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En vue de l'obtention du diplôme  
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Thème

**Élaboration d'emballages alimentaires à base de substances  
naturelles**

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# **Master's Thesis**

In order to obtain a Master's degree  
in Food Conservation and Packaging

## **Theme:**

**Development of Food Packaging Based on Natural  
Substances**

**Presented by:**

**MEKERLOUF Hossam & AMRANE Chaima**

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## List of abbreviations

B.C : Before Christ

CFU : Colony forming unit

DPPH : 2,2-diphényl 1-picrylhydrazyle

FDA : Food and Drug Administration

FTIR : Fourier transform infrared spectroscopy

MAP : Modified atmosphere packaging

MBC : Minimum bactericidal concentration

MIC : Minimum inhibitory concentration

OD : Optical density

PHA : Polyhydroxyalkanoates

PLA : Poly Lactic Acid

POPs : Persistent organic pollutants

ROP : Reduced Oxygen Packaging

SFAs : Saturated fatty acids

TPC : Total phenolic content

UFAs : Unsaturated fatty acids

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# Introduction



Food packaging serves as an indispensable tool to deliver a food product to the consumer. It increases the product's shelf life by providing a physical barrier from the adversities in the environment, such as microbial and chemical contaminants, together with facilitating the handling, storage, and transportation of the product (Perera et al., 2024).

Nowadays packaging materials used to preserve foods are widely spread in the food chain (industry, supermarkets, and restaurants) and contribute enormously to environmental pollution (Amin et al. 2022). A major part of the environmental concern from food packaging is derived from plastic materials that have extremely long degradation periods in the environment, with half-lives ranging from 2 to more than 2500 years (Chamas et al., 2020). The current balance between production and recycling of packaging materials is also not favorable for the environment. Data from the European Union illustrates the complexity of this immense challenge, plastic packaging accounts for more than 15 million tons in 2019, increase of 26% in comparison to 2009 (Amin et al, 2022). In October 2018, the European Parliament proposed the reducing of the impact of certain plastic products on the environment, so, the use of plastic materials in food packaging will be reduced from 2021. Hence the need to develop new technologies and new materials in the synthesis of food packaging (Jancikova et al. 2019).

There are various types of food packaging such as flexible packaging, aseptic packaging, vacuum packaging, semi-rigid packaging, rigid packaging, active and intelligent packaging, modified atmosphere packaging (MAP), biodegradable packaging and edible packaging. Unfortunately, none of them are made with natural substances that are beneficial to the consumer's health, such active packages which serve their protective function but also elevate food quality (Dodange et al., 2024).

Researchers have concentrated on innovative approaches to increase the shelf life of perishable food products and monitor their quality during storage and transportation as consumer demand for safe, environmentally friendly, and effective packaging develops (Ramakrishnan et al, 2024). Indeed, the use of the films made from the natural compounds is gaining popularity, due to their biodegradability and environmental friendliness.

Date pits are a major waste product of the date palm industry that present environmental and economic challenges. These substantial byproducts intensify the industry's environmental impact, necessitating sustainable waste management strategies (Musa and Elnou, 2024).

They are by-product of date palm fruit consumption, and offer a promising solution due to their abundance, biodegradability (Benmoussa et al.,2023) and their phytochemicals, minerals and energy composition (Gourchala et al., 2022).

Biodegradable films were previously produced from various plant seeds (Alqahtani et al. 2021), two recent studies have been conducted on the formulation of active packaging films using date seeds extracts (Elhadeb et al., 2024a; Elhadeb et al., 2024b). The first one is about an active film with date pits aqueous extracts and gelatin sodium alginate for extending raw minced beef shelf life and the second one is about the pH-sensitive films based on CMC with anthocyanins extract of date pits. As date pits are a low-cost source of antioxidants, it is interesting to investigate their potential in the manufacturing of antioxidant films. To the best of our knowledge, limited data are available on the application of date pits phenolic extract in the formulation of antioxidant films. Thus, the aim of this work was to develop a biodegradable and antioxidant films using by-products of two Algerian date pits (Deglet Nour and Degla Baidha). The objectives of the present study were, (i) extraction of phenolic compounds from dates pits by ultrasound assisted solvent extraction method and determination of their total phenolic content and biological activities (antioxidant and antibacterial activities); (ii) formulation of packaging films with incorporation of these extracts into CMC film, and evaluation of their physical parameters and their antioxidant capacity.

# Literature review

# Chapter I : Food packaging and generalities on date palm and seeds

## **Chapter I: Food packaging and generalities on date palm and seeds**

### **I.1. Definition of food packaging**

Packaging, an integral aspect of food processing, plays a crucial role in preventing spoilage and contamination while extending shelf life. It serves multiple functions, including containment (holding the product), protection (ensuring quality, safety, and freshness), providing information (through graphics and labels), and offering convenience. Beyond these basic benefits, packaging delivers even more advantages to both manufacturers and consumers. It safeguards food, can modify the atmosphere to extend shelf life, conveys messages, supports marketing efforts, enhances content security. Additionally, packaging helps with portion control to combat "portion distortion," offers ease of use, and facilitates convenient transport, benefiting both children and adult consumers (Vaclavik, 2021). It involves the design, production, and application of packaging solutions tailored to the specific requirements of different types of food products, including fresh produce, perishable goods, and processed items (Han, 2014, Li et al., 2024).

### **I.2. Food packaging history**

Packaging was in every historical period adapted to the civilizational and technological level of the society. There is information on packaging from 12 000 years ago. It was used by people who lived as nomads, hunters, and gatherers of fruits. At that time, hollow trees, tickles, animal bladders and skin were used as packaging materials. Flexible leaves were joined with flexible grass. Later, leather reusable bags and wicker bags were made. The historical development of food packaging is presented with particular emphasis on the last 200 years that began with the introduction of canned foods, followed by the industrialization of processes to make paper and paperboard packages and glass containers not only available on a large scale but also significantly cheaper. The development of packaging was gradual. Initially, the changes took place slowly, but with the faster development of the society, the subsequent changes also accelerated. The development of technology was slow and largely conditioned by the development of new materials (Table 1). In accordance with those processes, packaging was developed (Bolanča et al., 2018).

**Table 1 : Materials used in packaging through history**

<b>Materials</b>	<b>Packaging</b>
120th century BC \ Materials directly from nature	Objects found directly in the nature
Ceramic	Ceramic pots for liquids and solids
70th century BC \ Glass	Glasses, bowls
60th century BC \ Glass and wood	Bottles, chests
50th century BC	Various bowls
30th century BC \ Clay ties	Writing text
30th Century BC \ silk	wrapping
3rd century BC \ Transparent glass	Transparent and colored bottles
2nd century	Wrapping paper
4th to 6th century	Stone and bronze as printing forms
6th century \ paper	Paper bags
9th century \ woon, linen, hemp	Bags
9th century \ bronze	Bronze packaging
13th century \ metal	Metal coated packaging
14th century \ iron, tin, cellulose	Iron and tin coated cans, metal signs
15th century \ silk printing	Ductile packaging
16th century \ cardboard	Binderies, food is placed into cans
18th century \ paper machine	Label printing
19th century \ protected metal	Containers
19th century \ vinyl chloride	Machines for making bags, glass bottles, lithography
20th century \ bakelite, corrugated cardboard	Offset, transport packaging, plastic packaging
20th century \ polymeric materials	Plastic packaging boom
20th century \ sandwich materials	Diverse packaging

### **I.3. Types of food packaging**

There are various types of food packaging:

#### **I.3.1. Flexible packaging**

Flexible packaging is extensively used in the foodservice industry and is increasingly adopted at the retail level. It includes packaging for items such as bagged cereals, candies, poultry, red meat, and sliced deli meat (Bauer et al., 2021).

Non-rigid packaging containers like stand-up pouches, tubes, and zippered bags are popular for products such as peanuts, peanut butter, fresh-cut lettuce, and peeled baby carrots (Vaclavik, 2021).

### **I.3.2. Aseptic packaging**

Aseptic packaging is used to destroy *C. botulinum* spores and extend the shelf life of low-acid foods. This method involves independently sterilizing both the food and the packaging material, with assembly under sterile conditions. The packaging material typically consists of layers of polyethylene, paperboard, and foil. It is sterilized using heat (superheated steam or dry hot air) or a combination of heat and hydrogen peroxide, then roll-fed through the packer to create the typical brick or block shape (Salila Vijayalal Mohan et al., 2020; Vaclavik, 2021).

### **I.3.3. Vacuum packaging**

Vacuum packaging extends shelf life by removing oxygen from the package, thus modifying the atmosphere surrounding the food. According to the FDA's Guidelines for Reduced Oxygen Packaging (ROP), vacuum packaging reduces the amount of air in a package and hermetically seals it to maintain a near-perfect vacuum inside (Vaclavik, 2021; Lee et al., 2024).

### **I.3. 4. Semi-rigid packaging**

Semi-rigid packaging materials offer adequate mechanical strength and other functional properties. They can be made from cellulosic materials like paperboard or various paperboard packaging formats, such as folding cartons, lined cartons, and composite containers. Additionally, polymeric materials are used to create formats like thermoformed containers and multilayer squeezable tubes (Saha, 2022; Rojas et al., 2023).

### **I.3.5. Rigid packaging**

Rigid packaging materials are typically stiff and strong, often heavier and more durable than flexible packaging materials. They can be made from paper, wood, plastic, metal, and glass, providing robust protection for the contents (Saha, 2022; McLauchlin et al., 2023).

### **I.3.6. Active and intelligent packaging**

Active and intelligent packaging can "sense" changes in the internal environment and respond as necessary.

These packages may contain small packets that control elements like ethanol, oxygen, or microbes. Active packaging contributes to product development, controls maturation and ripening, helps achieve proper color development in meats, and extends shelf life.

Although termed "smart" or "interactive," this packaging generally does not actually sense environmental conditions and change accordingly.

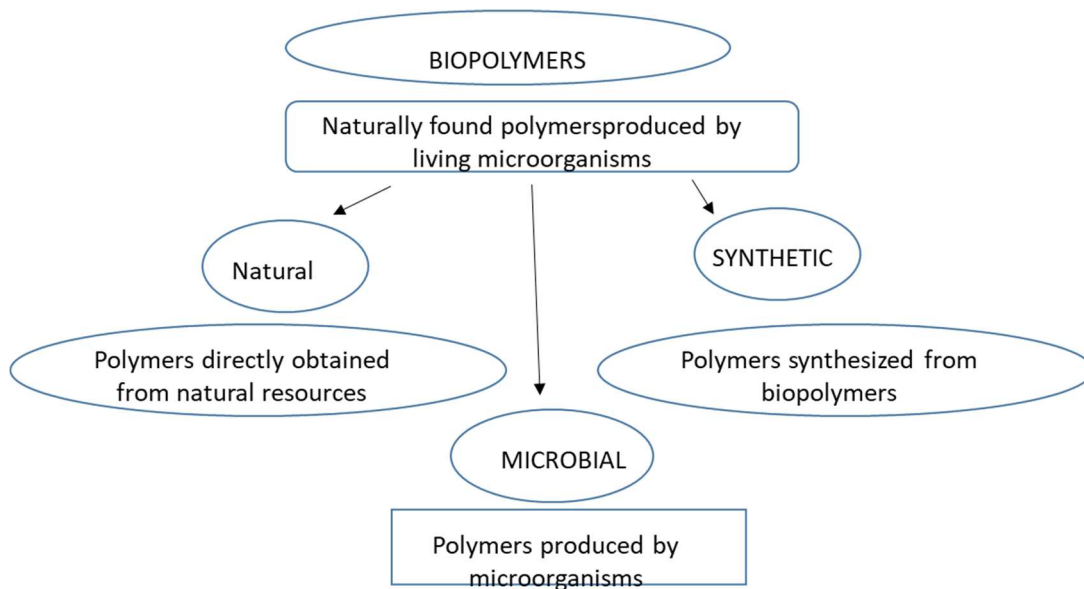
Examples include edible moisture or oxygen barriers, antimicrobial polymer films, odor scavengers, and oxygen scavengers (Vaclavik, 2021; Ramakrishnan et al., 2024).

