 41.1 and 58.8%. Juhaimi *et al*. (2012) reported 4.68-7.96% oil content in seven Saudi date cultivars, with oleic acid as the most abundant fatty acid (40.51-45.74%), followed by lauric (17.95-25.01%), meristic (12.07-13.94%), palmitic (10.45-11%), and stearic (2.15-2.73%) acids. Therefore, date seed oil exhibits potential for various industrial applications. Additionally, date seed oil acts as a UV protector against UV-A and UV-B radiations (Devshony *et al.*, 1992).

2. Materials and methods

2.1 Materials

The Seasons Ahsa Dates Factory (Al-Hufuf, Kingdom of Saudi Arabia) donated date seeds (kernels) of the Al-Khalas, Al-Raziz, and Ajwa date cultivars. While, the Ozog Taiba Dates Factory (Al-Medina Al-Munawarah City, Kingdom of Saudi Arabia) donated Al-Ajwa date seeds.

2.2 Sample preparation

Seeds of the three date cultivars were washed, dried at an approximate room temperature (25°C) for 24 hrs, and ground into a fine powder using a special milling machine (HOME ELEC, HC-G-350N; High-Speed Multi -Function Comminutor, China). Then, the powder was filtered through a standard sieve (opening size of 0.38 um) and tightly packed in polyethylene bags until use.

2.3 Oil extraction

Due to the unavailability of cold extraction devices, date seed oil was extracted via Soxhlet extraction (Serial No. 10887711, Device Type Electro-Thermal Engineering, UK) in the laboratory of the Date Palm Research Center of Excellence at the King Faisal University, Saudi Arabia Kingdom. Date seed powder (20 g) was placed in a cellulose thimble inside a condenser and oil was extracted using hexane and petroleum ether organic solvents at 69.1 and 35°C, respectively, as described by the Association of the Official Analytical Collaboration (AOAC) International (2010). The extracted oil was stored in a refrigerator at 4°C until use.

2.4 Fatty acid profile analysis

Free fatty acids in the three date seed oils were analyzed using a gas chromatograph-mass spectrometer (QP 2010 Plus; Shimadzu, Tokyo, Japan) equipped with an automatic injector (Model AOC-5000; Shimadzu). A capillary column (100 m × 0.25 mm id × 0.20 µm d; SP-2560 Supelco, Bellefonte, USA) was used as a stationary phase, with helium as the carrier gas at a flow rate of 1.59 mL/min. The samples were injected with an injector at a split ratio of 1:40, temperature of 250°C, and pressure of 300 k Pa.

2.5 Determination of the antioxidant activity

The antioxidant activities of the date seed oils were determined as described by Gorinstein *et al*. (2004). The oil sample (0.2 mL) was mixed with 2.8 mL of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (1.0 mole), and the mixture was kept in the dark for 30 min. Then, the absorbance of each oil sample and control was measured at 517 nm using a spectrophotometer (UV-1800-240 V; Shimadzu Corporation, Japan). The antioxidant activity of each sample was calculated using the following equation:

$$\text{Antioxidant (\%)} = ([A^1] - [A^0]) \times 100/A^0$$

Where A^0 = Absorbance of the control and A^1 = Absorbance of the oil sample

2.6 Determination of the total phenol content

The total phenol contents of the date seed oils were measured as described by Gokbulut *et al*. (2013). Briefly, 0.02 mL of date seed oil extract (oil: methanol = 1.0:6.0) and 1 mL of Folin and Ciocalteu's phenol reagent were added to a beaker. After 3 min, 1 mL of saturated sodium carbonate solution (99%) with 0.98 mL of distilled water was added to the same beaker. The mixture was kept in the dark for 2 hrs, and absorbance was measured at 725 nm. A calibration curve was plotted using several concentrations of Gallic acid (50, 100, 150, 250, and 500) as a standard curve. Then, phenolic concentrations of the samples were calculated using the following equation:

$$C = a \times y \times (v/m) \times 100$$

Where C = Phenol concentration (g/100 g Gallic acid), a = Dilution factor, y = Concentration determined

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from the standard curve (mg/mL), v = Volume of the oil extract and m = sample weight.

