ROYAUME DU MAROC

Université Mohammed V
- RABAT -

جامعة محمد الخامس
- الرباط -

Faculté des sciences
كلية العلوم

CENTRE D'ÉTUDES DOCTORALES - SCIENCES ET TECHNOLOGIES

N° d'ordre : **3928**

THÈSE

en vue de l'obtention du : **DOCTORAT**

Centre de Recherche : Sciences et Technologies (FS)
Structure de Recherche : Laboratoire des Matériaux, Nanotechnologies et Environnement (LMNE)
Discipline : Chimie
Spécialité : Chimie organique-phytochimie et analytique

Présentée et Soutenue le : 01 / 06 / 2024

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The valorization of "Phoenix Dactilifera L." date seeds

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Année Universitaire : 2023 - 2024

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ACKNOWLEDGEMETS

Initially; Praise and thank Allah, our Lord, and Almighty God, for granting me the will, patience, health, and courage to accomplish this modest work. Thank you for showing me the way to success.

I would like to express my sincere gratitude to Prof. Abdelkebir BELLAOUCHOU and Prof. Mohamed TABYAOUI my supervisors, for accepting me as a PhD student in the Laboratory of Materials, Nanotechnology, and Environment, and, for their great support and their continuous supervision during my entire studies, without which this work would have been difficult to finish with satisfaction. My thanks go to Prof. Abdelkader ZARROUK and Prof. Harhar for their time and advices. I would like to extend my sincere thanks to the Professors of LMNE and PhD students who supported me during my experimental work.

I would like to offer my special thanks to Prof. Abdelkebir BELLAOUCHOU for agreeing to chair the jury for this thesis, which is an honor for me.

My sincere thanks also go to Prof. Smail ZIDANE, Prof. Lhou MAJIDI and Prof. Abdellah FARAH for agreeing to examine this modest work. I am honored by their presence on the jury for this thesis.

Last but not least, a special word of gratitude to my friends and family; and I would like to express my sincere thanks to my mother and husband, without their support, my thesis couldn't have been finished smoothly.

Thank you

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General Introduction

The date palm (*Phoenix dactylifera* L.), an ancient and mythical tree, with its fruit—the date—harbors resources whose importance is well established. A symbol of oasis agriculture, it is the creator of life in arid regions and the source of invaluable economic, religious, moral, and ecological values.

The date palm is mentioned seventeen times in the Qur'an and has been cultivated for millennia, with a history spanning over 7,000 years. Native to the Euphrates basin, it spread westward across North Africa and eastward as far as India. Its cultivation later expanded into Western countries, first in Spain, and then in the eighteenth century, to other parts of Europe.

Today, the date palm is primarily cultivated for its fruit. It is a perennial, lignified plant **(DJERBI, 1994),** as its name suggests, belonging to a large family of palm trees and producing dates. The date palm is an arborescent monocot, classified in the plant kingdom alongside grasses and other herbaceous plants **(PEYRON, 2000).**

The oldest date palms date back to the Miocene. The date palm was cultivated in the warm regions between the Euphrates and the Nile around 4500 BCE. From there, its cultivation was introduced to Lower Mesopotamia around 2500 BCE, then it spread northward across the country, reaching the coastal region of the Persian plateau and eventually the Indian subcontinent (Munier, 1973). After Egypt, the cultivation techniques for the date palm spread to Libya, then gradually expanded to other Maghreb countries such as Tunisia, Algeria, and southern Morocco, eventually reaching the Adrar region of Mauritania. Today, date palm cultivation extends across the Northern Hemisphere.

Moreover, many studies have been conducted on the fruit of the date palm worldwide; however, few studies have been carried out on its seed, whether from a nutritional or toxicological perspective.

In our thesis topic, we focused on the identification of bioactive compounds as well as their antioxidant activity through in-vitro tests. This thesis is organized into three parts. The first is devoted to a literature review, which focuses broadly on the definition, history, and studies conducted. The second part presents the materials and methods used to prepare date seeds, the extraction protocol, and the analysis and identification methods for the bioactive compounds. Finally, it includes the evaluation of antioxidant activity and the toxicological study. The third part includes the results and discussion, organized into five chapters.

The first chapter is a comparative study of the physicochemical parameters and chemical composition of date seed oil from ten different varieties. Its objective is to identify the components of the extracted oil and determine its physicochemical properties as well as its quality.

The second chapter is an analysis of the bioactive compounds present in date seeds and the evaluation of their inhibitory and reducing capacities.

The third chapter presents a toxicological study to determine the level of oxalate ions, which are widely found in plants.

The fourth chapter focuses on the roasting of date seeds, optimizing the parameters to obtain a beverage rich in natural antioxidants.

The final chapter is a study on the stability of an oxidized oil enriched with a natural antioxidant and compares it with a standard antioxidant (ascorbic acid) against a control. This is done using evaluation and monitoring techniques over time.

Part 1:

Bibliography

A- Date Palm "Phoenix Dactilifera L.": History, Botanical Description, Production and Geographical distribution

I- History of the date palm

The date palm "Phoenix Dactilifera L." is considered one of the oldest fruit trees in the world. For thousands of years, it has been cultivated mainly in North Africa and the Middle East; its oasis extends from Morocco in the west to the Indo-Iranian border in the east, and from central Syria in the north to Yemen in the south. It aids in the construction of agricultural production centers in difficult environments as well as the creation of an artificial ecosystem that ensures the subsistence of a large portion of the population living in these areas. Despite its social and economic importance, its origin remains unknown. Some reports state that it has been used since 4000 BC in Mesopotamia and since 2000-3000 BC by the Egyptians. Other evidence suggests that the origins of oasis agriculture go back to prehistoric times in the Persian Gulf region [1],[2].

The scientific name of Phoenix Dactilifera L. comes from the Phoenician term. Phoenix means date palm, and Dactilifera is derived from the Greek word Dactulos which means finger.

There are about 2000 different cultivars of the date palm, but only a few of them have been used due to their agricultural productivity and fruit quality [3]. There are more than 100 million date palms in the world; their fruit production starts at the average age of 5 years and can last up to 60 years [4]. The average annual yield is between 400-600 and 100-150 kg/tree for fresh and dry harvests, respectively [4]. The quality and yield of date fruits depend on several factors such as pollination, fertilization, cultivar, and climate [5].

II-Botanical Description of Phoenix Dactilifera L.

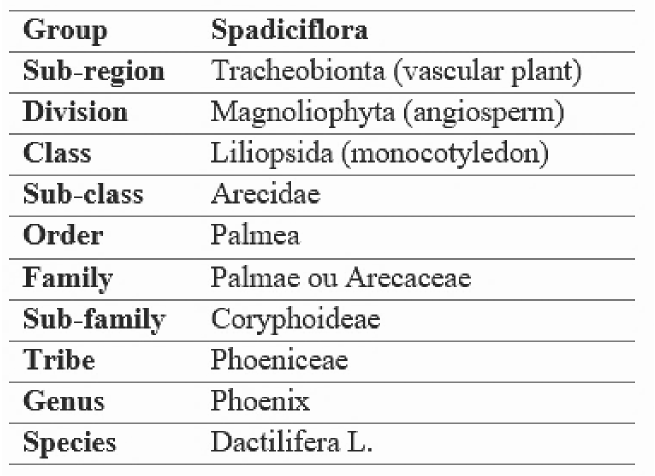
Botanically, the date palm is a monocotyledonous plant of the Arecaceae family (formerly known as Palmaceae or Palmae). The Palmaceae, often called Palmae, is a family of plants that includes 200 genera and about 2000 species [6].

The Phoenix genus has several species, but Phoenix Dactylifera L. is the most generally cultivated for its edible fruit, while the other species produce fruit that animals and birds may consume [7]. These species can be found in tropical or subtropical areas in the Middle East, North Africa, South Asia, North America, and Australia.

1- Taxonomy and Classification of Date Palm

The date palm (Phoenix Dactylifera L.) gets its name from the Phoenician term "Phoenix," which means "date palm" in Phoenician, and dactylifera comes from the Greek word "Dactulos," which means "finger," alluding to the fruit's shape [8]. Table 1 shows the botanical identification of the date palm defined by Djerbi in 1994.

__Table 1: Botanical classification of the date palm__



Group	**Spadiciflora**
Sub-region	Tracheobionta (vascular plant)
Division	Magnoliophyta (angiosperm)
Class	Liliopsida (monocotyledon)
Sub-class	Arecidae
Order	Palmea
Family	Palmae ou Arecaceae
Sub-family	Coryphoideae
Tribe	Phoeniceae
Genus	Phoenix
Species	Dactilifera L.

2- Morphology of the date palm

Considering the enormous number of current cultivars, this plant presents an important degree of morphological variety, whether in terms of port, palms, fruits, seeds, etc.

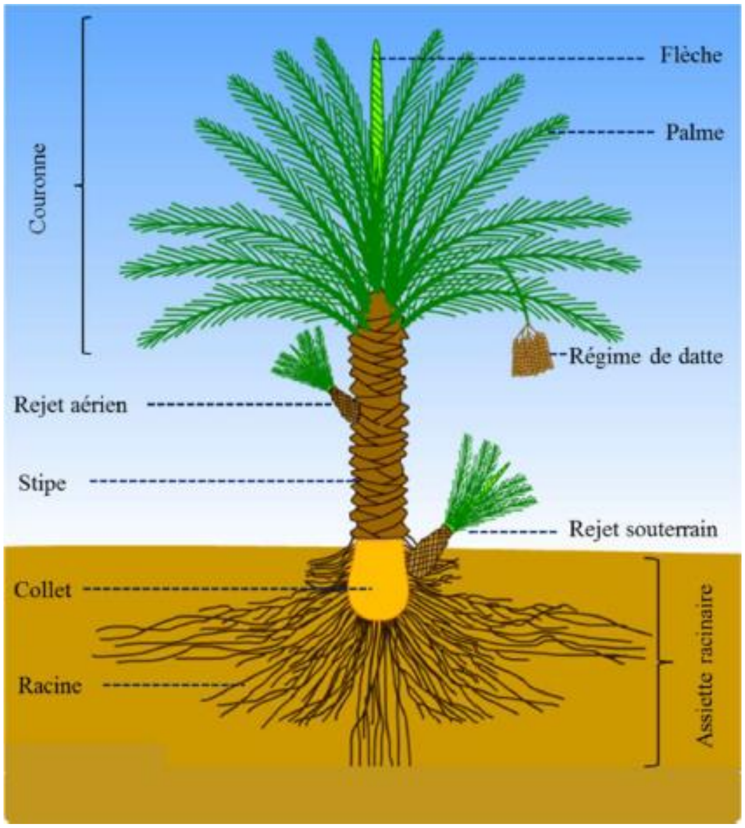


Figure 1 : Representative diagram of the different components of an adult date palm[9]

The date palm is a monocotyledonous angiosperm with an arborescent habit. It is made out of a stipe above a palm crown. Its height can reach 30 meters. Given the vast number of contemporary cultivars, this plant exhibits a significant degree of morphological variability, whether in terms of port, palms, fruits and seeds. Morocco, Algeria, and Tunisia worked together to establish a paper on the morphological properties of date palms [10].

However, they are rarely employed because the process is very detailed and tedious. Some countries have inventories of their biodiversity [11], but the agrobiodiversity of date palm is mostly unknown. Describing cultivars based on fruit characteristics (shape, size, weight, color, etc.) is an intriguing method since the fruit is an interesting, variable, and easily accessible part of the plant for study. However, these parameters are qualitative, as they are influenced by the environment and developmental stage, and their discriminatory potential is limited.

2.1- Root System

The date palm's root structure is fascicular, which means it does not branch. The bulb, or root plate, has only a few rootlets and protrudes slightly above the soil surface [8],[12]. The system is separated into four root types:

- *Zone n°1*, called respiratory zone: These are the respiratory roots, which can emerge on the ground at a depth of less than 0.25 m.
- *Zone n°2*, called nutritional zone: These are the nutrition roots, which reach a depth of 0.30 to 0.40 m.
- *Zone n°3*, called absorbing zone: These are the absorption roots, which can reach the phreatic level at a depth of 1 to 1.8 m.
- *Zone n°4*: The largest portion of this zone is dependent on underground water. At lesser depths, it is difficult to discern between Zone III and Zone IV since both types of roots are present. When the underground water is deep, the roots in this zone may reach larger depths. They are commonly shown as vessels with a positive geotropism (Figure 1).

2.2- Vegetative System

The date palm is made up mostly of the following components:

a. Trunk:

The trunk, also named a stipe has a cylindrical form and varies in size according to the variety and the climatic conditions for the same type. Its coronal section elongates due to the terminal bud or phyllophore. The stipe may grow to be up to 20 m long, its thickness remains consistent throughout the palm's life, and it can show minor shrinkage zones that generally correlate with dry seasons.

The trunk of date palm, also called a stipe or stem, is brown in colour and is a single vertical cylinder which can extend to 30 m in length. It is covered by leaf bases that are enclosed in fibre which protect the trunk from animals and insects, and in addition reduce loss of water [12], [13].

b. Leaves :

They are pinnate compound leaves that vary in length and flexibility depending on the cultivar and growing circumstances. The petiole is hard and relatively rigid, the leaflets are arranged in an oblique position along the rachis and have a life span of three to seven years (Figure 2).[14]

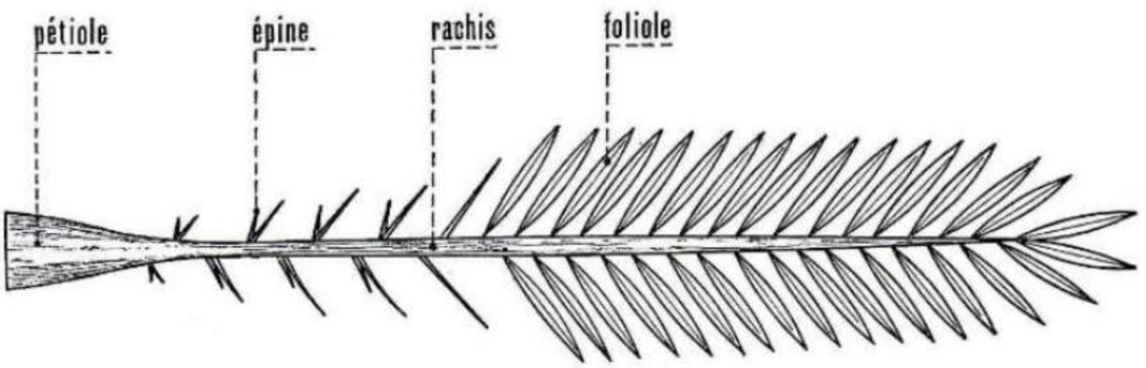


Figure 2: Representative diagram of a date palm leave

c. Inflorescences or flowers:

Since the palm is a dioecious plant, the sexes are split into a female palm that bears the fruit and a male palm that acts as a pollinator and produces pollen. It is required to wait 6 to 8 years for the induction of the first flowerings before determining the sex of the plants. [15]

d. Fruit :

The date is a fruit containing a single seed (Figure 3). It has a thin cellulose envelope, called epicarp, and a fleshy mesocarp with variable consistency, which adopts a peripheral zone of stronger color and compact texture, and another internal one of lighter color and fibrous texture, called endocarp, reduced to a parchment-like membrane that surrounds the seed or nucleus. The shape and firmness of the fruit varies from one variety to another. In terms of nutritional content, for example, a dry date contains about 287 calories per 100 g, which is comparable to other dried fruits.[12]

Figure 3: Morphology and anatomy of date palm fruit and seed.

3- Other Species of Phoenix

According to the literature, the palm family comprises over 2,400 species spread across Saharan, sub-Saharan, tropical and sub-tropical regions. The Phoenix genus is unique within the Phoenixae tribe, comprising 17 palm species: Phoenix dactylifera L., P. atlantica A., P. canariensis, P. Theophrasti, P. reclinata, P. Sylvestris, P. humilis , P. hanceana, P. roebelinii, P. farintfera, P. rupicola, P. acaulis, P. paludosa. Among these different Phoenix species, only P. canariensis, and P. theophrasti and P. reclinata are the most morphologically similar to date palms [16],[17](Figure 4).

Cretan date palm “*Phoenix theophrasti* ” **Canary Island Date Palm** “*Phoenix canariensis*”

Figure 4 : The two most prominent species of date palm

III-Growth and Maturity stages of Dates:

The date palm fruit matures in five stages after pollination. Each stage is distinguished by physical changes in flavor, color, and texture, as well as chemical changes in the fruit's composition. These stages are called Loulou or Hababouk, Kimri, Khalal, Rutab, and Tamr [18] (Figure 5).

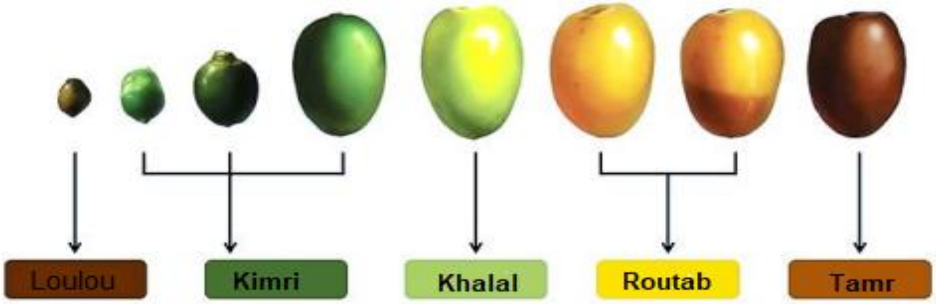


Figure 5: The appearance of the date palm fruit at the five stages of growth

a. First Stage: Loulou or hababouk

The stage of loulou or hababouk is considered the slowest stage of growth. After fertilization, it lasts from four to five weeks, and its end is marked by the fall of the two unfertilized carpels. At this stage, the fruit is completely covered by perianth and is characterized by slow growth. The shape of the fruit is ovoid with a point at the apex, and it is characterized by a color going from cream to light green. Its coloration turns bright green, which it keeps until the following stage, while its weight is inferior to 1g at the setting.

The loulou or hababouk stage is the slowest stage of development. It lasts four to five weeks after fertilization and is indicated by the fall of the two unfertilized carpels. At this stage, the fruit is entirely covered by perianth and is growing slowly. The fruit has an oval form with a tip to the apex and a tint that ranges from cream to light green. Its coloration then changes to a bright green that it retains until the next stage, while its weight is less than 1g at the setting.

b. Second Stage : Kimri

The Kimri stage lasts from nine to fourteen weeks and is characterized by a fast growth in fruit size and weight to a maximum weight of 5 to 12g. On the other side, it has the greatest humidity rate, the strongest acidity, and the largest concentration of tannins, starch, reducing sugars, total sugars, and dry matter. The fruit turns green before changing to the color associated with the following stage (Khalal).

c. Third Stage : Khalal

The khalal stage lasts between three and five weeks. It is distinguished by a slight decrease in the rate of fruit weight and size development, as well as a decrease in acidity, water, and starch content. On the other hand, the proportion of sucrose, total sugars, and dry matter increases rapidly. During this stage, the fruit's color goes from green to light yellow, then to yellow, pink, or red, depending on the variety.

d. Fourth Stage : Routab

The Routab stage takes two to four weeks to complete. It is characterized by fast moisture loss and enhanced enzymatic activity of pectinases. Pectinases are enzymes that can hydrolyze pectic compounds [19],[20]. During ripening and maturity, increased activity of these enzymes causes textural changes and softening of the fruit. Pectin esterase (PE), pectin methyl esterase (PME), and polygalacturonase (PG) are the names given to these enzymes depending on their mechanism of action. The PE enzyme is only capable of hydrolyzing the methyl ester group of polygalacturonic acid.

The PG enzyme is a depolymerizing enzyme that hydrolyzes the α-1,4 glycosidic bonds between galacturonic acid monomers at the non-reducing end of pectic substances, resulting in α-1,4-D-galacturonide and galacturonic acid correspondents [21],[22]. During this stage, the weight of the fruit decreases, sucrose is converted to invert sugar, and the color of the fruit changes from yellow or red to dark brown or black with a softer texture.

e. Fifth Stage: Tamr

The Tamr stage is the final stage of ripening; the fruit recognizes a massive decrease in moisture from about 84% in the second stage (Kimri stage) to about 24% in the Tamar stage (Ahmed et al., 1995; Al-Shahib & Marshall, 2003), which induces a high sugar/water ratio that prevents fermentation and ensures the preservation of the fruit, as well as a wrinkled pericarp and the characteristic dark brown color of dates. The fruit also loses tannins and acidity, which reduces its astringency [22],[4].

IV- Geographical distribution

1- Geographical distribution of date palm worldwide

The date palm is a xerophile, meaning it can only blossom and give fruit in hot weather. Indeed, it is found mostly in dry and semi-arid locations, from the Atlantic coast of North Africa to the south of the Mediterranean and from the southern edge of the Middle East to the southeast of Iran (Figure 6).

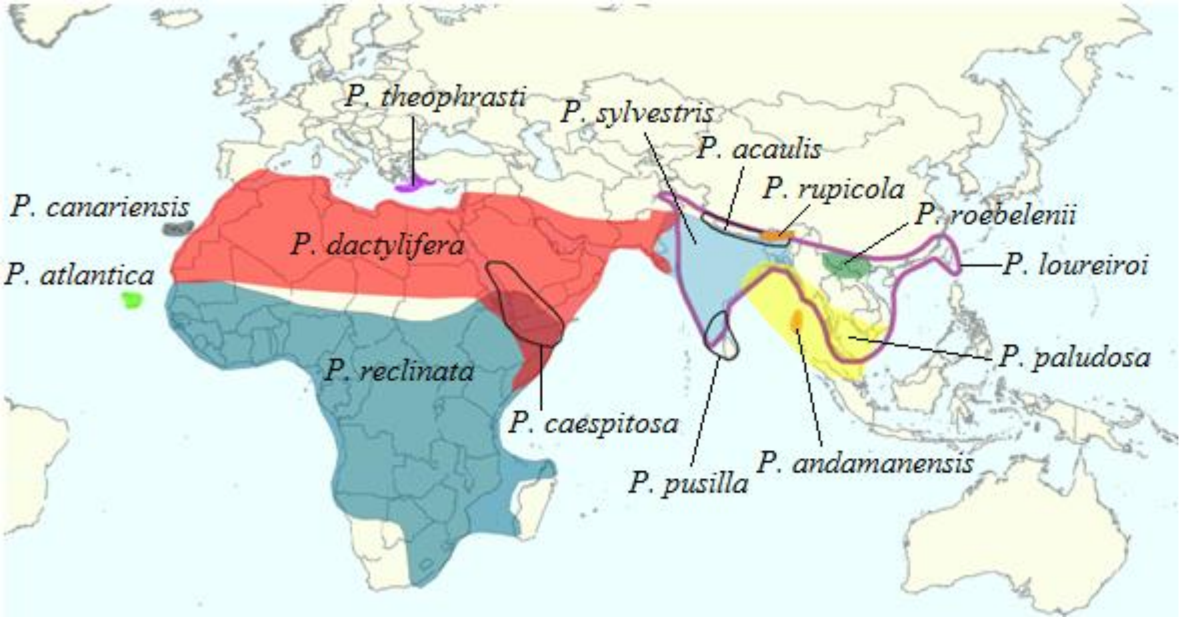


Figure 6: Geographical repartition du palmier of date palm in africa and middle east

The palm grove of Elche in Spain is Europe's only producer of dates. In America, the phoeniculture is mainly exploited in the United States (Arizona, California, and Texas). It is also found, albeit on a smaller scale, in Mexico, Colombia, Brazil, Argentina, Thailand, Namibia, South Africa, and Australia. [23]

2- Geographical distribution of the date palm in Morocco

Date palms are grown in several areas in Morocco, including the southern flank of the Atlas Mountains, along rivers such as the Drâa and Ziz, and around water points (e.g., springs and permanent wells) such as the Tafilalet plain and the palm groves of Figuig, Goulmim, and Alnif. The provinces of Ouarzazate, Errachidia, and Tata account for almost 90% of the total palm trees. [23]

V-Date Production

1- Date Production on an International Scale

According to FAO statistics (table 2), the world production of dates was around eight million tons in 2018. North Africa and the Middle East are the world's largest producers of dates. Saudi Arabia, Algeria, Iraq, Iran, Morocco, Niger, Pakistan, Tunisia, and Yemen are the main producers.

Table 2: Date production by country in 2018 (FAO 2018)

Country	**Valeur de la production brute (1000 US$)**
Algeria	3.689.880
Egypt	307.680
Iran	1.219.313
Iraq	248.577
Israel	236.396
Morocco	91.589
Pakistan	340.615
Saudi Arabia	3.755.833
Tunisia	441.428
United States of America	155.998
Yemen	52.850

2- Date Production on a national scale

Morocco is ranked seventh in terms of area, eleventh in terms of date palm number, and twelfth in terms of date production (FAOSTAT 2011). After citrus and other fruit crops, date cultivation comes third in terms of national arboriculture. The date palm is the primary foundation for the survival of the oasis ecosystem in the Saharan areas, and it plays important ecological, social, and economic functions. Indeed, date palm farming offers a microclimate for the underlying tree and herb crops, as well as prudent protection from the rigors of the external environment and the severity of its abrupt shifts. This protection helps to halt the process of desertification and mitigate the effects of the desert.

On a socio-economic level, the date palm sector contributes to the supply of dates for Moroccans. The annual national consumption of dates is about 3 kg per person and can reach 15 kg per person in manufacturing areas. With an average of 100,000 tons per year, date production ranks first among fruit oases and accounts for 20 to 60% of oasis farmers' income.

B-Natural substances found within a seed

I-Vegetable Oils

1- Generalities:

Humans have been using vegetable oils since ancient times, and their applications have evolved through time. Originally, oil was used as a fuel for illumination rather than for food purposes. The term "oil" refers to triglycerides that are liquid at room temperature. Many plants contain them, including legumes (such as peanuts and soybeans), seeds (such as rapeseed and sunflower), fruits (olives), and cereals (corn). Vegetable oil compounds are organic non-volatiles with hydrophobic and sometimes amphiphilic properties (phospholipids). They are water insoluble but soluble in apolar organic solvents.

In general, there are two main classes of vegetable oil:

- ✓ Fluid vegetable oils: rapeseed, corn, sunflower, soybean and olive oil.
- ✓ Concrete vegetable oils (fat): copra (coconut), palm oil.

Vegetable oils are considered as a privileged source of essential macronutrients such as linoleic acid C18:2 (Omega 6) and alpha-linoleic acid (Omega 3) as well as micronutrients like tocols (like vitamin E) and phytosterols [23].

2- Various Applications of Vegetable Oils

One-third of the world's fat production is destined for industrial use. While two-thirds of the output is destined for human consumption. Among the various industrial applications of fats are the manufacture of soaps, fatty acids, etc. Triglycerides are also a source of a wide range of chemical compounds that may be employed in the formulation of a variety of products such as cosmetics, medicines, paints, etc.

Indeed, the fatty acid composition of a vegetable oil dictates its utility. Corn, sunflower, and castor oils, for example, are rich in long-chain fatty acids, making them more appealing for the production of polymer precursors and lubricants. Coconut oil and palm oil, on the other hand, have captured the attention of the detergent industry due to their medium length chains containing 12 to 18 carbons [23].

3- Chemical Composition of a Vegetable Oil

A vegetable oil contains a wide range of compounds (Figure 7), which may be divided into two fractions, the saponifiable (95-99%) and unsaponifiable (1-5%) [24].

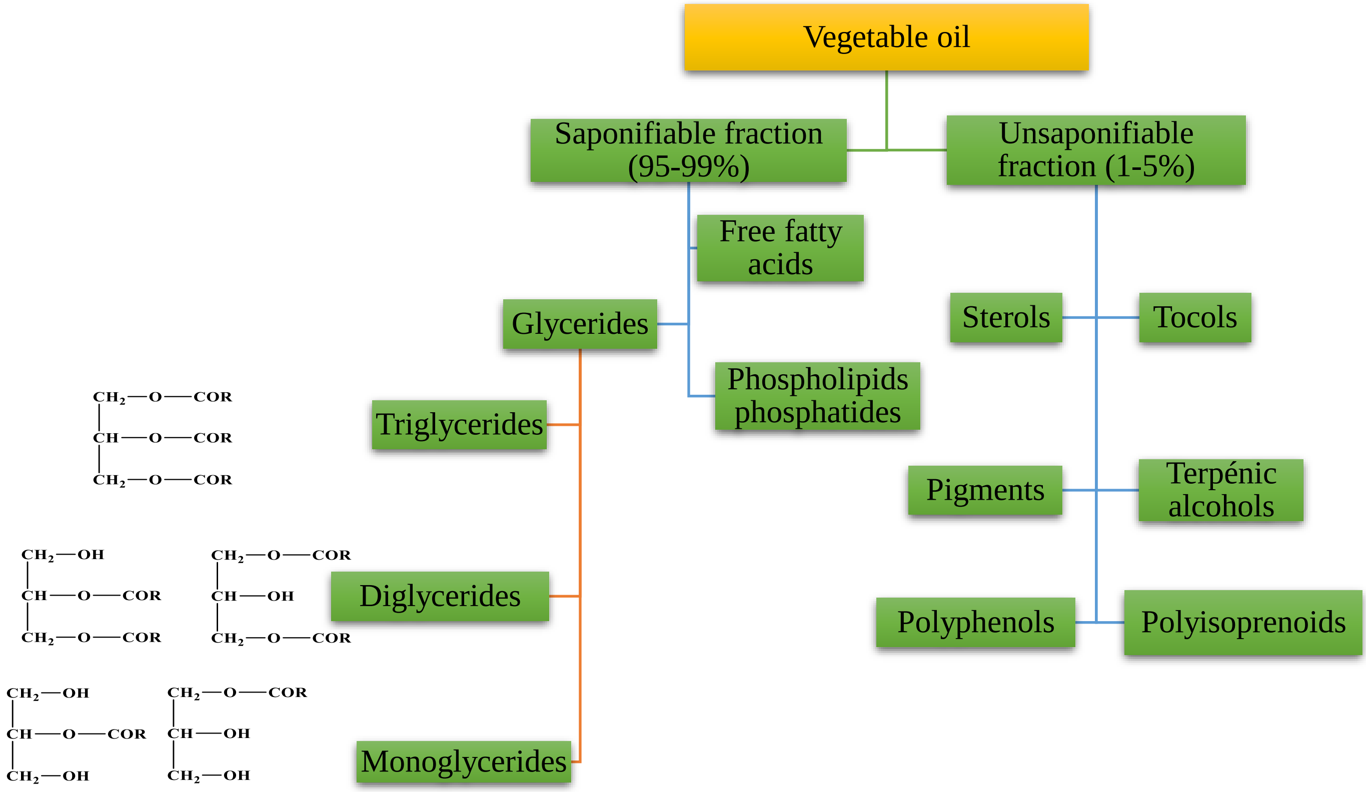


Figure 7: The components of a vegetable oil

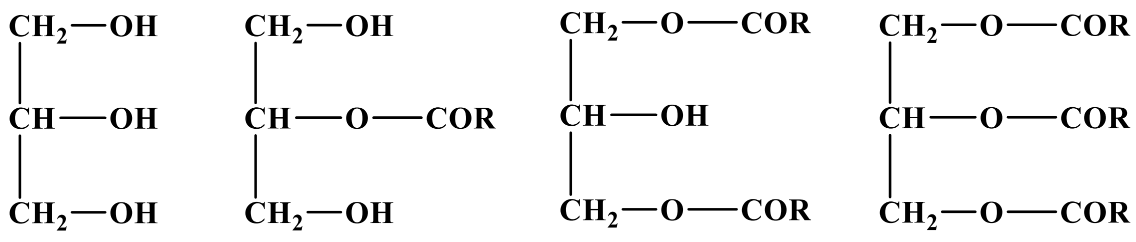
The saponifiable fraction is mostly constituted of triglycerides, which account for at least 95% of the weight of a crude oil and 98% of the weight of a refined oil. There is also minor polar components such as phospholipids (0.1-0.2%) and free fatty acids. The components of the unsaponifiable fraction are non-glyceride, they are present in small quantities and have a complex structure. Among these minor compounds we find phytosterols, tocols, pigments, etc. (Figure 7) [23].

3.1- Components of the saponifiable fraction of a vegetable oil:

3.1.1- Triglycerides:

Triglycerides, also known as triacylglycerols, comprise three fatty acid molecules (with a carboxyl function) and one glycerol molecule (with three hydroxide groups). These compounds are the primary components of fats and oils. In the human body, they serve as an energy reserve stored in fat cells as droplets in the cytosol [25].

Depending on the arrangement and combination of fatty acids on glycerol, the glyceride may have one of three structures: a monoglyceride (one fatty acid), a diglyceride (two fatty acids), or a triglyceride (three fatty acids) (Figure 8). Thus, the spatial arrangement of the glyceride may be either symmetrical or asymmetrical. In the case of a triglyceride, if glycerol is combined with three molecules of the same fatty acid, the triglyceride is homogeneous. Otherwise, the triglyceride is heterogeneous or mixed [26].



Glycérol **Monoglycéride** **Diglycéride** **Triglycéride**

Figure 8: Structure of a glycerol and a mono-, di- and triglyceride

3.1.2- Fatty Acids:

Fatty acids are organic compounds that are hydrophobic and not very abundant in free form in nature. They contain carbon, hydrogen, and oxygen in proportions of 76%, 12.7%, and 11.3% of their molecular weight, respectively. Fatty acids possess a carboxylic acid group (-COOH) and a chain of carbon atoms (known as aliphatic chain "R-") with a general structure of R-COOH (as shown in Figure 9) [27].

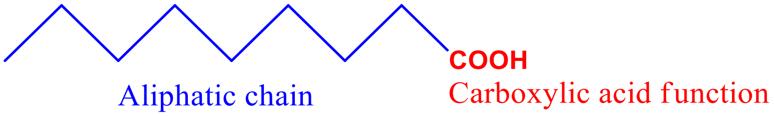


Figure 9: Skeletal structural formula of fatty acid

The aliphatic chain of a fatty acid might be saturated or contain one or more unsaturation. Indeed, the length of the carbon chain, as well as the quantity, position, and spatial structure (cis or trans) of the double bonds, can distinguish one fatty acid from another. This differentiation is indicated by the notation (Cn: X), where "n" denotes the number of carbons and "X" represents the number of double bonds. According to chain length, fatty acids fall into four categories:

- ✓ Volatile chain: contains two, three, or four carbon atoms.
- ✓ Short chain: has 6 to 10 carbon atoms.
- ✓ Medium chain: can be formed with 12 to 14 carbon atoms.
- ✓ Long chain: contains up to 16 carbon atoms.

The International Union of Biochemistry and Molecular Biology (IUBMB) and the International Union of Pure and Applied Chemistry (IUPAC) have developed an international standard nomenclature for fatty acids. The nomenclature begins by identifying the carbon number, with the carbon of the functional group receiving the number 1 and the remainder receiving order numbers. The carbon adjacent to carbon number 1 gets the Greek letter alpha (α), the following carbon beta (β), and the last carbon of the aliphatic chain, which is the terminal methyl group, will always be designated as omega (ω). Additionally, there is a second nomenclature known as the "omega nomenclature", commonly employed by nutritionists. In contrast to the international standard nomenclature, the carbon of the terminal methyl group is assigned the number 1 and the double bonds are sequenced according to it. For instance, linolenic acid will be denoted as C18:3 in the standard nomenclature and omega 3 in the omega nomenclature (Figure 10) [28].

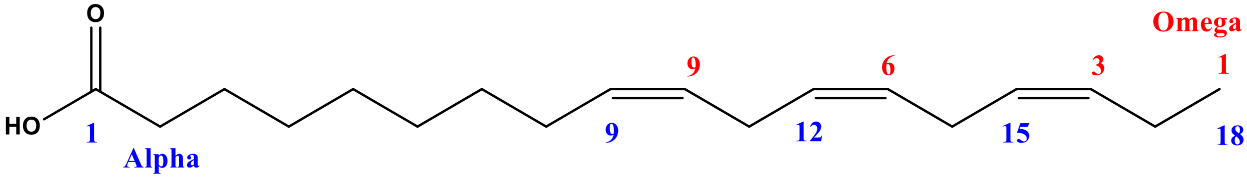


Figure 10: Standard and omega nomenclature of a fatty acid

Besides the classification by chain length, fatty acids are categorized into three groups based on the saturation or unsaturation of their aliphatic chains:

a. Saturated Fatty Acids

Saturated fatty acids contain a hydrocarbon chain with no double bonds but only single -C-C- bonds, with the typical formula CH3 - (CH2)n -COOH, where n is a number ranging from 2 to 22 (or more). At room temperature, they are generally solid and quite stable. Their structural formula is written as follows (Figure 11) and they are commonly shown as below (Figure 12) [29].

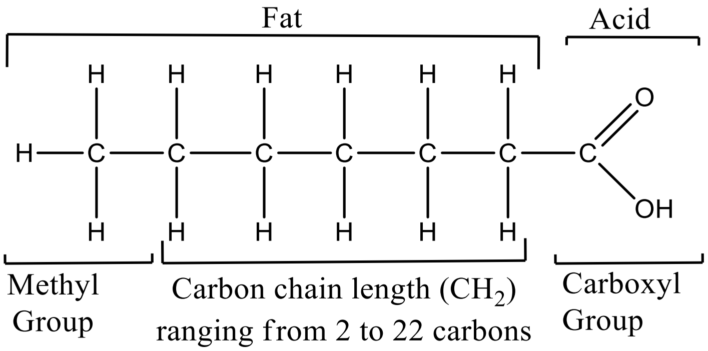


Figure 11: Essential features of a fatty acid

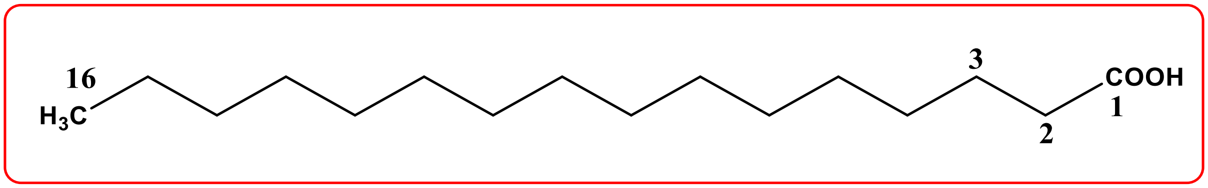


Figure 12: Skeletal formula of a fatty acid

b. Unsaturated Fatty Acids:

In their aliphatic chain**,** unsaturated fatty acids can have one to six double bonds. They are fluid at room temperature, unlike saturated fatty acids. They are divided into two categories based on the number of unsaturation:

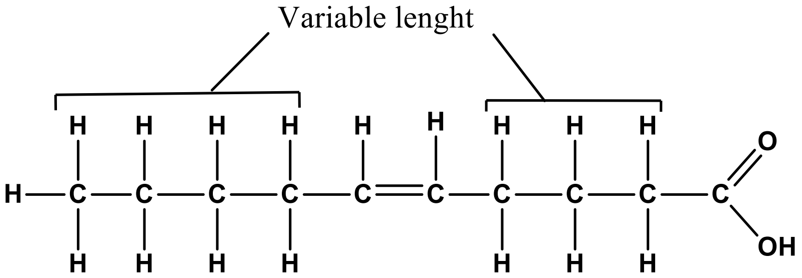
- ✓ "Mono-Unsaturated" Fatty Acids

They are characterized by a single unsaturation in their aliphatic chain. This double bond is usually found at carbon 9 or carbon 10, and its presence results in two possible configurations: the Z or Cis configuration, which most natural fatty acids adopt, and the E or Trans configuration, which is synthetic [30]. A monounsaturated fatty acid's general formula and developed structure are written as follows:

$$H_3C - (CH_2)\ n - HC = CH - (CH_2)\ p - COOH$$

Where "n" and "p" are numbers greater than or equal to zero.

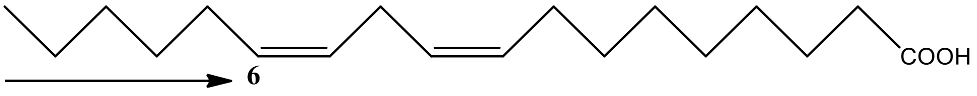
The developed structure :



- ✓ "Polyunsaturated" Fatty Acids

These acids contain a number of double bonds which is higher or equal to 2. The first double bond is also located at carbon 9 or carbon 10 and the following ones are conjugated or separated by a methyl group. In this category, there are two families of essential omegas: omegas 3 and omegas 6 (Figure 13). These families are based on two main fatty acids: linoleic acid (C18:2 n-6), a precursor of omega 6 , and -linoleic acid (C18:3 n-3), a predecessor of omega 3 [28].

Linoleic acid C18:2 (n-6)



Alpha-linolenic acid C18:3 (n-3)

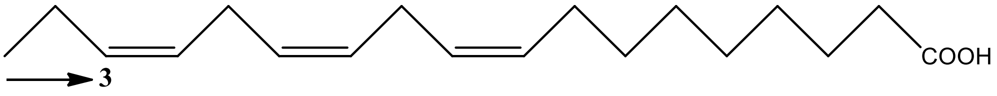


Figure 13: Skeletal formula of linoleic acid and linolenic acid

3.1.3- Phospholipids:

Phospholipids, also known as glycerophospholipids, are triesters formed by a glycerol molecule esterified by two fatty acids and a phosphoric acid, which is then linked by another ester bond to an amine (ethanolamine) or a sugar (inositol) (Figure 14). The phospholipid content varies depending on the crushing process or plant material. It can be very low or non-existent in some oils (palm oil) or represent a rate of 1.5 and 3% in rapeseed and soybean oil [31].

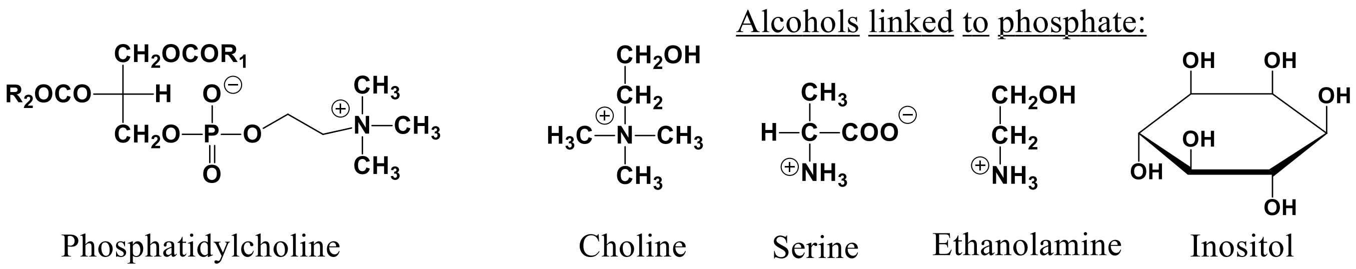


Figure 14: Structural formula of several forms of phospholipids

3.2- Components of the Unsaponifiable Fraction of a Vegetable Oil:

In addition to the saponifiable fraction, vegetable oils also contain an unsaponifiable fraction including non-glycerides and complex lipids called "minor constituents", as their name indicates they have a very low content in a vegetable oil.

3.2.1- Pigments

The photosynthetic pigments are colored compounds or dyes that absorb certain wavelengths (colors) of light during the photosynthesis process and convert the light energy into chemical energy to produce its primary metabolites [32].

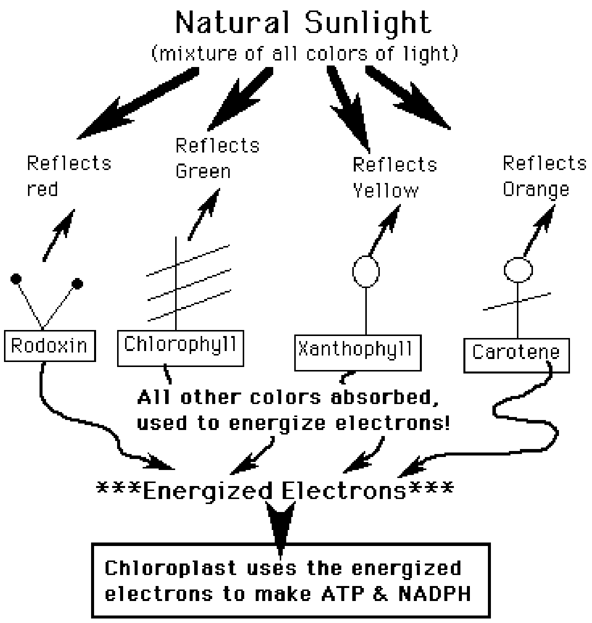
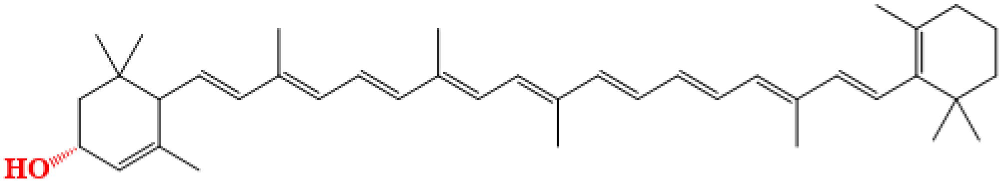


Figure 15: The process of the light energy's transformation into chemical energy and the reflection of the characteristic color by each pigment during photosynthesis

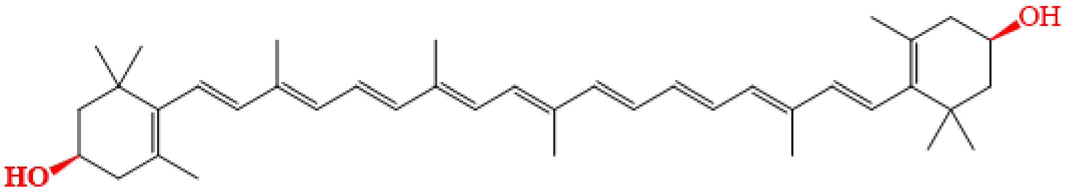
There are several types of pigments whose content varies from one plant to another. The most well-known are Chlorophylls (a to f), carotenoids (particularly carotenes and xanthophylls), pheophytins, and phycobilins. In vegetable oils Chlorophylls (a and b) and carotenoids (carotene and provitamin A) are commonly present (Figure 15).

3.2.1.1- Carotenoids

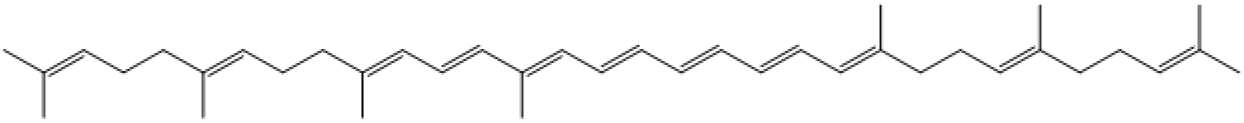
Carotenoids are lipophilic pigments from plants and microorganisms, they are sensitive to heat and ultraviolet radiation. They are divided into two large families that are xanthophylls and carotenes (Figure 16), of which there are around 600 present in nature. At the structural level, they have a long hydrocarbon chain containing several conjugated unsaturations with a chemical formula of $C_{40}H_{56}$ for carotenes and $C_{40}H_{(52-56)}O_{(1-4)}$ for xanthophylls, and presented in different types (α, β, γ, ε, ψ, ζ). This one attributes them an antioxidant power by deactivating the activated oxygen under particular conditions such as oxidation by photosensitization. Generally, vegetable oils contain only a few hundred mg/kg of these pigments, but they are present in significant concentrations of 1 to 2 g/kg in red palm oil [32].