**I.3.7. Modified atmosphere packaging (MAP)**

MAP modifies the internal atmosphere of the package by replacing air with nitrogen or carbon dioxide, potentially increasing the product’s shelf life by up to 200%. This process, involving gas flushing and sealing, reduces oxygen levels and slows down the respiration of vegetables. Unlike normal air (78.08% nitrogen, 20.96% oxygen, and 0.03% carbon dioxide), MAP offers a one-time modification of gas composition (Vaclavik, 2021; Xu et al., 2024).

**I.3.8. Biodegradable packaging**

In response to environmental concerns, biodegradable packaging materials have been developed as eco-friendly alternatives to synthetic, non-biodegradable polymers. Biopolymers, which are non-toxic, compostable, and biodegradable, are derived from renewable sources. They can be categorized into polymers chemically prepared from biobased monomers, those produced by microorganisms, and those extracted from natural resources (Malik, 2023; Lou et al., 2024).



**Figure 1 : Types of biopolymers**

**I.3.9. Edible packaging**

Edible packaging is advantageous for its barrier and mechanical properties, its ability to control the release of active ingredients, and its enhancement of sensory aspects.



Edible packaging, which can be consumed along with the food product, includes films, sheets, coatings, and pouches. Both films and coatings are made from the same formulations; however, films are applied as solid sheets, whereas coatings are applied as a liquid product (Malik, 2023; Laksanawati et al., 2024).

#### **I.4. Food packaging materials**

Packaging materials for food can vary between commercial and retail operations, though many of the same materials are used in both settings. Common packaging materials include paper, glass, plastic, and metal.

#### **I.5. Roles of food packaging**

The main role of packaging is to contain foodstuffs and protect them from external aggression, whether physical or biological.

##### **I.5.1. Physical protection**

Food packaging provides a physical barrier that protects food products from external elements such as physical damage, contamination, moisture, oxygen, light, and temperature fluctuations during transportation, handling, and storage. It prevents mechanical stresses, bruising, and breakage, thereby preserving the integrity and quality of the enclosed foods (Han, 2014; Maru and Goswami, 2020; Pascall et al., 2022).

##### **I.5.2. Containment**

The primary role of food packaging is to contain the product, ensuring that it remains secure during handling, transport, and storage. This containment function is critical to prevent spillage, contamination, and physical damage to the food product. Packaging provides a physical barrier between the food and the external environment, thereby maintaining the integrity and quality of the food throughout its shelf life (Robertson, 2016). Proper containment also aids in portion control and convenient distribution, making it easier for consumers to handle and store food products (Han, 2014).

##### **I.5.3. Preservation**

Packaging materials and technologies are selected to extend the shelf life of food products by creating optimal storage conditions. This includes features such as MAP, vacuum sealing, and antimicrobial coatings, which slow down microbial growth, enzymatic reactions, and oxidative

processes that lead to spoilage and degradation (Salila Vijayalal Mohan et al., 2020; Vaclavik, 2021; Laksanawati et al., 2024).

#### **I.5.4. Convenience**

Food packaging enhances consumer convenience by facilitating portion control, handling, and storage. It incorporates user-friendly features such as resealable closures, portioned servings, microwaveable trays, and easy-to-open packaging formats that streamline meal preparation, serving, and consumption, catering to the needs and preferences of modern lifestyles (Yam, 2009; Thurber and Curtzwiler, 2020).

#### **I.5.5. Information communication**

Packaging serves as a communication tool that provides essential information to consumers, including product identification, ingredients, nutritional content, allergen warnings, expiration dates, cooking instructions, and storage guidelines. Clear and accurate labeling, branding, and packaging graphics help consumers make informed purchasing decisions, ensure product safety, and comply with regulatory requirements (Yam, 2009; Burgess et al., 2024).

#### **I.5.6. Marketing and branding**

Packaging plays a crucial role in branding, product differentiation, and marketing strategies aimed at attracting consumers, building brand loyalty, and driving sales. It incorporates visual elements such as colors, logos, graphics, and typography that convey brand identity, quality, authenticity, and value propositions, influencing consumer perceptions, preferences, and purchasing behavior (Brody et al., 2015; Yan et al., 2022; Khandeparkar et al., 2024).

### **I.6. Problematic of plastic packaging**

#### **I.6.1 Environmental impact of plastic packaging**

The environmental impact of plastic food packaging is a significant concern due to its widespread use (Mousania et al., 2024), persistence in the environment, and detrimental effects on ecosystems. Here are some key points regarding the environmental impact of plastic food packaging (Geyer et al, 2017):

**Pollution:** Plastic food packaging contributes to pollution throughout its lifecycle. Improper disposal of plastic packaging, such as littering or inadequate waste management, results in plastic pollution in terrestrial and aquatic environments (Abate and Elofsson, 2024).

Plastics can persist in the environment for hundreds of years, accumulating in ecosystems and posing risks to wildlife through ingestion, entanglement, and habitat disruption (Phelan et al., 2022)

**Resource depletion:** The production of plastic packaging requires significant amounts of fossil fuels, primarily oil and natural gas, as raw materials. Extraction and processing of these resources contribute to greenhouse gas emissions and environmental degradation. Additionally, the manufacturing process of plastics consumes energy and water, further exacerbating resource depletion and environmental impacts (Islam et al., 2024).

**Microplastics:** Plastic food packaging can degrade over time into smaller fragments known as microplastics, which are less than 5 millimeters in size. Microplastics are ubiquitous in the environment and have been found in soil, water bodies, and even in the air.

They can enter the food chain through ingestion by marine organisms, terrestrial animals, and humans, posing potential health risks and ecological consequences (Gündoğdu et al., 2024).

**Chemical pollution:** Plastic food packaging may contain additives such as plasticizers, flame retardants, and colorants, which can leach into food or the environment, leading to chemical pollution. Some of these chemicals have been linked to adverse health effects, including endocrine disruption, reproductive issues, and carcinogenicity. Furthermore, persistent organic pollutants (POPs) can adsorb onto plastic particles, potentially transporting harmful chemicals across ecosystems (Gündoğdu et al., 2024).

**Waste management challenges:** Plastic food packaging contributes to the global waste management crisis due to its non-biodegradable nature and inefficient recycling rates. Most plastics end up in landfills or are incinerated, releasing greenhouse gases and toxic pollutants. Recycling of plastic packaging is limited by factors such as contamination, lack of infrastructure, and economic viability, exacerbating waste accumulation and environmental impacts (Mielinger and Weinrich, 2024).

### **I.6.2 Need for sustainable and eco-friendly alternative solutions**

The need for sustainable and eco-friendly alternative solutions to plastic food packaging is increasingly urgent due to the environmental challenges associated with plastic pollution and resource depletion. Several reasons why sustainable alternatives are crucial:

**Environmental protection:** Sustainable alternatives to plastic food packaging help reduce plastic pollution and mitigate its detrimental effects on ecosystems, wildlife, and human health.

By using materials that are biodegradable, compostable, or derived from renewable resources, the environmental impact of packaging can be minimized, contributing to ecosystem conservation and biodiversity preservation (Ellen MacArthur Foundation,2016; Dodange et al., 2024).

**Resource conservation:** Eco-friendly packaging solutions prioritize the use of renewable resources and minimize reliance on finite fossil fuels. By promoting materials such as plant-based plastics, biopolymers, recycled paper, and cardboard, sustainable packaging reduces resource depletion and energy consumption associated with traditional plastic production (European Commission,2018; Abatan et al., 2024).

**Waste reduction:** Sustainable packaging solutions aim to minimize waste generation and promote circularity by adopting principles of reduce, reuse, and recycle.

By designing packaging that is reusable, recyclable, or compostable, the volume of single-use plastic waste entering landfills and oceans can be significantly reduced, leading to a more sustainable waste management system preservation (Ellen MacArthur Foundation, 2016; Abatan et al., 2024)

**Climate change mitigation:** The production, use, and disposal of plastic packaging contribute to greenhouse gas emissions and climate change. Sustainable alternatives, such as bio-based plastics or packaging made from agricultural residues, have the potential to lower carbon footprints and mitigate the environmental impact of packaging throughout its lifecycle (United Nations Environment Programme, 2018; Abatan et al., 2024).

**Consumer preference and brand image:** With growing environmental awareness and concerns about plastic pollution, consumers are increasingly demanding sustainable and eco-friendly products, including food packaging. Brands that prioritize sustainability and offer environmentally responsible packaging solutions can enhance their reputation, attract environmentally conscious consumers, and gain a competitive edge in the market (World Economic Forum,2020; Shimul and Cheah, 2022).

**Regulatory compliance:** Governments and regulatory bodies are implementing policies and regulations to address plastic pollution and promote sustainable packaging practices.

By adopting eco-friendly alternatives and complying with regulatory requirements (Jones and Head, 2023), businesses can avoid potential penalties, litigation, and reputational risks associated with non-compliance (United Nations Environment Programme, 2018).

**Innovation and collaboration:** The transition to sustainable packaging requires collaboration among stakeholders across the value chain, including manufacturers, retailers, consumers, and policymakers (Tapiola et al., 2023).

Innovation in materials science, packaging design, and waste management technologies plays a crucial role in developing sustainable alternatives and driving systemic change toward a more circular and sustainable economy (World Economic Forum,2020; Leta et al., 2024).

## **I.7 Alternative materials for food packaging**

Bio-sourced and biodegradable materials are increasingly being explored and utilized as alternatives to traditional petroleum-based plastics and packaging materials. These materials offer several advantages, including reduced environmental impact, renewable sourcing, and potential biodegradation at the end of their lifecycle.

### **I.7.1. Bio-sourced Materials**

Bio-sourced materials are derived from renewable resources such as plants, algae, agricultural residues, or microbial sources, examples of bio-sourced materials include bio-based plastics (bioplastics), bio-based polymers, and natural fibers (Gururani et al., 2023). Bio-sourced materials can be produced through various processes, including fermentation, extraction, and chemical synthesis from renewable feedstocks (Narancic & O'Connor, 2019). These materials offer the potential to reduce reliance on finite fossil fuels, lower carbon emissions, and promote sustainable resource management (Chilabade et al., 2024).

### **I.7.2. Biodegradable materials**

Biodegradable materials are capable of undergoing degradation by biological processes, such as microbial action, enzymes, or natural environmental conditions, into simpler compounds like water, carbon dioxide, and biomass. Biodegradable materials can be derived from both bio-sourced and petroleum-based sources, depending on their chemical composition and degradation mechanisms.

Examples of biodegradable materials include certain types of bio-based plastics (e.g., PLA, PHA), biodegradable polymers, and natural materials such as cellulose, starch, and chitosan (Hussain et al., 2024). Biodegradable materials offer the potential to reduce plastic pollution, minimize waste accumulation in landfills and oceans, and contribute to a more sustainable waste management system (Mong et al., 2024).

### **I.7.3. Properties and applications**

Bio-sourced and biodegradable materials exhibit a wide range of properties depending on their chemical composition, processing methods, and intended applications. These materials can be engineered to possess specific characteristics such as mechanical strength, barrier properties, flexibility, and thermal stability, making them suitable for various packaging, consumer goods, agricultural, and biomedical applications (Cheng et al., 2024).

### **I.7.4. Challenges and opportunities**

Despite their potential benefits, bio-sourced and biodegradable materials face challenges such as cost competitiveness, scalability of production, performance limitations, and compatibility with existing infrastructure (Plastics Europe, 2019). Collaboration among stakeholders across the value chain, including industry, academia, government, and civil society, is essential to accelerate the development and adoption of bio-sourced and biodegradable materials and promote a transition to a more sustainable and circular economy (Geyer et al., 2017).

Indeed, bio-sourced and biodegradable materials offer promising alternatives to traditional plastics, contributing to environmental sustainability, resource conservation, and waste reduction (Marturano et al., 2023). Continued advancements in research, technology, and collaboration are essential to realize the full potential of these materials and address global challenges related to plastic pollution and environmental degradation.

## **I.8. Generalities about date palm and pits**

### **I.8.1. Generalities about date palm**

According to Djaoudene et al. (2019), the date palm (*Phoenix dactylifera* L.) is a key fruit crop in arid and semiarid regions such as North Africa and the Middle East. The fruit consists of a fleshy pericarp and a seed, which together account for 10 to 15% of the date's total weight.

Beyond their nutritional value, dates are used medicinally in various traditional systems of medicine to treat a wide range of ailments. Dates are a vital and staple element of the daily diet and the most traditional because they have valuable nutritional components and health benefits (Djaoud et al., 2024, Messaoudi et al., 2021).

