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Thème

Exploring the biochemical composition and antioxidant activity of date palm(*Phoenix dactylifera* L.)seed oil.

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Dedication

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Summary

Acknowledgements	
Dedication	
Summary	
Tables	Ι
Figures	II
Abreviations	
Introduction	1
First part: Review of literature	
Chapter I : Generality on the date palm tree (Phoenix dactylifera L.)	
1. Date palm tree	3
1.1 Historical and origin	3
1.2 Botanical Description	3
1.3 Taxonomy	4
1.4 Cultivation of date palm in Algeria	5
2. Production of date palm	5
2.1 In the world	5
2.2 In Algeria	6
3. The fruit of the date palm is called "date "	7
3.1 Morphology	7
Chapter II: Date palm seed and seed oil	
1. Date Palm Seed (DPS)	8
1.1 Description of date palm seed	8
1.2 Composition of Date Palm Seed	8
1.2.1 biochemical composition	8
2. Date palm seed oil (DPSO)	9

2.1 Importance of vegetable oils	9
2.1.1 Biochemical Comoposition	9
3. Potential Applications	11
3.1 Cosmetic applications	11
3.2 Culinary applications	11
3.3 Health Beneficial applicqtions	11
Second part: Articles synthesis	
Chapter I : Material and methods	
1. Plant material	12
2. Methods	13
2.1 Preparation of seed	13
2.2 Oil extraction	13
2.2.1 Different methods for extraction oil from date palm seed	14
2.2.2 Solvents used for extraction	14
2.3Methods of biochemical analysis	15
2.3.1 Fatty Acids	15
2.3.2 Tocopherol	16
2.3.3 Carotenoids	16
2.3.4 Polyphenols	16
2.3.5 Sterols	17
2.3.6 triacylglycerols TAG	17
2.4 Antioxidant Activity assays	18
2.4.1 DPPH assay	18
2.4.2 ABTS assay	18
Chapter II: Results and discussion	
1. Oil extraction	19
2. Oil yield	20

2.1 The yield of date palm seed oil in the latest studies (Algeria and Morocco)	21
2.2 The effect of the solvent used on the production of date seed oil	22
2.3 The effect of extraction methods used on the production of date seed oil	24
3. Biochemical components of date palm seed oil	25
3.1 Fatty Acids	25
3.1.1 Fatty acids dosage	26
3.1.2 Fatty acids composition	27
3.2 Tocopherols (Vitamin E)	28
3.2.1 Tocopherols dosage	28
3.2.2 Tocopherols Composition	30
3.3 Carotenoids	31
3.3.1 Carotenoids dosage	31
3.3.2 Carotenoid Composition	32
3.4 Polyphenols	34
3.4.1 Polyphenols dosage	34
3.4.2 Polyphenols Composition	35
3.5 Sterols	36
3.5.1 Sterols dosage	36
3.5.2 Sterols Composition	37
3.6 triacylglycerols	38
3.6.1 Triacylglycerols dosage	38
3.6.2 Triacylglycerols Composition	39
4. Antioxidant Activity	41
4.1 In vitro evaluation of antioxidant activity	41
4.1.1 DPPH (α,α-diphenyl-β-picrylhydrazyl)	41
4.1.2 ABTS (2,2 -azinobis (3-ethylbenzothiazoline-6-sulfonic acid))	42

Conclusion

Reference

Tables list

Table 1. Chemical composition of the date seeds10
Table 2. Source of date palm seed material used for oil extraction
Table 3. The solvents used for extraction16
Table 4. The effect of the solvent used in extraction on oil yield
Table 5. Different extraction methods used in producing date seed oil
Table 6: Fatty acids identified in oil extracted from Deglet Nour cultivar using different
methods and solvents
Table 7. Tocopherols identified in the date seed oil of some varieties studied (mg/100g)32
Table 8. Carotenoids detected in date seed oil of 18 varieties
Table 9: Identified polyphenols in the date palm seed oil of some studied varieties37
Table 10: Sterols identified in the date seed oil of 3 varieties studied
Table 11: Triacylglycerols (TAG) identified in the date seed oil of 3 varieties studied42

Figures list

Figure 1. Schematic presentation of a date palm tree (Munier, 1973)4
Figure 2. Date palm growing regions in Algeria (Messar, 1996)5
Figure 3. Area harvesting date palm producing countries % . (Khan et Prathapar,2022)6
Figure 4. Date palm production (qx/year) in Algeria (MADR, 2019)7
Figure 5. Morphological presentation of the date fruit (Richarde, 1972)
Figure 6. Date palm seed oil (Olowokere et <i>al.</i> , 2019)21
Figure 7. Oil yield of date palm seed varieties from different cultivars
Figure 8. Results of oil content (%) from different varieties of Algerian date palm seed (Biskra)
Figure 9: Results of oil content (%) from different varieties of Moroccan date palm seed24
Figure 10. Total percentage of free fatty acids of some varieties
Figure 11. Total tocopherol content of date palm seed oil from different varieties
Figure 12. Total Carotenoid content of date palm seed oil from different varieties
Figure 14. Total Content of sterols in palm seed oil of different varieties
Figure 15. Total content of TAG in palm seed oil of different varieties40
Figure 16. Evaluation of antioxidant activity the different varieties of test DPPH43
Figure 17: Evaluation of antioxidant activity the different varieties of test ABTS45

Abreviations

ABTS: 2,2 -azinobis (3-ethylbenzothiazoline-6-sulfonic acid)
DPPH: α,α-diphenyl-β-picrylhydrazyl
DPS: Date Palm Seed
DPSO: Date Palm Seed Oil
FAMEs: Fatty Acids Methyl Esters
GC-FID: Gas Chromatography-Flame Ionization Detector
GC-MS: Gas Chromatography-Mass Spectrometry
H2O2: Hydrogen peroxide
HPLC : High-Performance Liquid Chromatography
Nd: Not Detected
SC-CO2: Supercritical CO2
TAGs: Triacylglycerols
UV-A: Ultrasound Violet A
UV-B: Ultrasound Violet B

Introduction

Introduction

The date palm has long been one of the most important fruit, where dates are the main income source and staple food for some local populations. In addition, this crop is not only a source of income from an economic point of view, but also a key for fixing populations and creating or maintaining centers of life (Mrabet et *al.*, 2020).

The date palm (*Phoenix dactylifera* L.) is the most important crop in arid and semi-arid areas. It is a tree of great interest due to its high productivity, the nutritional quality of its highly sought-after fruits, and its ability to adapt to Saharan regions. In addition to its ecological and social roles, it holds significant value. This importance stems from the fact that all parts of the tree can be used either directly as human and animal feed or indirectly through industrial and traditional processes (Sedra, 2003).

Algeria is ranked among the leading date-producing countries. It holds the 4th position globally with a 14% market share. The cultivation of date palms is primarily concentrated in the Saharan provinces. It is estimated that there are 10 million date palm trees, out of which 76% are productive, resulting in an annual production of approximately 270,000 tons of dates. Among these dates, 45% are of the Deglet Nour variety (Chehma et *al.*, 2001).

Dates are the leading agricultural product exported by the country. In recent years, the date industry has shown significant dynamism, resulting in a substantial increase in production. Algerian dates represent a true "source" of foreign currency for the country (Benziouche et *al.*, 2012).

Indeed, the date palm (*Phoenix dactylifera* L.).) is a species of flowering plant that produces sweet, edible fruits (Venkatachalam et *al.*, 2016). It is vital for desert regions and serves as a foundation for the survival of their populations (AL.Hooti et *al.*, 2002).

The by-products of the date palm tree such as leaves, trunk, pits, pedicels, etc., have various uses in the Saharan regions. Date pits, in particular, are used as livestock feed when they are not simply discarded. However, their utilization in human consumption remains poorly known, especially in our country (Boussena et Khali, 2016; Djerbi, 1994).

Numerous research studies are dedicated to the valorization of date pits in various forms, such as : activated carbon (Hazourli, 2007; Alhamed, 2009; El Nemr et *al.*, 2008).supplement in livestock feed (Hussein et Alhadrami, 2003). preparation of citric acid and proteins

(Abouzeid et *al.*, 1983). In traditional medicine for its antimicrobial and antiviral properties (Hamada et *al.*, 2002).

The objective of this study is to identify and analyze the biochemical composition and he antioxidant properties of date palm seed oil extracted from various date palm varieties from different counties. Additionally, knowing their extraction methods and solvent used.

This study includes:

First part. This section provides general information about date palm in the first chapter and about date palm seed and the extracted oil in the second chapter.

Second part. This part consists of two chapter:

- Materials and Methods. It provides information on the preparation of date palm seed material, as well as the methods and techniques used to investigate its biochemical compounds and antioxidant properties.
- Results and Discussion. Analyzing and discussing the biochemistry findings from selected studies on date palm seed oil and comparing them with the results documented in current literature.

First part. Review of literature

Chapter I. Generality on the date palm tree (*Phoenix dactylifera* L.)

1. Date palm tree

1.1 Historic and origin

The oldest palm trees date back to the Miocene era. The date palm was cultivated in the hot areas between the Euphrates and the Nile around 4500 years. From there, its cultivation was introduced in Lower Mesopotamia around 250 years. It then spread northward and reached the coastal region of the Iranian plateau, followed by the Indus Valley (Munier, 1973).

The cultivation techniques for date palms progressed towards Libya and then to other countries in the Maghreb, such as southern Morocco, Tunisia, Algeria, and eventually reached the Adrar region in Mauritania (Bouguedoura et *al.*, 2015)

1.2 Botanical description

The scientific name of the palm tree is "*Phoenix dactylifera* L." It belongs to the monocotyledonous family Arecaceae (Palmaceae). This diploid and perennial primitive fruit plant has persistent leaves and is dioecious. The name "Phoenix" in Greek means violet or red (referring to the fruit), while "dactylifera" refers to the finger-like shape of the fruit cluster (Chao et Krueger, 2007).

It is a tall palm tree with a trunk measuring 20 to 30 meters in height, and it bears a crown of leaves or fronds that are 4 to 7 meters long (Figure 1). Each frond is pinnate, and the leaflets are transformed into spines at the base. The species is dioecious, producing separate male and female inflorescences. Dates are formed from the development of the female flower (Bouguedoura et Bennaceur, 2016).

