# Chapter 4
# Chemical Composition of Date Pits: Potential to Extract and Characterize the Lipid Fraction


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**Abstract** Date palm *Phoenix dactylifera* L., is recognized as an oldest plant and is grown for its palatable fruit and pit in various Arab countries for centuries. Its fruit is considered as an important source of dietary carbohydrates, fibers, antioxidant compounds, definite unique profile of vitamins and minerals especially the pits are tremendous reservoirs of lipids and protein components. The considerable amount of oil fraction in date pits is not only characterized with stability but also with biological activities and potential health benefits. A variety of techniques are available in scientific literature for extraction of date pit lipid fraction demonstrated significant amount of neutral fats, high molecular weight triglycerides, and sterol contents. Fractionation of pits lipids by Gas-liquid chromatography revealed more unsaturated fatty acids (e.g. oleic acid), less saturated fatty acids (lauric acid) and average amounts of myristic, palmitic and linoleic acids. The greater oil proportion of date seeds make it efficient use as renewable resource with significant value addition to the agricultural products and most commonly recognized for cosmetics, and food products usage. Presence of bioactive substances in this oil makes it a suitable candidate for variety of nutraceuticals and value-added food products.

**Keywords** Date pits · Lipid extraction · Liquid · Saturated fatty acids · Sterols · Unsaturated fatty acids · Lauric acid · Fibers · Fractionation · Gas-liquid chromatography

## 4.1 Date Palm

Date palm usually recognized as Phoenix dactylifera is considered as an ancient (5500–3000 BCE) cultured date crop that possess nutraceutical, economical and ornamental advantages. It is most commonly cultivated in arid and semi-arid areas worldwide because of religious, traditional, ecological, and social expansion of

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M. Naushad, E. Lichtfouse (eds.), *Sustainable Agriculture Reviews 34*,
Sustainable Agriculture Reviews 34, https://doi.org/10.1007/978-3-030-11345-2_4

individuals present. Especially the Middle East and African countries are leading regions regarding date palm cultivation, production and export (Vayalil 2012). The fruit of date palm is generally considered as monocotyledonous and its tree usually found at a highness of 1500 m in well prepared drained loams. Currently, areas of many countries like Iraq, Iran, Saudi Arabia, Algeria, Egypt, Libya, Pakistan, Morocco, and Oman are famous for date palm cultivation. However, due to unique nutraceutical characteristics, date is consumed as major food item in numerous areas of Asian, Arabian, and various African countries (Assirey 2015). Date palm possess unique characteristic compared to other fruit crops due to its consumption at any of the various ripeness stages such as Rutab, Khalal and Tamar. It can be consumed both in fresh and dried form but on commercial level dried dates are mostly preferred because of their extensive shelf life. The ripening stages and choice of the varieties have a great influence on the sensory (taste, texture) nutritional and phytochemical properties that varies greatly in various regions. It was estimated that there are almost more than 5000 date varieties cultivated in various areas worldwide, among which Aseel, Dhakki, Zahidi, Majdool, Mabrook, Halawi, Lasht, Deggla and Bamy are considered as the most common ones (Khalid et al. 2016, 2017a, b).

## 4.2 Development of Date Pit as Value Added Product

There is a growing trend for cultivation of date palm across the globe due to increased utilization of date palms by the different food processing industries. Rising demands for dates and its products enhanced its production that touches to an amount of about 7.2 million tons in 2016, (Al-Alawi et al. 2017) and this accounts almost 720,000 tons of date seeds (10% of total datet palm). Moreover, about 1.3 billion tons of foodstuff and products waste formed annually all through the supply chain could fed almost two billion persons devoid of any detrimental effect on atmosphere as significantly recognized by FAO (Rodrigo et al. 2014). Nowadays, food waste is considered as “one of the great paradoxes of our times”, and it is the best deteriorating resources to bring into account for producing various other valuable food products. Various research studies demonstrated the use of different food industry waste in formulation of value added products and could also efficient source of economic profits for industry, farmers, food safety and product sustainability. In past, due to insufficient research on date pits, these are mostly used as feed for livestocks or as fertilizers. Today in modern times, extensive scientific research on date pits and their extracts make it possible to open up new avenues for its use. Modern scientific literature on date pits indicated wide range of applications in food, cosmetics and pharmaceutical products (Salah et al. 2012). For instance, date flesh and pits can be used to make nutritious granola bar that could be a great source of dietary fibers, antioxidants and other nutrients (Abu-Qaoud 2015; Ahmad et al. 2017).

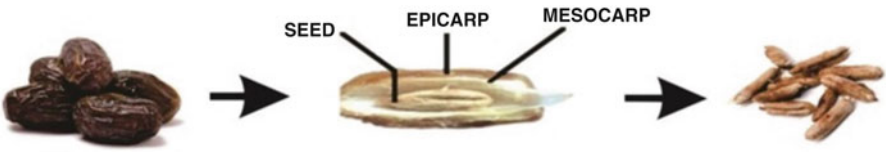


**Fig. 4.1** Date palm and seed parts

Generally, the date palm fruit is comprising of fleshy pericarp and pit or seed (Fig. 4.1). Besides date flesh, seeds are recognized to possess high extractable value-added constituents. Various research studies on chemical composition of dates proved that pits are largely wasting byproducts of industries due to their biological and technological transformation. Consequently, a larger proportion of date palm pits might effortlessly be collected from various date based treating productions or by employing their wasted byproducts (Hossain et al. 2014; Khalid et al. 2017b).

Date pits often underutilized and considered as low value agricultural by-products. Their actual potential was not discovered in last century, now it is considered as good source of nutritional components having vast applications in products formulation. For example, date pits are considered as a good precursor for the formulation of carbon, used as most popular and effective adsorbent in various industries (Yaish and Kumar 2015). It is also good in galactomannan, glucomannan and heteroxylan that had been extracted and characterized from date seeds. Ample amounts of carbohydrates (62.90%), crude fiber (19.99–39.66%) and lipids (7.87–9.76%) are also available in date pits. (Al-Humaid et al. 2010; Khalid et al. 2016). However, nowadays major consideration was given towards the lipids or oil extraction of date seeds due to their potential industrial and biological applications. Some studies demonstrated a diversified physiochemical configuration and fatty acid description in date pit oils. Date seeds oil was found to possess higher amounts of oleic acid (47.66%) and lauric acid 17.39%, however linoleic (10.54%), palmitic acid (10.20%) and myristic acid (10.06%) were also found in moderate to low levels (Elbasheer et al. 2012; Khalid et al. 2017a).

## 4.3 Nutritional Composition of Date Flesh and Pits

Date fruits and pits are recognized as enriched with various nutritional characteristics. The nutritive configuration of date palm flesh and pit has been described by various research studies. It was found that dates flesh and seed contains higher amounts of reducing sugars in addition to other amino acids, proteins and fats. Whereas, the date pits possess greater proportions of proteins, crude fat and crude fiber as compared to date flesh (Khalid et al. 2017a, b). Recent studied specified the

proximate configuration of date flesh and pits and depicted the presence of moisture, ash, glucose, fructose, galactose and maltose, protein, fats and fibers in date pits and flesh. The details of these chemical constituents are as under:

### 4.3.1 Carbohydrates

Date fruits are considered as an energy dense food because of their rich carbohydrate constituents. Glucose and fructose are among the chief reducing sugars while sucrose as non-reducing and other polysaccharides such as starch and cellulose contribute as minor carbohydrate composition. The content of glucose, fructose and sucrose in date flesh varies and mainly depend upon date varieties, maturity stages and moisture contents. Studies suggested that date sugar content increases during Kimri, Khalal and Tamar stages, as the total soluble solids and sugars significantly increases with reduction in moisture levels (Abbès et al. 2011). Carbohydrate content gain special importance in date fruits as it is regarded as significant characteristic of both fresh and dried fruits on commercial basis. Sugar content comprises 50–80% of total date weight as 1 kg of dates provides 3000 calories. Dates are classified as sweeter or less sweet on basis of reducing or non-reducing content of dates. Comparing with date flesh, date seeds or pits comprises of almost 83.0% of carbohydrates among which, 42% of cellulose, 8% of hemicellulose, 25% of total sugar and other components are present (Ahmed and Theydan 2012). Date seeds signify almost 10–15% of total date fruit of which major contributors are insoluble fiber type carbohydrates (cellulose, hemicellulose). Similarly, carbohydrates are considered as major storage reservoirs in date seed endosperm mostly in (1 → 4) β-D mannan form. Several other hemicellulose portions that were recognized in date pits are mainly comprises of water-soluble gluco- and galacto-mannans and an alkali soluble heteroxylans. An alkali-soluble heteroxylans in date pits are generally comproses of xylose (82%) and 4-O-methylglucuronic acid (17%) with minor quantity of arabinoses and very few traces of galactose, glucose and mannose contents (Assirey 2015).