In 2005, there were more than 180 million date palm trees in the world, spread across around thirty countries on three continents : Asia, Africa and America. Asia largely dominated

production, with 125.5 million palm trees, or around 70% of the world total. Iran was the top producer with 25 million trees, followed by Iraq with 21 million. Africa had 52.6 million palm trees, mainly concentrated in North Africa. America was only a distant third with around 6 million palm trees, shared between the United States and Mexico. Some popular varieties include Ajwa, Abel, Al-Barakawi, Assel, Bireir, Barhi, Bamy, Dabbas, Dayri, Deglet-Noor, Dhakki, Dora, Degla, Ftimi, Hilali, Hallawi, Khunaizi, Khalas, Khodri, Khadrawy, Kentichi, Lasht, Munifi, Medjool, Ruthana, Ruchdi, Sukkary, Sefri, Segae, Thoory, and Zahidi (Deshpande & Deshpande, 2017).

Algeria stands out as a major player in date production, ranking fourth according to FAO statistics in 2013. In 2015, the Ministry of Agriculture and Rural Development recorded an area of 167,000 hectares dedicated to date palm cultivation in Algeria. This arboreal heritage included more than 18.6 million palm trees, allowing an annual production of dates of all varieties estimated at nearly 990,000 tonnes (Rekis, 2021).

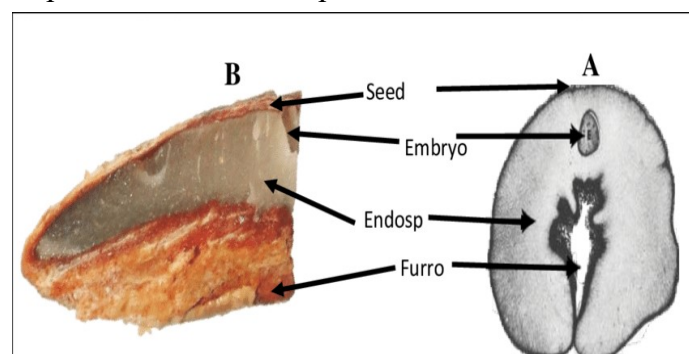
### **I.8.2. Generalities about date palm seeds**

Date pits, also known as seeds, kernels, stones, or pips, are by-products of date processing factories. Despite the valuable nutritional composition of date pits as a source of carbohydrates, dietary fiber, protein, oil, natural antioxidants, and bioactive polyphenols, they remain underutilized and are widely treated as a waste product, date pits consist of several layers.

**Seed Coat (Testa):** The outermost layer of the date pit that protects the inner embryo and endosperm. This layer is often hard and resistant to environmental factors.

**Endosperm:** The nutrient-rich tissue inside the seed coat that supports the developing embryo. In mature seeds, it may serve as the main storage tissue for starches, oils, and proteins.

**Embryo:** The young plant contained within the endosperm. It holds the genetic material required for the development of a new date palm tree when conditions are favorable (Espiard,



**Figure 2 : Composition of date pits**

2002). Date pits play a crucial role in the reproduction of date palm trees, as they contain the genetic material required for germination and seedling development.

They are also a source of various nutrients, including carbohydrates, proteins, and fats. Additionally, date pits have been utilized in traditional medicine and culinary practices in some cultures (Al-Khalili et al., 2023).

### **I.8.2.1 Biochemical composition of date pits**

Date pits contain valuable and potentially valuable ingredients (Oladipupo Kareem et al., 2021). They are analyzed for their chemical composition to determine suitable uses (Shi et al., 2014). Al-Khalili et al. (2022) have examined the mineral composition of date pit powder and noted the absence of major heavy metals such as arsenic, cadmium, chromium, lead, and mercury. However, they do contain minerals like nickel, copper, and zinc at levels of 1.4, 9.6, and 20.5 mg/kg, respectively. Lenntech (2013) suggests daily recommended allowances for these minerals are <1, 2, and 15 mg/day, respectively. Exceeding these limits would require unrealistic consumption levels, as toxicity thresholds are not easily reached.

Date pits are utilized based on their content of fibers, oil, protein, polyphenols, and antioxidant compounds in various food products. The principal components are illustrated in the table below.

**Table 2 : Chemical composition of date pits (Al-Khalili et al., 2023)**

Chemical component		g/100 g sample
Fiber	<b>Insoluble</b>	
	Crude	60–80
	Cellulose	20.0–46.8
	Hemicellulose	17.5–55.0
	Lignin	11.0–30.6
	<b>Soluble</b>	5.0
Protein		4.8–12.5
Oil	Crude	3.9–13.8
	<b>Unsaturated fatty acids (UFAs) (g/100 g oil)</b>	
	Oleic	39.5–55.4
	Linoleic	6.2–19.8
	Linolenic	0.3–8.1
	<b>Saturated fatty acids (SFAs)(g/100g oil)</b>	



	Lauric	6.793–45.4
	Palmitic	2.64–12.6
	Stearic	1.3–47.9
	Capric	0.25–11
	Myristic	0.04–12.8
	Arachidic	0.02–0.39
	<b><math>\alpha</math>-tocotrienol</b>	30.2–37.4
Polyphenols	g GAE/100 g	5.1–9.5

**I.8.2.2 Physical and mechanical properties of date palm seeds**

The physical and mechanical properties of date seeds are important for any application or conversion to useful products (Table 3).

**Table 3 : Physical properties of date pits**

<b>Property</b>	<b>Range/Typical Value</b>
Seed Size (length x width x thickness)	15-30 mm x 8-15 mm x 5-10 mm
Seed Weight	1-3 g per seeds
Bulk Density	600-800 kg/m <sup>3</sup>
Porosity	40-50%
Color	Light brown to dark brown
Surface Texture	Smooth to slightly rough

Palm date seeds become a promising choice as an enforcement material in a bio-composite structure, thanks to their mechanical properties (Table 4).

**Table 4 : Mechanical properties of date pits (Al-Zahrani et al., 2022)**

<b>Property</b>	<b>Typical Value</b>
<b>Hardness</b>	<b>50-100 N</b>
<b>Compressive strength</b>	<b>20-50 MPa</b>
<b>Tensile strength</b>	<b>5-15 MPa</b>

<b>Property</b>	<b>Typical Value</b>
<b>Modulus of elasticity</b>	<b>1-3 GPa</b>

### **I.8.2.3. Antioxidant activity of date seeds**

*In vitro* and *in vivo* studies on date seeds have shown their effective antioxidant properties. Previous studies have reported that date seed extracts had high scavenging activity against ABTS, DDPH, and hydroxyl radicals.

Date seeds have also shown higher antioxidant properties than date flesh, making them a viable natural source of antioxidants (Manai et al., 2024). Oral administration of date seed extracts was found to lower oxidative stress damage and improve the defense systems of the organs (Manai et al., 2024).

Antioxidant activity of date seeds was also reported by Benouamane et al. (2022). Thanks to its bioactive composition, nanoencapsulated date seeds extract in yogurt displayed interesting radical scavenging properties and alleviated oxidative stress contributing to the good preservation capacity of the product (Manai et al., 2024).



# Experimental Work

# Chapter II : Materials and Methods

## **Chapter II : Materials and Methods**

The purpose of this study was to develop antioxidant film for food preservation using bioactive compounds extracted from two varieties of date pits: Deglet Nour and Degla Baidha. This section outlines the materials and the methodologies used to achieve the preparation of extracts, their phytochemical analysis and then their application in the proposed biofilm.

The materials and methods described here provide a comprehensive overview of the processes and analytical techniques used to ensure the reproducibility and reliability of the results.

### **II.1. Samples treatment**

Dates were purchased from a local Bejaia store, we selected two varieties of dates Deglet Nour and Degla Baidha.

The choice of these two varieties is based on the fact that they are varieties which undergo industrial transformations and which generate by-products called date kernels, stones, pits or seeds. Indeed, these two varieties are processed to product date syrup, date pasta or filled dates with dried fruits, chocolate, etc.

The date pits were cleaned with tap water to remove any residual pulp, dust, or contaminants that might interfere with subsequent extraction procedures. Then, they undergo a second cleaning using distilled water to ensure no additional impurities were introduced. After that, they were dried in a ventilated drying oven at temperature of 40 °C for one week. Once dried, the date pits were subjected to mechanical blending. A high-speed commercial blender (Power 3000 Watts, Brand *Silver Crust*) was used for this purpose. The blender was pre-cleaned to avoid cross-contamination from previous uses. The dried date pits were added to the blender in small batches to ensure uniform particle size reduction and to prevent overloading the machine (Alsahli et al., 2021).

The blending process was conducted in cycles: 2 min of blending followed by 1 min of rest, repeated until a homogenous coarse powder was obtained. The blending was carried out under ambient conditions to maintain the integrity of these compounds (Bashir et al., 2019).



**Figure 3 : Photography showing the different blending steps using a 3000 Watts rotator blender**

The blended date pit powder was then sieved using a stainless steel sieve with a mesh size of 250  $\mu\text{m}$ . Sieving ensured uniform particle size, which is crucial for maximizing the surface area during the subsequent extraction process. Uniform particle size enhances the efficiency of solvent penetration and bioactive compound extraction (Ahmed et al., 2020).

The sieving process involved gently shaking the sieve to allow the finer particles to pass through while retaining larger particles for re-blending. This step was repeated until all the date pit powder passed through the 250  $\mu\text{m}$  mesh. The two powders were packed in dark bottles and stored at room temperature for further analysis.

## **II.2. Evaluation of moisture content of the sample**

Thermal drying method is used in the determination of moisture content of the sample. 10g of each sample were placed in an oven at 105°C for 3 H (Boulekbache-Makhlouf et al., 2013). The moisture content (MC) was calculated by expressing the weight loss upon drying as a fraction of the initial weight of sample used.

$$\text{MC \%} = \frac{W_0}{W_i} \times 100$$

Where  $W_0$  correspond to the loss in weight (g) on drying and  $W_i$  correspond to the initial weight of sample (g).

### II.3. Extraction of phenolic compounds

To extract bioactive compounds from the date pits, 20 g of finely ground date pit powder from each variety were accurately weighed. This initial mass was chosen based on preliminary studies indicating optimal extraction efficiency (Amin et al., 2020). Phenolic compounds were extracted using the methodology of Elhadeb et al. (2024a), with some modifications. The date pit powders were transferred to separate extraction flasks, and 400 mL of 80% aqueous ethanol were added. Ethanol was selected as the solvent due to its efficacy in extracting polyphenols and other bioactive compounds while being relatively safe and environmentally friendly (Xu et al., 2017). Indeed, the 80% ethanol concentration balances the polarity required for optimal solubility of both hydrophilic and hydrophobic compounds (Sulaiman et al., 2011). The mixtures were then subjected to ultrasound-assisted extraction. Each flask was placed in an ultrasound bath set at a temperature of 25°C during 60 min. This method utilizes ultrasonic waves to create cavitation bubbles in the solvent, which collapse and disrupt the plant cell walls, enhancing the release of bioactive compounds into the solvent (Altemimi et al., 2017; Dhanani et al., 2013). The controlled temperature ensures that the integrity of heat-sensitive compounds is maintained (Wang & Weller, 2006). Following the ultrasound extraction, the mixtures were centrifuged at 5000 revolutions per min (rpm) for 15 min using a high-speed centrifuge. Centrifugation facilitated the separation of solid residues from the liquid extract, ensuring a clear supernatant for further processing (Ibrahim et al., 2019). Then the supernatants were decanted and filtered through filter paper. Filtration is a crucial step to prevent any solid residues from interfering with the subsequent drying and analysis processes (Saleh et al., 2011).

The filtered extracts were concentrated to dryness under reduced pressure by rotary evaporation at 40 °C. This low-temperature drying method ensured the gentle evaporation of the solvent, thereby preserving the bioactivity of the extracted compounds (Cheok et al., 2018). Slow drying prevents thermal degradation and ensures the stability of sensitive polyphenols and other bioactive compounds (Shah et al., 2020). After that, the vials were stored in a dark environment. Indeed, proper storage is essential to maintain the integrity and efficacy of the extracts for subsequent analyses and applications (Kumar et al., 2019).

The extraction yield was calculated as follows:

$$\text{Yield (\%)} = \text{W1/W2} \times 100,$$

Where W1 correspond to the loss in weight (g) on drying and W2 correspond to the initial weight of sample (g).



