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Original Article

# Extraction and Identification of Cactus *Opuntia Dillenii* Seed Oil and its Added Value for Human Health Benefits

**Alya Jameel Ali Alsaad[1], Ammar B Altemimi[1]*, Salah Naji Aziz[1], Naoufal Lakhssassi[2]**

**ABSTRACT**

Cactus *Opuntia dillenii* presents multiple health benefits. The current study aims to investigate the seed composition and content of prickly pear fruits from Iraq. Results obtained showed that *Opuntia dillenii* contained 9.5% of seeds of the entire fruit while extracted oil presented 6.5% of total seed composition. Fatty acid analysis revealed that the polyunsaturated linoleic acid (72.9%), the saturated palmitic acid (15.12%) and stearic acid (7.51%) presented the main seed fatty acids of *Opuntia dilleniid*. Other essential oils were detected but at low percentage. Interestingly, stearic acid content in Cactus oil presented 7.51%, which is much higher than soybeans (~3%) that are considered as the largest source of animal protein feed and the second largest source of vegetable oil worldwide. Stearic acid presents neutral effects on the concentration of blood serum LDL cholesterol and does not exhibit cholesterolemic effects on human health. The analysis of cactus seed oil demonstrated a strong antioxidant ability estimated by their capability to reduce oxidation. Treated cake with BHT (butylated hydroxytoluene) at concentration of 0.02 mg/100g of butter from cactus seed-oil exhibited lower peroxide values ranging from 0.67 to 1.5 milli-equivalents (meq) peroxide per 1 kg of oil throughout 15 days of storage time at 4 °C. In contrast, treated cake with 0.11 mg/100g of butter from cactus seed-oil presented lower peroxide values ranged from 0.69 to 2.5 meq peroxide per 1 kg of oil among all treatments. Because of its high-saturated fatty acid composition (>22%) and rich linoleic acid (72.9%) composition, *Opuntia dillenii* present an alternative source with several health benefits by lowering cholesterol risks and for biodiesel production.

**Key words:** fatty acid, cactus, seed-oil, GC-MS, cake, peroxide value.

**Alya Jameel Ali Alsaad[1], Ammar B Altemimi[1]*, Salah Naji Aziz[1], Naoufal Lakhssassi[2]**

*[1]Department of food science-college of Agriculture-University of Basrah, IRAQ.*
*[2]Department of Plant, Soil and Agricultural Systems, Southern Illinois University, Carbondale, IL 62901, USA.*

**Correspondence**

**Ammar B Altemimi**

Department of food science-college of Agriculture-University of Basrah, Iraq

Tel. +9647735640090

E-mail: ammaragr@siu.edu

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## INTRODUCTION

Cactus *Opuntia dillenii* is massively grown in the South of Iraq, especially in Basrah city (Al-Fao). Cactus belongs to the dicotyledonous angiosperm *Cactaceae* family that includes about 1500 species of cactus worldwide. Due to its acidic taste and the presence of a large number of seeds within the fruit, cactus is not widely used for consumption. However, recent studies have highlighted the presence of multiple benefits for human health and medicine in natural cactus.[1,2] It has been shown that *Opuntia dillenii* has anti-inflammatory, analgesic, anti-hyperglycemia, and hypocholesterolemic effects.[3,4] During the last decade, consumer demand for food with high nutritional value has increased as a result of their high-fat (fatty acids) content, as well as health benefits, which have created a new category known as "functional foods".[5,6] Cactus is rich in minerals with inorganic nutrients including calcium, phosphorus, iron, magnesium, copper, and zinc usually required in small amounts (1 to 2500 mg per day). Humans and other vertebrates need large amounts of calcium because it is necessary for bone and for normal function of nerves and muscles.[7] Adenosine triphosphate (ATP) and acid-base balance does not occur without the presence of phosphorus, which is essential for nucleic acid composition.[8] In addition, red blood cells cannot function properly without iron in the hemoglobin. Iron is also an important component of the cytochromes that function in the cellular respiration process.[7] Other minerals like magnesium, copper, and zinc are important cofactors that can be found in the structure of certain enzymes and indispensable in numerous biochemical pathways.[9]

Oil-seed content from *O. ficus-indica* (prickly pears) ranges from 5.0% to 14.4% according to the Turkish varieties *Ortaoren* or *Eskioba* Matth¨aus. The seeds of *Opuntia dillenii* were used as high-quality edible oil with health benefits as they contains high amounts of unsaturated fatty acids. The main fatty acids of the 17 cactus oil samples from Morocco are palmitic acid and linoleic acid.[10] Cactus Pear seed-oil contains saturated and unsaturated fatty acids, with higher linoleic acid content, while myristic, palmitoleic, hexa-decadienoic, and margaric are present in minimal amounts. *Opuntia dillenii* seed-oil also includes phenolic acids, flavonoids, and tannins that are considered as antioxidants for the pharmaceutical industry.[11] The fatty acid composition of this oil is an essential indicator for its added nutritional value.[12] Supplementation of bakery products like cake, that present a rich source of energy and protein, with butter of Cactus seed-oil will further help in improving its nutritional value and chemical qualities. Development of value added products from diverse raw ingredients is receiving the prime focus of the food processing industry and by researchers.[13,14]

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The main objectives of this study were (1) determination of minerals compounds from dry seeds Cactus *Opuntia dillenii* grown in Basrah city, (2) analyze the extracted seed-oil by GC-MS to determine their fatty acid composition and other essential oils that may be present, (3) explore the feasibility of development of added cake value from essential oils, and (4) determination of the antioxidant activity of Cactus seed-oil by measuring the peroxide value of the cake to understand the inhibitory oxidation effect of extracted Cactus seed-oil after storage by refrigeration.