(3R)-β,β–carotene-3-ol ou la provitamine A



(3R,3'R)-β,β–carotene-3,3'-diol



ζ-carotene

Figure 16: Skeletal formula of beta-carotene (alicyclic carotene), zeaxanthin (β,β-carotene-3,3'-diol), and zeta-carotene (aliphatic carotene)

3.2.1.2- Chlorophylls

Chlorophyll pigments are green, fat-soluble pigments present in all algae, higher plants and cyanobacteria that carry out photosynthesis (Figure 17). When food containing chlorophyll is consumed, it is converted after ingestion by the body into pheophytin, pyropheophytin and pheophorbide. These bioactive compounds have an antimutagenic effect and are therefore likely to play an important role in the prevention of cancer, particularly by inhibiting the multiplicity of myeloma cells via pheophorbide.

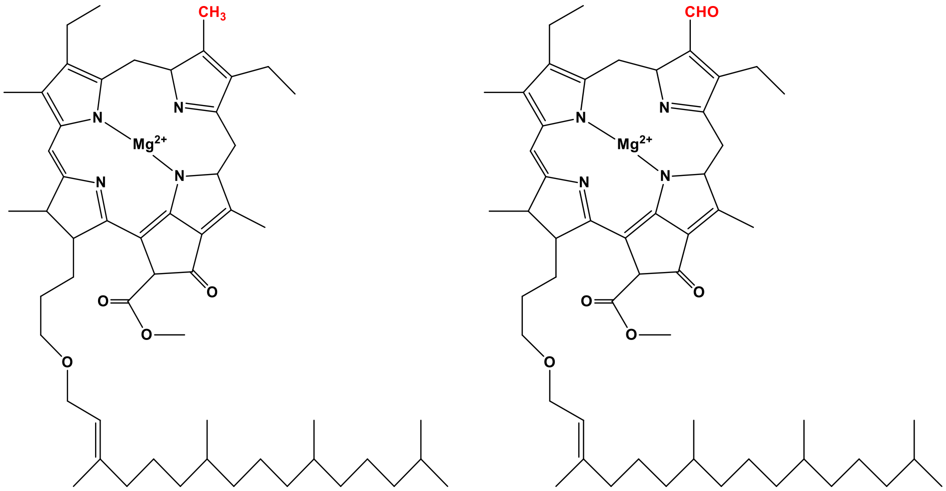


Figure 17: Skeletal formula of chlorophyll a (CH_3) and b (CHO)

Chlorophyll pigments, by far the most abundant, have two absorption bands (blue and red) in the light spectrum, which results in a maximum reflectance value around green, hence the green color of plants (Figure 18).[32]

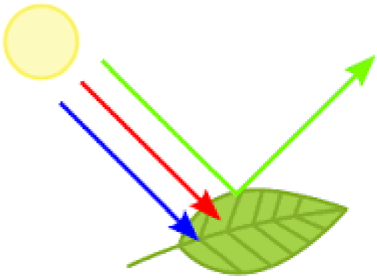


Figure 18 : the green color plant's absorption and reflectance

3.2.2- Sterols or phytosterols:

Phytosterols are plant-derived sterols, of which all vegetable oils contain 0.1 to 0.5% on average, and their molecular structure is strikingly similar to that of cholesterol. They are beneficial to human health. They inhibit the absorption of dietary cholesterol in the intestine, leading in a decrease in the amount of cholesterol released into the blood, resulting in a hypocholesterolemic effect. Furthermore, phytosterols inhibit cell proliferation, which contributes to their anti-cancer effect. They have also demonstrated numerous immunological actions due to their anti-inflammatory activity [33].

Sterols are an amphiphilic rigid molecule composed of four rings (A, B, C, and D) that form a hydrophobic skeleton and a hydrophilic head characterized by an alcoholic function on carbon 3 in β position (Figure 19) [32].

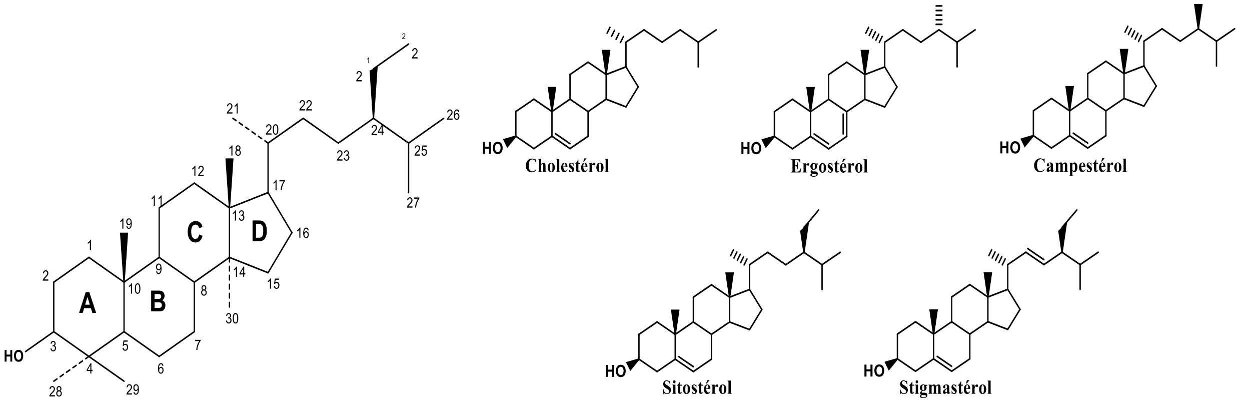


Figure 19: Skeletal structure of the main phytosterols and numbering of the carbon skeleton according to the I.U.P.A.C

Because of this structure, sterols are placed between two lipidic layers of a plasma membrane, allowing the apolar skeleton to interact with the phospholipid fatty acids through Van der Walls linkages (Figure 20). These cause a condensation of the membrane and thus its reinforcement. Furthermore, by improving its fluidity, they prevent it from stiffening at low temperatures. This characteristic is critical for plants whose temperature is affected by their surroundings [32].

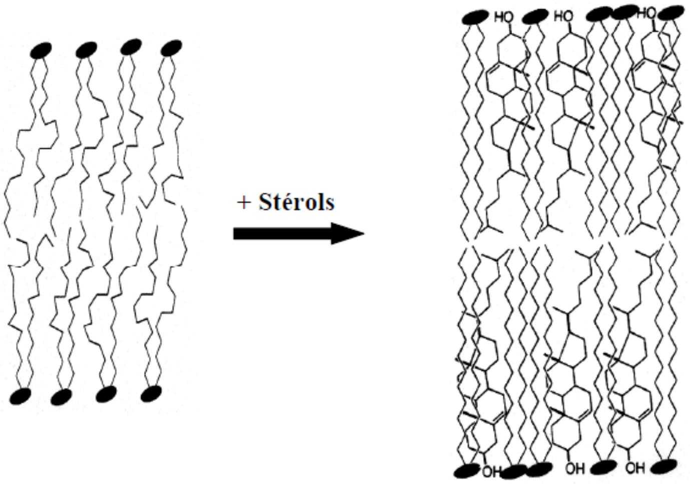
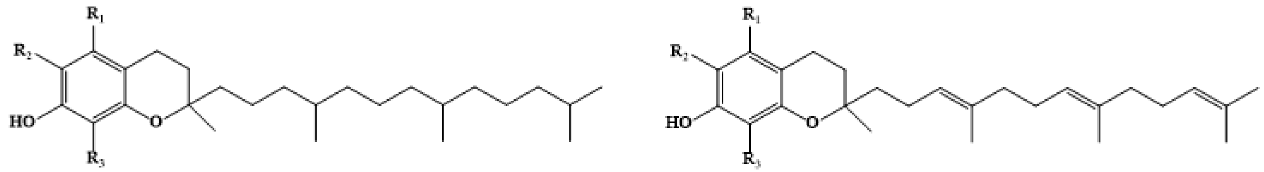


Figure 20: Sterol integration in a plasma membrane's lipid bilayer

3.2.3- Tocols

The tocols are known under the general term of vitamin E, they are grouped in two large families which are the tocopherols and the tocotrienols. Each family has four different forms (alpha-, beta-, delta- and gamma-). These forms have a similar structure (they can be structural or positional isomers) with a chromanol ring linked to a saturated (tocopherol) or unsaturated (tocotrienol) phytyl side chain (Figure 21).



Tocopherol	R1	R2	R3
α-tocopherol (5,7,8-trimethyltocol)	CH_3	CH_3	CH_3
β-tocopherol (5,7,8-trimethyltocol)	CH_3	H	CH_3
γ-tocopherol (5,7,8-trimethyltocol)	H	CH_3	CH_3
δ-tocopherol (5,7,8-trimethyltocol)	H	H	CH_3

Tocotrienol	R1	R2	R3
α-tocotrienol (5,7,8-trimethyltocol)	CH_3	CH_3	CH_3
β-tocotrienol (5,7,8-trimethyltocol)	CH_3	H	CH_3
γ-tocotrienol (5,7,8-trimethyltocol)	H	CH_3	CH_3
δ-tocoptrienol (5,7,8-trimethyltocol)	H	H	CH_3

Figure 21: Chemical structure of tocopherols and tocotrienols

Each form might reflect a different biological property from another. For example, for tocopherols, alpha-tocopherol is the most active form whereas the other three forms have a weaker activity (beta and gamma-tocopherol) or very weak (delta-tocopherol). Tocotrienols, unlike tocopherols, have three unsaturations in their side chain, allowing them to be more flexible and to permeate cells more efficiently. In fact, Watson and Preedy demonstrated that the advantages of tocotrienols far outweigh those of α-tocopherol [24].

Tocols have biological properties such as neoprotection, anticancer, anti-inflammatory, cardioprotection, immunostimulant, antidiabetic, hepatoprotective, and nephroprotection. According to Anderson et al. tocotrienols in cell culture generally increase the levels of IKAP (a multifunctional protein that contributes to many processes in the cell), they have proposed it as a treatment for patients with familial dysautonomia and suggest using 50-200 mg/day. In addition, tocols protect the oil from free radical damage, thus contributing to their stability. They are more effective than the synthetic antioxidant butylated hydroxytoluene (BHT) [34].

II-Secondary Metabolites:

1- Generalities:

Plants contain two kinds of metabolites: primary and secondary. In the presence of water, mineral salts, light energy, oxygen and/or carbon dioxide, plants produce primary metabolites through photosynthesis, which contribute to the fundamental structure and functioning of the plant cell. These metabolites include amino acids, nucleic acids, organic acids, proteins, and simple sugars.

Secondary metabolites, on the other hand, are formed from primary metabolites and derive from subsequent chemical processes rather than being formed directly during photosynthesis. Depending on the stage of development, these metabolites are frequently synthesized in one specific zone of the plant and stored in another.

A secondary metabolite's role is often linked to its location within the plant. Furanocoumarins, for example, are accumulated on the leaf surface of some plants, where they act as a protective chemical barrier against bacteria and fungus. Likewise, they protect the plant against herbivores, pathogens, and parasites, as well as preventing competitive plants from germinating and developing. Secondary metabolites thus help plants in adapting to their surroundings.

Many secondary metabolites adopt a wide structural variety that can be simple or complex and serve as the basis for the bioactive compounds found in medicinal plants. Indeed, they have diverse biological and therapeutic activities such as antibiotics, anticancer agents, antioxidants, antifungals, herbicides, and insecticides.

Secondary metabolites are divided into three major families. The first family is made up of phenolic compounds such as coumarins, lignins, flavonoids, and other compounds containing at least one hydroxyl group connected to an aromatic ring. The second includes terpenoids (terpenes) and steroids, which are molecules consisting of several isoprene-type units. Finally, the alkaloids are compounds that contain one or more intracyclic nitrogen atoms [34].

2- Phenolic compounds:

Phenolic chemicals are secondary plant metabolites found throughout plant tissues and organs. They are made up of a wide range of structurally varied compounds that are difficult to identify. The term "polyphenol" was introduced in 1980 to replace the older term "plant tannin." Tannins were the former name for polyphenols.

In general, these compounds are characterized by six-carbon aromatic ring (benzene) with one or more hydroxyl groups linked to it, either free or in an ester, ether, or heteroside function. Their classification (Figure 22) is based on the following criteria:

- ✓ the complexity of their basic skeleton, which ranges from simple to very complex polymeric structures.
- ✓ The extent to which the skeleton has been modified: one or more hydroxyl functions.
- ✓ The interaction with other molecules (carboxylic acids, amines, lipids, etc.) results in ten distinct classes.

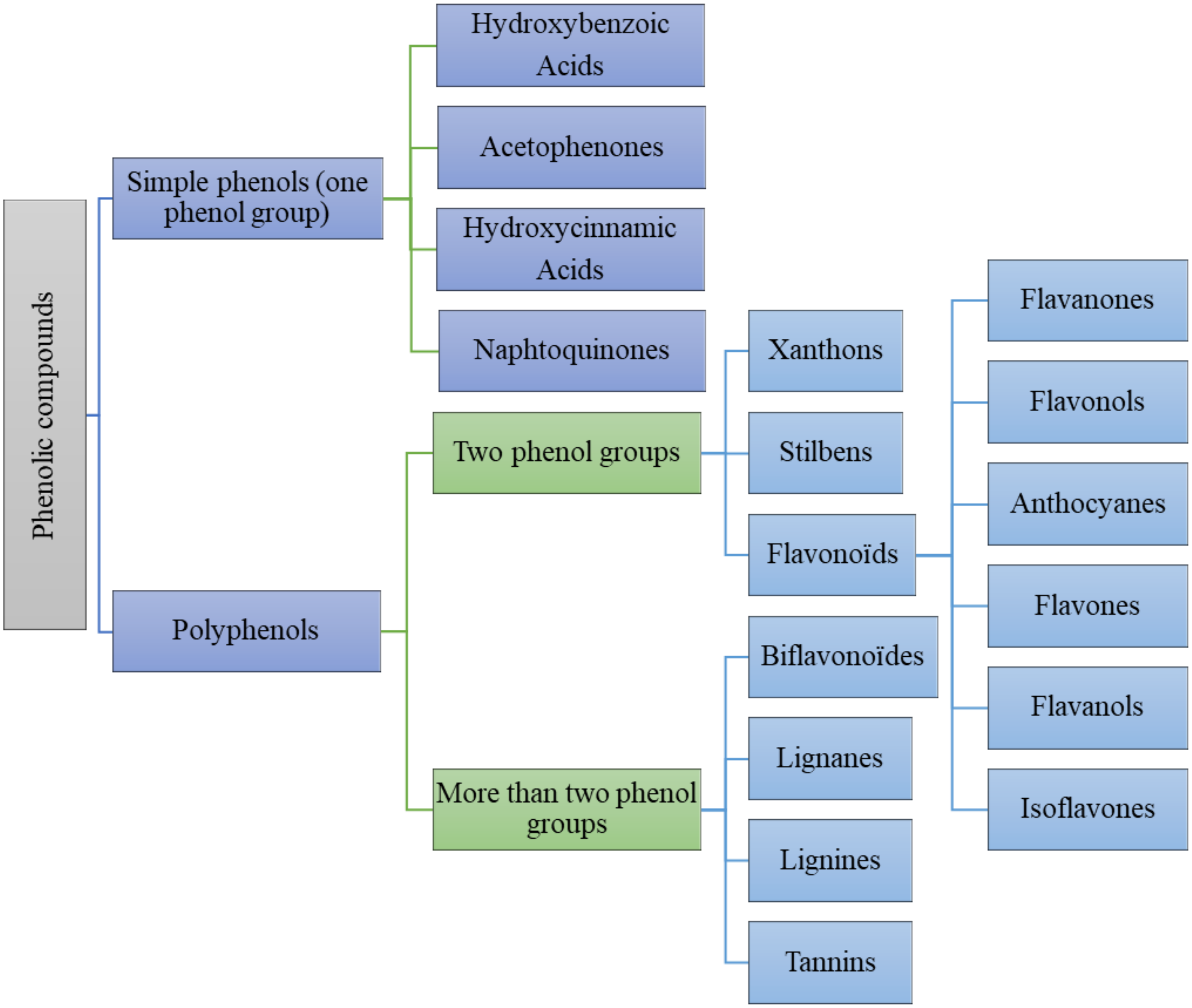


Figure 22: Classification of the phenolic compounds

The biosynthesis of phenolic compounds is a fundamental process in plant biochemistry. There are two biosynthetic pathways. The first is the shikimate pathway, which leads from glycose to aromatic amino acids (phenylalanine, tyrosine, and tryptophan). These are the precursors of phenylpropanoid biosynthesis, whose cyclization leads to the formation of coumarins, dimerization to lignans, polymerization to lignins, and the elongation of their side-chain leads to flavonoids and stilbenes [35]. The second one is the acetate pathway, which consists of multiple acetate units condensation to β-keto derivatives that tend to cyclize. This pathway also produces quinones, naphthodianthrones, orcinols, and phloroglucinols. On the other hand, polyacetates can interact with aromatic compounds from the shikimate pathway to give so-called mixed substances (flavonoids) [36].

3- Terpenoids and steroids: Isoprenoids

Terpenoids and steroids are two of the most diverse families of secondary metabolites, obtained from the same precursors and generated by the assembly of branched five-carbon units derived from 2-methylbutadiene (isoprene polymers). Squalene, cholesterol, sesquiterpenes, and diterpenes are found in animals and are not specific to plants. However, the tremendous diversity of terpenoids found in plants contrasts with the small number found in animals.

Terpenes are categorized according to the number of isoprenic units containing five carbon atoms in their fundamental structure. Hemiterpenes are at C5, monoterpenes are at C10, sesquiterpenes are at C15, diterpenes are at C20, sesterpenes are at C25, triterpenes are at C30, and tetraterpenes are at C40.[34]

4- Alkaloids: nitrogenous compounds

Alkaloids are a complex family of low-molecular-weight, nitrogen-containing compounds (formed mainly from amino acids) present in around 20% of plants [37]. They are nitrogen-containing bases that react with acids to form salts. Before the advent of chromatography, alkaloids could be easily separated and identified due to their ability to react with complex metal ions (Mayer and Dragendorf reagents). Plants use alkaloids as a defense against herbivores and pathogens. Likewise, for humans, many alkaloids are used as drugs, stimulants, narcotics, and poisons due to their powerful biological activity. Alkaloids are classified according to their ring systems, including indolizidine, quinolozidine, quinoline, quinazoline, and acridone systems. Most alkaloids are derived from aromatic amino acids such as phenylalanine, tyrosine, and tryptophan (Figure 23) [38].

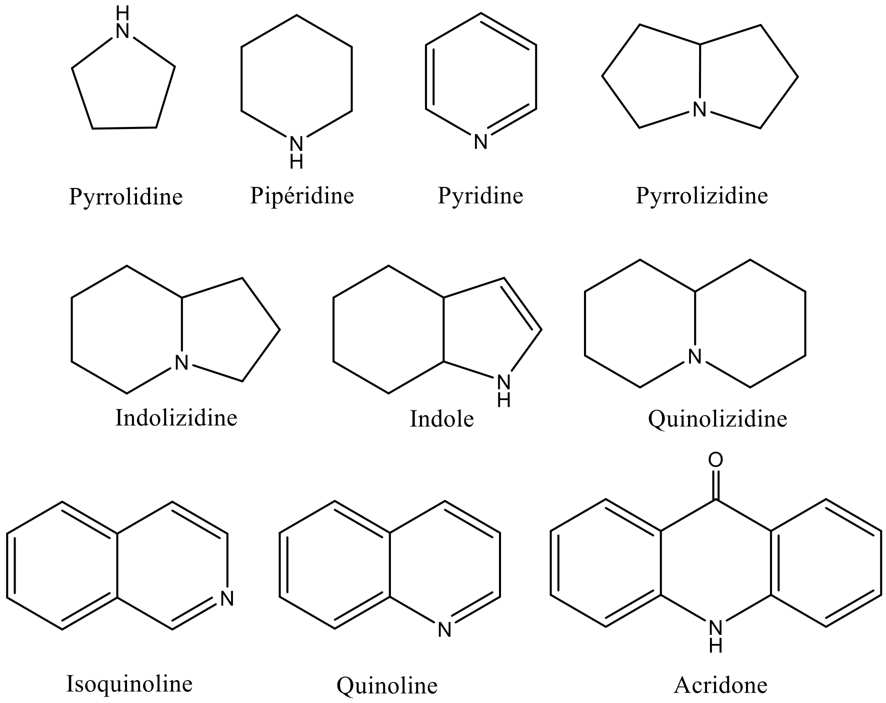


Figure 23 : Skeletal structures of various types of alkaloids

The endoplasmic reticulum is where alkaloids are synthesized and then concentrated in the vacuole. In general, alkaloids are formed in growing tissues, such as young leaves and roots. They are then moved to new sites, where they can undergo alterations. Nicotine, for example, is synthesized in the roots and migrates to the leaves, where it is demethylated. In many plants, alkaloids are located in the floral parts, fruits, or seeds [39].

C-Oxidative stress, prooxidants and antioxidants

Scientific research is increasingly centering on the antioxidant capacity of bioorganic compounds, which has been shown to be closely linked to the study of biological activities. In this chapter, the notions of pro-oxidant and antioxidant will be defined, along with the agents, conditions, or molecules that cause oxidative stress and the ones that prevent it.

I- Oxidative stress and prooxidants

1- Generalities

The study of free radicals in biology is gaining increased attention for their involvement not only in acute events like trauma or ischemia, but also in various chronic age-related disorders such as cancer, cardiovascular and inflammatory diseases, and immune system deterioration. Conversely, these radicals are essential for a variety of physiological activities such as cell communication, growth control, and phagocytosis. Likewise, antioxidant defense systems exist to maintain free radicals at non-cytotoxic levels by eliminating them or limiting their formation [40].

2- Definition

Oxidative stress is defined as an imbalance between prooxidants and antioxidants in a cell or organism. This imbalance can be caused by the overproduction of prooxidants, (such as prolonged exposure to sunlight, smoking, alcohol, pollution, intense and prolonged exertion, successive medication, etc.) or by an antioxidant deficiency (a lack of antioxidant micronutrients, enzyme inhibitors, etc.) [41].

3- Free Radicals

Prooxidants, also known as free radicals, are molecules or atoms with one or more unpaired electrons in their valence layers. This gives them great instability, resulting in highly reactive properties and extremely short half-lives (10-9 milliseconds) [42]. Free radicals are categorized into two types:

- ✓ ***Reactive Oxygen Species (ROS),*** including superoxide anions (O^{2-}), hydroxyl radicals (OH^-), hydroperoxyl radicals (HOO^-), peroxyl radicals (ROO^-), and alkoxyl radicals (RO^-).
- ✓ ***Reactive Nitrogen Species (RNS),*** including nitric oxide (NO^-) and nitrogen dioxide (NO^{2-}), as well as other free radicals like DPPH (1,1-diphenyl-2-picrylhydrasyl) radicals and $ABTS^{+\cdot}$ cation radicals, can be utilized to evaluate antioxidant activity.[43]

II- Antioxidant capacity and antioxidants

An antioxidant is a more or less complex chemical compound found within an organism able to neutralize prooxidants. It has a preventive action against prooxidant formation by inhibiting the initiation of reaction chains, deactivating ROS directly, and chelating metal ions.

1- Antioxidant: mechanisms of action

Depending on their mode of action, antioxidants can be classified into four categories:

- ✓ ***Enzymatic systems***, such as catalase and glutathione peroxidase, as well as vitamins A and E...
- ✓ ***Oxidizing enzyme inhibitors*** like xanthine oxidase, lipoxygenases, flavonoids, and also vitamins A and E...
- ✓ ***Metal chelators*** such as phenolic compounds...
- ✓ ***Free radical scavengers*** including phenolic compounds and vitamin C.

The body's endogenous mechanisms are dedicated to this protective activity, yet this defense mechanism quickly becomes exhausted. Consuming exogenous antioxidants, which are abundantly found in the diet, provides invaluable support in the battle against oxidation. They can be found in fruit (such as apples, pears, red berries, etc.), vegetables (such as broccoli, onions, etc.), beverages (such as coffee, tea, wine, etc.), spices, chocolate and cereals. Antioxidants are recognized for their ability to react directly with free radicals and "neutralize" them through a reduction process. Antioxidants compose a diverse group that includes endogenous, enzymatic, and non-enzymatic antioxidant systems, vitamins, trace elements, and polyphenols [44].

2- Involvement of phenolic compounds in defensive mechanisms

Phenolic compounds possess an aromatic ring with delocalized electrons, which stabilizes them in their radical form. Two classes of phenolic compounds, namely phenolic acids and flavonoids, are credited with exhibiting antioxidant activity [44].

The antioxidant properties of flavonoids are determined by the nature and placement of their substituents, the number of hydroxyl groups, and their ability to terminate radical chains through electron and proton transfer processes [45]. In addition, flavonoids have the ability to terminate radical chains via electron and proton transfer processes, as well as to bind transition metal ions, which can act as catalysts for lipid peroxidation [46] (Figure 24).

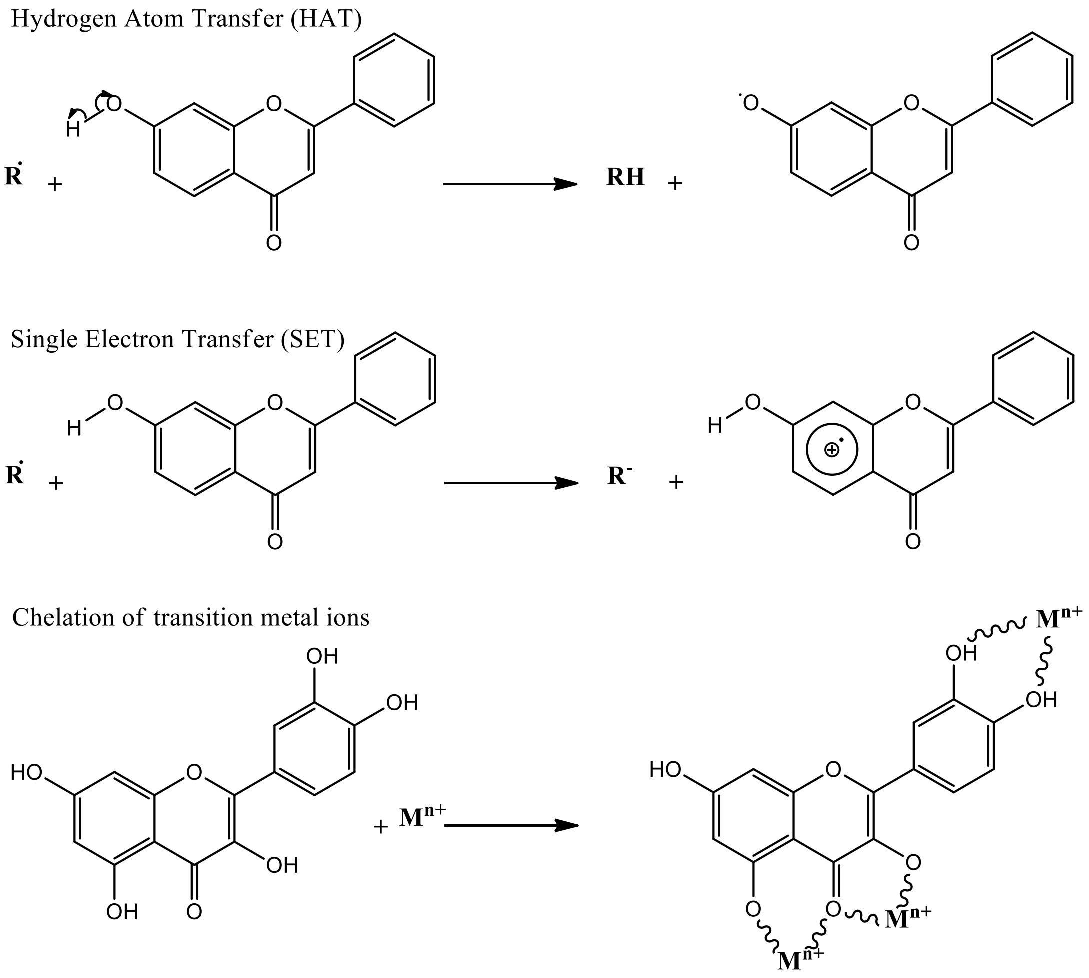


Figure 24: Flavonoids' antioxidant activity mechanism[46]

In the case of phenolic acids, hydroxycinnamic acid has a high antioxidant capacity compared with its counterparts. The presence of a propionic side chain contributes to the superior antioxidant activity of hydroxycinnamic derivatives, and the double bond stabilizes the phenoxy radical, enhancing their antioxidant activity [44].

3- Direct scavenging of free radicals by phenolic compounds

The transfer of hydrogen (ArO-H) to the radical (R°) is the process by which phenolic compounds capture prooxidants.

ArO----H + R° -------→ ArO° + RH

Several mechanisms can be used to stabilize the new radical (ArO°), including the delocalization of Pi electrons from the aromatic ring, further hydrogen transfer from ArO-H, or another free radical from R°. A flavonoid, for example, can trap a free radical, forming a flavonol radical (on ring B) that stabilizes via quinone production [47] (Figure 25).

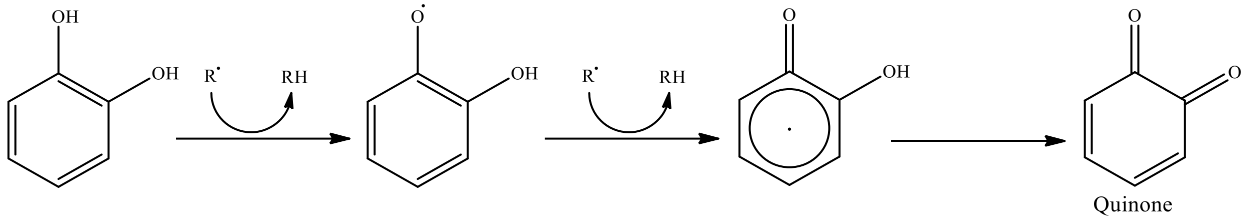


Figure 25: Mechanism of prooxidant scavenging by a flavonoid

4- Metal ion chelation

According to the Fenton reaction, metal ions such as iron or copper may generate extremely reactive hydroxyl radicals from H_2O_2 within human bodies:

$$H_2O_2 + Fe^{2+}(Cu^{+}) \rightarrow OH^{-} + OH^{\circ} + Fe^{3+}(Cu^{2+})$$

Through the complexation process, phenolic compounds can neutralize these ions. The structure of flavonoids (Figure 26) includes three chelation sites. The B ring, which contains the catechol nucleus, the C ring which has a hydroxyl group at position 3 and an oxo group at position 4, and the area between rings A and C where ring A has a hydroxyl group at position 5 and ring C has an oxo group at position 4 [48].

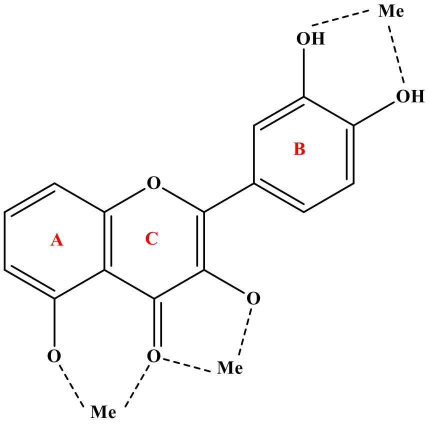


Figure 26: The three essential metal ion chelation sites in flavonoids

5- Lipid peroxidation inhibition

Lipid peroxidation is a fatty acid chain degradation process. It causes oxidative stress and decreases membrane fluidity, both are linked to certain diseases. However, phenolic compounds reduce lipid peroxidation through two mechanisms: direct scavenging of alkoxyl and peroxyl radicals or suspension of the propagation process with the cessation of chain oxidation, and regeneration of alpha-tocopherol during alpha-tocopheryl radical reduction [49],[50].

D-“Phoenix Dactilifera L.” date seeds, description, chemical composition and biological properties

Date palm seeds are a byproduct of numerous industrial processing companies and are usually either discarded or partially incorporated into animal feed in most date-producing nations. While there are a handful of conventional applications, the potential for human nutrition remains widely unexplored.

I- Date seed morphology

The date pit or seed is a reproductive organ that accounts for 7 to 30% of the weight of the fruit [51]. A membrane endocarp surrounds it. It might be elongated, oblong, rounded, oval, or even spherical in form. It might be smooth, voluminous, or have lateral protuberances. It has a cellulose envelope and a diametrically opposite embryo with a firm, horny albumen (endosperm)[14] (Figure 27).

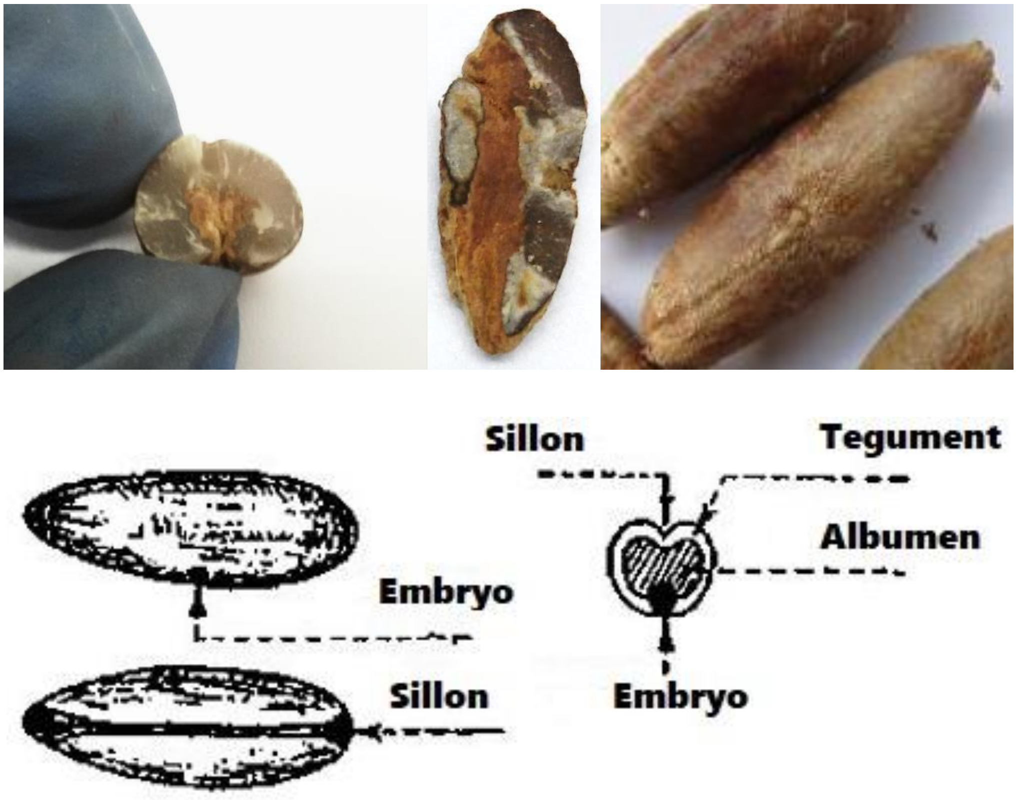


Figure 27: Morphology of date palm seed

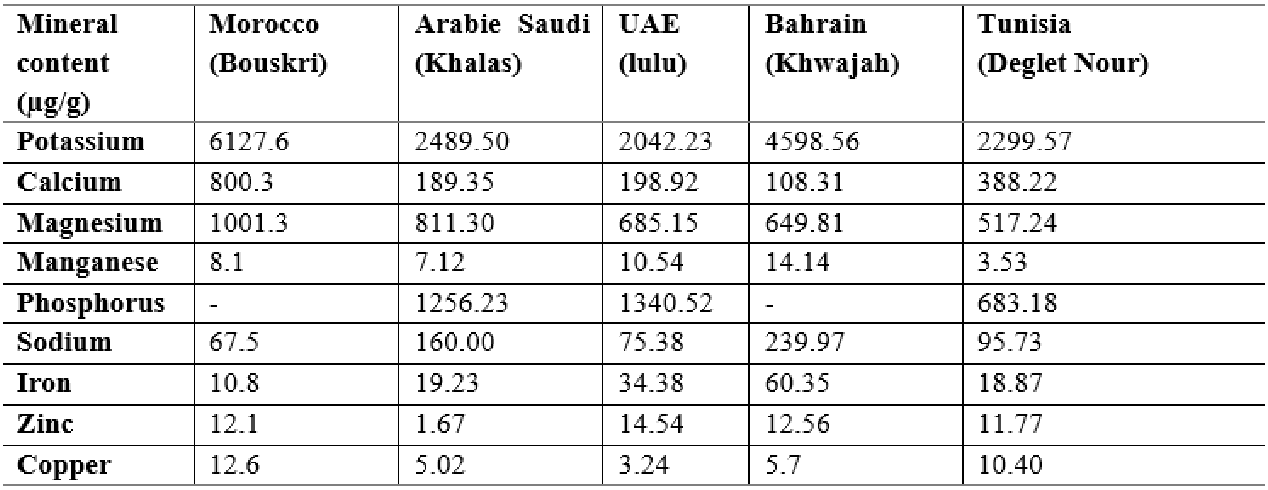
II- Biochemical composition and nutritional value of date seeds

Research on date pits' chemical composition in literature shows the presence of minerals like K, P, Ca, Na, Fe, Mn, Zn, and Cu, as well as substantial levels of proteins, carbohydrates, lipids, and phenolic compounds.

1- Mineral content

Studies have demonstrated that date pits contain a diverse variety of minerals, including potassium, calcium, magnesium, phosphorus, sodium, iron, manganese, zinc, and copper. Potassium, phosphorus, magnesium, calcium, and sodium have the largest concentration in date pits when compared to other minerals. In addition, iron, manganese, zinc, and copper are present in high concentrations, classifying them as microelements (Table 3).

Table 3: Mineral content of date seeds from different countries and varieties [52,53,54,55,56,57]



Mineral content (µg/g)	Morocco (Bouskri)	Arabie Saudi (Khalas)	UAE (lulu)	Bahrain (Khwajah)	Tunisia (Deglet Nour)
Potassium	6127.6	2489.50	2042.23	4598.56	2299.57
Calcium	800.3	189.35	198.92	108.31	388.22
Magnesium	1001.3	811.30	685.15	649.81	517.24
Manganese	8.1	7.12	10.54	14.14	3.53
Phosphorus	-	1256.23	1340.52	-	683.18
Sodium	67.5	160.00	75.38	239.97	95.73
Iron	10.8	19.23	34.38	60.35	18.87
Zinc	12.1	1.67	14.54	12.56	11.77
Copper	12.6	5.02	3.24	5.7	10.40

Date seeds are a good source of minerals for both human and animal nutrition. In addition, due to their high potassium content and low sodium levels, they are highly recommended for individuals with high blood pressure following a low-sodium diet [3].

2- Protein and amino acid content

Proteins can be found in date pits in amounts of up to 10.6%. Bouaziz et al. discovered and identified seventeen different kinds of amino acids, the most well-known are lysine, isoleucine, leucine, methionine, threonine, valine, and phenylalanine. Glutamic acid (Glu) was the most abundant, accounting for 17% of the total, followed by arginine (Arg), aspartic acid (Asp), leucine (Leu), lysine (Lys), valine (Val), glycine (Gly), alanine (Ala), and phenylalanine (Phe). Date seed proteins have a better biological value than egg proteins due to their high concentration of essential amino acids. Their molecular weights range between 22 and 70 kDa [58] [59].

3- Lipid content

Date seeds have a fat level ranging from 3.2% to 11.5%, which is greater than the pulp, which has a fat content ranging from 0.21% to 0.33%. Their oil is a complex combination of triglycerides

(90-95%), diglycerides (1-2%), free fatty acids (approx. 0.5%), phospholipids, and minor elements including phytosterols, tocols, and pigments [52].

4- Natural antioxidant content

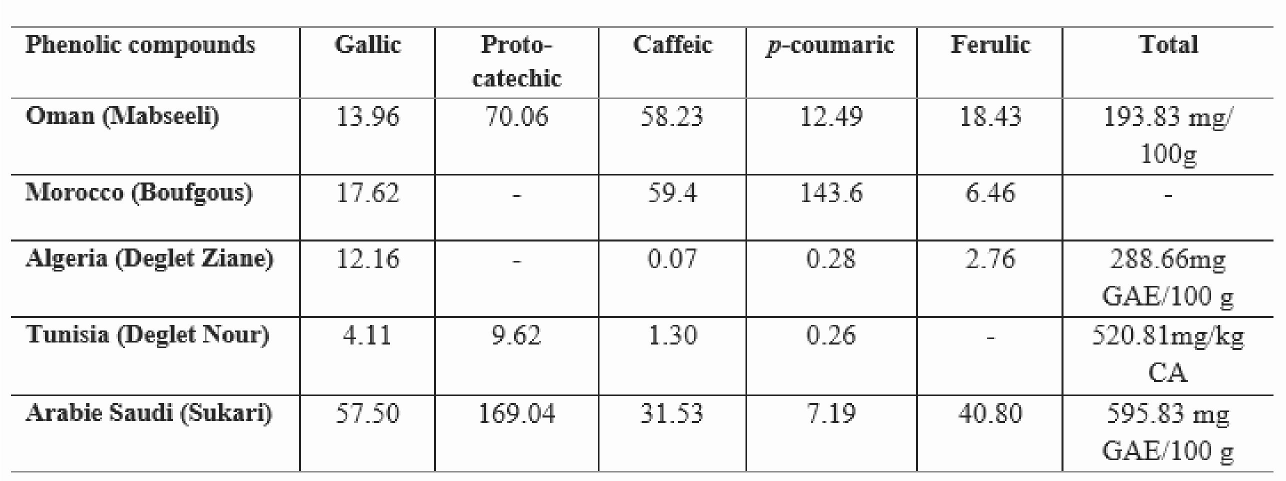
Date stones are high in antioxidants such as phenolics, alkaloids, steroids, and tocols. They have strong antioxidant action, even surpassing synthetic antioxidants.

4.1- Phenolic content

The antioxidant capacity of phenolic compounds, as well as their capacity to interact with numerous enzymes, are well recognized. They protect against some illnesses by having anti-free radical, anti-tumor, anti-allergic, anti-inflammatory, analgesic, antibacterial, venotonic, and hepatoprotective properties. Ferulic acid, a derivative of cinnamic acid, is used in cosmetics and gets its name from the Latin word "Ferula," which means "giant fennel." It is commonly used in creams, lotions, serums, and other products to treat photodamage, skin irritation, and redness caused by sun exposure [60].

Babiker et al. [61] discovered sixteen different kinds of phenolic compounds in date seed. Table 4 shows how the content of each category varies by variety and place of origin. For instance, protocatechic acid is predominant in the Mabseeli variety (Oman), while p-coumaric acid is prevalent in the Boufgouss variety in Morocco. This variation also holds for the total polyphenol content.

Table 4: phenolic compounds in date seed [61,62, 63, 64,65]

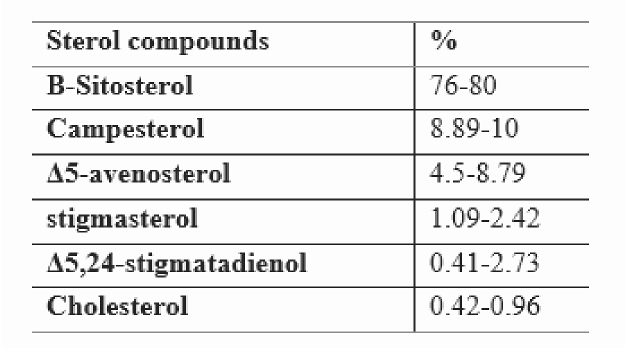


Phenolic compounds	Gallic	Proto-catechic	Caffeic	*p*-coumaric	Ferulic	Total
Oman (Mabseeli)	13.96	70.06	58.23	12.49	18.43	193.83 mg/ 100g
Morocco (Boufgous)	17.62	-	59.4	143.6	6.46	-
Algeria (Deglet Ziane)	12.16	-	0.07	0.28	2.76	288.66mg GAE/100 g
Tunisia (Deglet Nour)	4.11	9.62	1.30	0.26	-	520.81mg/kg CA
Arabie Saudi (Sukari)	57.50	169.04	31.53	7.19	40.80	595.83 mg GAE/100 g

4.2- Phytosterol content

Sterols are present in date seed oil in their esterified form and are among the main components of the unsaponifiable fraction. The β-sitosterol, campesterol, and Δ5-avenasterol are the major compounds, with their content ranging from 470 to 845mg/100g (Table 5). These sterols are crucial for the functional properties of the oil, specifically its ability to resist oxidation. Furthermore, several studies have stated numerous health benefits associated with sterols [66].

Table 5: Sterol compounds in date seed oil

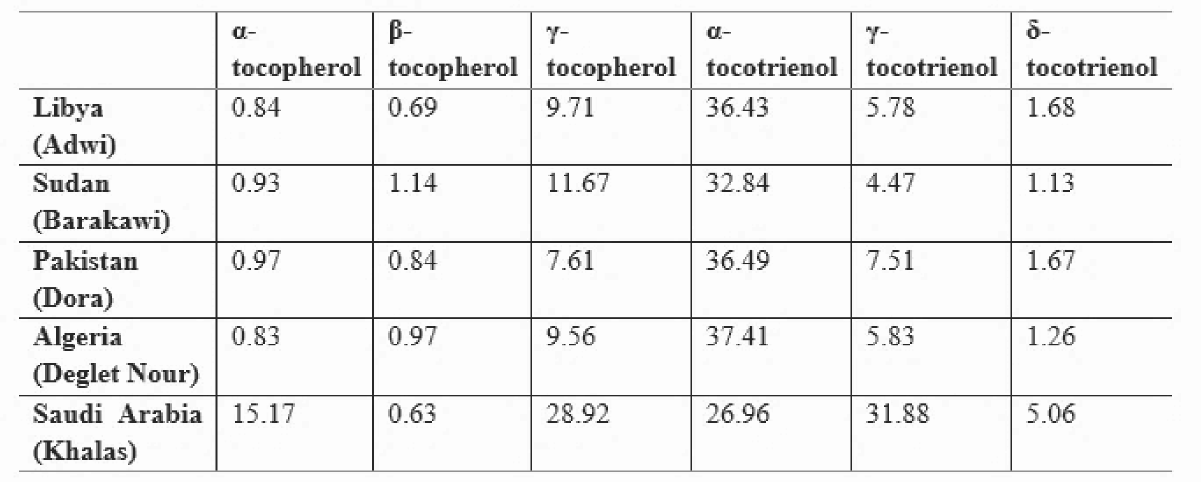


Sterol compounds	%
B-Sitosterol	76-80
Campesterol	8.89-10
Δ5-avenosterol	4.5-8.79
stigmasterol	1.09-2.42
Δ5,24-stigmatadienol	0.41-2.73
Cholesterol	0.42-0.96

4.3- Tocols content

Tocopherols and tocotrienols, natural antioxidants known as vitamin E, are abundant in date seeds. Date seed oil has a tocopherol concentration of 74.1 mg/100g, which is greater than olive oil (23.39 mg/100g) and the kernel oil of its nearby species, Phoenix canariensis (50 mg/100g). According to several research on the characterization of tocols in date seed oil, tocotrienols are more abundant than tocopherols. However, the quantitative and qualitative differences in tocopherol composition vary among cultivars based on the country of origin and environmental circumstances (Table 6) [34],[67],[54].