## **II.4. Phytochemical analysis**

To reconstitute the dry extracts obtained from the date pits, a 40% dimethyl sulfoxide (DMSO) solution was prepared using distilled water. DMSO was chosen for its excellent solvent properties, particularly its ability to dissolve both hydrophilic and hydrophobic compounds, making it ideal for reconstituting bioactive extracts for various bioassays (Agarwal et al., 2017).

The preparation involved diluting 40 mL of pure DMSO in 60 mL of distilled water to achieve the desired concentration (Ali et al., 2022). Then 10 mg of each dry extract were extracted with 10 mL of the previously prepared 40% DMSO solution, resulting in a final concentration of 1 mg/mL. This concentration was chosen based on preliminary experiments indicating optimal solubility and bioactivity of bioactive compounds (He et al., 2020, El-Mageed et al., 2019).

### **II.4.1. Determination of total phenolic content (TPC)**

The analysis of TPC in the date pit extracts was conducted using the Folin-Ciocalteu method, a widely recognized and reliable assay. This method involves a colorimetric reaction where polyphenols reduce the Folin-Ciocalteu reagent, resulting in a blue color whose intensity is proportional to the polyphenol concentration (Everette et al., 2010; Swain & Hillis, 1959). For each extract, three replicate tubes were prepared, along with one blank tube for calibration and control purposes.

To each of the tubes, including the blank, 50  $\mu$ L of the respective extract were added. The blank tube received the same volume of distilled water instead of the extract to account for any absorbance due to the reagents themselves (Singleton et al., 1999). Then, 3 mL of distilled water were added to each tube. This step ensures adequate dilution of the extract for a clearer reaction with the Folin-Ciocalteu reagent. Following this, 250  $\mu$ L of Folin-Ciocalteu reagent were added to each tube. The Folin-Ciocalteu reagent, a mixture of phosphomolybdate and phosphotungstate, reacts with the polyphenols to form a blue complex (Ainsworth & Gillespie, 2007).

After the addition of the Folin-Ciocalteu reagent, 750  $\mu$ L of 20% sodium carbonate solution were added. Sodium carbonate neutralizes the acids in the Folin-Ciocalteu reagent, promoting the reduction of the reagent by the polyphenols, which is necessary for the colorimetric change (Waterhouse, 2002). To standardize the volume in all tubes to 5 mL, an additional 950  $\mu$ L of distilled water were added. This step ensures uniform reaction conditions across all samples

and the blank. The solutions were then incubated for 2 H at ambient temperature, protected from light.

This incubation period allows the reaction between the polyphenols and the Folin-Ciocalteu reagent to reach completion, resulting in the maximum color development. Keeping the tubes in the dark prevents any light-induced degradation of the polyphenol compounds, ensuring the accuracy and reliability of the assay (Prior et al., 2005).

After the incubation period, the absorbance of each sample was measured using a spectrophotometer at a wavelength of 760 nm. This wavelength is optimal for detecting the blue complex formed by the reaction of polyphenols with the Folin-Ciocalteu reagent. The absorbance readings were used to determine the total polyphenol content by comparing them to a standard curve prepared with known concentrations of gallic acid (Dewanto et al., 2002).

#### **II.4.2. Determination of antioxidant activity**

The antioxidant activity of the date pit extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, a widely used method for assessing the free radical scavenging ability of bioactive compounds. To prepare the DPPH solution, 4 mg of DPPH were dissolved in 10 mL of methanol. This solution serves as the stable free radical required for the assay (Brand-Williams et al., 1995). 9 sets of dilution tubes were prepared for each extract, for the first set of Deglet Nour extract, 1.5 mL of the extract were mixed with 1.5 mL of distilled water in one tube. Once the serial dilutions were prepared, each tube received 500  $\mu$ L of the extract mixture. Following the distribution of the extract, 500  $\mu$ L of methanol and 75  $\mu$ L of the prepared DPPH solution were added to each tube. The addition of methanol serves to maintain the consistency of the solvent environment, while the DPPH solution provides the necessary free radicals for the assay (Chen et al., 2013).

Each tube was then vortexed to ensure thorough mixing of the contents. This step is crucial for the uniform distribution of DPPH radicals and extracts, which facilitates accurate interaction and subsequent measurement of antioxidant activity. After that, the tubes were incubated for 1.5 H in the dark at ambient temperature. This incubation period allows sufficient time for the reaction between the DPPH radicals and the antioxidant compounds in the extracts to reach completion.

Incubating the samples in the dark prevents light-induced degradation of the DPPH radicals, which could otherwise affect the reliability of the assay results (Blois, 1958; Sharma & Bhat, 2009). At the end, the absorbance of each sample was measured using a spectrophotometer at a wavelength of 517 nm.

The decrease in absorbance is indicative of the scavenging activity of the extracts against the DPPH radical, with lower absorbance values corresponding to higher antioxidant activity.

The results were compared against a control and expressed as the percentage of DPPH radical inhibition (Alam et al., 2013), the absorbance readings were used to calculate the percentage of DPPH inhibition using the following equation:

$$\text{DPPH inhibition (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where  $A_0$  is the absorbance of the DPPH solution without the extract, and  $A_1$  is the absorbance of the DPPH solution with the extract. Then the IC<sub>50</sub> values were calculated as the concentration of extracts causing a 50% inhibition of DPPH radical, a lower IC<sub>50</sub> value corresponds to a higher antioxidant activity of sample.

### II.4.3. Determination of antibacterial activity

#### II.4.3.1. Bacterial strains

The antibacterial activity of the date pit extracts was evaluated against seven bacterial strains: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Proteus mirabilis*.

#### II.4.3.2. Agar diffusion method

The antibacterial activity was assessed using the well diffusion method using Mueller–Hinton agar. The wells were then filled with different concentrations of the date pit extracts, prepared by serial dilution: 1/2, 1/4, 1/8, 1/16, and 1/32. The initial solution of the extracts served as the undiluted control.

#### II.4.3.3. Standardization and bacterial culture preparation

The bacterial cultures were standardized by measuring the absorbance to ensure an optical density (OD) between 0.08 and 0.13 at 600 nm, corresponding to a bacterial population of

approximately  $10^8$  CFU/mL. This standardization ensures the consistency and reliability of the antibacterial assay results (CLSI, 2018).

Young bacterial cultures were prepared by inoculating fresh medium and incubating until they reached the desired optical density. The standardized bacterial suspension was then used to seed the Petri dishes containing the MH agar and wells with the date pit extracts. Then, the seeded Petri dishes were incubated at 37°C for 24 H.

After the incubation period, the antibacterial activity was assessed by measuring the zone of inhibition (mm) around each well. Larger zones of inhibition indicate higher antibacterial activity of the extracts.

#### **II.4.4.4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**

The MIC and MBC of the extracts were determined using a microdilution method. Serial dilutions of the extracts were prepared in microplates, and the bacterial cultures were added. The plates were incubated at 37°C for 24 H. The MIC was defined as the lowest concentration of the extract that inhibited visible bacterial growth. The MBC was determined by subculturing the samples from the MIC wells onto fresh agar plates and observing for bacterial growth after 24 H of incubation. The MBC was the lowest concentration that resulted in no bacterial growth, indicating bactericidal activity (Wiegand et al., 2008; CLSI, 2018).

### **II.5. Biofilm preparation**

The biofilm was prepared using CMC, which is an environmentally friendly and readily available cellulose derivative that can be employed to manufacture films for food packaging due to its hydrophilic and biodegradable properties (Ramakrishnan et al., 2024).

To prepare the film-forming solution, 4 g of CMC was dissolved in 100 mL of distilled water (Elhadeif et al., 2024a). The solution was heated to 95°C for 30 min to ensure complete dissolution and homogeneity (Saini et al., 2016). Once the CMC solution was prepared, glycerol was added as a plasticizer. The amount of glycerol used was 0.90 g, constituting 30% (w/w) of the total mixture. The addition of glycerol was performed at 95°C, and the solution was mixed for 20 min to ensure uniform distribution of the plasticizer throughout the solution. Glycerol enhances the flexibility and mechanical properties of the biofilm (Shankar et al., 2018).

For the CMC/date pits biofilms, 1.5% (w/w) of date pits extracts were added to the prepared CMC-glycerol solution. Specifically, 1.6 mg of Deglet Nour date extract and 3 mg of Degla Baidha extract were used based on values determined from the DPPH assay. The extracts were blended into the mixture for 10 min to ensure a uniform distribution. This process was conducted for two separate biofilms: one with Deglet Nour extract and another with Degla Baidha extract. A control film was also prepared for comparison (Mahcene et al., 2020).

After the incorporation of the extracts, 9 mL of the film-forming solution were poured into Petri dishes. The films were then subjected to drying at 40°C for a day to allow the solvent to evaporate completely, forming a stable biofilm. This controlled drying process is crucial to achieving uniform thickness and consistent properties in the biofilms (Bof et al., 2016). Once dried, the resulting films were carefully peeled off from the Petri dishes. These films were then stored in desiccators at 25°C to maintain their integrity and prevent moisture absorption until further analysis. The desiccator storage ensures that the biofilms remain dry and stable, preserving their mechanical and functional properties (Elhadef et al. 2024a).

## **II.6. Film characterization**

### **II.6.1. Antioxidant capacity**

The antioxidant capacity of the biofilm was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay, following the method described by Dou et al. (2018). This method is widely recognized for evaluating the antioxidant potential of various materials due to its simplicity and sensitivity.

30 mg of the film sample (either containing Deglet Nour extract, Degla Baidha extracts, or the control film without any extract) was immersed in 10 mL of distilled water. This step allowed the extraction of active compounds from the film into the water (Dou et al., 2018). A 0.5 mL aliquot of the resulting sample solution was taken and mixed with 0.5 mL of 0.1 mM DPPH solution. The DPPH solution was prepared freshly to ensure its stability and reactivity. The reaction mixture was incubated in the dark at 25°C for 30 min to allow the DPPH radicals to interact with the antioxidants present in the film samples (Dou et al., 2018). This step is crucial to prevent light-induced degradation of the DPPH radicals. After the incubation period, the absorbance of the mixtures was measured at 517 nm using a UV-Vis spectrophotometer. The decrease in absorbance at this wavelength indicates the scavenging of DPPH. The DPPH radical scavenging activity was calculated as described in the section II.4.2.

**II.6.2. Fourier transform infrared spectroscopy (ftir) analysis**

For the FTIR analysis, three different film samples were prepared: a control film containing only CMC and glycerol, a Deglet Nour film containing CMC, glycerol, and Deglet Nour date extract, and a Degla Baidha film containing CMC, glycerol, and Degla Baidha extract. Additionally, two powder samples were prepared using pure extracts of Deglet Nour dates and Degla Baidha.

Each film sample was analyzed by placing three drops on the ATR crystal of the FTIR spectrometer, recording the spectra in the range of 4000 to 400  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$ , and performing 32 scans per sample. For the powder samples, 0.08 g of potassium bromide (KBr) was finely ground with 0.002 g of each extract, and the mixture was subjected to high pressure (60 kN) for 1-2 min using a hydraulic press to form thin, transparent pellets. The FTIR spectra for these pellets were recorded under the same conditions as the film samples.

The spectra were then analyzed to identify characteristic peaks corresponding to various functional groups. This method ensured accurate identification of the functional components in both the film and powder samples (Smith, 2018; Ferraria, Marques, & Botelho do Rego, 2020; Dong, Jia, & Wang, 2018).

**II.6.3. Water solubility assessment**

The water solubility (WS) of the film samples was assessed in accordance with the procedure outlined by Chaari et al. (2022). Initially, film samples were dried at 105°C for 24 H and weighed to evaluate their initial solid content ( $W_i$ ).

Pre-weighed film samples ( $2 \times 2 \text{ cm}^2$ ) were then immersed in 30 mL of distilled water for 24 H at 25°C. After immersion, the film fragments were dried again at 100°C until a consistent final dry mass ( $W_f$ ) was attained. The WS percentage of the films was calculated using the formula:

$$\text{WS\%} = [(W_i - W_f) / W_i] \times 100$$

Where  $W_i$  represents the initial film weight (g) and  $W_f$  represents the final film weight (g). This method provided a reliable measure of the films' water solubility, indicating their potential for various applications (Chaari et al., 2022; Khan, Anwar, & Afridi, 2019; Martins et al., 2020).