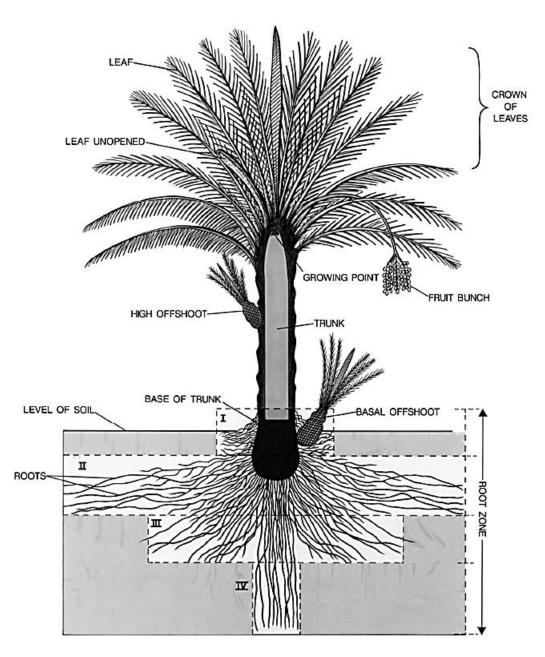


Figure 1: Schematic presentation of a date palm tree (Munier, 1973).

1.3 Taxonomy

The date palm is an arboreal and diploid monocotyledonous plant (2n=36). According to Djerbi (1994), the botanical classification of the date palm is as follows:

Group: Spadiciflore

Phylum: Angiospermae (Flowering plants)

Class: Liliopsida (Monocotyledons)

Order: Arecales (Palms)

Family: Arecaceae (Palm family)

Tribe: Phoeniceae

Genus: Phoenix

Species: Phoenix dactylifera L.

1.4 Cultivation of date palm in Algeria

The cultivation of date palms is carried out in the Saharian regions of Algeria, including Biskra, Ghardaïa, Béchar, Khenchella, Batna, Djelfa, Laghouat, and El-Bayedh.

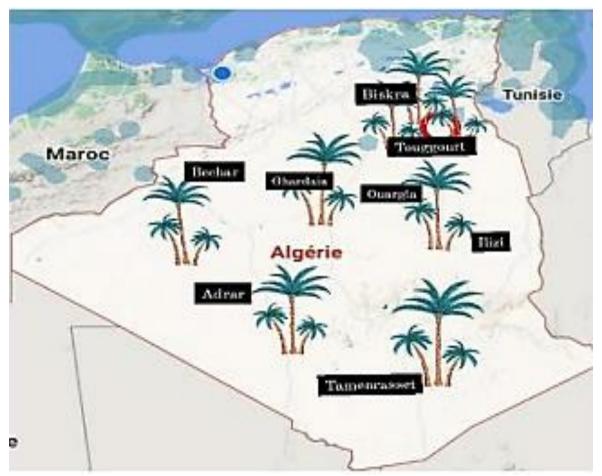
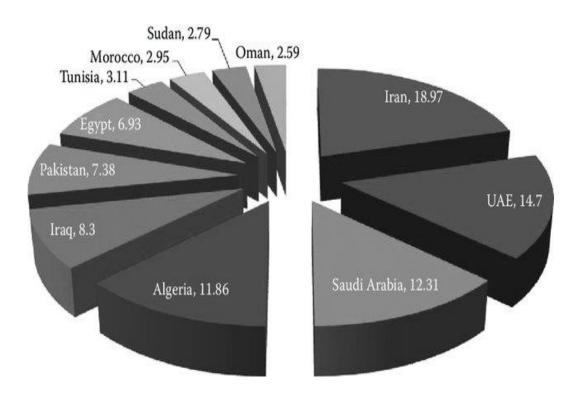


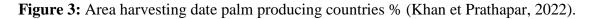
Figure 2: Date palm growing regions in Algeria (Messar, 1996).

2. Production of date palm

2.1 In the world

According to a recent study by(Khan and Prathapar, 2022), global date palm production is distributed as follows: Asia ranks first with a percentage of 64.25% (including Iran, UAE, Saudi Arabia, Iraq, Pakistan, and Oman). Africa ranks second (including Algeria, Egypt, Tunisia, Morocco, and Sudan). This information is detailed in figure 3.





2.2 In Algeria

According to Fares' study, date production in Algeria is distributed as follows: Biskra ranks first in terms of production with a value of 4723000qx. The lowest value is recorded in the Naama region with 6505qx quintals. This information is detailed in figure 4.

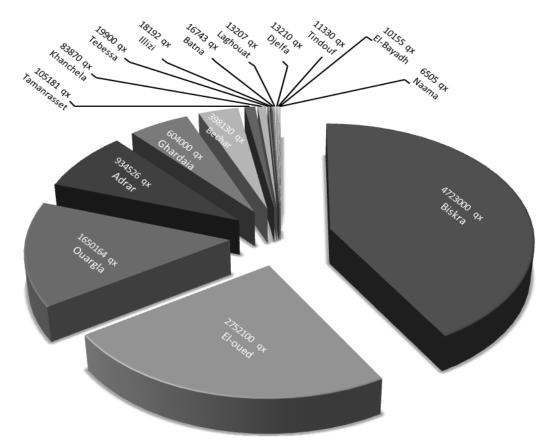


Figure 4: Date palm production (qx/year) in Algeria (MADR, 2019).

3. The fruit of the date palm is called "date "

3.1 Morphology

The date is a berry with a generally elongated, oblong, or ovoid shape, although spherical dates can also be found. Its size varies according to the variety, ranging from 1.5 to 8 cm in length, and its weight ranges from 2 to 20 g Retima (2015). The color of dates can range from pale yellowish-white to very dark almost black, including shades of amber, red, and brown. The date contains a single seed referred to as the "seed" (Figure 05).

The edible part of the date is called the "flesh" or "pulp," and it consists of:

An outer layer called the "epicarp," which is a thin, cellulose-like envelope referred to as the skin.

A usually fleshy "mesocarp" that varies in consistency depending on its sugar content and has a rich color.

A lighter-colored and fibrous-textured "endocarp," sometimes reduced to a parchment-like membrane surrounding the seed.

The non-edible part of the date is elongated, often bulky, and smooth or provided with lateral protrusions in ridges or wings. It has a fairly deep ventral groove and a dorsal embryo. Its consistency is hard and horny (Retima, 2015).

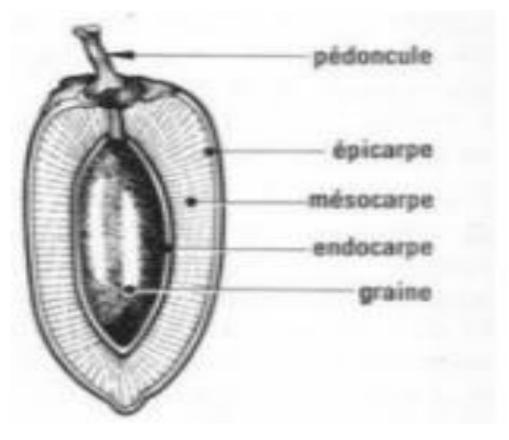


Figure 5: Morphological presentation of the date fruit(Richarde, 1972).

Chapter II. Date palm seed and seed oil

1. Date Palm Seed (DPS)

1.1 Description of date palm seed

The word "date" comes from the Greek "dactylos," meaning "finger," because dates resemble fingers. Dates are the edible fruits of the date palm tree and are typically 4 to 6 cm long with a grooved seed inside. Dates are berries, consisting of a hard, inedible seed and a soft, edible pulp. The edible part includes a thin outer layer (pericarp), a variable-textured and colored middle layer (mesocarp), and a lighter, fibrous inner layer (endocarp) around the seed. (Adrar, 2016)

As Acourene et Tama (1997) suggest, the physical attributes of date palm seed, including their length, diameter, and weight, can vary significantly between different cultivars and even within the same cultivar from the same plantation. The researchers also found that Algerian date palm seeds (Ziban) exhibit a weight range of 0.6 to 1.69 g, a length range of 2.9 to 3.15 cm, and a diameter range of 0.58 to 1 cm. Moreover, these variations may be influenced by cultural practices, particularly those related to pollination and fertilization techniques (Harrak et Boujnah, 2012; Khalifa et *al.*, 1980).

"The seeds of dates are long and come in different sizes. They usually weigh around one gram and make up 7 to 30% of the total weight of a date (Acourene et Tama, 1997). Inside the date seed, there's a hard, tough substance called albumen, and it's covered by a thin layer made of cellulose (Khali et al., 2015; Adrar, 2016).

1.2 Composition of date palm seed

1.2.1 Biochemical composition

biochemical analysis of the DPS from several varieties of dates from Saudi Arabia has revealed the presence of proteins, carbohydrates, lipids, and minerals such as potassium (K), phosphorus (P), calcium (Ca), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) (Ben Abbes, 2018).

Compounds	Content	Authors
Glucose	20,1%	Khalid et <i>al.</i> , (2016)
Fructose	16,1%	
Sucrose	2,8%	
Oleic acid	48.67%	(Guido, et al., 2011; Nehdi et al.,
Lauric acid	17.26%	2018)
Stearic acid	10.74%	
Linolenic acid	8.13%	
Alpha-tocotrienol	19.07%	
Alpha-tocopherol	17.52%	
Carbohydrates	8,70-9,54g/100g	Alem et <i>al.</i> , (2017)
Lipids	5,66-6,97g/100g	
Protein	4,30-6,14mg/100g	
Dietary fiber	15,84-19,9mg/100g	
Ash	1,09-1,3mg/100g	
Essential amino acids		Assirey (2015)
Glutamate	265mg/100g	
Asparagine	225mg/100g	
Arginine	60mg/100g	

2. Date palm seed oil (DPSO)

2.1 Importance of vegetable oils

Vegetable oils, also known as fixed oils, are non-volatile compounds found in the oilbearing cells of plants, such as seeds or pits. They play a significant role in global daily consumption. From 1996 to 2000, the average annual worldwide production of vegetable oil was 105 million tonnes, and from 2016 to 2020, it was expected to exceed 184.7 million tonnes Wang et *al.*, (2012). Generally, vegetable oil consists of a wide variety of components. However, these oils remain expensive to extract and are characterized by their limited availability.

2.1.1 Biochemical Composition

a. Fatty acid

Research indicates that date pit oil contains a significant amount of fat, with studies by various authors (Barreveld, 1993; Abdel Nabey, 1999; Besbes et *al.*, 2005) finding the fat

content to range from 7% to 13%. This fat content supports the potential value and usefulness of date Palm seed oil.

According to studies by Besbes et *al.*, (2004) and Besbes et *al.*, (2005), the oil extracted from the pits of two Tunisian date varieties, Deglet Nour and Allig, predominantly features a monounsaturated fatty acid profile. The fatty acid composition of date palm seed oil can vary, encompassing both saturated and unsaturated fatty acids, depending on the specific type of date palm seed.

Further research on fourteen different date varieties has shown that date palm seed oil contains 14 distinct types of fatty acids. In contrast, the fruit pulp of dates only contains 8 types of fatty acids, and at lower overall concentrations (Besbes et *al.*, 2004).

b. Tocopherols

Date seed oil is a significant source of tocopherols, which are antioxidant compounds. It contains 30 g of tocopherols per 100 g of oil, with α -tocopherol being the main molecule; the other stereoisomers (β and δ) are present only in trace amounts (Besbes et *al.*, 2004).