Table 6: Tocol content in date seed oil (mg/100g)



	α-tocopherol	β-tocopherol	γ-tocopherol	α-tocotrienol	γ-tocotrienol	δ-tocotrienol
Libya (Adwi)	0.84	0.69	9.71	36.43	5.78	1.68
Sudan (Barakawi)	0.93	1.14	11.67	32.84	4.47	1.13
Pakistan (Dora)	0.97	0.84	7.61	36.49	7.51	1.67
Algeria (Deglet Nour)	0.83	0.97	9.56	37.41	5.83	1.26
Saudi Arabia (Khalas)	15.17	0.63	28.92	26.96	31.88	5.06

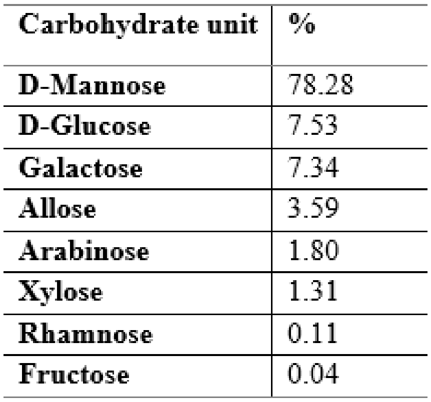
5- Fiber content

According to Al Farsi et al.'s research, the seeds contain the most fiber, their content varies per variety, ranging from 64% to 84% with the predominant was insoluble fiber. Indeed, the total fiber content of the Mabseeli variety is 83.50 g/100g, of which 1.53 g/100g is soluble (pectins, inulins, and gums) and 81.97 g/100g is insoluble (cellulose and hemicellulose) [62].

6- Carbohydrate content

Date pits contain 18% of raw polysaccharides, with separation providing an acid and neutral component (Table 7). The acidic fraction accounts for over 5% of the total and is mostly made up of arabinose, galactose, rhamnose, and glucose. The neutral fraction accounts for around 46% of the total, including mostly mannose, glucose, arabinose, and rhamnose. Date pit polysaccharides are made up of mannose in the form of mannans, galactomannans, and glucomannans [68].

Table 7: Chemical composition of date seeds carbohydrate content



Carbohydrate unit	%
D-Mannose	78.28
D-Glucose	7.53
Galactose	7.34
Allose	3.59
Arabinose	1.80
Xylose	1.31
Rhamnose	0.11
Fructose	0.04

III- Date seed processing and utilization

1- Incorporation into cattle feed

The value of integrating date pits into cattle feed has been demonstrated in studies conducted under this area. Date pits are reduced to powder and put to a 20% concentration of nursing goat feed[69]. This raises body weight by 10% and milk production by 16%, owing to the high casein content. Casein is a protein that boosts muscular strength and keeps blood amino acid levels high for up to 8 hours after consumption[70].

2- Date seed powder Incorporation into bread flour

Date seeds are an excellent source of total dietary fiber, making them perfect for bread production. Furthermore, their powder contains up to 26.54% protein, compared to 13.3% for other powders, and is abundant in essential minerals[71]. At 10% ratio, date kernel powder can be used to substitute other types of non-cereal fiber, such as wheat bran. This is particularly significant in countries where growing such products is challenging due to climate constraints, making date production a vital alternative[72].

3- Mayonnaise production from date seed oil

Date seed oil is used as a replacement for standard oils in the manufacture of mayonnaise. According to the findings of Basuny et al., mayonnaise prepared with date seed oil outperforms mayonnaise made with corn oil in terms of sensory properties. Furthermore, date seed oil has higher oxidative stability than other vegetable oils, allowing for better preservation [53].

4- Decaffeinated coffee made from date seed powder

Nowadays, many individuals drink coffee and, as a result, suffer from health problems associated with excessive caffeine consumption. Date seed coffee, on the other hand, is a nutritious, user-friendly alternative beverage high in carbs, reducing sugars, and polyphenols, which are effective natural antioxidants [73].

5- Traditional uses in southern Morocco

In the Southern Moroccan folklore, date seeds are commonly used for non-caffeinated coffee or for cosmetic purposes such as eyeliner kohl and hair coloring. Research reveals that date seed powder can, in fact, may be used as a cosmetic product (eyeliner) with no adverse effects on the eyes[74].

6- Activated carbon

Date-seed charcoal has proven to be a very efficient filtering aid in the removal of oxidation products from fried oils. It may also be used in the treatment of drinking water and wastewater, particularly for the removal of organochlorine pesticides, azo dyes, and rhodamine b, as well as the sorption of metal ions such as Cu(II), Ni(II), and Cr(VI)[75].

V- Pharmacological and cosmetic use

Date pits have numerous therapeutic benefits. They are abundant in natural antioxidants and can safeguard the kidneys and liver from hepatotoxicity or damage. Additionally, they provide benefits in diabetes cases and possess the ability to protect DNA and combat various viral diseases.

1- Pharmacological actions

a. Antiseptic function

According to Jassim et al., date kernel extract can restore normal liver functioning and provide protection against drug-induced liver toxicity[76].

b. Antiviral activity

Date seeds possess antiviral properties against various human pathogenic viruses, contributing to the treatment and prevention of viral infections. For instance, a study conducted by Jassim et al.

discovered that their extract had a preventive action against the ineffectiveness of the Pseudomonas phage ATCC 14209-B1 and bacterial lysis[77].

c. DNA damage preventive activity

Date seeds have a protective effect against chemically-induced liver damage, oxidative DNA damage, and oxidative damage in vivo, according to a research by Yasin et al. This is owing to their anti-free radical and antioxidant properties[78].

d. Preventing Chronic Kidney and liver Disease

Due to its high content of proanthocyanidins, one study suggests that date kernel extract provide protection against chemical toxicity in the liver and kidneys[79].

e. Regulation of blood glucose levels

The bioactive compounds found in date pits alleviate oxidative stress in pancreatic beta cells, thereby restoring insulin production. In fact, consuming date seeds can actually reduce blood sugar levels in hyperglycemic patients and maintain the glycemic index stable in healthy individuals [80].

2- Cosmetic use

a. Protective effect against dermal diseases

Regarding the non-therapeutic utilization of date pits, a recent study suggests that date seed extract and oil is effective in decreasing melanin, as well as treating eczema, acne, and dry patches. Furthermore, this treatment leads to a significant improvement in skin hydration and elasticity [74].

b. Skin and scalp protection

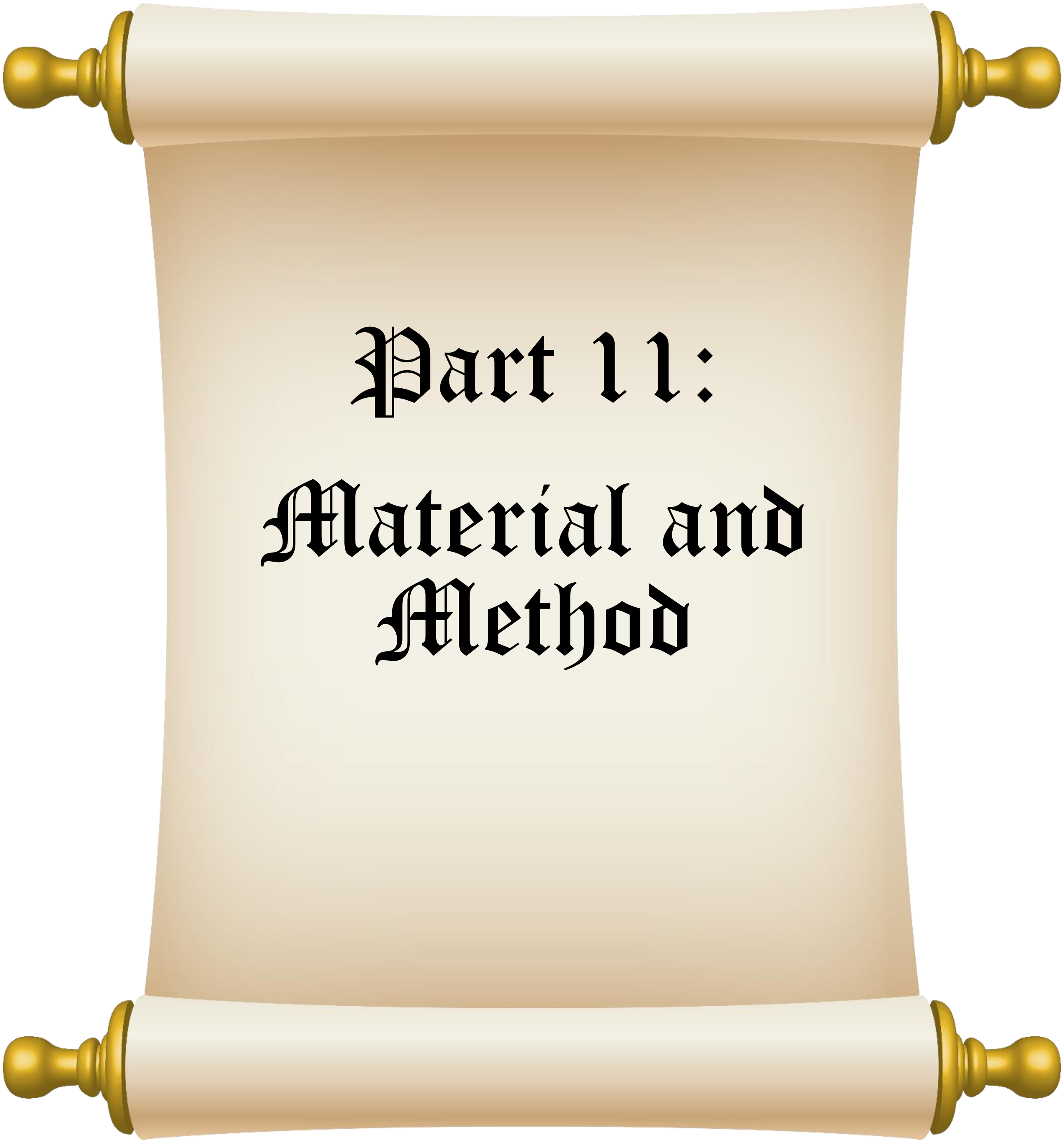
Date seeds have anti-aging properties that aid in wrinkle reduction, thanks to their essential components. They have the capacity to strengthen hair and skin, prevent premature graying of hair, limit the formation of wrinkles, and give the skin a youthful appearance [74].

c. Protective effect against UV radiation

Other research on UV exposure indicates that skin cultures containing date kernel oil experienced four times less DNA damage than those without, when exposed to the same level of irradiation [81].

Conclusion

Date palm seeds (*Phoenix Dactilifera L.)* are an underutilized byproduct of the date industry, yet they offer significant nutritional, medicinal, and industrial value. Comprising 7–30% of the fruit’s weight, the seeds are rich in essential minerals (such as potassium, magnesium, calcium, and iron), proteins with a high concentration of essential amino acids, lipids including beneficial triglycerides and phytosterols, and potent antioxidants like phenolics and tocols. They also contain up to 84% dietary fiber—mostly insoluble—and a variety of complex carbohydrates, primarily mannans and galactomannans. These components contribute to a wide range of applications: as additives in animal feed and bakery products, substitutes for caffeine in coffee, and ingredients in cosmetics and pharmaceuticals. Studies show that date seed derivatives can support liver and kidney health, regulate blood glucose levels, protect against DNA and UV-induced skin damage, and promote skin and hair vitality. Additionally, they are used in traditional remedies and as activated carbon in environmental treatments, highlighting their versatile benefits across multiple sectors.



Part 11:

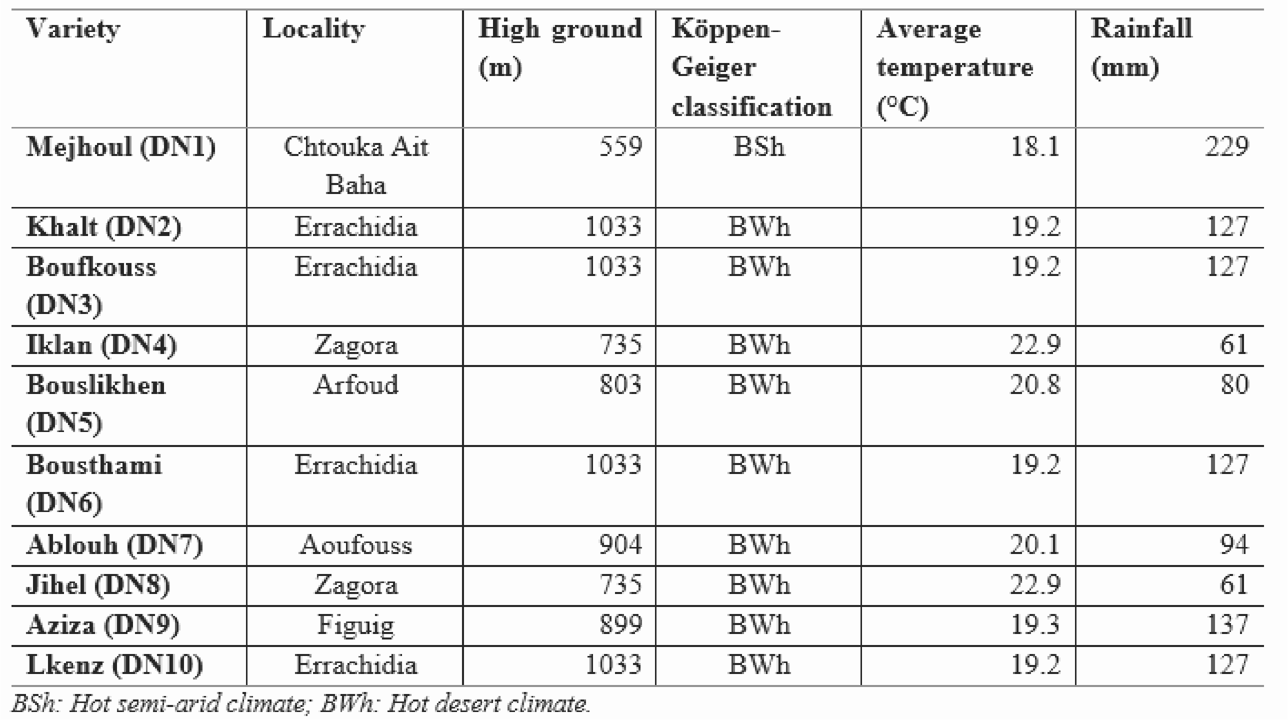
Material and Method

A- Raw material

I- Preparation of date seeds

The Moroccan date varieties were all obtained at the final growth stage (Tamr stage). They come from the districts of Sous-Massa, Darâa Tafilalet, and Oriental regions (see Table 8). The seeds are separated from the pulp and meticulously steeped in distilled water to eliminate all traces of pulp. After drying overnight at 50°C, they were grounded thoroughly with an electric grinder and kept in a freezer (-16°C) until extraction or analysis. The soxhlet technique was used to extract the lipid fraction, which was initially extracted using an apolar solvent. Subsequently, an alcoholic extract was obtained using a polar solvent.

Table 8 : Geographic Information on Fruit Harvesting Sites Date.



Variety	Locality	High ground (m)	Köppen-Geiger classification	Average temperature (°C)	Rainfall (mm)
Mejhoul (DN1)	Chtouka Ait Baha	559	BSh	18.1	229
Khalt (DN2)	Errachidia	1033	BWh	19.2	127
Boufkouss (DN3)	Errachidia	1033	BWh	19.2	127
Iklan (DN4)	Zagora	735	BWh	22.9	61
Bouslikhen (DN5)	Arfoud	803	BWh	20.8	80
Bousthami (DN6)	Errachidia	1033	BWh	19.2	127
Ablouh (DN7)	Aoufouss	904	BWh	20.1	94
Jihel (DN8)	Zagora	735	BWh	22.9	61
Aziza (DN9)	Figuig	899	BWh	19.3	137
Lkenz (DN10)	Errachidia	1033	BWh	19.2	127

BSh: Hot semi-arid climate; BWh: Hot desert climate.

II-Experimental process of solid-liquid extraction using the soxhlet apparatus

Extraction is commonly employed to selectively extract one or multiple compounds from a starting mixture based on their physical or chemical properties. It entails transferring one or more compounds from one phase to another, for example, from a solid phase to a liquid phase or from a liquid phase to another liquid phase with different chemical properties. This process enables the separation of specific compounds from an animal or plant organism using various techniques.

1- Principle of solid-liquid extraction

Solid-liquid extraction is a gradual method which transfers compounds from the solid phase (sample) to the liquid phase (extraction solvent). The classic solid-liquid extraction methods comprise infusion, maceration, and decoction, whereas the most common methods utilized in laboratories are Soxhlet and Kumagawa extractors. It should be noted that these methods cannot extract a single compound regardless of the selectivity of the solvent used; however, other compounds are concurrently transferred. Conversely, the polarity of the organic solvent can be used to extract more or less selectively, employing successive extractions with increasing polarity solvents [82].

2- Soxhlet extraction principle

The Soxhlet extractor is a device/ tool utilized in analytical chemistry to efficiently extract compounds from solids (sample). It is named after Franz Von Soxhlet, its inventor. The method is straightforward, allowing for the extraction cycle to be repeatedly performed with fresh solvent until the solute in the raw material is depleted (Figure 28). This study employed Soxhlet extraction to examine the impact of supercritical pretreatment on the raw material [83].

3- Extraction Experimental process

The ground powder is placed in a cartridge, and the distillation flask is a 500 ml flask filled with 300 ml of solvent: n-hexane to extract the vegetable oil, selected for its apolar nature and high yield, and ethanol for the alcoholic extract. Methanol can also be utilized, however due to its toxicity, it is not suggested. The extracted solution is filtered using filter paper with a desiccant ($MgSO_4$) to eliminate any trace of water, then the solvent is evaporated under reduced pressure using a Rotavapor, and the recovered extract is weighed to determine the yield[84].

Figure 28: Soxhlet apparatus

B- Plant oil analysis protocol

I- Physicochemical analysis: Oil quality indexes

1- Measurement of primary and secondary oxidation products: K_{232} and K_{270}

a. Definition

The detection of specific spectrophotometric absorbances in the UV range is an important indicator of oil oxidation. Specific absorbances, denoted by the letter E, are measured in the UV light spectrum and correspond to absorption maxima. The specific extinction at 232 nm may be used to determine the "freshness" of the oil, but the value at 270 nm is more difficult to understand and indicates both the development of oxidation by-products and the probable inclusion of refined oils. In general, the existence of conjugated diene structures correlates to the maximum absorption at 232 nm, whereas conjugated triene structures absorb at 270 nm. These chemicals are generated as a result of the oxidation of unsaturated hydroperoxides [85].

b. Method principle :

The sample fat is dissolved in cyclohexane, a suitable solvent for the determination of absorbance using a UV spectrophotometer within specific wavelength ranges from 232nm to 270nm. The recorded absorbances at 232nm and 270nm indicate the existence of conjugated diene and triene systems, respectively.

c. Experimental process :

0.1 grams of the sample are dissolved in 10 milliliters of cyclohexane. The reading is taken at two wavelengths (232 nm and 270 nm) using quartz cuvettes.

d. Expression of results:

The extinction coefficients, K_λ for wavelengths 232 nm and 270 nm, are calculated using the following methods:

$$K_\lambda = {}^{A_\lambda}/_{(C.S)}$$

With:
K_λ : Wavelength specific extinction λ.
A_λ: Absorbance measured at the wavelength λ.
C: Concentration in grams per 100ml.
S: cuve thickness in centimeters.

2- Free fatty acid content

a. Definition

The chemical or microbiological decomposition of triacylglycerols results in the formation of free fatty acids. These must be neutralized during processing since they tend to lower the smoke point of frying fats and oxidize quickly, imparting a rotten taste to the oil. The quantity of free fatty acids can be used to determine the quality of the seeds utilized. A high amount (>1.0% oleic acid) indicates that the seeds are damaged and of low (or poor) quality [86].

b. Method principle

Free acidity is measured by dissolving a specimen in a solvent mixture and then titrating with ethanoic sodium hydroxide solution (NaOH/EtOH). The acidity is usually presented as a percentage by mass of oleic acid.

$$\underset{\text{Fatty Acid}}{RCOOH} + \underset{\text{Sodium Hydroxyde}}{NaOH} \longrightarrow \underset{\text{Soap}}{RCOOK} + \underset{\text{Water}}{H_2O}$$

Chemical equation n° 1 : Fatty Acid reaction with NaOH

c. Experimental process:

5g of oil is added to 25ml of an equivalent volume (v/v) combination of diethyl ether and ethanol in an Erlenmeyer flask, followed by a few drops of phenolphthalein as a color indicator. Using a burette, the sample is titrated by progressively adding a solution of sodium hydroxide (ethanolic NaOH) of Normality 0.1N. Titration is complete when a persistent pink color appears (Figure 29).

Figure 29: FFA's experimental test

d. Expression of results:

The result is expressed as a percentage of oleic acid using the following formula:

$$FFA\ (\%) = \frac{V\ \times N\ \times 282}{10\ \times WS}$$

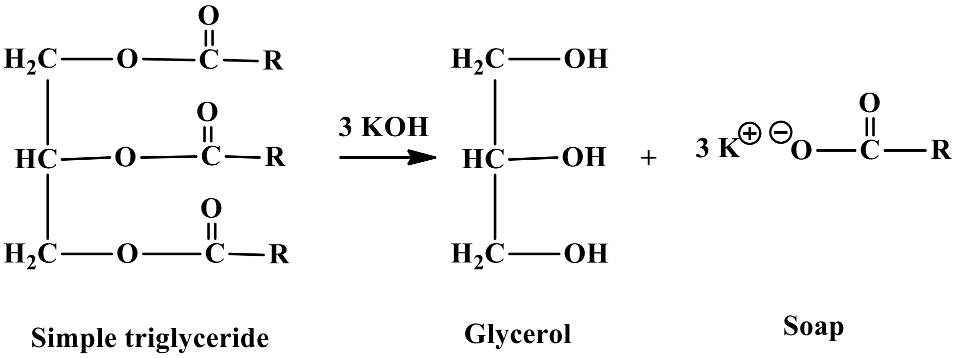
With :

V: volume poured (ml)
N: normality of NaOH solution
WS: test sample (g)
282: molecular weight of oleic acid.

3- Saponification index

a. Definition

The saponification index (S.I.) is the quantity in milligrams of potassium hydroxide required to saponify a defined quantity (1g) of oil. This parameter gives an indication of the nature of the fatty acids containing the fat, and depends on the average molecular weight of these fatty acids. It is also useful for comparative studies on the chain length of fatty acids in oils [86].



Chemical Equation n°2 : Saponification reaction of a simple triglyceride with KOH

The saponification index is linked to other quality indices such as the acid and ester indices by the following formula:

$$Saponificaton\ Index = \text{Ester}\ Index + Acide\ Index$$

The amount of KOH potash used in IS measurement varies with the molar mass of the fatty acids. The higher the molar mass, the lower the saponification index.

b. Method principle:

This is a backflush method, in which an oil solution is reacted with an excess of potassium hydroxide (KOH). This excess is then dosed with a solution of hydrochloric acid (HCl).

c. Experimental process :

In a conical flask, 5g of oil is dissolved in 25 ml of an ethanoic solution of potassium hydroxide (KOH/EtOH). A condenser is then placed over the flask and the mixture is heated to 80°C for one hour, with occasional stirring to ensure that saponification takes place, after which 3 to 5 drops of phenolphthalein are added as a color indicator. The container is cooled for a while before the contents are titrated with HCl (0.5 N) until the pink color disappears completely. The test solution consists of 25 ml KOH/EtOH only.

d. Expression of results:

The saponification index is expressed in milligrams of potash (KOH) per 1g of fatty substance. The formula used for the calculation is as follows:

$$S.I(mg\ KOH/g\ of\ oil) = \frac{(V_0 - V_1) \times 56.1\ \times N}{WS}$$

V0 : volume of HCl poured into the sample solution (ml)
V1 : volume of HCl added to the test solution (ml)
N : normality of HCl solution (ml)
WS : test sample (g)
56.1 : molecular weight of KOH.

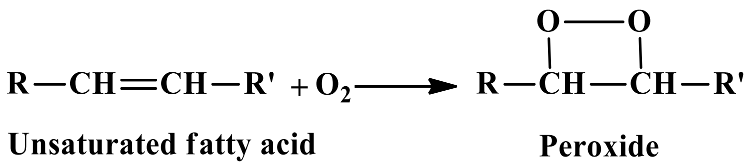
4- Peroxide index

a. Definition

Autoxidation is a reaction between lipids and an oxygen molecule. This phenomenon is responsible for oil degradation. The consequence of auto-oxidation is essentially the production of unstable hydro-peroxides, which spontaneously decompose into a series of products, notably mixtures of volatile aldehydes. The peroxide index (P.I.) is therefore a very useful and sensitive criterion for assessing the early stages of oxidative deterioration of fat [86].

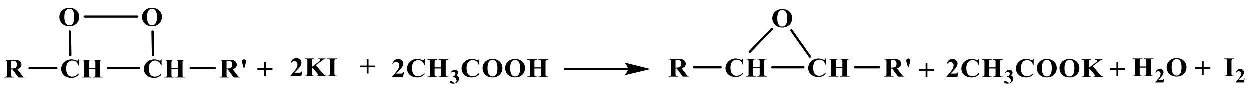
b. Method principle:

The peroxide index of a fatty substance is the number of micrograms of active oxygen of the peroxide contained in one gram of oil, or the number of milliequivalents of active oxygen per kilogram of fatty substance oxidizing potassium iodide with iodine release under the conditions of method NF T 60-220. In the presence of atmospheric oxygen, unsaturated fatty acids oxidize to produce peroxides according to the following reaction:



Chemical equation n°3 : the oxidation of an unsaturated fatty acid to peroxide

The PI is the quantity of active O_2 per kilogram of oil, capable of oxidizing potassium iodide with the release of iodine. It is expressed in milliequivalents of active O_2 per 1 kg of fat. One oxygen molecule is attached to a peroxide molecule. Of the two oxygen atoms attached, only one is active and capable of oxidizing iodides according to the following reaction:



Chemical equation n°4 : Epoxidation of a peroxidized fatty acid

c. Experimental process :

About 1g of oil is weighed into a 250 ml Erlenmeyer flask, to which 10 ml of chloroform and 15 ml of acetic acid are added, followed by 1 ml of a saturated aqueous potassium iodide solution. Shake for one minute, then place in the dark for 5 minutes at a temperature of 15-25°C. Add 75 ml distilled water, stirring vigorously, and a few drops of starch as an indicator. The 0.01 N sodium thiosulfate solution is then added. A blank test without the fatty substance is performed at the same time. The experimental approach is the same as that for the oil sample.

d. Expression of results :

The PI is expressed in milliequivalents of oxygen per kg of oil and is given by the following formula:

$$PI\ (meq\ O_2/kg) = \frac{(V - V_0)\ \times 1000\ \times N)}{WS}$$

V: volume in ml of the sodium thiosulfate solution used for the test
V_0: volume in ml of sodium thiosulfate solution used for blank test
WS: Test sample of oil to be analyzed in g
N: Normality of sodium thiosulfate solution

5- Iodine index

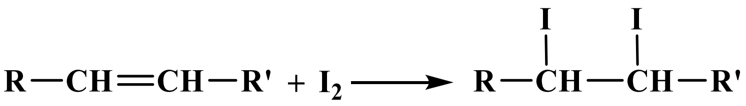
a. Definition

The iodine index is an indicator of the total quantity of unsaturated fatty acids present in fats and oils. By definition, it is a measure of the number of grams of iodine absorbed per 100g of sample.

It is constant for a particular oil. It is also a useful parameter in the study of oxidative rancidity of oils, as the higher the unsaturation, the greater the potential for rancidity [85].

b. Method principle :

The iodine index can be determined by 16 different methods. Whatever halogen reagent is used, the principle is the same. Ethyl bonds, particularly those of fatty acids, bind halogens. Iodine binds to the unsaturations of fatty chains, saturating them as follows:



$$R{-}CH{=}CH{-}R' + I_2 \longrightarrow R{-}\underset{}{\overset{I}{\overset{|}{CH}}}{-}\overset{I}{\overset{|}{CH}}{-}R'$$

Chemical Equation n°5: Iodine reaction with unsaturated fatty acid

Due to the dangerous nature of the reagents used, the iodine value is generally calculated from the fatty acid composition of the oils [87]. Thus, the calculation method used gives results in line with Wijs' method.

c. Experimental process:

The iodine index is determined from the chemical composition of fatty acids according to the Cd 1c-85 method recommended by the American Oil Chemists Society (AOCS).

d. Expression of results:

The iodine Index is determined by the following equation:

$$I.I\ (g\ I_2/100\ of\ oil) = \frac{12.69\ \times N\ \times (V_0 - V_1)}{WS}$$

With:
I.I: Iodine Index
V_0: Volume of sodium thiosulfate used for blank titration
V_1: Volume of sodium thiosulfate solution
N: Normality of thiosulfate solution
WS: Weight sample

II- Measurement of oil pigments: carotenoids and chlorophylls

a. Definition

A pigment is a colored chemical compound that reflects a certain wavelength in visible light. It is the agent responsible for the coloring of various plants such as flowers, leaves, fruit, etc. A specific pigment absorbs one or more distinct wavelengths of electromagnetic radiation and refracts its inverse, which is visible to the eye.

Chlorophyll is a group of pigments characterized by a green coloration (they absorb yellow and blue wavelengths to reflect a green color), which are present in photosynthetic organisms such as plants, algae and so on. These pigments have the ability to capture light energy in order to produce carbohydrates. They generally comprise four types of chlorophyll: chlorophyll a, b, c and d. Chlorophyll a and b are the most common, and are mainly involved in photosynthesis.

Carotenoids are pigments that reflect the colors yellow, orange and red. There are two main types of carotenoids: carotenes and xanthophylls. Xanthophylls are the yellow pigments, while carotenes are orange to yellow. Lycopene, on the other hand, is also a red carotenoid pigment, and is responsible for the coloring of tomatoes.

Unlike chlorophylls, carotenoids are unable to transfer absorbed light directly into the photosynthetic pathway, but they can transfer it to chlorophylls and assist photosynthesis.

b. Method principle :

The content of pigmented phytoconstituents in the oil is determined using a UV spectrophotometer by the x method. First, the sample to be analyzed is dissolved in a suitable organic solvent (usually cyclohexane), then the absorbance of the sample is determined at two wavelengths characteristic of each pigment [88].

c. Experimental process :

7.5 grams of oil are dissolved in 25 ml of cyclohexane. The solution is vortexed and the absorbance measured in a UV spectrophotometer. To determine chlorophyll content, the wavelength is set at 670 nm, and for carotenoid content the wavelength is set at 470 nm, using a 1cm-wide quartz cuvette.

d. Expression of results:

Chlorophyll content is expressed as mg of alpha pheophytin per Kg of oil, while carotenoid content is expressed as mg of lutein per Kg of oil. Values are calculated using the following two equations:

$$\text{Chlorophylls}\ (\text{mg}.\text{kg}^{-1}) = (\text{Abs}_{670} \times 10^{6})/(613 \times 100 \times \text{cuve lenght})$$

$$\text{Carotenoids}\ (\text{mg}.\text{kg}^{-1}) = (\text{Abs}_{470} \times 10^{6})/(2000 \times 100 \times \text{cuve lenght})$$

III- Chemical composition of a vegetable oil

1- Fatty acid analysis

a. Definition

Oils contain a saponifiable glyceride fraction and an unsaponifiable fraction. The glyceride fraction is made up of esters of glycerol and fatty acids. The carbon chain of the latter may be saturated, containing one or more double bonds. They are referred to as saturated (SFA), monounsaturated (MUFA) or polyunsaturated (PUFA) fatty acids respectively [84].

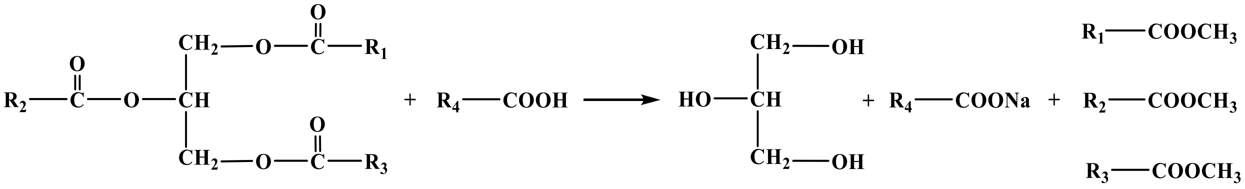
b. Preparation of methyl esters :

Fatty acids are identified according to this method. It is based on the esterification of fatty acids to methyl esters in two successive steps:

First step: Basic methanolysis

In this step, the neutral glycerol components are converted to methyl esters and any free fatty acids are transformed into soaps.

In this step, all molecules with a glycerol function are converted into methyl esters, while any free fatty acids present are converted into soaps, as illustrated in the following reaction:



Chemical equation n°6: Alkaline methanolysis with sodium methoxide

The second step: Acid esterification

Due to an excess of hydrochloric methanol, the free fatty acids previously transformed into soaps in the basic methanolysis reaction are esterified by acidifying the medium.

$$R_4{-}COONa \longrightarrow R_4{-}COOCH_3 + NaCl$$

Chemical equation n°7: Esterification of a fatty acid

c. Chromatographic analysis of methyl esters:

Pre-prepared methyl esters are analyzed by gas chromatography using the CEE/2568/91 method. The chromatograph is coupled to a CPWax 52CB capillary column (30m× 0.25mm) with an initial and final temperature of 170°C and 230°C, gradually increased at 4°C/min. Helium was used as the carrier gas, with a column flow rate of 1 milliliter per minute. Results were processed by Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA).

d. Expression of results :

Using a previously analyzed control, methyl esters are identified by comparing the retention times of the sample with the retention times of the control. Results are expressed as percentage attributed to each fatty acid.

$$\text{Fatty acid \%} = \left(\frac{S}{\sum S}\right) \times 100$$

Where:
S: area of the peak corresponding to the fatty acid in question.
∑**S**: sum of the areas of all the peaks of a chromatogram corresponding to the fatty acids in the mixture.

2- Sterol analysis by gas chromatography (ISO 6799)

a. Definition

Phytosterols are generally found in esterified forms in oils [89], and the determination of these phytochemical compounds is used to analyze oil quality and detect commercial fraud. They are presented as the unsaponifiable fraction of the major components [90].

b. Choice of method :

Unsaponifiable matter content is determined in accordance with NF ISO 6799 by gas chromatography. Capillary gas chromatography (GC) is the technique of choice for the analysis of sterols and related compounds. Capillary columns offer shorter analysis times and less interference between peaks, improved component resolution and greater thermal stability than packed columns [91].

c. Sterol analysis of date kernel oil:

About 250 mg of DSO is weighed into a 25 ml flask, and 1.0 ml of an internal standard solution (Betulin in acetone, 1.0 mg/ml) is added. Then, 5 ml of a potassium hydroxide solution (1N in ethanol) is added. The mixture is heated under reflux for 15 minutes until it becomes clear.

The unsaponifiable matter is isolated using solid-phase extraction with an aluminum oxide column that retains fatty acid anions while eluting the unsaponifiable content with 5 ml of ethanol followed by 30 ml of ether. The sterol fraction is separated from the unsaponifiable matter via thin-layer chromatography (TLC) on silica gel, using a mobile phase of n-heptane and distilled diethyl ether (50:50 v/v).

The sterol fraction is scraped off, dissolved in 10 ml of chloroform, filtered, and evaporated to convert the sterols into silylated derivatives (TMS) using a silylation agent (50 µl of methylimidazole dissolved in MSHFBA). The sterol composition is then determined by gas chromatography (GC). The compounds are separated on an SE 54 CB column (Macherey-Nagel, Germany; 50 m long, 0.32 mm diameter, 0.25 µm film thickness).

Other parameters include helium as the carrier gas, an injection and detection temperature of 320°C, and a temperature program of 245–260°C at 5°C/min.

d. Identification and quantification:

Sterols were identified using standard compounds (β-sitosterol, campesterol, stigmasterol), sterols from rapeseed oil (brassicasterol), and sunflower oil (Δ7-avenasterol, Δ7-stigmasterol, Δ7-campesterol), according to their retention times. The results were expressed in mg/100g of oil.

3- HPLC tocols analysis

a. Definition

HPLC is the most widely used technique to analyze tocols, and both normal-phase (NP) and reversed-phase (RP) chromatography are applied. Tocols are stable under HPLC conditions, easy to dissolve in appropriate solvents, and there are several detectors that can be combined with HPLC to detect tocols. Fluorescence detection (FLD) and ultraviolet detection (UV) are the most commonly used in food and feed analyses. Gas chromatography (GC) could also be used to analyze tocols, but that would need derivatization of the analytes and might include a risk for decomposition due to high temperatures. Yet GC is still sometimes being used for tocol analysis [92].

a. Analysis of date kernel oil tocols :

Tocols are determined using the ISO 9936 method. Using High-Performance Liquid Chromatography (HPLC) equipped with a silica column (25 cm 4 mm) and a fluorometric detector. Excitation and emission wavelengths were 290nm and 330nm respectively. The eluent was prepared from a mixture of isooctane/isopropanol (99:1) (V/V), with a flow rate of 1.2 ml/min.

b. Identification and quantification:

Quantification was carried out using an external standard and reference data from the American Oil Chemists' Society (AOCS). Authentic tocopherol standards are commercially available, and thus it is relatively easy to identify them from chromatograms obtained by FLD or UV responses. Elution orders of different tocopherols can also easily be found in literature. Tocotrienol standards, however, are not available as pure compounds, and thus their identification is usually done by using palm oil or cereal grain extracts that are known to contain tocotrienols.

C- Extract analysis Experimental process

I- Phytochemical screening :

Date seed powder and extract are analyzed for polyphenols, flavonoids, tannins, carotenoids, saponins, cardiotonic glycosides, anthocyanins and leucoanthocyanins according to standard methods [93] [94] [95] [96] [97].

Polyphenol test [Ferric chloride test]:

To 1ml of alcoholic extract, add 2ml of distilled water, then a few drops of 10% aqueous ferric chloride solution. The formation of a blue or green color indicates the presence of polyphenols.

Flavonoid test

To 0.5ml of alcoholic extract, add 5 to 10 drops of HCl diluted with a small amount of Mg, then boil for a few minutes. A reddish-pink or brown color indicates the presence of flavonoids.

Test for tannins

Approx. 0.5 g of extract is boiled in 10 ml of water, then filtered. The addition of a few drops of 0.1% ferric chloride reveals a brownish-green color indicating the presence of condensed (catechic) tannins, or a blue-black color indicating the presence of gallic tannins.

Salkowski test: Detection of terpenoids

To 0.5 g of the extract 2 ml of chloroform were added. Next, 3 ml of concentrated H2S04 were very carefully added to form a layer. The appearance of a reddish-brown coloration at the interface indicates the presence of terpenoids.

Carotenoid test

A mixture of 20g powder and 150ml distilled water is filtered. 3 ml of HCl are added to the filtrate, followed by 3 ml of H2S04. The appearance of a blue-green coloration reveals the presence of carotenoids.

Alkaloid test: Alkaloid precipitants

Wagner's reagent: A few drops of Wagner's reagent were added to 3m of sample. The appearance of a brownish precipitate indicates the presence of alkaloids (Figure 30 on the left).

Mayer's reagent: A few drops of Mayer's reagent were added to 3ml of extract. The formation of a precipitate indicates the presence of alkaloids (Figure 30 on the right).

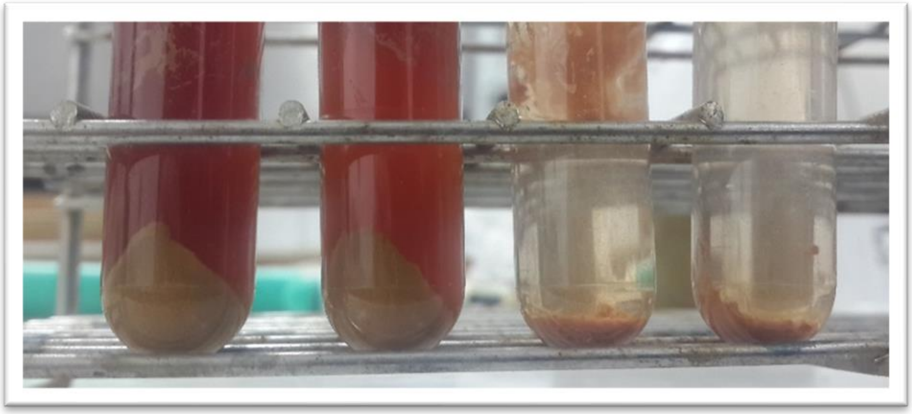


Figure 30: Alkaloids precipitation by Wagner's and Mayer's reagent

Test for saponins

In a test tube, 2.5 g of extract were added to 5 ml of boiled distilled water. The solution was shaken vigorously. The formation of a stable, persistent foam for half an hour indicates the abundant presence of saponins (Figure 31).

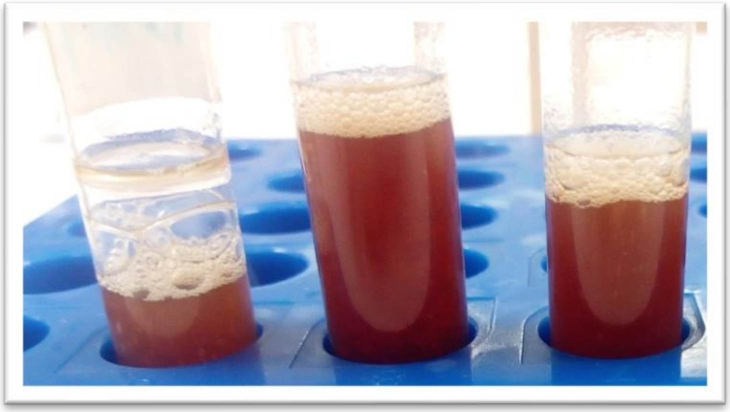


Figure 31: Saponins test

Detection of cardiac glycosides [Keller Killiani test]:

Approximately 0.1g of methanolic extract was dissolved in 1ml glacial acetic acid containing a drop of ferric chloride solution. Then 1ml of concentrated H2S04 was added. The presence of a deoxy sugar characteristic of cardenolides was detected by a brown ring at the interface.

Anthocyanin detection:

2 ml of 25% hydrochloric acid solution is added to 2 ml of methanolic extract. The appearance of a purplish-blue-pink coloration on addition of a 25% ammonia solution indicates the presence of anthocyanins.

Detection of leucoanthocyanins:

To 2 ml of methanolic extract is added 2 ml of concentrated HCl. The mixture is placed in a boiling water bath for around twenty minutes. The appearance of a red color indicates the presence of leucoanthocyanins.

II- Quantitative analysis of bioactive compounds:

1- Determination of polyphenols content

a. Definition

Quantitative analysis of total polyphenols is carried out by UV spectrophotometer using the method described by Zhang et al. [98], using the Folin-Ciocalteu reagent. This method is widely used for characterizing plant extracts. It is based on quantification of the total concentration of hydroxyl groups present in the sample [99].

b. Method principle :

Folin Ciocalteu reagent is an acidic yellow solution, consisting of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). When it oxidizes phenolic compounds to phenolate ions, it is reduced to a mixture of blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_4). This blue coloration (Figure 32) has a maximum absorption around 750 nm, which is proportional to the quantity of phenols present in a sample.

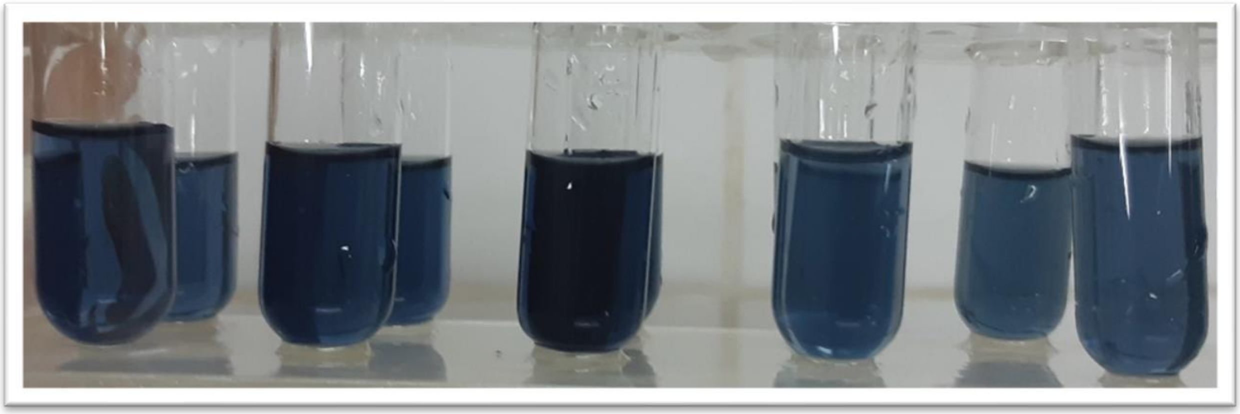


Figure 32: polyphenols content's experimental test

c. Experimental process :

In test tubes, 0.5 ml of each sample to be analyzed (the concentration of the stock solution is 1000 µg/ml) is introduced with 4 ml of a 7.5% sodium carbonate (Na_2CO_3) solution. After stirring, 2.5 ml of 10% Folin-Ciocalteu solution is added. The mixture is then incubated in a water bath at 45°C for 30 minutes. Absorbance readings are taken at 765nm against an extract-free control. Analyses are performed in triplicate. Gallic acid is used as a standard in a range of concentrations (0 to 1000 µg/ml), applying the same experimental process.

d. Expression of results:

To calculate polyphenol content, a calibration curve (linear: $y = ax + b$) is established with the standard (gallic acid). Results are expressed in equivalent milligrams of gallic acid per gram of sample (mg GAE/g).

2- Determination of flavonoids content:

a. Definition :

Determination of flavonoid content is estimated by the colorimetric method described by Oubihi *et al.* using aluminum trichloride ($AlCl_3$) and sodium hydroxide (NaOH). The latter forms a complex with yellow and orange flavonoids respectively (Figure 33), and absorbs in the visible range at 510 nm [100].

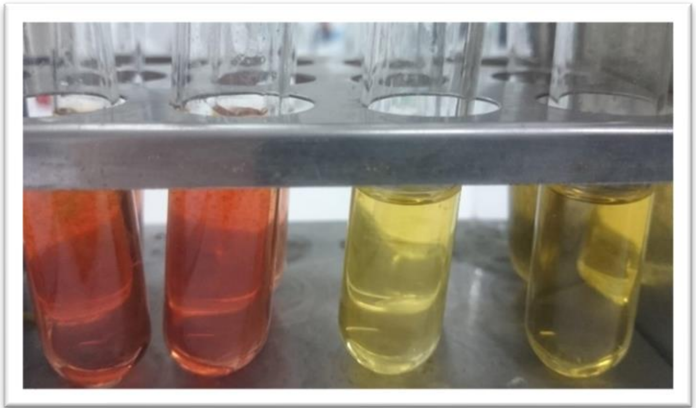
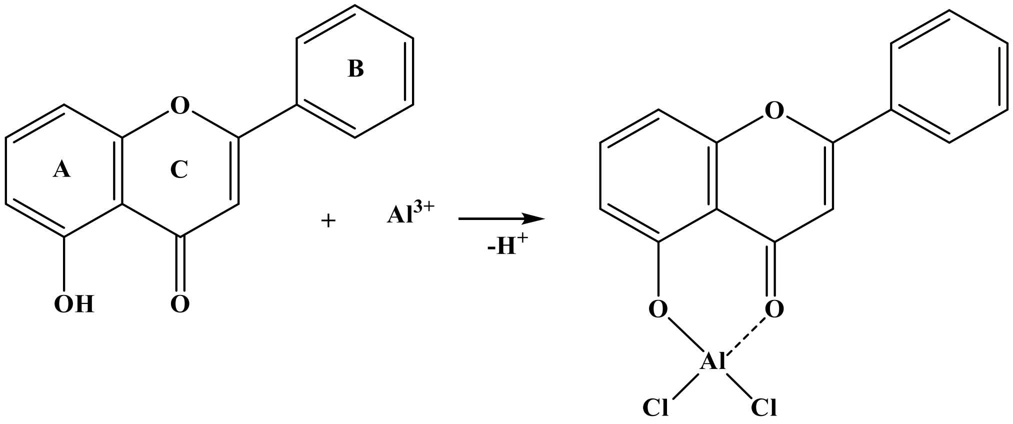


Figure 33: flavonoids content's experimental test

b. Method principle :

Determination of flavonoid content by Wambui et al.[101] method is based on the formation of stable acid complexes between aluminum chloride and the ketone group at carbon 4 (C-4) and the hydroxyl group at carbon 3 (C-3) or carbon 5 (C-5) of flavones or flavonols. The aluminum trichloride complex reveals a yellow coloration, then the addition of sodium hydroxide gives a pink to red-orange coloration of flavonoid content-dependent intensity, which can be measured at 510 nm [102].