### 4.3.2 Dietary Fibers

Date palm kernel or seeds are good source of total, soluble and insoluble dietary fibers. Dietary fiber in date flesh can be characterized as insoluble fiber that comprises of cellulose, hemicellulose, lignocellulose, lignin, and insoluble proteins. Dietary fiber signify almost 6.4–11.5% of total date weight of which insoluble fiber ranges between 84% and 94%. However, the cellulose, hemicellulose, and lignin contribute 1.55%, 1.28%, and 2.01% of total date fiber, respectively (Breil et al. 2016). The nutritional constituents of date especially fiber greatly varies with

different varieties and ripening stages. For example, the total fiber content of Deglet Nour is 8.09% and in Kentichi 20.25%. It significantly rises during kimri stage (6.1–12.3%) compared to the tamer stage (2.01–3.10%) of maturity. Not only date flesh but seeds are also regarded as excellent source of dietary fibers 77.8–80.2 g/100 g that constitute 10–20% of fresh date weight (Al-Daihan and Bhat 2012). The dietary fiber content of date pits has higher concentrations than date flesh. For example, Ajwa date palm has TDF constituents ranges between 6.20% and 8.70% while the Ajwa date pit has total dietary fiber of 26.4–33.9% (Khalid et al. 2017b). The various studies conducted on chemical analysis of dietary fiber demonstrates some important functional properties in food industry greatly associated with date fiber. For example: possession of higher water-binding capability, higher oil-holding capacity, emulsifying properties, pseudoplasticity behavior of suspensions, and gel formation. Moreover, to improve the nutraceutical properties in food products, dietary fiber can extensively be employed as an important constituent in many foodstuffs (dairy, soup, meat, bakery products and jams) generally recognized to alter texture related properties and enhance the high fat foods stability (Saafi-Ben Salah et al. 2012; Khalid et al. 2016).

### *4.3.3 Protein*

The protein and fat contents of dates gain special importance due to their high concentrations and biological values. Dates provide almost 1–7% of total protein content and essential amino acids that play important role in human body. Date protein content deliver most important 16–20 types of amino acids, that are not even existing in fruits (orange, apples and banana) and other products. The most important amino acids present in date palm cultivars comprises of histidine, lysine, arginine, aspartic acid, threonine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine (Samad et al. 2016). Moreover, dates isoleucine content is 800 times greater than apple and 5000 times greater lysine content than oranges. Not only date flesh but date seeds also contains considerable amount of protein constituent. Soluble proteins like albumin, globulin, prolamine and glutelin constitute main portion of date seeds that is 5–6% of total date proteins (Zhang et al. 2013).

Majority of essential and sulphur containing amino acids (methionine, cystine) comprises relatively considerable amount of date seed proteins compared to other seed protein products (soybean, peanuts, cottonseed). Glutamic acid, aspartic acid and arginine specified almost half of the total amino acids that are extracted from Ruzeiz and Sifri cultivars. Lysine is present in greater concentrations whereas the tryptophan is the only amino acid that is present in limited amounts in date seed fractions (Akasha et al. 2012). A brief composition of date flesh and pits are demonstrated in Table 4.1.

**Table 4.1** Nutritional composition of date palms and pits

| Components | Date flesh % | Date pits % | References |
|---|---|---|---|
| Moisture | 9.7–17.7 | 8.6–12.5 | Sadiq et al. (2013) and Parvin et al. (2015) |
| Lipids | 0.5–3.3 | 5.7–8.8 | Al-Orf et al. (2012) and Assirey (2015) |
| Protein | 1.1–3.0 | 4.8–6.9 | Deng et al. (2012) |
| Ash | 1.4–2.0 | 0.8–1.1 | Saleh et al. (2011) and Paranthaman et al. (2012) |
| Dietary fiber | 5.9–18.4 | 67.6–74.2 | Basuny and Al-Marzooq (2011) |
| Carbohydrates | 72.8–85.4 | 2.4–4.7 | El Arem et al. (2014) and Baliga et al. (2011) |
| Energy | 357.95 | 399.69 | Eid et al. (2013) and Agboola and Adejumo (2013) |

### *4.3.4 Minerals and Vitamins*

Dietary minerals are recognized as indispensable chemical constituents which are vital for numerous human functions like skeletal structure maintenance, cellular and biochemical reactions and functions. Hence the adequate amount of these minerals is necessary for optimum growth and maintenance of human physiological and metabolic functions. Regarding their importance dates fruit (flesh and pits) could highly considered as excellent sources of mineral constituents. Several studies reported that potassium content in date flesh of several varieties could be as higher as 0.9%. However, the most important minerals in date fruits are Ca, K, P and Mn (Kumar et al. 2014; Khalid et al. 2016). Dates are also preferred to various other fruits because of their high content of potassium, phosphorus and iron. Several studies demonstrated the higher percentage of iron content as compared to zinc and copper which is considerably present in lower amounts in date fruit. The beneficial fluorine content of date flesh has advantageous affects for preventing tooth decay. Similarly, selenium play important role in cancer prevention, providing antioxidant properties and improvement of human immune system (Sun et al. 2013). Date seeds are also found to be enriched with greater amounts of various minerals like Mg, Ca, P, Na, K, Al, Cd, S and Pb in various proportions. Additionally, in some of the varieties potassium contribute 0.5% of total date seed minerals. Whereas iron, manganese, zinc and copper confer major concentrations regarding micronutrients. Various studies stated that the major differences in the minerals content of date flesh and seeds are due to various date varieties, soil type and quantity of fertilizer. As the Saudi Arabian dates comprises of minor quantity of phosphorus ranges (0.19–0.26%) whereas the selenium component contribute 1.48–2.96 mg/g of total minerals proportion compared to other date varieties (Habib and Ibrahim 2009).

The important vitamins like vitamin A (exist as β- carotene or provitamin A), vitamin C (ascorbic acid), vitamin B1 (thiamine), niacin and riboflavin are reported in date fruits. Among various date cultivars Ajwa dates comprises of relatively high provitamin A and vitamin C content. Due to these important vitamin components, dates are regarded as an ideal food because they proposed important nutritional components with significant potential health benefits (Habib et al. 2014).

### *4.3.5 Lipids*

The lipid contents are regarded as most important components of date's fruits and gain special importance due to their nutritional significance. Although the date flesh comprises of only a small fraction of fat contents but date seeds are considered as much more concentrated source of lipids. Previous research studies demonstrated the narrow range of lipid contents in different date varieties but significantly varies along with maturity stages. For example the fat content generally declines from 0.5% to 0.1% from the Kimri to the Tamer maturity stages. The total lipid content in date seeds ranges from 5.05% to 12% while the oil contents that are 30–45% of total seed lipids have potential applications at industrial and pharmaceutical level (Al-Humaid et al. 2010; Akbari et al. 2012).

#### 4.3.5.1 Free Fatty Acids

The most important fatty acids determined in date pits oil were comprising of oleic (C18:1), linoleic (C18:2), palmitic (C16:0), myristic (C14:0), and lauric (C12:0) acid that altogether contribute almost 90–95% of the total fatty acids. However, most of the fatty acids profile composition described in oil of the date pits may significantly differs among various varieties, and along with climatic and growing conditions (Amani et al. 2013). Similarly, among the major SFA (saturated fatty acids) possess by date–pits are: lauric acid (0.10–38.71 g), myrsitic acid (3.11–18.13 g), palmitic acid (0.41–15.19 g), and stearic acid (1.56–6.04 g) are present in greater concentrations. Likewise, palmitoleic acid (0.07–1.51 g) and oleic acids (32.15–54.10 g) are the chief MUFA (monounsaturated fatty acids) while linoleic acids (4.32–21.10 g) and linolenic acids (0.02–1.68 g) are the major PUFA (polyunsaturated fatty acids) but are present in relatively lower amounts (Akbari et al. 2012). Regarding the beneficial fatty acid profile, the date seed oil usually has low degree of unsaturation that exhibited good flavour and frying stability. Oleic acid in date pits as dominant component could considered advantageous to human health owing to its low saturated level and high potential to decrease LDL cholesterol along with greater oxidative constancy although the linoleic acid and linolenic acid is essential regarding growth of healthy human skin yet these posses less oxidative stability (Farooq et al. 2013).

