

## PHYTOCHEMICAL STUDY OF DATE SEEDS LIPIDS OF THREE FRUITS (*PHOENIX DACTYLIFERA L*) PRODUCED IN OUARGLA REGION

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### ABSTRACT

Algeria is considered as one of the major date producing countries. The annual production reaches up to 468,000 tons; most of it is consumed locally. The seeds or date stones which represent a relatively high weight are considered in most cases as waste and hence the present work is a contribution to their valorization.

The physico-chemical characteristics and the fatty acid composition of date seed oil of three date palm (*Phoenix dactylifera L*), Deglet-Nour, Ghars, and Tamdjouhert, originating from south eastern of Algeria (Ouargla region). The oil contents of these seeds range from 5.05 to 6.08%.

The physico-chemical properties of these oils indicated that acid values range from 1.35 to 1.38 mgKOH/g. The saponification values and Iodine values are in the range 204.84 – 215.87 mgKOH/g ; 67.22-74.80 (g Iodine /100g) respectively. The refractive index of date seeds oil was found to be between 1.4778 and 1.4792. The Specific gravity of these oils range from 0.8836 to 0.9295.

The amount of sterols has been determined by a simple spectrophotometric method. The total sterol contents were found to be between 5.417 and 7.884 mg/g. However, the Physico-chemical properties are within the same range of some edible oils.

### Keywords

Date Seed, *Phoenix dactylifera L*, Oil, fatty acid composition, physico-chemical properties, Sterols.

## 1. Introduction

The (*Phoenix dactylifera L.*) is a monocotyledoneous woody perennial belonging to the Arecaceae family, which comprises 200 genera and 3000 species. The beneficial health and nutrition values of date palm, for human and animal consumption, have been claimed for centuries [1].

Algeria is the sixth important countries in date world production. During 2007, 468000 metric tons were produced in Algeria. The Algerian dates represented about 7.28% of the total world production as reported by FAO [2].

The date is composed of a fleshy pericarp and seed. Date pits (known also as date stones, kernels, or seeds) are a waste product of many date processing plants producing pitted dates, date powders, date syrup, date juice, chocolate coated dates and date confectionery [3]. At present, pits are used mainly for animal feeds in the cattle, sheep, camel and poultry industries. However, value can be added in several food products. An additional function includes roasting the date pits and making a caffeine-free drink which can substitute coffee when caffeine is a concern but a coffee-related flavor is desired [3,4]. Such a drink is used in the Arabic world for quite a while. Thus a traditional beverage is obtained by roasting and grinding date seeds in a similar way as for coffee beans. A commercial product (Date Pits Powder – Coffee Substitute) has also been introduced recently to the market [3].

The seed powder is also used in some traditional medicines [1]. Date pits have been studied as ingredients for fish feeds (as food carp (*Cyprinus Carpio L*)) [5].

Furthermore, the use utilization of date pits in the production of citric acid and protein by *Candida lipolytica*, *Apergillus oryzae* and *Candida utilis* were also investigated [6]. *Ishrud et al.* (2001) have isolated and studied polysaccharides from seed of date [7]. Studies aiming at the extraction and characterisation of the different fractions of date are limited to oil, polyphenol and dietary fibre from date seeds [8, 9].

In terms of dry weight, the chemical composition of date pits has been reported as containing 5–10% moisture, 5–7% protein, 7–12% oil, 10–20% crude fiber, 55–83% carbohydrates and 1–2% [10, 11].

As well as (*Sawaya .W.N et al.* 1984; *Al-Whaibl .M.H et al.* 1985) Most current literature was limited to proximate and mineral compositions[10,12].

The pits represent about 6–15% of the total weight of the mature date [1, 9, 11, 13]. In Algeria, lipid fraction of date seed could amount to over 2300 tons annually, assuming a mean yield of 10 % of seed in date fruit and 5 % oil content of seeds. Some scientists have studied chemical characteristics and fatty acid composition of date seed oil. The chemical characteristics of seed oil from six Libyan date cultivars were as follows: iodine number  $\approx$  54.8, saponification value  $\approx$ 207 and acid value  $\approx$ 1.75 [14].