Chemical equation n°8: flavonoids and $AlCl_3$

c. Experimental process :

In a test tube, 1ml of sample is introduced with 6.4ml of distilled water and 0.3ml of sodium nitrite ($NaNO_2$) 5% (w/v), the mixture is vortexed and incubated for 5 min at room temperature. Next, 0.3ml of 10% (w/v) aluminum trichloride is added and the mixture undergoes a second incubation for 6 minutes. Next, 2 ml of 1M sodium carbonate is added and the mixture is incubated for a final 30 minutes. Using a spectrophotometer, absorbances are read at a wavelength of 510 nm.

d. Expression of results :

Flavonoid content is calculated using the quercetin calibration curve (the standard). Results are expressed in milligram equivalent of quercetin per gram of sample (mg QE/g).

3- Determination of tannins content:

a. Definition

The vanillin method with HCl is a colorimetric method adopted for the determination of tannin content [103].

b. Method principle :

This method consists of reacting the vanillin molecule with a terminal flavonoid group of condensed tannins to form complexes characterized by a red color, due to the ability of tannins to transform into red anthocyanidols.

c. Experimental process :

0.1ml sample is mixed with 3ml 4% vanillin (alcoholic solution) and 1.5ml chloridric acid is added, vortexed and incubated for 20 minutes. Absorbance readings are taken at 500nm.

d. Expression of results :

The concentration of tannins in each sample is calculated using a calibration curve established with catechin as the standard. All concentrations are expressed in milligrams of catechin equivalent per gram of sample (mg CE/g).

4- Determination of alkaloids content:

a. Method principle:

The conduction of alkaloid content was carried out using the gravimetric alkaline precipitation method as described by Harbone et al.[104]

b. Experimental process:

Five grams of sample are weighed, and 50ml of 10% acetic acid solution in ethanol is added. The mixture is then stirred and left to stand for four hours. Afterward, the mixture is filtered and evaporated in a water bath to reduce it to a quarter of the initial volume. The alkaloid in the extract is precipitated by adding ammonium hydroxide (NH_4OH) dropwise until completely turbid. The precipitate is collected, washed with 1% ammonia solution, and dried in an oven.

c. Expression of results :

The alkaloids content is determined and expressed as a percentage of the sample analyzed using the following formula:

$$\%Alkaloids = \frac{W_{alk}}{W_{\mathrm{Sample}}} \times 100$$

With :

W_{Alk}: Weight of alkaloids

W_{Sample}: Sample weight

5- Determination of total sugars content:

a. Definition

Oses and their methylated derivatives react with phenol and concentrated sulfuric acid to give an orange-yellow colored product (Figure 34) that is stable for several hours, with absorption maxima at 490 nm for hexoses and 480 nm for pentoses [105].

Figure 34: sugars content's experimental test

b. Method principle :

When oses and their methylated derivatives come into contact with concentrated sulfuric acid and phenol, they react to form stable compounds characterized by a yellow-orange coloration. The intensity of this coloration is measured by a spectrophotometer at a wavelength of 480nm.

c. Experimental process :

First, take 1ml of each sample and add 1ml of 5% phenol and 5ml of concentrated sulfuric acid (H_2SO_4). The samples are then carefully shaken to allow them to stand for 10 min at room temperature. The samples are then placed in a water bath at 30°C for 20 min. Finally, the absorbances of the samples are measured at λ=480nm.

a. Expression of results:

The total sugar content of samples is determined using a calibration curve established with glucose as the standard. Sugar content is expressed in milligrams of glucose equivalent per gram of sample.

6- Determination of terpenes:

a. Definition

Terpenes are essential to the fluidity of cell membranes, as they include triterpenes, which act as a precursor molecule to cholesterol. Cholesterol serves as a precursor to steroid hormones such as progesterone, estradiol and testosterone. Estradiol and progesterone play an essential role in female reproductive cycle processes such as ovulation, implantation and maintenance of pregnancy [106].

b. Method principle:

Using a spectrophotometer, terpenes can be determined via their maximum absorbance of 420nm.

c. Experimental process:

1 gram of each sample was added to 10 ml petroleum ether and extracted for 15 minutes. The solution was filtered and absorbance measured at 420nm.

d. Expression of results:

Absorbances measured are expressed as a total percentage of terpene content:

$$\% \, Terpene = [Measured \; Abs]$$

7- Saponin determination:

a. Method principle:

The saponin content of the samples was determined by the gravimetric double solvent extraction method, as described by Awomukwu et al. [107].

b. Experimental process:

Five grams of powdered sample are weighed and mixed with 50 ml of 20% aqueous ethanol solution. The mixture is heated in a water bath with periodic stirring for 90 min at 55°C. The mixture is then filtered and the residue undergoes a second extraction with 50ml of 20% ethanol. Finally, the two extracts are combined together. The combined extracts are reduced to 40ml using a water bath at 90°C. They are then transferred to a 250ml separating funnel and 40ml diethyl ether is added and shaken vigorously and carefully. This process of purifying the aqueous phase is repeated two or three times until the aqueous phase becomes clear. Next, the saponins are extracted with 60ml of n-butanol (two extractions). The combined n-butanol extract is washed twice with 5% sodium chloride solution, then evaporated and dried in an oven to constant weight. The saponin content is determined and expressed as a percentage of the analyzed weight according to the following formula:

$$\%Saponine = \frac{W_{Sapo}}{W_{\mathrm{Sample}}} \times 100$$

With :
W_{Sapo}: Weight of saponins
W_{Sample}: Sample weight

III- Toxicity study: determination of oxalate ions by titration

Quantitative oxalate analysis is determined using the method described by Onwuka [108] and Ejikeme et al. [109] Determination involves three main steps: digestion, oxalate precipitation and permanganate titration.

1- Digestion:

For 2.5g of sample, 20ml of HCl (0.3M) is added. The whole is put under constant magnetic stirring for one hour at a temperature of 50°C. Extraction was performed three times. Then filtered.

2- Oxalate precipitation:

For oxalate estimation, 1ml ammonium hydroxide (5M) is added to 5ml extract to ensure alkalinity, along with 2 drops phenolphthalein indicator, 3 drops glacial acetic acid and 1ml calcium chloride (5%) to make the mixture acidic, before allowing it to stand for 3 hours. Then centrifuge the mixture at 3000 rpm for 15 minutes. The precipitate is collected and washed three times with hot water, mixing carefully with each centrifugation. Next, 2ml of 3M tetraoxosulfate (VI) acid is added to each tube to dissolve the precipitate by heating in a water bath at 70°C.

3- Permanganate titration :

A solution of potassium permanganate (0.01M) is freshly prepared to titrate the contents of each tube at room temperature until the color pink appears. The solution is left to stand until colorless, then heated on an electric hotplate at 70°C for 3 minutes, and titrated again until a pink color appears and persists. The titration reaction and the oxalate ion content of the sample are shown below:

The titration reaction : $5\ C_2O_4^{2-} + 16H^+ + 2MnO_4^- \rightarrow 2Mn^{2+} + 10\ CO_2 + 8\ H_2O$

Calculation of oxalate ion content : $Oxalate\ \ g/100g = \frac{P_{KMnO_4}}{2.5} \times T$

IV- Antioxidant activity

Many methods were used to test the antioxidant capacity of natural compounds. There are two free radicals that are widely employed to evaluate this capacity known as DPPH and ABTS radicals.

1- Evaluation of the inhibitory capacity of extracts by DPPH and ABTS radicals:

1.1- DPPH antioxidant test

a. Method principle:

DPPH (1,1-Diphenyl-2-picrylhydrazyl) (Figure 30) is a stable, purplish-colored free radical that absorbs at 517nm. In the presence of free radical scavengers, the DPPH radical is reduced, changing color to yellow (Figure 35). Absorbances measured at 517 nm are used to calculate the percentage inhibition of the DPPH radical, which is proportional to the anti-free radical power of the sample.

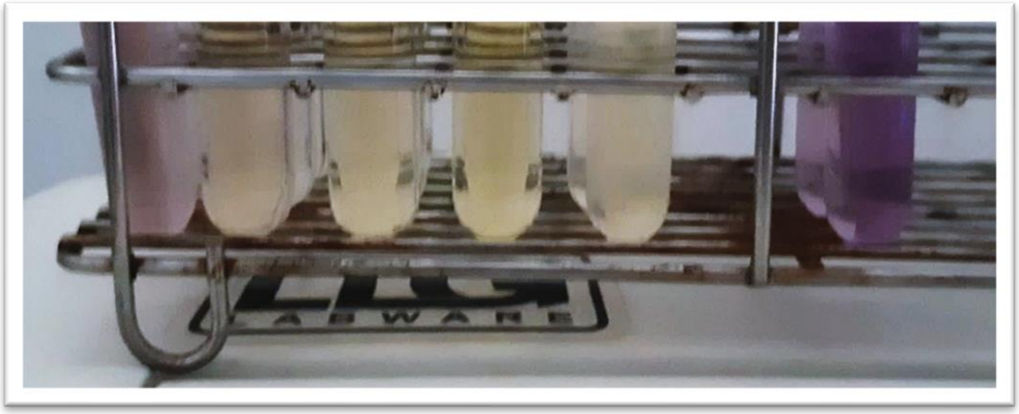


Figure 35: DPPH experimental test

This method is based on measuring the ability of antioxidants to trap the DPPH radical. The effect of our species' extract on DPPH is measured by the procedure described by Benhammou et al. [110].

b. Experimental process:

To 2.5ml of sample we add 0.5ml of a 0.2mMol DPPH solution in test tubes using a concentration range. Each tube is vortexed and incubated for 30min at room temperature, protected from light. Absorbances are measured with a spectrophotometer against a blank.

c. Calcul of IC_{50}

IC_{50} or 50% inhibitory concentration (also known as EC_{50} for Efficient concentration 50), is the concentration of test sample required to reduce 50% of the DPPH radical. IC_{50}s are calculated graphically using plots of inhibition percentages as a function of different concentrations of the fractions tested.

1.2- ABTS antioxidant test

a. Method principle :

This method measures the ability of antioxidants to scavenge the radical ABTS. This radical has a blue-green color (Figure 36) and is produced by reacting potassium persulfate or potassium permanganate (powerful oxidizing agents) with the ABTS salt [111].

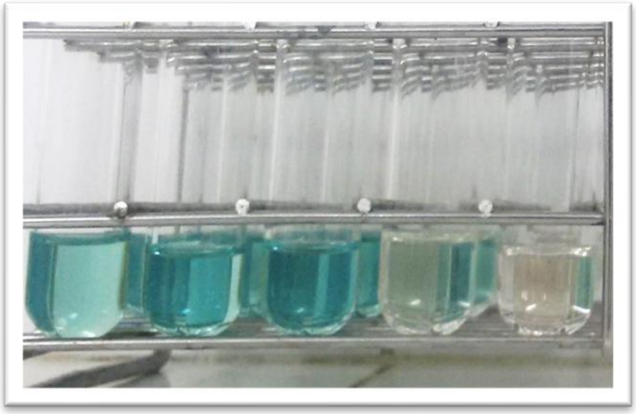


Figure 36: ABTS experimental test

b. Experimental process:

An aqueous solution of ABTS (7mM) and potassium persulfate (2.45mM) were reacted to generate the ABTS radicals cations ($ABTS^+$). The mixture was stirred for 12–16 h in the dark at room temperature, then was diluted with methanol to an absorbance of 0.700 (±0.02) at 734 nm. In test tubes, 2ml of ABTS solution are added with 200µl of sample at different concentrations. After 30min of incubation, the absorbance is measured at 734nm.

c. Calculation of IC_{50}:

The percentage inhibition of absorbance at 734 nm is calculated and plotted as a function of antioxidant. Trolox were used for the standard reference data.

$$\%\ \text{Inhibition}_{(\text{ABTS})} = \left(\frac{\text{Abs}_{\text{negative control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{negative control}}}\right) \times 100$$

2- Evaluation of the reducing capacity of extracts using the FRAP method:

a. Method principle:

The process of determining the reducing power of a substance or solution involves a redox reaction between the reducing agent(s) in the medium and the reagent $K_3Fe(CN)_6$ (Potassium hexacyanoferrate III or Potassium ferricyanide) [112].

b. Experimental process :

Assessment of the reducing power of samples is based on the ability to reduce ferric ions (Fe^{3+}) from the potassium ferrocyanide complex $K_3Fe(CN)_6$ to ferric ions (Fe^{2+}). This reaction reveals a yellow (Fe^{3+}) to blue-green (Fe^{2+}) coloration, measured at 700nm. About 0.5ml of sample at various concentrations is mixed with 1.25ml 0.2M phosphate buffer (PH 6.6) and 1.25ml of 1% potassium ferricyanide $K_3Fe(CN)_6$.

- ✓ Incubation (water bath) at 50°C for 20 min, then cooling.
- ✓ 2.5ml 10% trichloroacetic acid is added to stop the reaction, followed by centrifugation at 3000 rpm for 10 min.
- ✓ 1.25ml of the supernatant is added to 1.25ml ED and 250ul of a freshly prepared 0.1% ferric chloride solution.
- ✓ Read against a blank at 700nm.

The blank: replacing the extract with distilled water to calibrate the instrument.

c. EC_{50} value calculation

The change in absorbance is directly related to the combined or "total" reducing power of the antioxidants (electron donating) present in the reaction mixture (Figure 37). EC_{50} is defined as the concentration of a sample needed to reduce 50% of Fe^{3+}.

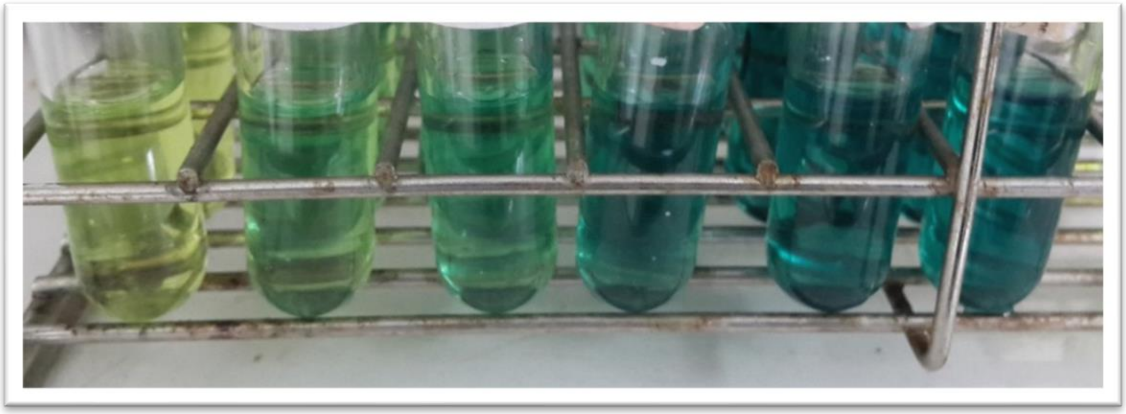


Figure 37: FRAP experimental test

D- Statistical Analysis

1- Data Analysis

The data collected from the experimental extraction is analyzed using the IBM SPSS software Statistics 23 and XLSTAT 2014 software. The product model is examined using three-factor multivariate variance analysis, followed so significantly by a post-Tukey test. Differences are considered to be significant if $p<0,001$.

2- Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA)

a. For oil analysis

The purpose of principle component analysis in this study is to establish the existence of a correlation between total sterol, total tocopherol, Chlorophyll, Carotenoid, Linoleic (C18:2), and linolenic (C18:2) acids in various Palm dates seeds varieties (10 samples), then between total sterol, total tocopherol, chlorophyll, carotenoid, linoleic (C18:2), and linolenic (C18:3). The results of Physicochemical parameters quality (Acid Value, Saponification Value, K270, K232 and Iodine Value, Peroxide Value) and total sterol, total tocopherol, chlorophyll, carotenoid, Linoleic (C18:2) and linolenic (C18:3) acid, which represented 12 variables, as well as those of the 10 samples oils from Palm dates, were subjected to principal component analysis. HCA was used to pursue the interrelatedness between all samples oils as a cluster characteristic, moreover, the dendrogram was shaped using the Ward cluster technique, and the squared Euclidean distance was considered as a coefficient of similarity.

b. For extract analysis

The purpose of PCA in this study is to show that there is a link between phytochemical compounds (TPC, TFC, and TTC) and antioxidant activity (DPPH, ABTS, and FRAP). Furthermore, this approach allows us to investigate the underlying factors that contribute to the difference in phytochemical component quality based on ten date seed samples. HCA was used to investigate the interrelatedness of all samples using a cluster characteristic. The Ward cluster technique dendrogram and the squared Euclidean distance were also used to calculate a similarity coefficient.

c. For roasting parameters

PCA was used to construct correlations between the responses (TPC, TFC, TTC, and DPPH ($1/IC_{50}$)) and the 11 extracts. This technique can be used to represent the variation of responses from 11 extracts according to the nature of roasting factors in graphs [113]. The software used in this study were JMP 11(SW) (CDD approach), XLSTAT 2014 (PCA), and IBM SPSS Statistics 21.

They were applied to express the data in means ±standard error of the mean and for the signification of data (Tukey test at p-value= 0.05) [114].

HCA was used to analyze the interconnectedness of all extracts into clusters based on determined bioactive component properties. Furthermore, the XLSTAT 2014 program generates dendrograms to depict the commonalities among the examined extracts. Square Euclidean measurements were used to calculate similarities between extracts. The gaps between clusters are calculated using an analysis of variance approach.

3- Correlation Matrix

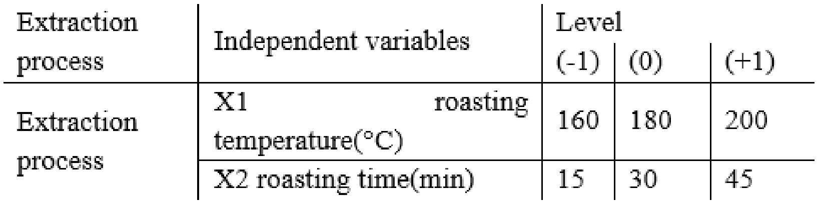
The PCA was carried out on a matrix that summarized the experimental result of antioxidant compounds and antioxidant properties. Ten date seed samples are used to represent each individual.

4- Experimental design for roasting parameters

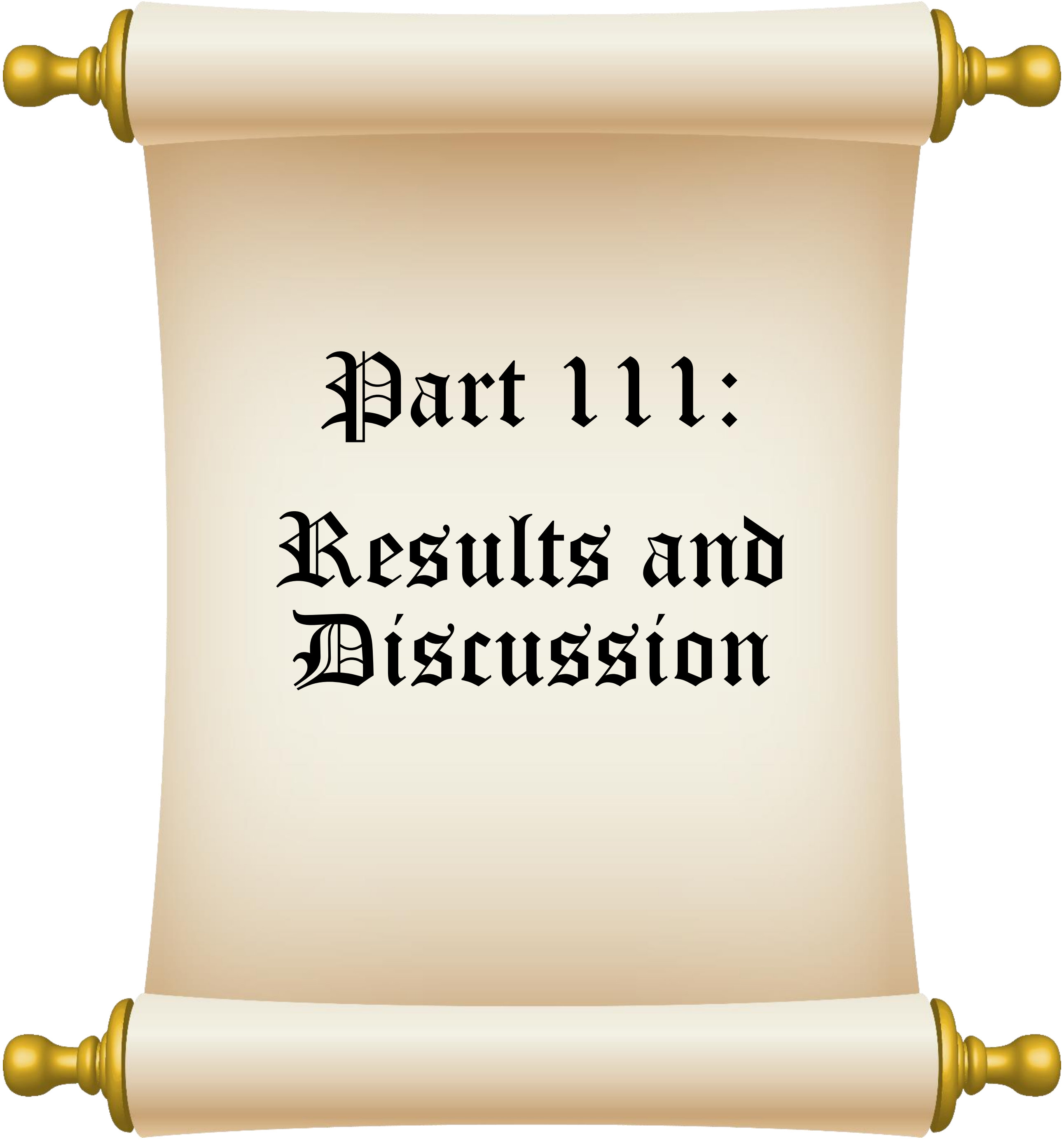
In this study, the CDD technique was employed to optimize the influence of roasting variables on the responses (TPC, TFC, TTC, and DPPH (IC_{50})) of the ' 'model's design (Y) employed in this work. This approach consists of eleven experimental assays (Table 9). Table 9 shows three levels (-1 .0. +1) of the independent variables (roasting temperature and roasting time). Fitting the experimental data to a second-order polynomial model yielded the regression coefficients (β), which were then used to construct the response analysis surface shown below.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

Table 9: Levels of variables of the roasting condition by CDD.



Extraction process	Independent variables	Level		
		(-1)	(0)	(+1)
Extraction process	X1 roasting temperature(°C)	160	180	200
	X2 roasting time(min)	15	30	45



Part 111:

Results and Discussion

Chapter 1 : Comparative study of the physico-chemical parameters and chemical composition of date seed oil from ten different varieties

The purpose of this chapter is to determine the chemical composition of the oil of ten varieties of date pits as well as to evaluate its quality using the qualitative analytical techniques indicated by the AOCS's official methodologies and recommended practices. Simultaneously to investigate the impact of date seed variety on these qualitative parameters, on extraction yields, and on the chemical composition of pigments, fatty acids, sterols, and tocols.

A- Evaluation of physicochemical parameters of date seed oil

I- Extraction yield

The extraction yield of date seed oil of different varieties studied is done by the soxhlet method using an apolar solvent, which in our case is n-hexane; the results are grouped in Figure 38. The extraction yield ranges from 2.94% to 8.06%, with Lkenz variety having the greatest yield of 8.06%, followed by Bousthammi at 4.97%, and Ablouh having the lowest yield of 2.94%. According to Ben-Youssef et al. and Jadhav et al. they found similar extraction yields to our results, which are respectively around 4.44% and 8.5% for the same experimental conditions.

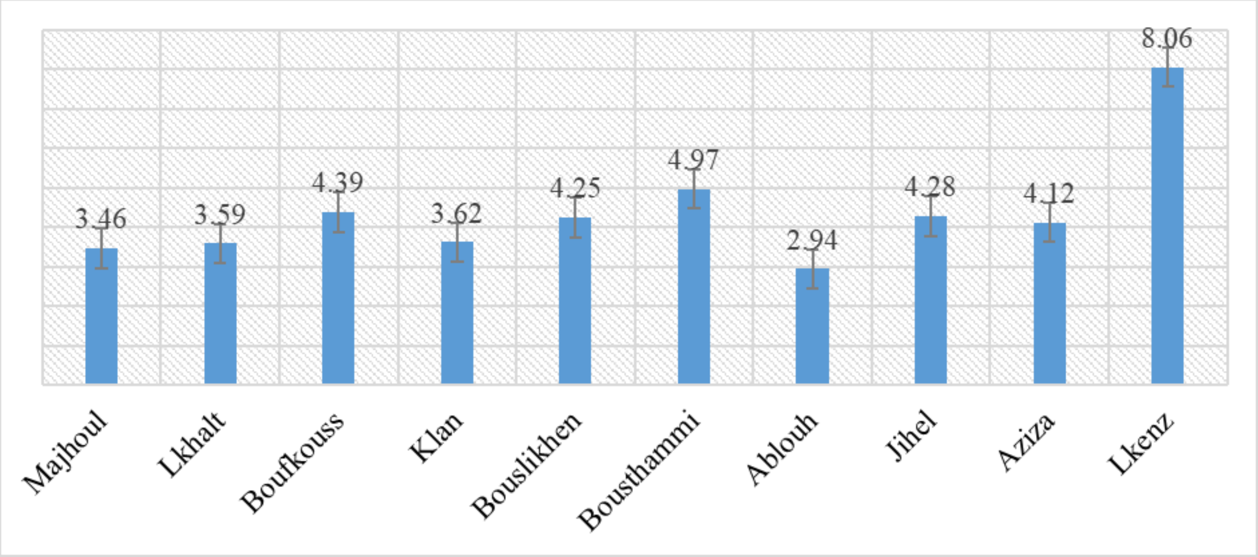


Figure 38: Extraction yield (%) of ten varieties of date seed oil

II- The influence of the variety of date seeds on the physicochemical parameters

1- Determination of the extinction coefficients of the primary and secondary oxidation products : K232 et K270

In order to study the presence of oxidation products, we used two coefficients K232 and K270 known as "specific UV extinction coefficients". They are also appropriate for assessing a vegetable oil's quality and preservation procedure.

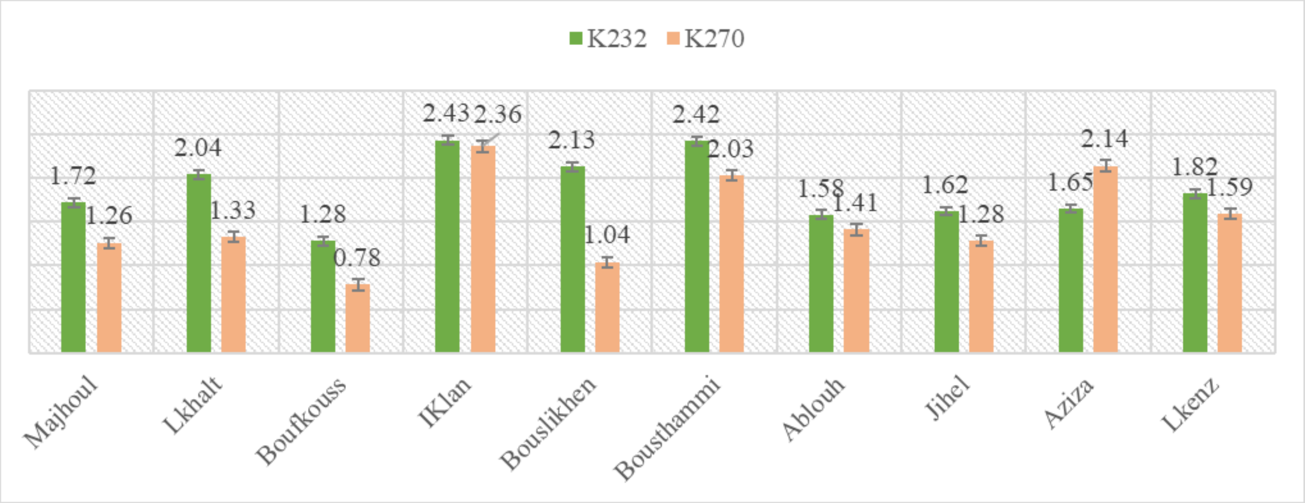


Figure 39: Extinction coefficients for primary and secondary oxidation products (K232 and K270)

The coefficient K232 is related to the presence of Primary Oxidation Products ie hydroperoxides conjugated, the experimental values of date seed oil range from 1.28 to 2.43. Based on the results of Figure 39, we can see that six varieties (Boufkouss, Ablouh, Jihel, Aziza, Majhoul and Lkenz) have a coefficient below 2. On the other hand, the four remaining varieties (Lkhalt, Bouslikhen, Bousthammi and Iklan) have recorded a value greater than 2. It can be concluded that the six varieties have a lower content of primary oxidation products than the four remaining varieties. In addition, the K232 coefficient of all varieties remains lower than that set by the CODEX standard for extra virgin and virgin olive oil (2.5 and 2.6 respectively).

On the other hand, the K270 coefficient is connected to the existence of free fatty acids, ketones and aldehydes known as secondary oxidation products. The experimental value of the coefficient K270 varies from 0.78 to 2.14. There are eight varieties that have the K270 coefficient lower than 2 of which the Boufkouss variety is lower than 1, and the two remaining varieties have a coefficient higher than 2. Thus, all the experimental values of the K270 coefficient are lower than the established limit (≤ 2.50). Therefore, not all varieties recorded a high content of secondary oxidation products.

From the analysis of the two specific UV extinction coefficients K232 and K270, it can be seen that the experimental values are below the established limit. Therefore, we conclude that the oil samples analyzed are weakly oxidized.

2- Determination of date seed oil acidity degree

The degree of acidity of the oils tested was analyzed via the determination of the acid index and the saponification number.

a. FFA determination

The FFA has an effective impact on the quality of the oil, especially on the organoleptic aspect. The analysis of this index exhibits identical values that are above 2% for all varieties studied, ranging from 2.11 to 2.54% (Figure 40). Except for the two varieties Jihel and Lkenz, which have values of 1.52 and 1.10% respectively, they are less than 2%. These findings are congruent with those of Besbes et al.[115], Nehdi et al.[67], and Devshony et al.[116].

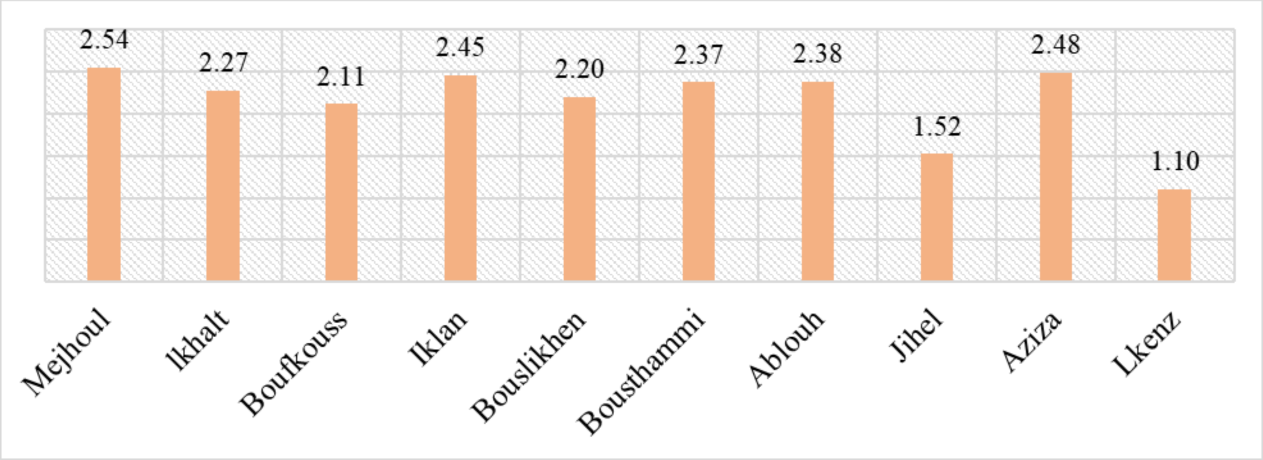


Figure 40: FFA (%)

Based on the results, it can be deduced that all samples contain a small amount of free fatty acids. This indicates that date kernel oil is suitable for consumption and has a longer shelf life compared to many other edible oils.

b. Determination of saponification index

The Saponification Index (SI) refers to the mass of potassium hydroxide (KOH) in mg that neutralizes all free fatty acids and saponifies the esters present in one gram of oil.

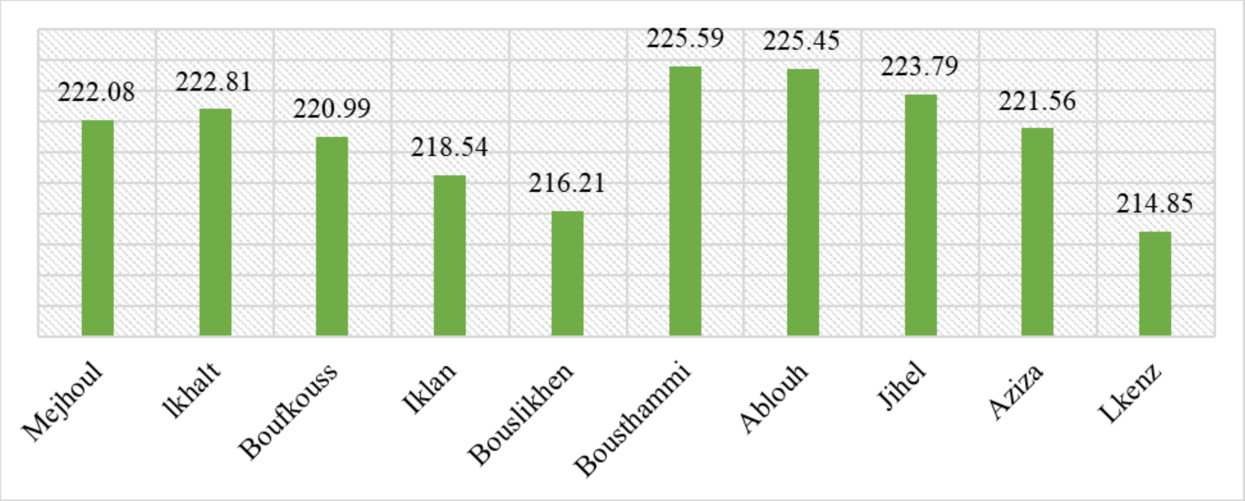


Figure 41: Saponification Index (mg KOH/g oil)

The lowest and highest experimental records of this index were 214.85 mg KOH/g oil for the Lkenz variety and 225.59 mg KOH/g oil for the Bousthammi variety (Figure 41). A high SI indicates a high number of carbon atoms in the fatty acids present in the oil, which means that after hydrogenation, date seed oil can be a substitute for conventional oil. According to Bart et al.(Bart et al., 2010) the SI of coconut oil was 242-263 mg KOH/g oil, for babassu 241-253 mg KOH/g oil, palm kernel 240-257 mg KOH/g oil, and palm 200-205 mg KOH/g oil.

3- Determination of date seed oil’s degree of unsaturation

The degree of unsaturation of our oils was evaluated by determining the oxygen content (peroxide value) and the number of grams of iodine (iodine value).

a. Determination of peroxide index

The peroxide index is a measure of the amount of active oxygen (epoxide or hydroperoxide) in the organic chains contained in the oil. The experimental contents of our samples are between 3.96 and 6.33 meq O_2/kg (Figure 42).

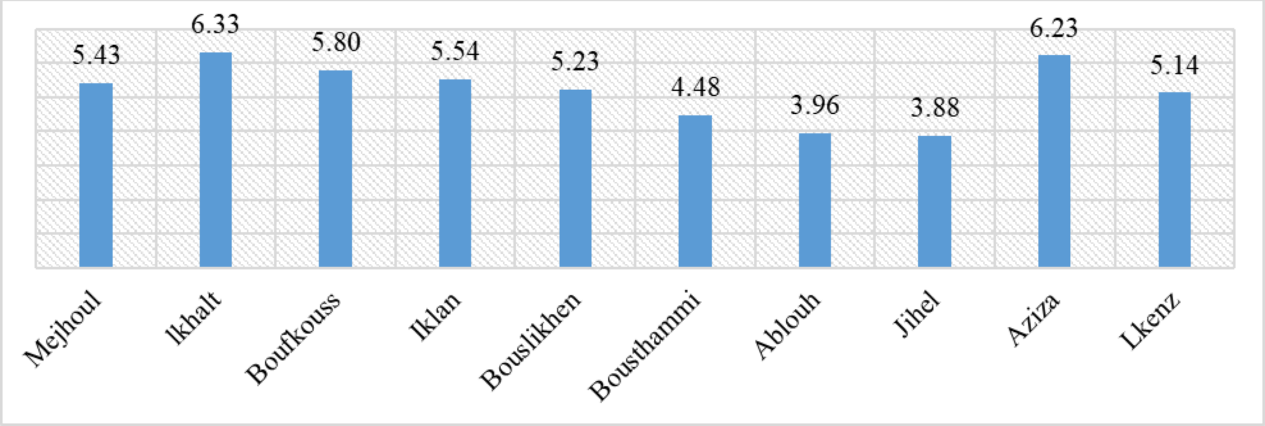


Figure 42: Peroxide index(mg O_2/kg oil)

For instance, Besbes et al.[117] found higher contents than our results, in the order of 16 and 25 mg O_2/100g for the Tunisian varieties of Deglet Nour and Allig, respectively. On the other hand, Abdalla et al.[118], Nehdi et al.[67], and Al Juhaimi[63] et al. obtained peroxide contents that seem lower than our measured contents, ranging from 4.8 to 7.4 mg O_2/kg, 3.26 to 5.62 mg O_2/kg, and 1.14 to 3.44 mg O_2/kg, respectively. Probably, these variations are due to various factors, such as the storage or packaging process, exposure to light, the degree of unsaturated fatty acids present in the oil, or the content of metals or bioactive compounds that protect against oxidation. The determined peroxide index is less than 30 mg O_2/kg, indicating that the date seed oil is fresh. Furthermore, it has a low risk of autoxidation and hence can be edible.

b. Determination of iodine index

The iodine value is a measure of the degree of unsaturation of fats and oils, it is expressed in number of grams of iodine per 100g of sample. The content of date seed oil samples is between 48.84 and 60.59 g I_2/100g oil, for the variety of Bousthammi and Bouslikhen respectively (Figure 43).

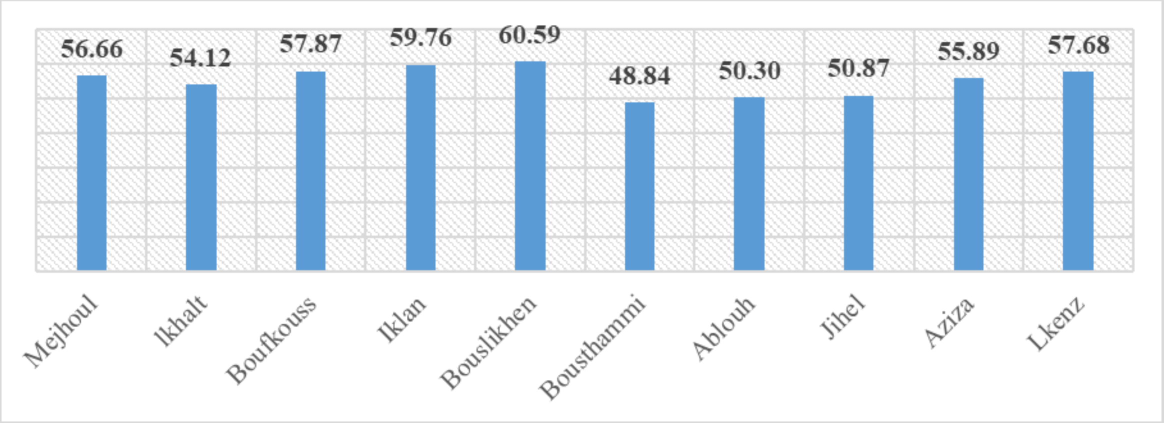


Figure 43: Iodine index (g I_2/100 g oil)

As an example from the literature, the iodine value of conventional oils is 34-40 g I_2/100 g oil for cocoa butter, 50-55 g I_2/100 g oil for palm oil, 100-115 g I_2/100 g oil for cottonseed, 118-141 g I_2/100 g oil for sunflower seed, and 48-60 g I_2/100 g oil for date seed. Thus, the measured contents of date kernel oil indicate that this oil can be classified as a highly unsaturated and non-drying oil.

B- Evaluation of the chemical composition of date seed oil

I- The influence of the variety of date seeds on oil pigment content :

The two main pigments that have been analyzed are chlorophylls and carotenoids. The results are grouped in Figure 44 and the contents are expressed in mg/kg of oil.

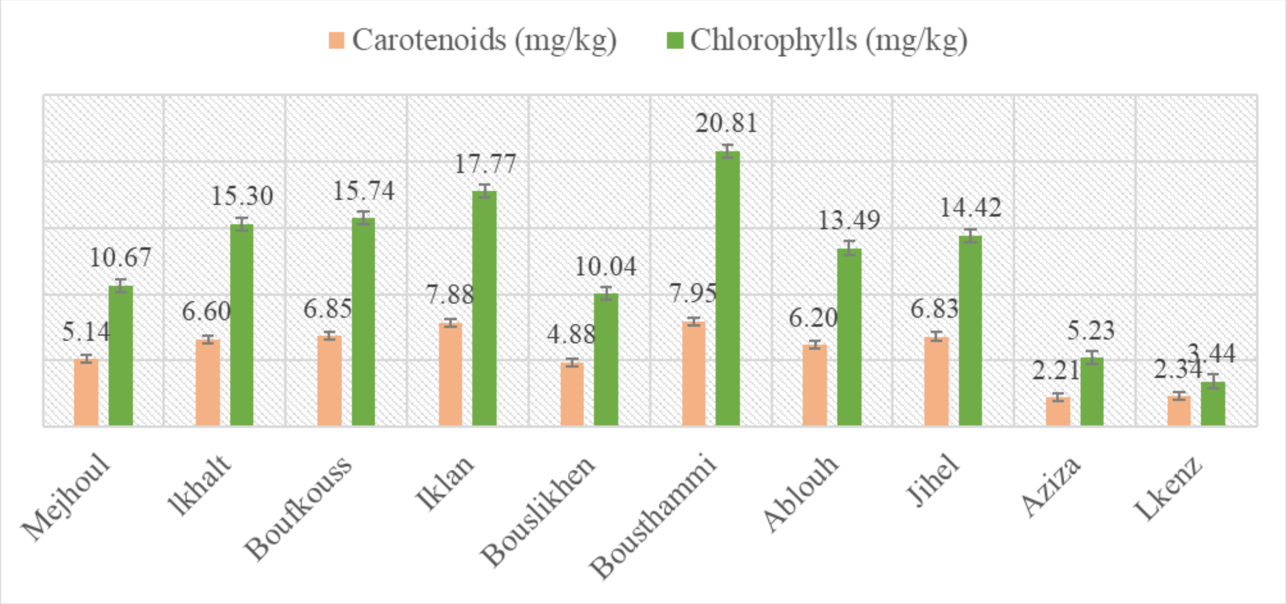


Figure 44: Chlorophyll and carotenoid content

1- Determination of chlorophylls content:

The results of chlorophyll content are expressed in Figure 44. The highest chlorophyll content was for the variety of Bousthammi and Iklan with 20.81 mg/kg and 17.77 mg/kg. Then the varieties Boufkouss, Lkhalt, Jihel, Ablouh had a similar content of 15.74 15.30 14.42 and 13.49 mg/kg. Then the variety Mejhoul and Bouslikhen which had a content around 10 mg/kg (10.67 and 10.04 mg/kg respectively). Finally the lowest content (<10 mg/kg) was recorded for both varieties of Aziza and Lkenz with 5.23 and 3.44 mg/kg.

2- Determination of carotenoid content:

According to the results grouped in Figure 44, the ranking of carotenoid and chlorophyll contents of date seed oil varieties was approximately similar. Indeed, Bousthammi and Iklan have a high content of 7.95 and 7.88 mg/kg, then a content of 6.85 6.83 6.60 and 6.20 mg/kg respectively for Boufkouss, Jihel, Lkhalt, Ablouh. At the bottom of the ranking we find the variety of Lkenz and Aziza with 2.34 and 2.21 mg/kg.

II- The influence of the variety of date seeds on the chemical composition of oil :

In this part we analyzed the chemical composition in fatty acids, sterols and tocols of date kernel oil of all the varieties studied. Then, an interpretation of the results and their comparison with the data of the literature.

1- Date seed oil’s chemical composition of fatty acids

The fatty acids composition of ten studied date seeds varieties is shown in Table 10. Overall, 14 fatty acids were detected in all date seed oil samples. Eight of them were saturated fatty acids, called, caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), margaric (C17:0), stearic (C18:0), and arachidic (C20:0) acids. Then the four monounsaturated were palmitoleic (C16:1), margaroleic (C17:1), oleic (C18:1), and gadoleic (C20:1) acids. While the two left were polyunsaturated fatty acids, named, Linoleic (C18:2) and linolenic (C18:3) acids. The results of the fatty acid analysis will be interpreted separately according to their degree of unsaturation.