#### 4.3.5.2 Sterols

The studies based on GC analysis of date pits oil demonstrated the extraction of trimethylsilyl derivatives of sterols. The major sterol components determined comprises of cholesterol, campesterol, stigmasterol, β-sitosterol, avenasterol and stigmastadienl. Moreover, the β-sitosterol and campesterol were among the most important constituents of date seed oils that contribute almost 90% of total sterol

fractions. Among which β-sitosterol add 83.31% and campesterol 9.10% of total sterols in seed oils. In some date varieties avenasterol is comparatively higher than other sterols e.g. Allig and Deglet Nour seed oil contains 4.50 against 0.45% of other sterols and greatly related with antioxidant effects (Al-Mssallem et al. 2013). Phytosterols was also found ranges from 0.50% to 0.90% for Allig and Deglet Noor varieties respectively. Phytosterols from date seed oils along with dietary fibers of date seed have a capacity to significantly lower the total and LDL cholesterol levels in hyperlipidemic individuals by preventing the intestinal cholesterol absorption. Despite this, date seed oil sterols can be highly beneficial for use as therapeutic agents for hypercholesterolemia treatments (Nigam and Singh 2011).

## *4.3.6 Antioxidant Compounds*

### 4.3.6.1 Phenolic Contents

Date pits are considered as an excellent reservoirs of phenolics constituents (3112–4420 mg gallic acid) and antioxidants (570–919 μmol) (Ahmad et al. 2012). Various studies reported that comparing with various other edible oils (olive oil) date seed oils are regarded as rich source of phenolic contents (21–62 mg gallic acid) when extracted with numerous solvents (acetone–water, ethanol–water, methanol–water). Among several date varieties, Iranian date seeds had comparatively highest antioxidant and radical scavenging activity that could be employed for various pharmaceutical and commercial purposes (Suresh et al. 2013). Among various date seed phenolics gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, m-coumaric acid and o-coumaric acid were recognized to be present in variable concentrations. While the hydroxybenzoic acid (9.89 mg), protocatechuic acid (8.84 mg), and m-coumaric acid (8.42 mg) were the phenolic constituents that contribute major antioxidant potential of date seeds (Waly et al. 2015).

### 4.3.6.2 Flavonoids

The flavonoids are regarded to be most abundant phenolic constituents present in date fruits. These polyphenols are mostly existing in date flesh with enormous health advantages such as antioxidant as well as radical scavenging activities. Due to antioxidant potential these phenolic compounds are operative in reducing cardiovascular and chronic diseases with positive response towards damaged cells proliferation. Date fruit is enriched with various flavonoids that mostly comprises of quercetin, isoquercetin, and rutin and can be determined using HPLC technique (Hossain et al. 2014; Kaleem et al. 2016). The various antioxidant flavonoids vary within different varieties same is true for date palm kernel or pits. However, the total flavonoids contents of Ajwa date palm is 2.79–4.35 mg/100 g whereas the date pit

consists of 1.35–3.67 mg/100 g of quercetin content as predominant flavonoid. Similarly, the various amounts of these compounds significantly differ among pits and flesh of date fruits due to variations in genetic considerations, experimental situations, and hydration levels. Phytochemicals (phenolics and flavonoids) in date fruit have strong antioxidant potential and possess major health advantages like reducing diabetes, prevention of cancerous cells, and cardiovascular disorders. The therapeutic potential of date pits flavonoids is comparable to other fruits flavonoids in combating microbial and viral infections (Biglar et al. 2012; Ahmad et al. 2015; Khalid et al. 2017a).

## 4.4 Extraction of Lipids from Date Pits

There are different methods employed for lipid extraction from date pits either using different solvents like hexane and petroleum ether or by using microwave and ultrasonic extraction. However, solvent extraction methods consume more time with high usage of solvents. Modern technologies make use of techniques like microwave and ultrasound extraction for optimum extraction of lipids. Thus more beneficial in terms of time, cost reduction, solvent consumption and power effectiveness. These methods could also improve stability of compounds to be extracted, process simplification, and quality and quantity of extraction and extract respectively. The total lipids or oil extracted through these methods could possess better oxidative stability than most of the vegetable oils even used in comparison with olive oil. Due to its unique characteristics date pit oils possess wide variety of applications in pharmaceuticals, cosmetics, and food products (Jassim and Naji 2010). Some other methods used for lipids extraction of date pits are explained as follows:

### *4.4.1 Folch Method*

Combination of various organic solvents have been suggested to use for extraction of lipids from date pits and other sources. Folch method is one of this kind of method based on extraction of fats by employing mix of solvents. Lipids from the endogenous cells are extracted using chloroform–methanol (2:1 by volume). In detail, the one-fourth of the saline solution was equilibrated with the normal homogenized cells with efficient mixing. The resulted mixture formed as a result of this combination was placed for some time for the separation of two layers and as a result lipids settled down in the top layer phase. This method was introduced in past for lipids extractions and after some modifications it formed the basics of all extraction procedures. The same procedure with some amendments is still employed for the assessment of date pits lipid extraction spectrophotometrically. Usually, the large number of samples are rapidly and easily processed by using this method. However, it lacks sensitivity in results in comparison with other modern processes (Ullah et al. 2011).

### 4.4.2 Bligh and Dyer Method

Bligh and Dyer method is another useful technique for lipid extraction and partitioning, protein exist at interface of two liquid phases in this technique. This method also have some similarity to Folch method, but the only difference is found between solvent-solvent and solvent-tissues ratio. During this process lipids from standardized cells suspension were extracted by employing 2:1 (v/v) methanol-chloroform (Breil et al. 2017). Then the desired lipids are extracted using chloroform phase and processed by numerous procedures. However, the above gravimetric method is most extensively employed by the date pits processors for lipids and oil estimation on commercial level. There are some basics improvements which have been made in order to modify this method. The major kind of modification is the prevention of acidic and denatured lipids binding by the addition of 1 M sodium chloride instead of water. Also the basic reason behind the preference of this method over others is that during lipid extraction from date pits, further addition of 0.2 M phosphoric acid and hydrochloric acid (Reis et al. 2013) to the NaCl solution resulted in progressive lipid recovery with very short time duration. Correspondingly, another kind of modification is the accumulation of 0.5% acetic acid to the water phase which significantly resulted in increased reclamation of acidic phospholipids. A more recent report suggested that such type of lipid extraction was verified to be the most proficient method for the date pits lipids extraction (Yao and Schaich 2015).

### 4.4.3 Ultrasonic-Assisted Extraction

The Ultrasound assisted extraction is considered as substitute to other lipid extraction methods, without problems that are associated with other conventional lipid extraction methods of cell disruption. This method is regarded as more simple that may result product of high purity. This technique offer an ecofriendly, economical, less time consuming, and product with greater quality. Comparatively, lower temperatures adopted in this technique ensure lower energy inputs (Chemat and Khan 2011). The ultrasonic waves generated in this process cause quick cycles of compression/decompression, resulted in the production of extremely confined power waves, that disrupts lipid containing cells in date pits. Sonication with high frequency waves resulted in cracking of cell membrane for lipids fractionation. The sonicators that are most commonly used during this process are hot and bath types that are most commonly employed for batch as well as for continuous operations. In the bath type processors, usually the shape and capacity of vessel determines the quantity and organization of transducers (Lee et al. 2012). The basic benefit related to this procedure as compared to microwave reactors is its formation and generation of low temperature and energy, thus resulted in less denaturation of heat sensitive lipid molecules. Additionally, the beads and chemical substances does not required

to be excluded in the end time process that cast off additional cost. Though, extended sonication some time resulted in free radical formation, that may be detrimental to the extracted oil quality (Hosikian et al. 2010).