2.7 Determination of the vitamin E content

Vitamin E contents of the date seed oils were measured using UV-HPLC (Thermo Scientific TRACE 1300 Series, USA), as described by Biglar *et al.* (2012). All chemicals used in this study were of high purity and obtained from Sigma Aldrich (Germany). Briefly, 0.2 g of date seeds oil was weighed, mixed with 5 mL of ascorbic acid solution (0.1 M) and 20 mL of potassium hydroxide s**o**lution (2 M), and heated at 60°C. After filtering through 10 mL of a saturated sodium chloride solution, 10 mL of butanol in hexane (5 mg/L) was added to the mixture, stirred for 1 min, and anhydrous sodium sulfate was added to dry the sample. Then, the sample was re-dissolved in 1 mL of methanol, filtered, and stored at –20°C. Finally, tocopherol content was determined at 294 nm using UV-HPLC (Thermo Scientific TRACE 1300 Series).

2.8 Statistical analyses

All experiments were conducted in triplicate. The results were expressed as the mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance with the Minitab software version 19.2020.1 (64-bit) 2020 (LLC All rights reserved, USA). Statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1 Extraction of date seed oil

Table 1 and Figure 1 show the oil yields as percentage of date seed oil extracted from the Al-Khalas, Al-Raziz, and Al-Ajwa Saudi date cultivars using hexane and petroleum ether. No significant differences ($P \leq 0.05$) were observed in the oil yields of the three cultivars when extracted with hexane and petroleum ether on a wet-weight basis. In contrast, significant differences were observed in the oil yields of the three cultivars when extracted separately with hexane or petroleum ether. The oil yield of the Al-Raziz and Al-Ajwa seeds was 10.21-12.69% when extracted with petroleum ether and 10.38-12.65% when extracted with hexane. Notably,

Table 1. Yields of the date seed oils extracted from three Saudi date cultivars with hexane and petroleum ether indicated as g/100 g seeds (wet weight).

Date cultivar	Hexane (g/100 g)	Petroleum ether
Al-Khalas	11.10 ± 0.08^{b}	11.03 ± 0.15^{ab}
Al-Raziz	10.38 ± 0.10^{a}	10.21 ± 0.25^{a}
Al- Ajwa	12.65 ± 0.13^{c}	12.69 ± 1.58^{c}

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are statistically significantly different at $P \leq 0.05$.

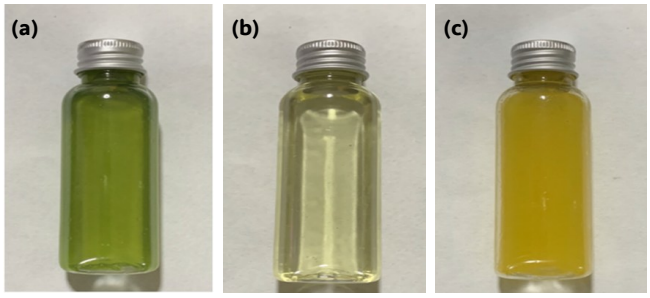

Figure 1. Date seed oils extracted from the Al-Khalas (A), Al-Raziz (B), and Al-Ajwa (C) Saudi date cultivars.

the date seed oil yield in this study was higher than that (4.68-7.96%) reported by Juhaimi *et al*. (2012) and Nehdi *et al*. (2018). This variation in the oil yields of different date seed cultivars may be attributed to the particle size of the date seed powder and oil extraction period. Al-Ajwa date seeds exhibited the highest oil yield, followed by Al-Khalas and Al-Raziz date seeds when extracted with hexane and petroleum ether.

3.2 Fatty acid profiles of the Al-Khalas, Al-Raziz, and Al -Ajwa Saudi date seed oils

Table 2 shows the fatty acid profiles of the Al-Khalas, Al-Raziz, and Al-Ajwa date seed oils. The most abundant fatty acid in the three date seed oils was oleic acid (49.8, 51.2, and 48.2%, respectively), followed by lauric (14.7, 13.4, and 14.7%, respectively), myristic (11.3, 10.3, and 12.2%, respectively), and palmitic (10.7, 11.0, and 11.7%, respectively) acids. Nehdi *et al*. (2018) reported oleic (48.67%) and lauric (17.26%) acids as the most abundant fatty acids in 6 Saudi date seed cultivars. Our results are consistent with those obtained by El-Massry *et al*. (2019), who revealed oleic (38.50-39.72%) and lauric (38.50-39.72%) acids as the most abundant fatty acids in two Saudi date cultivars. Moreover, Reddy *et al*. (2017) revealed that oleic acid-containing date seed oils exhibit good flavor and high stability during frying.