Tocopherols have important antioxidant activity by preventing the action of singlet oxygen, which initiates lipid peroxidation (Chan, 1998). Due to its hydrophobic nature, α -tocopherol can integrate into biological membranes and neutralize peroxyl radicals (LOO°). Additionally, this tocopherol has a synergistic effect with β -carotene by protecting it from oxidation (Abbane, 2023).

c. Polyphenols

Date palm seed oil is rich in phenolic compounds. The amount of phenols in DPSO depends on the storage conditions (Marinova et Yanishlieva, 2003; Besbes et *al.*, 2004).

d. Sterols

According to Salvador et *al.*, (2001), the sterols in date seed oil (3000 to 3500 mg/kg) are higher than those in olive oil (1500 mg/kg). According to Besbes et *al.*, (2004), the oil extracted from date palm seed contains different types of sterols such as cholesterol, campesterol, stigmasterol, β -sitosterol, Δ 5-avenasterol, and Δ 5.2,4-stigmastadienol. Among these components, β -sitosterol and campesterol are the main ones, making up about 90 g per 100 g of the total sterols.

3. Potential Applications

3.1 Cosmetic applications

Here are some possible uses of date seed oil in cosmetics, such as body creams, shaving soap, and shampoos, Date seed oil is very stable and can be stored for a long time without going bad. It can protect the skin from UV-A and UV-B light, which can cause cell damage, and also from damage caused by hydrogen peroxide. Additionally, date seed oil can help repair the skin because of its antioxidant properties. These qualities make it good for use in cosmetics (Afiq et *al.*, 2013).

The oils with a high concentration of free fatty acids require more [catalysts] to counterbalance their acidity (Afiq et *al.*, 2013). A study investigated the potential use of date seed oil in soap production. The results indicated that incorporating 30% or more date seed oil led to enhanced [efficiency] of the soap [foam]. All the soap samples demonstrated [effects] on human skin that were consistent with the [control] Ahmed, (1999). Additionally, the date seed oil has been reported to exhibit [antimicrobial] activity against Escherichia coli, [alpha] and [beta] hemolytic streptococci, Staphylococcus aureus, and Aspergillus fumigates (Ekpa et Ebana, 1996).

3.2 Culinary applications

Date seed oil has a good chemical makeup and physical properties that make it useful as edible oil. It is high in oleic acid, which is important for nutrition. The oil also contains saturated fatty acids and many antioxidants like phenolic compounds, tocols, and carotenoids, making it very stable against going bad and heat. This makes it suitable for cooking, frying, or seasoning, and even as a substitute for palm olein. The carotenoids in the oil give a natural yellow color, which is good for making margarine without artificial colorants. Basuny et Al-Marzooq (2011) used date seed oil instead of corn oil to make mayonnaise, which had better taste and texture. However, any use of date seed oil for eating needs to be fully checked for safety. (Mrabet et *al.*, 2020)

3.3 Health Beneficial applications

In a study by Mrabet et *al.*, 2020 date seed oil was tested for its effects on human sperm motility and viability after oxidative damage caused by H2O2. They found that date seed oil protected sperm, especially after 24 hours. These studies show the importance of adding

natural products with high antioxidants to our diet and to cosmetics or dietary supplements. Date seed oil is a promising dietary product with proven antioxidant effects, especially when extracted using eco-friendly methods.

The chemical composition of date seed oil obtained by slow pyrolysis was studied using GC/MS. Compounds like triterpenoids and steroids found in the oil could be valuable for their health benefits. The stearic, palmitic, and oleic acids in the oil make it useful for anti-inflammatory pharmaceutical products, not as active ingredients, but as helpers that improve the absorption of other anti-inflammatory drugs. (Mrabet et *al.*, 2020)

Second part. Articles synthesis

Chapter I. Material and methods

1. Plant material

The plant material under study is the date palm seed. seed used were from several varieties of dates originating from different regions and countries (Table 2), selected under standard conditions: harvested at the final stage of maturity (Tamer), of uniform size, of the same color, and free from physical damage, insect injuries, and fungal infections.

Country	Oasis	Studies
Algeria	Ouargla,Biskra	(Boukouada et al., 2014; Boussena et Khali, 2016; Al-
	Ghardaïa	Juhaimi et al., 2018; Laghouiter et al., 2018; Laouer
		et al., 2019; Harkat et al., 2022)
Morocco	/	(Alem et al., 2017; Al-Juhaimi et al., 2018; Ourradi
		et al., 2021)
Tunisia	Degach, Tozeur	(Besbes et al., 2004; Saafi et al., 2008; Herchi et al.,
		2014; Benyoussef et <i>al.</i> , 2017)
Sudan	Omdurman	(Abdalla et al., 2012; Al-Juhaimi et al., 2018)
Libia	/	Al-Juhaimi et al., (2018)
Pakistan	/	Raza et <i>al.</i> , (2019).
Malaysia	/	(Al-Juhaimi et al., 2018; Mohd Jaih et al., 2019).
Saudi Arabia	Al-Hasa, Riyadh	(Basuny et Al-Marzooq, 2011; Nehdi et al., 2018;
		Mohd Jaih et al., 2019; Raza et al., 2019).
Oman	(Sohar)	Al-Sumri et <i>al.</i> , (2016).
UAE	/	Habib et <i>al.</i> , (2013).
-		
Iraq	/	Ali et <i>al.</i> , (2015).
Iran	Qom, Karaj,	(Mehran et Filsoof, 1975; Akbari et al., 2012; Biglar
	Bushehr,	et al., 2012; Kazemi et Dadkhah, 2012).
	Khuzestan	
India	/	Jadhav et al., (2016).

Table 2: Source of date palm seed material used for oil extraction

2. Methods

2.1 Preparation of seed

Before proceeding with the extraction, approximately 30 g to 1000 g of mature date palm seed from each variety were used for oil extraction (Ourradi et *al.*, 2021; Harkat et *al.*, 2022; Benyoussef et *al.*, 2017).

The preparation of date palm seed powder is carried out following the following steps:

- Dehusking: The separation of pulp and seed (Harkat et *al.*, 2022).
- Washing: The seed are washed to remove foreign particles and traces of pulp using distilled water (Ourradi et *al.*, 2021).
- Drying: After washing, the seed are air-dried at room temperature, and then ovendried at 50 °C for 12 h them for grinding (Ourradi et *al.*, 2021; Benyoussef et *al.*, 2017).
- Grinding: Then seeds of each variety were grinded using the hammer mill with 1 mm (Benyoussef et *al.*, 2017).
- Sieving: Sieve and preserved at 20 °C until extractions (Benyoussef et al., 2017).

2.2 Oil extraction

The most commonly used extraction method is soxhlet extraction with a suitable solvent, typically hexane. The extraction process continues for several hours.

Samples extracted, and evaporated under reduced pressure. The obtained dry samples were reextracted with hexane ,centrifuged and then the hexane was evaporated under reduced pressure. The obtained dry extracts were stored in a freezer (-20 °C) for subsequent analyses (Benyoussef et *al.*, 2017; Jadhav et *al.*, 2016; Harkat et *al.*, 2022).

2.2.1 Different methods for extraction oil from date palm seed

There are several other extraction methods that have been recently used to increase yield and reduce extraction time. These include:

supercritical CO2 (SC-CO2): CO2 with a purity of 99.99% is continuously fed into an extractor with a fixed solvent flow rate regulated by an automated back-pressure regulator. (Laouer et *al.*, 2019).

<u>Micro-Wave:</u> Microwaves heat the solvent until it reaches its boiling point. The solvent vapors then penetrate the seeds, and condensation occurs on a condenser (reflux extraction). Benyoussef et *al.*, 2017).

<u>Maceration:</u> A mixture of the sample and solvent is subjected to mechanical agitation for several hours in a glass reactor with a thermostatic double-jacket. The mixture is then centrifuged and filtered. (Herchi et *al.*, 2014; Jadhav et *al.*, 2016; Benyoussef et *al.*, 2017).

<u>Ultrasound</u>: The mixture of seeds and solvent is irradiated using a 20 kHz sonicator in a glass reactor with a thermostatic double-jacket. (Jadhav et *al.*, 2016; Benyoussef et *al.*, 2017).

2.2.2 Solvents used for extraction

Several solvents have been used to determine which one provides the highest yield recently All of this information is outlined in the following table:

solvent	Authors
2-Propanol	Ali et <i>al.</i> , 2015
Chloroform	Ali et <i>al.</i> , 2015
Toluen	Ali et <i>al.</i> , 2015
Methanol	Ali et <i>al.</i> , 2015
Hexane	Ali et <i>al.</i> , (2015) Laouer et <i>al.</i> , (2019) Harkat et <i>al.</i> , (2022)
Ethanol	Al-Sumri et al., (2016)
Acétone	Al-Sumri et al., (2016)

Table 3: the solvents used for extraction

2.3 Methods of biochemical analysis

2.3.1 Fatty Acids

The method used for the determination of fatty acids in all studies is Gas chromatography coupled with flame ionization detector (GC-FID) First, the fatty acids from the oil need to be converted into their methyl esters to enhance volatility (Benyoussef et *al.*, 2017; Ourradi et *al.*, 2021; Harkat et *al.*, 2022).

a. Preparation of fatty acids methyl esters (FAMEs)

Acid-catalyzed transmethylation was used in order to prepare fatty acids methyl esters (FAMEs) from the extracted oil as described by Li et al Morrison et Smith (1964). Briefly, a determined quantity of the extracted oil was mixed with 1 mL of a methanolic-sulfuric acid (5%) solution. Triheptadecanoin (C17:0 TAG) was used as internal standard. The obtained mixture was heated at 85 °C for 90 min. After that, the solution was cooled at room temperature and was mixed with 1.5 mL of sodium chloride (0.9%) solution and 1 mL of n-hexane; then it was shook vigorously for 30 s. The organic layer was recovered and transferred to a vial for a gas chromatography coupled with flame ionization detector (GC-FID) analysis. ..(Benyoussef et *al.*, 2017)

b. FAMEs analysis

Gas chromatography coupled with flame ionization detector (GC-FID) technique was used to analyze FAMEs prepared from the extracted oils. This step was carried out by an Agilent..(Benyoussef et *al.*, 2017; Ourradi et *al.*, 2021; Harkat et *al.*, 2022).

2.3.2 Tocopherol

Tocopherol composition was determined according to the method described by Nehdi et *al.*, (2018). Tocopherol isomers were separated using a normal HPLC system (Agilent, Kyoto, Japan) equipped with an Inertsil ODS-3 normal phase column ($250 \times 4.6 \text{ mm}$, 5 µm) and SPD-M20A photodiode-array detector. Prior HPLC analysis, 0.2 g of oil was diluted in 2 mL hexane. The identification and quantification of tocopherol isomers were assured by comparing the peak areas with the external standards,(Harkat et *al.*, 2022).