### 4.4.4 Microwave Extraction Method

Earlier, the microwave radiations applications were only restricted to samples digestion for estimation of metals and organic contaminants extraction. The microwave technology has greatly recognized as quick, safe and cost effective method for lipids extraction (Kim et al. 2012). In this method usually polar material or dielectric is introduced in electric field generated by microwaves, resulted in heat release due to various frictional forces among inter and intra molecular surfaces. During intracellular heating in this method, high energy water vapors formed, resulted in disruption of lipids containing cells, opening of cell membranes thereby intercellular lipid metabolites are extracted (Amarni and Kadi 2010). Consequently, the quick pressure and heating system generated within biological structure, forced compounds out from cell matrix, significantly resulted in best-quality extracts production along with recovery of better lipids compounds. It was suggested that microwave treated lipids cells of date pits possess greater oil yields, because of numerous cell wall cracks, resulted in extraction and transesterification of oils into biodiesels (Ghasemi et al. 2016).

The microwave extraction method is preferred compared to other methods because of its low equipment cost, less reaction time, small operating cost involved, and efficient extraction. This method is demonstrated to be more suitable for biodiesel recovery from reaction mixture in a very short time of 15–20 min, as compared to conventional time consuming (6–7 h) heating method. Conversely, the drawback associated with microwave method is the high cost involved on commercial level applications (Lee et al. 2010).

### 4.4.5 Nile Red Fluorescence Extraction of Lipids

The Nile red is considered as an intense fluorescent lipophilic dye highly employed to determine the intracellular lipids quantity in algae, selective novel herbs and date pits. Generally the efficiency of Nile red is effected by various cell membrane diffusion levels and greatly depends on the time required for highest fluorescence emission (Sitepu et al. 2012). Moreover, the Nile Red is recognized as the most appropriate method of oil extraction and estimation, highly used to determine the accumulated lipids through fluorescence microscopy after the accumulation of Nile Red in cellular lipids. This method is more easy, simple, modernized, efficient and preferred, that used fluorescence plate reader for lipids quantification and highly recognized for most of the lipids containing seeds including date pits, algae and

yeasts (Rumin et al. 2015; Takeshita et al. 2015). Similarly, several studies demonstrated that this method provides more stable values by using 20% dimethyl sulfoxide during its growth phase. However, the fluorescence power capabilities highly reduced with enhancing dimethyl sulfoxide concentrations. This method demonstrated to added up more lipids fraction with passage of time using sodium chloride solution. Conversely, the penetration of Nile Red across the cell membrane highly depends on process conditions and type of cell for lipids extraction (Hounslow et al. 2017). Generally, the fluorometric process does required lipid to be extracted; but for the fluorometric dye penetration through the material is necessary. This method is also used for algal and yeast lipids extraction but for oilseeds like date pits, its efficiency is highly enhanced by adding dimethyl sulfoxide and Nile Red along with acetone (Natunen et al. 2015).

### *4.4.6 Expeller Press Oil Extraction*

Expeller press is considered as one of the oldest and simplest oil extraction method from raw seeds. This conventional technique is equally effective in case of dried dates seeds (Demirbaş 2008). The oil extraction is proportional to higher mechanical pressure that is applied to date seeds mass to break down the cell structure, then to rupture them for lipid extraction. Higher extraction efficacy can only be achieved by applying pressure in a specific range, too much high pressure effect the lipids quality due to accumulation of high heat and also cause obstruction problems (Boldor et al. 2010). This technique can be combined with use of chemicals/solvents for enhanced recovery. The limitations with these extraction technique is the longer time of extraction and initial high costs. Additionally, for mechanical pressing, input material of low moisture content is desirable that require additional drying and thus incur extra energy and production costs (Ranjith et al. 2015). Another important drawback during date seed oil extraction is hindrance of oil due to rigid cell wall structure that is mostly not happens in most of the vegetables oil solvent extraction. By adopting mechanical expression process, several natural pigments also get their way into the crude oil that have to remove during refining process by using either solvent extraction or carbon adsorption method, that yet again responsible for additional cost. Other problems associated with this type of method comprises of higher energy and maintenance costs, skilled labors requirement, and less efficiency in comparison with other methods (Johnson and Wen 2009).

### *4.4.7 Bead Beating Extraction*

Another mechanical method of cell disruption most commonly used is bead beating, where cell is directly damaged by high rated spinning of seed extract slurries along with quality beads. All cell types including date seeds could be processed by cell

disruption using grinding beads opposite to seed cells. Moreover, due to usage of multiple shaking vessels in a bead mill with vibrant platform, such kind of bead extraction method is regarded as best for seed samples that required same handling settings for disruption. (Lee et al. 2012). However, improved oil extraction efficiencies could be acquired, when beads were agitated along with cell culture. The cooling jacket often used inside the mill to protect the heat sensitive cell molecules, and also to minimize the heat generated during entire process. Numerous types of beads are usually employed for varied cell categories, however the beads made up of zirconium oxide, or titanium carbide could significantly increase the distraction rates and extraction efficacy of date seeds probably due to their greater density and hardness (Šoštarič et al. 2012). The overall comparison of all lipids extraction methods is demonstrated in Table 4.2.

### *4.4.8 Superior Solvent Extraction Methods*

In this type of solvent extraction method usually chloroform is used as the main lipid extracting solvent that enhances its effectiveness and efficiency. However, on commercial level during large-scale lipids extraction employing these procedures is excluded due to ecological and health threats. Consequently, many researchers have been developing the least-toxic, but more operative, alternatives such as by using ethanol, isopropanol, butanol, acetic acid esters, hexane, and numerous solvents in combinations for date pitsoil extraction (Sheng et al. 2011). Moreover, the usage of above described solvents highly dependent on the lipids classes required to be extracted. Though, in a latest research studies, the extracted lipid fraction was highly recovered by using 2-ethoxyethanol (2-EE) in comparison with other common solvent extraction methods (chloroform, methanol and hexane) (Jones et al. 2012). A general description of date pit oil extraction steps along with solvent fractionation is presented in Fig. 4.2.

To further improve the efficiency of extraction process, ASE (accelerated solvent extraction) process, employing heat and pressure, was introduced. This method highly shortens the extraction time, and thus resulted in efficient solvent recovery for re-usage, thus minimize the operating costs. Recently, most of the modern solvent extraction as well as combined solvent/physical extraction methods are being developed for lipid extraction by various researchers all over the world. Although, the organic solvent extraction methods have possess various disadvantages when executed on commercial level (Cooney et al. 2009).

### *4.4.9 Extraction of Lipids Classes*

The better and efficient method was developed by (El-Sharnouby and Al-Eid 2009) for extraction of various lipid classes, that is a modified form of the Folch/Bligh and

**Table 4.2** Comparison of different methods for lipid extraction from date pits

| Method | Efficiency | Cost involved | Energy requirements | Safety concerns | References |
|---|---|---|---|---|---|
| Use of organic solvents (chloroform/ methanol) | Moderate | High due to use of solvents | Energy intensive | Health and environmental hazards | Habibi-Najafi (2011) and Wang et al. (2013) |
| Pressurized solvent extraction | High | High because of solvent used and pressurized nitrogen | High energy requirements | Environmental and regulatory issues | Juhaimi et al. (2014) and Al-Mssallem et al. (2013) |
| Isotonic extraction | Moderate high | Higher cost of solvents | Energy intensive | Less hazardous | Kelley et al. (2015) and Al-Harrasi et al. (2014) |
| Expeller pressing | Low moderate | Higher cost | Energy intensive | High heat generation and possible damaged to compounds | Sadiq et al. (2013) and Orabi and Shawky (2014) |
| Bead beating | Moderate | Cost-effective | Energy intensive, high pressure usage | Difficult to scale up | Halaby et al. (2014) and Bonsegna et al. (2011) |
| Microwave | Very high | High maintenance and initial setup cost | Too high energy demands (for cooling) | Easy to scale up, but yet need to be standardized at commercial level | Camus et al. (2013) and Chang et al. (2015) |
| Sonication method | High | High cost | Energy intensive –energy requires for cooling and sonication | Poor product quality due to the loss during the process | Filipe and McLauchlan (2015) and Umate (2012) |
| Electroporation | Very high | High maintenance cost | Less energy | Favorable but detailed pilot-scale studies need to be carried out | Wahlroos et al. (2015) and Besbes et al. (2004) |