## MATERIALS AND METHODS

### Sample collection and preparation

Mature fruits of prickly pear, Cactus *Opuntia dillenii,* were collected 6 weeks after blossoming in August 2017 from Ma'Amir, Al-Fao (Basra, Iraq) (Figure 1). The cultivated cactus are grown at Ma'Amir (30.027148 deg latitude, 48.436624 deg altitude and 3 feet elevation), at al-Faw Peninsula in the Persian Gulf, located in the extreme southeast of Iraq, and is part of a delta for the Shatt al-Arab river. Temperatures typically vary from 49 °F to 109 °F with very low chance of rain through out the year. type of soil is silt and sand. Cactus seeds were studied during fruit ripening.

Fruits were peeled, and seeds were isolated by pressing the edible pulp. Next, the seeds were washed with distilled water and dried at room temperature to calculate the percentage of seeds in the edible fraction (pulp) by taking the weight of the pulp prior to seed-weight. Seeds were macerated to a fine powder, passed through a sieve with particle size of 0.425 mm and stored at -20 °C until use.[9]

### Determination of mineral compounds

In order to determine the mineral compounds of *Opuntia dillenii*, 1g weight of dry powder seed was placed in silica dish and then in a muffle furnace. Moreover, samples were burned to ash at 550 °C for 4 h. Next, samples were cooled and ash dissolved in 5 ml of 2N $HNO_3$, then filtered and diluted to 50 ml volume in distilled water. The samples were analyzed in three replicates, and mineral compounds were determined for calcium, magnesium, sodium, potassium, iron, phosphorus, copper, and zinc by Atomic Absorption Spectrophotometry (AAS, USA) as previously described.[15]

### Essential oil extraction

The essential oil samples were obtained by hydro distillation for 4 h and 30 min in a Clevenger-type apparatus using 50 g of dried seeds in 1 L of distilled water, with three technical replicates. After extraction, the essential oil samples were centrifuged at 5,000 rpm for 2 min to separate the residual water from the oil. To calculate the essential oil yield content, the total mass of the essential oil sample to be analyzed was measured using an analytical balance (accurate to 0.0001 g).

### Determination of antioxidant activity (scavenging activity of DPPH radical)

The method was carried out as described previously.[16] The essential oils were dissolved in methanol at different concentrations (10, 50, 100,500 and 1,000 µg/mL). The assay mixture (total volume of 1 mL) contained 500 µL of the oil, 125 µL prepared DPPH (1 mM in methanol) and 375 µL solvent (methanol). After 30 min incubation at 25 °C, the decrease in absorbance was measured at λ = 517nm. Ascorbic acid was used as comparative sample. The radical scavenging activity was calculated from the equation: % DPPH = $Abs_{control} - Abs_{sample} / Abs_{control} \times 100$

((absorbance of control), $Abs_{sample}$ (absorbance of sample $Abs_{control}$

**Figure 1.** The Different plant tissue tested in the current study. Left to right are cultivated Cactus *Opuntia dillenii* plant, fresh fruits, dry fruit, seeds, extracted oil.

### Preparation of fatty acid methyl esters (FAME)

Total fatty acid content and fatty acid composition were determined simultaneously in the fruit's seeds-oil samples. Fatty acid analysis was performed in triplicate and consisted of two consecutive steps; (1) preparation of fatty acid methyl ester (FAME) and (2) chromatographic analysis. The AOAC (1996) method was followed to esterify the lipid extract. FAME was prepared from the lipid extracted samples by heating with the methanolic NaOH and then with BF3 absolute Methanol for esterification. Next, 5 ml n-heptane was added to recover the methyl esters. organic phase, saturated with NaCl solution, was added to the mixture, and the aqueous and organic layers were separated using a profile-separating funnel. The upper n-heptane phase was pipetted out into 10 ml glass vials and then stored at -20 °C until performing GC-MS analysis.

### Fatty acid methyl ester (FAME) analysis by gas chromatography mass spectrometer (GC MS)

The gas chromatography analysis of methylated fatty acids was performed on a Shimadzu QP2010 quadrupole Gas Chromatography Mass Spectrometer (GC-MS) instrument equipped with a carbowax (30 m × 0.25 mm ID; 0.25 (µm film thickness) capillary column (Intercut DB5MS, Japan). One microliter of sample was injected into the capillary column. Helium was used as the carrier gas. Injector and detector temperatures were set at 280 °C. Injection was performed in split mode (1:30). The column temperature was programmed initially at 50 °C for 1 minute, then to increase at a rate of 5 °C per min at final temperature of 280 °C. Fatty acid methyl esters were separated at constant pressure (100 kPa) and peaks were identified by comparing the mass spectra with the mass spectral database. The identification of compounds was based on the comparisons of their mass spectra with NIST Library 2008.

### Application of cactus seed-oil in cake

#### *Cakes preparation*

200 g of flour, 120 g of sugar, 100 ml of skimmed milk, 80 g of fresh whole eggs, 100 g of butter, 8 g of baking powder, and 2 g of vanilla were used. All ingredients were mixed during 10 min at normal speed using a Kitchen-Aid Professional Mixer. All of the cake ingredients were placed into a metallic mold (100 mm diameter and 50 mm height), lightly coated with vegetable corn oil and next baked in an electric oven for 30 min at 180 °C. Butter was substituted by Cactus seed-oil at 0% (control), 5%, 10%, and 15%. After baking, cakes were removed from the mold and left 30 min for cooling to room temperature. Then, cake samples were placed on coded white glass plates for the sensory evaluation.