The aim of this study is was to evaluate the lipid profiles of date seeds from three important cultivars grown in south eastern of Algeria and to determine fatty acid composition as well as the physicochemical characteristics. Furthermore, the total sterol content was also measured for date seeds oils. The date seeds which represent a relatively high yield are considered in most cases as waste and hence the present work was planned with the aim of making use to them and to study the possible economic and medicinal potentialities of this crop in the Algeria.

## 2. Materials and methods

### 2.1. Seed material

Three different Algerian date varieties Deglet-Nour (DN), Ghars (Gh), and Tamdjouhert (Tam) were obtained from a (palms grove) local in Ouargla region The different varieties were identified in the (Agronomic National Institute of Ouargla).

The seeds (pits) were manually separated from the flesh. Their relative percentage weight compared with the weight of the fresh fruits was about 9.82% for the Deglet Nour, 9.72% for the Ghars variety, and about 13.13% for the Tamdjouhert variety. Then, The seeds were rinsed clear of any flesh by water and dried for 48 hours at 60°C [13].and then ground to a fine powder by grinder to pass 1-2 mm [9,11].

### 2.2. Chemicals and reagents

All chemicals were purchased from Sigma, Aldrich (Milwaukee), Fluka Chemie Sigma-Aldrich (Germany and Merck), Riedel-dhaen, Prolabo.

### 2.3. Extraction

The dry seed powder was extracted in a soxhlet apparatus using Hexane as a solvent for 6 hours. After extraction the solvent was removed using a Rotary vacuum evaporator at a temperature not exceeding 40 °C and then the oils obtained stored in a freezer at 6 °C and analyzed[15].

### 2.4. Physicochemical characteristics of oils

The different chemical characteristics of the seed oils used in this study (iodine value, saponification value, and acid value) were determined according to the methods of *AFNOR* (1984) [16].

The Refractive index and Specific gravity were determined according to the methods of the *AOAC* (1975) [17].

### 2.4.1. Physical Analysis

#### Refractive Index:

The refractometric value (index) of seed oil was read at 20° C with a digital refractometer ATAGO DR-A1

#### Specific Gravity:

The Specific gravity of the sample oil was measured using specific gravity bottle . Specific gravity was determined at ambient temperature of 20 °C.

### 2.4.2. Chemical Analysis

**Acid value:** The Acid value was evaluated by the French norm numbered **AFNOR NFT 60-204 (1984)**

**saponification value:** The saponification value was evaluated by the French norm numbered **AFNOR NF T 60-206 (1984)**

**Iodine value:** The Iodine value was evaluated according to the French norm numbered **AFNOR NF T 60-203 (1984)**

### 2.5. Analysis of oil extract

#### 2.5.1. Fatty acid composition.

The oils were converted to methyl esters using a methanolic potassium hydroxide (1 M). The mixture was refluxed during 30 minutes. 10 ml of distilled water was then added. Then, the product

GC analyses were performed on a Delsi gaz-chromatography equipped with a flame ionization detector (FID), and a capillary column (MEGA 10, 25m × 0.32mm × 0.25µm). The column temperature was programmed from 150 to 200 °C at 2 °C/min and both the injector and detector temperature were set at 250 °C. Helium was the carrier gas.

#### 2.5.2. Fatty acid identification

The fatty acids methyl esters were identified by comparing the retention time of the samples and appropriate fatty acids methyl esters standards (Fig. 1-3).

### 2.6. Determination of Sterols content

The total content of sterols was determined using the Liberman-Burchard assay [18, 19]. 1 ml of diluted sample solutions was mixed with 2 ml of Liberman-Burchard Reagent.. The mixture was incubated in the dark for 30 min and green color formed is measured at 550 nm using a spectrophotometer Shimadzu UV-VIS 1700. Cholesterol was used as the standard for the calibration curve (Fig. 4).

### 3. Results and discussion

At 20° C all the seed oils examined are Semi solids. The colour of the oils varies from green yellow to Brown yellow.