Table 10: Fatty acid composition (%) of DSO

Fatty Acides	Mejhoul	Ikhalt	Boufkouss	Iklan	Bouslikhen	Bousthammi	Ablouh	Jihel	Aziza	Lkenz
Saturated fatty acid										
C8	0.35±0.05^{a}	0.23±0.03^{b}	0.24±0.02^{b}	0.13±0.02^{c}	0.02±0.01^{d}	0.27±0.05^{b}	0.22±0.02^{b}	0.35±0.07^{a}	0.34±0.03^{a}	0.28±0.04^{b}
C10	0.40±0.05^{a}	0.35±0.03^{b}	0.31±0.04^{b}	0.20±0.02^{c}	0.3±0.06^{b}	0.41±0.05^{a}	0.44±0.07^{a}	0.45±0.03^{a}	0.43±0.03^{a}	0.32±0.09^{b}
C12	19.39±0.61^{a}	19.90±0.83^{a}	18.88±0.68^{a}	15.90±0.25^{b}	15.62±0.17^{b}	23.00±0.13^{c}	22.80±0.36^{c}	20.76±0.51^{a}	19.51±0.71^{a}	17.69±0.93^{d}
C14	10.49±0.15^{a}	11.34±0.09^{b}	10.14±0.20^{c}	10.47±0.11^{a}	8.63±0.17^{d}	12.70±0.09^{e}	12.31±0.20^{e}	11.20±0.18^{b}	9.93±0.13^{c}	9.95±0.23^{c}
C16	9.87±0.17^{a}	10.95±0.13^{b}	9.64±0.25^{a}	10.33±0.16^{c}	10.47±0.21^{c}	10.19±0.27^{c}	10.15±0.20^{c}	10.73±±0.23^{b}	10.49±0.22^{c}	9.37±0.28^{a}
C17	0.07±0.02^{a}	0.08±0.01^{b}	0.06±0.02^{c}	0.07±0.02^{a}	0.07±0.01^{a}	0.05±0.01^{c}	0.06±0.01^{c}	0.07±0.02^{a}	0.09±0.01^{b}	0.05±0.01^{c}
C18	4.09±0.21^{a}	3.67±0.18^{b}	3.59±0.13^{b}	3.30±0.15^{c}	3.33±0.1^{c}	3.72±0.19^{b}	3.64±0.14^{b}	4.21±0.11^{a}	3.88±0.15^{b}	3.80±0.10^{b}
C20	0.47±0.05^{a}	0.37±0.03^{b}	0.52±0.09^{ac}	0.41±0.07^{b}	0.54±0.05^{c}	0.54±0.06^{c}	0.46±0.03^{a}	0.64±0.08^{d}	0.47±0.05^{a}	0.40±0.03^{b}
Total of SFA	45.13±1.31^{a}	46.89±1.33^{b}	43.38±1.43^{c}	40.81±0.8^{d}	38.71±0.78^{e}	50.83±0.85^{f}	50.08±1.03^{f}	48.41±1.23^{b}	45.14±1.33^{a}	41.86±1.71^{d}
Monounsaturated fatty acid										
C16:1	0.16±0.03^{a}	0.16±0.02^{a}	0.11±0.02^{b}	0.12±0.01^{b}	0.12±0.04^{b}	0.09±0.01^{c}	0.09±0.01^{c}	0.10±0.02^{bc}	0.09±0.02^{c}	0.07±0.01^{d}
C17:1	0.03±0.01^{a}	0.03±0.01^{a}	0.04±0.01^{b}	0.04±0.01^{b}	0.04±0.01^{b}	0.03±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}	0.05±0.01^{c}	0.03±0.01^{a}
C18:1	45.45±0.33^{a}	44.83±0.57^{b}	47.23±0.42^{c}	50.27±0.45^{d}	52.60±0.38^{e}	42.13±0.25^{f}	42.43±0.31^{f}	45.23±0.29^{a}	45.58±0.21^{a}	45.70±0.50^{a}
C20:1	0.28±0.05^{a}	0.28±0.03^{a}	0.45±0.15^{b}	0.44±0.17^{b}	0.49±0.11^{b}	0.43±0.09^{b}	0.39±0.08^{b}	0.42±0.14^{b}	0.44±0.11^{b}	0.28±0.28^{a}
Total of MUFA	45.92±0.42^{a}	45.30±0.63^{b}	47.83±0.6^{c}	50.87±0.64^{d}	53.25±0.54^{e}	42.68±0.36^{f}	42.94±0.41^{f}	45.78±0.46^{a}	46.16±0.35^{a}	46.08±0.8^{a}
Polyunsaturated fatty acid										
C18:2	8.87±0.06^{a}	7.61±0.10^{b}	8.59±0.08^{c}	8.12±0.08^{d}	7.45±0.14^{e}	6.18±0.13^{f}	6.84±0.11^{g}	5.76±0.09^{h}	8.33±0.06^{d}	9.27±0.15^{i}
C18:3	0.02±0.01^{a}	0.11±0.02^{b}	0.07±0.02^{cdf}	0.08±0.02^{dc}	0.07±0.02^{cdf}	0.03±0.01^{ae}	0.03±0.01^{ae}	0.04±0.01^{e}	0.06±0.01^{ef}	0.06±0.01^{ef}
Total of PUFA	8.87±0.07^{a}	7.72±0.12^{b}	8.66±0.10^{a}	8.20±0.10^{c}	7.52±0.16^{b}	6.21±0.14^{d}	6.87±0.12^{e}	5.80±0.10^{f}	8.39±0.07^{g}	9.33±0.16^{h}

**SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid*

a. Analysis of saturated fatty acids (SFA)

The total of SFA is between 38.71% and 50.83%. The oils samples of Bousthammi and Ablouh variety have a total ≥ 50% of SFA, which means that they are rich in SFA. On the other hand, a total ≥ 45% of SFA is attributed to four varieties including Jihel, Lkhalt, Aziza, Mejhoul. And finally, the last four varieties (Boufkouss, Lkenz, Iklan, Bouslikhen) have a total ≤ 42% SFA with the lowest percentage being 38.71% for Bouslikhen which indicates that they possess a low SFA content. Among the SFA, the predominant is lauric acid (C12:0) for all varieties including particularly the variety of Bousthammi with a rate of 23%. Followed by palmitic acid (C14:0) and myristic acid (C16:0) with the exception of the variety Bouslikhen and Aziza whose percentage of myristic acid is higher than palmitic.

b. Analysis of monounsaturated fatty acids (MUFA)

The total of MUFA is higher than the total of SFA except for the variety Bousthammi, Ablouh, Jihel and lkhalt of which they reverse. The varieties Bouslikhen and Iklan have a total ≥ 50% of MUFA, followed by six varieties with a total of MUFA between 50% > total ≥ 45%, then Bousthammi and Ablouh possessing the lowest MUFA content with 42.68% and 42.94%, respectively. The analysis of MUFA reveals that oleic acid (C18:1) is very abundant with a rate higher than 40% for all varieties while gadoleic (C20:1), palmitoleic (C16:1), and margaroleic (C17:1) acids do not exceed 0.5%.

c. Analysis of polyinsaturated fatty acids (PUFA)

The PUFAs of the samples have a total ranging from 5.80 to 9.33% which is much lower than the total of SFAs and MUFAs. In addition, it is noted that there is no significant difference between the varieties (a difference of 3.5%). In addition, in this group there are only two fatty acids, linoleic acid (C18:2) which is in the majority, ranging from 5.76% to 9.27%, and linolenic acid (C18:3) which is between 0.03% and 0.11%.

d. Analysis and interpretation of the set

According to the analysis of the total of the three types of fatty acids, it can be seen that the oil of Bouslikhen seeds contains the highest content of unsaturated fatty acids with a total of MUFA+PUFA= 60.77%. On the other hand, the most abundant oil in SFA was Bousthammi with 50.83%. Conversely, the oil of Bouslikhen has the lowest content of SFA with 38.71% while the oil of Bousthammi has only 48.89% of unsaturated fatty acids (MUFA + PUFA). Among the fatty acids identified, oleic acid (C18:1), lauric acid (C12), palmitic acid (C14:0) and myristic acid (C16:0) occupy more than 80% of the total fatty acid constitution of the oil. According to the literature, when oleic acid and lauric acid are predominant, the oil analyzed is therefore considered as an oleic-lauric type. As an example, date seed oil in Iran is also an oleic-lauric type[120]. That of Oman is an oleic-myristic type[121]. On the other hand, that of the United Arab Emirates can be an oleic-linolenic type or an oleic-linolenic type[122].

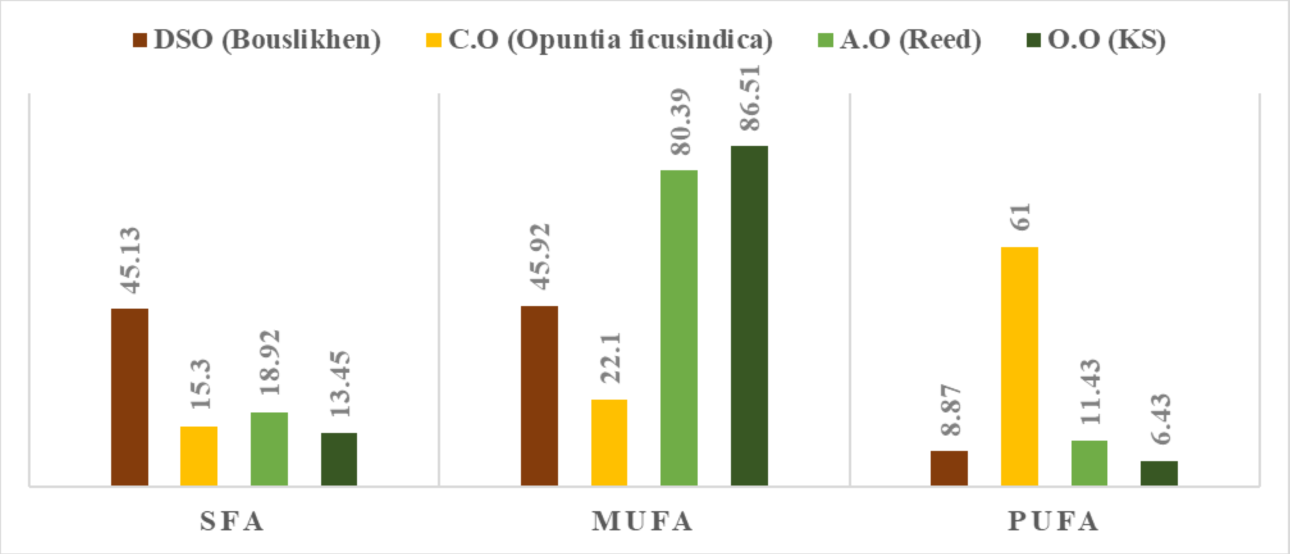


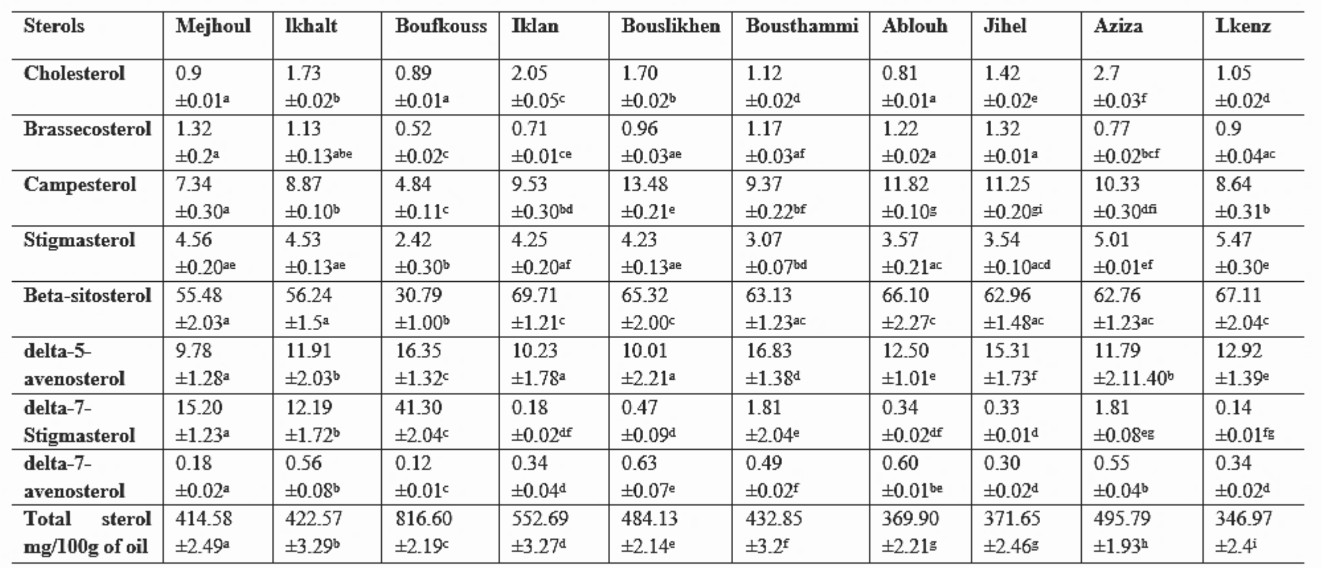
Figure 45: a comparison of SFA,MUFA and PUFA of DSO with conventional oil

If we compare DSO with other conventional oil (Figure 45), DSO contains more than twice the amount of SFA (45.13%), have approximately the same amount of PUFA content as olive oil and avocado oil, while Cactus oil is six times higher by 61%. However, DSO has an average amount of MUFA content of 45.92% whereas olive and avocado oil have 86.51% and 80.39%, respectively.

2- Sterol composition of date seed oil

Table 11 summarizes the sterol composition of ten date seed oil varieties. A total of eight sterols were identified in all varieties. Among them are cholesterol, Brassecosterol, Campesterol, Stigmasterol, Beta-sitosterol, Delta-5-avenosterol, Delta-7-stigmasterol and Delta-7-avenosterol.

Table 11: Sterolic composition (%) of ten varieties of DSO



Sterols	**Mejhoul**	**Ikhalt**	**Boufkouss**	**Iklan**	**Bouslikhen**	**Bousthammi**	**Ablouh**	**Jihel**	**Aziza**	**Lkenz**
Cholesterol	0.9 $\pm0.01^{a}$	1.73 $\pm0.02^{b}$	0.89 $\pm0.01^{a}$	2.05 $\pm0.05^{c}$	1.70 $\pm0.02^{b}$	1.12 $\pm0.02^{d}$	0.81 $\pm0.01^{a}$	1.42 $\pm0.02^{e}$	2.7 $\pm0.03^{f}$	1.05 $\pm0.02^{d}$
Brassecosterol	1.32 $\pm0.2^{a}$	1.13 $\pm0.13^{abe}$	0.52 $\pm0.02^{c}$	0.71 $\pm0.01^{ce}$	0.96 $\pm0.03^{ae}$	1.17 $\pm0.03^{af}$	1.22 $\pm0.02^{a}$	1.32 $\pm0.01^{a}$	0.77 $\pm0.02^{bcf}$	0.9 $\pm0.04^{ac}$
Campesterol	7.34 $\pm0.30^{a}$	8.87 $\pm0.10^{b}$	4.84 $\pm0.11^{c}$	9.53 $\pm0.30^{bd}$	13.48 $\pm0.21^{e}$	9.37 $\pm0.22^{bf}$	11.82 $\pm0.10^{g}$	11.25 $\pm0.20^{gi}$	10.33 $\pm0.30^{dfi}$	8.64 $\pm0.31^{b}$
Stigmasterol	4.56 $\pm0.20^{ae}$	4.53 $\pm0.13^{ae}$	2.42 $\pm0.30^{b}$	4.25 $\pm0.20^{af}$	4.23 $\pm0.13^{ae}$	3.07 $\pm0.07^{bd}$	3.57 $\pm0.21^{ac}$	3.54 $\pm0.10^{acd}$	5.01 $\pm0.01^{ef}$	5.47 $\pm0.30^{e}$
Beta-sitosterol	55.48 $\pm2.03^{a}$	56.24 $\pm1.5^{a}$	30.79 $\pm1.00^{b}$	69.71 $\pm1.21^{c}$	65.32 $\pm2.00^{c}$	63.13 $\pm1.23^{ac}$	66.10 $\pm2.27^{c}$	62.96 $\pm1.48^{ac}$	62.76 $\pm1.23^{ac}$	67.11 $\pm2.04^{c}$
delta-5-avenosterol	9.78 $\pm1.28^{a}$	11.91 $\pm2.03^{b}$	16.35 $\pm1.32^{c}$	10.23 $\pm1.78^{a}$	10.01 $\pm2.21^{a}$	16.83 $\pm1.38^{d}$	12.50 $\pm1.01^{e}$	15.31 $\pm1.73^{f}$	11.79 $\pm2.11.40^{b}$	12.92 $\pm1.39^{e}$
delta-7-Stigmasterol	15.20 $\pm1.23^{a}$	12.19 $\pm1.72^{b}$	41.30 $\pm2.04^{c}$	0.18 $\pm0.02^{df}$	0.47 $\pm0.09^{d}$	1.81 $\pm2.04^{e}$	0.34 $\pm0.02^{df}$	0.33 $\pm0.01^{d}$	1.81 $\pm0.08^{eg}$	0.14 $\pm0.01^{fg}$
delta-7-avenosterol	0.18 $\pm0.02^{a}$	0.56 $\pm0.08^{b}$	0.12 $\pm0.01^{c}$	0.34 $\pm0.04^{d}$	0.63 $\pm0.07^{e}$	0.49 $\pm0.02^{f}$	0.60 $\pm0.01^{be}$	0.30 $\pm0.02^{d}$	0.55 $\pm0.04^{b}$	0.34 $\pm0.02^{d}$
Total sterol mg/100g of oil	414.58 $\pm2.49^{a}$	422.57 $\pm3.29^{b}$	816.60 $\pm2.19^{c}$	552.69 $\pm3.27^{d}$	484.13 $\pm2.14^{e}$	432.85 $\pm3.2^{f}$	369.90 $\pm2.21^{g}$	371.65 $\pm2.46^{g}$	495.79 $\pm1.93^{h}$	346.97 $\pm2.4^{i}$

The results are expressed in the form of an average of 2 individual repetitions (n = 2e ± SEM). The mean followed by similar letters exposing in the column are not different ($P < 0.05$).

a. Analysis of total sterol content

The first group has two varieties of date seed oil whose total content exceeds 500 mg/100g of oil, this is the case of the varieties of Iklan and Boufkouss with 552.69 and 816.60 mg/100g of oil. Then, the total content of the second group is between 400 and 500 mg/100g oil, comprising five varieties such as Mejhoul (414.58 mg/100g oil), lkhalt (422.57 mg/100g oil), Bousthammi (432.85 mg/100g oil), Bouslikhen (484.13 mg/100g oil) and Aziza (495.79 mg/100g oil). Then, the last group contains three varieties with a total of less than 400mg/100g of oil including the variety of Lkenz with 346.97 mg/100g of oil, Ablouh with 369.9 mg/100g of oil, and Jihel with 371.65 mg/100g.

By comparing our total contents with those of the literature, we identify a similarity with the neighboring species, which is the seed oil of Phoenix Canariensis with a content of 336 mg/100g oil[63], then with Tunisian date seed oil, which has a content of 350 mg/100g oil[56]. Finally, with two commonly used oils, such as rapeseed oil and soybean oil, which have 500 mg/100g oil and 900 mg/100g oil[123].

b. Analysis of identified sterols

The oil extracted from various date seed varieties contains eight sterols, with the highest content being β-sitosterol, ranging from 30.79 to 69.71%, except for the variety of Boufkouss, which has Δ 7-stigmasterol as the predominant sterol. The second place is occupied by Δ5-avenasterol for the varieties of Iklan, Bouslikhen, Bousthammi, Ablouh, Jihel, Aziza and Lkenz. On the other hand, the Δ7-stigmasterol does not exceed 2% for these varieties while it is the second in the ranking for the varieties of Mejhoul and lkhalt. Following the ranking, we find campesterol, stigmasterol, brassecosterol, cholesterol and Δ7-avenasterol occupying the same positions for all varieties.

β-sitosterol is an important phytosterol in the human diet. It is synthesized by melonic acid in plants. It has a wide range of pharmaceutical activities, including antipyretic, antacid, angiogenic, antioxidant, immunomodulatory, antihyperlipidemic, anti-atherosclerotic, and cholesterol levels. It also affects the central nervous system, liver function, gastrointestinal tract, reproductive system which may be the basis of its use for the treatment of diseases or disorders of this system[124].

3- Tocols composition

The analysis of the tocols composition reveals that date seed oil contains six isomers of vitamin E. Among them are α-, β- and γ-tocopherol (abbreviated as AT, BT and GT) and α-, β- and γ-tocotrienol (denoted as AT3, BT3 and GT3) grouped in Table 12 with the total tocols content.

Table 12: Tocols composition (mg/kg) of DSO.

Tocol	AT	AT3	BT	BT3	GT	GT3	Total tocol mg/kg of oil
Mejhoul	279.38 ±1.00^{a}	33.99 ±1.00^{a}	65.83 ±1.00^{a}	37.82 ±1.00^{a}	14.26 ±1.05^{a}	46.56 ±1.00^{a}	3686.27 ±2.00^{a}
Ikhalt	309.19 ±0.10^{b}	1406.66 ±2.02^{b}	109.44 ±1.00^{a}	65.65 ±1.00^{a}	105.57 ±2.00^{b}	432.12 ±2.02^{b}	2650.29 ±1.10^{b}
Boufkouss	248.17 ±1.12^{c}	29.39 ±0.30^{a}	63.14 ±1.00^{a}	49.11 ±1.00^{a}	12.89 ±0.9^{a}	37.85 ±1.10^{a}	3844.06 ±1.13^{c}
Iklan	622.60 ±0.3^{d}	547.42 ±1.10^{c}	127.58 ±1.00^{a}	194.68 ±1.00^{a}	27.70 ±0.60^{c}	78.10 ±1.12^{c}	2539.31 ±1.20^{d}
Bouslikhen	553.10 ±1.70^{e}	737.99 ±1.00^{d}	113.23 ±1.00^{a}	74.06 ±1.00^{a}	167.25 ±2.10^{d}	335.67 ±2.11^{d}	2079.56 ±1.32^{e}
Bousthammi	561.81 ±1.14^{f}	596.88 ±1.99^{e}	124.70 ±1.00^{a}	204.35 ±1.00^{a}	154.13 ±1.00^{e}	456.05 ±2.05^{e}	2157.06 ±2.06^{f}
Ablouh	522.36 ±2.00^{g}	509.81 ±0.11^{f}	121.88 ±1.00^{a}	176.50 ±1.00^{a}	147.66 ±1.12^{e}	392.66 ±1.13^{f}	1937.14 ±1.04^{g}
Jihel	786.3 ±1.11^{k}	740.12 ±2.02^{d}	158.69 ±1.00^{a}	262.69 ±1.00^{a}	36.75 ±1.17^{f}	105.93 ±2.03^{g}	3165.97 ±2.02^{h}
Aziza	477.17 ±1.10^{l}	914.01 ±1.00^{g}	197.40 ±1.00^{a}	73.44 ±1.00^{a}	45.60 ±1.10^{g}	97.79 ±1.12^{g}	3486.64 ±2.04^{k}
Lkenz	1584.57 ±1.12^{m}	30.51 ±1.20^{a}	-	-	272.92 ±2.02^{h}	318.54 ±2.3^{h}	2275.63 ±0.97^{l}

The results are expressed in the form of an average of 2 individual repetitions (n = 2e ± SEM). The mean followed by similar letters exposing in the column are not different (P < 0.05).

a. Analysis of tocols content

Date seed oil contains a high amount of tocols. Indeed, four Date Seeds Oil (DSO) varieties (Boufkouss, Mejhoul, Aziza, and Jihel) had a total content exceeding 3000 mg/kg. Next, we find that half of the DSO varieties evaluated have a total between 2000mg/kg and 3000 mg/kg, including Lkhalt, Iklan, Lkenz, Bousthammi, and Bouslikhen. Finally, there is only one variety, named Ablouh, that has a content below 2000 mg/kg of oil.

b. Analysis of identified tocols

The predominant tocols was AT3 (1406.66-596.88 mg/kg oil) for Lkhalt, Bouslikhen, Bousthammi and Aziza, whereas AT (1584. 57 to 248.17 mg/kg oil) was the predominant tocols for Mejhoul, Boufkouss, Iklan, Ablouh, Jihel and Lkenz. The GT3 is the second most abundant isomer in half of the examined varieties (Lkhalt, Bouslikhen, Bousthammi, Ablouh, and Lkenz) ranging from 318.54 to 456.05 mg/kg oil, they had also the highest amount of GT compared to other varieties (147.66-272.92 mg/kg oil). However, two varieties had BT3, named Ilkan and Jihel, with 194.68 and

262.69 mg/kg oil, respectively. Finally, the BT was abundant for three varieties named Mejhoul, Boufkouss, and Aziza (63.14-197.40 mg/kg oil).

According to the results of Nehdi et al.[119] the Bahri variety had a similar value to our variety Lkenz, with GT 289.2 mg/kg oil and 272.92 mg/kg oil respectively. Then GT3 with 318.8 mg/kg oil and 318.54 mg/kg oil. Unlike the study of Al Juhaimi et al.[63] they recorded a lower value of GT with 82.6 and GT3 with 118.4 mg/kg oil. There is a significant difference in tocopherol and tocotrienol contents according to variety, maturity and location.

c. Interpretation of the set

Tocopherols are well known to have a strong antioxidant capacity, like vitamin E who offers a good protection of body against oxidation reactions or radicals that carry cholesterol and lipoproteins to membranes. It is also react as a screening reagent to prevent UV-induced skin damage and aging, suppress cancer cell growth, and act as a protective agent against cirrhosis by lowering cholesterol levels [67],[125].

C- Statistical analysis : Correlation Matrix, PCA and HCA

I- Correlation Matrix

Table 13 shows the correlation coefficients of the various physicochemical indicators of oil quality (acid value, Saponification Value, K232, K270, Iodine Value, Peroxide Value), total sterols, total tocols, chlorophyll, carotenoid, linoleic (C18:2) and linolenic (C18:3). In addition, Table 14 shows the correlation matrix coefficient p-values.

Table 13: Pearson's correlation matrix coefficient between different parameters used.

Variables	Total sterols	Total tocols	Carotenoids	Chlorophylls	Linoleic C18:2	Linolenic C18:3	IV	FFA%	PV	SV	K232	K270
Total sterols	1	0.508	0.251	0.273	0.304	0.322	0.439	0.259	0.456	-0.627	-0.264	-0.236
Total tocols	0.508	1	-0.116	-0.115	0.361	-0.130	0.195	0.130	0.426	-0.294	-0.598	-0.242
Carotenoids	0.251	-0.116	1	0.979	-0.565	0.034	-0.339	0.303	-0.305	-0.061	0.327	-0.024
Chlorophylls	0.273	-0.115	0.979	1	-0.579	0.036	-0.401	0.382	-0.245	-0.022	0.378	0.067
Linoleic C18:2	0.304	0.361	-0.565	-0.579	1	0.156	0.736	-0.046	0.681	-0.198	-0.224	-0.034
Linolenic C18:3	0.322	-0.130	0.034	0.036	0.156	1	0.402	-0.103	0.588	-0.279	0.211	0.023
IV	0.439	0.195	-0.339	-0.401	0.736	0.402	1	-0.018	0.610	-0.212	0.046	-0.103
FFA%	0.259	0.130	0.303	0.382	-0.046	-0.103	-0.018	1	0.300	0.520	0.215	0.242
PV	0.456	0.426	-0.305	-0.245	0.681	0.588	0.610	0.300	1	-0.066	0.017	0.067
SV	-0.627	-0.294	-0.061	-0.022	-0.198	-0.279	-0.212	0.520	-0.066	1	0.507	0.598
K232	-0.264	-0.598	0.327	0.378	-0.224	0.211	0.046	0.215	0.017	0.507	1	0.601
K270	-0.236	-0.242	-0.024	0.067	-0.034	0.023	-0.103	0.242	0.067	0.598	0.601	1

Table 14: p-values of the correlation matrix coefficient.

Variables	Total sterols	Total tocols	Carotenoids	Chlorophylls	Linoleic C18:2	Linolenic C18:3	IV	FFA%	PV	SV	K232	K270
Total sterols	0	0.134	0.485	0.446	0.392	0.364	0.204	0.470	0.185	0.052	0.461	0.512
Total tocols	0.134	0	0.750	0.752	0.305	0.721	0.589	0.720	0.220	0.410	0.068	0.500
Carotenoids	0.485	0.750	0	< 0.0001	0.089	0.925	0.339	0.394	0.392	0.868	0.356	0.947
Chlorophylls	0.446	0.752	< 0.0001	0	0.079	0.920	0.251	0.276	0.495	0.952	0.282	0.854
Linoleic C18:2	0.392	0.305	0.089	0.079	0	0.667	0.015	0.899	0.030	0.583	0.533	0.926
Linolenic C18:3	0.364	0.721	0.925	0.920	0.667	0	0.250	0.777	0.074	0.435	0.559	0.949
IV	0.204	0.589	0.339	0.251	0.015	0.250	0	0.961	0.061	0.557	0.900	0.777
FFA%	0.470	0.720	0.394	0.276	0.899	0.777	0.961	0	0.400	0.123	0.550	0.500
PV	0.185	0.220	0.392	0.495	0.030	0.074	0.061	0.400	0	0.856	0.964	0.855
SV	0.052	0.410	0.868	0.952	0.583	0.435	0.557	0.123	0.856	0	0.135	0.068
K232	0.461	0.068	0.356	0.282	0.533	0.559	0.900	0.550	0.964	0.135	0	0.066
K270	0.512	0.500	0.947	0.854	0.926	0.949	0.777	0.500	0.855	0.068	0.066	0

Table 13 shows the Pearson correlation, which was used to examine the connections between the various factors examined in this research. Table 14 also shows the p-values of the correlation matrix coefficients for all variables. As a result, we found a substantial positive connection (p-value < 0.0001) between chlorophyll and carotenoid ($r^2 = 0.979$). Furthermore, with $r^2 = 0.736$, there was a positive connection (p-value < 0.05) between linoleic (C18:2) and the Iodine Value. linoleic (C18:2) and peroxide Value ($r^2 = 0.681$) also had a strong positive connection (p-value < 0.05).

II- Principal Component Analysis (PCA)

Total sterol, total tocopherol, chlorophyll, carotenoid, linoleic acid (C18:2), linolenic acid (C18:3), and oil quality characteristics are all considered variables. The PCA uses the F1-F2 factorial design to project them (Figure 37). The first main component (F1) accounts for 31.92 percent of the total data, while the second main component (F2) accounts for 20.47 percent. The linear combination is representative of the variables since the cumulative proportion of the two initial main components is more than 50% (52.39 percent). In reality, the first two axes are adequate for representing the whole data set. The plane created by axes F1 and F2 in Figure 38 depicts the correlation between the variables. The positive connection between total tocopherol, chlorophyll, carotenoid, K270, and K232 forms the foundation of the F1 axis. The positive connection between linoleic (C18:2), peroxide, and Iodine Value forms Axis F2.

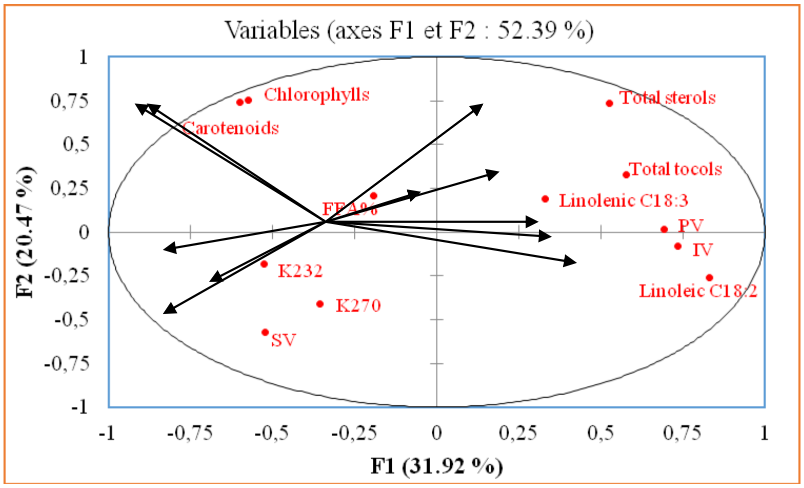


Figure 46: PCA factorial plan was used to analyze the values of several physicochemical properties of various DSO samples.

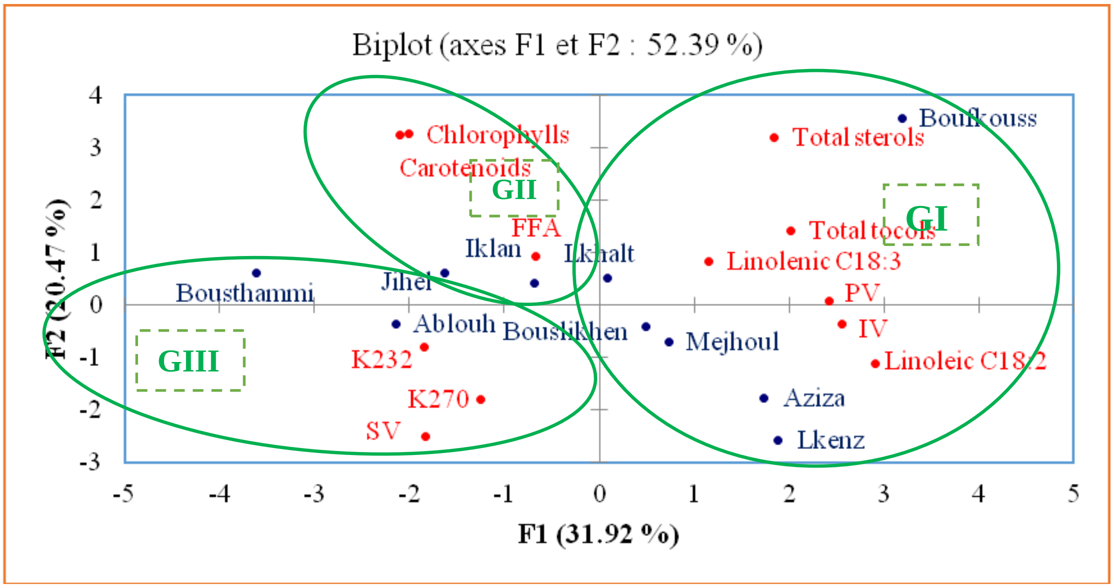


Figure 47: Individual projection on the factorial scheme (F1F2). GI stands for Group I, GII for Group II, and GIII for Group III.

The projection of our variables onto the factorial plan (F1-F2) resulted in their being split into three distinctive groups, as illustrated in Figure 38. Boufkouss, Lkenz, Mejhoul, and Aziza are the four types that comprise Group I. They have a high concentration of total tocol, total sterol, linoleic C18:2, and linolenic acid C18:3, as well as a high iodine and peroxide value. They did, however, have lower levels of carotenoid and chlorophyll than Group III. Iklan and Lkhlat belong to Group II, and they have the greatest specific UV extinction coefficient (K232 and K270), FFA percent, and saponification value. But also, they had an average content of carotenoids and chlorophyll. The last group (Group III) has four species: Bouslikhen, Ablouh, Jihel, and Bousthammi, with a high content of carotenoid and chlorophyll. Their oil recorded the lowest values of the physicochemical parameters of oil quality. On other hand, the total of sterol and tocol is lower than Group I.

III- Hierarchical clustering analysis (HCA)

In order to assess the similarity measure, 10 variants of DSO were categorized using the Wards technique and the squared Euclidean, as shown in the dendrogram in Figure 39. HCA is used to evaluate the correlation between all samples, and then to illustrate their similarities using data from physicochemical characteristics of oil quality and chemical composition of oil, such as total sterol, total tocol, chlorophyll, carotenoid, linoleic (C18:2) and linolenic (C18:3).

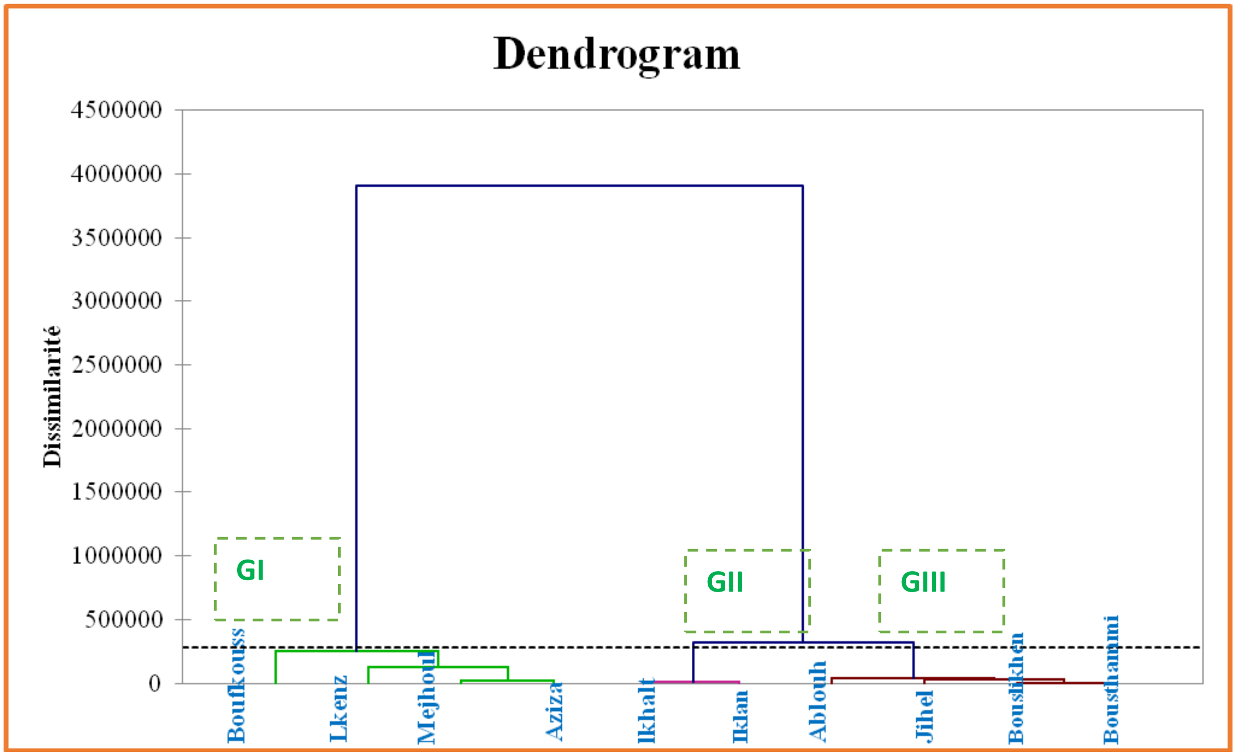


Figure 48: Dendrogram of the sample's oils studied obtained by cluster analysis (Ward and Euclidean distance).

<u>Table 15:Parameter's data of each cluster.</u>

Class	Total sterols (mg/100 g oil)	Total tocols (µg/g of oil)	Carotenoids (mg/kg)	Chlorophylls (mg/kg)	Linoleic C18:2 (%)	Linolenic C18:3 (%)	IV (g I_2/100 g oil)	FFA%	PV (meq O_2/kg)	SV(mg KOH/g oil)	K232	K270
1	524.656	3545.737	5.257	11.513	7.888	0.043	0.553	2.165	5.330	229.465	1.564	1.363
2	487.630	2594.797	7.238	16.533	7.865	0.095	0.569	2.350	5.930	263.195	2.230	1.840
3	408.463	2112.348	5.345	11.945	7.435	0.048	0.544	2.010	4.698	245.405	1.988	1.515

Ten varieties of DSO have been divided into three clusters. Cluster I contains four varieties of DSO (Boufkouss, Lkenz, Mejhoul, and Aziza). They account for 40% of the total. They are characterized by a high mean value of total sterol with 524.656 mg/100 g oil (Table 15), total tocol with 3545.737 µg/g of oil, linoleic acid C18:2 with 7.888%, and Iodine Value of 0.553 g I_2/100 g. But compared to other clusters data, they have a lower mean value of carotenoid (5.257 mg/kg) and chlorophyll (11.513 mg/kg). Cluster II is only formed by Iklan and Lkhalt and it has a high mean value of linolenic (C18:3) of 0.095%. They also recorded the highest parameters of oil quality; the FFA was 2.26%, and the mean saponification value was 263.195 mg KOH/g oil, the same for the specific UV extinction coefficients with 2.230 in K232 and 1.840 in K270 (Table 15). Cluster III represents 40% and contains four DSO varieties named Bouslikhen, Bousthammi, Ablouh, and Jihel respectively. This cluster is characterized by the highest mean values of chlorophyll (20.81 mg/kg), carotenoid (7.95 mg/kg), and a medium mean value of FFA (2.37%), and saponification value (263.55 mg KOH/g oil). These results are in agreement with the data from the PCA, where the distribution of all extracts on the score plot indicates a similar trend. Furthermore, the PCA results were consistent with those of HCA.

Conclusion

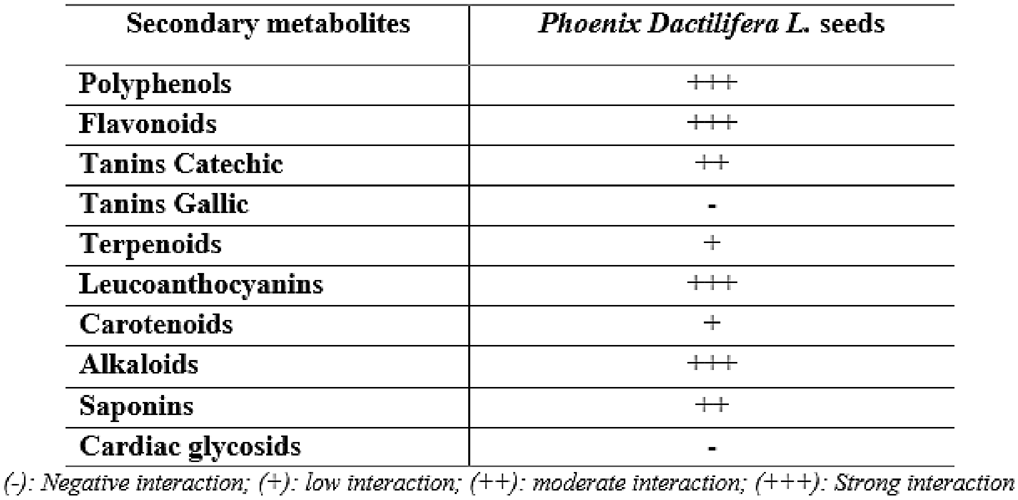
The study evaluates date seed oil (DSO) extracted from 10 varieties of Phoenix dactylifera L. grown in Morocco, analyzing its physicochemical properties, fatty acid composition, sterols, and tocopherols (vitamin E components). It identifies DSO as an oleic-lauric type oil due to its significant oleic acid content (42%-53%) and high levels of sterols (370-817 mg/100g) and tocopherols (1937-3844 µg/g). These properties indicate DSO's potential for food, pharmaceutical, and cosmetic applications. The oil demonstrates excellent oxidative stability and quality parameters, supporting its industrial and health-related applications. Further safety testing is recommended for broader industrial applications.

Chapter II : Qualitative and quantitative analysis of Phoenix Dactilifera L. Seeds extract, toxicological study and evaluation of antioxidant activity

A- Qualitative analysis: Phytochemical screenings

In the present study, the preliminary phytochemical analysis indicated the presence of polyphenols, flavonoids, tannins, terpenoids, leucoanthocyanins, carotenoids, alkaloids, saponins and cardiac glycosides in Phoenix Dactilifera L. seeds powder and extract. (Table 16)

Table 16: Phytochemical screenings of *Phoenix Dactilifera L.* seeds



Secondary metabolites	*Phoenix Dactilifera L.* seeds
Polyphenols	+++
Flavonoids	+++
Tanins Catechic	++
Tanins Gallic	-
Terpenoids	+
Leucoanthocyanins	+++
Carotenoids	+
Alkaloids	+++
Saponins	++
Cardiac glycosids	-

(-): Negative interaction; (+): low interaction; (++): moderate interaction; (+++): Strong interaction

The various phytochemical compounds detected renowned to possess useful importance in medical science. For instance, in keeping with Khurana & al study [126] polyphenols defend the vascular system and performance as antioxidants. forestall substances from being regenerate to harmful change chemical states, including low-density lipoprotein (LDL), additionally called as bad cholesterol. The tannins have high-temperature stability. They can decrease protein digestibility in man and animals, by inducing proteins to be partly unprocurable or inhibiting digestive enzymes and increasing fecal nitrogen [127]. Saponins show loads of useful effects in humans. In keeping with revealed studies[128129], flavonoids have inhibitor activity and therefore the ability to scavenge free radicals. They'll play a role within the hindrance of coronary cardiopathy and anti-cancer activity, however even have the potential for human immunological disorder virus functions.

B- Determination phytochemical compounds and antioxidant activity

I- Yields of extracts

After delipidation with hexane using a soxhlet apparatus, the following extraction was performed to get an alcoholic extract. Subsequently, we determined the extraction yields for each variety. The extraction yields are grouped in the following Figure 49.

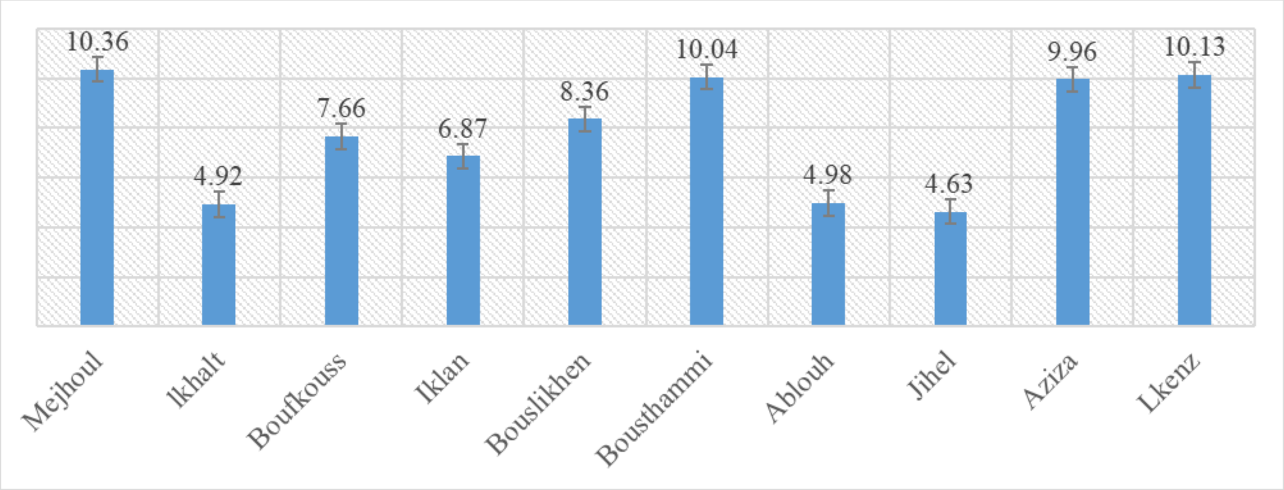


Figure 49: Extraction yield (%) by a polar solvent (alcohol) after a delipidation

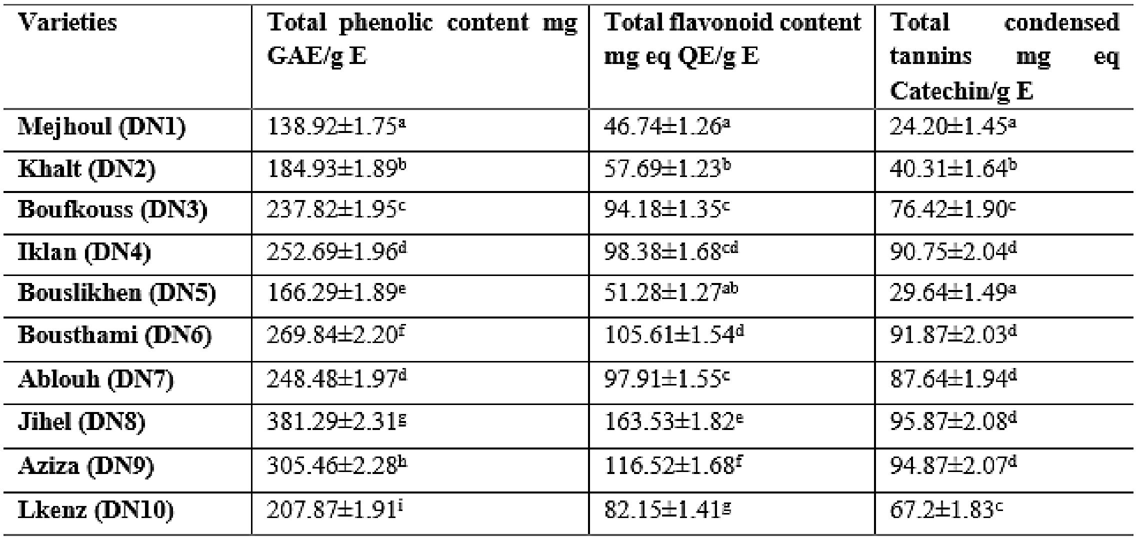
The alcohol extraction yields are clearly higher than the oil extraction yields. Then we see a considerable variation between varieties. Indeed, the varieties Mejhoul, Lkenz, and Bousthammi had high yields of 10.36%, 10.13%, and 10.04%, respectively, whereas Jihel had the lowest yield of 4.63%. Furthermore, the yield variation might be linked to the variety's origin, area, or climatic conditions.

