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Thème

**Elaboration d'un emballage actif à base de
coproduits issus de l'industrie de
transformation des dattes**

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co-products from the date processing industry**

Presented by: Ms. BENBERKANE Lina & Ms. OUCHEN Riha

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Dedications

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Abstract

This study highlights the valorization of date pits (*Phoenix dactylifera L.*) into a biodegradable and bioactive packaging material. Physicochemical characterization of two Algerian cultivars (Degla Baidha and Deglet El Ghers) confirmed their suitability for biofilm development, with the Deglet El Ghers extract exhibiting remarkable antioxidant activity (IC_{50} : 6.91 $\mu\text{g/mL}$), surpassing that of the Trolox standard. A bioactive film was optimized using Response Surface Methodology (RSM) to evaluate the effects of date pit powder, agar, and glycerol on tensile strength (TS) and antioxidant activity (AA). The optimal formulation (0.47 g powder, 0.56 g agar, 0.30 mL glycerol) achieved predicted values of 31.07 MPa (TS) and 47.59% (AA), with a high desirability index of 0.84. The resulting films demonstrated significant antimicrobial activity against *Pseudomonas aeruginosa*, with a synergistic effect observed in composite films containing both powder and extract (inhibition zone: 15.5 ± 0.5 mm). These findings confirm that date pits represent a promising resource for the production of biodegradable and functional packaging, thereby contributing to the advancement of a circular bioeconomy.

Keywords: Date pits; Biodegradable packaging; Active films; Antioxidant activity; Response Surface Methodology; Antimicrobial activity; Circular bioeconomy.

Résumé

Cette étude met en évidence la valorisation des noyaux de dattes (*Phoenix dactylifera L.*) en un matériau d'emballage biodégradable et bioactif. La caractérisation physicochimique de deux cultivars algériens (Degla Baidha et Deglet El Ghers) a confirmé leur aptitude à l'élaboration de biofilms, l'extrait de Deglet El Ghers présentant une activité antioxydante remarquable (IC_{50} : 6,91 $\mu\text{g/mL}$), supérieure à celle du Trolox. Un film bioactif a été optimisé par la méthodologie de surface de réponse (RSM) afin d'évaluer l'effet de la poudre de noyaux de dattes, de l'agar et du glycérol sur la résistance à la traction (TS) et l'activité antioxydante (AA). La formulation optimale (0,47 g de poudre, 0,56 g d'agar, 0,30 mL de glycérol) a permis d'obtenir des valeurs prédites de 31,07 MPa (TS) et 47,59 % (AA), avec un indice de désirabilité élevé (0,84). Les films obtenus ont montré une activité antimicrobienne significative contre *Pseudomonas aeruginosa*, renforcée par un effet synergique dans les films composites contenant à la fois poudre et extrait (zone d'inhibition : $15,5 \pm 0,5$ mm). Ces résultats confirment que les noyaux de dattes constituent une ressource prometteuse pour la production d'emballages biodégradables et fonctionnels, contribuant ainsi au développement d'une bioéconomie circulaire.

Mots-clés : Noyaux de dattes ; Emballage biodégradable ; Films actifs ; Activité antioxydante ; Méthodologie de surface de réponse ; Activité antimicrobienne ; Bioéconomie circulaire.

Glossary

Glossary

A

- **Active Packaging** – An advanced packaging technology that interacts with the food and its environment to improve preservation, extend shelf life, and enhance safety and quality. This is achieved by absorbing undesirable compounds (e.g., oxygen, moisture) or releasing beneficial substances (e.g., antimicrobials, antioxidants).

B

- **Biodegradable Material** – A material capable of being broken down by the action of living organisms, primarily microorganisms, into natural elements such as water, carbon dioxide, and compost.
- **Carbone Dioxide (CO₂) Generating/Absorbing System** – An active packaging technology designed to either emit or remove carbon dioxide to inhibit microbial growth and modify the internal atmosphere of the package, thereby extending the shelf life of specific food products.
- **Conventional Packaging** – Traditional packaging that acts primarily as a passive barrier to contain and protect food products from external factors like moisture, light, and physical damage, without actively interacting with the food.

D

- **Date Pit (Kernel/Seed)** – The hard, oblong seed found within the date fruit (*Phoenix dactylifera L.*), a by-product of date processing. It is composed of a seed coat (testa), an endosperm, and an embryo, and is recognized for its nutritional and functional properties, including a high content of phenolic compounds and antioxidants.
- **DPPH Assay** – A common method used to evaluate the free radical scavenging (antioxidant) activity of a substance by measuring its ability to donate an electron to the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, resulting in a color change.

E

- **Ethylene Absorber** – An active packaging system that removes ethylene gas, a plant hormone that accelerates ripening and senescence in fruits and vegetables, from the packaging headspace to prolong freshness.