The amounts and percentages of total lipids in date palm seeds are presented in Table 1. The lipid contents of date seeds were found to be between 5.05 and 6.08%. The percentages of lipid content were highest in Tamdjouhert (6.08%) and lowest in Ghars (5.05%).

The total lipid contents of Deglet-Nour (5.20%). However, this is in agreement with previous reports [13, 20]. The Although the date seeds cannot be considered as an oil-bearing as in peanut, olive, sunflower and cotton seeds which possess (30–45% oil content), its oil content was in the

range of other vegetable materials that are used for their health components or their industrial or pharmaceutical applications, as in the case of wheat germs (less than 10% fat content) [15].

### 3.1. Physicochemical properties of seed oil

As for the physical properties, the Refractive index and Specific gravity were studied at room temperature 20°C.

The refractive index of date seeds oil was found to be between 1.4778-1.4792, Specific gravity of these oils range 0.8836-0.9295 (Table 1).

The physicochemical parameters of the oils are shown in Table 1

Acid value is the number of milligrams of potassium hydroxide necessary to neutralise the free acids in 1 g of sample. This value can be used for a purity check of oil and may be already started decomposition reactions [21]. Acid values of oil from date seeds are between (1.35 -1.38 mg KOH/g).

The acid value was low in the three oils, which indicates that the oils contain a small amount of free fatty acids, and could explain the decline in the acid value of oil perhaps due to the small exposure of the seeds to the air during the maturity of the fruits of the dates. The low free fatty acid (FFA) content of the oil shows that it is edible and could have a long shelf life.

Iodine value is a measure of the unsaturation of fats and oils and is expressed in terms of the number of gram of iodine absorbed per 100 gram sample [22]. Iodine values of oil from date seeds are between (67.22 and 74.8 g Iodine /100g) were highest in seeds oil of Ghars (74.8 g Iodine /100g); the lowest value was in seeds oil of Tamdjouhert (67.22 g Iodine /100g)

The relatively high iodine value in the four oils may be indicative of the presence of many unsaturated bonds and would certainly contain more unsaturated fatty acids and can thus be classified as drying oils

The iodine values of (67.22 - 74.8 g Iodine /100g) indicate that this oil is non-drying, highly unsaturated oil.

Oils are classified into drying, semi drying and non- drying according to their iodine values. Since the iodine value of date seed oil is lower than 100 it could only be classified as a non drying oil.

Saponification value is expressed as number of milligrams of potassium hydroxide required to saponify 1 g of the sample. The saponification value is an indication of the average molecular mass of fatty acids present in oil [23]. Saponification values of the analysed rapeseed oils are in the range of 204.84 mg KOH g oil<sup>-1</sup> and 215.87 mg KOH g oil<sup>-1</sup> and were highest in seeds oil of Tamdjouhert variety (215.87 mg KOH/g); the lowest value was in seeds oil of Deglet-Nour (204.84 mg KOH/g).

Because there is an inverse relationship between saponification value and weight of fatty acids in the oils, it can be assumed that the oils hold fatty acids with 16–18 carbon atoms with a significant amount of saturated fatty acids in the case of the date seeds oil.

The saponification value was compared to the values for some common oils like palm oil (196-205mgKOH/g), groundnut oil (188-96mgKOH/g), corn oil (187-196mgKOH/g), coconut oil (253mgKOH/g) and palm kernel oil (247mgKOH/g) [24]. However, the saponification values are within the same range of some edible oils.