#### *Sensory evaluation of cake*

Evaluation of baked cake quality characteristics was carried out following cooling to room temperature. Sensory evaluation was performed by twenty-specific evaluators from the Department of Food Science at the College of Agriculture at Basrah University. Cakes were randomly assigned to each panelist. A 10-point hedonic scale

score was applied to evaluate important parameters including color, color, texture, taste, odor and overall acceptability.[17] The designated number 10 was given to "I like it extremely well" while number 1 was given to "I dislike it extremely".

### Peroxide value determination

The number of peroxide value milli-equivalents (meq) peroxide per 1 kg of oil was measured as described earlier.[18] Control and treated cake with different concentrations of seeds oil 0.03, 0.05, 0.07, 0.09, and 0.11 mg/100g of butter and BHT (butylated hydroxytoluene) at concentration of 0.02 mg/100 g of butter, was stored at different times 0, 5, 10, and 15 days at 4 ˚C. Next, 1 g of the sample was boiled with 1 g potassium iodide and 20 mL of solvent mixture (Glacial acetic acid and chloroform [2:1] v/v) for 30 s and then vigorously for another 30 s before being filtrated to collect the leachate. Leachate was next poured into 20 mL of 5% potassium iodide and the boiling tube washed twice with 25 mL of distilled water. Titration was carried out with 0.002 M of the $Na_2S_2O_3$ using starch indicator. Blank was similarly titrated. Peroxide value determination was calculated as follow:

$$\text{Peroxide value} = \frac{1000\,(V2-V1)T}{M}$$

Where M = mass of oil taken (1 g); $V_2$ = volume of 0.1 N Na2S2O3; $V_1$ = volume of 0.1 N $Na_2S_2O_3$ used as blank; and T = normality of $Na_2S_2O_3$ (0.1 N).

### Statistical analysis

Analysis of variance (ANOVA) was carried out using SPSS program (version. 16). The cake characteristics with or without seed oil were analyzed using ANOVA. Means and standard deviation of three replicates were calculated. Analysis of variance (ANOVA) was performed to determine any significant differences ($p < 0.05$).

## RESULTS AND DISCUSSION

### Yield percentage of seeds in pulp

Yield percentage analysis showed that the seeds contained in the pulp of *Opuntia dillenii* constituted about 9.5% of the fruit pulp. The obtained seed content was in agreement with those found in the literature,[19] which ranges between 2 to 10% of the fruit pulp. Prickly pear seeds presented 10-15% of the edible pulp and are usually discarded as waste after extraction of the pulp as described by Sáenz.[20] The edible part of the fruit contains a relatively large number of seeds, which amount can vary from 30% to 40% on a dry weight basis. These seeds are usually discarded while proper utilization of these waste products could lead to an important new source of oil and meal.[21] Prickly pear seeds are 10-15% of the edible pulp and are usually discarded as waste after extraction of the pulp seeds from *Opuntia sp.* and were shown to be rich in polyphenols, flavonoids, and tannins. The concentrations of those molecules were shown usually to be higher than in the fruit pulp.[22]

### Determination of mineral compounds

Analysis of the mineral compounds reveals that Cactus fruit seeds are rich in minerals, with a predominance of calcium, phosphorus, and potassium at 280.81, 243.90, and 181.96 mg/100g respectively, as shown in Table 1. Percentage of magnesium, sodium, and Zinc was 156.94, 28.01, and 52.90 mg/100g, respectively. Copper and iron contained less amounts with 1.36 and 3.63 mg/100g, respectively. Our results showed that the macro elements contents of Cactus *Opuntia dillenii* seeds were less than those reported in the literature by Ghazi *et al.* (2015), where Phosphorus was found to be the major element at 970.15 mg/100 g dry seeds followed by calcium at 408.28 mg/100g, magnesium at 240.30 mg/100g, potassium at 201.96 mg/100g, and sodium at a lower content 18.18mg/100g, in Cactus *Opuntia dillenii* seeds.

**Table 1: Distribution and content of minerals in Cactus *Opuntia dillenii* seeds expressed as mg/100 g dry weight.**

| OD Mineral (mg/100 g dry weight) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Minerals** | **Macro elements** | | | | | **Trace elements** | | |
| | Ca | Mg | Na | K | P | Fe | Cu | Zn |
| | 280.81 | 156.94 | 28.01 | 181.96 | 243.90 | 3.63 | 1.36 | 52.90 |

### Essential oil yield

The present study shows that yield percentage of extracted oil from Cactus *Opuntia dillenii* was about 6.5%. Stintzing[23] reported that seeds are a relatively untapped source of the lipid fraction, presenting 7 to 15% by weight of the whole seed and is characterized by a high degree of unsaturation, wherein the linoleic acid is the main fatty acid and ranged between 56,1% to 77%. Labuschagne and Hugo[24] reported that oil content in Cactus *Opuntia dillenii* seeds from South Africa was 5.69 %, while Chang[25] reported that oil content in Cactus pear seed oil from China was 6.01%. The two results were close to the obtained results from the present study. However, oil content from Italian cultivar was 9.14%,[26] and oil content was 11.05% from another cultivar from Tunisia.[9] Compared to other oil-seed crops, *Opuntia dillenii* presented lower oil content. Indeed, higher amounts were recovered from cotton seeds 15–24%, soybean seeds 17–21%, grape seeds 6–20%, and olive 20–25%.[27]