F

- **Flavor/Odor Absorbers/Releasers** – Active packaging technologies designed to either remove undesirable off-flavors and odors from the package or to release pleasant aromas and flavors to enhance product appeal and sensory perception.

I

- **IC₅₀ Value** – The half-maximal inhibitory concentration; a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. In antioxidant

assays, it represents the concentration of an extract required to scavenge 50% of free radicals, with a lower value indicating higher antioxidant activity.

M

- **Microwave-Assisted Extraction (MAE)** – An advanced extraction technique that uses microwave energy to heat solvents and plant matrices rapidly and efficiently, leading to the rupture of cell walls and enhanced release of intracellular compounds like phenolics and antioxidants.
- **Moisture Absorber** – An active packaging component (e.g., silica gel, clays) used to control excess water or humidity within a package, preventing microbial growth, condensation, and quality degradation in water-sensitive foods.

O

- **Organic Matter Content** – The mass of material in a sample derived from living organisms, including carbohydrates, proteins, lipids, and bioactive compounds. In date pits, a high organic matter content indicates a significant presence of these valuable components.
- **Oxygen Scavenger** – A system incorporated into packaging to remove residual oxygen from the headspace. This prevents oxidative reactions that cause spoilage, such as lipid oxidation, color changes, and the growth of aerobic microorganisms.

P

- **Phytochemicals** – Bioactive compounds found in plants, such as phenolic acids, flavonoids, proanthocyanidins, and tannins, which have therapeutic or health-beneficial properties, including antioxidant activity.
- **Proanthocyanidins** – A class of polyphenolic compounds, also known as condensed tannins, known for their strong antioxidant properties. They are polymers of flavan-3-ol units and contribute to the astringency of plant materials.

T

- **Tensile Strength (TS)** – A key mechanical property of a film material that measures the maximum stress it can withstand while being stretched or pulled before breaking. It is expressed in Megapascals (MPa).
- **Total Phenolic Content (TPC)** – A measure of the total concentration of phenolic compounds in a sample, quantified colorimetrically (e.g., using the Folin-Ciocalteu method) and expressed in milligram Gallic Acid Equivalents per gram of dry weight (mg GAE/g DW).

Z

- **Zone of Inhibition (ZOI)** – The clear area around an antimicrobial test sample (e.g., a film disk) on an agar plate where microbial growth has been inhibited. The diameter of this zone is measured to evaluate the antimicrobial efficacy of the sample.

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Introduction

Introduction

At the intersection of major economic, environmental, and public health challenges, food packaging today requires a collective effort from all stakeholders to drive the development of materials and technologies that are more respectful of both the environment and consumers. In recent years, packaging has been endowed with active and intelligent functionalities (**Realini & Marcos, 2014 ; Gontard, 2017**). These functionalities go far beyond those of conventional packaging, aiming not only to preserve food quality and safety but also to reduce losses and waste (**Ravindran, 2019**).

Active packaging represents an advanced category that, unlike traditional packaging, actively contributes to food preservation rather than merely serving as a passive barrier. This innovative form of packaging interacts with both the food and its environment, employing different mechanisms to inhibit spoilage (**Yildirim et al., 2018; Envases Activos, 2022**). Such systems may incorporate compounds including oxides, acids, alcohols, peptides, polyphenols, polysaccharides, and fatty acids, which can absorb or release gases, modulate humidity, regulate temperature, or act as antioxidants and antimicrobials. Within this framework, two important approaches are distinguished: smart packaging and edible coatings. Smart packaging integrates sensors capable of transmitting information about the presence of specific substances, as well as indicators of temperature, freshness, and ripening (**Vanderroost, 2014**). Conversely, coatings act primarily as gas barriers and can be enriched with antioxidant or antimicrobial agents to mitigate food spoilage (**Fathi, 2020**).

Date pits (DP), also known as date kernels (DK), are the hard seeds found inside dates, recognized for their nutritional and functional potential. They are abundant, inexpensive by-products rich in energy and phenolic compounds, traditionally used in animal feed and increasingly explored for their role in biodegradable materials (**Habib & Ibrahim, 2009; Platat et al., 2014**). Research suggests they could serve as an alternative energy source in ruminant feeding by reducing methane production and improving feed degradability (**Sabry et al., 2021**). Furthermore, their high polyphenol content makes them a promising candidate for antioxidant-rich bio-based materials (**Ardekani et al., 2010**).