2.3.3 carotenoids

The carotenoid content of the oils was measured by the calorimetric method of Minguez-Mosquera et *al.*, (1991) 0.6 g of each sample was diluted in 2 ml cyclohexane. After agitation, the carotenoid content was determined at 470 nm using a spectrophotometer (JASCO V-630). The content was calculated according to the formula:

Carotenoid(mg / kg) = A470 \times 106 / E0 \times 100 \times d

Where A470: absorbance at 470 nm, E0: specific extinction of lutein, which is the majority compound of the carotenoids fraction, d: the thickness of the spectrophotometer quartz cell 1 cm, (Herchi et *al.*, 2014; Ourradi et *al.*, 2021).

The identification of carotenoids was made by high-performance liquid chromatography, the lipid extract was reconstituted in 1 mL of chloroform and injected into an HPLC system. The system operated in isocratic condition with 100% mobile phase and a flow rate of 0.8 ml/min for an operating time of 20 min(Miller et *al.*, 1984).

2.3.4 Polyphenols

The total phenolic content in each oill was measured by calorimetric method described by Salvador et *al.* (2001), 0.1 ml of methanolic extract was mixed with 0.5 ml Folin-Ciocalteu reagent and incubated at room temperature for 5 min, then 1 ml of sodium carbonate 35% (w/v) was added and mixed, then 5 ml of distilled water was added. After 1 h of incubation in the dark a room temperature. The absorbance was read at 725 nm, the total polyphenol content was expressed as mg Gallic acid equivalent per 100 g oil.(Herchi et *al.*, 2014; Ourradi et *al.*, 2021; Basuny et AlMarzooq, 2011).

Phenolic compounds identification was performed using high-performance liquid chromatography (HPLC). As mobile phases, a 0.05% mixture of acetic acid with water (A) and acetonitrile (B) was used. The flow rate of the mobile phase and injection volume were set at 1 mL/min and 20 mL, respectively, at 308°C. Peak records were obtained at 280 nm and 330 nm. The total run time for each sample was 60 minutes (Singleton et *al.*, 1999).

2.3.5 Sterols

The total content of sterols was determined using the Lieberman-Burchard assay Barreto (2005) 1 ml of diluted sample solutions was mixed with 2 ml of Lieberman-Burchard Reagent (Liebermann reagent constituted of 60 ml anhydrous acetic +10 ml concentric sulfuric acid +30 ml acetic acid). The mixture was incubated in the dark for 30 min and the green color formed was measured at 550 nm using a UV/VIS-1800 Shimadzu spectrophotometer (Laghouiter et *al.*, 2018).

The identification of sterols was carried out by gas chromatography mass spectrometry (GC-MS) according to the method of AOCS (1997) Ca 6b-53.

The sterol fraction was diluted in pure chloroform (1:10, v/v) then separated from the other unsaponifiables by thin layer chromatography (TLC) previously activated at 103° C over a period of 30 min. The solvent used for sterol elution was hexane/diethyl ether (70:30, v/v). After homogenization, the sterols were analyzed with a gas chromatography system coupled to a mass spectrometer.

Helium was the carrier gas with a flow rate of 1 mL/min. The column temperature was programmed from 180 to 280°C at 5°C/min, and the injector and detector temperatures were set at 280°C (Besbes et *al.*, 2004).

2.3.6 Triacylglycerols (TAG)

TAG analyses were performed by means of a gas chromatograph (Agilent 7820 A), equipped with a flame ionization detector at 360 °C and a capillary column. The initial temperature was 285 °C for 35 min before increasing at 10 °C/min, to 310 °C, and was kept for 10 min. The carrier gas was helium, and the flow rate was 0.5 mL/min. To ensure homogeneity, 100 mg of oil was melted and vortexed and then dissolved in 10 mL n-heptane (Turkish Food Codex Communique on olive oil and pomace oil, communique number 2010/36). The solution was transferred to a vial for GC analysis.

The comparison between the oil's triacylglycerol retention times and those of the standard TAG mixtures permitted determining the triacylglycerol of DPSO (Harkat et *al.*, 2022).

2.4. Antioxidant activity assays

2.4.1 DPPH assay

The DPPH radical scavenging assay was performed following the procedure outlined in reference Zhu et *al.*, (2006). To summarize, 1 mL of ethanolic DPPH solution was combined with 1 mL of the samples dissolved in ethanol at various concentrations. The reaction mixture was then shaken and left to incubate in the dark at room temperature for 30 minutes. After incubation, the absorbance of the mixture was measured at 517 nm against a blank. (Boukouada et *al.*, 2014; Laghouiter et *al.*, 2018)

A control group using " α -tocopherol" was prepared in the same manner as the test group, except that the antioxidant solution was replaced with the corresponding extraction solvent. In the control group, the inhibition percentage was determined to be IC50 = 0.034 g/L.

The inhibition of the DPPH radical by the sample was calculated using the following formula:

Inhibition % =
$$(1 - A/A_0) \mathbf{x} \ 100\%$$

Here, A_0 represents the absorbance value of the control, and A represents the absorbance value of the tested sample. (Boukouada et *al.*, 2014).

2.4.2 ABTS assay

The ABTS 2,20 -azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assay was used to determine the DPSO radical scavenging power, according to the method described by Pellegrini et *al.* (1999). A total of 7 and 2.45 Mm of final concentrations of ABTS stock solution and potassium persulfate, respectively, were transformed to a volumetric flask. The solution was left for 16 h in the dark for the formation of ABTS+ radical stock solution. Then, an appropriate quantity of ethanol was added to adjust the absorbance of the ABTS+ stock solution to 0.700 at 765 nm.

The radical scavenging activity of oil against ABTS+ was determined after reacting 100 μ L of the diluted oil in ethanol, with 2.9 mL of ABTS+ solution, measuring the reduction of the absorbance at 765 nm after 6 min. The results are expressed as μ g Trolox equivalent per 100 g of oil μ g TE/100 g oil (Harkat et *al.*, 2022).

Chapter II. Results and discussion

The synthesis draws from over 30 scientific articles that specifically examine date palm seed oil (*Phoenix dactylifera* L.) from different countries. These articles were gathered, analyzed, compared, and deeply studied to enhance the "Results and Discussion" section.

1. Oil extraction

The most commonly used extraction method is Soxhlet extraction using hexane as the solvent. However, some authors have employed other methods with different solvents, which influenced the oil yield. In addition to the solvent influence, there are other parameters that have an effect on the yield, such as particle size, extraction time, and temperature. These parameters can indeed affect the content of the targeted compounds and their solubility during the extraction process (Benyoussef et *al.*, 2017; Nehdi et *al.*, 2018; Harkat et *al.*, 2022).

All extracted date palm seed oils had a viscous liquid appearance at room temperature and a semi-solid consistency at temperatures below 10°C, with a pale yellow color and a pleasant odor. Figure 6 depicts the date palm seed oil.



Figure 6: Date palm seed oil (Olowokere et al., 2019)

2. Oil yield

The most commonly used extraction method is solid-liquid extraction using the Soxhlet apparatus, with a duration of 6 hours and employing hexane as the solvent due to its nonpolar properties, which give it a high affinity for lipids. This method is widely used for the extraction of fats and oils.

The synthesis on oil yield is based on twelve articles from seven different countries. Figure 7 presents the results of studies that examined the oil yield using the soxhlet apparatus and hexane as the solvent. Figure 7 presents the oil yield of different varieties of date palm seed from mul tiple authors ten varieties of different origins.

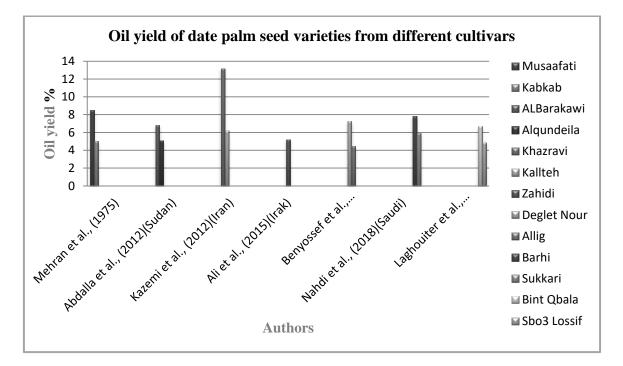


Figure 7: Oil yield of date palm seed varieties from different cultivars.

Figure 7 presents the oil yield obtained from Soxhlet extraction using hexane from different cultivars. The results are expressed as a percentage. The highest oil yield, with a value of 13.2%, was achieved by the Khazravi variety from Qom, Iran, as reported by Kazemi et Dadkhah (2012). On the other hand, the lowest oil yield was reported by Benyoussef et *al.*, (2017), from Tunisia, with a value of 4.44% for the Allig variety from Tozeur, Tunisia. the Iranian studies conducted by Mehran et Filsoof (1975) and Kazemi et Dadkhah (2012) demonstrate significant differences in the oil yield between extracts from two regions within the same country. The variety Khazravi, studied by Kazemi et Dadkhah (2012), obtained a much higher oil yield of 13.2% compared to the Musaafti and Kabkab varieties from Karaj, which yielded 8.5% and 5% respectively. However, the Musafti variety showed a higher yield

than the Kallteh variety from Qom, which had a yield of 6.2%. The study conducted by Abdalla et *al.*, (2012) showed nearly equal productivity for the ALBarakawi and ALqundeial varieties, similar to the study presented by Laghouitera et *al.*, (2018) on the Bint Qbala and Sbo3 Lossif varieties, as well as similar to the study presented by Ali et *al.*, (2015) on the Allig variety. While we can observe that the study conducted by Nehdi et *al.*, (2018) and his team yielded slightly higher returns compared to the study presented by (Abdalla et *al.*, 2012).

These differences in oil yield can be attributed to various factors such as the different varieties, regions of cultivation, hexane concentration, or extraction conditions. Indeed, date palm seed are a valuable source of oil that has the potential to be utilized in various domains.

2.1 The yield of date palm seed oil in the latest studies (Algeria and Morocco)

The production of date palm seed oil has been studied in two different regions, involving several different varieties of dates. Figure 8 and 9 presents the results of studies focusing on the production of oil in the two regions. the results indicated in percentage.

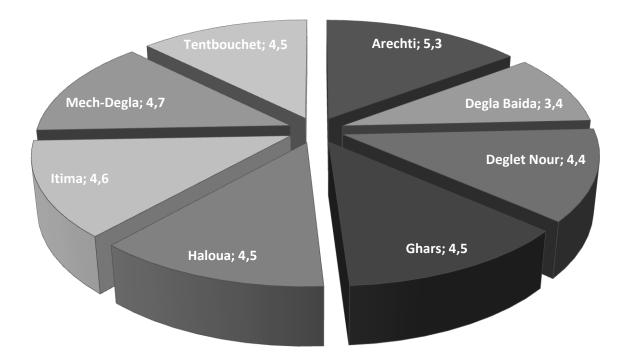


Figure 8: Results of oil content (%) from different varieties of Algerian date palm seed (Biskra).