### Antioxidant activity

The potential antioxidant activity of the oil was determined on the basis of DPPH free radical scavenging activity. The analysis of cactus seed oil demonstrated a strong antioxidant abilities estimated by their capability to reduce oxidation. In addition, the obtained results showed the potent scavenging activity of cactus seed oil compared to the control (ascorbic acid). The obtained DPPH scavenging activities were 36.5-78.1% and 46.5- 81.3% for cactus seed oil extraction and ascorbic acid at concentrations (10, 50, 100, 500, and 1,000 µg/mL), respectively (Figure 2). The statistical analysis results showed that there was no significant difference ($p > 0.05$) between cactus seed oil extraction and ascorbic acid at concentrations 10, 50, and 100 µg/m) for DPPH scavenging activities. However, there was significant difference ($p < 0.05$) between cactus seed oil extraction and ascorbic acid at concentration at 500 and 1000 µg/mL for DPPH scavenging activities. The scavenging activities increased significantly while increasing the concentration. In fact, cactus seed oil represents a strong electron donor and could react with free radicals to convert them to more stable products and terminate the radical chain reaction. This observed effect could be associated with high content of phenolic components such as Tocopherols, a natural occurring antioxidants presenting biological activity.[28] The extracted *Opuntia stricta* oil using supercritical (SC)-$CO_2$ method showed high antioxidant activities due to enriched polyphenols (172.2 ± 11.9 µg gallic acid equivalents (GAE) $g^{-1}$ oil), a process that led to more compounds.[29] The cactus seed oil extracts have an important role in treating several diseases including hypoglycemic effects, anti-tumoral, and antioxidant activities.[30] DPPH scavenging activities increased significantly with increasing the concentration of the cactus seed oil and fruit juices from 5 to 20 µL/mL. It has ranged between 24.84 - 53.15% for *O. Ficus* while *O. dillenii* values ranged from 21.04 - 42.6% and ascorbic acid ranged from 21.02 to 63.85%.[31]

### Analysis of *Opuntia dillenii* seeds-oil by gas chromatography-mass spectrometry (GC-MS)

Gas-chromatography coupled with mass spectrometry analysis revealed the presence of an interesting profile of fatty acids contained in extracted seed oil from *Opuntia dillenii* (Figure 3 and Table 2). The current study reveal that the main fatty acids were 9,12-Octadecadienoic acid methylester (linoleic acid) with 72.9%, Hexadecanoic acid methyl ester (palmitic acid) with 15.12%, and the Octadecanoic acid methyl ester (stearic acid) with 7.51%. Other essential oils were detected but at low percentages, these include the 1,4-Benzenedicarboxylic acid dimethyl ester, Methyl tetradecanoate, 9-Hexadecenoic acid methyl ester, cis-