As shown in Table 2, total saturated fatty acid contents were 42.2, 40.1, and 43.4% and total unsaturated fatty acid contents were 57.8, 59.0, and 56.6% in the Al-Khalas, Al-Raziz, and Al-Ajwa date seed oils, respectively. Moreover, polyunsaturated fatty acid contents were 7.96, 8.7, and 8.36% in the Al-Khalas, Al-Raziz, and Al-Ajwa date seed oils, respectively. Sum percentages of the omega-3 (0.51, 7.45, and 49.8%), omega-6 (0.53, 8.17, and 51.2%), and omega-9 (0.44, 7.92, and 48.2%) fatty acids in the Al-Khalas, Al-Raziz, and Al-Ajwa date seed oils are also shown in Table 2. Ramadan *et al*. (2005) reported that dietary oil rich in unsaturated fatty acids prevents cardiovascular and inflammatory diseases. Beebe *et al*. (2005) reported that date seed oil with low unsaturated fatty acids is highly stable against fat oxidation.

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Table 2. Fatty acid profiles of the Al-Khalas, Al-Raziz, and Al-Ajwa date seed oils.

Fatty acid (%)	Date palm cultivar			Statistics value	
	Al-Khalas	Al-Raziz	Al-Ajwa	P value	R^2 (%)
Caprylic acid	0.25±0.002[b]	0.26±0.002[a]	0.21±0.001[c]	0.000	99.69
Capric acid	0.35±0.003[b]	0.31±0.002[b]	0.27±0.191[a]	0.000	100.00
Lauric acid	14.70±0.104[a]	13.40±0.104[b]	14.70±0.095[a]	0.002	98.67
Myristic acid	11.30±0.080[b]	10.30±0.073[c]	12.20±0.086[a]	0.000	99.48
Palmitic acid	10.70±0.076[c]	11.00±0.078[b]	11.70±0.083[a]	0.002	98.28
Stearic acid	3.61±0.026[a]	3.66±0.026[a]	3.37±0.024[a]	0.003	98.09
Oleic acid	49.80±0.341[b]	51.20±0.362[a]	48.20±0.341[c]	0.008	96.08
Linoleic acid	7.45±0.053[c]	8.17±0.058[a]	7.92±0.056[b]	0.002	98.32
Arachidic acid	0.63±0.004[a]	0.53±0.004[b]	0.50±0.004[c]	0.000	99.75
Behenic acid	0.46±0.003[a]	0.43±0.003[b]	0.36±0.003[c]	0.000	99.75
Lignoceric acid	0.30±0.002[a]	0.21±0.002[b]	0.16±0.001[c]	0.000	99.96
Omega-3 (n-3)	0.51±0.004[b]	0.53±0.004[a]	0.44±0.003[c]	0.000	99.59
Omega-6 (n-6)	7.45±0.056[c]	8.17±0.058[a]	7.92±0.053[b]	0.002	98.32
Omega-9 (n-9)	49.80±0.352[b]	51.20±0.352[a]	48.20±0.341[c]	0.008	96.08
Saturated fat (SAFA)	42.30±0.299[b]	40.10±0.284[c]	43.40±0.307[a]	0.003	97.74
Monounsaturated fat (MUFA)	49.80±0.352[b]	51.20±0.352[a]	48.20±0.341[c]	0.008	96.08
Polyunsaturated fat (PUFA)	7.96±0.056[c]	8.70±0.062[a]	8.36±0.059[b]	0.003	98.16
Total unsaturated fat (TUFA)	57.80±0.409[b]	59.90±0.424[a]	56.60±0.400[b]	0.009	95.70

Values are presented as mean±SD, n = 3. Values with different superscripts within the same row are statistically significantly different at $P \leq 0.05$.

3.3 Antioxidant activities of the Al-Khalas, Al-Raziz, and Al-Ajwa Saudi date seed oils

Next, the antioxidant activities of the Al-Khalas, Al-Raziz, and Al-Ajwa date seed oils were investigated using the DPPH radical scavenging assay (Table 3). Al-Raziz date seed oil exhibited the highest antioxidant activity (63.2%), followed by the Al-Khalas (52.78%) and Al-Ajwa (41.1%) date seed oils. Kazemi and Dadkhah (2012) revealed that the antioxidant activity of the date seed oils of 15 Iranian date cultivars was 5.1-55.5%, with an average of 30.1%. Interestingly, the highest antioxidant activity of the Iranian date seed cultivars is intermediate between that of the two Saudi date cultivars, Al-Khalas (52.78%) and Al-Raziz (63.2%), investigated in this study. However, the antioxidant activity of the Al-Ajwa date seed oil (41.1%) was higher than the average antioxidant activity of the Iranian date seed oils (30.1%).