Based on the study conducted by Harkat et *al.*, (2022). in the Biskra region of Algeria, which illustrates the oil content of different varieties of date palm seed, it is observed that the oil production percentages among the varieties were close. The highest value was recorded in the Arechit variety at 5.3%, while the lowest value was in the Degla Baida variety at 3.4%. Comparing these results to the previously studied varieties by Benyoussef et *al.*, (2017). from

Tunisia, the highest value was obtained in the Daglet Nour variety at 7.24%, while the lowest value was recorded in the Allig variety at 4.44%. The variations in the oil content percentage extracted from the date palm kernels among the studied varieties can be attributed to cultural conditions and the type of solvent used in the extraction process.

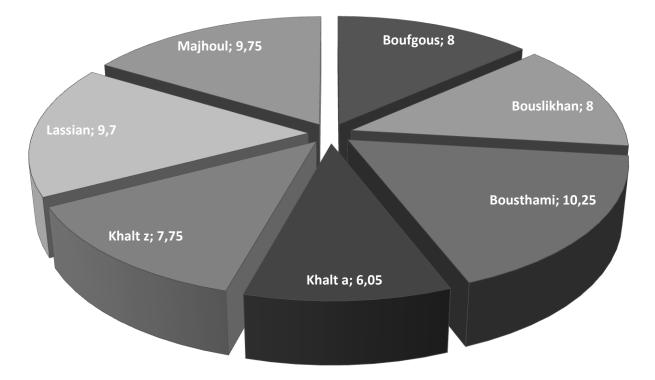


Figure 9: Results of oil content (%) from different varieties of Moroccan date palm seed.

The oil content of date palm seed in figure 9 Ourradi et *al.*, (2021). showed relatively close variations across all studied varieties. Furthermore, the oil content of the date palm seed ranged from (6.05% to 10.25%). The highest value was observed in the Bousthami variety at 10.25%, while the Khalt- a variety recorded the lowest oil content at 6.05%. Alem et *al.*, (2017). reported a range of oil content ranging from(5.66% to 6.97%) in four varieties that utilized hexane as a solvent for extraction. However, Besbas et *al.*, (2004). indicated higher values (10.19% to 12.67%) in two Tunisian varieties Saafi et *al.*, (2008).

2.2 The effect of the solvent used on the production of date seed oil:

Several research teams conducted experiments to test the extraction of the same quantity of date palm seed powder from various varieties of different origins using different origin using different solvents to evaluate the oil yield (see Table 4). The results are expressed in percentage.

Table 4 : The effect of the solvent used in extraction on oil yield.

Authors	Country (Region)	Varieties	2-Propanol (%)	Chloroform (%)	Toluen (%)	Methanol (%)	Hexane %	Ethanol (%)	Acétone (%)	petroleum ether
AlSumri et <i>al.</i> , (2016)	Oman (Sohar)	AlFarth	/	/	/	/	/	10	9,6	/
Harkat et <i>al.</i> , (2022)	Algerian	Arechti	/	1	/	/	5,30	1	/	
	Aigenaii	Degla- baida		/	/		3,41			/
Ali et <i>al.</i> , (2015)	Iraq (Karbala)	Zahidi	0,9	3,9	6,1	0,7	5	/	/	/
Ourradi et <i>al.</i> ,	M	Bousthami	,		,	1	,	,	/	10,25
(2021)	Moroccan	Khalt a	/	/	/	/	/	/	/	6,05

The most cost-effective extraction solvents, based on the mentioned studies, are as follows:

Petroleum ether, reported by the team Ourradi et *al.*, (2021), is the most profitable solvent for the Bousthami variety from the Moroccan region, with a yield of 10.25%.

Ethanol, according to AlSumri et *al.*, (2016), is the second most cost-effective solvent for the AlFarth variety from the Sohar region in Oman, with a yield of 10%.

Acetone is the third most profitable solvent for the same AlFarth variety, with a yield of 9.6%.

Methanol, as reported by Ali et *al.*, (2015), is the least profitable solvent, with only a 0.7% yield for the Zahidi variety from the Karbala region in Iraq.

To further enrich this information, Ramadan et *al.*, (2014) claim that ethanol is a better extraction solvent than methanol.

In the study conducted by Ali et *al.*, (2015) on the Zahidi variety, the highest oil yield was found with toluene at 6.1%. In contrast, methanol and 2-propanol recorded the lowest values at 0.9% and 0.7% respectively.

In the Algerian study conducted by Harkat et *al.*, (2022)., hexane exhibited the highest productivity with a yield of 5.30% for the Arechti variety. This is in contrast to the yield of 5% reported by Ali and al. for the Zahidi variety.

2.3 The effect of extraction methods used on the production of date seed oil

Besides the influence of solvents, the utilization of various extraction methods has demonstrated an impact on the yield (see Table 5). The outcomes are expressed as percentages.

Table 5 : Different extraction methods used in producing date seed oil.

Authors	Benyoussef et <i>al.</i> , (2017)	Louaer et <i>al.</i> , (2019)	Jadhav et <i>al.</i> , (2016)				
Country (Region)	Tunisie (Tozeur)	Algérie (Biskra)	India				
Varieties	Deglet Nour	Ghars	/				
Method extraction	Micro-Wave	SC-CO2	Soxhlet	Maceration	Ultrasound		
Solvent	Hexane	/	Hexane	Hexane	Hexane		
Yield of DSO %	5,52	14	8,5	4,2	8,5		

As shown in the table, the supercritical CO2 method used by Louaer et *al.*, (2019) and their Algerian team (2019) on the Ghars variety from Biskra showed the highest yield value at 14%.

Regarding the study conducted by Jadhav et *al.*, (2016) and their team in India, the results of the Soxhlet and Ultrasound methods were equal in this study, both yielding 8.5%, which was the highest compared to the Maceration method, which recorded a percentage of 4.2, the lowest value in the study.

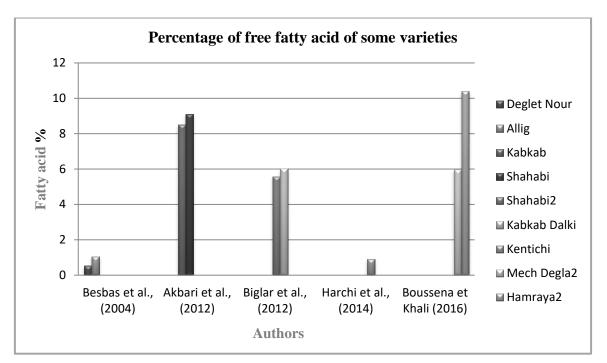
On the other hand, the study conducted by Benyoussef et *al.*, (2017) and their team on the Deglet Nour variety in Tunisia, using the Microwave method with Hexane as the solvent, yielded 5.52%.

These results highlight the superior performance of the supercritical CO2 method in terms of yield compared to the other methods discussed in the study

3. Biochemical components of date palm seed oil

3.1 Fatty Acids

Date palm seed oil contains various fatty acids, including saturated fatty acids such as lauric acid, myristic acid, and palmitic acid, as well as unsaturated fatty acids like oleic acid and linoleic acid (Nahdi et *al.*, 2010; Akbari et *al.*, 2012)



3.1.1 Fatty acids dosage

Figure 10: Total percentage of free fatty acids of some varieties.

A significant disparity is observed in the fatty acid content of date palm seed oil. The lowest content is recorded for the Tunisian variety Deglet Nour at 0.53%, as reported by Besbas et *al.*, (2004), while the highest content is attributed to Biskra (Algeria) at 10.39%, as reported by Boussena et Khali (2016).

Variability in fatty acid content is also evident among varieties from the same country, as shown in Tunisian studies by Harchi et *al.*, (2014) on the Kentichi variety and Besbas et *al.*, (2004) on the Allig variety, reporting values of 0.90% and 1.05%, respectively, in addition to the previously mentioned value for Deglet Nour. Similar variations are observed in the works of Biglar et *al.*, (2012) on the Shahabi and Kabkab Dalki varieties from the Bushehr region in Iran, with values of 5.57% and 6.01%, respectively, which are lower than those reported by Akbari et *al.*, (2012) for the Kabkab Dalki and Shahab varieties from the same region, at 8.5% and 9.1%.

The low levels of free fatty acids in date palm seed oils indicate their edibility and potential for extended shelf life Boukouada et *al.*, (2014). The difference in free fatty acid content can be attributed to cultural differences, variety, climatic conditions, and/or region and harvesting period.

3.1.2 Ftty acids composition

Table 13 presents the percentage (%) composition of different fatty acids determined in the oil of the "Dokkar Nour" variety, studied in two different regions using different extraction methods and solvents, as reported by Benyoussef et *al.*, (2017) and Harkat et *al.*, (2022).

Table 6: Fatty acids identified in oil extracted from Deglet Nour cultivar using different methods and solvents

Authors									
	Harkat et <i>al</i> .,		Benyoussef et al., (2017)						
	(2022).								
Country	Algérie			Tur	nisie				
(Region)	(Biskra)			(Toz	zeur)				
Varieties	Deglet Nour			Degle	et Nour				
Method	Soxhlet	Mace	eration	Ultra	sound	Micro	-Wave		
extraction									
Solvent	Hexane	Hexane	MeTHF	Hexane	MeTHF	Hexane	MeTHF		
Caprylic									
C8 :0 (%)	0.42	nd	nd	nd	nd	nd	Nd		
Capric									
C10:0(%)	0.45	0.39	0.51	0.48	0.48	0.51	0.49		
Lauric									
C12 :0 (%)	22,03	20.05	22.64	22.19	22.22	23.10	22.25		
Myristic									
C14 :0 (%)	8,83	11.26	10.55	10.17	10.20	10.29	8.88		
Palmitic									
C16 :0 (%)	9,11	10.73	9.68	9.02	9.03	10.19	9.07		
Stearic									
C18 :0 (%)	3.36	4.59	3.59	3.47	3.47	3.42	3.50		
Oleic									
C18 :1 (%)	46.18	44.02	46.9	45.56	45.49	44.84	45.40		
Linoleic									
C18 :2 (%)	7,15	8.97	6.13	9.11	9.12	8.96	9.10		
Arachidic									
C20 :0 (%)	0.66	Nd	nd	Nd	nd	nd	Nd		

The fatty acids identified in the oil extracted from Deglet Nour date kernels using different methods and solvents from the Deglet Nour variety in two different countries are as follows:

The most important fatty acids identified in all the methods used are oleic acid and lauric acid, together representing over 68% of the total fatty acid composition of the extracted oils (ranging from 44.02-46.9% for C18:1 and 20.05-23.10% for C12:0).

Oleic acid, present in the oil extracted through the maceration method with MeTHF and the Soxlet method with hexane, exhibited the highest content at 46.9% and 46.18%, respectively. For lauric acid, the oil extracted through the Microwave method showed the highest content at 23.10%.