The goal of the present study is to valorize this agri-food by-product, currently considered waste, by transforming it into a functional material for active food packaging. More specifically, we aim to address the question: *Is it possible to develop biodegradable active packaging using date pits?*

Bibliographic synthesis

Bibliographic synthesis

1 Food packaging :

1.1 Food packaging materials:

According to the European Directive 2004/12/EC, packaging refers to any product designed to contain, protect, and present goods, while facilitating their transport and handling. Traditional food packaging fulfills several essential functions, including safeguarding food against moisture, light, gases, microbial contamination, and physical damage, thereby ensuring preservation and extending shelf life (**Hu, 2022 ;Karate, 2024**). It also serves as a communication tool, providing nutritional information and supporting brand identity through design, while offering consumer convenience with features that simplify handling and distribution . Despite its effectiveness, traditional packaging—particularly plastic-based—raises ecological and health concerns, which has encouraged the transition toward sustainable alternatives that balance functionality with reduced environmental impact (**Gandhi, 2022 ; Arcot, 2024**).

Food packaging plays a crucial role in preserving food quality and safety while facilitating distribution, and it traditionally relies on materials such as plastics, metals, glass, paper, and cardboard. Plastics are valued for their adaptability and protective properties, including applications in modified atmosphere packaging, whereas metals provide excellent barriers against light and oxygen, making them ideal for cans. Glass, being inert and recyclable, is highly effective for preserving aromas and preventing contamination, while paper and cardboard offer ecological, biodegradable, and recyclable options, especially suited for dry or non-perishable products. Recent advances in packaging also emphasize sustainable alternatives, such as bio-based materials, edible films, and biodegradable plastics, aimed at reducing environmental impact (Piergiovanni, 2015).

1.2 Food Packaging Manufacturing Process

The manufacturing process of food packaging involves several key steps to ensure functionality and safety. It begins with the preparation and selection of materials according to the desired barrier properties (Yumeng, 2021; Hyo, 2020), followed by printing and coating, where techniques such as rotogravure are used to apply designs and information, while adhesives guarantee sealing and folding (**Yumeng, 2021 ;Ho, 2020**). The next stage includes laminating, which enhances durability and barrier efficiency, and shaping, where films are cut and molded

into pouches or bags (**Hu Zuolin, 2019 ;Ho, 2020**). Quality checks are carried out throughout the process to minimize defects and ensure reliability (**Ho, 2020**). Despite its effectiveness, this process raises environmental concerns due to the extensive use of plastics and non-biodegradable materials, highlighting the need for sustainable alternatives in the food packaging sector.

1.3 Environmental Impacts of Traditional Food Packaging

Traditional food packaging, particularly those based on plastics such as polyethylene and polystyrene, has significant ecological consequences due to its persistence in the environment and contribution to pollution. These materials can take centuries to degrade, leading to long-term waste accumulation and environmental contamination (**Phillips, 2024**). Their breakdown also generates microplastics that infiltrate aquatic systems and food chains, posing risks to ecosystems and human health (**Mafe, 2025**). Moreover, the production and disposal of single-use plastics release substantial amounts of greenhouse gases, further aggravating climate change (**Hilmarsdóttir, 2024**). In contrast, alternative packaging derived from edible sources and biopolymers presents more sustainable solutions, offering biodegradability and reduced carbon emissions, thereby mitigating some of the major environmental challenges linked to conventional packaging (**Mafe, 2025**).

1.4 Active Packaging: Definition, Principles, and History

Active packaging is recognized as one of the most dynamic technologies for extending the shelf life of packaged foods (**Bentayeb, 2007**). It is defined as packaging that incorporates additives into films or containers to maintain and prolong product quality (**Day, 2008**), going beyond passive protection by interacting with the food or its environment (**Wagner, 1999 ; Kerry , 2006; Day , 2008**) and, in some cases, communicating information about quality (**Brody, 2001**). Its principle lies in embedding active agents within the packaging that can absorb undesirable compounds such as oxygen, moisture, or ethylene, or release beneficial substances like antimicrobials and antioxidants, thereby enhancing food safety, sensory quality, and shelf life (**Vermeiren, 1999; Gontard, 2000; Dainelli, 2008**). The concept gained recognition after Dr. Theodore Labuza's 1987 presentation at an EU conference in Iceland (**Labuza, 1989**), although earlier patents from 1938 and 1943 already proposed oxygen absorption using metal powders, and a 1955 U.S. patent described catalytic hydrogen-to-water conversion for oxygen removal in milk powder packaging (**Robertson, 2012**). The first large-scale commercial application emerged in Japan in 1979 with iron-based oxygen scavenging sachets, while intelligent

packaging, including time–temperature indicators (TTIs), actually predates active packaging, with applications dating back to 1971 (**Robertson, 2012**).