II- Phytochemical determination of the bio-compounds present in the alcoholic extract of date pits:

1- Determination of the phenolic content

The phytochemical assay is a necessary step in order to quantify and analyze the content of bioactive compounds present in an extract. The main compounds analyzed are the polyphenols, which represent the largest family of phenolic compounds. Then, the subfamily is the flavonoids, which have a wide range of applications. Finally, the condensed form of phenolic compounds that are the catechic tannins. The contents are determined using a specific standard and are grouped in the following table n°17.

Table 17: Phytochemical analysis of date seeds extracts



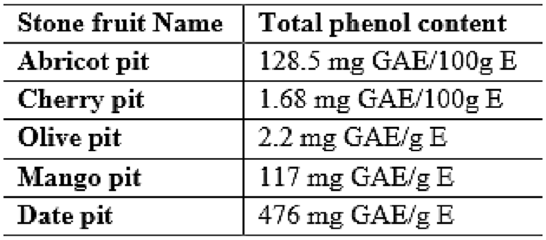
Varieties	Total phenolic content mg GAE/g E	Total flavonoid content mg eq QE/g E	Total condensed tannins mg eq Catechin/g E
Mejhoul (DN1)	138.92 ± 1.75^{a}	46.74 ± 1.26^{a}	24.20 ± 1.45^{a}
Khalt (DN2)	184.93 ± 1.89^{b}	57.69 ± 1.23^{b}	40.31 ± 1.64^{b}
Boufkouss (DN3)	237.82 ± 1.95^{c}	94.18 ± 1.35^{c}	76.42 ± 1.90^{c}
Iklan (DN4)	252.69 ± 1.96^{d}	98.38 ± 1.68^{cd}	90.75 ± 2.04^{d}
Bouslikhen (DN5)	166.29 ± 1.89^{e}	51.28 ± 1.27^{ab}	29.64 ± 1.49^{a}
Bousthami (DN6)	269.84 ± 2.20^{f}	105.61 ± 1.54^{d}	91.87 ± 2.03^{d}
Ablouh (DN7)	248.48 ± 1.97^{d}	97.91 ± 1.55^{c}	87.64 ± 1.94^{d}
Jihel (DN8)	381.29 ± 2.31^{g}	163.53 ± 1.82^{e}	95.87 ± 2.08^{d}
Aziza (DN9)	305.46 ± 2.28^{h}	116.52 ± 1.68^{f}	94.87 ± 2.07^{d}
Lkenz (DN10)	207.87 ± 1.91^{i}	82.15 ± 1.41^{g}	67.2 ± 1.83^{c}

a. Analysis of total polyphenol content

Table 17 shows the total phenolic content (TPC) measured in several Date Seeds Extract (DSE) varieties. These results can be divided into three groups depending on the quantity of DSE at phenolic compounds. The first group contains the Jihel and Aziza varieties with more than 300 mg GAE/g E (milligrams of gallic acid equivalents per gram of extract). Next comes the second group with over 200 mg GAE/g E for the varieties Bousthammi, Iklan, Ablouh, and Boufkouss. The third group containing less than 200 mg GAE/g E includes Lkhalt, Bouslikhen, and Mejhoul. This large variety of total phenolic content depends on the seed variety and crop area.

According to our and different study’s result (Table 17 and Table 18), the total phenolic content in stone fruit or drupe such as Apricot pit, cherry pit, Mango pit, olive pit and date pit, had shown important value but date seed had shown a higher value for the same measuring method and experimental conditions.

Table 18: Total polyphenol content of five different types of fruit seeds [130, 131, 132, 133, 134]



Stone fruit Name	Total phenol content
Abricot pit	128.5 mg GAE/100g E
Cherry pit	1.68 mg GAE/100g E
Olive pit	2.2 mg GAE/g E
Mango pit	117 mg GAE/g E
Date pit	476 mg GAE/g E

b. Analysis of total flavonoid content

The widest class of polyphenols are flavonoids; they have a common structure of diphenylpropanes with two aromatic rings linked by three carbons. Flavonoids have protective effects against many diseases particularly cardiovascular disease by their capacity of scavenging free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing alpha-tocopherol radicals, and inhibiting oxidases.

The amount of the total flavonoids content TFC in the ten studied varieties of DSE is shown in Table 17. The highest amount of total flavonoids was found in the variety of Jihel (163.53 mg QE/g E), followed by Aziza (116.52 mg QE/g E), Bousthammi (105.61 mg QE/g E), while the lowest amount was found in Khalt (57.69 mg QE/g E), Bouslikhen (51.28 mg QE/g E) and Mejhoul (46.74 mg QE/g E). According to the results of Djaoudene et al.[134] and Adeosun et al.[135], the total amount of flavonoid in DSE was lower than our results with 6.52 mg QE/g E and 24.71 mg QE/g E.

c. Analysis of total condensed tannins content

Tannins are a polyphenols of molecular weight went from 500 to 3000 Da. They can be in complexes with proteins, alkaloids and polysaccharides with a molecular weight up to 20.000 Da. Tannins are commonly classified as hydrolysable and nonhydrolyzable tannins (condensed tannins). Condensed tannins are the most abundant in the nature while the hydrolysable can be found in few dicotyledon species or in combination with condensed tannins in different proportions [136].

In our study, the determination of total condensed tannins content TTC in DSE had shown a significant value (Table 17). Indeed, there is a widely similar value between varieties, from DN6 to DN9 and DN4 was ranged between 95.87 and 87.64 mg CE/g E, followed by DN3 and DN10 with 76.42 and 67.2 mg CE/g E, while the lower one was DN1 with 24.20 mg CE/g E. Based on the results of Adeosun et al.[135], DSE contains a very low amount of tannins with 133.2 mg CE/g E. Likewise in Thouri et al.[137] results, the quantity of tannin in DSE was greater than Mejhoul, Bouslikhen with more than 30 mg CE/g E.

2- Determination of alkaloids content

Extraction of alkaloid from date seeds could serve as a cheap and steady means of providing this secondary metabolite in industries where they are greatly needed. The determination of alkaloids content indicates that Date seeds has an important amount. In fact, four varieties were ranged from 10.20% to 12.50%, three were from 21.40% to 33.20%, and two had the highest content with 40.50% and 46.30%, respectively for the variety of Iklan and Mejhoul (Figure 50).

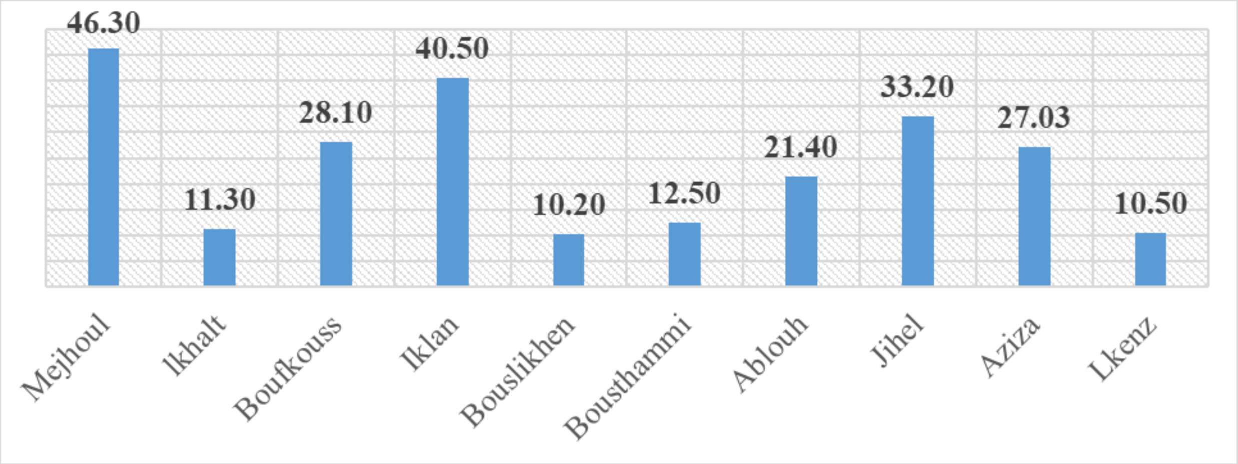


Figure 50: Alkaloids content (%)

According to literature, alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine), antibacterial (e.g., chelerythrine), and antihyperglycemic activities (e.g., piperine). Alkaloids have equally been exploited for their importance in traditional pharmaceutical usage. Other alkaloids possess psychotropic (e.g., psilocin) and stimulant activities (e.g., cocaine, caffeine, and nicotine) and have been used as recreational drugs. Although alkaloids carry out many metabolic activities in humans and other animals, they almost uniformly evoke a bitter taste[138, 139].

3- Determination of saponin content in DSE

Saponins are glycosides widely present in various plants, and are characterized by bitter taste and foaming in aqueous solution. Akindahunsi et al.[140] clearly pointed out that saponins have tumor inhibitory effect on animals. Asl & Hossein[141] also reported that there is evidence that traditional medicinal preparations contain saponins, and oral administration may lead to hydrolysis of glycosides in terpenoids.

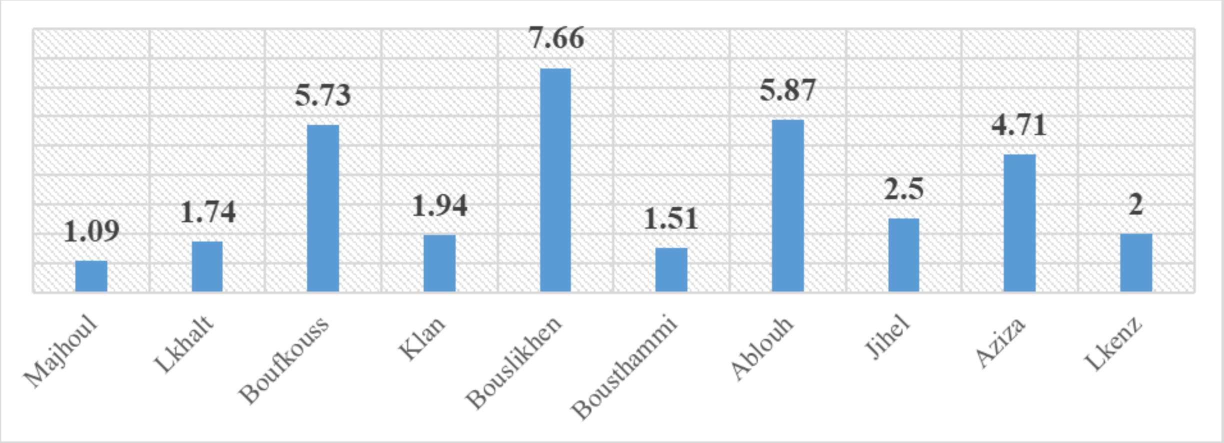


Figure 51: DSE's saponins content (%)

Quantitative analysis showed that saponins were present in all samples, but at different concentrations (Figure 51). In fact, four extracts contained greater than 4.5%, while the other six contained less than 2.5%. Although saponins are non-toxic, their median lethal dose is 200mg/kg body weight. The Bouslikhen variety had the highest saponin content of 7.66%, and for Mejhoul variety had the lowest saponin content of 1.09%, which did not the lethal dose.

4- Determination of sugar content in DSE

The sugar content of DSE varied from variety to another ranging from 373.96 to 826.84 mg D-GluE/g extract (Figure 52). Six varieties (Iklan, Lkenz, Ablouh, Mejhoul, Bouslikhen and Bousthammi) varied from 506.84 to 605.96 mg D-GluE/g extract and three varieties (Aziza, Boufkouss and Lkhalt) ranged from 373.96 to 417.51 mg D-GluE/g extract, whereas the Jihel variety stood out from the others with the highest content.

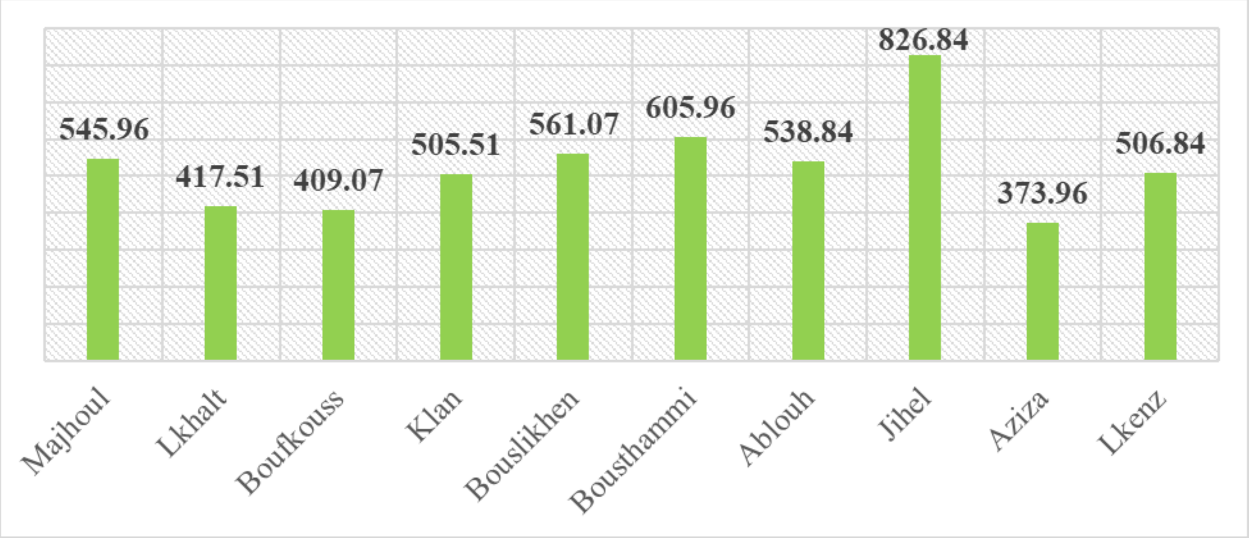


Figure 52: Total sugar content of DSE

III- Evaluation of antioxidant activity

The FRAP, ABTS, and DPPH assays were used to measure antioxidant activity. The FRAP test detects the presence of antioxidants by reducing ferric ions to ferrous ions (from Fe^{3+} to Fe^{2+}). The ABTS and DPPH assays are based on a sample's capacity to scavenge the free radicals 2,20-azino-bis (ethylben zothiazoline-6-sulfonic acid) ($ABTS^{+\cdot}$) and 1,1-diphenyl-2-picrylhydrazyl (DPPH). Table 19 summarizes the findings of all of these tests.

Table 19: Results of antioxidant tests by DPPH, ABTS and FRAP

Variety	DPPH IC$_{50}$ µg/mL	ABTS IC$_{50}$ µg/mL	FRAP EC$_{50}$ µg/mL
Mejhoul (DN1)	4.78 ± 0.17^{a}	46.62 ± 1.56^{a}	161.26 ± 1.66^{a}
Khalt (DN2)	4.5 ± 0.13^{a}	34.92 ± 1.38^{be}	131.82 ± 1.38^{b}
Boufkouss (DN3)	3.8 ± 0.1^{c}	27.38 ± 1.17^{cf}	116.95 ± 1.02^{c}
Iklan (DN4)	2.44 ± 0.49^{d}	26.62 ± 0.80^{cf}	104.29 ± 0.79^{d}
Bouslikhen (DN5)	4.72 ± 1.62^{a}	39.51 ± 1.46^{b}	148.26 ± 1.56^{e}
Bousthami (DN6)	1.9 ± 0.04^{e}	24.25 ± 0.75^{cd}	97.51 ± 0.69^{f}
Ablouh (DN7)	3.01 ± 0.07^{f}	27.31 ± 0.91^{cf}	107.45 ± 0.82^{d}
Jihel (DN8)	1.13 ± 0.02^{g}	18.90 ± 0.63^{d}	69.25 ± 0.5^{g}
Aziza (DN9)	1.52 ± 0.03^{eg}	22.13 ± 0.74^{cd}	88.98 ± 0.62^{h}
Lkenz (DN10)	4.29 ± 0.11^{ac}	31.44 ± 1.26^{ef}	127.30 ± 1.27^{b}
Ascorbic acid	1.91	-	45.8
Trolox	-	29.01	-

1- Assessment of the antioxidant capacity by inhibition of DPPH and ABTS radicals

The antioxidant capacity of DSE was evaluated under their donation of the hydrogen atom or electron to free radicals from forming a stable intermediate. The antioxidant activities of various DSE are shown in Table 7. All varieties showed a good scavenging activity for both radical's DPPH and ABTS. Therefore, there is a significant different result from DSE to another. Likewise, Jihel and Aziza seeds registered a better neutralization of the DPPH radical with IC$_{50}$=1.13 µg/ml and IC$_{50}$=1.52 µg/ml while the lowest was shown in Bouslikhen and Mejhoul seeds with IC$_{50}$= 4.72 µg/ml and IC$_{50}$= 4.78 µg/ml. ABTS assay results showed also a strong inhibition for DSE of Jihel with IC$_{50}$=18.9 µg/ml, and a similar inhibition for DSE of Aziza, Bousthammi, Iklan, Ablouh, and Boufkouss ranged from 22.13 µg/ml to 27.38 µg/ml. Although Lkhalt, Bouslikhen, and Mejhoul had the lowest activity, it varied from 34.92 µg/ml to 46.62 µg/ml. The antioxidant activity of DSE had the same range as reported by Djaoudene et al.[134] from eight different date seed varieties, the IC$_{50}$ varied from 13.89 to 32.31 µg/ml.

2- Evaluation of the antioxidant capacity by reduction of iron ions (FRAP)

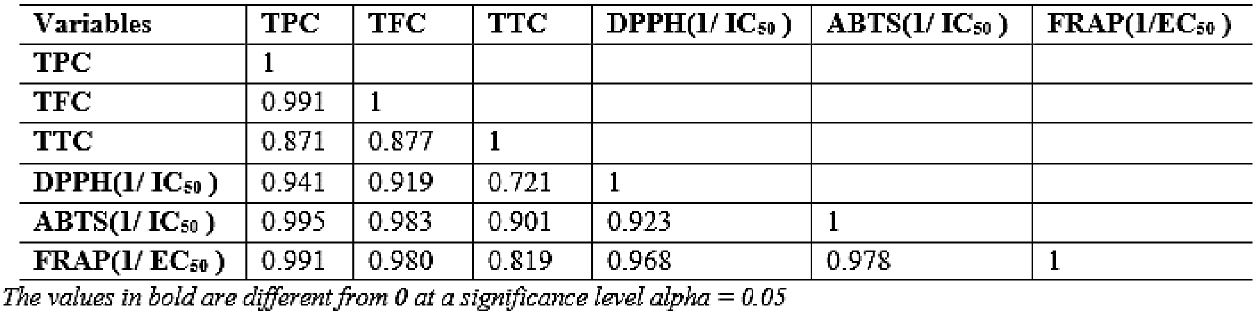
The results of FRAP assay showed a good reducing power of ferric ions with EC$_{50}$=69.25µg/ml for Jihel Followed by Aziza with EC$_{50}$= 88.98 µg/ml, then Bousthammi, Iklan, Ablouh, Boufkouss, Lkenz and Lkhalt went from EC$_{50}$= 97.51 µg/ml to EC$_{50}$= 131.82 µg/ml while the lowest reducing power was for Mejhoul with EC$_{50}$= 161.26 µg/ml. Based on the results of the reduction power of Thouri et al. [137], the aqueous extracts reduction capacity of Korkobbi and Arechti date seeds was close to Boufkouss and Mejhoul respectively with 120 µg/ml and 190 µg/ml, whereas their methanolic extract showed a low reduction ranging from 270 µg/ml to 260 µg/ml.

IV- Statistical analysis : Correlation Matrix, PCA and HCA

1- Correlation Matrix

The correlation coefficients are shown in Table 20, and their p-values were presented in Table 21. The antioxidant activities of DPPH, ABTS, and FRAP were represented by the inverse of IC_{50} and EC_{50}.

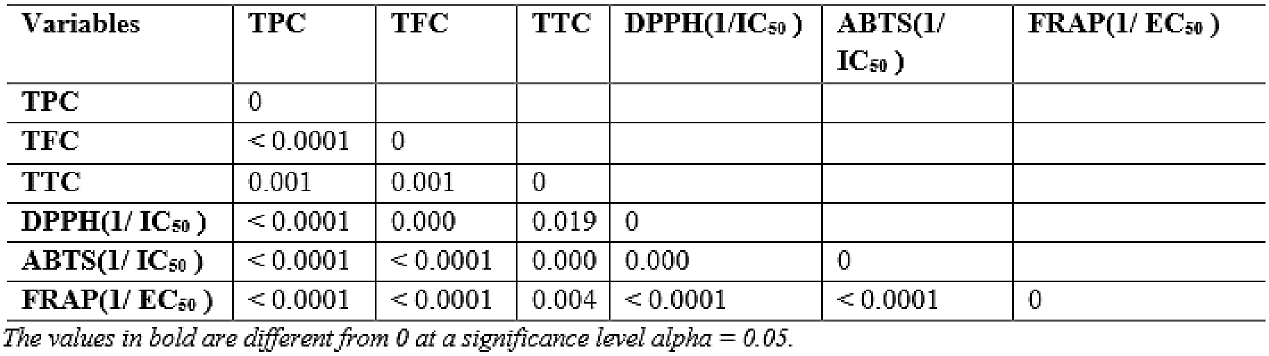
Table 20: Pearson's correlation matrix coefficient for Physico-chemical characteristics and antioxidants substances.



Variables	TPC	TFC	TTC	DPPH(1/ IC_{50})	ABTS(1/ IC_{50})	FRAP(1/EC_{50})
TPC	**1**					
TFC	0.991	**1**				
TTC	0.871	0.877	**1**			
DPPH(1/ IC_{50})	0.941	0.919	0.721	**1**		
ABTS(1/ IC_{50})	0.995	0.983	0.901	0.923	**1**	
FRAP(1/ EC_{50})	0.991	0.980	0.819	0.968	0.978	**1**

The values in bold are different from 0 at a significance level alpha = 0.05

Table 21: p-values of the correlation matrix coefficient.



Variables	TPC	TFC	TTC	DPPH(1/IC_{50})	ABTS(1/ IC_{50})	FRAP(1/ EC_{50})
TPC	0					
TFC	< 0.0001	0				
TTC	0.001	0.001	0			
DPPH(1/ IC_{50})	< 0.0001	0.000	0.019	0		
ABTS(1/ IC_{50})	< 0.0001	< 0.0001	0.000	0.000	0	
FRAP(1/ EC_{50})	< 0.0001	< 0.0001	0.004	< 0.0001	< 0.0001	0

The values in bold are different from 0 at a significance level alpha = 0.05.

According to Tables 20 and 21, the Pearson correlations between bioactive compounds and their antioxidant activities showed that the TPC, TFC and TTC had strong positive correlation significant (p-value < 0.0001) with the antioxidant capacities by DPPH, ABTS, and FRAP assay. The correlation results of total polyphenol content (TPC) were 0.941, 0.995, and 0.991 with DPPH, ABTS and FRAP assays respectively. These results indicate that the antioxidant activity of the samples examined can be attributed to the presence of phenolic compounds.

Likewise, the TFC had also a strong positive correlation between DPPH with r^2= 0.919, ABTS with r^2= 0.983, and FRAP with r^2= 0.980. Furthermore, the TTC had a positive correlation significant (p-value < 0.05) between the antioxidant activity, but they are low compared to those of TPC and TFC. These results showed that the flavonoids and phenolic compounds contribute strongly in this antioxidant activity more than the Tannins.

In addition to that, all three assays of antioxidant activities were correlated ($p\text{-value} < 0.05$) with each other. Therefore, these high correlations indicate that the bioactive compounds, which provide free radical scavenging power by ABTS and DPPH assays, are themselves, who contribute to the reducing power of ferric ions by FRAP assay.

2- Principal Component Analysis (PCA)

In this segment, the variables include the various phytochemical contents and antioxidant activity results of ten samples (Figure 53). The PCA was used to design them based on the F1-F2 factorial plan.

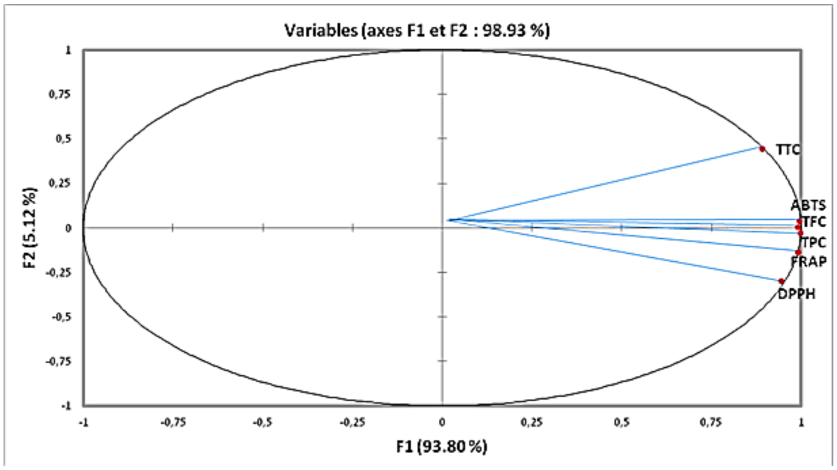


Figure 53: PCA factorial design for bioactive compounds and antioxidants activities of samples.

The first (F1) and second (F2) primary components account for 93.80% and 5.12% of the overall information.. Furthermore, the cumulative proportion of these components is 98.93%, implying that the linear combination is representative of the variables due to its value up to 50%. Thusly, the first two axes are appropriate for representing the data as a whole. Figure 53 exhibits the plane generated by axes F1 and F2, which shows the correlation between the variables. The F1 axis is primarily defined by the positive connection between bioactive chemicals and their antioxidant properties.

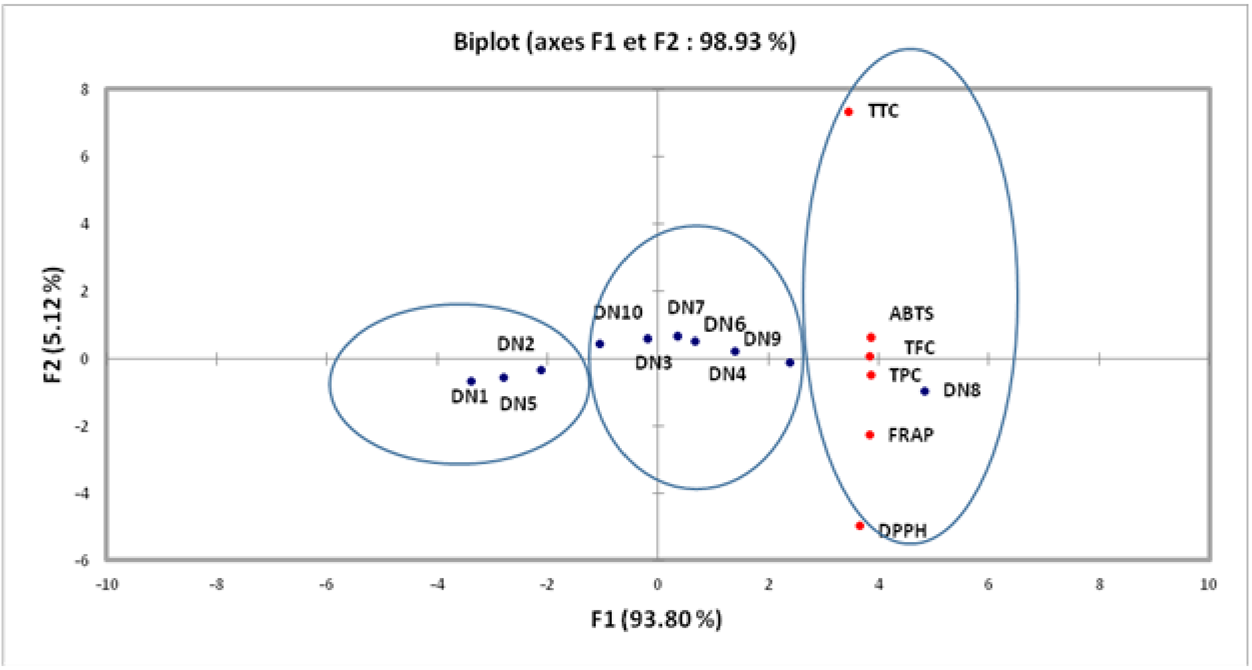
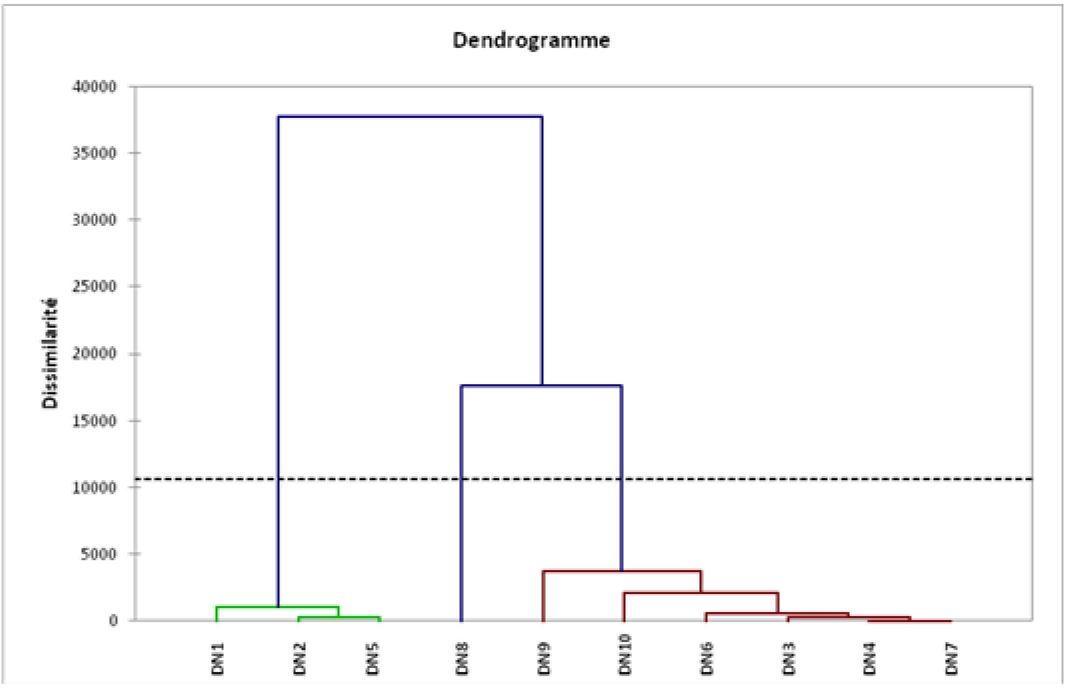


Figure 54: Projection of individuals on the factorial plan (F1×F2). GI: Group I; GII: Group II.

As shown in Figure 54 the ten varieties of DSE are spread into 3 main groups (GI, GII and GIII). The GI group consists of 3 DSE (DN1, DN2, DN5) named Mejhoul, Khalt, and Bouslikhen respectively. These samples were characterized by a lower value of bioactive compounds (TPC, TFC, TTC), as well as, they had a lower antioxidant power compared to other groups. The GII group is made up of 6 samples (DN3, DN4, DN6, DN7, DN9, DN10) named Boufkouss, Iklan, Bousthami, Ablouh, Aziza, and Lkenz respectively. They can be qualified as a medium-high value of antioxidant content, as well as, by a medium-high antioxidant activity over three assays DPPH, ABTS, and FRAP. GIII group is taken shape by one sample named Jihel (DN8). It is characterized by a high value of phytochemical contents. Likewise, it had the strongest antioxidant activity by the same assays.

3- Hierarchical clustering analysis (HCA)

The ten samples (varieties) of DSE were sorted out by the Ward's method and the squared Euclidean for evaluating the similarity measure. HCA was utilized to assess the connection among samples and to demonstrate their similarity on bioactive content and antioxidant activity using a dendrogram. According to the results of ten samples, they were classified into three clusters (Figure 55).



CI: Cluster I; CII: Cluster II; C III; Cluster III

Figure 55: Dendrogram of the studied samples generated by cluster analysis (Ward and Euclidean distance).

The Cluster I held 3 samples of DSE (DN1, DN2, DN5) named Mejhoul, Khalt, and Bouslikhen respectively, representing 30 % of the total samples. They are characterized by the lowest mean value of TPC 163.38 mg GAE/g, TFC 51.90 mg QE/g and TTC 31.38 mg CE/g, along with a lower antioxidant power compared to cluster II and III. The Cluster II was formed by 6 samples (DN3, DN4, DN6, DN7, DN9, DN10), and accounted 60 % of total samples. These samples commonly held a medium mean value of TPC 253.69 mg GAE/g, TFC 99.12 mg QE/g and TTC 84.79 mg CE/g with a medium antioxidant activity. Cluster III contained one sample named Jihel (DN8) which represents approximately 10 % of the total samples. This cluster is described by a higher value mean of TPC 381.29 mg GAE/g of, TFC 163.53 mg, TTC 95.87 mg CE/g.

Likewise, this sample recorded the highest antioxidant power compared to cluster I and II. The overview of these results shows some affinity with those of the PCA, which the distribution of all samples on the score plot shows a similar trend. The analysis of the outcomes indicates that there is consistency between the data in the PCA score chart and the HCA scores.

C- Successive solvent extraction for the evaluation of phytochemical compounds and antioxidant activity

The phytochemical contents of date seed were extracted successively by seven different solvents ranging in polarity from 0.009 (n-hexane) to 1 (water). This technique was used to ensure that a wide polarity range of compound could be extracted.

I- Physical aspect

The successive extraction with increasing polarity solvents produced a different types of extracts with different physical aspects and chemical properties. Table 22 summarizes the results from the extracts analyzed. When the polarity of the extraction solvent is increased the physical aspect of the extract changes from an oily state to a very viscous solid with a color shift from yellow to dark brown.

Table 22: Physical aspects of the different extracts obtained (Aziza variety)

Extraction solvents	**Relative polarity***	**Physical aspect**	**Coloration**	
Hexane	0.009	Oily	Yellow	
Ethyl acetate	0.228	Oily and viscous	Dark orange	
Chloroform	0.259	Oily and viscous	Yellow orange	
Acetonitrile	0.460	Pasty solid	Chocolate brown	

Isopropanol	0.546	Crystallized solid	Dark garnet red	
Ethanol	0.654	Solid	Dark garnet red	
Water	1	Highly viscous	Dark brown	

** Relative polarity values are normalized from solvent shift measurements and absorption spectra* [142]

II- UV spectroscopic analysis of oil extracts : Carotenoid and chlorophyll content

The chlorophyll and carotenoid content was determined in extracts with an oily appearance such as the hexanic, ethyl acetate and chloroform extract. The results of this analysis were summarized in the table and Figure 56 below.

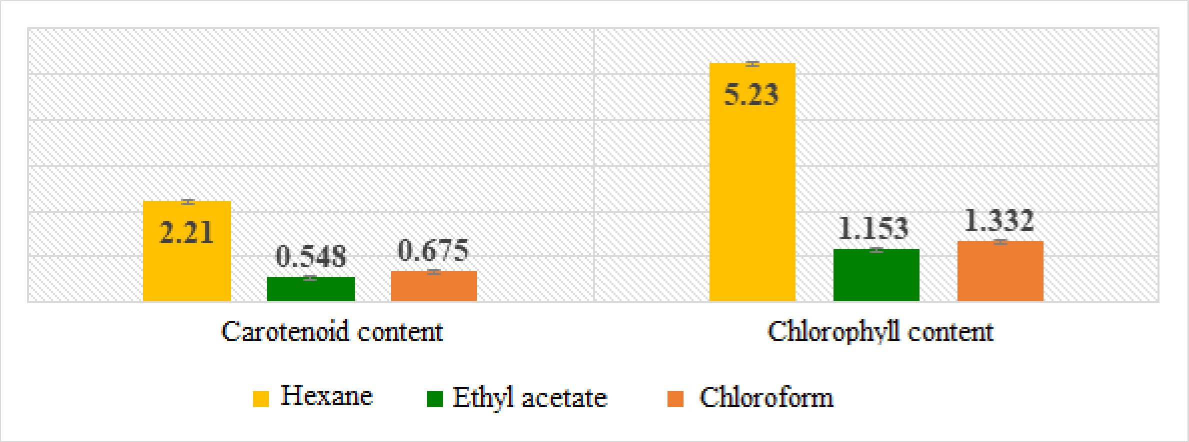


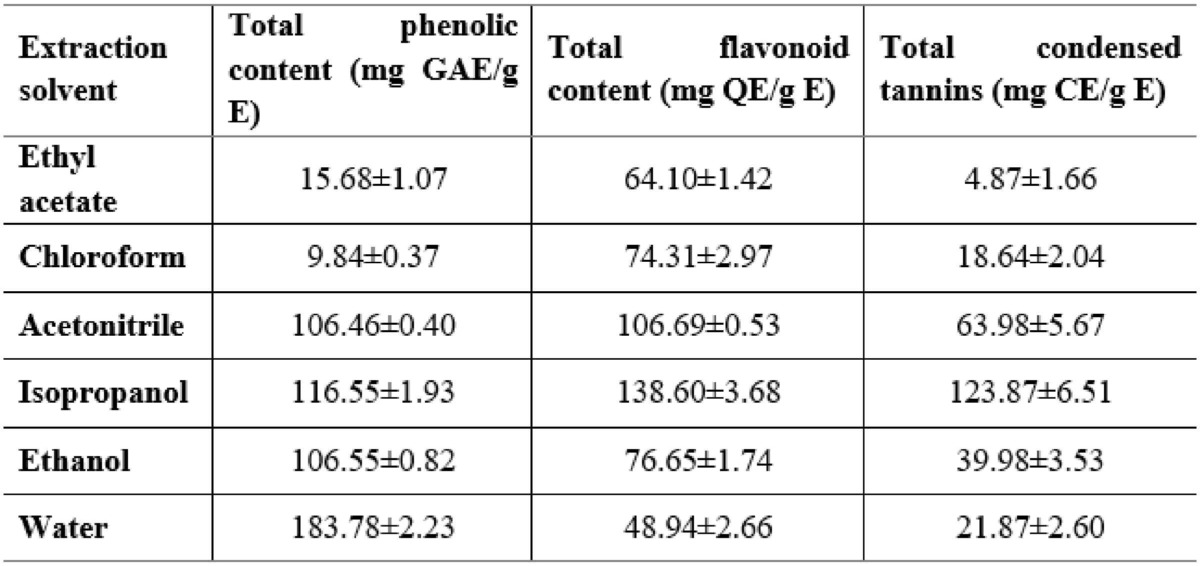
Figure 56: Carotenoid and chlorophyll content of the hexanic, ethyl acetate and chloroform extract

We can clearly notice that the hexanic extract has four times more chlorophylls and three times more carotenoids than the ethyl acetate and chloroform extracts. On the other hand, the chlorophyll and carotenoid contents of the chloroform extract are slightly higher than those of the ethyl acetate extract. We can thus affirm that the majority of these compounds are extracted by the hexane nevertheless a non-negligible quantity of chlorophylls and carotenoids remains unextractable by the hexane.

III- Phytochemical determination of the extract of each solvent

The determination of phenolic compounds showed significant variation and content in each extract. The results of the phytochemical assay are shown in Table 23.

Table 23: Determination of polyphenol, flavonoid et tannin content



Extraction solvent	Total phenolic content (mg GAE/g E)	Total flavonoid content (mg QE/g E)	Total condensed tannins (mg CE/g E)
Ethyl acetate	15.68±1.07	64.10±1.42	4.87±1.66
Chloroform	9.84±0.37	74.31±2.97	18.64±2.04
Acetonitrile	106.46±0.40	106.69±0.53	63.98±5.67
Isopropanol	116.55±1.93	138.60±3.68	123.87±6.51
Ethanol	106.55±0.82	76.65±1.74	39.98±3.53
Water	183.78±2.23	48.94±2.66	21.87±2.60

1- Analysis of total polyphenols content

Based on the analysis of polyphenol content results (Table 23) , the polyphenol content of the two oily extracts was very low, varying between 9.84 to 15.68 mg EAG/g E. On the other hand, the content increased and exceeded 106.46 mg EAG/g E (acetonitrile extract), to a maximum of 183.78 mg EAG/g E (water extract). When the nitrile group was replaced by hydroxyl group, the polyphenol content remained unchanged. Therefore, as the polarity of the solvent increases, the extraction of polyphenols becomes more favorable and the content of the latter increases.

2- Analysis of total flavonoids content

The flavonoid content indicated that all extracts possess significant amounts ranging from 48.94 up to 138.60 mg EQ/g extract. The highest flavonoid content was in the isopropanol extract and the lowest in the water extract. According to table 23, flavonoids were extracted from ethyl acetate; subsequently, by increasing the polarity, the extraction became favorable and it reached its extremum during the extraction by isopropanol. Then, despite increasing the polarity of the solvent, the content dropped sharply.

3- Analysis of total condensed tannins content

Determination of condensed tannins revealed significant differences between each extracts (table 23). In fact, the contents varied between 4.87 and 123.87 mg CE/g extract. The aqueous extract, chloroform and ethyl acetate contained less than 30 mg CE/g extract. Followed by, ethanol and acetonitrile extracts varying from 30 to 65 mg CE/g extract. Finally, only the isopropanol extract contained more than 100 mg CE/g extract. Based on this results, the tannin content ranges from a very low level in the ethyl acetate extract (4.87 mg CE/g extract) to a 25 times higher level in the isopropanol extract (123.87 mg CE/g extract). Tannins are profusely extracted from acetonitrile and then decrease significantly during ethanolic and aqueous extraction.

4- Interpretation of the set

At the beginning of this successive extraction process, we found that polyphenols and tannins were low in the first two extractions, but flavonoids could be extracted with ethyl acetate and chloroform (Figure 57). According to the literature, these two solvents enable the selective extraction of flavonoids during liquid-liquid extraction or HPLC column separation. Subsequently, by passing through acetonitrile all contents increase considerably. In addition, the polyphenol content will continue to increase until the last solvent with the highest content is recorded. In the case of flavonoids and tannins, on the other hand, its peak ends at the fourth extraction with isopropanol and then drops to the last solvent, where the flavonoid content is lowest, the tannins content was 5.6 times lower than the maximum content.

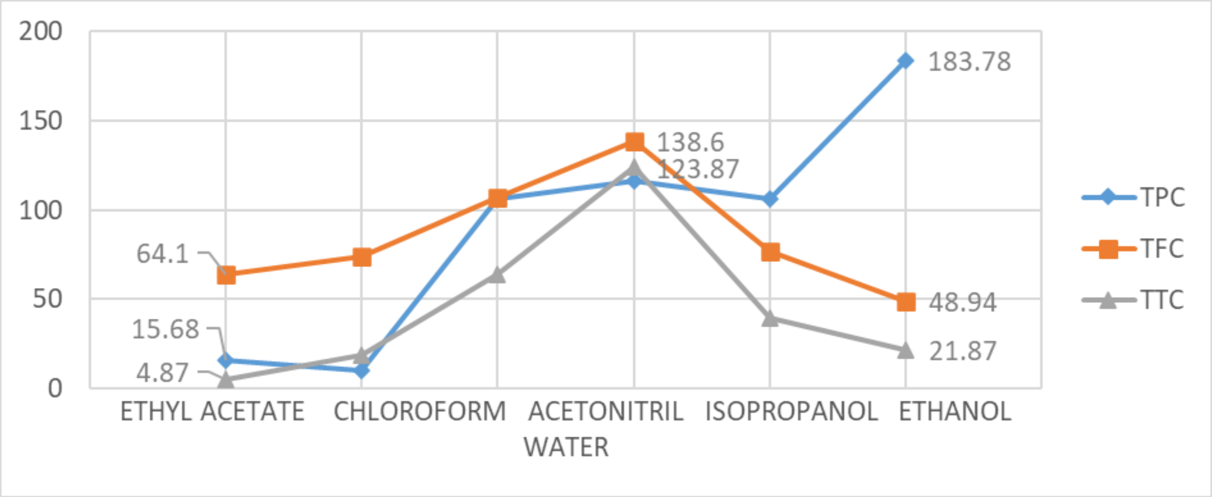


Figure 57: Polyphenol, flavonoid et tannin content of all extracts

Conclusion

The phytochemical screening of *Phoenix Dactilifera L.* (date) seed revealed the presence of several bioactive compounds. Among these, polyphenols, flavonoids, tannins, terpenoids, leucoanthocyanins, carotenoids, alkaloids, saponins, and cardiac glycosides were identified. Polyphenols, flavonoids, and leucoanthocyanins were found in notably high concentrations, while tannins, saponins, and terpenoids were present in moderate amounts. Notably, cardiac glycosides were not detected in any of the seed extracts.

In the quantitative analysis, the total phenolic content (TPC) varied significantly across the different date seed varieties. The Jihel and Aziza varieties exhibited the highest TPC values, exceeding 300 mg GAE/g extract. This suggests that these varieties have a particularly high concentration of phenolic compounds. When compared to other seeds fruits like apricot, cherry, and mango, the date seeds displayed a higher phenolic content, reinforcing their potential as a rich source of bioactive compounds. Similarly, the total flavonoid content (TFC) was highest in the Jihel variety, while the lowest flavonoid concentrations were observed in the Khalt and Bouslikhen varieties. The study also quantified tannins, particularly condensed tannins, which were most abundant in the Bousthammi, Iklan, and Aziza varieties.

The antioxidant activity of the date seed extracts was assessed using three different methods: DPPH, ABTS, and FRAP assays. These tests demonstrated the strong antioxidant capacity of the extracts, with the Jihel variety showing the best free radical scavenging activity in the DPPH assay, as evidenced by the lowest IC_{50} value. Similarly, the Jihel and Aziza varieties exhibited the highest inhibition of the ABTS radical. In terms of reducing power, the Jihel variety also demonstrated the strongest activity, showing the highest reducing power in the FRAP assay.

A correlation analysis revealed a significant positive relationship between the phytochemical content (including TPC, TFC, and TTC) and the antioxidant activities measured by the DPPH, ABTS, and FRAP assays. This suggests that the antioxidant potential of date seed extracts is closely linked to their phenolic and flavonoid content, with these compounds playing a key role in the observed antioxidant effects.

To further explore the relationship between the different date seed varieties and their bioactive compounds, principal component analysis (PCA) and hierarchical clustering analysis (HCA) were employed. Both methods indicated that the varieties clustered into three distinct groups based on their phytochemical content and antioxidant activity. These findings highlight the diversity among the date seed varieties in terms of their bioactive compound profiles and antioxidant potentials.

Finally, the study demonstrates that date seeds, particularly those from the Jihel and Aziza varieties, contain significant amounts of polyphenols, flavonoids, and tannins, which contribute to their potent antioxidant properties. This makes date seeds a promising source of functional ingredients for use in food, cosmetics, and nutraceuticals, with potential health benefits attributed to their high antioxidant activity and rich bioactive profile.