Traces of caprylic acid, capric acid, and arachidic acid were found using the Soxlet method, as well as other methods for capric acid.

Thus, all the extracted date kernel oils were considered as oleic-lauric oils. In contrast to Besbas et *al.*, (2004), they were the only ones who found that the oil from the Tunisian Allig variety can be considered as oleic-linoleic type due to its content of 47.7% oleic acid, 21% linoleic acid, and 5.8% lauric acid.

The two main fatty acids identified have numerous health benefits. Oils containing a high amount of oleic acid have shown frying stability and good flavor due to their low saturation level, trans-isomer content, high oxidation stability, and ability to reduce LDL cholesterol in the blood.

Saafi et *al.*, (2008) demonstrated that lauric acid is a good substitute for polyunsaturated hydrogenated fats in margarine preparation, resulting in a favorable lipid profile for reducing the risk of cardiovascular diseases.

3.2 Tocopherols (Vitamin E)

3.2.1 Tocopherols dosage

Date palm seed oil is a good source of tocopherols, which are a form of vitamin E (Habib et *al.*, 2013; Sen et *al.*, 2010) Tocopherols have antioxidant properties and help protect the body against oxidative stress(Kamaleldin et Appelqvist, 1996)

Tocopherols have a potential role in the prevention of various human diseases due to their powerful antioxidant and anti-radical properties (Fryer, 1992).

Second part, Chapter II

A synthesis was conducted on the dosage and identification of tocopherols in the studied varieties, based on 5 articles from six different countries. The concentrations of tocopherols in the oil derived from the various studies discussing this subject are represented in Figure 11.

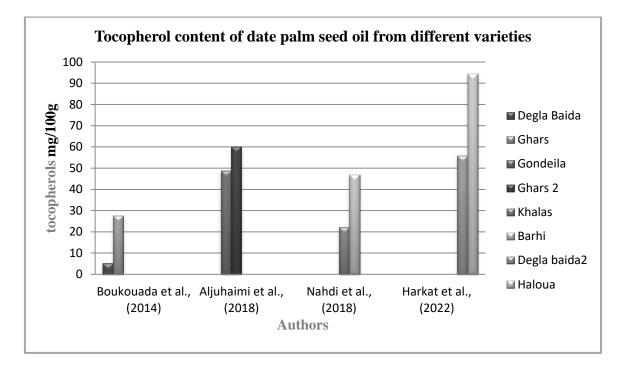


Figure 11: Total tocopherol content of date palm seed oil from different varieties.

The values presented in the above figure represent the results of colorimetric analysis of tocopherols. The results are expressed in mg/100g.

The results show significant variations in tocopherol content among different varieties of date palm. The highest value of tocopherol was recorded for the variety "Haloua" by the Algerian team of Harkat et *al.*, (2022). from the Biskra region, with a value of 94.6. On the other hand, the lowest value was recorded for the variety "Degla Baida" by the Algerian team of Boukouada et *al.*, (2014).

Variability in tocopherol content is also observed within the same variety originating from the same country but different regions. For example, in the Saudi study conducted by Nehdi et *al.*, (2018). on the Khalas variety, and the study by Al Juhaimi et *al.*, (2018), the highest value was reported for the white Ghars variety with a value of 60.25, while the Barhi variety from Nehdi study recorded a lower value of 46.93

These differences can likely be attributed to analytical conditions, genetic factors, varieties, and origins.

Tocopherols, due to their ability to neutralize free radicals in cell membranes, play an important role in the prevention of Alzheimer's disease and cancer. They have been widely used in human nutrition, pharmaceuticals, cosmetics, and resins (Yong et Salimon, 2006).

3.2.2. Tocopherols composition

The identification of tocopherols present in the DPSO, was conducted to detect the tocopherols present in each variety of the studied works. Please refer to Table 6 for more details on the specific tocopherols identified in each variety.

Authors	Nehdi et <i>al.</i> , (2018)		Harkat et <i>al.</i> , (2022)	Al Juhaimi et <i>al.</i> , (2018)			
Country (Region)	Arabie	Saoudite	Algérie	Lybie	Maroc	Pakistan	Sudan
Varieties	Barhi	Khalas	Ghars	Adwi	Allig	Dora	Gondeila
α-Tocophérol	15.17	9.18	0.81	0.84	0.69	0.97	0.54
β-Tocophérol	0.63	1.20	1.17	0.69	0.71	0.84	0.83
δ-Tocophérol	2.21	0.26	1.40	1.68	2.82	1.67	1.18
γ-Tocophérol	28.92	11.69	11.84	9.71	10.84	7.61	8.61
α -Tocotrienol	26.96	15.95	36.77	36.43	33.76	36.49	31.76
γ-Tocotrienol	31.88	5.11	8.26	7.58	5.78	7.51	5.84
δ-Tocotrienol	5.06	1.34	Nd	nd	nd	nd	Nd

Table 7: Tocopherols identified in the date seed oil of some varieties studied.(mg/100 g)

The identification of tocopherols present in the oil was conducted using the highperformance liquid chromatography (HPLC) method. The results obtained are indicated in Table 04, with values expressed in mg/100 g.

As shown in the table, the identified tocopherols were found in all varieties, except for δ -Tocorientol, which was not detected in Ghars, Adwi, Allig, Dora, and Dondeila. The predominant tocopherols are α -tocotrienol with a value of 36.77 mg/100 g for the Algerian

variety Ghars estimated by Al Juhaimi et *al.*, (2018), followed by γ -Tocorientol with 31.88 mg/100 g for the Saudi variety Barhi by Nehdi et *al.*, (2018), and then γ -tocopherol with 28.92 mg/100 g for the same variety. The lowest value was recorded for δ -tocopherol of the Khalas2 variety by the Saudi team of Nehdi et *al.*, (2018).

As for α and β -tocopherol, they were detected in trace amounts in all varieties except for the Saudi varieties Barhi and Khalas, which recorded α -tocopherol values of 15.17 and 9.18 mg/100 g, respectively.

These differences can likely be attributed to the variability of the studied varieties, genetic factors, and geographical distribution.

3.3 Carotenoids

3.3.1 Carotenoids dosage

Date palm seed oil contains carotenoids, which are natural pigments responsible for the oil's yellow to reddish color. Carotenoids are known for their antioxidant properties and potential health benefits. Some of the carotenoids present in date palm seed oil include beta-carotene, lutein, and zeaxanthin(Sassi et *al.*, 2020)

The synthesis on the dosage and identification of the carotenoids present in date palm seed oil is carried out based on 5 articles from 3 different countries. The works dealing with the carotenoid content Figure 12 are written as follows:

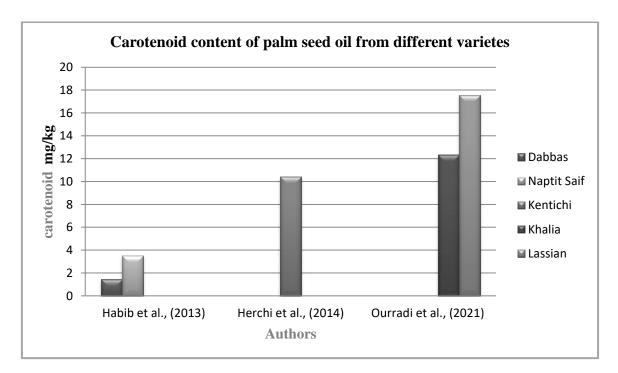


Figure 12: Total Carotenoid content of date palm seed oil from different varieties. The values presented in the above figure represent the results of colorimetric analysis of carotenoid. The results are expressed in mg/kg.

Regarding the percentage of carotenoids, a wide range has been observed among different varieties. In fact, the maximum value was obtained for the Lassian variety from the Moroccan region, measuring 17.57mg/kg, according to Ourradi et *al.*, (2021). On the other hand, the minimum value was found in the Dabbas Al-A'in variety from the United Arab Emirates, with a percentage of 1.46mg/kg, according to Habib et *al.*, (2013). In contrast, the Harshi variety and others recorded a moderate percentage of 10.41mg/kg, for the Kentichi variety, according to Harchi et *al.*, (2014).

It's important to note that carotenoid content can vary depending on factors such as the variety, growing conditions, and analytical methods used for measurement. Carotenoids are responsible for the vibrant colors in fruits and vegetables and have been associated with various health benefits due to their antioxidant properties.

In general, the composition and total pigment content of oils are important qualities as they are correlated with color, which is an attribute for evaluating oil quality, as seen in the case of olive oil (Aparicio et *al.*, 1999; Uluata, 2016).

3.3.2 Carotenoid composition

The identification of carotenoids present in the DPSO was conducted by the Emirati team of Habib et *al.*, (2013), on 18 date varieties using the high-performance liquid chromatography (HPLC) method. The following table presents the different carotenoids detected in the 18 varieties, with results expressed in mg/kg.

Second part, Chapter II

Results and discussion

Varieties	Luteine	Echinenone	Lycopene	α-Carotene	β-Carotene	γ-Carotene
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Khalas	0.07	0.10	0.01	0.07	2.14	0.27
Barhe	0.14	0.15	0.01	0.08	2.68	0.13
Lulu	0.11	0.11	0.02	0.05	1.92	0.11
Shikat alkahlas	0.11	0.11	nd	Nd	1.78	0.17
Sokkery	0.09	0.08	nd	Nd	1.34	0.14
Bomaan	0.19	0.09	0.01	0.06	1.88	0.18
Sagay	0.09	0.13	nd	Nd	2.24	0.11
Shishi	0.14	0.10	nd	0.03	1.49	0.20
Maghool	0.06	0.09	nd	Nd	1.57	0.13
Sultana	0.14	0.18	nd	Nd	2.09	0.26
Fard	0.13	0.10	nd	Nd	1.60	0.20
Maktoo mi	0.25	0.13	0.03	0.03	1.73	0.36
Naptit saif	0.26	0.19	0.01	0.03	2.42	0.49
Jabri	0.27	0.09	nd	0.06	1.25	0.12
Khodary	0.23	0.07	nd	nd	1.34	0.11
Dabbas	0.11	0.06	nd	Nd	1.18	0.03
Raziz	0.11	0.09	nd	Nd	1.43	0.16
Shabebe	0.23	0.17	nd	nd	2.11	0.40

Table 8: Carotenoids detected in date seed oil of 18 varieties.

Most of the carotenoids identified by Habib et *la*., (2013) and his team were found in trace amounts. Lutein, echinenone, β -carotene, and γ -carotene were detected in all 18 varieties, while lycopene was not detected in Sokkery, Shishi, Maghool, Dabbas, and Raziz. Additionally, α -carotene was not detected in 10 of the varieties, namely Shikat alkahlas, Sokkery, Sagay, Maghool, Sultana, Fard, Khodary, Dabbas, Raziz, and Shabebe.