**II.7. Statistical analysis**

All experiments were conducted in triplicates and results are expressed as mean  $\pm$  standard deviation (SD).

Analysis of variance was performed by ANOVA procedure with one factor for all tests. IC50 value were determined by regression analysis. Statistical analysis was performed in the software jmp Pro 17, differences were considered to be significant at  $p < 0.05$ .

# Chapter III : Results and Discussion



**Chapter III: Results and Discussion**

The increasing demand for sustainable and functional packaging solutions has driven research into bio-based films with antioxidant and antimicrobial properties. This study focuses on the development of antioxidant biofilms using extracts from Deglet Nour and Degla Baidha date pits. Date pits, a byproduct of the date industry, are rich in polyphenols and exhibit significant antioxidant and antimicrobial activities, making them suitable for biofilm applications (Ammar et al., 2021; Mahcene et al., 2020).

**III.1. Moisture content of sample****III.1.1. Moisture content**

The water content of Deglet Nour and Degla Baidha pits was assessed to determine their moisture levels, which significantly influence their drying behavior and storage stability as well as the extraction procedure. Indeed, high humidity is a source of antioxidant degradation, water can affect the solubility during extraction and even the stability of the active compounds (Owen and Johns, 1999). For Deglet Nour pits, the water content was determined to be  $5.8\% \pm 0.2\%$ . Degla Baidha pits exhibited a slightly higher water content of  $6.2\% \pm 0.3\%$ . These values are lower than those reported by Messaoudi et al. (2021) for the methanolic and aqueous extracts, who found 8.652 and 7.128%, respectively.

The results suggest that Degla Baidha pits contain more moisture compared to Deglet Nour pits, implying that they might require more energy or longer drying times to achieve similar dryness levels. This difference in water content can be attributed to the intrinsic structural and compositional differences between the two types of date pits, which affect their moisture retention capacity (Elhadeif et al., 2024a).

These findings are crucial for optimizing the extraction and storage processes of bioactive compounds from date pits, ensuring their effectiveness in bioactive packaging materials (Benmoussa et al., 2023; Elhadeif et al., 2024).

### III.2. Extraction yield and phenolic content

The obtained results (Table 5) indicate that Deglet Nour date pits have a higher extraction yield compared to Degla Baidha, which can be attributed to the specific composition of their polyphenolic compounds and the efficiency of the ethanol extraction method. Ethanol is effective in breaking down plant cell walls and dissolving polyphenolic compounds, and the use of an ultrasound bath at 25°C further enhances the extraction efficiency by improving mass transfer and reducing extraction time (Mohd Zaid & Husain, 2019; Jimenez et al., 2012). The significant yields of polyphenols from both types of date pits highlight their potential as valuable sources for developing bioactive packaging materials with antioxidant properties, crucial for extending the shelf life and safety of food products (Singh et al., 2019; Mlalila et al., 2018).

Our results are consistent with those reported by previous studies on polyphenol extraction from date pits, which generally report yields in the range of 5-20%, depending on the extraction method and conditions used.

**Table 5 : Extraction yield and polyphenol content of date pit extracts**

Extracts	Yield (%)	TPC (mg GAE/g DW)
Deglet Nour	15.6%	45.2±0.752 <sup>A</sup>
Degla Baidha	13.2%	37.2±0.622 <sup>B</sup>

Values are averages ± standard deviation of triplicate analysis; different letters indicate significant difference ( $p < 0.05$ ). Results are ranked in ascending order; A > B.

The higher yield from Deglet Nour could be attributed to the specific composition of their polyphenolic compounds, which may be more readily extractable under the given conditions.

Furthermore, the optimal extraction conditions, including the use of an ultrasound bath and a relatively low temperature (25°C), likely contributed to the preservation and efficient extraction of polyphenols from both types of date pits.

Previous research has demonstrated that ultrasound-assisted extraction can significantly enhance the yield of bioactive compounds from plant materials by improving mass transfer and reducing extraction time (Jimenez et al., 2012).

The extraction of polyphenols from Deglet Nour and Degla Baidha date pits yielded promising results, with Deglet Nour showing a higher extraction yield. These findings highlight the potential of utilizing date pits as a valuable source of polyphenols for developing bioactive packaging materials.

The total polyphenols content of the date pit extracts was determined using the Folin-Ciocalteu method. Each extract was tested in triplicate, using a calibration curve of gallic acid as a standard; the results were expressed in mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW). The results are shown in Table 5.

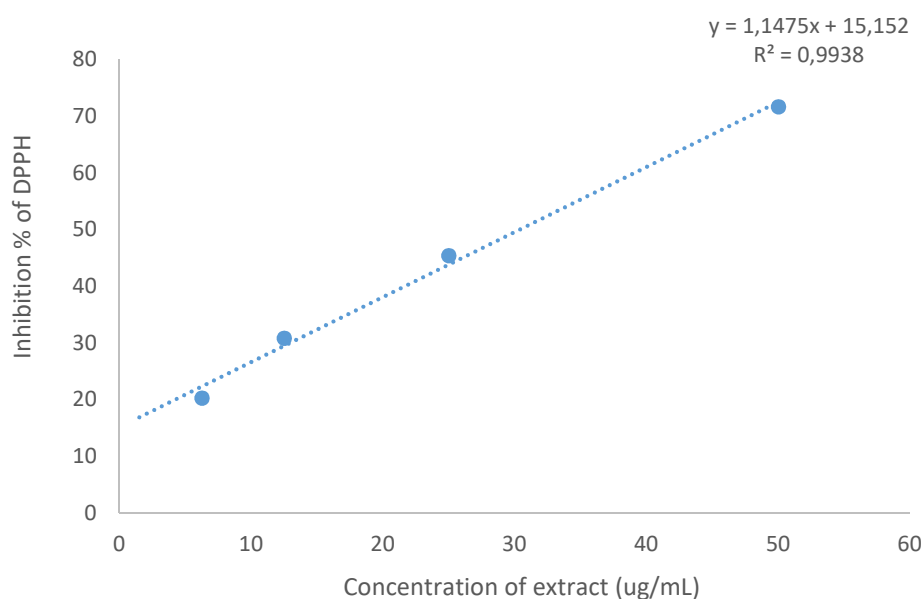
The polyphenol content of the Deglet Nour and Degla Baidha extracts showed significant differences ( $p < 0.05$ ). The Deglet Nour extract exhibited higher polyphenol content, which aligns with previous studies that have reported higher content in Deglet Nour dates compared to other varieties (Al-Farsi & Lee, 2008; Ammar et al., 2021). Indeed, phenolic content of date pits can vary significantly based on the variety and cultivation conditions (Al-Farsi & Lee, 2008; Biglari et al., 2008).

Several studies have been conducted on the date pits phenolic composition. Recent works have been conducted on phenolic compounds of date pits, e.g. a content of 14.5% GAE was reported by Manai et al. (2024). Another work conducted by Souli et al. (2022) found 198 mg GAE/g DW in methanolic extract of Deglet Nour pits (Manai et al., 2024). The richness of this variety compared with Degla Baidha has already reported by Algerian team researchers (Messaoudi et al., 2021) who has reported values of  $1.5892 \pm 0.0171$  and  $2.1477 \pm 0.0026$ ;  $3.7727 \pm 0.0485$  and  $4.6591 \pm 0.0111$  mg GAE /100 g DW, in aqueous and methanolic extracts of Degla Baidha, respectively. As we can see, methanol is better for the extraction of phenolics from date pits compared with water.

This result confirms that the extraction yield depends on the operating conditions and the solvent used. However, we can't compare these results with those obtained in the present study, because they are expressed differently (mg GAE /100 g DW and mg GAE/g dry extract, respectively).

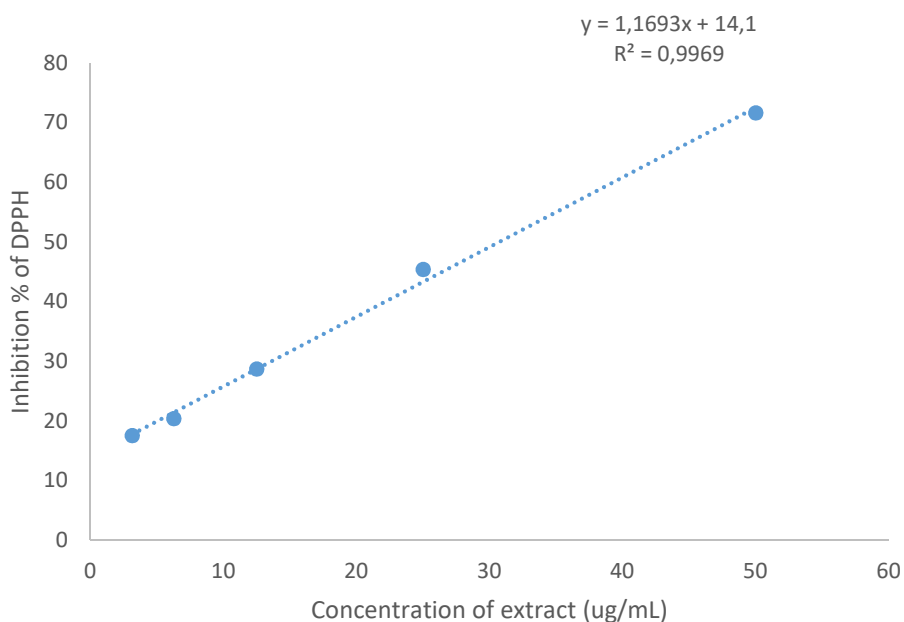
### III.3. Antioxidant Activity

The antioxidant activity of the date pit extracts was evaluated using the DPPH assay, which measures the ability of the extracts to scavenge free radicals. The results are expressed as the percentage inhibition of DPPH (figure 4 and 5) and the IC<sub>50</sub> values (Table 6). The antioxidant activity was tested at various concentrations (50, 25, 12.5, 6.26, 3.125, and 1.52 µg/mL) for both Deglet Nour and Degla Baidha extracts. The average results from three independent experiments are presented below.



**Figure 4 : DPPH Inhibition for Deglet Nour Date pit extract**

Figures 4 and 5 illustrate the dose-dependent increase in DPPH inhibition for both extracts. At the highest concentration (50 µg/mL), Deglet Nour extract achieved an inhibition of approximately 70±0.2%, whereas Degla Baidha extract reached around 64±0.02% these values are significantly different. This trend underscores the superior efficacy of Deglet Nour extract in scavenging free radicals across all tested concentrations.



**Figure 5 : DPPH● Inhibition for Degla Baidha date pit extract**

Both extracts exhibit substantial radical scavenging capabilities. However, the Deglet Nour extract demonstrated a significantly higher effect ( $p < 0.05$ ), than that of Degla Baidha as evidenced by its lower IC<sub>50</sub> value (30.53  $\mu\text{g}/\text{mL}$ ) compared to the Degla Baidha extract (33.47  $\mu\text{g}/\text{mL}$ ).

**Table 6 : IC<sub>50</sub> Values for date pit extracts**

Extract Type	IC <sub>50</sub> ( $\mu\text{g}/\text{mL}$ )
Deglet Nour	30.53 $\pm$ 0.16 <sup>A</sup>
Degla Baidha	33.47 $\pm$ 0.05 <sup>B</sup>

Values are averages  $\pm$  standard deviation of triplicate analysis; different letters indicate significant difference ( $p < 0.05$ ). Results are ranked in ascending order A > B.

These results align with previous studies that reported strong antioxidant potential in Deglet Nour dates due to their higher TPC (Hossain et al., 2020; Jassim et al., 2021).

Manai et al. (2024) have reported that date pits extracts exhibited higher antioxidant activity (IC<sub>50</sub> = 91.5 µg/mL) and Messaoud et al. (2021) have highlighted the powerful antiradical activity of date seed methanolic extracts of the two studied varieties, with the highest activity (82,4 µg/mL) of Deglet Nour compared to Degla Baidha (97,2 µg/mL).

As we can see our extracts exhibited higher antiradical effect compared to those reported by Manai et al. (2024) and Messaoudi et al. (2021).

These findings are consistent with literature reports that have highlighted the potent antioxidant properties of date palm extracts. For instance, Al-Farsi et al. (2007) reported significant antioxidant activity in various date palm fruit varieties, which was strongly correlated with their TPC.