**Table 1:** Physicochemical characteristic of date seeds oils

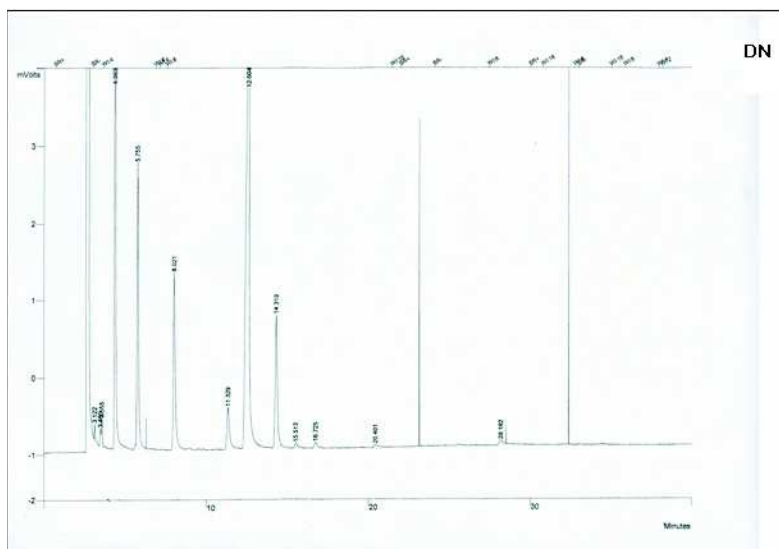
Parameter	Cultivars		
	DN	Gh	Tam
Iodine Value (g Iodine /100g)	70.16	74.80	67.22
Saponification Value (mg KOH /g)	204.84	212.85	215.87
Acid Value (mg KOH/g)	1.35	1.36	1.38
Refractive index ( 20 °C )	1.4778	1.4792	1.4789
Specific gravity (20 °C)	0.8994	0.8836	0.9295
Color	greenish yellow	Brownish yellow	greenish yellow
Total lipid content (%)	5.20	5.05	6.08
State at Room temperature	Semi liquid	Semi liquid	Semi liquid

**3.2. Fatty acid composition**

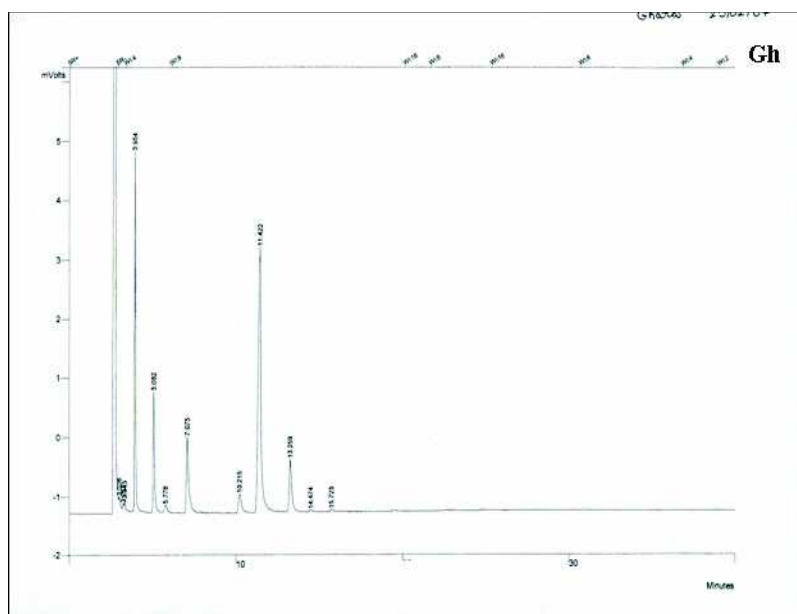
The FAMES composition of the oils of the species is shown in Table 2.

The most abundant fatty acids of date seed oil were oleic (C<sub>18:1</sub>), linoleic (C<sub>18:2</sub>), palmitic (C<sub>16:0</sub>), myristic (C<sub>14:0</sub>), and lauric (C<sub>12:0</sub>) which together composed about 90-95% of the total fatty acids. The major fatty acid found in those cultivars was oleic acid (40.66-43.91%), ranging from 42.54% for Deglet Nour seed oil , 43.91 % for Ghars seed oil, and 40.66% for Tamdjouhert seed oil. followed by lauric acid (21.03-25.66%), myristic acid (10.28-11.66%), palmitic acid (9.11-10.53%), linoleic acid (7.05-7.80%), and Stearic acid (3.10-3.63%) . Linolenic acid (0.42-0.51%) and Arachidic acid (0.38-0.54%) were present in low amounts. These results are in general agreement with those of [9-11, 13, 20, 25].The fatty acid profiles of the three date seed oils were similar with only minor differences. The degree of unsaturation of Tamdjouhert, Deglet Nour, and Ghars date seed of was 47.71%, 50.85%, and 53.27% respectively.