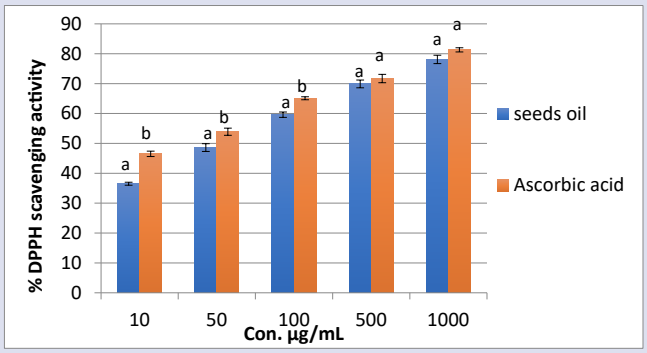


**Figure 2:** Antioxidant activity of Cactus seed-oil.

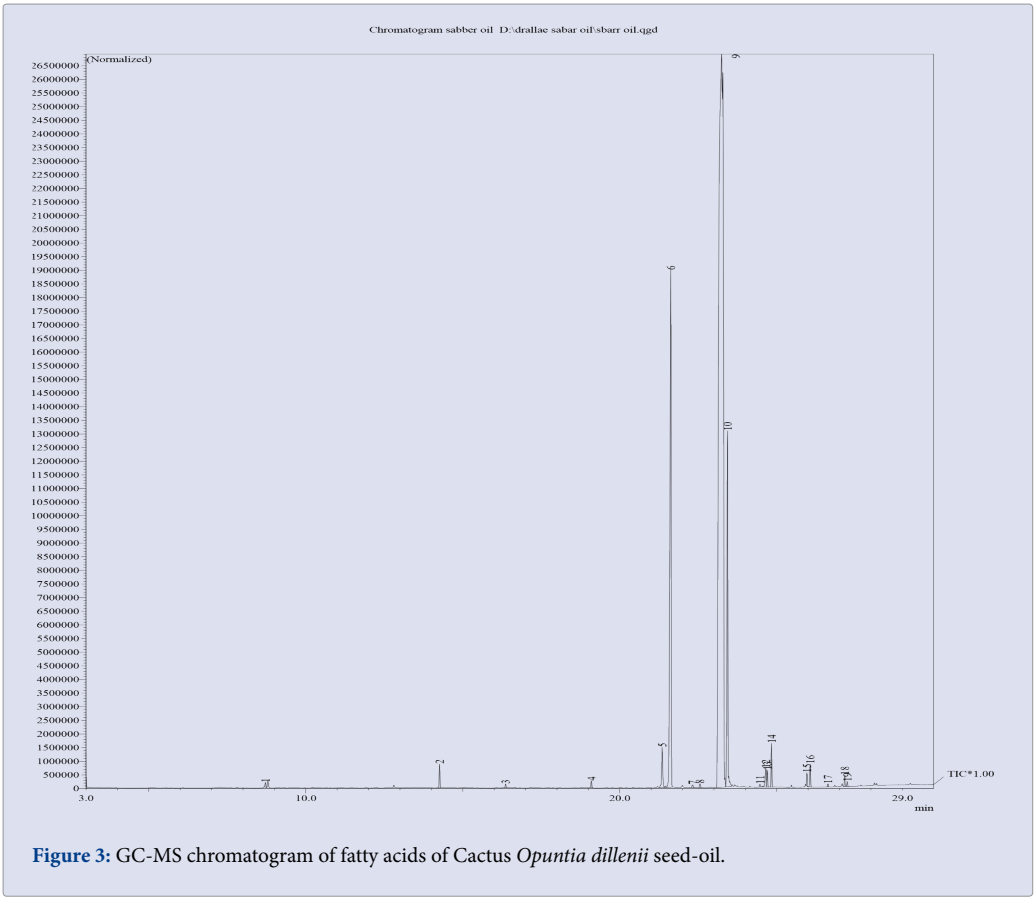


**Figure 3:** GC-MS chromatogram of fatty acids of Cactus *Opuntia dillenii* seed-oil.

11-Eicosenoic acid methyl ester, cis-13-Eicosenoic acid methyl ester, Methyl 18-methylnonadecanoate, and Methyl 11-docosenoate. The obtained results showed that Cactus *Opuntia dillenii* seed-oil was rich in fatty acids. The saturated fatty acids identified include palmitic acid (C16:0) and stearic acid (C18:0) with levels containing up to 22.63% saturated fatty acids, while high levels of the poly-unsaturated omega-6 linoleic acid (C18:2) exceeding 72.9% were obtained.

Filip[32] reported that the level of fatty acids in *Opuntia dillenii* seed oil is higher than in sunflower seed oil, grape seed oil, or sesames seed oil. Omega-6 like linoleic acid and arachidonic acid present a hypocholesterolemic effect and present important inhibitory properties against colon cancer metastatic cells.[33] Three types of sunflower seed oil were developed; high-oleic, mid-oleic and low-oleic (FAO). Consequently, the linoleic acid content is high in low oleic germplasms but is low in high oleic sunflower seed oil. Differences between the oil content of cactus seeds from different locations can be explained by differences in growing locations and environmental conditions[28] *Opuntia dillenii* seed-oil presents higher proportion of poly-unsaturated fats (linoleic acid) compared to certain conventional edible vegetable oils such as olive oil (3.5 - 21%), soybean oil (49.7%), corn oil (47.7%), sesame oil (44.5%), sun flower oil (49.7%), and cotton oil (50.0%).[34-36] These characteristics illustrate that *Opuntia dillenii* may be an interesting natural source of edible oil containing high amounts of healthy fatty acids. The obtained seed-oil contents were in agreement with those found in the literature.[37] Although poly-unsaturated fatty acids like (linoleic acid) are predominant, many reports have shown that saturated fatty acids (i.e. palmitic and stearic acids) were found in percentages ranging from (16 - 17%). These results were in agreement with recently published studies by Ghazi[8] where the linoleic acid was the dominating fatty acid with an exceptional level up to 79.83%, followed by palmitic acid (13.52%), and stearic acid (2.75%) resulting in 16.27% saturated fatty acids. The current study shows that Cactus *Opuntia dillenii* contains similar amounts of linoleic acid levels (72.9%), but

**Table 2. Fatty acid content identified in Cactus *Opuntia dillenii* seed-oil.**

| P | R.Tim | Area | Area% | Name | Formula | MW | Ret Index |
|---|---|---|---|---|---|---|---|
| 1 | 8.71 | 3466 | 0.09 | Eucalyptol | C10H18O | 154 | 1059 |
| 2 | 14.26 | 16639 | 0.46 | 3-Cyclohexene1-methanol,.alpha.,.alpha.,4-trimethyl-, acetate | C12H20O2 | 196 | 1333 |
| 3 | 16.36 | 2600 | 0.07 | 1,4-Benzenedicarboxylic acid, dimethylester | C10H10O4 | 194 | 1440 |
| 4 | 19.09 | 4946 | 0.14 | Methyltetradecanoate | C15H30O2 | 242 | 1680 |
| 5 | 21.35 | 35624 | 0.98 | 9-Hexadecenoic acid, methylester,(Z)- | C17H32O2 | 268 | 1886 |
| **6** | **21.62** | **552445** | **15.12** | **Hexadecanoic acid, methylester** | **C17H32O2** | **268** | **1886** |
| 7 | 22.32 | 2704 | 0.07 | cis-10-Heptadecenoic acid, methylester | C18H34O2 | 282 | 1986 |
| 8 | 22.55 | 3303 | 0.09 | Heptadecanoic acid, methylester | C18H36O2 | 284 | 1978 |
| **9** | **23.24** | **2662843** | **72.90** | **9,12-Octadecadienoic acid(Z,Z)-, methylester** | **C19H34O2** | **294** | **2093** |
| **10** | **23.43** | **274318** | **7.51** | **Octadecanoic acid, methylester** | **C19H38O2** | **298** | **2077** |
| 11 | 24.46 | 2295 | 0.06 | Cyclopropaneoctanoic acid, 2-[[2-[(2- | C22H38O2 | 334 | 2266 |
| 12 | 24.65 | 19462 | 0.53 | cis-11-Eicosenoic acid, methylester | C21H40O2 | 324 | 2284 |
| 13 | 24.70 | 10616 | 0.29 | cis-13-Eicosenoic acid, methylester | C21H40O2 | 324 | 2284 |
| 14 | 24.83 | 27387 | 0.75 | Methyl18-methylnonadecanoate | C21H42O2 | 326 | 2212 |
| 15 | 25.960 | 7648 | 0.21 | Methyl11-docosenoate | C21H40O2 | 324 | 2284 |
| 16 | 26.06 | 14292 | 0.39 | Methyl20-methyl-heneicosanoate | C23H46O2 | 354 | 2411 |
| 17 | 26.62 | 2069 | 0.06 | Methyl20-methyl-docosanoate | C24H48O2 | 368 | 2510 |
| 18 | 27.16 | 6757 | 0.19 | Tetracosanoic acid, methylester | C25H50O2 | 382 | 2674 |
| 19 | 27.242 | 675771 | | Terephthalic acid, di(2-ethylhexyl)ester | C24H38O4 | 390 | 2704 |
| | | 3652550 | 100.00 | | | | |