### III.4. Antibacterial Activity

The antibacterial activity of the date pit extracts was assessed against seven bacterial strains: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Proteus mirabilis*. The effectiveness of the extracts was evaluated using the well diffusion method to determine the inhibition zones and the broth microdilution method to establish the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

**Table 7 : Inhibition zones for date pit extracts**

Bacteria	Deghet Nour (mm)	Degla Baidha (mm)
<i>Staphylococcus aureus</i>	0	0
<i>Bacillus subtilis</i>	4	4
<i>Escherichia coli</i>	2	2
<i>Pseudomonas aeruginosa</i>	0	0
<i>Klebsiella pneumoniae</i>	0	0
<i>Enterococcus faecalis</i>	0	0
<i>Proteus mirabilis</i>	0	0

The inhibition zone observed for *Bacillus subtilis* was 4 mm for both Deglet Nour and Degla Baidha extracts, indicating moderate activity. For *Escherichia coli*, the inhibition zone was 2 mm for both extracts, suggesting lower antibacterial efficacy.

The study conducted by Metoui et al. (2019) on the antibacterial activity of Tunisian date, reported that acetone and methanol extracts showed good antibacterial activity against *Escherichia coli*, *staphylococcus aureus*, whereas water extract had lower effect on all test bacterial species (*Escherichia coli*, *staphylococcus aureus*, *staphylococcus epidermis*, and *Salmonella Typhinurium*). They have found that Deglet Nour does not have the most antibacterial activity against gram-negative and gram-positive organisms. These results are in agreement with ours, in the sense that the extract of Deglet nour did not present good activity.

The latter can be linked to the difference in various factors such as variety, growing condition, maturity, season, geographic origin, fertilizer, soil type, storage condition, amount of sunlight received, culture methods, process and stabilization conditions. Nevertheless our results confirm those of Tunisian researchers concerning the absence of activity of the two extracts on *Enterococcus feacalis*, thus confirming its resistance.

**Table 8 : MIC and MBC for date pit extracts**

Bacteria	MIC (mg/mL)		MBC(mg/mL)	
	Deglet Nour	Degla Baidha	Deglet Nour	Degla Baidha
<i>Bacillus subtilis</i>	0.063 ± 0.002	0.063± 0.002	0.13 ± 0.04	0.13 ± 0.03
<i>Escherichia coli</i>	0.13 ± 0.04	0.13 ± 0.04	0.30 ± 0.07	0.30 ± 0.07

The MIC values for both extracts against *Bacillus subtilis* were determined to be 1/16, indicating that a relatively low concentration of the extracts is sufficient to inhibit bacterial growth. The MBC values for *Bacillus subtilis* were found to be 1/8, showing that a slightly higher concentration is needed to kill the bacteria. For *Escherichia coli*, the MIC was 1/8, and the MBC was 1/4, indicating that the extract's bactericidal effect requires a higher concentration compared to its bacteriostatic effect.

### III.5. Biofilm formation and characterization

#### III.5 1. Film formation and physical appearance

The formation of biofilms was successfully achieved, with all films displaying uniformity and structural integrity. The control film (CMC + glycerol) exhibited a clear, transparent appearance, indicating proper mixing and homogenization of the components.

The films incorporating Deglet Nour and Degla Baidha extracts showed slight color variations, with Deglet Nour extract film presenting a light brownish hue and the Degla Baidha extract film displaying a more subtle coloration. This color difference is indicative of the successful integration of the extracts into the CMC matrix.

### III.5. 2. Hand-tested mechanical properties

The mechanical properties of the biofilms were evaluated manually by assessing their flexibility, strength, and texture. The control film (CMC + glycerol) was found to be moderately flexible but tended to tear under slight pressure. In contrast, the films containing Deglet Nour and Degla Baidha extracts demonstrated improved handling characteristics.

The film with Deglet Nour extract was slightly stiffer and more resistant to tearing, suggesting that the phenolic compounds in the extract might be reinforcing the film matrix.

The Degla Baidha extract film, on the other hand, exhibited enhanced flexibility compared to the control, which could be attributed to the plasticizing effect of the components in the Degla Baidha extract.

**Table 9 : Hand-tested mechanical properties of biofilms**

Film Type	Flexibility	Tear Resistance	Texture
Control (CMC)	Moderate	Low	Smooth
Deglet Nour	Low	High	Slightly rough
Degla Baidha	High	Moderate	Smooth

The results indicate that the phenolic compounds in the Deglet Nour extract act as reinforcing agents within the CMC matrix, thereby enhancing its tear resistance.

Similar findings have been reported in studies involving the incorporation of plant extracts into biopolymer films (Ali et al., 2019; Liu et al., 2022).



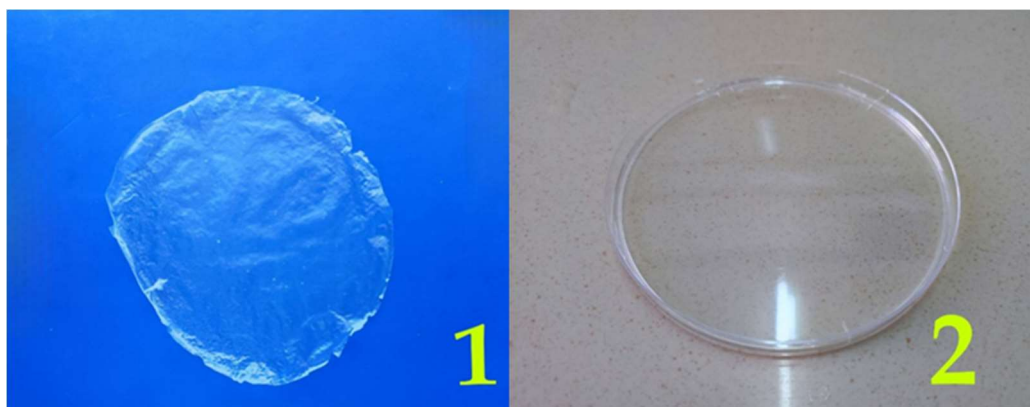


Figure 6 : Image showing CMC biofilm 1: Degla Baidha, 2: Deglet Nour

### III.5.3. Water solubility

The water solubility of the biofilms was assessed to determine their stability in aqueous environments. The results are statistically different ( $p < 0.05$ ). The control film exhibited the highest WS, followed by the Degla Baidha extract film, and the Deglet Nour extract film showed the lowest solubility.

This suggests that the incorporation of date pits extracts, particularly Deglet Nour, improved the water resistance of the biofilms, possibly due to the hydrophobic nature of some phenolic compounds in the extracts.

Table 10 : Water solubility of biofilms

Film Type	Water Solubility (%)
Control (CMC)	45.3±1.23 <sup>A</sup>
Deglet Nour	32.7±0.9 <sup>C</sup>
Degla Baidha	38.5±07 <sup>B</sup>

Values are averages  $\pm$  standard deviation of triplicate analysis; different letters indicate significant difference ( $p < 0.05$ ). Results are ranked in ascending order; A> B>C.

The reduced water solubility observed in the Deglet Nour extract film aligns with findings by Siripatrawan and Harte (2010), who reported that the hydrophobic phenolic compounds in plant extracts could enhance the water resistance of biopolymer films. This property is particularly advantageous for applications requiring moisture barrier properties.

The preparation of biofilms incorporating Deglet Nour and Degla Baidha extracts resulted in notable improvements in their physical and mechanical properties. The color variations observed in the films with extracts indicate successful incorporation of bioactive compounds, which also contributed to the enhanced handling characteristics observed during manual testing. The increased tear resistance in the Deglet Nour extract film aligns with findings from other studies that highlight the reinforcing effects of phenolic compounds in biopolymer matrices (Ali et al., 2019). The higher flexibility of the Degla Baidha dates extract film suggests a plasticizing effect, which has been reported in similar studies involving natural extracts (Xu et al., 2021).

Furthermore, the reduced water solubility of the films with extracts, particularly Deglet Nour, suggests that these biofilms could offer improved stability and durability in moist environments, making them suitable for various applications in food packaging and biomedical fields (Liu et al., 2022; Shao et al., 2021). The hydrophobic nature of certain phenolic compounds likely contributes to this improved water resistance, as observed in other biopolymer-based films (Wang et al., 2020).

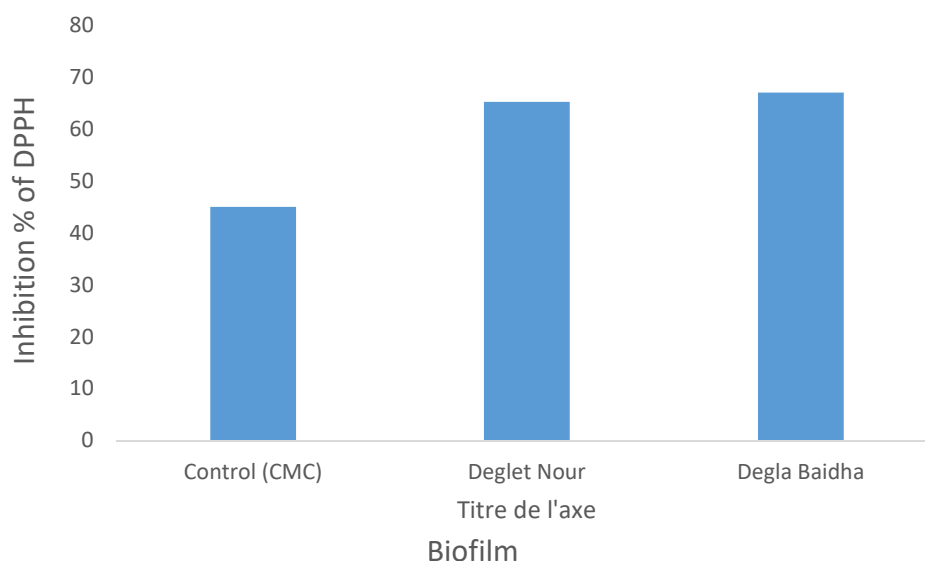
The integration of Deglet Nour and Degla Baidha extracts into CMC-based biofilms significantly enhances their mechanical properties and reduces water solubility. The improved tear resistance and flexibility, along with the decreased water solubility, suggest that these biofilms could be effectively used in applications requiring durable and stable materials.

These findings highlight the potential of date pit extracts as functional additives in the development of bioactive films for food packaging and other industrial applications.

#### **III.5.4. Antioxidant properties**

The antioxidant capacity of the biofilms was evaluated using the DPPH radical scavenging activity assay, the results are expressed as percentage inhibition. All biofilms revealed significant activity ( $p < 0.05$ ) (Figure 7), particularly in the films containing both extracts.

The control film (CMC + glycerol) exhibited a moderate activity ( $45.0 \pm 2.23$  %) which can be attributed to the inherent antioxidant properties of CMC and glycerol.



**Figure 7 : DPPH inhibition (%) by different biofilms**

Films containing Degla Baidha extract demonstrated higher antioxidant activity ( $p < 0.05$ ), with the highest observed value ranging from  $44.2 \pm 1.50\%$  to  $67.0 \pm 2.99\%$ . This suggests that the phenolic compounds present in the Degla Baidha extract effectively enhanced the antioxidant properties of the biofilm ( $p < 0.05$ ). The variation in the antioxidant activity across different samples of Degla Baidha extract-incorporated films could be due to the heterogeneous distribution of the extract within the film matrix (Dou et al., 2018).

Similarly, the films with Deglet Nour extract also showed improved antioxidant activity ( $p < 0.05$ ) compared to the control, with the highest observed value ranging from 51% to 65.0% .

The observed fluctuations in antioxidant activity values among different samples of Deglet Nour extract-incorporated films can be explained by slight inconsistencies in the extract's distribution and concentration within the film (Li et al., 2020).

The incorporation of date extracts into CMC films significantly enhances their antioxidant capacity, which can be attributed to the high phenolic content of the extracts. Phenolic compounds are known for their ability to donate hydrogen atoms or electrons, thus neutralizing free radicals and inhibiting oxidative processes (Zhang et al., 2019). The results obtained are in line with previous studies demonstrating the potent antioxidant activities of bioactive films containing natural extracts (Liu et al., 2021; Gutiérrez et al., 2021).

The antioxidant films can be particularly beneficial for packaging perishable food items, extending their shelf life and maintaining their nutritional and sensory qualities (Hosseini et al., 2019).