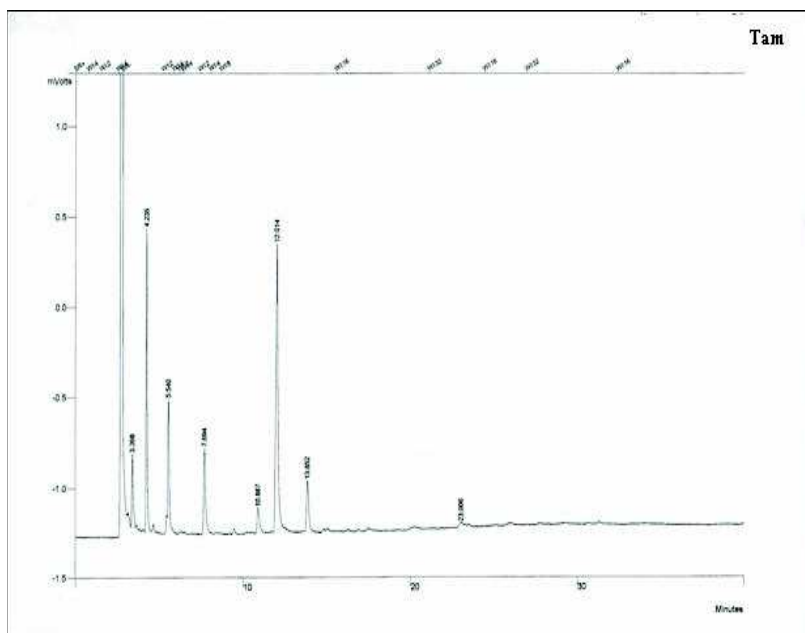
When compared with the commonly consumed vegetable oils, the date seed oil has a relatively lower degree of unsaturation. Despite this, date seed oil has a good potential for use in human and/or animal diets.



**Fig. 1:** Chromatogram of the fatty acids composition of Deglet Nour seed oil



**Fig. 2:** Chromatogram of the fatty acids composition of Ghars seed oil



**Fig. 3:** Chromatogram of the fatty acids composition of Tamdjouhert seed oil

**Table 2:** Fatty acid composition (%) of date seeds oils

Fatty acid	Relative amount (%)		
	DN	Gh	Tm
Capric C <sub>10:0</sub>	-	-	6.26
Lauric C <sub>12:0</sub>	25.66	22.17	21.03
Myristic C <sub>14:0</sub>	10.73	10.28	11.66
Myristoleic C <sub>14:1</sub>	-	1.14	-
Palmitic C <sub>16:0</sub>	9.11	10.53	9.70
Stearic C <sub>18:0</sub>	3.10	3.36	3.63
Oleic C <sub>18:1</sub>	42.54	43.91	40.66
Linoleic C <sub>18:2</sub>	7.80	7.80	7.05
Linolenic C <sub>18:3</sub>	0.51	0.42	-
Arachidic C <sub>20:0</sub>	0.54	0.38	-
ΣSFA	49.15	46.73	52.29
ΣUFA	50.85	53.27	47.71
U/S	1.034	1.14	0.912

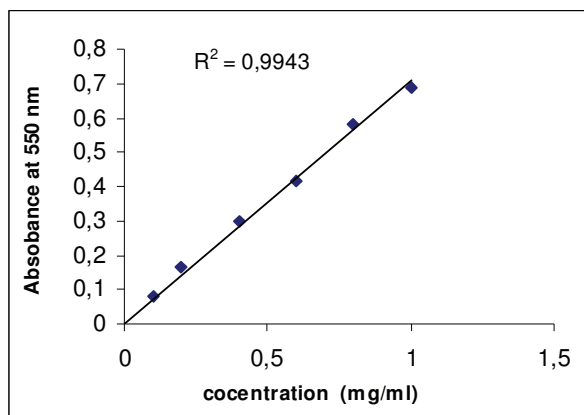
Σ SFA: sum of Saturated Fatty Acid, Σ UFA: sum of unsaturated fatty acids.