The only predominant carotenoid is β -carotene, which ranged from 1.18 to 2.68 mg/kg. The Barhe variety exhibited the highest value, while the Dabbas variety recorded the lowest value.

The variation in concentrations among the 18 varieties could be attributed to differences in cultivars, degree of maturity, latitude, environmental conditions, processing techniques, and storage conditions. In general, the composition and total carotenoid pigment content of oils are important qualities as they are correlated with color, which is an attribute for evaluating oil quality, as seen in the case of olive oil Aparicio et *al.*, (1999) and (Uluata.2016).

3.4 Polyphenols

3.4.1 Polyphenols dosage

Date palm seed oil contains polyphenolic compounds, which have antioxidant and antiinflammatory properties. Polyphenols are associated with various health benefits, such as reducing the risk of cardiovascular diseases and supporting overall well-being (Fernánde zlópez et *al.*, 2022).

The provided information indicates the total polyphenol content in date palm seed oil from different studies conducted by seven scientific teams in seven different countries, involving different varieties of dates. The data is listed as follows Figure 13:

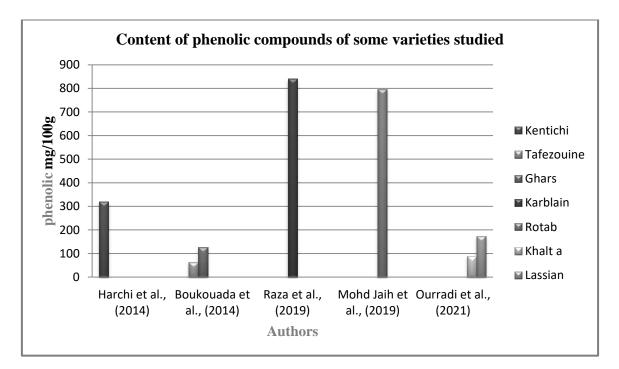


Figure 13:Total phenolic Content of date palm seed oil from different varieties.

The values presented in the figure above are the results of the polyphenol dosage, carried out using the Folin ciocalteu calorimetric method. The results are expressed in mg/100g.

The polyphenol level in the presence of the oil reaches its maximum value in a Pakistani study by Raza et *al.*, (2019). with a value of 840.07 for the Khalas variety. as for its minimum value, it is observed in Algerian research by Boukouada et *al.*, (2014) in the Ouargla region with 64 mg/100g for the Tafezouine variety. Therefore, it is clear that the date palm seed oil from Pakistani varieties is richer in polyphenols compared to the other varieties mentioned by the authors.

In contrast, the Malaysian study by Mohd Jaih et *al.*, (2019). on the Rotab variety recorded a high value of 796mg/100g, compared to the study conducted by Harchi et *al.*, (2014). In Tunisia, specifically in the Tozeur region, on the Kentichi variety, which reached a value of 319mg/100g. Meanwhile, Ourradi et *al.*, (2021). reported a value of 127.94mg/100g for the Lassine variety and a value of 89.34mg/100g for the Khalt a variety in their study conducted in Morocco.

3.4.2 Polyphenols composition

The identification of total polyphenols present in DPSO was done using the highperformance liquid chromatography (HPLC) method. The results obtained are indicated in Table 8, and the values are expressed in percentages (%).

Authors	Besbas et <i>al.</i> , (2004)		Al Juhaimi et <i>al.</i> , (2018)					
Country (Region)	``	Tunisie		Libia	Maroc	Pakistan	Sudan	
Varieties	Deglet	Allig	Algérie Ghars	Adwi	Allig ²	Dora	Gondeila	
	Nour							
Hydroxytyrosol (%)	10.22	6.94	nd	nd	nd	nd	Nd	
Gallic acid (%)	4.11	2.48	4.31	5.84	6.69	6.91	3.56	
Protocatechic acid (%)	9.62	4.26	0.051	0.071	0.053	0.049	0.077	
3,4dihydroxyphenylacetic	nd	1.56	nd	nd	nd	nd	Nd	
acid (%)								
Tyrosole (%)	4.50	8.10	nd	nd	nd	nd	nd	
Cafeic acid (%)	1.30	4.59	1.40	3.43	2.81	1.67	1.18	
p-coumaric acid (%)	0.26	0.22	nd	nd	nd	nd	Nd	
Oleuropein (%)	0.18	0.11	nd	nd	nd	nd	nd	
Syringic acid (%)	nd	Nd	3.76	4.86	3.43	1.56	2.78	
Rutin (%)	nd	nd	1.07	1.23	0.97	0.93	1.18	
Catechin (%)	nd	nd	7.23	5.43	3.18	2.86	6.61	

Table 9: Identified polyphenols in the date palm seed oil of some studied varieties.

Firstly, the highest content is that of hydroxytyrosol with 10% in the Tunisian Deglet Nour variety according to Besbas et al., (2004),the lowest value is attributed to Protocatechuic acid with 0.049% for the Dora variety originating from Pakistan in the study by Al Juhaimi et al., (2018). The presented results thus show a significant disparity in the concentrations of identified phenolic compounds.

A closer observation highlights the variability existing among phenolic compounds within the same country. As shown by Besbas et *al.*, (2004), the same phenolic compounds were found in both Tunisian varieties, except for 3,4-dihydroxyphenylacetic acid, which was only found in the Allig date palm seed oil.

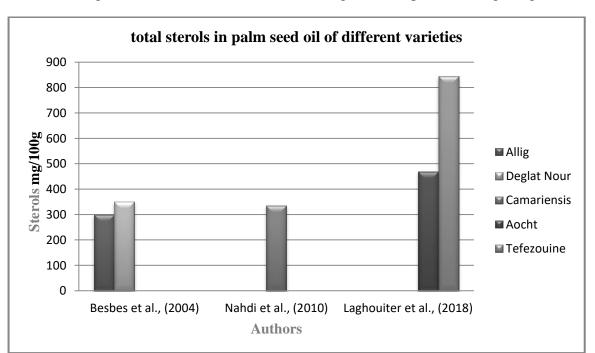
These identified polyphenols (Hydroxytyrosol, Tyrosol, p-coumaric acid, and Oleuropein) were not detected by Al Juhaimi et *al.*, (2018).who identified three completely different compounds: syringic acid, rutin, and catechin. There is, therefore, a significant difference in the presence of each compound in the studied varieties.

Flavonoids are natural bioactive compounds with a high ability to prevent diseases, including cancer and cardiovascular disease. These benefits are attributed to their antioxidant activity (Williams et *al.*, 2004).

3.5 Sterols

3.5.1 Sterols dosage

The sterol fraction is indeed one of the most studied components of the unsaponifiable fraction.the synthesis on the quantification and identification (Besbas et al., 2004; Laghouiter et al., 2018).of sterols in the studied varieties is based on six articles from different countries.



The dosage results obtained are illustrated in Figure 14, expressed in mg/100g.

Figure 14: Total Content of sterols in palm seed oil of different varieties.

Based on the figure, a high level of sterols was identified in the date palm kernel oil of the Tefewouine variety, reported by Laghouitera et *al.*, (2018). in the Ghardaia region of Algeria, with a value of 845mg/100g. On the other hand, the Tunisian variety,Allig recorded a lower value with a content of 300mg/100g, according to Besbas et *al.*, (2004). Furthermore, a study conducted by Nahdi et *al.*, (2010). on the P-Camariensis variety yielded a peak of 336.07mg/100g. Through comparing the presented studies, it can be inferred that studies conducted within the same country tend to have very close results.

It was reported by Salvador et *al.*, (2001) that sterols are important constituents of oil as they relate to its quality and are widely used to verify its authenticity.

3.5.2 Sterols composition

The identification of sterols present in the oil was performed using the Gas Chromatography-Mass Spectrometry (GC-MS) method. The results obtained are presented in Table 10, and the values are expressed in (%).

Author	Besbas et <i>al.</i> , (2004)		Basuny et Al-Marzooq
			(2011)
Country (Region)	Tunisie	(Degach)	Arabie Saoudite (Al-Hasa)
Varieties	Deglet Nour	Allig	Khalas
Cholesterol (%)	0,96	0.58	Nd
Campesterol (%)	9.10	10.19	8.70
Stigmastérol (%)	2.42	2.29	3.48
β-Sitosterol (%)	83.31	78.66	25.83
Δ 5-Avenasterol (%)	0.45	4.5	Nd
Δ 5.2,4-Stigmastadienol (%)	0.23	0.41	Nd

Table 10: Sterols identified in the date seed oil of 3 varieties studied.

According to the table, β -Sitosterol is the major component of sterols in all varieties. Tunisian varieties recorded the highest values with 83.31% and 78.66% for Deglet Nour and Allig varieties, respectively, while the lowest value was reported by Basuny et Al-Marzooq (2011). in the Khalas variety with 25.83%. Campesterol ranks second, constituting approximately 90% of the total sterols, along with β -Sitosterol. Stigmasterol is also present, accompanied by trace amounts of cholesterol, Δ 5-Avenasterol, and Δ 5,2,4-Stigmastadienol in Tunisian varieties. The existing differences in sterol compositions make them suitable for determining the botanical origin of oils and detecting adulteration among vegetable oils (Aparicio et Aparicio-Ruiz, 2000).

3.6 Triacylglycerols

3.6.1 Triacylglycerols dosage

Date palm seed oil contains triacylglycerols (TAGs), which are the main components of its lipid fraction (Nahdi et *al.*, 2010).

The profile of TAGs in date palm seed oil contributes to its nutritional and functional properties, such as texture, stability, and oxidative stability. The specific composition of TAGs can also influence the oil's taste, aroma, and potential health benefits (Nahdi et *al.*, 2010; Fakhfakh et *al.*, 2019).

In the study described in Figure 15, we will discuss determining the values of TAGs in date seed oil for several different varieties. the study will be limited to four articles for four different regions.

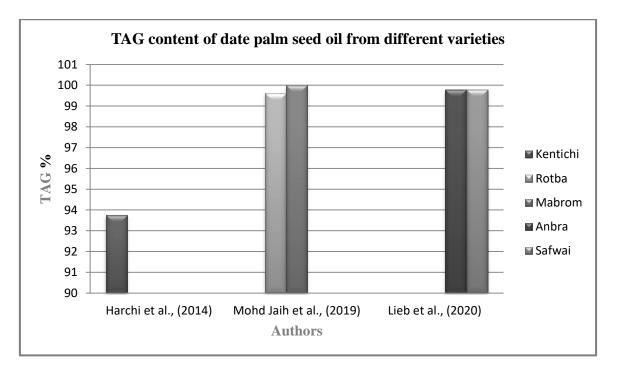


Figure 15: Total content of TAG in palm seed oil of different varieties.