3.4 Total phenol contents of the Al-Khalas, Al-Raziz, and Al-Ajwa Saudi date seed oils

Table 4 presents the phenol contents of the Al-Khalas, Al-Raziz, and Al-Ajwa date seed oils. Significant differences were observed in the phenol contents of the date seed oils. Al-Khalas seed oil had the highest (302.53 mg/100 g), whereas Al-Raziz seed oil had the lowest (274.17 mg/100 g) phenol content. Herch *et al*. (2014) reported that the phenol content of Tunisian date seed oil is 312 mg/100 g. However, phenol contents of two types of Algerian date seed oils (64-85 mg/100 g) are lower than those of Tunisian and Saudi date seed oils (Boukouada *et al*., 2014). Besbes, Blecker, Deroanne, Lognay *et al*. (2004) reported that oils extracted from date seeds contain different concentrations of eight phenolic compounds; they also attributed the high stability of one seed oil to its high phenol concentration. Date seed oil prevents hydrogen peroxide-induced

Table 3. Antioxidant activities (%) of the Al-Khalas, Al-Raziz, and Al-Ajwa Saudi date seed oils.

Date Seed Oil	Antioxidant activity (%)
Al-Khalas	52.78±0.2[b]
Al-Raziz	63.2±0.2[c]
Al- Ajwa	41.1±0.1[a]

Values are presented as mean±SD, n = 3. Values with different superscripts are statistically significantly different at $P \leq 0.05$.

Table 4. Total phenol contents (mg/100 g) of the Al-Khalas, Al-Raziz, and Al-Ajwa Saudi date seed oils.

Date Seed Oil	Total phenol content (mg/100 g)
Al-Khalas	302.53±0.25[c]
Al-Raziz	274.17±0.32[a]
Al- Ajwa	285.73±0.32[b]

Values are presented as mean±SD, n = 3. Values with different superscripts are statistically significantly different at $P \leq 0.05$.

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oxidative damage (Ines *et al*., 2010). Concentrations of phenolic compounds and tocopherols significantly affect the flavor, shelf life, oxidation resistance, and stability of seed oils. Particularly, date seed oil has a higher phenol content than olive oil, thus serving as an important source of natural phenols (Besbes, Blecker, Deroanne, Bahloul *et al*., 2004).

3.5 Vitamin E contents of the Al-Khalas, Al-Raziz, and Al -Ajwa Saudi date seed oils

Vitamin E (tocopherols and tocotrienols) is a natural antioxidant important for human health owing to its lipoperoxyl radical-scavenging activity (Adhikari *et al*., 2008; Nehdi *et al.*, 2013). Table 5 shows the vitamin E contents of the Al-Khalas, Al-Raziz, and Al-Ajwa date seed oils. Significant differences were observed in the vitamin E contents of the three date seed oils. Al-Raziz seed oil had the highest (19.22 mg/100 g), whereas Al-Khalas seed oil had the lowest (16.68 mg/100 g) vitamin E content. Roy *et al.* (2002) reported that the vitamin E family consists of eight types of tocopherols. Among them, alpha (α) and gamma (γ) tocopherols account for 60% of the total vitamin E content in vegetable oils (Besbes *et al*., 2004). Vitamin E contents of the three Saudi date seed oils determined in this study were consistent with those reported by Nehdi *et al*. (2018). Biglar *et al*. (2012) reported that the tocopherol content of 14 types of Iranian date seed oils is 22.04–0.06 mg/100 g.

Table 4. Total phenol contents (mg/100 g) of the Al-Khalas, Al-Raziz, and Al-Ajwa Saudi date seed oils.

Date Seed Oil	Vitamin E content (mg/100 g)
Al-Khalas	16.68 ± 0.00^{c}
Al-Raziz	19.22 ± 0.03^{a}
Al- Ajwa	17.36 ± 0.02^{b}

Values are presented as mean±SD, n = 3. Values with different superscripts are statistically significantly different at $P\leq0.05$.

4. Conclusion

From the results obtained in this study, it can be concluded that the production of date seed oils from Al-Raziz, Al-Khalas and Al-Ajwa date cultivars will have high potential possible industrial utilization for the oil in food, medicine, pharmaceutics and cosmetic products, based on its chemical and physical properties. Moreover, the utilization of date seeds in the oil industry not only will support the economic, social and industrial developments of the Saudi date fruits sector but also, reduce the risk of date seed environmental pollution around date manufacturing areas.

Conflict of interest

The authors declare no conflict of interest.

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