U/S: ratio Unsaturated /Saturated fatty acids.

### 3.3. Sterols content

Levels of phytosterols (ST) in vegetable oils are used for the identification of oils, oil derivatives and for the determination of the oil quality [26].

Sterol fraction was considered as the major unsaponifiable fraction in many oils. A high level of sterols were determined in both date seed oils, The Sterol contents of date seed oils were found to be between 5.41 and 7.88 mg/g which made up about 5.41 mg/g Tam seed oil, 7.84 mg/g DN seed oils, and about 7.88 mg/g Tam seed oil (Table 3). The sterol content was in the range of other vegetable materials; Corn (23 mg/g), Soybean (9 mg/g), Rapeseed (5 mg/g) and Coconut (0.8 mg/g) [27]. The Sterol content was higher in the other Tunisian species of date seeds [28].





**Fig. 4.**Standard graph of cholesterol**Table 3:** Sterol content of date seeds oils

Cultivars	Sterol (mg/g)
DN	7.84
Gh	7.884
Tam	5.417

#### 4. Conclusion

Three date palm (*Phoenix dactylifera* L) seed samples originating from Ouargla region were analyzed, and the characteristics and constituents of the seed oils were determined, including physico-chemical properties, specific gravity, refractive index, Saponification value, Acid value, and Iodine value. Total fatty acid composition was determined by gas liquid chromatography (GLC), The amount of sterols has been determined by a simple spectrophotometric method. The study of lipid profiles of date seeds oils may help their industrial application. Thus, waste products, such as seeds, from date industry, could serve as a source of edible oil. These results indicate that date seed oil could be used in cosmetics, pharmaceuticals and food

#### References

- [1]Sabah A. A. Jassim and Mazen A. Naj; In vitro Evaluation of the Antiviral Activity of an Extract of Date Palm (*Phoenix dactylifera* L.) Pits on a *Pseudomonas* Phage, *eCAM* (2007)1-6.
- [2]FAO (2009); Statistical Databases; <http://faostat.fao.org>, accessed March 3, 2009.
- [3]M.S. Rahman , S. Kasapis, N.S.Z. Al-Kharusi, I.M. Al-Marhubi and A.J. Khan ; Composition characterisation and thermal transition of date pits powders, *Journal of Food Engineering* 80 (2007) 1–10.
- [4]Al-Qarawi A.A., Abdel-Rahman H., Ali B.H., Mousa H.M. and S.A. El-Mougy; The ameliorative effect of dates (*Phoenix dactylifera* L.) on ethanol-induced gastric ulcer in rats, *Materials and methods Journal of Ethnopharmacology* 98 (2005) 313–317.
- [5]Al-Asgah N.A.; Date Palm Seeds as Food for Carp (*Cyprinus carpio* L.), *J. Coll Sci, King Soud Univ*, 19 (1) (1988)59-64.
- [6]Abou-Zeid A. Abou-Zeid, Ahmed O. Baghlaif, Jalauldin A. Khan, Saleh S. Makhashin; Utilization of date seeds and cheese whey in production of citric acid by *Candida lipolytica*, *Agricultural wastes* 8 (1983)131-142.
- [7]Ishrud O., Zahid M., Zhou H. and Pan Y.; A water-soluble galactomannan from the seeds of *Phoenix dactylifera* L. *Carbohydrate Research*, 335(4) (2001).297–301.
- [8]Mohamed Ali Al-Farsi and Chang Yong Lee; Optimization of phenolics and dietary fibre extraction from date seeds, *Food Chemistry* 108 (2008) 977–985.
- [9]Besbes S., Blecker C., Deroanne C, Lognay G., Drira N.E. and Attia H.; Heating effects on some quality characteristics of date seed oil, *Food Chemistry* 91 (2005) 469-476.
- [10]Sawaya W.N., Khalil J.K. and Saf W.J.; Chemical composition and nutritional quality of date seeds, *J. Food Scie.*, 49 (2) (1984)617-619.