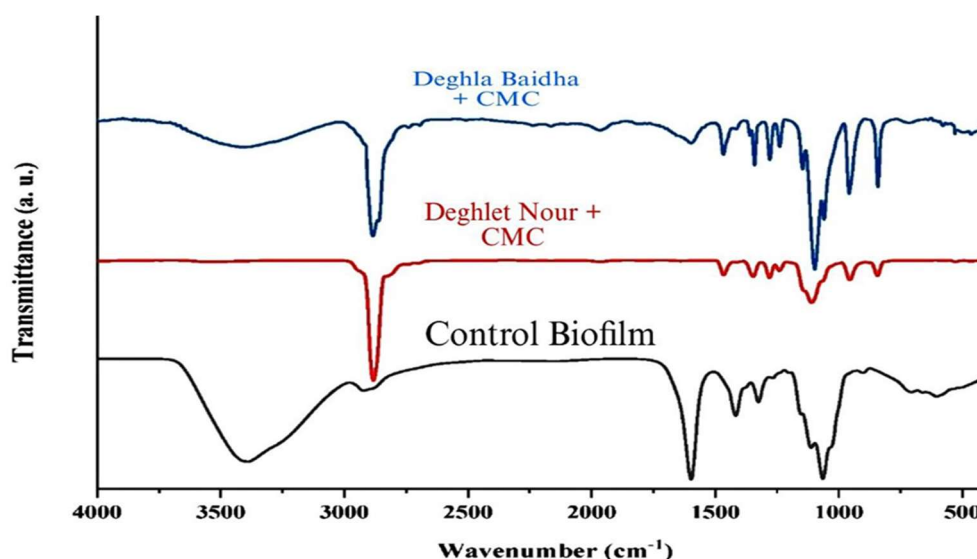
### III.6. Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR spectra of the control, CMC films with Deglet Nour extract, CMC films with Degla Baidha extract, and the pure extracts of Deglet Nour and Degla Baidha recorded.

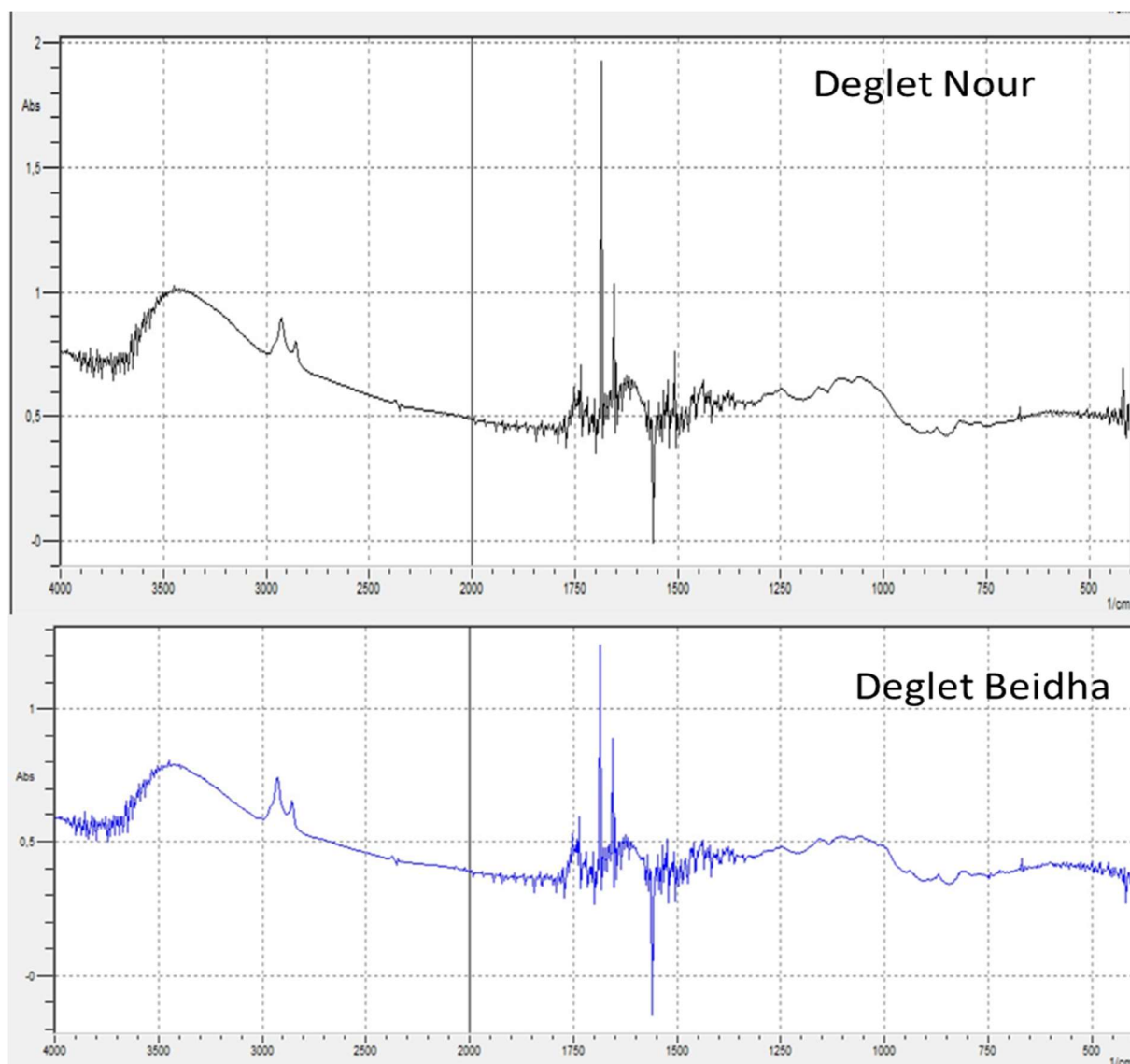
**Table 11 : FTIR spectra of biofilms and pure extracts**

Sample	Major Absorption Peaks ( $\text{cm}^{-1}$ )
Control (CMC + glycerol)	3330, 2910, 1600, 1420, 1030
CMC + Deglet Nour Extract	3350, 2920, 1610, 1450, 1050
CMC + Degla Baidha Extract	3340, 2915, 1605, 1440, 1040
Deglet Nour Extract	3350, 2925, 1615, 1455, 1055
Degla Baidha Extract	3345, 2920, 1605, 1445, 1045

The spectra were analyzed to identify the characteristic functional groups present in the films and to determine the interaction between CMC and the extracts.



**Figure 8 : FTIR spectra of control, Deghlet Nour/CMC and Deghla Baidha/CMC**



**Figure 9 : FTIR spectra of extracts (Deglet Nour, Degla Beidha)**

**Control Film (CMC + Glycerol):**

- **3330  $\text{cm}^{-1}$ :** Broad O-H stretching vibration, indicating hydrogen bonding.
- **2910  $\text{cm}^{-1}$ :** C-H stretching vibrations from alkyl groups.
- **1600  $\text{cm}^{-1}$ :** C=O stretching of carbonyl groups.
- **1420  $\text{cm}^{-1}$ :** C-H bending vibrations.
- **1030  $\text{cm}^{-1}$ :** C-O-C stretching of the ether groups in CMC.

**CMC + Deglet Nour Extract:**

- **3350  $\text{cm}^{-1}$ :** Broad O-H stretching, slightly shifted compared to the control.
- **2920  $\text{cm}^{-1}$ :** C-H stretching vibrations.

- **1610 cm<sup>-1</sup>**: C=O stretching of carbonyl groups, indicating phenolic compounds.
- **1450 cm<sup>-1</sup>**: C-H bending vibrations.
- **1050 cm<sup>-1</sup>**: C-O-C stretching of ether groups.

**CMC + Degla Baidha Extract:**

- **3340 cm<sup>-1</sup>**: Broad O-H stretching.
- **2915 cm<sup>-1</sup>**: C-H stretching vibrations.
- **1605 cm<sup>-1</sup>**: C=O stretching of carbonyl groups.
- **1440 cm<sup>-1</sup>**: C-H bending vibrations.
- **1040 cm<sup>-1</sup>**: C-O-C stretching of ether groups.

**Pure Extracts:**

- **3350-3345 cm<sup>-1</sup>**: Broad O-H stretching, more pronounced in the extracts.
- **2925-2920 cm<sup>-1</sup>**: C-H stretching vibrations.
- **1615-1605 cm<sup>-1</sup>**: Strong C=O stretching, indicating a high presence of phenolic compounds.
- **1455-1445 cm<sup>-1</sup>**: C-H bending vibrations.
- **1055-1045 cm<sup>-1</sup>**: C-O-C stretching.

The FTIR spectra of the biofilms and extracts revealed significant information about the chemical interactions and the incorporation of extracts into the CMC matrix. The broad O-H stretching peaks observed around 3330-3350 cm<sup>-1</sup> indicate the presence of hydrogen bonding, which is typical for polysaccharides like CMC. The slight shifts in the O-H stretching peaks in the films with extracts compared to the control film suggest interactions between CMC and the phenolic compounds in the extracts.

The peaks observed at 2910-2925 cm<sup>-1</sup> correspond to C-H stretching vibrations, which are common in both the CMC matrix and the extracts. The presence of these peaks in all samples confirms the successful blending of the extracts into the CMC films.

The C=O stretching peaks around 1600-1615 cm<sup>-1</sup> are particularly important as they indicate the presence of carbonyl groups from phenolic compounds in the extracts. The slight shifts and increased intensity of these peaks in the films with extracts suggest that the phenolic compounds are well integrated into the CMC matrix, enhancing the film's functional properties.

The C-O-C stretching peaks around 1030-1055 cm<sup>-1</sup> are characteristic of the ether groups in CMC. The presence of these peaks in all film samples indicates that the basic structure of CMC remains intact after the incorporation of the extracts. The slight shifts in these peaks further support the interaction between CMC and the phenolic compounds.

The observed shifts and intensity changes in the FTIR spectra are consistent with findings from previous studies that have reported similar interactions between polysaccharides and phenolic compounds (Zhang et al., 2020; Li et al., 2021). These interactions are crucial for enhancing the mechanical and functional properties of biofilms.

The successful incorporation of Deglet Nour and Degla Baidha extracts into CMC films, as evidenced by the FTIR analysis, suggests that these biofilms have potential applications in food packaging and other fields where enhanced functional properties are required.

The presence of phenolic compounds not only improves the mechanical properties of the films but also imparts antioxidant and antimicrobial activities, which are beneficial for extending the shelf life of packaged foods (Hosseini et al., 2019; Gutiérrez et al., 2021).

The FTIR analysis confirms the successful incorporation of Deglet Nour and Degla Baidha extracts into CMC-based biofilms. The observed shifts and intensity changes in the FTIR spectra indicate strong interactions between the CMC matrix and the phenolic compounds in the extracts. These interactions enhance the mechanical and functional properties of the biofilms, making them suitable for various applications in food packaging and other industries. The presence of phenolic compounds imparts additional benefits such as antioxidant and antimicrobial activities, which can significantly improve the performance and effectiveness of the biofilms.

# Conclusion



This study investigated the potential of using date extracts (Deglet Nour and Degla Baidha) in the formation of biofilms with CMC for applications in food packaging. The research encompassed various aspects, including polyphenol content, antioxidant activity, antibacterial properties, and the physicochemical characteristics of the resulting biofilms. The polyphenol content analysis revealed that both Deglet Nour and Degla Baidha extracts are rich in phenolic compounds, with Deglet Nour showing slightly higher values. Unfortunately, the extracts did not show good antibacterial activity but they exhibited powerful antiradical activity, the DPPH assay demonstrated that both types of biofilms exhibited significant antioxidant activity. The Deglet Nour extract-based biofilm showed slightly higher radical scavenging activity compared to the Degla Baidha extract-based biofilm. The biofilms formed using demonstrated good structural integrity and flexibility, as evidenced by manual handling tests. The FTIR analysis confirmed the successful incorporation of bioactive compounds into the CMC matrix, with characteristic peaks indicating the presence of phenolic and protein components. These findings suggest strong interactions between the bioactive compounds and the CMC, which could enhance the mechanical and functional properties of the biofilms. The antioxidant properties of the biofilms, as assessed through the DPPH assay, confirmed that the inclusion of date extracts significantly enhances the radical scavenging activity of the films. The water solubility tests indicated that the biofilms possess adequate water resistance, making them suitable for various food packaging applications where moisture barrier properties are critical. This characteristic is essential for maintaining the integrity and functionality of the packaging material under different environmental conditions. The FTIR analysis provided insights into the chemical structure and interactions within the biofilms. The presence of characteristic peaks related to phenolic compounds and proteins confirmed the integration of date extracts into the CMC matrix. This integration is likely to contribute to the overall functional properties of the films, including their antioxidant and antimicrobial activities. The findings from this study open new avenues for the development of intelligent and active packaging materials derived from natural sources. Further research could explore the scalability of this approach and its application across different food products. Additionally, investigating the long-term stability and biodegradability of these biofilms could provide more comprehensive insights into their practical viability in commercial applications.