Dyer method. This procedure provides improved recovery of nearly all chief lipid classes and is based on using MTBE(Methyl-tert-butyl ether) as a solvent, and offers the most precise lipidemic profile. This can only be possible to acquire due to the development of a lipid-containing low density organic upper phase, that resulted in more easy and accurate lipid extractions. Briefly, 1.5 ml of methanol was mixed rigorously for 200 ml sample (vortexing), moreover after adding 5 ml of MTBE, the solution was incubated for 1 h at normal room temperature (Saura-Calixto 2012). Likewise, phase separation during the process was developed by the addition of 1.25 ml of water to the solution and permitted to stand at room temperature. The top

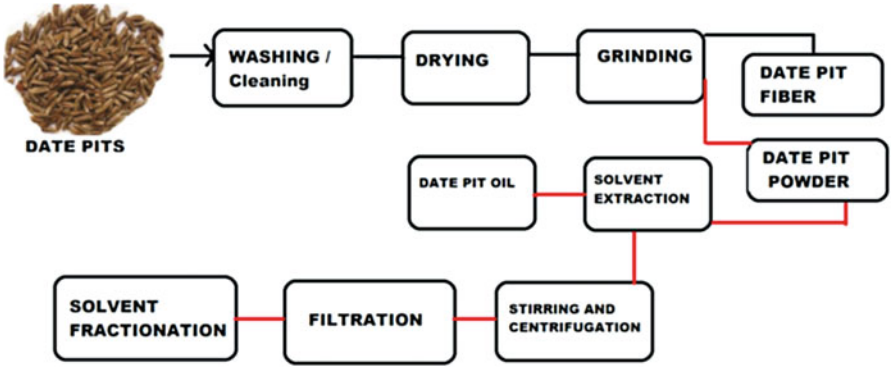


**Fig. 4.2** Extraction method for date pit oil and bioactive substances

organic phase was extracted by centrifugation and the lower phase was re-extracted by adding 2 ml of MTBE/methanol/water (10/3/2.5, v/v/v) to accomplish complete recovery of lipidic profile (Lemine et al. 2014). However, the excessive solvent remained could be easily drained off by vacuum drying of both the organic phases including lipid extract. Additionally, the resultant extracted lipids were retained for better storage by dissolving in 200 ml of chloroform/methanol/water (60/30/4.5, v/v/v) or can be directly employed for further studies (Mahmoud and El-Bana 2013). All these type of procedures are appropriate for lipid extraction from all kinds of lipid-bearing cells, containing date pits (El-Sohaimy and Hafez 2010).

### *4.4.10 Hydrolysis of Lipids by Supercritical Transesterification*

Transesterification is recognized as an efficient method to make lipids extracts in the form of solid and liquid fractions. Generally, in this method wet lipids fractions from date seeds are processed for lipids extraction followed by trans esterification. Briefly, wet lipids mass was retained in stainless steel reactors, submerged in isothermic fluidized bath for specific time followed by water cooling process (Shin et al. 2014). Continuous hydrolysis reaction between two reactors carried out, followed by drying of pits extract and again mixed with water in huge reactors and the reaction was persistent for 1 h at 250 °C. Repeatedly continuous dehydration and drying process transforms the wet lipidic biomass into compact fraction, consequently the appropriate filters were used under light vacuum conditions for efficient separation of aqueous and solids phase (Han et al. 2011). Additionally, the detailed experimental procedure was carried out for the determination of special effects resulted by reaction time, temperature, ethanol filling yield and crude biodiesel configuration. Although, the resulted seed oil was checked to verify its quality characteristics to meet the standards by using appropriate methods. Yet, the described process has to be verified for its commercial utilization for date pits (Zhao and Zhang 2013).

### *4.4.11 Hexane: Isopropanol (HIP) Lipid Extraction*

The hexane based lipid extraction method was used to quantify the lipid contents found in tissues using mixed solvent as hexane and isopropanol, followed by washing of extract and removal of non-lipid residues using sodium sulphate solution. Various studies demonstrated the use of HIP method was better and offer less residual level of solvents in final product. Gravimetric methods were usually recognized as best methods for the determination of lipids content weight directly, however the hexane-isopropanol was considered to be efficient for polar lipids extraction (Shimada et al. 2014). Moreover, the basic advantages of gravimetric methods are: total fat measurement, more quantitative process, least equipment requirements, less workers required, simpler and easier to accomplish. However, this method have several limitations including less accuracy of lipid weight, compositional analysis of fats, lowest detection level of lipids. This method require shorter time and resemble Folch method that required much shorter time for oil extraction as compared to gravimetric and soxhlet method (Murphy 2012).

## 4.5 Future Perspectives

Date seeds are considered as byproducts of various date fruit industries. Numerous studies demonstrated the potential of date seeds as a source of edible oil. In addition to oil, some other nutritional constituents like dietary fiber, protein, minerals, phenolic that present in significant amount make date seeds as an effective functional food ingredient. Hence the potential usage of date seeds in numerous industries is favorable for developing value added products. Having favorable lipids and fatty acid profile in date pits, it is recommended to use it for development of nutraceutical products, as an edible cooking oil, and for formulation of different oil based products (margarines), because of its higher oxidative stability and thermal resisting properties and have good shelf life capabilities for extensive storage time period. Furthermore, as date seed oil possess unique unsaturation degree in comparison to other vegetable oils, therefore highly recommended as a vegetable oil substitutes in many food industries.

Besides possessing wide variety of applications date seed offers great number of challenges. As there are less information regarding date seed oils, more research studies are required for determination of oil safety regarding its use as important constituent in pharmaceutical and food industry for the development of edible and non-edible products. Additionally, there is great need to meet the challenges of using suitable extraction methods from date seeds to attain the high oil production. Although date pits are also accessible at very low or without any cost, so the seed pits oil extraction could be possible and possess important worth considerations on commercial level. As the date seeds are recognized as discarded byproducts and have possess high probability to be used as ingredients for various food products

formulation, for extraction of beneficial bioactive components, water sanitization, and biomass production. However, the future research studies require to be concentrated on technological development for commercialized production and biomaterials specification for their safety concerns and functionality needs. Presently the systematic health entitlements regarding date pits are at its initial stages so more research studies are requires for establishment of their safety and efficiency. Different studies were demonstrated to extract the date pits extract using numerous solvents, though it is more essential to improve more technological advancements for oil and other bioactive components extracted compounds to be employed for cosmetics, pharmaceutical and food products.

## 4.6 Conclusion

The various functional components of Date palm *Phoenix dactylifera* L., and pits described in this chapter constitute promising alternatives to various other crops as it is recognized an oldest plant and cultured for its desirable fruit and pits in various Arab countries for centuries and also preferred because of its nutraceutical and economical benefits. Compared to date flesh, pits are highly recognized as an important source of dietary carbohydrates, proteins, fibers, antioxidant compounds, definite indispensable vitamins and minerals. Especially the lipid fraction of date pits constitute major portion along with higher amounts of beneficial oleic acid and lauric acid, however linoleic acid, palmitic acid and myristic acid were present in lower amounts respectively. The different extraction methods described here establish favorable alternates to recover date pit lipid fraction either using different solvents like hexane and petroleum ether or by using microwave and ultrasonic extraction. However, solvent extraction methods are less prefer because of excessive time consumption and usage of greater amount of solvents. Also the basic reason behind the less usage of organic solvents for oil extraction is highly related to the harmful effects associated with these compounds. Consequently, various type of modern technologies like microwave, expeller pressing and ultrasound extraction provide more benefits in terms of time and cost reduction, solvent consumption and power effectively. These methods could also improve stability of compounds to be extracted, process simplification, and quality and quantity of extraction and extract respectively.

An important drawback in the research studies of date oil extraction is the process scale-up, comprising the process cost and efficiency analysis. The most of the research studies regarding oil extraction from date seeds have been accomplished on lab scale. That out comes about a very little information regarding availability and development of this process on large scale. Consequently, additional studies should be conducted using greater amounts of date seeds biomass to analyses the oil extraction efficacy and to compared with results studies previously identified for lab-scale process.