Based on figure 15, which represents the quantity of TAG (Triacylglycerol) in date seed oil for different varieties, we observe that the percentage of TAG is very close across all varieties. The highest value, with minimal differences, was recorded in the study conducted by Mohd Jaih et *al.*, (2019),on the variety "Mabrom" with a value of 100%. On the other hand, the lowest value was found in the study conducted by Harchi et *al.*, (2014),on the variety "kentichi" with a value of 93.75%. Additionally, we found the same value of 99.8% for two varieties, "Anbra" and "Sfwai," respectively, in the study conducted by Lieb et *al.*, (2020).

3.6.2 Triacylglycerols composition

The triacylglycerol (TAG) composition was analysed using a high-performance liquid chromatography (HPLC), the results obtained for six different varieties in the study conducted by Mohd Jaih et *al.*, (2019) are presented in Table 10, expressing the results in percentages.

Table 11: Triacylglycerols (ATG) identified in the date seed oil of 3 varieties studied.

Second part, Chapter II

Results and discussion

TAG	Variety of date seed oil (%)						
	Mabroum	Rotab	Safawi				
LaLaLa	3.95	1.7	0.76				
LaLaM	4.24	2.43	4.29				
LaLaO	1.54	1.97	1.98				
LaLaP + LaMM	10.96	10.83	8.19				
LaMO	3.23	1.64	1.77				
LaMP	4.91	3.76	5.8				
MLL + LLL + LaLO	13.12	11.77	11.92				
LaOPd	3.28	ND	ND				
SSCI	ND	1.19	ND				
LaOO	16.52	15.54	18.44				
LaPO + POLn	11.29	11.73	9.47				
OOL	2.33	2.33	4.33				
МОО	4.31	9.24	8.96				
LaSO + SSCp + SOLn + POL	4.75	6.95	5.67				
000	4.07	4.81	8.08				
POO + PGL + ALL	3.11	7.04	5.79				
РОР	2.11	3.66	2.35				
SOO + POS	6.3	3.04	2.19				

The present work revealed that LaOO was the dominant TAG in the date palm seed oil, which ranged between 15.5 and 18.4%. In contrast, LaOPd and SSCl were only detected in Mabroum and Rotab seed oil, In another study, Nahdi et *al.*, (2010) found LaLaP + LaMM as the two dominant TAGs in their P. canariensis oil. Therefore, the highest tri-unsaturated and tri-saturated TAG in the five DPSO in the present work were obtained by OOO and LaLaP + LaMM, respectively, which is comparable to the DPSO studied by Nahdi et *al.*, (2010). However, the report by Abdul Afiq et *al.*, (2013). highlighted the lack of study conducted to determine the triacylglycerol composition of P. dactylifera seed oil. (Mohd Jaih et *al.*, 2019).

4. Antioxidant Activity

4.1 In vitro evaluation of antioxidant activity

Date palm seed oil contains natural antioxidants, such as tocopherols (vitamin E). These antioxidants help protect the body against oxidative stress and free radical damage, thereby exerting potential health benefits.

4.1.1 DPPH (α,α-diphenyl-β-picrylhydrazyl)

The evaluation of the antioxidant activity in the studied works was determined using the DPPH. radical scavenging test. The synthesis addressing this evaluation of the studied varieties is based on four articles from two different countries.

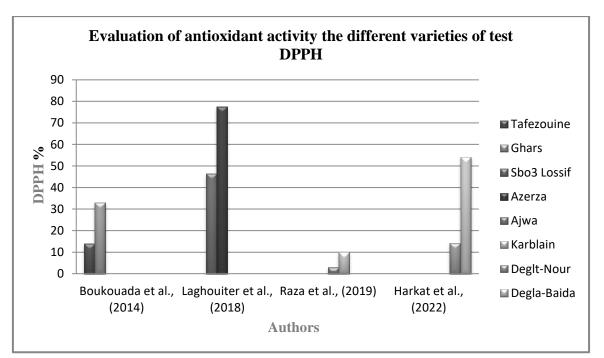


Figure 16 represents the results of the DPPH test of the different varieties

Figure 16: Evaluation of antioxidant activity the different varieties of test DPPH

Figure 16 highlights the variation in content reported by the Boukouada et *al.*, (2014) Algerian, for different varieties from the same country but different regions. They recorded contents of 14% and 33% for varieties from the regions of Ouargla, Tafzouat and Ghars variety, respectively. These values are much lower than those reported by Laghouitera et *al.*, (2018) for the Azerza variety, which showed the highest content of 77.58% in Ghardaia. On the other hand, Raza et *al.*, (2019) reported the lowest content of 3% for the Pakistani variety Ajwa. Additionally, the study conducted by Harkat et *al.*, (2022). in Biskra, Algeria, revealed the highest value of 54.17% for the white Degla-Baida variety, which is considered high compared to previous studies.

The results of these studies have shown that date palm seed oil can be a good source of antioxidants. A strong correlation between phenolic content and radical-scavenging efficacy has been found. This indicates that the antioxidant activity of the oil may be mainly attributed to the presence of water-soluble compounds with potent free radical scavenging effects.

4.1.2 ABTS (2,20 -azinobis (3-ethylbenzothiazoline-6-sulfonic acid))

The evaluation of the antioxidant activity in the studied works was determined using the ABTS. radical scavenging test. The synthesis addressing this evaluation of the studied varieties is based on two articles from two different countries, For olive oil and date seed oil.

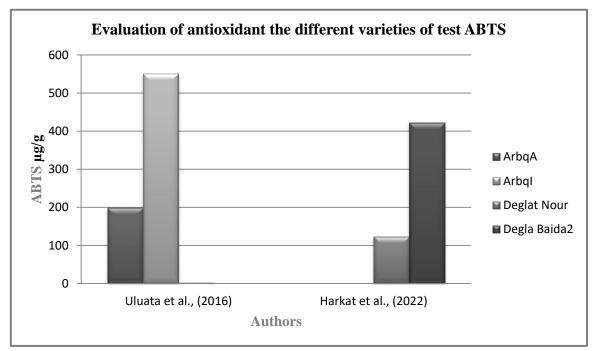


Figure 17 represents the results of the ABTS test of the different varieties

Figure 17: Evaluation of antioxidant activity the different varieties of test ABTS

Based on figure 17, which represents the evaluation of antioxidant activity in different varieties using the ABTS assay for olive oil and date seed oil, it is observed that the study conducted by Uluata et *al.*, (2016) in Turkey on olive oil recorded a higher value (551,38 μ g/g) compared to the study conducted by Harkat et *al.*, (2022) in Algeria on date seed oil recorded a highest value (423 μ g/g).

Therefore, we can conclude from the following study that the antioxidant activity in the ABTS test is higher in olive oil compared to date seed oil.

Conclusion

Conclusion

Date palm seed waste, which has traditionally been considered a byproduct with limited use, is now being recognized for its valuable applications. Recent research has shown that date seeds are rich in dietary fiber, essential fatty acids, and antioxidants, making them a potential source of health benefits. In particular, studies have focused on the nutritional composition of date palm seed oil, revealing its significant potential as a valuable natural resource.

The oil is rich in beneficial bioactive compounds such as unsaturated fatty acids (oleic acid and linoleic acid), tocopherols (α and γ -Tocophérol ; α and γ -Tocotrienol), carotenoids, polyphenols (gallic acid, caffeic acid, syringic acid and catechin), sterols (Stigmasterol and sitosterol) and triacylglycerols, which gives a strong antioxidant capacity and make it suitable for various applications.

This discovery presents an opportunity to utilize date palm seed oil as an underutilized byproduct of the date industry with significant untapped potential. Further research and development in extraction, characterization, and application of this natural oil can lead to the creation of innovative, sustainable, and value-added products. Ultimately, this can contribute to the diversification of the agricultural economy by creating new opportunities for utilizing what was previously considered waste.

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Abstract

Date palms, scientifically known as *Phoenix dactylifera* L., play a significant role as a crop in dry and semi-dry regions, offering dates as a valuable food source and income. This research aims to elucidate the outcomes of biochemical composition and antioxidant properties of date palm seed oil examined in diverse countries. The findings encompass the analyses of fatty acid composition, tocopherols, polyphenols, and sterols in date palm seed oil. Variations among different date palm varieties are also discussed. Furthermore, the potential uses of date palm seed oil in cosmetics, food, and health-related sectors are investigated. This study adds to the comprehension of the characteristics and potential applications of date palm seed oil, which may be of interest to professionals working with plant-based oils and their diverse uses.

Key words: Date palm; seed oil; bioactive compounds; antioxidant activity.

ملخص

تلعب نخيل التمر، المعروفة علمياً باسم Phoenix dactylifera L. ورًا هامًا كمحصول في المناطق الجافة وشبه الجافة، حيث تقدم التمور كمصدر غذائي قيم ومصدر للدخل. يهدف هذا البحث إلى شرح نتائج الخاصة بالمكونات البيوكيميائية والخصائص المضادة للأكسدة لزيت بذور نخيل التمر المدروس في أوساط متنوعة. تشمل النتائج تحليل تركيب الأحماض الدهنية، والتوكوفيرولات، والبوليفينولات، والستيرولات في زيت بذور نخيل التمر. كما يتم مناقشة الاختلافات بين مختلف أصناف نخيل التمر. علاوة على ذلك، يتم استقصاء الاستخدامات المحتملة لزيت بذور نخيل التمر في مجالات التجميل، والغذاء، والقطاعات ذلت الصلة بالصحة. يضيف هذا البحث إلى فهم الخصائص والتطبيقات المحتملة لزيت بذور نخيل التمر في مجالات التجميل، والغزاء، والقطاعات ذلت الصلة بالصحة. المعتمدة على النباتات واستخدامات المحتملة الذيت بذور نخيل التمر في مجالات التجميل، والغزاء، والقطاعات ذلت الصلة بالصحة.

كلمات مفتاحية: نخيل التمر، زيت البذور، مركبات حيوية، نشاط مضاد للأكسدة.

Résumé

Le palmier dattier, connus scientifiquement sous le nom de *Phoenix dactylifera* L., jouent un rôle significatif en tant que culture dans les régions sèches et semi-arides, offrant des dattes comme source alimentaire et de revenus précieuse. Cette recherche vise à élucider les résultats de la composition biochimiques et les propriétés anti-oxydantes de l'huile de graines de palmier dattier examinée dans divers pays. Les résultats englobent les analyses de la composition en acides gras, de tocophérols, de polyphénols et de stérols dans l'huile de graines de palmier-dattier. Les variations entre différentes variétés de palmiers-dattiers sont également discutées. De plus, les utilisations potentielles de l'huile de graines de palmier dattier dans les secteurs des cosmétiques, de l'alimentaire et de la santé sont étudiées. Cette étude contribue à la compréhension des caractéristiques et des applications potentielles de l'huile de graines de palmier-dattier, qui peuvent intéresser les professionnels travaillant avec des huiles végétales et leurs utilisations diverses

Mots clé : Palmier dattier ; huile de graine ; composés bioactives ; activité antioxydante.