- [11]Besbes S.; Blecker C., Deroanne C., Drira N.E. and Attia H.; Date seeds: Chemical composition and characteristic profiles of the lipid fraction, *Food Chemistry* 84 (2004) 577–584.
- [12]Al-Wahaibi M.H., Basalah M.O. and Al-Ackhal I.E.; Chemical Composition of Some Date-Palm Seeds, *J. Coll Sci, King Saud Univ*, 16 (1) (1985)23-29.
- [13] Al-Showiman Salim S.; Chemical Composition of Some Date Palm Seeds. (*Phoenix dactylifera L.*) in Saudi Arabia, *Arab Gulf J. Scient. Res.*, 8 (1) (1990) 15-24.
- [14]El-Shurafa M. Y., Ahmed H. S. and Abou-Naji S.E.; Organic and inorganic constituent of dates palm pit (seeds), *Journal of Date Palm*, 2 (1982)275–284.
- [15]Charef M., Yousfi M., Saidi M. And Stocker P.; Determination of the Fatty Acid Composition of Acorn (*Quercus*), *Pistacia lentiscus* Seeds Growing in Algeria, *J Am Oil Chem Soc* 85(2008) 921–924.
- [16]AFNOR (1984) ; Association Française de Normalisation ; Recueil de normes françaises des corps gras, graines oléagineuse, produits dérivés, 3ème édition.
- [17]American Oil Chemists Society (AOAC) (1975). *Official and Tentative Methods*. (3rd edn), revised AOCS, Champaign, Illinois
- [18]Naudet N. and Hautfenne A. ; "Méthode Normalisée pour la Détermination des Stérols Totaux dans les Huiles Et Graisses", *Rev. Fr. Corps Gras*, 33 (1986) 167.
- [19]Barreto M. Carma; Lipid extraction and cholesterol quantification, *J. Chem. Educ.* 82(2005) 103-104.
- [20]Mehran M. And Filsoof M.; Characteristics of Date Pit Oil; *Department of food science College of agriculture Karaj, Iran* (1974).
- [21]Manuela Guderjan, Pedro Elez-Martínez and Dietrich Knorr; Application of pulsed electric fields at oil yield and content of functional food ingredients at the production of rapeseed oil , *Innovative Food Science and Emerging Technologies* 8 (2007) 55–62.
- [22]Omer Adam Omer El Tom and Abo El-Gasim Ahmed Yagoub; Physicochemical properties of Processed Peanut (*Arachis hypogaea L.*) Oil in Relation to Sudanese standards: A case study in Nyala; South Darfur State; Sudan, *Journal of Food Technology* 5(1) (2007)71-76.
- [23] Ayo R.G., Audu O.T. and Amupitan J.O.; Physico-chemical characterization and cytotoxicity studies of seed extracts of *Khaya senegalensis* (Desr.) A. Juss, *African Journal of Biotechnology*. 6 (7) (2007) 894-896.
- [24]Akinhanmi T.F. , Atasié V.N., Akintokun P.O.; Chemical Composition and Physicochemical Properties Of Cashew nut (*Anacardium occidentale*) Oil and Cashew nut Shell Liquid, *journal of Agricultural, Food and Environmental Sciences*, 2(2008)1.
- [25]Al-Wahaibi M.H. and Basalah M.O.; Fatty Acids in Seeds of Four Cultivars of Date Palm Trees, *J. Coll Sci. King Saud Univ*. 17 (1) (1986) 27-35.
- [26]Fatnassi Saloua, Nehdi Imed Eddine and Zarrouk Hedi; Chemical composition and profile characteristics of Osage orange *Maclura pomifera* (Rafin.) Schneider seed and seed oil, *Industrial Crops and Products*, 29 (2009) 1-8.
- [27]Syed Mubbasher Sabir, Imran Hayat and Syed Dilnawaz Ahmed Gardezi; Estimation of Sterols in Edible Fats and Oils, *Pakistan Journal of Nutrition* 2 (3) (2003) 178-181.
- [28]Besbes S.; Blecker C.; Deroanne C., Bahloul N., Lognay G., Drira N.E. and Attia H.; Date seed oil: phenolic, tocopherol and Sterol profiles, *Journal of Food Lipids* 11 (2004) 251–265.