## References

Abbès F, Bouaziz MA, Blecker C, Masmoudi M, Attia H, Besbes S (2011) Date syrup: effect of hydrolytic enzymes (pectinase/cellulase) on physico-chemical characteristics, sensory and functional properties. LWT Food Sci Technol 44:1827–1834. https://doi.org/10.1016/j.lwt.2011.03.020

Abu-Qaoud H (2015) Date palm status and perspective in Palestine. In: Date palm genetic resources and utilization. Springer, Boca Raton, pp 423–439. https://doi.org/10.1007/978-94-017-9707-8

Agboola OS, Adejumo A (2013) Nutritional composition of the fruit of the Nigerian wild date palm, Phoenix dactylifera. World J Dairy Food Sci 8:196–200. https://doi.org/10.5829/idosi.wjdfs.2013.8.2.81178

Ahmad T, Danish M, Rafatullah M, Ghazali A, Sulaiman O, Hashim R, Ibrahim MNM (2012) The use of date palm as a potential adsorbent for wastewater treatment: a review. Environ Sci Pollut Res 19:1464–1484. https://doi.org/10.1007/s11356-011-0709-8

Ahmad A, Kaleem M, Ahmed Z, Shafiq H (2015) Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections – a review. Food Res Int 77:221–235. https://doi.org/10.1016/j.foodres.2015.06.021

Ahmad A, Irfan U, Amir RM, Abbasi KS (2017) Development of high energy cereal and nut granola bar. Int J Agric Bio Sci 1(3):13–20

Ahmed MJ, Theydan SK (2012) Equilibrium isotherms, kinetics and thermodynamics studies of phenolic compounds adsorption on palm-tree fruit stones. Ecotoxicol Environ Saf 84:39–45. https://doi.org/10.1016/j.ecoenv.2012.06.019

Akasha IA, Campbell L, Euston SR (2012) Extraction and characterisation of protein fraction from date palm fruit seeds. World Acad Sci Eng Technol 70:292–294. https://doi.org/10.1999/1307-6892/3421

Akbari M, Razavizadeh R, Mohebbi G, Barmak A (2012) Oil characteristics and fatty acid profile of seeds from three varieties of date palm (Phoenix dactylifera) cultivars in Bushehr-Iran. Afr J Biotechnol 11:12088–12093. https://doi.org/10.5897/AJB12.1084

Al-Alawi RA, Al-Mashiqri JH, Al-Nadabi JS, Al-Shihi BI, Baqi Y (2017) Date palm tree (Phoenix dactylifera L.): natural products and therapeutic options. Front Plant Sci 8:845. https://doi.org/10.3389/fpls.2017.00845

Al-Daihan S, Bhat RS (2012) Antibacterial activities of extracts of leaf, fruit, seed and bark of Phoenix dactylifera. Afr J Biotechnol 11:10021–10025. https://doi.org/10.5897/AJB11.4309

Al-Harrasi A, Rehman NU, Hussain J, Khan AL, Al-Rawahi A, Gilani SA, Al-Broumi M, Ali L (2014) Nutritional assessment and antioxidant analysis of 22 date palm (Phoenix dactylifera) varieties growing in Sultanate of Oman. Asian Pac J Trop Med 7:S591–S598. https://doi.org/10.1016/S1995-7645(14)60294-7

Al-Humaid A, Mousa H, El-Mergawi R, Abdel-Salam A (2010) Chemical composition and antioxidant activity of dates and dates-camel-milk mixtures as a protective meal against lipid peroxidation in rats. Am J Food Technol 5:22–30. https://doi.org/10.3923/ajft.2010.22.30

Al-Mssallem IS, Hu S, Zhang X, Lin Q, Liu W, Tan J, Yu X, Liu J, Pan L, Zhang T (2013) Genome sequence of the date palm Phoenix dactylifera L. Nat Commun 4:ncomms3274. https://doi.org/10.1038/ncomms3274

Al-Orf SM, Ahmed MH, Al-Atwai N, Al-Zaidi H, Dehwah A, Dehwah S (2012) Review: nutritional properties and benefits of the date fruits (Phoenix dactylifera L.). Bull Nat Nutr Inst Arab Rep Egypt 39:97. https://doi.org/10.9755/ejfa.2016-01-104

Amani MA, Davoudi MS, Tahvildari K, Nabavi SM, Davoudi MS (2013) Biodiesel production from Phoenix dactylifera as a new feedstock. Ind Crop Prod 43:40–43. https://doi.org/10.1016/j.indcrop.2012.06.024

Amarni F, Kadi H (2010) Kinetics study of microwave-assisted solvent extraction of oil from olive cake using hexane: comparison with the conventional extraction. Innov Food Sci Emerg Technol 11:322–327. https://doi.org/10.1016/j.ifset.2010.01.002

Assirey EAR (2015) Nutritional composition of fruit of 10 date palm (Phoenix dactylifera L.) cultivars grown in Saudi Arabia. J Taibah Univ Sci 9:75–79. https://doi.org/10.1016/j.jtusci.2014.07.002

Baliga MS, Baliga BRV, Kandathil SM, Bhat HP, Vayalil PK (2011) A review of the chemistry and pharmacology of the date fruits (Phoenix dactylifera L.). Food Res Int 44:1812–1822. https://doi.org/10.1016/j.foodres.2010.07.004

Basuny AMM, Al-Marzooq MA (2011) Production of mayonnaise from date pit oil. Food Nutr Sci 2:938. https://doi.org/10.4236/fns.2011.29128

Besbes S, Blecker C, Deroanne C, Drira N-E, Attia H (2004) Date seeds: chemical composition and characteristic profiles of the lipid fraction. Food Chem 84:577–584. https://doi.org/10.1016/S0308-8146(03)00281-4

Biglar M, Khanavi M, Hajimahmoodi M, Hassani S, Moghaddam G, Sadeghi N, Oveisi MR (2012) Tocopherol content and fatty acid profile of different Iranian date seed oils. Iran J Pharm Res IJPR 11:873

Boldor D, Kanitkar A, Terigar BG, Leonardi C, Lima M, Breitenbeck GA (2010) Microwave assisted extraction of biodiesel feedstock from the seeds of invasive Chinese tallow tree. Environ Sci Technol 44:4019–4025. https://doi.org/10.1021/es100143z

Bonsegna S, Bettini S, Pagano R, Zacheo A, Vergaro V, Giovinazzo G, Caminati G, Leporatti S, Valli L, Santino A (2011) Plant oil bodies: novel carriers to deliver lipophilic molecules. Appl Biochem Biotechnol 163:792–802. https://doi.org/10.1007/s12010-010-9083-0

Breil C, Meullemiestre A, Vian M, Chemat F (2016) Bio-based solvents for green extraction of lipids from oleaginous yeast biomass for sustainable aviation biofuel. Molecules 21:196. https://doi.org/10.3390/molecules21020196

Breil C, Abert Vian M, Zemb T, Kunz W, Chemat F (2017) "Bligh and dyer" and Folch methods for solid–liquid–liquid extraction of lipids from microorganisms. Comprehension of solvatation mechanisms and towards substitution with alternative solvents. Int J Mol Sci 18:708. https://doi.org/10.3390/ijms18040708

Camus G, Vogt DA, Kondratowicz AS, Ott M (2013) Lipid droplets and viral infections. Methods Cell Biol 116:167–190. https://doi.org/10.1016/B978-0-12-408051-5.00009-7. Elsevier

Chang W, Zhang M, Zheng S, Li Y, Li X, Li W, Li G, Lin Z, Xie Z, Zhao Z (2015) Trapping toxins within lipid droplets is a resistance mechanism in fungi. Sci Rep 5:15133. https://doi.org/10.1038/srep15133

Chemat F, Khan MK (2011) Applications of ultrasound in food technology: processing, preservation and extraction. Ultrason Sonochem 18:813–835. https://doi.org/10.1016/j.ultsonch.2010.11.023

Cooney M, Young G, Nagle N (2009) Extraction of bio-oils from microalgae. Sep Purif Rev 38:291–325. https://doi.org/10.1080/15422110903327919

Demirbaş A (2008) Production of biodiesel from algae oils. Energy Sources Part A Recover Util Environ Eff 31:163–168. https://doi.org/10.1080/15567030701521775

Deng G-F, Shen C, Xu X-R, Kuang R-D, Guo Y-J, Zeng L-S, Gao L-L, Lin X, Xie J-F, Xia E-Q (2012) Potential of fruit wastes as natural resources of bioactive compounds. Int J Mol Sci 13:8308–8323. https://doi.org/10.3390/ijms13078308