Salts formed from oxalic acid are called oxalates such as calcium oxalate, which is widely found in plants. Oxalic acid forms strong bonds with various other minerals such as calcium, magnesium, sodium and potassium. This chemical combination leads to the formation of oxalate salts.

Some oxalate salts, such as sodium and potassium, are soluble, while calcium oxalate salts are generally insoluble. The insoluble calcium oxalate tends to precipitate (or coagulate) in the kidneys or urinary tract, forming sharp calcium oxalate crystals at sufficiently high concentrations. These crystals can form kidney stones in the urinary tract when the acid is excreted in the urine.

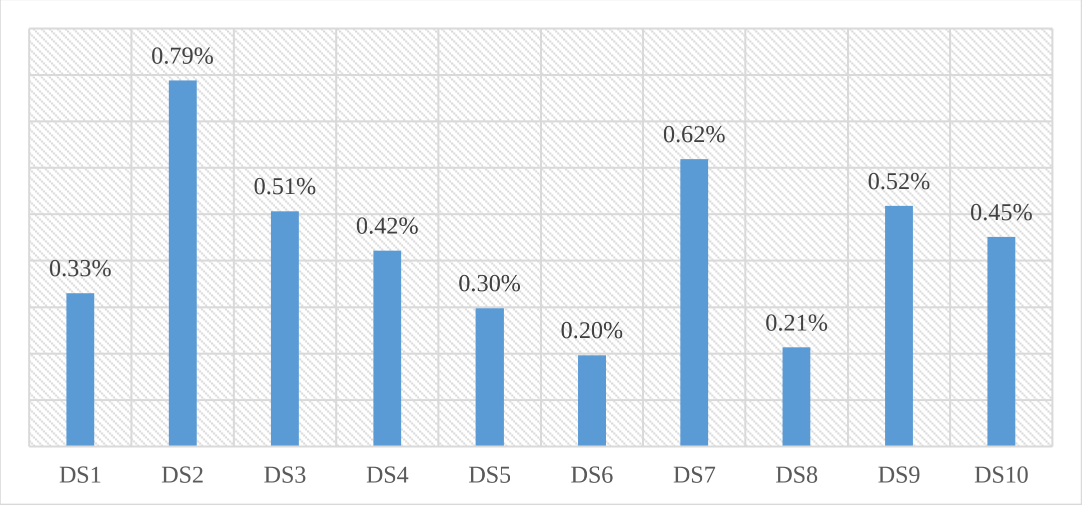


Figure 58: oxalate ions content in date seed extract

According to our study results, there is a significant difference between all the samples. The range was between 0.20 -0.79%. The variety of Lkhalt showed the highest value of 0.79% and the variety of Bousthammi had the least value of 0.20% (Figure 58).

The amount of oxalate ingested can lead to a significant risk of developing idiopathic calcium oxalate kidney stones (nephrolithiasis). In fact, too much soluble oxalate in the body interferes with the absorption of soluble calcium ions. This is because oxalate binds with calcium ions to form an insoluble complex of calcium oxalate.

Therefore, people who are prone to kidney stone formation should avoid foods rich in oxalates. On the other hand, it is recommended to consume oxalates in moderation for people suffering from coronary heart disease should as it helps lower blood cholesterol levels.[143]

Conclusion

Based on our study results, there is a significant difference in oxalate levels among the samples, with values ranging from 0.20% to 0.79%. The Lkhalt variety exhibited the highest oxalate content at 0.79%, while the Bousthammi variety had the lowest at 0.20%. Excessive oxalate intake can increase the risk of developing idiopathic calcium oxalate kidney stones (nephrolithiasis), as oxalate binds with calcium to form an insoluble complex, preventing calcium absorption. Consequently, individuals prone to kidney stones should avoid oxalate-rich foods. However, moderate oxalate consumption is recommended for people with coronary heart disease, as it can help reduce blood cholesterol levels.

Chapter IV : Roasting study: determination of the degree of roasting of date palm seed as a coffee substitute rich in bioactive compounds

The date seeds are used as a substitute for a decaffeinated coffee. In previous chapters, the study of their chemical composition showed that they contain phytosterols, rich in phenolic compounds and tocotrienols. The aim of this chapter is to optimize the roasting time and temperature of the date pits in order to determine the highest content of bioactive compounds and to evaluate the antioxidant capacity, and then make a comparison with three types of coffee.

I- Roasting of date pits and analysis of the physical aspect and extraction yields

1- Analysis of the physical aspect of the roasted seeds

The date pits powder is roasted in a carbolite chamber furnace at three distinct temperatures (160°C- 180°C- 200°C) and three different times (15min, 30min, and 45min) (Table 24). The color of the powder became darker as the temperature and time rise. This is due to the non-enzymatic browning and pyrolysis reactions that occur during the roasting process, which promote the development of brown pigments, resulting in a darker coloration.

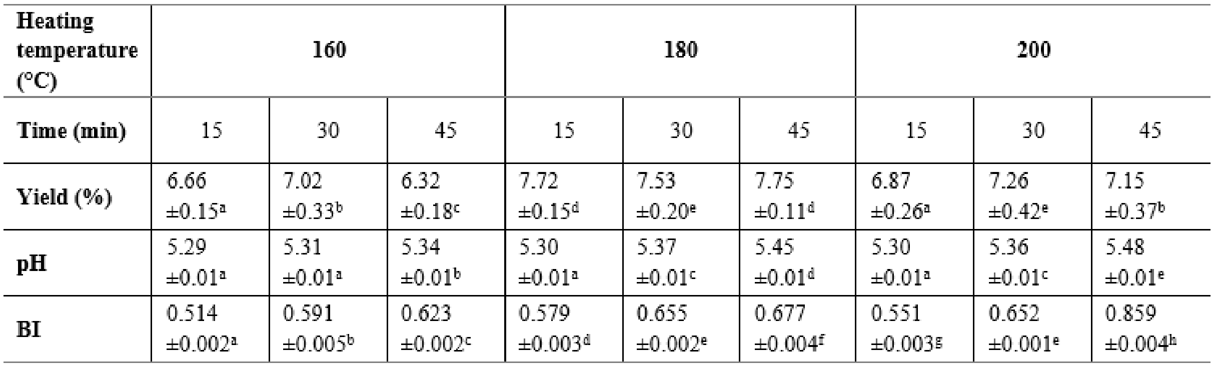
Table 24 : Color changes of the roasted date seeds at different roasting parameters

Heating temperature (°C)	**Time (min)**		
	15	30	45
160			
180			
200			

2- Analysis of the extraction yields, pH and Browning Index (BI)

Table 25 illustrates the color changes, extraction yield, pH value, and Browning Index (BI) of RDS at three different temperatures (160, 180 and 200°C) depending on three roasting times (15, 30, and 45min). The highest extraction yield was obtained for roasting conditions of 180°C for 45 min at 7.75%. The pH changed slightly by a value of 5.29±0.01 to 5.48±0.01. However, extraction yield and pH rose slightly as the temperature of the heat increased. This can perhaps be attributed to roasting conditions that break up the insoluble polymers and soften the pit texture.

Table 25: Extraction yield, pH and Browning Index (BI) of roasted date seed

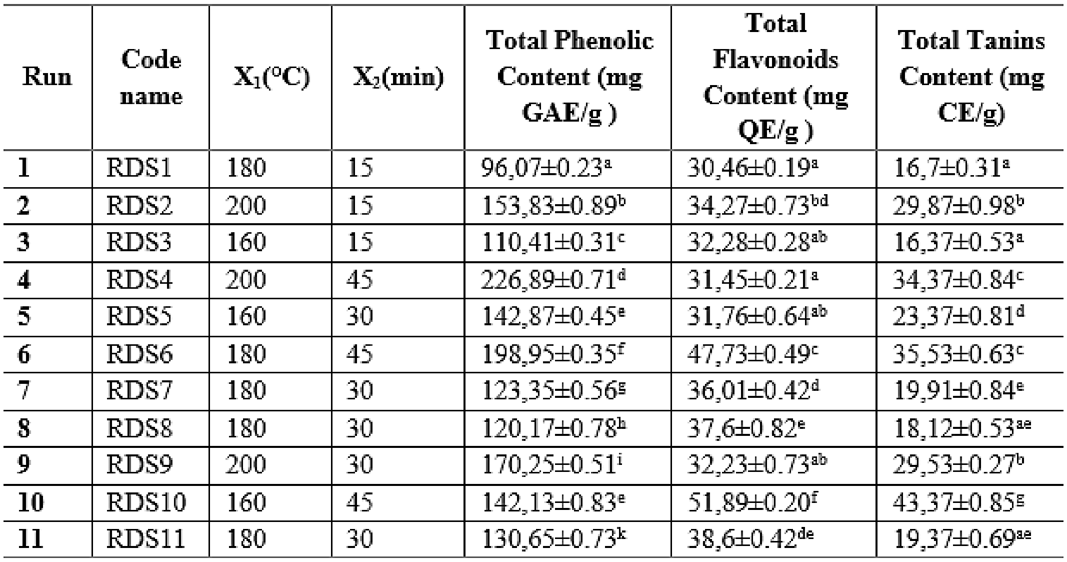


Heating temperature (°C)	**160**			**180**			**200**		
Time (min)	15	30	45	15	30	45	15	30	45
Yield (%)	6.66 ± 0.15^{a}	7.02 ± 0.33^{b}	6.32 ± 0.18^{c}	7.72 ± 0.15^{d}	7.53 ± 0.20^{e}	7.75 ± 0.11^{d}	6.87 ± 0.26^{a}	7.26 ± 0.42^{e}	7.15 ± 0.37^{b}
pH	5.29 ± 0.01^{a}	5.31 ± 0.01^{a}	5.34 ± 0.01^{b}	5.30 ± 0.01^{a}	5.37 ± 0.01^{c}	5.45 ± 0.01^{d}	5.30 ± 0.01^{a}	5.36 ± 0.01^{c}	5.48 ± 0.01^{e}
BI	0.514 ± 0.002^{a}	0.591 ± 0.005^{b}	0.623 ± 0.002^{c}	0.579 ± 0.003^{d}	0.655 ± 0.002^{e}	0.677 ± 0.004^{f}	0.551 ± 0.003^{g}	0.652 ± 0.001^{e}	0.859 ± 0.004^{h}

II- Study of the phenolic compounds content in alcoholic extracts

The phenolic compound concentration was determined by measuring the total polyphenols, flavonoids, and tannins contents of all samples under the circumstances specified in the table below (Table 26).

Table 26: Effect of roasting Phenolic compounds content of roasted date seed extracts



Run	**Code name**	**X_1(°C)**	**X_2(min)**	**Total Phenolic Content (mg GAE/g)**	**Total Flavonoids Content (mg QE/g)**	**Total Tanins Content (mg CE/g)**
1	RDS1	180	15	$96,07 \pm 0.23^{a}$	$30,46 \pm 0.19^{a}$	$16,7 \pm 0.31^{a}$
2	RDS2	200	15	$153,83 \pm 0.89^{b}$	$34,27 \pm 0.73^{bd}$	$29,87 \pm 0.98^{b}$
3	RDS3	160	15	$110,41 \pm 0.31^{c}$	$32,28 \pm 0.28^{ab}$	$16,37 \pm 0.53^{a}$
4	RDS4	200	45	$226,89 \pm 0.71^{d}$	$31,45 \pm 0.21^{a}$	$34,37 \pm 0.84^{c}$
5	RDS5	160	30	$142,87 \pm 0.45^{e}$	$31,76 \pm 0.64^{ab}$	$23,37 \pm 0.81^{d}$
6	RDS6	180	45	$198,95 \pm 0.35^{f}$	$47,73 \pm 0.49^{c}$	$35,53 \pm 0.63^{c}$
7	RDS7	180	30	$123,35 \pm 0.56^{g}$	$36,01 \pm 0.42^{d}$	$19,91 \pm 0.84^{e}$
8	RDS8	180	30	$120,17 \pm 0.78^{h}$	$37,6 \pm 0.82^{e}$	$18,12 \pm 0.53^{ae}$
9	RDS9	200	30	$170,25 \pm 0.51^{i}$	$32,23 \pm 0.73^{ab}$	$29,53 \pm 0.27^{b}$
10	RDS10	160	45	$142,13 \pm 0.83^{e}$	$51,89 \pm 0.20^{f}$	$43,37 \pm 0.85^{g}$
11	RDS11	180	30	$130,65 \pm 0.73^{k}$	$38,6 \pm 0.42^{de}$	$19,37 \pm 0.69^{ae}$

Data are the mean of three repetition ± SEM, the means followed by similar letters exposingin the same colum are not different (P<0.05). TPC (mg GAE/g extract); TFC(mg QE/g extract) , TTC(mg QAE/g extract) ; X_1 *roasting temperature(°C) ;* X_2 *roasting time(min).*

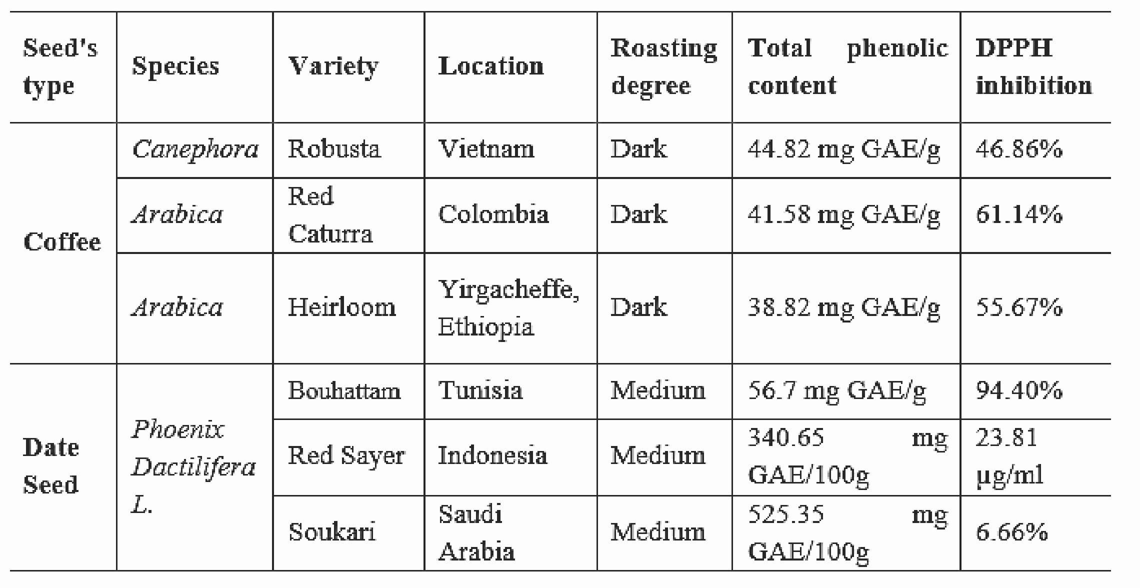
During the roasting process one of the occurrence reactions is the Maillard reaction, it involves sugars and amino compounds by leading to toxic compounds such as acrylamide from foods rich on starch, heterocyclic amines from protein-rich food and high levels of advanced glycation end products from dairy and baked products. In fact, they have shown mutagens and carcinogens characters, therefore date seed polyphenols can act as powerful inhibitors of harmful substances.

1- Determination of total Phenolic content (TPC)

The level of phenolic content shows a significant difference between samples (RDS1 to RDS11) ranging from 96.07 to 226.89 mg GAE/g. According to Table 16, the sample RDS4 had the highest content of phenolic compounds with 226.89 mg GAE/g followed by RDS6 with 198.95 mg GAE/g and RDS9 with 170.25 mg GAE/g. Then the RDS1 and RDS3 recorded the lowest content, with 96.07mg GAE/g and 110.41 mg GAE/g, respectively.

The total phenols increased with the increase in roasting temperature and time. This hypothesis pertains to all samples. In fact, three samples (RDS4, RDS6 and RDS9) showed the highest phenolic content since they received the highest processing time and temperature.

Table 27: Total phenolic content of medium roasting Coffee and date seed [144, 145, 146,61]



Seed's type	Species	Variety	Location	Roasting degree	Total phenolic content	DPPH inhibition
Coffee	*Canephora*	Robusta	Vietnam	Dark	44.82 mg GAE/g	46.86%
	Arabica	Red Caturra	Colombia	Dark	41.58 mg GAE/g	61.14%
	Arabica	Heirloom	Yirgacheffe, Ethiopia	Dark	38.82 mg GAE/g	55.67%
Date Seed	*Phoenix Dactilifera L.*	Bouhattam	Tunisia	Medium	56.7 mg GAE/g	94.40%
		Red Sayer	Indonesia	Medium	340.65 mg GAE/100g	23.81 µg/ml
		Soukari	Saudi Arabia	Medium	525.35 mg GAE/100g	6.66%

At dark roasting degree, the coffee seeds of Robusta, Red Caturra, and Heirloom had a TPC of 44.82 mg GAE/g, 41.58 mg GAE/g and 38.82 mg GAE/g, respectively (Table 27). Compared to our results at the same roasting degree, date seeds contain five times more phenolic compounds than an aqueous extract of roasted coffee seeds. Other studies indicate that there is a difference in TPC from one variety to another. This difference may be due to variety, geographic origin, maturity, soil type, and extraction process.

2- Determination of total Flavonoid Content (TFC):

In Table 26, the total flavonoid content of RDS extract varied from 30.46 mg CE/g to 51.89 mg CE/g. The RDS10 had the highest level of flavonoid content with 51.89 mg CE/g, followed by the RDS6 with 47.73 mg CE/g, the RDS11 with 38.60 mg CE/g, and the RDS2 with 34.27 mg CE/g. The five remaining samples (RDS3, RDS9, RDS5, RDS4 and RDS1) showed a similarity in the flavonoid content, which ranged from 30.46 to 32.28 mg CE/g. According to Kreicbergs et al.[147], roasted coffee of Heirloom and Robusta had 50 and 60 mg QE/ 100g in total flavonoid content, which is very low compared to TFC in RDS.

3- Determination of total tannins content (TTC):

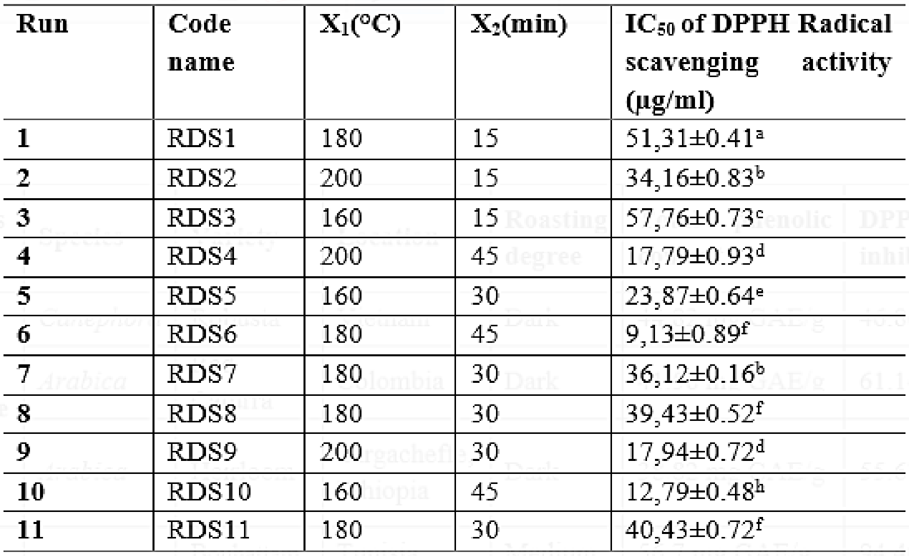
The results of TTC in Table 26 can be divided into three distinct groups. The first group was the most predominant, with 34.37 mg CE/g, 35.53 mg CE/g and 43.37 mg CE/g for RDS2, RDS10 and RDS3. Then the second group, ranging from 23.37 to 29.87 mg CE/g containing three samples coded RDS5, RDS8 and RDS6. Finally, the last group with the lowest content of TTC has RDS9, RDS4, and RDS7 with 16.37 mg CE/g, 16.70 mg CE/g and 18.12 mg CE/g.

In a study of eighteen brands of roasted coffee conducted by Kreicbergs et al.[147], the TTC registered the highest value of 83.13 CE/100g yet it remains significantly lower than those recorded for RDS samples.

III- Evaluation of the antioxidant activity via the inhibition of DPPH radicals

The DPPH test was performed to evaluate the antioxidant activity of roasted date seed extracts. The results were regrouped in Table 28.

Table 28: DPPH radical scavenging activity of roasted date seed

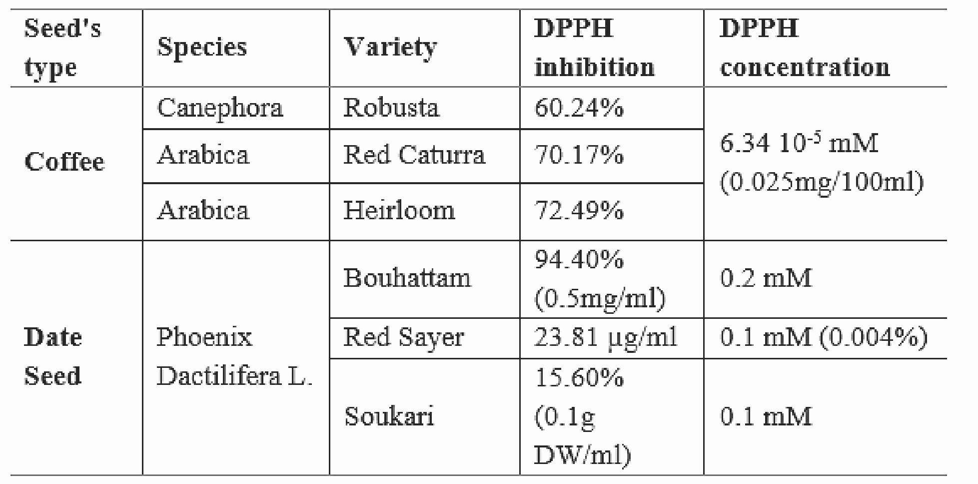


Run	Code name	X_1(°C)	X_2(min)	IC_{50} of DPPH Radical scavenging activity (µg/ml)
1	RDS1	180	15	51,31±0.41^a
2	RDS2	200	15	34,16±0.83^b
3	RDS3	160	15	57,76±0.73^c
4	RDS4	200	45	17,79±0.93^d
5	RDS5	160	30	23,87±0.64^e
6	RDS6	180	45	9,13±0.89^f
7	RDS7	180	30	36,12±0.16^b
8	RDS8	180	30	39,43±0.52^f
9	RDS9	200	30	17,94±0.72^d
10	RDS10	160	45	12,79±0.48^h
11	RDS11	180	30	40,43±0.72^f

Data are the mean of three repetitions ± SEM, the means followed by similar letters exposing in the same column are not different ($P<0.05$). DPPH IC_{50} (µg/ml); X_1 roasting temperature(°C) ; X_2 roasting time(min).

The RDS6 shows the strongest inhibition with an IC_{50} of 9.13µg/ml followed by the RDS10 with 12.79 µg/ml, then RDS4 and DRS9 with 17.79 and 17.94 µg/ml, respectively. The average inhibition was recorded for RDS4, DRS9 and RDS5 with 17.79 µg/ml, 17.94 µg/ml and 23.87 µg/ml. At the same time, the lowest was for RDS3 and RDS1 with 51.31 and 57.76 µg/ml.

Table 29: DPPH inhibition of roasted Coffee and date palm seed from the literature [144,145,146,61]



Seed's type	Species	Variety	DPPH inhibition	DPPH concentration
Coffee	Canephora	Robusta	60.24%	6.34 10^{-5} mM (0.025mg/100ml)
	Arabica	Red Caturra	70.17%	
	Arabica	Heirloom	72.49%	
Date Seed	Phoenix Dactilifera L.	Bouhattam	94.40% (0.5mg/ml)	0.2 mM
		Red Sayer	23.81 µg/ml	0.1 mM (0.004%)
		Soukari	15.60% (0.1g DW/ml)	0.1 mM

At first sight, the concentrations of DPPH in the Table 29 are different. In fact, the evaluation of antioxidant in coffee seeds required a deficient concentration of DPPH solution compared to date seeds in the range of 6.34 10^{-5} mM. Unlike coffee seed, the DPPH assay of date seeds contains a higher concentration of 0.1 and 0.2mM showing a good inhibition of 94.40% for the Bouhattam variety and an IC_{50} of 23.81µg/ml for the Red Sayer variety.

IV- Surface model's response interpretation

1- TPC response surface model interpretation.

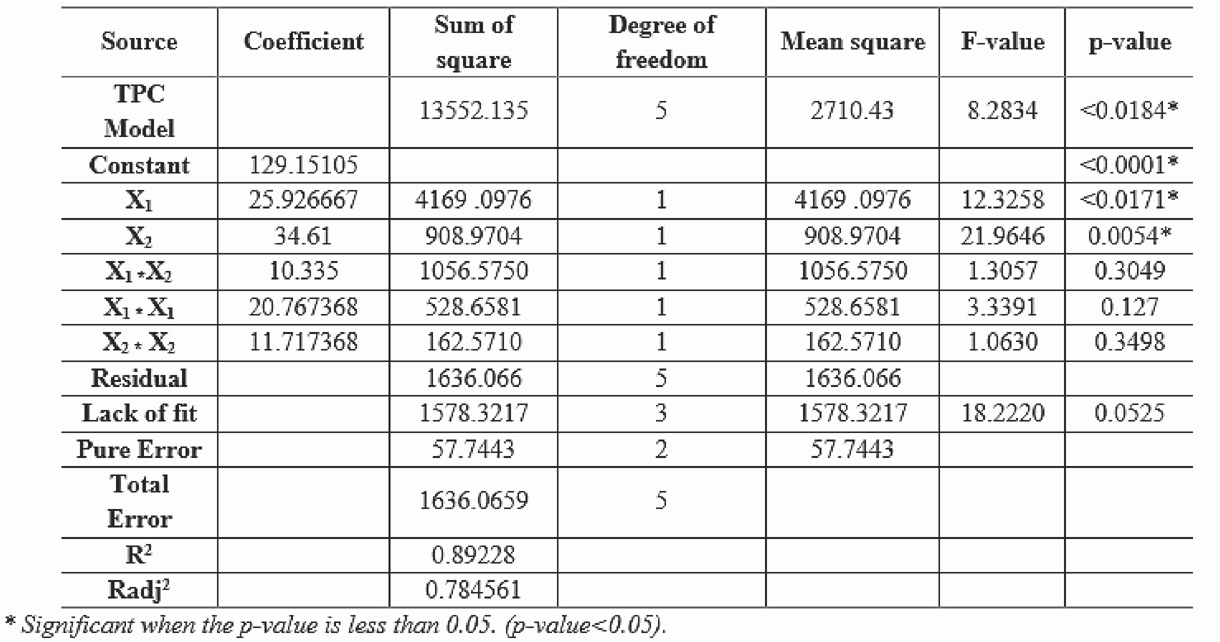
a. Model of second-order polynomial equation.

TPC levels in RDS extracts varied from 96.07±1.02 to 226.89±1.94 mg GAE /g extract in the current study. Moreover, the model was significant ($p\text{-value} < 0.0184$) based on the ANOVA findings for TPC (Table 30). The lack of fit was not statistically significant ($p\text{-value} = 0.0525$), suggesting that the model equation was adequate for predicting response values. In addition, the R^2 value was 0.89228, and the adjusted R_{adj}^2 coefficient of determination was 0.784561, indicating that the model accurately represented the real combination of all components investigated.

According to Li et al.[148], the model is appropriate if the coefficient of determination is larger than 0.75. A simplified regression model was used to develop the equation (Eq1), which predicts the influence of the factor variables on TPC content.

$$TPC = 129.15105 + 25.926667X_1 + 34.61X_2 + 10.335X_1{*}X_2 + 20.767368X_1{*}X_1 + 11.717368X_2{*}X_2 \quad \text{(Eq1)}$$

Table 30: Anova data of the regression coefficient and the terms of the model.



Source	Coefficient	Sum of square	Degree of freedom	Mean square	F-value	p-value
TPC Model		13552.135	5	2710.43	8.2834	<0.0184*
Constant	129.15105					<0.0001*
X_1	25.926667	4169 .0976	1	4169 .0976	12.3258	<0.0171*
X_2	34.61	908.9704	1	908.9704	21.9646	0.0054*
$X_1 * X_2$	10.335	1056.5750	1	1056.5750	1.3057	0.3049
$X_1 * X_1$	20.767368	528.6581	1	528.6581	3.3391	0.127
$X_2 * X_2$	11.717368	162.5710	1	162.5710	1.0630	0.3498
Residual		1636.066	5	1636.066		
Lack of fit		1578.3217	3	1578.3217	18.2220	0.0525
Pure Error		57.7443	2	57.7443		
Total Error		1636.0659	5			
R^2		0.89228				
$Radj^2$		0.784561				

** Significant when the p-value is less than 0.05. (p-value<0.05).*

The roasting temperature (X_1) exhibited a substantial positive linear effect ($p\text{-value} < 0.0171$) on TPC, whereas the quadratic $X_1{*}X_1$ had no notable effect ($p\text{-value} > 0.05$). The linear impact of roasting time X_2 was likewise clearly positive ($p\text{-value} < 0.05$). However, the interaction among the two investigated factors, $X_1{*}X_2$, had no major influence on TPC ($p\text{-value} > 0.05$).

b. Analysis of TPC using the response surface methodology (RSM).

On the resulting 3D response surface plot shown in Figure 65, the effect of roasting temperature, and time, as well as their interaction on TPC, can be viewed. Under the static roasting time (X_2), the TPC content rose when the roasting temperature (X_1) increased, and as the roasting duration (X_2) exceeded 40 minutes, the TPC content also increased rapidly, as shown in Figure 67. Therefore, maximal TPC extraction was discovered at a high roasting temperature and time.

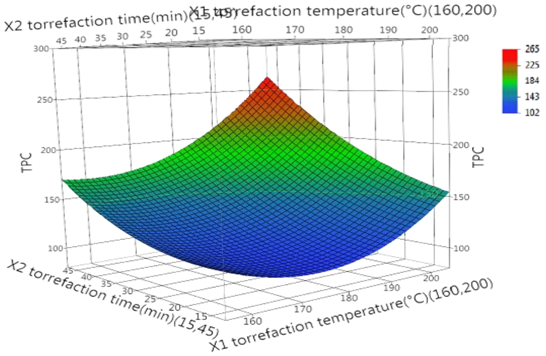
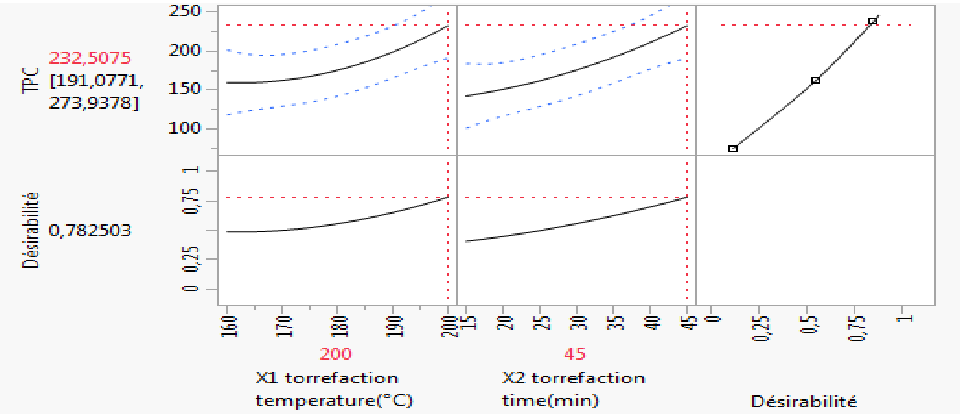


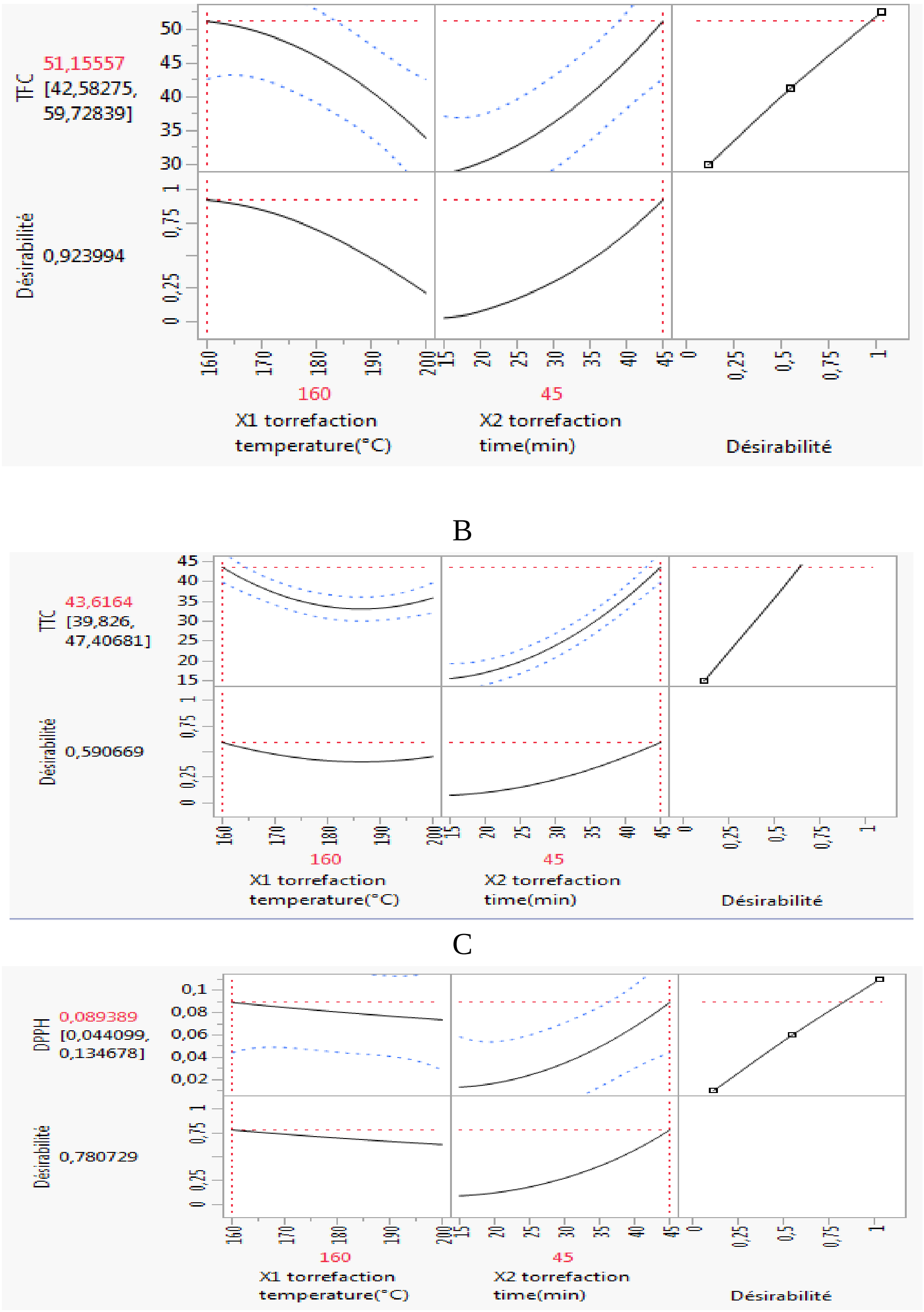
Figure 59: TPC response surface as a function of roasting parameters.

To obtain a global optimization of all variables tested, the function (d) of desirability was used to achieve the highest response in the TPC of RDS, resulting in the greatest response accuracy when the desirability was near 1 (Figure 68)[149,150,151].

As a result, the JMP predictive profiler calculated the best circumstances. The optimal roasting conditions by soxhlet extraction were when the desirability value (d=0.78) was near 1 (Figure 68) with roasting parameters of 200°C and 45 minutes and a predicted value of 232.51 mg GAE/g extract.



A



B

C

D

Figure 60: Desirability results for (A) TPC; (B) TFC; (C) TTC; (D) DPPH.

The predicted and experimental (226.89±1.94mg GAE/g extract) responses were nearly identical. As a result of these findings, the model appears to be valid for predicting TPC from RDS soxhlet extraction. These findings corroborate previous investigations.

According to Chandrasekara et al. [152], roasting at a high temperature (130°C) for 33 minutes enhanced the TPC compared to unprocessed seed. Yu et al. [114] discovered that roasting at 175°C for five minutes raised the TPC (in either aqueous or alcoholic solvent) from peanut skin by around 35.9% compared to the crude. According to Arlorio et al. [153] and Yin et al. [154], the TPC of a soluble extract of hazelnut skin raised more at a roasting time of 20 minutes than 10 minutes (at 180°C), and the TPC grew about 3.4 times at a temperature between 120°C and 140°C for 180 minutes, and it improved more at 140°C.

Furthermore, Jeong et al. [155] demonstrated that heat treatment raised TPC considerably. As a result, the increase in phenolic compound content can be attributed to the fact that during the roasting process, the phenolic substances can be degraded or polymerized, revealing the formation of new compounds.

2- TFC response surface model interpretation.

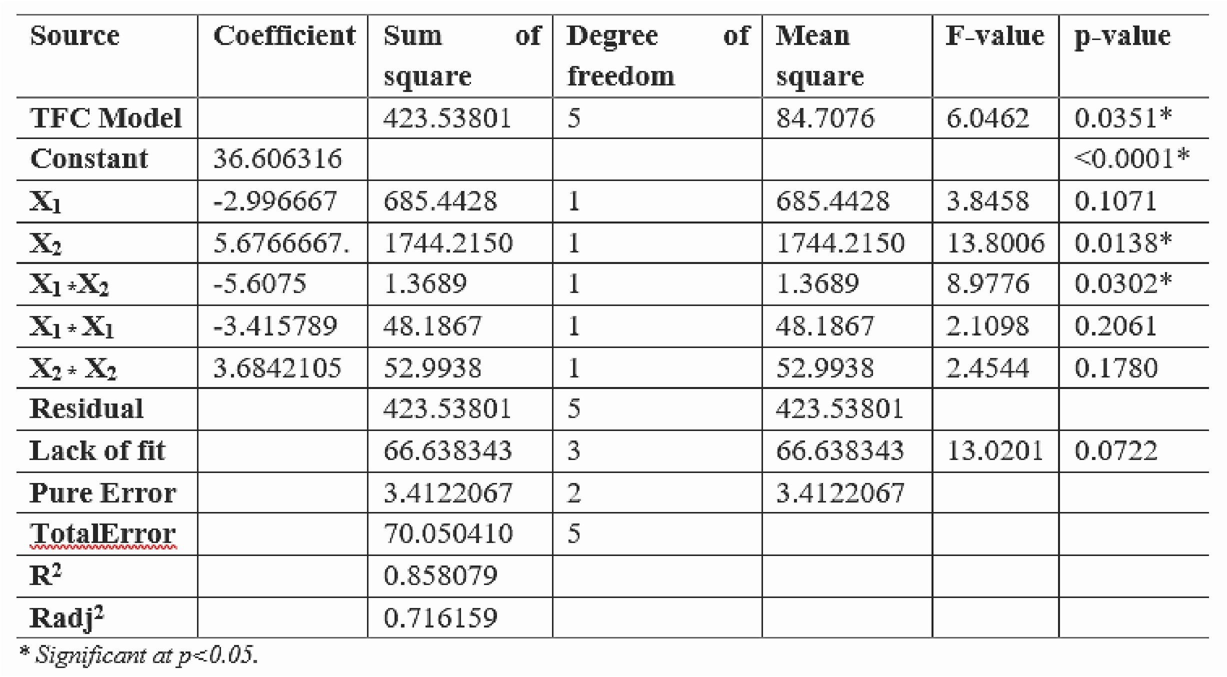
a. Model of second-order polynomial equation.

The analysis of variance (ANOVA) was performed to validate the model's adequacy and significance. Table 31 displays a high F-value (6.0462) and a low p-value (0.0351), indicating that the model was confirmed.

Furthermore, the lack of fit was not significant, having a p-value of 0.0722, suggesting that there was a significance for TFC prediction of the RDS; the inability of the model was due to the lack of fit value[156]. The coefficients of determination, R2 and adjusted Radj2, were high, being 0.858079 and 0.716159, respectively. Such values indicated that the quality of the model was valid. In fact, these numbers implied that the quality of the approximation of the model was accurate. It is given by the formula (Eq2) that expresses the relationship of the variables and predicts TFC.

$$TFC=36.606316-2.996667X_1+5.6766667X_2-5.6075X_1*X_2-3.415789X_1*X_1+3.6842105X_2*X_2 \quad (Eq2)$$

Table 31: ANOVA data for the regression coefficient and model terms.



Source	Coefficient	Sum of square	Degree of freedom	Mean square	F-value	p-value
TFC Model		423.53801	5	84.7076	6.0462	0.0351*
Constant	36.606316					<0.0001*
X_1	-2.996667	685.4428	1	685.4428	3.8458	0.1071
X_2	5.6766667.	1744.2150	1	1744.2150	13.8006	0.0138*
$X_1 * X_2$	-5.6075	1.3689	1	1.3689	8.9776	0.0302*
$X_1 * X_1$	-3.415789	48.1867	1	48.1867	2.1098	0.2061
$X_2 * X_2$	3.6842105	52.9938	1	52.9938	2.4544	0.1780
Residual		423.53801	5	423.53801		
Lack of fit		66.638343	3	66.638343	13.0201	0.0722
Pure Error		3.4122067	2	3.4122067		
TotalError		70.050410	5			
R^2		0.858079				
$Radj^2$		0.716159				

** Significant at $p<0.05$.*

In table Table 31, the roasting time (X_2) showed a significant positive linear influence on TFC with a p-value of 0.0138 and under 0.05, but no significant quadratic effect with a p-value of 0.1780. Furthermore, there was no significant negative linear influence of roasting temperature (X_1) nor a quadratic effect with p-values of 0.1071 and 0.2061, respectively. Finally, the interaction between the roasting time and roasting temperature (X_1*X_2) on TFC content was significant, with a p-value of 0.0302.

b. Analysis of TFC using RSM

Figure 69 shows the response surface plot of roasting temperature and roasting time on total flavonoid content.

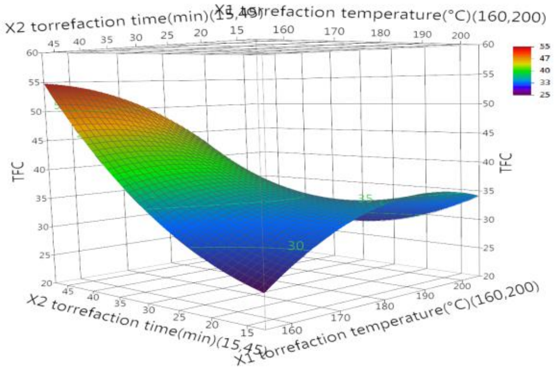


Figure 61:TFC response surface as a function of roasting parameters.

The TFC increased with the increased both the roasting time and temperature. Accordingly, the higher TFC yield was detected in regions of medium roasting temperature (160°) and long roasting time. Consequently, the optimum extraction of TFC was at: roasting temperature 160°C and roasting time 45 min, and it was assigned for the predicted response was 51.15 (mg QE/g extract) with the desirability was d=0.92 (Figure 66.B).

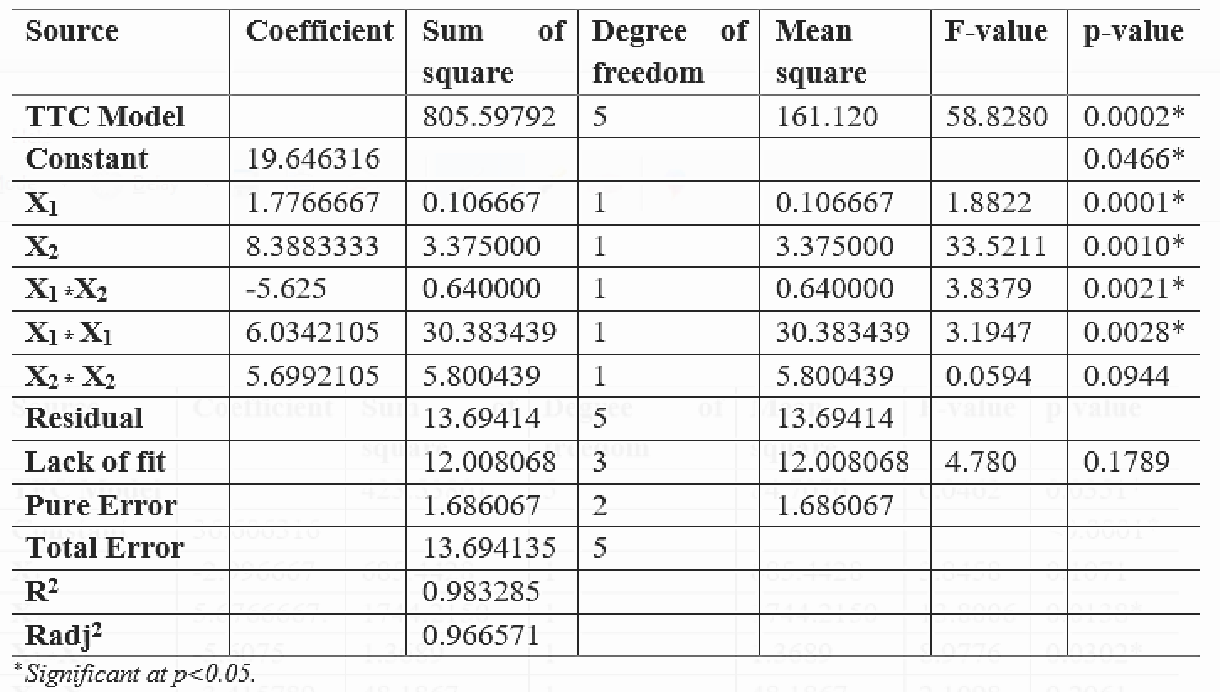
3- TTC response surface model interpretation.

a. Model of second-order polynomial equation.

The regression coefficients and their significance for tannin concentration are shown in Table 32. The regression model was significant in statistical terms, with a p-value of 0.0002. Additionally, the R2 coefficient (0.983285) and the lack of fit values (0.1789) generated from for the TTC response were not significant (p > 0.05), indicating that the model can elucidate all outcomes. As a result, the optimal roasting parameters incorporated the response variable, and the Radjs2 was 0.966571, showing that the model accounts for 96.65% of the variability. Therefore, the equation (Eq 3) was applied as a model of a second-order polynomial.

$$TTC=19.646316+1.7766667X_1+8.3883333X_2-5.625X_1*X_2+6.0342105X_1*X_1-5.6992105X_2*X_2$$
(Eq3)

Table 32: Anova data of the regression coefficient and the terms of the model.



Source	Coefficient	Sum of square	Degree of freedom	Mean square	F-value	p-value
TTC Model		805.59792	5	161.120	58.8280	0.0002*
Constant	19.646316					0.0466*
X_1	1.7766667	0.106667	1	0.106667	1.8822	0.0001*
X_2	8.3883333	3.375000	1	3.375000	33.5211	0.0010*
$X_1 * X_2$	-5.625	0.640000	1	0.640000	3.8379	0.0021*
$X_1 * X_1$	6.0342105	30.383439	1	30.383439	3.1947	0.0028*
$X_2 * X_2$	5.6992105	5.800439	1	5.800439	0.0594	0.0944
Residual		13.69414	5	13.69414		
Lack of fit		12.008068	3	12.008068	4.780	0.1789
Pure Error		1.686067	2	1.686067		
Total Error		13.694135	5			
R^2		0.983285				
$Radj^2$		0.966571				

*Significant at $p<0.05$.