**Table 3: The total saturated and unsaturated Fatty acid content unsaturated/saturated fatty acid ratio identified in Cactus *Opuntia dillenii* seed-oil.**

| | Total major saturated FA | Total major unsaturated FA | Unsaturated/Saturated FA ratio |
|---|---|---|---|
| Cactus *Opuntia dillenii* | 22.63% | 72.9% | 3.22% |

higher amounts of total saturated fatty acids 22.63%, with unsaturated/saturated major fatty acid ratio of 3.22 (Table 3). Moreover, in another study, it has been reported that linolenic acid constituted the main fatty acid (66.56%), followed by palmitic acid (19.78%), stearic acid (9.01%), and linoleic acid (2.65%) in Cactus *Opuntia dillenii*.[33] The profile of fatty acid extracted from different Cactus *Opuntia dillenii* varieties grown in different regions worldwide is more likely due to the interaction between genetics and different environment which impact very much the fatty acid content of different oil seed plants.

In the fatty acid biosynthesis pathway, the oleic acid content, which is synthesized from stearic acid by the Stearoyl-Acyl Carrier Protein Desaturase (SACPD) and at the same time is considered as the precursor of the linoleic acid (by the Fatty acid desaturases (FAD2), was very low in Cactus *Opuntia dillenii* seeds. This could be explained by the high activity of the *FAD2* enzyme in converting most of the synthesized oleic acid to linoleic acid. It has been shown that both *SACPD* and FAD2 activity (down-regulation or up-regulation) have a major effect on controlling levels of stearic and oleic acids in oil seed plants including soybeans (due to induced and/or natural occurring mutations) and olives.[38-40] In fact, duplicated oleaster *FAD2* genes were found to be regulated by an siRNA derived from a transposable element-rich region responsible for suppressing levels of *FAD2* gene expression. Neofunctionalization of *SACPD* gene family members has been shown to increase expression of *SACPD2*, 3, 5, and 7 resulting in an increased desaturation of stearic acid.[41] Thus, the accumulation of exceptionally high levels of linoleic acid in in Cactus *Opuntia dillenii* seeds may be likely be explained by an increase/decrease of *SACPD/FAD2* expression.

Linoleic acid is an essential fatty acid and is the precursor of the very long polyunsaturated fatty acids like the arachidonic (ARA) that are beneficial for human health. ARA (20:4) is present in phospholipids of membranes of the body's cells, abundant in the brain, and liver.[42]

Leguminous plants like soybeans present also higher amounts of polyunsaturated linoleic acid (55%), followed by oleic acid (20%), while the saturated fatty acids like palmitic and stearic acids present 10% and 3%, respectively.[36] Stearic acid content in Cactus oil (7.51%) was much higher than in soybeans, considered the largest source of animal protein feed and the second largest source of vegetable oil in the world. Stearic acid presents neutral effect on the concentration of blood serum LDL cholesterol and does not exhibit cholesterolemic effects on human health. Many efforts have been made by the soybean community and industry to increase the stearic acid content in soybeans by EMS mutagenesis and mutational breeding.[43] However, all developing soybean lines with high stearic acid content (10 to 20%) presented poor agronomic performance because the non-healthy nodules.[43] Therefore, extracted natural cactus seed-oil present additional value for human health benefits compared with soybeans.

The fatty acid contents obtained in this study were quite similar to those found in the *Opuntia ficus-indica* seed-oil studied by Ozcan and Al Juhaimi.[43] In fact, linoleic acid and palmitic acid contents were 67% and 16%, respectively. The environmental conditions such as heat and humidity, in addition the fruit maturity conditions may explain the observed differences in the oil concentrations.[44]

## Sensory evaluation of cake

The sensory evaluation results are shown in Table 4. The five sensory indexes of 5, 10, and 15% Cactus seed-oil treatment was evaluated and compared between different treated samples and the control sample. The obtained data showed that all the five sensory of 5% Cactus seed-oil treatment samples had a significant difference when compared to the control. However, no significant difference ($p > 0.05$) in the two sensory indices (Texture, Taste) of cakes prepared with Opuntia dillenii seed-oil was found between 5% and 10% of Cactus seed-oil treatment samples. The two sensory indexes (Texture, Taste) of cakes prepared

with Opuntia dillenii seed-oil using 15% of Cactus seed-oil treatment had significant differences ($p < 0.05$) compared to 5% and 10% of Cactus seed-oil treatment samples. Similarly, significant difference ($p < 0.05$) regarding overall acceptability of cakes prepared with Opuntia dillenii seed-oil was found between 5% and 10% of Cactus seed-oil treatment samples. But there was no significant difference ($p > 0.05$) between 5% and 10% of Cactus seed-oil treatment samples regarding overall acceptability index. These results were in agreement with Hafez (2012) who showed the absence of any significant differences among the samples with and without marjoram powder substitution for the liking scores of crump color, texture, and overall acceptability.