Eid NM, Al-Awadi B, Vauzour D, Oruna-Concha MJ, Spencer JP (2013) Effect of cultivar type and ripening on the polyphenol content of date palm fruit. J Agric Food Chem 61:2453–2460. https://doi.org/10.1021/jf303951e

El Arem A, Thouri A, Zekri M, Saafi EB, Ghrairi F, Zakhama A, Achour L (2014) Nephroprotective effect of date fruit extract against dichloroacetic acid exposure in adult rats. Food Chem Toxicol 65:177–184. https://doi.org/10.1016/j.fct.2013.12.023

Elbasheer EE, Abdalla RS, Elhussein AR, Gadkariem EA (2012) Physico-chemical characteristics of date seed oil grown In Sudan. Am J Appl Sci 9(7):993–999. https://doi.org/10.3844/ajassp.2012.993.999

El-Sharnouby GA, Al-Eid SM (2009) Utilization of enzymes in the production of liquid sugar from dates. Afr J Biochem Res 3:041–047. https://doi.org/10.5897/AJBR

El-Sohaimy S, Hafez E (2010) Biochemical and nutritional characterizations of date palm fruits (Phoenix dactylifera L.). J Appl Sci Res 6:1060–1067. https://doi.org/10.11648/j.ajls.20150304.14

Farooq M, Ramli A, Subbarao D (2013) Biodiesel production from waste cooking oil using bifunctional heterogeneous solid catalysts. J Clean Prod 59:131–140. https://doi.org/10.1016/j.jclepro.2013.06.015

Filipe A, McLauchlan J (2015) Hepatitis C virus and lipid droplets: finding a niche. Trends Mol Med 21:34–42. https://doi.org/10.1016/j.molmed.2014.11.003

Ghasemi NF, González González LM, Chan W, Schenk PM (2016) Progress on lipid extraction from wet algal biomass for biodiesel production. Microb Biotechnol 9:718–726. https://doi.org/10.1111/1751-7915.12360

Habib HM, Ibrahim WH (2009) Nutritional quality evaluation of eighteen date pit varieties. Int J Food Sci Nutr 60:99–111. https://doi.org/10.1080/09637480802314639

Habib HM, Platat C, Meudec E, Cheynier V, Ibrahim WH (2014) Polyphenolic compounds in date fruit seed (Phoenix dactylifera): characterisation and quantification by using UPLC-DAD-ESI-MS. J Sci Food Agric 94:1084–1089. https://doi.org/10.1002/jsfa.6387

Habibi-Najafi MB (2011) Date seeds: a novel and inexpensive source of dietary fiber. In: ICFEB 2011. pp 62-B018

Halaby MS, Farag MH, Gerges AH (2014) Potential effect of date pits fortified bread on diabetic rats. Int J Nutri Food Sci 3:49–59. https://doi.org/10.11648/j.ijnfs.20140302.16

Han Y, Wen Q, Chen Z, Li P (2011) Review of methods used for microalgal lipid-content analysis. Energy Procedia 12:944–950. https://doi.org/10.1016/j.egypro.2011.10.124

Hosikian A, Lim S, Halim R, Danquah MK (2010) Chlorophyll extraction from microalgae: a review on the process engineering aspects. Int J Chem Engg 2010:1–11. https://doi.org/10.1155/2010/391632

Hossain MZ, Waly MI, Singh V, Sequeira V, Rahman MS (2014) Chemical composition of date-pits and its potential for developing value-added product – a review. Pol J Food Nutri Sci 64:215–226. https://doi.org/10.4236/fns.2011.29128

Hounslow E, Noirel J, Gilmour DJ, Wright PC (2017) Lipid quantification techniques for screening oleaginous species of microalgae for biofuel production. Eur J Lipid Sci Technol 119:1500469. https://doi.org/10.1002/ejlt.201500469

Jassim SA, Naji MA (2010) In vitro evaluation of the antiviral activity of an extract of date palm (Phoenix dactylifera L.) pits on a Pseudomonas phage. Evid Based Complement Alternat Med 7:57–62. https://doi.org/10.1093/ecam/nem160

Johnson MB, Wen Z (2009) Production of biodiesel fuel from the microalga Schizochytrium limacinum by direct transesterification of algal biomass. Energy Fuel 23:5179–5183. https://doi.org/10.1021/ef900704h

Jones J, Manning S, Montoya M, Keller K, Poenie M (2012) Extraction of algal lipids and their analysis by HPLC and mass spectrometry. J Am Oil Chem Soc 89:1371–1381. https://doi.org/10.1007/s11746-012-2044-8

Juhaimi FA, Ghafoor K, Özcan MM (2014) Physicochemical properties and mineral contents of seven different date fruit (Phoenix dactylifera L.) varieties growing from Saudi Arabia. Environ Monit Assess 186:2165–2170. https://doi.org/10.1007/s10661-013-3526-3

Kaleem M, Ahmad A, Khalid S, Azam MT (2016) HPLC condition optimization for identification of flavonoids from Carissa opaca. Sci Int (Lahore) 28(1):343–348

Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ (2015) The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 10:845. https://doi.org/10.1038/nprot.2015.053

Khalid S, Ahmad A, Masud T, Asad M, Sandhu M (2016) Nutritional assessment of Ajwa date flesh and pits in comparison to local varieties. J Plant Anim Sci 26:1072–1080

Khalid S, Khalid N, Khan RS, Ahmed H, Ahmad A (2017a) A review on chemistry and pharmacology of Ajwa date fruit and pit. Trends Food Sci Technol 63:60–69. https://doi.org/10.1016/j.tifs.2017.02.009

Khalid S, Ahmad A, Kaleem M (2017b) Antioxidant activity and phenolic contents of Ajwa date and their effect on lipo-protein profile. Funct Foods Health Dis 7(6):396–410. https://doi.org/10.31989/ffhd.v7i6.337

Kim Y-H, Choi Y-K, Park J, Lee S, Yang Y-H, Kim HJ, Park T-J, Kim YH, Lee SH (2012) Ionic liquid-mediated extraction of lipids from algal biomass. Bioresour Technol 109:312–315. https://doi.org/10.1016/j.biortech.2011.04.064

Kumar A, Negi YS, Choudhary V, Bhardwaj NK (2014) Characterization of cellulose nanocrystals produced by acid-hydrolysis from sugarcane bagasse as agro-waste. J Math Phys Chem 2:1–8. https://doi.org/10.12691/jmpc-2-1-1

Lee J-Y, Yoo C, Jun S-Y, Ahn C-Y, Oh H-M (2010) Comparison of several methods for effective lipid extraction from microalgae. Bioresour Technol 101:S75–S77. https://doi.org/10.1016/j.biortech.2009.03.058

Lee AK, Lewis DM, Ashman PJ (2012) Disruption of microalgal cells for the extraction of lipids for biofuels: processes and specific energy requirements. Biomass Bioenergy 46:89–101. https://doi.org/10.1016/j.biombioe.2012.06.034

Lemine FMM, Samb A, Zein el Abidine OB, Ahmed MVOM, Djeh T-KO, Boukhary AOMSO (2014) Assessment of physicochemical diversity in fruit of Mauritanian date palm (Phoenix dactylifera L.) cultivars. Afr J Agric Res 9:2167–2176. https://doi.org/10.5897/AJAR2013.8250

Mahmoud A, El-Bana H (2013) Evaluation of olive and palm byproducts in feeding camels. Pak J Nutr 12:879. https://doi.org/10.3923/pjn.2013.879.885

Murphy DJ (2012) The dynamic roles of intracellular lipid droplets: from archaea to mammals. Protoplasma 249:541–585. https://doi.org/10.1007/s00709-011-0329-7

Natunen K, Seppälä J, Schwenk D, Rischer H, Spilling K, Tamminen T (2015) Nile Red staining of phytoplankton neutral lipids: species-specific fluorescence kinetics in various solvents. J Appl Phycol 27:1161–1168. https://doi.org/10.1007/s10811-014-0404-5

Nigam PS, Singh A (2011) Production of liquid biofuels from renewable resources. Prog Energy Combust Sci 37:52–68. https://doi.org/10.1016/j.pecs.2010.01.003

Orabi SH, Shawky SM (2014) Effect of date palm (Phoenix dactylifera) seeds extracts on hematological, biochemical parameters and some fertility indices in male rats. Int J Sci Basic Appl Res 17:137–147. https://doi.org/10.3923/ajb.2014.119.130