With a p-value of 0.05, the quadratic impact of roasting heat X_1 * X_1 was positively meaningful for TTC, while its linear effect was also significant (p-value = 0.001). Likewise, roasting time X_2 for TTC was significant (p-value = 0.0001) but not its quadratic impact (p-value =0.0944).

b. Analysis of TTC using RSM

The 3D response surface of a regression equation (Eq3) was created via RSM to demonstrate the influence of roasting heat and duration on TTC (Figure 68). The tannin concentration climbed once the roasting heat reached 180°C, then swiftly declined. The same phenomenon applies when the roasting duration ranges from 25 to 45 minutes.

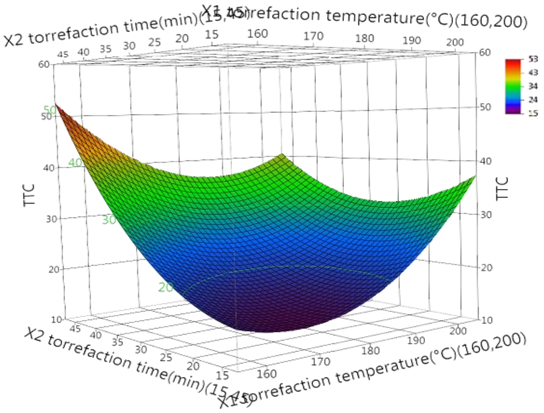


Figure 62:Response surface graph of TTC influenced by roasting parameters.

As a result, TTC extraction was optimal at roasting parameters of 160°C for 45 minutes that prompted predicted responses to be 43.61 mg QE/g extract and a desirability of 0.59 (Figure 68). These findings support those of Li et al.[148] who found a significant concentration of condensed tannins in the ethanol extract, obtained after roasting at 200°C for 20 minutes.

4- DPPH assay response surface model interpretation.

a. Model of second-order polynomial equation.

The ANOVA findings in Table 33 summarize the DPPH test done from RSM design. This model had a significance feature with a p-value of 0.0174. Furthermore, R2 and Radj2 are also 0.894724 and 0.789449, respectively, which confirms the model is adequate as it is greater than a threshold limit of 0.75. Furthermore, the lack of fit, with a p-value of 0.0568, confirmed the model's adequacy in predicting the antioxidant capacity of RDS. As a result, a second-order polynomial model was used (Eq4).

$DPPH(IC_{50})=34.836842-4.088333X_1-17.25333X_2+7.15X_1*X_2 8.197105X_1*X_1+1.1178947X_2*X_2$ (Eq4)

Table 33: Anova data of the regression coefficient and the terms of the model.

Source	Coefficient	Sum of square	Degree of freedom	Mean of square	F-value	p-value
DPPH Model		2264.1738	5	452.835	8.4989	<0.0174*
Constant	34.836842					<0.0001*
X_1	-4.088333	100.2868	1	100.2868	1762.768	<0.0001*
X_2	-17.25333	1786.0651	1	1786.0651	510.5521	<0.0001*
$X_1 * X_2$	7.15	204.4900	1	204.4900	86.3369	0.0002*
$X_1 * X_1$	-8.197105	170.2211	1	170.2211	38.6788	0.0016*
$X_2 * X_2$	1.1178947	3.1659	1	3.1659	0.3606	0.5743
Residual		266.4087	5	266.4087		
Lack of fit		256.23131	3	256.23131	16.7843	0.0568
Pure Error		10.17740	2	10.17740		
Total Error		266.40871	5			
R^2		0.894724				
$Radj^2$		0.789449				

According to the ANOVA statistical analysis for the DPPH test, the roasting heat (X_1) and roasting duration (X_2) showed a substantial negative linear influence on the IC_{50}, with p-values both under 0.0001. Furthermore, with a p-value of 0.0002, their interaction was significant, but their quadratic impact ($X_2 * X_2$) was not significant since their p-value was above 0.005.

b. Analysis of IC_{50} (DPPH test) using the RSM.

The RSM was used to create the 3D graph of equation 75 (Eq. 5) to show the influence of roasting heat (X1) and duration (X2) with their interaction (X1*X2) on the IC_{50} (Figure 71). The effect of IC50 values on antioxidant potential is known to be inversely proportional.

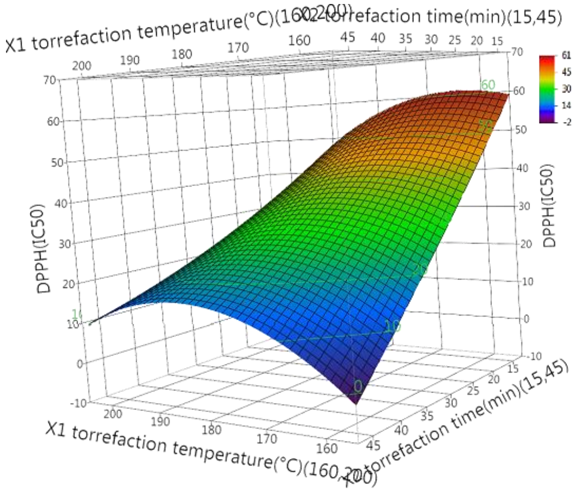


Figure 63: Response surface graph of IC50 (DPPH test) influenced by roasting parameters.

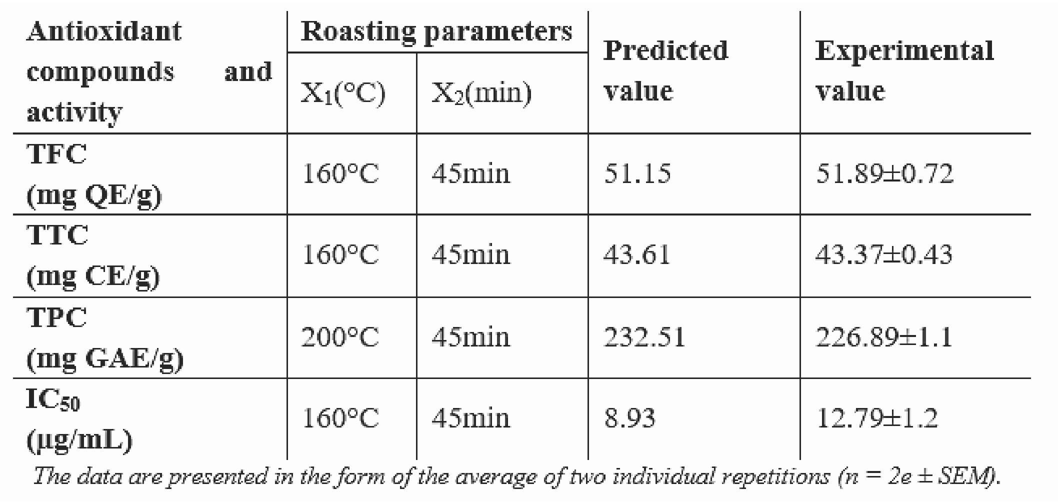
Better antioxidant capacity was observed as the increase in temperature and time of roasting ensued. Therefore, the DPPH test had the maximum antioxidant capacity at the roasting parameters of 160°C for 45 minutes, with a response that is predicted at 8.93 µg/mL and a desirability of 0.78 (Figure 68). Lin et al. [157] also revealed that ethanol extracts of almond (Prunus duclis) exhibited strong antioxidant capacity at high temperatures of 200°C for 20 minutes.

Furthermore, Chandrasekara et al. [152] observed that the scavenging of DPPH radicals by the soluble phenolic extract from testa seeds rose drastically with higher roasting temperatures, and this increment was related to the byproducts of the Maillard reaction. In fact, the reaction between reducing sugars and amino acids occurs through the roasting process, creating new substances that contribute to the food's flavor, color, and TPC [152].

5- Collation of experimental and predicted data.

The verification studies of our responses are summarized in Table 34: DPPH inhibition capacity (IC_{50}), TPC, TFC, and TTC. These tests were carried out under optimal response settings and experimental ranges. These findings indicate that the experimental response is close to the expected value.

Table 34: Predicted and experimental outcomes under optimal circumstances.



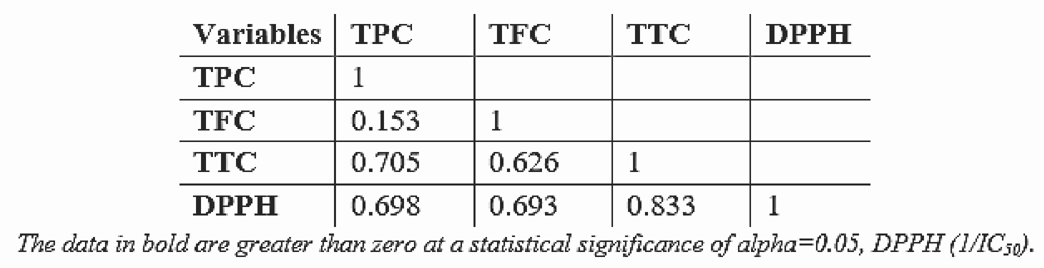
Antioxidant compounds and activity	Roasting parameters		Predicted value	Experimental value
	X_1(°C)	X_2(min)		
TFC (mg QE/g)	160°C	45min	51.15	51.89±0.72
TTC (mg CE/g)	160°C	45min	43.61	43.37±0.43
TPC (mg GAE/g)	200°C	45min	232.51	226.89±1.1
IC_{50} (µg/mL)	160°C	45min	8.93	12.79±1.2

The data are presented in the form of the average of two individual repetitions (n = 2e ± SEM).

6- Matrix of correlation.

Table 35 shows the correlation coefficient data among all examined responses, and Table 36 presents the p-value of these correlation coefficients. Furthermore, the DPPH ($1/IC_{50}$) represents the ability to inhibit the free DPPH radicals.

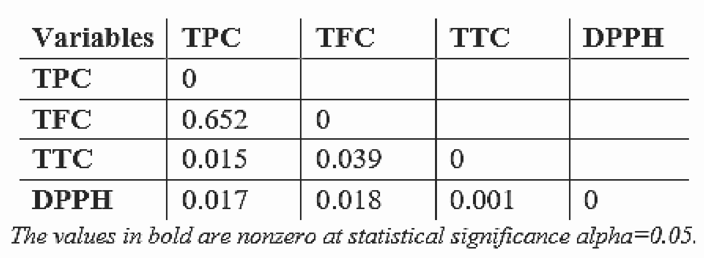
Table 35:Pearson coefficients were generated via a correlation matrix of antioxidant capacity and compounds present in RDS extracts.



Variables	TPC	TFC	TTC	DPPH
TPC	1			
TFC	0.153	1		
TTC	0.705	0.626	1	
DPPH	0.698	0.693	0.833	1

The data in bold are greater than zero at a statistical significance of alpha=0.05, DPPH (1/IC_{50}).

Table 36: p-values of all variables' correlation matrix coefficients.



Variables	TPC	TFC	TTC	DPPH
TPC	0			
TFC	0.652	0		
TTC	0.015	0.039	0	
DPPH	0.017	0.018	0.001	0

The values in bold are nonzero at statistical significance alpha=0.05.

We discovered a considerable positive correlation between TPC and antioxidant efficacy in Tables 35 and 36. The correlation coefficients of TPC with TTC and the scavenger DPPH were 0.705 and 0.698, respectively. These positive correlations are predicated on the notion that antioxidant efficacy in RDS is dependent on the availability of phenolic substances, with results that are consistent with those reported in other publications [46,47,48]. TFC and DPPH were likewise shown to have a substantial positive connection, with a correlation value of 0.693. As a result, the substantial positive correlation (with a p-value under 0.05) across TFC and TTC validated these findings (r^2 = 0.626). TTC with antioxidant efficacy also had a significant p-value (p-value under 0.05), indicating that tannins assist in this bioactivity.

7- Principal component analysis (PCA).

The factorial plan shown in Figure 72 was used to project the studied and experimental responses for analysis. The overall percentage (93.65%) implies that it is representative of the variable since it surpasses 50%. Furthermore, both axes could explain all the findings, with the first (F1) and second (F2) principal components explaining 72.43% and 21.53% of the data, respectively. The plan generated by the F1 and F2 axes explains the connections between all variables evaluated. Moreover, the F1 axis shows positive correlations among TPC, TFC, TTC, and DPPH.

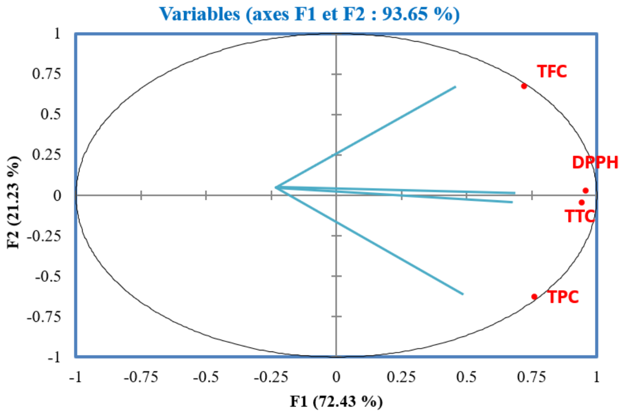


Figure 64: Factorial plan for performing PCA on the concentrations (TPC, TFC, TTC, and DPPH) of several date palm seeds Phoenix Dactilifera L. extracts.

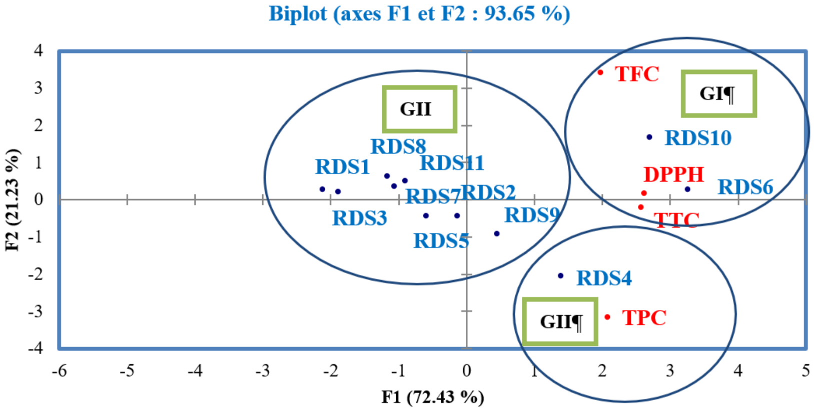


Figure 65: Individual variable projection on the factorial plan (F1xF2). DPPH (1/DPPH IC50); GI: Group I; GII: Group II, GII Group III.

Generally, according to responses given (Figure 73), RDS extracts were clustered into three groups. Two extracts having the maximum values for TFC and TTC, and whose were highly active with DPPH, were included in Group I (RDS6 and RDS10).

Group II contained one extract (RDS4) characterized by a strong TPC value. Therefore, its antioxidant activity was moderate compared to the first group. Group III consisted of eight extracts (RDS1, RDS2, RDS3, RDS5, RDS7, RDS8, RDS9, and RDS11) characterized by low TPC and TFC levels and low antioxidant capacity when compared to the other groups' extracts. Finally, Group I extracts have a long roasting time (45 minutes) and a temperature range of 160°C to 180°C, which are ideal conditions for the extraction of bioactive components with the highest antioxidant potential.

8- Hierarchical clustering analysis HCA.

Extracts were categorized using the squared Euclidean and Wards techniques to determine consistency measures, according to HCA. As shown in Figure 74, HCA was employed to examine the relationship and similarity among the eleven extracts based on antioxidant activity results from the DPPH test. Eleven extracts were split into three clusters based on this data.

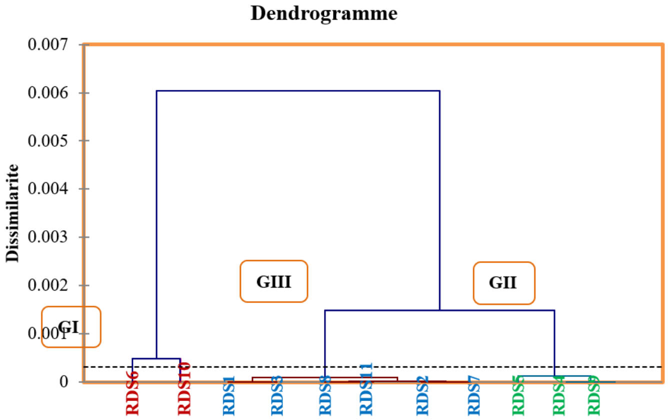


Figure 66: Dendrogram of the extracts tested found by HCA using antioxidant capacity. CI: Cluster I; CII: Cluster II; C III; Cluster III.

Cluster I contains two extracts named RDS10 and RDS6, both constituting 18.18% of the total extracts, with high TFC and TTC averages and high antioxidant capacity compared to Cluster II. Cluster II consisted of three extracts, RDS5, RDS4, and RDS9, accounting for 27.27% of the total extracts, and the mean value TPC of these extracts was the highest. They also have moderate antioxidant activity as measured by the DPPH assay. From the cluster analysis, Cluster III contained six extracts, named RDS1, RDS2, RDS3, RDS7, RDS8, and RDS11, accounting for 54.54% of the total extracts, with low mean values of the antioxidant compounds TPC, TFC, and TTC, respectively, and low antioxidant activity compared to Clusters I and II. These findings are consistent with the PCA data, showing similar trends in the distribution of all extracts on the score plots. Moreover, the PCA results corroborated the HCA results.

9- Chi-squared automatic interaction detector (CHAID).

CHAID is a very useful technique for finding out which variables are most helpful in separating the samples under study into known sets. Here, the CHAID method was used to analyze eleven extracts using antioxidant activity, providing a model to differentiate these extracts (Figure 75).

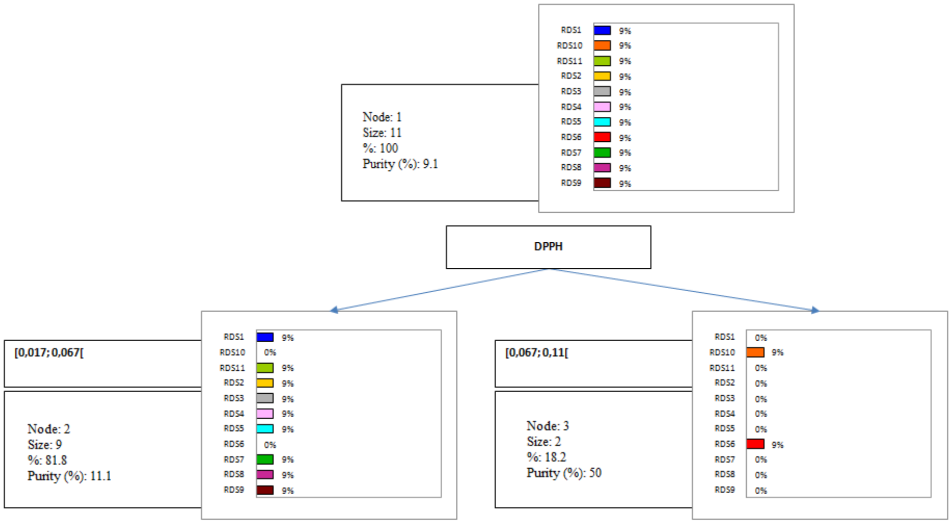


Figure 67: A classification tree was obtained from CHAID for the samples extracted.

Using antioxidant activity, CHAID achieved a correct classification rate of 18.18% for these extracts. The process uses a basic algorithm to build a non-binary tree that relies on a chi-square test to find the best split. Regarding the findings, it should be emphasized that the RDS6 and RDS10 extracts have significant antioxidant capacity compared to the other extracts. The great interest in the CHAID analysis stems from the fact that the correct classification was made according to the adjacent classification of samples based on antioxidant activity.

Conclusion

In this study, various analysis of roasted date seeds were focused such as extraction yields, pH, Browning Index, phenolic compounds content, flavonoid content, tannin content, and antioxidant activity.

The analysis of extraction yields, pH, and Browning Index was conducted at three different roasting temperatures (160°C, 180°C, and 200°C) and three roasting times (15, 30, and 45 minutes). The results showed that the highest extraction yield of 7.75% was achieved at 180°C for 45 minutes. Although the pH value slightly increased from 5.29 to 5.48, the roasting process led to slight increases in both extraction yield and pH. These changes were likely due to the breakdown of insoluble polymers and the softening of the date seed texture under high heat conditions.

The study also investigated the phenolic compounds in the roasted date seeds, including total polyphenols, flavonoids, and tannins. The total phenolic content varied significantly across samples, ranging from 96.07 to 226.89 mg GAE/g, with the highest content found in RDS4 (226.89 mg GAE/g). Thus, phenolic content increases tannin and flavonoid concentrations at higher roasting temperatures and for longer roasting times. The roasted date seeds contained much higher levels of phenolic compound content compared to the roasted coffee seeds. The flavonoids were determined in a lower range (30.46-51.89 mg CE/g), where a sample containing the highest content was RDS10. Tannin content varied from 16.37 mg CE/g to 43.37 mg CE/g, with some samples showing significantly higher levels than those found in roasted coffee.

The antioxidant activity of the roasted date seed extracts was evaluated using the DPPH radical scavenging test. The RDS6 sample showed the strongest antioxidant inhibition, with an IC50 value of 9.13 µg/ml, followed by RDS10 and RDS4. In comparison, the antioxidant activity of date seeds was found to be significantly higher than that of coffee seeds, suggesting that date seeds are a potent source of antioxidants.

In conclusion, the roasting of date seeds at higher temperatures for more extended periods significantly increases the phenolic and antioxidant content of the seeds. These findings allow us to conclude that roasted date seeds can be a valuable source for bioactive compounds that can have significant health effects, including antioxidant activity.

Chapter V : Effect of date seed Phoenix Dactilifera L. extract as antioxidant on the quality and the oxidative stability of cactus seed oils Opuntia Ficus Indica during storage

The second chapter's results demonstrate that date seed extract has significant antioxidant activity, even better than ascorbic acid. So the purpose of the study described in this chapter is to employ a natural antioxidant to enhance an oil that tends to oxidize easily and compare it with a standard antioxidant (ascorbic acid) against a control. Thus we will evaluate the oxidative stability of the studied oil using quality indexes and evaluating its chemical composition in fatty acids, sterols and tocols.

I- Schaal oven test

An oven test at 60°C was conducted to study thermo-oxidation effects on quality parameters and chemical composition of cactus oil enriched with antioxidant. Three different types of samples were stored for four weeks (Figure 59).

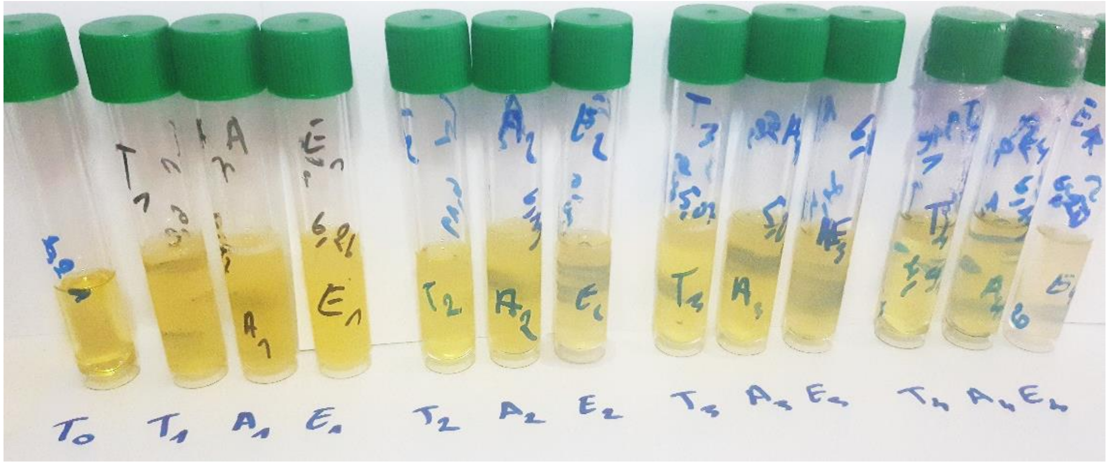


Figure 68: COO, COAA and CODSE samples

The first one is a control sample with Cactus Oil Only (COO). The second was a Cactus Oil fortified with 50 ppm of Ascorbic Acid (COAA). The final one was a Cactus Oil with 50ppm of Date Seed Extract (CODSE). Sampling was done at 0, 1, 2, 3 and 4 weeks.

II- Evolution of cactus seed oil’s quality parameters during the thermal process

1- Determination of quality indexes

The oxidative stability was assessed by determining oil quality indicators using peroxide value (PV), iodine value (IV), saponification value (SV), FFA, K_{232} and K_{270}.

a. Free fatty acids Index (FFA)

After one week, the FFA of COO and COAA increased by 0.26% (0.037%/day) and 0.22% (0.031%/day) while CODSE only increased by 0.06% (0.008%/day). A significant increase was then observed for all samples (Figure 60). At the end of the process, the COO's FFA was 2.41% (0.103%/day), the COAA had 1.99% (0.05%/day), and the CODSE has 1.61% (0.006%). From this it can be concluded that the FFA of COO and COAA has reached the limit of 2% for cold-pressed oils, whereas CODSE has not exceeded (Codex Alimentarius Commission 2009).

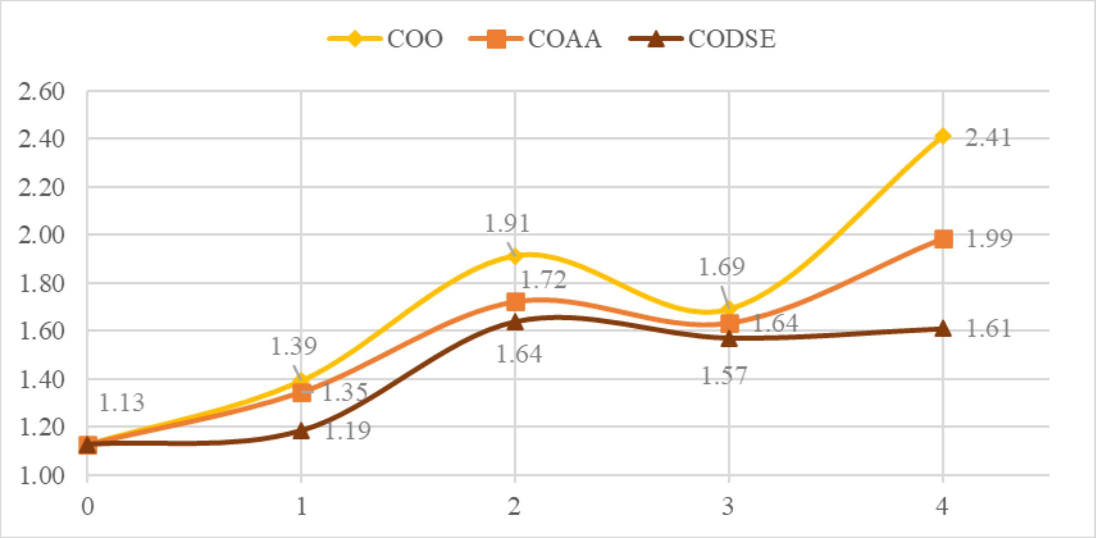


Figure 69: Evolution of FFA (%) during storage for four weeks

b. Peroxide value (PV)

Peroxide value (PV) measures the level of hydroperoxide in the oil and is a valuable tool for the indication of a commencement period of oxidation. Furthermore, PV reaches the highest level during the progression of oxidation and following this stage (secondary oxidation), the decomposition rate of hydroperoxides surpasses the rate of their development.

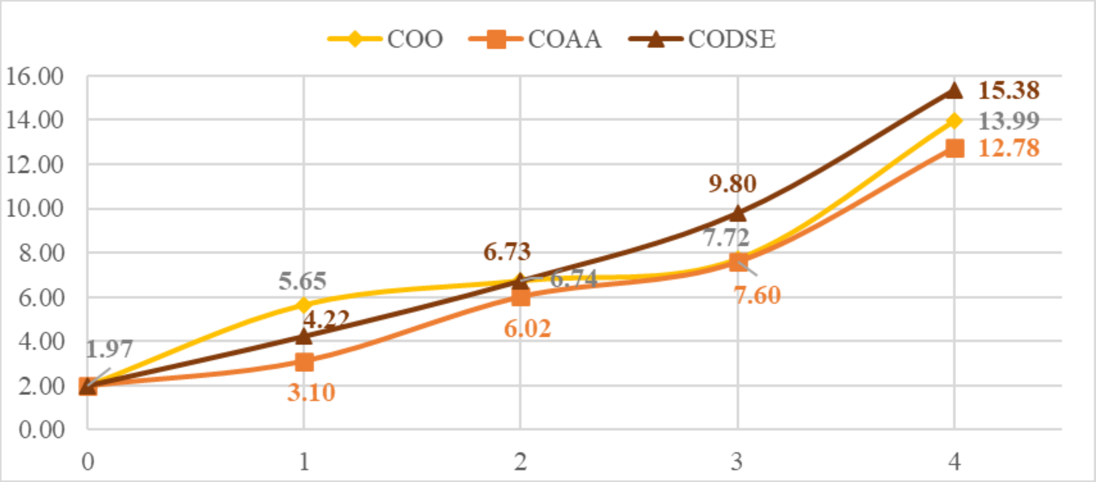


Figure 70: Peroxyde Value (meq O_2/kg) evolution during storage

Peroxide value is also used to test oxidative rancidity in oils and fats. PV started from 1.97 meq O_2/kg and finished with 12.78 meq O_2/kg as the lowest value recorded for oil enriched with ascorbic acid (Figure 61).

2- Determination of primary and secondary oxidation products

a. Determination of conjugated dienes : K_{232}

The formation of primary compounds of oxidation such as hydroperoxides coincided with the increase of absorptivity at 232nm.

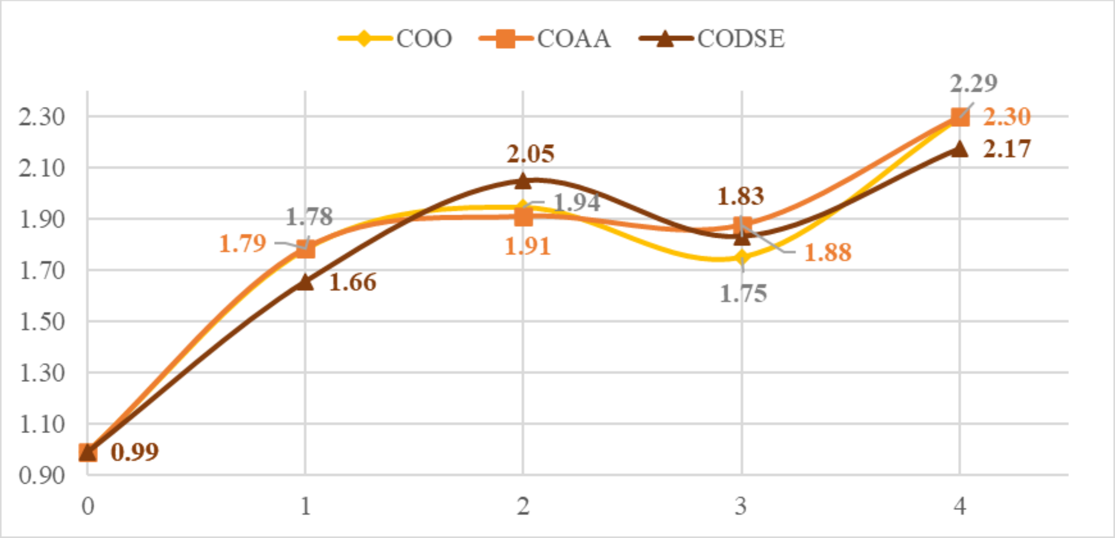


Figure 71: Evolution of K_{232} during storage

Formation of primary compounds of oxidation occurred initially at a lower rate. In fact, the extinction coefficient at 232 nm passed from ~0.99 to ~2.30. In fact, after 28 days in an oven, specific extinction at 232 nm became much higher for COO and COAA, while CODSE showed the least increase at the same time., supporting its role in retarding primary oxidation. (Figure 62).

b. Determination of secondary oxidation products : K_{270}

The primary products of oxidation are not stable under heating and then their degradation could promote the formation of secondary product of oxidation that absorb at about 270 nm. The most abundant secondary products are compounds with carbonyl group such as aldehyde and ketone, their formation becomes more favorable during the thermal process.

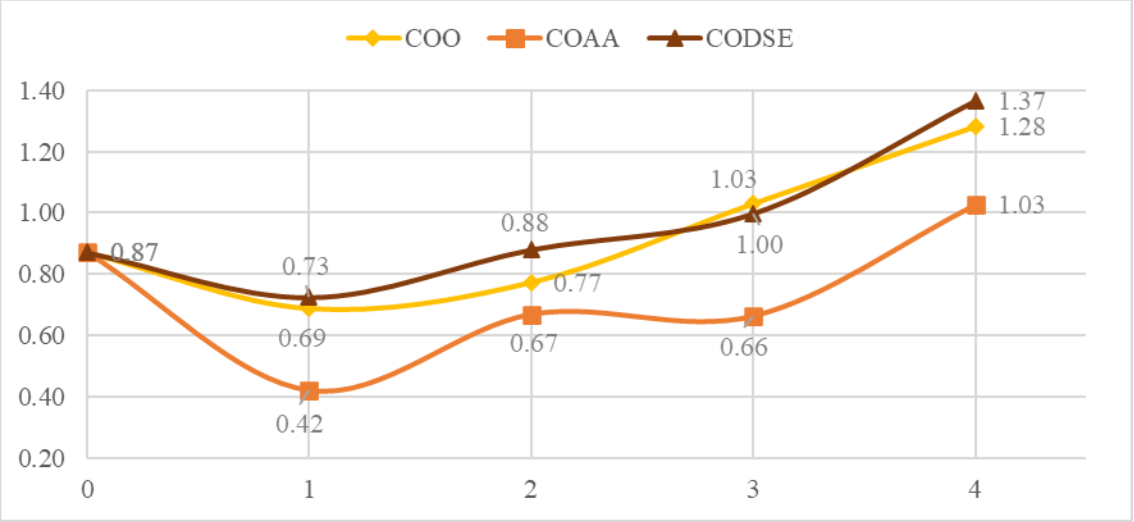


Figure 72: : Evolution of K_{270} during storage

After two weeks, all samples showed a good oxidative stability (Figure 63). However, from the third week, there was a significant increase for COO and CODSE until the last week, with the highest values of 1.28 and 1.37, respectively. On the other hand, the COAA didn't show a significant increase and the final week recorded a value of 1.03, suggesting that ascorbic acid may delay secondary oxidation.

III- Evolution of cactus seed oil's chemical composition during the thermal process

1- Fatty acids composition

Oxidative stability of oils depends mainly on their fatty acids composition, i.e. degree of unsaturation, and presence of antioxidant. Cactus seed oil is mainly composed of linoleic (C18:2) and oleic (C18:1) known as omega 6 and omega 9, they constitute more than 80% of fatty acids (Figure 64 and Figure 65).

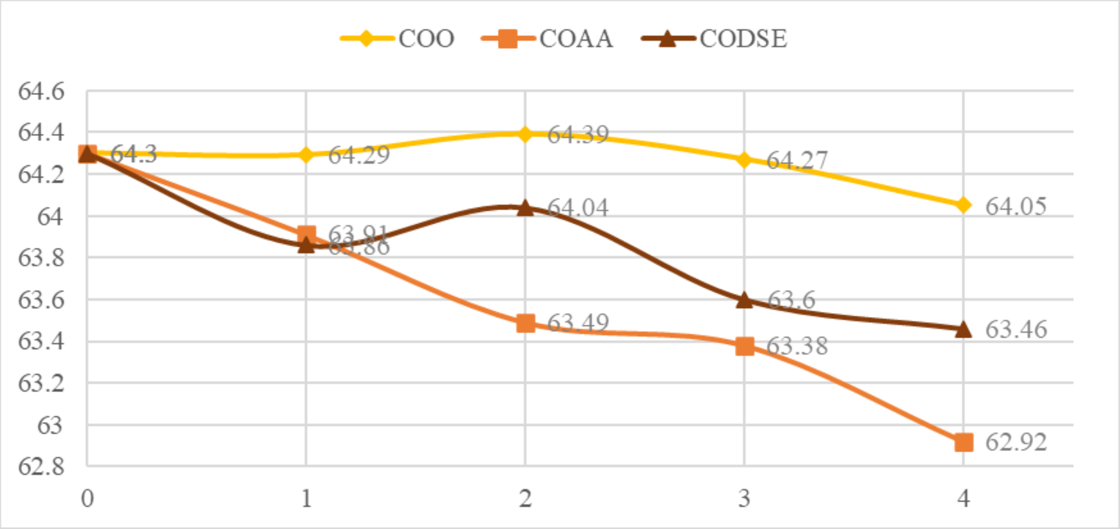


Figure 73: The evolution of omega 6 during storage

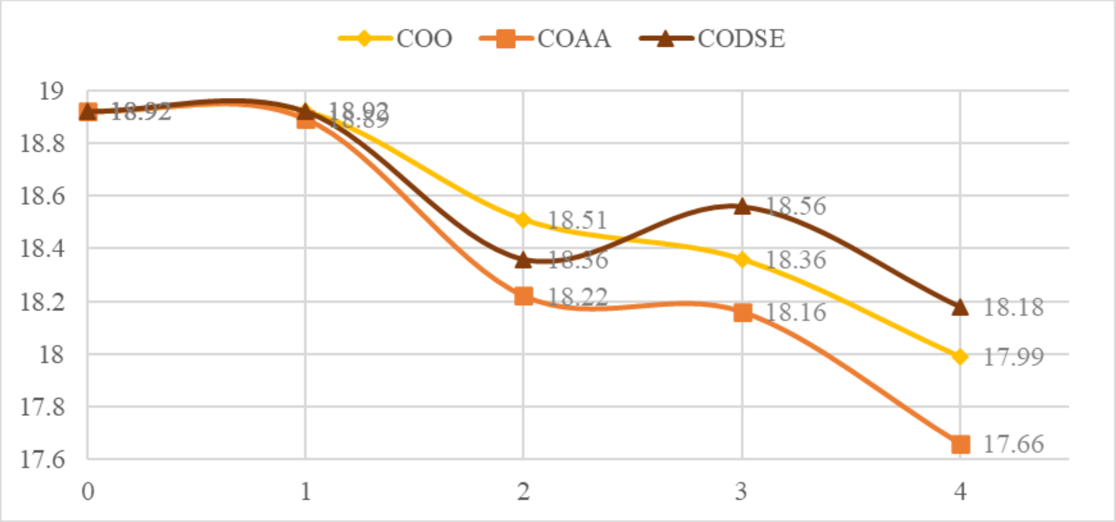


Figure 74: The evolution of omega 9 during storage

Generally, high level of unsaturation especially high level of linoleic acid (omega 6) in conjunction with the absence of linolenic acid (omega 3), affect adversely the stability of the oil. For COAA, it showed the same tendency for both omega 6 and 9, but recorded the lowest value for both at the end of the process. Meanwhile, for CODSE the omega 6 decreased with 0.84% and recorded the highest value for omega 9 with 0.74%, implying that the date seed extract helped stabilize monounsaturated fatty acids more effectively.

2- Tocopherol composition

The evolution of oil oxidation along the 28 days experiments at 60°C, is represented in Figure 66.

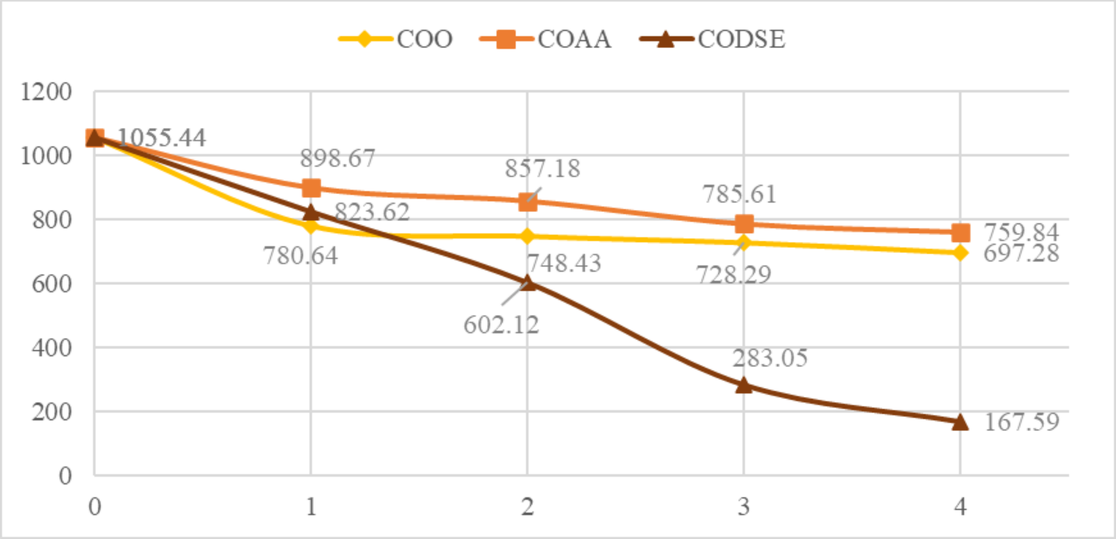


Figure 75: The evolution of gamma-tocopherol during storage

A significant decrease in gamma-tocopherol content was observed during the thermal process for all samples. However, the decrease in gamma-tocopherol content was 66% for COO, 72% for COAA and more pronounced for CODSE with 84%. This decrease is related to the increase in oxidation products (K_{232} and K_{270} values) in the oils.

Conclusion

Date seed extract at 50 ppm demonstrated a strong protective effect against oil oxidation, outperforming both the control and the sample enriched with ascorbic acid. Its ability to preserve fatty acids, delay FFA and peroxide formation, and reduce primary oxidation markers supports its potential as a natural antioxidant in oil preservation.

General Conclusion and Perspective

From the results of our study, we found that date seed oil is rich in bioactive compounds with therapeutic and nutritional properties. In fact, date seed oil is an oleic-lauric type that has a preventive effect on cardiovascular illnesses. The fatty acid analysis revealed a high amount of saturated (38.71% - 50.83%) and monounsaturated (42.68% - 53.25%) fatty acids, and a medium content of polyunsaturated fatty acids (6.21% - 9.33%). Furthermore, date seed oil has a significant amount of sterols, the most abundant of which is beta-sitosterol. Meanwhile, this by-product also possesses a rich source of tocols, ranging from 1937.14 µg/g of oil to 3844.06 µg/g of oil. The analysis of the physicochemical parameters such as acid value (AV), peroxide value (PV), saponification value (SV), iodine value (IV), K232, and K270 shows that date seed oil is edible and has a low probability of autoxidation, which implies that it can be considered safe for being consumed by humans. This examination indicates that DSO has high oxidative stability (can be easily conserved), can be considered as a non-drying oil, and may be used as a substitute for some conventional oils. According to these and several other results, date seed oil has a potential application in several cosmetic formulations, such as body creams, shaving soaps and shampoos, and pharmaceutical products. However, more tests of safety should be conducted before it is used by the factories that make food and cosmetics.

The extract of date seed have various bioactive compounds. Indeed, the phytochemical screening revealed the presence of these compounds while the phytochemical assay allowed us to quantify their content for each variety. Then, the antioxidant activity showed a strong inhibiting and reducing capacity of all the extracts. This prompted us to use this antioxidant power to enrich an oil subject to oxidation to make it even more resistant.

Our study reveals a significant variation in oxalate levels among the different samples, with the Lkhalt variety having the highest oxalate content (0.79%) and the Bousthammi variety the lowest (0.20%). Given the potential health risks associated with excessive oxalate intake, particularly in individuals prone to calcium oxalate kidney stones, it is crucial to monitor and regulate oxalate consumption. Future studies could explore the impact of different oxalate concentrations on various health conditions, particularly focusing on individuals with kidney stones or coronary heart disease. Understanding the balance between oxalate intake and its potential benefits or risks could help refine dietary guidelines and improve patient care strategies for those with specific health conditions.

The best roasting time and conditions was for RDS6 and RDS10. They showed the highest values of TFC and TTC and strong antioxidant activity in the DPPH assay, these extracts had the longest roasting time of 45 minutes and a temperature of 160 and 180°C respectively. These findings suggest that the antioxidant activity of extracts required a higher roasting temperature. However, bioactive compound and antioxidant activity levels in coffee seeds samples remained significantly lower than those in the RDS samples. In fact, the high phenolic content may have a nutritional effects as antioxidants or anti-nutritional effects as metal chelation lowering iron bioavailability and acting as phytoestrogens. As a result, date seed should be taken with caution, especially from people that consuming it a lot as a coffee substitutes.

The findings of this study highlight the promising role of date seed extract as a natural antioxidant in enhancing the oxidative stability of cactus oil. Compared to ascorbic acid, the date seed extract provided more comprehensive protection against both primary and secondary oxidation, maintained better fatty acid integrity, and effectively reduced hydrolytic degradation. Its ability to preserve key oil quality attributes under accelerated thermal conditions suggests significant potential for commercial applications in food preservation, cosmetics, and nutraceutical formulations.

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Résume *(200 mots max.)*

Le palmier dattier (Phoenix Dactylifera L.) joue un rôle économique, social et écologique très important pour les populations des régions arides et semi-arides. Son fruit est un aliment de base pour des millions de personnes, il est constitué essentiellement d'eau, de sucres réducteurs, d'une valeur énergétique très importante, et aussi des protéines et des acides aminés indispensables chez l'homme. En revanche, son noyau est considéré comme un déchet ou utilisé en alimentation du bétail. Dans le cas de notre travail nous avons effectué une analyse phytochimique afin de détecter et doser quantitativement les substances chimiques présentes dans ces noyaux. Ensuite, nous avons étudié la composition chimique en acides gras, stérols et tocophérols de l'huile végétale obtenue, tout en utilisant les indices de qualité recommandée par Codex Alimentarius afin de déterminer la qualité de l'huile. Et finalement, nous avons évalué leur pouvoir antioxydant par différentes méthodes telles que le pouvoir d'inhibition par DPPH et ABTS, et le pouvoir de réduction par FRAP. Nous avons testé son utilité dans notre vie quotidienne via l'identification de ses paramètres optimaux de torréfaction afin de l'utilisé comme un succédané de café et l'enrichissement d'une huile végétale susceptible de s'oxyder afin de maintenir sa qualité.

Mots-clefs (5) : Dosage phytochimique, huile végétale, *Phoenix Dactylifera L.*, pouvoir antioxydant, torréfaction.

Abstract

The date palm (Phoenix Dactylifera L.) has a significant economic, social, and ecological impact on the residents in arid and semi-arid area. Its fruit is a staple food for millions of people, consisting mainly of water, reducing sugars and a high energy content, as well as proteins and amino acids essential for human consumption. In contrast, the seed is considered a waste product or used as animal feed. In our study, we performed a phytochemical investigation to identify and quantify the chemical compounds found in these seeds. Next, we examined the chemical composition of fatty acids, sterols, and tocopherols in the resulting vegetable oil, using the Codex Alimentarius quality indices to assess the oil's quality. Finally, we examined their antioxidant capacity using a variety of techniques, including DPPH and ABTS inhibition and FRAP. We tested its usefulness in our daily lives by identifying its optimum roasting parameters for use as a coffee substitute and enhancing a vegetable oil prone to oxidation in order to preserve its quality.

Keywords (5) : Antioxidant activity, *Phoenix Dactylifera L.*, phytochemical content, roasting, vegetable oil.