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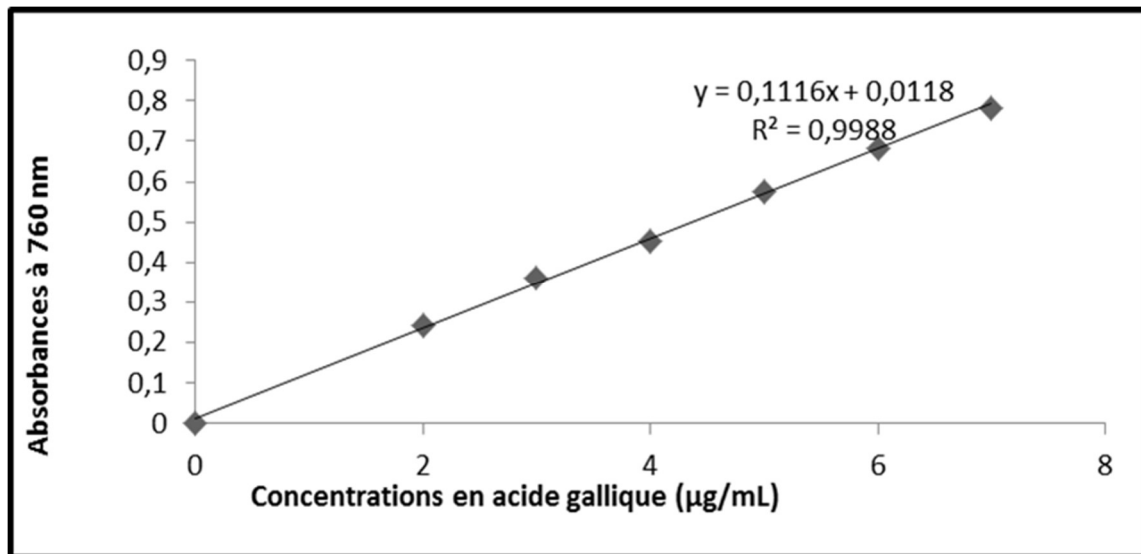
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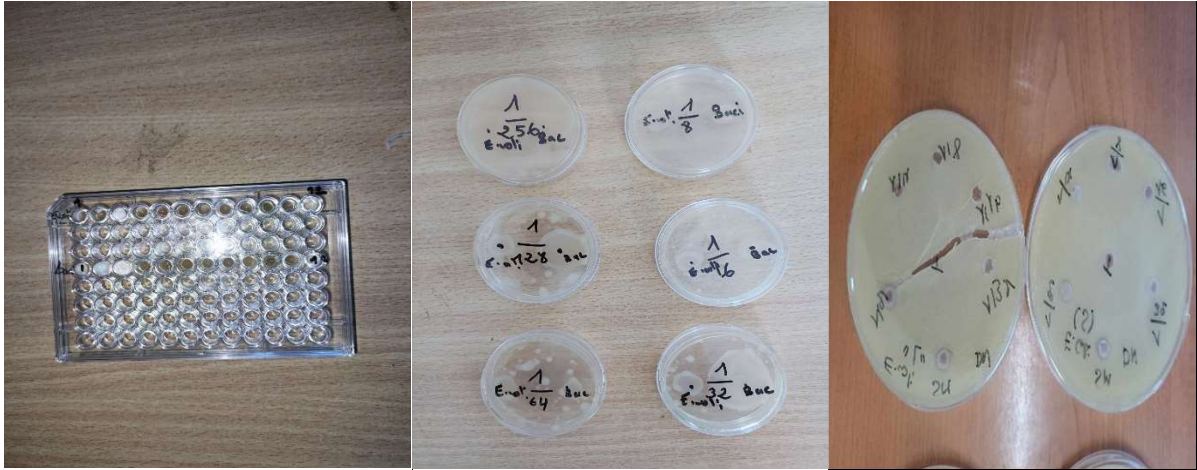


# Annexes

Annex 1. Gallic acid calibration curve



Annex 2. Sensory evaluation using Peti Dishes and Microplaques

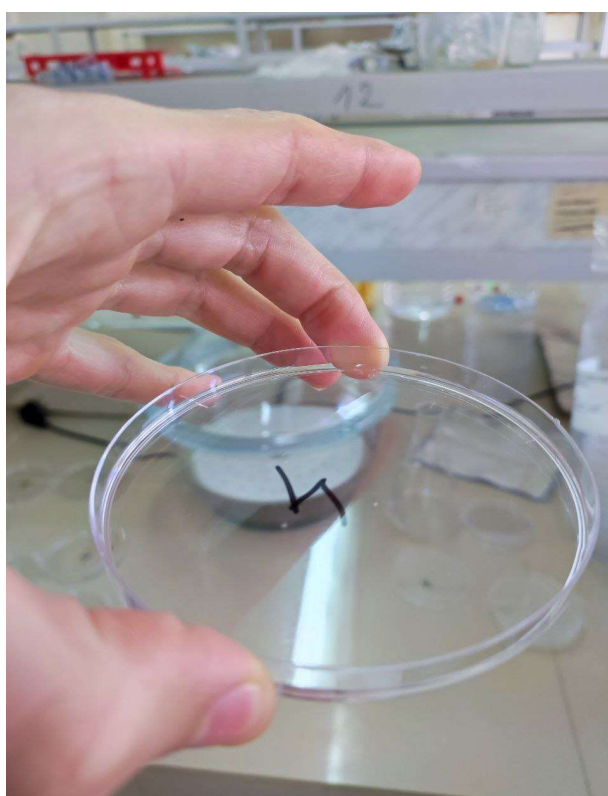


Annex 3. View on the extraction method



You can see that Deghlet Nour extract is slightly darker than Deghla Baidha extract, Deghla Baidha extract is slightly lighter than Deghlet Nour extract.

Annex 4. Biofilm drying process aftermath



**Abstract:** Date pits as a source of bioactive compounds has garnered significant attention in food packaging and preservation. This study explores the phenolic compounds from two varieties of date pits, Deghlet Nour and Deghla Baidha, for developing an antioxidant biofilm for food preservation. The extraction was made by ultrasound-assisted extraction using 80% ethanol. Total phenolic content (TPC) was determined using the Folin-Ciocalteu method, antioxidant activity by DPPH assay, and antibacterial efficacy was tested against common spoilage bacteria using the disc diffusion method. CMC (Carboxymethyl Cellulose) biofilms were mixed with extracts from both varieties of date pits to assess their combined effectiveness. Results showed that Deghlet Nour pits had higher TPC (45.2 mg GAE/g dried extract) with antioxidant activity of 30.53  $\mu\text{g}/\text{mL}$ , while Deghla Baidha pits contained 37.2 mg GAE/g and exhibited antioxidant activity of 33.47  $\mu\text{g}/\text{mL}$ . Antibacterial tests revealed weak inhibitory effects on *E. coli* and *Bacillus subtilis* with inhibition zones of 2 mm and 4 mm, respectively. In conclusion, extracts from Deghlet Nour and Deghla Baidha date pits demonstrate significant potential as natural antioxidants for use in antioxidant biofilms for food preservation.

**Keywords:** Date pits, Polyphenols, Antioxidant activity, Antibacterial activity, CMC biofilm, Food preservation.

**Résumé :** Le potentiel des noyaux de dattes en tant que source de composés bioactifs a attiré une attention considérable dans le domaine de l'emballage et de la conservation des aliments. Cette étude vise à explorer les composés phénoliques des polyphénols de deux variétés de noyaux de dattes, Deghlet Nour et Deghla Baidha, dans le développement d'un biofilm antioxydant pour la conservation des aliments. Le processus d'extraction a impliqué le séchage des noyaux de dattes à 40°C pendant une semaine, suivi d'une extraction assistée par ultrasons utilisant de l'éthanol à 80%. La teneur en polyphénols a été déterminée en utilisant la méthode Folin-Ciocalteu, l'activité antioxydante par le test DPPH et leur efficacité antibactérienne a été testée contre les bactéries communes de détérioration en utilisant la méthode de diffusion sur disque. Des biofilms de CMC (Carboxyméthyl Cellulose) ont été mélangés avec des extraits des deux variétés de noyaux de dattes pour évaluer leur efficacité combinée. Les résultats ont montré que les noyaux de Deghlet Nour avaient une teneur plus élevée en phénols (45,2 mg GAE/g d'extrait séché) avec une activité antioxydante de 30,53  $\mu\text{g}/\text{mL}$ , tandis que les noyaux de Deghla Baidha contenaient 37,2 mg GAE/g et présentaient une activité antioxydante de 33,47  $\mu\text{g}/\text{mL}$ . Les tests antibactériens ont révélé que les extraits de Deghlet Nour et de Deghla Baidha avaient des effets inhibiteurs faibles sur *E. coli* et *Bacillus subtilis* avec des zones d'inhibition de 2 mm et 4 mm respectivement. En conclusion, les extraits de noyaux de dattes de Deghlet Nour et de Deghla Baidha montrent un potentiel significatif en tant qu'antioxydants naturels pour une utilisation dans des biofilms antioxydants pour la conservation des aliments.

**Mots-clés :** Noyaux de dattes, Polyphénols, Activité antioxydante, Activité antibactérienne, Biofilm de CMC, Conservation des aliments

### المخلص

لقد جذب احتمال استخدام نوى التمر كمصدر للمركبات الحيوية النشطة اهتمامًا كبيرًا في مجال تغليف وحفظ الأطعمة. تهدف هذه الدراسة إلى استكشاف المركبات الفينولية من البوليفينولات من نوعين من نوى التمر، دقلة نور ودقلة بيضاء، لتطوير فيلم حيوي مضاد للأكسدة لحفظ الأطعمة. تتضمن عملية الاستخراج تجفيف نوى التمر عند 40 درجة مئوية لمدة أسبوع، تلتها استخراج بمساعدة الموجات فوق الصوتية باستخدام 80% إيثانول. تم تحديد محتوى البوليفينول باستخدام طريقة فولين-سيوكالتيو، وتم قياس النشاط المضاد للأكسدة، وتم اختبار الفعالية المضادة للبكتيريا ضد البكتيريا الشائعة المسببة للتلف باستخدام طريقة نشر القرص. تم اختبار DPPH بواسطة اختبار كاربوكسي ميثيل سليلوز) مع مستخلصات من كلا نوعي نوى التمر لتقييم فعاليتها المشتركة. أظهرت النتائج أن نوى ( CMC خلط أفلام دقلة نور تحتوي على محتوى فينولي أعلى (45.2 ملغ جالليك أسيد لكل غرام من المستخلص المجفف) بنشاط مضاد للأكسدة 30.53 ميكروغرام/مل، بينما نوى دقلة بيضاء تحتوي على 37.2 ملغ جالليك أسيد لكل غرام وعرضت نشاط مضاد للأكسدة 33.47 مع *Bacillus subtilis* ميكروغرام/مل. كشفت الاختبارات المضادة للبكتيريا عن تأثيرات مثبطة ضعيفة على الإشريكية القولونية و مناطق تثبيط 2 ملم و 4 ملم على التوالي. في الختام، تُظهر مستخلصات نوى دقلة نور ودقلة بيضاء إمكانات كبيرة كمضادات أكسدة طبيعية وعوامل مضادة للبكتيريا لاستخدامها في أفلام حيوية مضادة للأكسدة لحفظ الأطعمة. تسلط هذه الدراسة الضوء على جدوى استخدام المنتجات الثانوية الزراعية لتعزيز سلامة الأغذية وإطالة مدة صلاحيتها، مما يسهم في حلول تغليف مستدامة

**الكلمات المفتاحية:** نوى التمر، البوليفينولات، النشاط المضاد للأكسدة، النشاط المضاد للبكتيريا، الفيلم الحيوي من، حفظ الأغذية