Paranthaman R, Kumar PP, Kumaravel S (2012) HPLC and HPTLC determination of caffeine in raw and roasted date seeds (Phoenix dactylifera L). J Chromatogr Sep Tech 1:249–253. https://doi.org/10.1002/jsfa.6387

Parvin S, Easmin D, Sheikh A, Biswas M, Sharma SCD, Jahan MGS, Islam MA, Shovon M, Roy N (2015) Nutritional analysis of date fruits (Phoenix dactylifera L.) in perspective of Bangladesh. Am J Life Sci 3:274–278. https://doi.org/10.11648/j.ajls.20150304.14

Ranjith KR, Hanumantha Rao P, Arumugam M (2015) Lipid extraction methods from microalgae: a comprehensive review. Front Energy Res 2:61. https://doi.org/10.3389/fenrg.2014.00061

Reis A, Rudnitskaya A, Blackburn GJ, Fauzi NM, Pitt AR, Spickett CM (2013) A comparison of five lipid extraction solvent systems for lipidomic studies of human LDL. J Lipid Res 54:1812–1824. https://doi.org/10.1194/jlr.M034330

Rodrigo R, Libuy M, Feliu F, Hasson D (2014) Polyphenols in disease: from diet to supplements. Curr Pharm Biotechnol 15:304–317. https://doi.org/10.2174/1389201015041408251138 15

Rumin J, Bonnefond H, Saint-Jean B, Rouxel C, Sciandra A, Bernard O, Cadoret J-P, Bougaran G (2015) The use of fluorescent Nile red and BODIPY for lipid measurement in microalgae. Biotechnol Biofuels 8:42. https://doi.org/10.1186/s13068-015-0220-4

Saafi-Ben Salah EB, Flamini G, El Arem A, Issaoui M, Dabbou S, BenYahia L, Ferchichi A, Hammami M, Achour L (2012) Compositional characteristics and aromatic profile of date palm seeds from seven varieties grown in Tunisia. Int J Food Sci Technol 47:1903–1908. https://doi.org/10.1111/j.1365-2621.2012.03049.x

Sadiq I, Izuagie T, Shuaibu M, Dogoyaro A, Garba A, Abubakar S (2013) The nutritional evaluation and medicinal value of date palm (Phoenix dactylifera). Int J Mod Chem 4:147–154

Salah EBS-B, El Arem A, Louedi M, Saoudi M, Elfeki A, Zakhama A, Najjar MF, Hammami M, Achour L (2012) Antioxidant-rich date palm fruit extract inhibits oxidative stress and nephrotoxicity induced by dimethoate in rat. J Physiol Biochem 68:47–58. https://doi.org/10.1007/s13105-011-0118-y

Saleh EA, Tawfik MS, Abu-Tarboush HM (2011) Phenolic contents and antioxidant activity of various date palm (Phoenix dactylifera L.) fruits from Saudi Arabia. Food Nutr Sci 2:1134. https://doi.org/10.4236/fns.2011.210152

Samad MA, Hashim SH, Simarani K, Yaacob JS (2016) Antibacterial properties and effects of fruit chilling and extract storage on antioxidant activity, total phenolic and anthocyanin content of four date palm (Phoenix dactylifera) cultivars. Molecules 21:419. https://doi.org/10.3390/molecules21040419

Saura-Calixto F (2012) Concept and health-related properties of nonextractable polyphenols: the missing dietary polyphenols. J Agric Food Chem 60:11195–11200. https://doi.org/10.1021/jf303758j

Sheng J, Vannela R, Rittmann BE (2011) Evaluation of methods to extract and quantify lipids from Synechocystis PCC 6803. Bioresour Technol 102:1697–1703. https://doi.org/10.1016/j.biortech.2010.08.007

Shimada TL, Takano Y, Shimada T, Fujiwara M, Fukao Y, Mori M, Okazaki Y, Saito K, Sasaki R, Aoki K (2014) Leaf oil body functions as a subcellular factory for the production of a phytoalexin in Arabidopsis. Plant Physiol 164:105–118. https://doi.org/10.1104/pp.113.230185

Shin H-Y, Ryu J-H, Bae S-Y, Crofcheck C, Crocker M (2014) Lipid extraction from Scenedesmus sp. microalgae for biodiesel production using hot compressed hexane. Fuel 130:66–69. https://doi.org/10.1016/j.fuel.2014.04.023

Sitepu I, Ignatia L, Franz A, Wong D, Faulina S, Tsui M, Kanti A, Boundy-Mills K (2012) An improved high-throughput Nile red fluorescence assay for estimating intracellular lipids in a variety of yeast species. J Microbiol Methods 91:321–328. https://doi.org/10.1016/j.mimet.2012.09.001

Šoštarič M, Klinar D, Bricelj M, Golob J, Berovič M, Likozar B (2012) Growth, lipid extraction and thermal degradation of the microalga Chlorella vulgaris. New Biotechnol 29:325–331. https://doi.org/10.1016/j.nbt.2011.12.002

Sun X, Lu C, Zhang W, Tian D, Zhang X (2013) Acetone-soluble cellulose acetate extracted from waste blended fabrics via ionic liquid catalyzed acetylation. Carbohydr Polym 98:405–411. https://doi.org/10.1016/j.carbpol.2013.05.089

Suresh S, Guizani N, Al-Ruzeiki M, Al-Hadhrami A, Al-Dohani H, Al-Kindi I, Rahman MS (2013) Thermal characteristics, chemical composition and polyphenol contents of date-pits powder. J Food Eng 119:668–679. https://doi.org/10.1016/j.jfoodeng.2013.06.026

Takeshita T, Takeda K, Ota S, Yamazaki T, Kawano S (2015) A simple method for measuring the starch and lipid contents in the cell of microalgae. Cytologia 80:475–481. https://doi.org/10.1508/cytologia.80.475

Ullah SR, Murphy B, Dorich B, Richter B, Srinivasan K (2011) Fat extraction from acid-and base-hydrolyzed food samples using accelerated solvent extraction. J Agric Food Chem 59:2169–2174. https://doi.org/10.1021/jf104001d

Umate P (2012) Comparative genomics of the lipid-body-membrane proteins oleosin, caleosin and steroleosin in magnoliophyte, lycophyte and bryophyte. Genomics Proteomics Bioinformatics 10:345–353. https://doi.org/10.1016/j.gpb.2012.08.006

Vayalil PK (2012) Date fruits (Phoenix dactylifera Linn): an emerging medicinal food. Crit Rev Food Sci Nutr 52:249–271. https://doi.org/10.1080/10408398.2010.499824

Wahlroos T, Soukka J, Denesyuk A, Susi P (2015) Amino-terminus of oleosin protein defines the size of oil bodies-topological model of oleosin-oil body complex. J Plant Biochem Physiol 3:155. https://doi.org/10.4172/2329-9029.1000155

Waly MI, Al-Ghafri BR, Guizani N, Rahman MS (2015) Phytonutrient effects of date pit extract against azoxymethane-induced oxidative stress in the rat colon. Asian Pac J Cancer Prev 16:3473–3477. https://doi.org/10.7314/APJCP.2015.16.8.3473

Wang W, Wu Z, Dai Z, Yang Y, Wang J, Wu G (2013) Glycine metabolism in animals and humans: implications for nutrition and health. Amino Acids 45:463–477. https://doi.org/10.1007/s00726-013-1493-1

Yaish MW, Kumar PP (2015) Salt tolerance research in date palm tree (Phoenix dactylifera L.), past, present, and future perspectives. Front Plant Sci 6:348. https://doi.org/10.3389/fpls.2015.00348

Yao L, Schaich K (2015) Accelerated solvent extraction improves efficiency of lipid removal from dry pet food while limiting lipid oxidation. J Am Oil Chem Soc 92:141–151. https://doi.org/10.1007/s11746-014-2568-1

Zhang C-R, Aldosari SA, Vidyasagar PS, Nair KM, Nair MG (2013) Antioxidant and anti-inflammatory assays confirm bioactive compounds in Ajwa date fruit. J Agric Food Chem 61:5834–5840. https://doi.org/10.1021/jf401371v

Zhao S, Zhang D (2013) A parametric study of supercritical carbon dioxide extraction of oil from Moringa oleifera seeds using a response surface methodology. Sep Purif Technol 113:9–17. https://doi.org/10.1016/j.seppur.2013.03.041