Cactus *Opuntia dillenii* seeds contained higher amounts of healthy fatty acids. Moreover, polyunsaturated fatty acids may be natural source of edible oil and contribute to the reduction of both total and LDL cholesterol and significant decrease in HDL cholesterol.[45] *Opuntia dillenii* seed-oil presents a higher proportion of polyunsaturated fatty acids (linoleic acid) compared to conventional edible vegetable oils such as olive oil, soy oil, corn oil, sesame oil, sunflower oil, and cotton oil.[35] Fatty acids play a natural preventive role in cardiovascular diseases and in alleviation of some other health problems.[46]

## Peroxide value determination

The peroxide value of treated cake (Figure 4) at 4 ˚C with 0.03, 0.05, 0.07, 0.09, and 0.11 mg/100g of butter from Cactus seed-oil showed no significant differences among the samples. However, there was a significant difference between the samples and the control. The treated cake with BHT at concentration 0.02 exhibited lower peroxide values ranged from (0.67 to 1.5) milli-equivalents (meq) peroxide per 1 kg of oil throughout 15 days of storage time at 4˚C. In contrast, the treated cake with 0.11 mg/100g of butter from Cactus seed-oil presented lower peroxide values ranged from (0.69 to 2.5) (meq) peroxide per 1 kg of oil among all treatments. There was no significant difference ($p>0.05$) between BHT and the treatment of 0.11 (mg/100g of butter). In addition, the statistical analysis showed that there was a significant difference ($p< 0.05$) between the control sample and all treatments at the same conditions.

These results suggested that seed-oil was effective in suppressing the oxidation of cakes. The anti-oxidative effect may have contributed to the oxidative stability of cakes with addition of natural antioxidants when added. These added antioxidants prevent the lipid peroxides formation during storage and delay oxidation. This could be due to the slow permeation rate of antioxidant components into lipid layer of the cakes. One of the most important changes that may occur to food is lipid oxidation. Lipid oxidation lowers the quality and nutritional value of food.[47] The susceptibility of lipids to oxidation is one of the major causes of oxidative stresses, resulting in the development of rancidity, unpleasant tastes and odors, as well as changes in color.[48] The concentration and effectiveness of these oils may vary among cultivars or varieties, crop environmental factors (i.e. light, temperature, and type of soil nutrients), or methods and solvents used for their extraction.[49] Fruit seed-oils are of great interest because they present high degree of unsaturation and with antioxidant radical scavenging properties.[50] The oil from plants can be potentially used by the food industry for the manufacturing of "natural" or "green" safe foods and for extend shelf-life.[51,52] The peroxide value is of the order of 1.43 for *Opuntia ficus indica* oil from cold pressure extraction, and 1.84 for hexane extraction. These peroxides index values are less than 10 meq O2 / kg oil which characterize most conventional oils.[53,54] Indeed, lower peroxide index values 10 mEq O2 / kg oil is generally regarded as indicating an acceptable level of oxidation.[6,41]

# CONCLUSION

Cactus fruit seeds are rich in minerals, with a predominance of calcium, phosphorus and potassium at 280.81, 243.90, and 181.96 mg/100g respectively. *Opuntia dillenii* seed-oil was found to contain highest unsaturated fatty acid levels. In fact, linoleic acid presented 72.9%

**Table 4: Sensory evaluation of cakes prepared with *Opuntia dillenii* seed-oil replacement of cake butter at (5, 10, 15) % of Cactus seed-oil.**

| Parameters | Control | 5% | 10% | 15% |
|---|---|---|---|---|
| Color | 8.79 ± 1.0$^a$ | 8.04± 0.30$^b$ | 8.63± 0.70$^c$ | 8.89± 0.20$^c$ |
| Texture | 8.43± 1.0$^a$ | 7.20± 1.80$^b$ | 7.12± 2.60$^b$ | 8.01± 0.86$^c$ |
| Taste | 8.01± 1.30$^a$ | 6.23± 2.00$^b$ | 6.11± 1.40$^b$ | 7.01± 0.80$^c$ |
| Odor | 8.30± 2.0$^a$ | 7.01± 0.81$^b$ | 6.02± 0.85$^c$ | 5.21± 0.78$^d$ |
| Overall Acceptability | 8.08± 0.21$^a$ | 7.60± 1.68$^b$ | 7.53± 1.80$^c$ | 7.50± 1.87$^c$ |

* Means within each raw with the same superscript letter (a,b,c) are not significantly different.

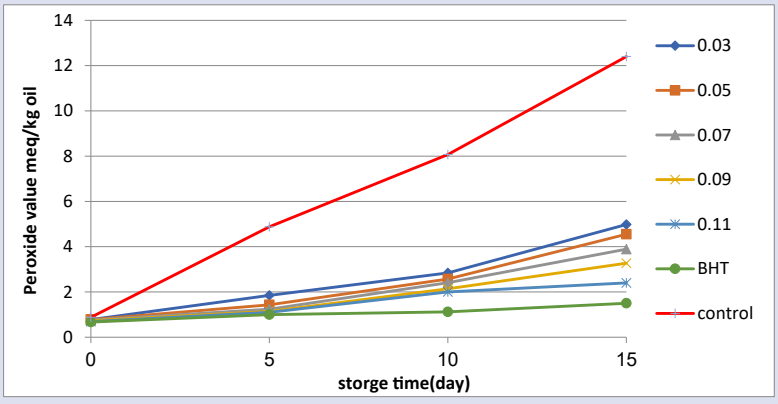


**Figure 4.** Peroxide value of treated cake with 0.03, 0.05, 0.07, 0.09, and 0.11 (mg/100g of butter) of Cactus seed-oil during 15 days storage.

while saturated fatty acids presented 15.12% (palmitic acid) and 7.51% (stearic acid) (22.63 total saturated fatty acids), much higher than the total of saturated fatty acids (~14%) contained in soybean [36,40,54]. The high-saturated fatty acid composition (>22%) and high linoleic acid contents (72.9%) in *Opuntia dillenii* have positive impact to explore this plant as an alternative source for healthy oil by lowering cholesterol risks and for biodiesel production. The other essential oils were present at low percentage. The degree of sensory evaluation of the control cake and the substitution of butter with cactus seed-oil were very high in all qualitative elements. The treated cake with BHT at concentration 0.02 exhibited lower peroxide values ranged from (0.67 to 1.5) milli-equivalents (meq) peroxide per 1 kg of oil throughout 15 days of storage time at 4 ˚C. In contrast, the treated cake with 0.11 mg/100g of butter of Cactus seed-oil presented lower peroxide values ranged from 0.69 to 2.5 meq peroxide per 1 kg of oil among all treatments. These results further highlight the benefits of using Cactus seed-oil as a natural source for added nutritional value and use in industrial and/or pharmaceutical sectors.

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## AUTHOR CONTRIBUTIONS

A.JA. and S.N.A designed and planned the major experiments. A.A and N.L carried out and wrote-up the main manuscript text.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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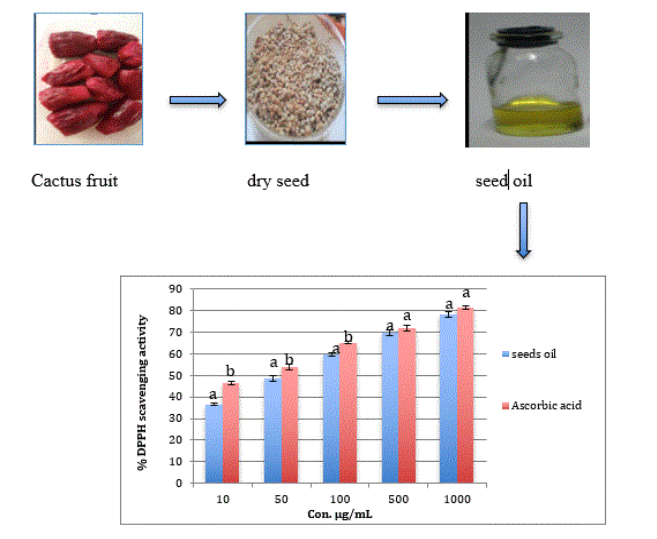
## GRAPHICAL ABSTRACT




## ABOUT AUTHORS

Dr. Alya Jameel Ali Alsaad a lecturer at Department of Food Science, Faculty of College of Agriculture, University of Basrah, Iraq. She has experience in the area of food chemistry and natural products.

Dr. Ammar Altemimi worked as Lecturer (2009 - present) in Department of foodscience and Biotechnology, University of Basrah, Iraq. He is now the chair of food science department, college of Agriculture, University of Basrah. He taught biochemistry and biotechnology for undergraduate, food chemistry, dairy products. Developing academic programs, monitor students educational progress, train and motivate other non-teaching staff; manage career counseling and other student service. He has published more than 11 papers in reputed journals such as Ultrsonic Sonochemistry Journal and Molecules Journal; and he has been serving as an editorial board member and reviewers of repute Journals.

Dr. Salah N. Aziz
A lecturer at Department of Food Science, College of Agriculture, University of Basrah, Iraq. He has experience in the area of molecular genetic and Biotechnology.

Dr. Naoufal Lakhssassi is currently an Associate Scientist at the Department of Plant Soil and Agricultural Systems, Agricultural College, Southern Illinois University Carbondale, USA. He holds a PhD in the field of Biochemistry and Molecular Biology from Malaga University, Spain, in the year 2011. He has been working on different biology projects related to microbiology, plant genetics, biochemistry, biotechnology, plant developmental biology, plant evolution, and bioinformatics. He served as an ad-hoc reviewer for several scientific journals including BMC Genomics, Plos1, Plant Cell Reports, and Crop Science, etc. He is serving on the editorial board of the Soil Science and Plant Health Journal, in addition to the Plant Science Journal. He handled several research projects during these years mainly working with Arabidopsis thaliana, tomato, and soybean with a major focus on soybean disease resistance and seed composition using mutation screening. Dr. Naoufal Lakhssassi's research includes characterization of considerable lines with increased seed oleic and stearic acids content, high protein content, tocopherols, desirable carbohydrates, high yielding varieties, in addition to the characterization of several genes involved in seed germination, and plant development.

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