1.5 Active Packaging Technologies and Applications

Active packaging technologies represent a major advancement over traditional passive systems by dynamically interacting with food and its environment to improve quality, safety, and shelf life (Table1) (**Robertson, 2012**). These systems can function indirectly through sachets or pads or directly by incorporating active compounds into packaging materials, and are generally classified into absorber systems, releaser systems, and intelligent sensors (**Ahvenainen, 2003; Kerry, 2006; Robertson, 2012**). Among absorbers, oxygen scavengers are widely used to limit oxidative spoilage, microbial growth, and nutrient loss while reducing the need for preservatives (**Brody, 2001; Kerry, 2006; Mohan, 2008**). Carbon dioxide generators help suppress microbial growth but must be carefully balanced to avoid adverse sensory and structural effects (**Scannell, 2000 ;López-Rubio, 2004**), while ethylene scavengers such as potassium permanganate delay ripening and senescence in fresh produce (**Abe, 1991 ;López-Rubio, 2004**). Moisture absorbers maintain product integrity by reducing microbial proliferation and preserving texture and flavor (**Rooney, 1995; Nielsen, 1997**). Other systems focus on sensory attributes, with controlled release of aromas and flavors to enhance consumer perception (**Almenar, 2009; Cobiella, 2019**). Antimicrobial packaging incorporates or releases bioactive agents to reduce pathogen and spoilage risks, complementing traditional preservation methods (**Cutter, 2002; Coma, 2008; Cobiella, 2019**), while antioxidant packaging integrates scavengers or agents to prevent lipid oxidation, maintaining sensory, nutritional, and functional properties (**Pereira de Abreu, 2010; Robertson, 2012**). Collectively, these technologies address critical issues such as oxidation, microbial contamination, moisture loss, and sensory degradation, providing innovative solutions for modern food preservation.

Table 1: Common active packaging systems, their mechanisms, and food applications (**Day, 2008**)

Active packaging system	Mechanisms Food	Applications
Oxygen absorbers	Iron-based, metal/acid, metal (e.g., platinum) catalyst, ascorbate/metallic salts,	bread, cakes, cooked rice, biscuits, pizza, pasta, cheese, cured meats and fish, coffee, snack foods, dried foods and

	enzyme-based and nylon MXD6	beverages coffee, fresh meats and fish, nuts and other snack foods and sponge cakes
Carbon dioxide emitters/absorbers	Iron oxide/calcium hydroxide, ferrous carbonate/metal halide, calcium oxide/ activated charcoal and ascorbate/sodium bicarbonate	Coffee, fresh meats and fish, nuts and other snack foods and sponge cakes
Ethanol emitters	Potassium permanganate, activated carbon and activated clays/zeolites	Pizza crusts, cakes, bread, biscuits, fish and bakery products
Ethylene absorber	Potassium permanganate, activated carbon and activated clays/zeolites	Fruits and vegetables
Moisture absorbers	Poly (vinyl acetate) blanket, activated clays and minerals and silica gel	Fish, meats, poultry, snack foods, cereals, dried foods, sandwiches, fruits and vegetable
Flavor / odor absorbers	Cellulose triacetate, acetylated paper, citric acid, ferrous salt/ascorbate and activated carbon/clays/zeolites	Fruit juices, fried snack foods, fish, cereals, poultry, dairy products and fruits

2 The dates

2.1 Date palm fruit

The date palm is a species that has been exploited and cultivated for several millennia in the Middle East and Africa. It is mainly cultivated in arid or semi-arid areas because the maturation of its fruit requires a long, hot summer with very low humidity. (Houssni, 2022)

The date is generally an elongated berry, though its shape and color can vary depending on the species. The edible part, called the flesh, consists of a thin cellulosic skin (the pericarp), a fleshy and deeply colored mesocarp (with a consistency that varies based on sugar content), and a lighter-hued, fibrous endocarp that sometimes forms a parchment-like membrane surrounding the pit (Noui, 2016)



Figure 1: Date palm

2.2 Chemical composition of date fruit:

The date fruit contains a wide range of functional nutritional components. It is rich in easily digestible sugars such as glucose and fructose. It represents a good source of fiber and trace elements such as potassium, phosphorus, magnesium, calcium, selenium, and iron, as well as vitamins such as ascorbic acid, niacin, and pyridoxine. It also contains bioactive components such as anthocyanin, phenolic compounds, carotenoids, procyanidins, and flavonoids that offer protection against oxidative stress (Ramchoun, 2017).

Table 2 : Date fruit content (Ramchoun, 2017)

Component	Content
Water	The water content depends on the varieties. It generally varies between 8 and 30% of the weight of the fresh flesh.
Carbohydrates	The date contains three major sugars: fructose, glucose, and sucrose, which account for approximately 44 to 88% of the dry weight of the pulp.
Proteins	Different varieties of dates are a good source of protein and provide a range of amino acids. However, depending on the stage of ripeness, the amino acid content varies significantly.
Lipids	The date contains a small amount of fat, about 1%. It is related to the variety and the stage of ripeness
Fibers	The date is relatively rich in fiber. It provides 6.5 to 11.5%, a large part of which corresponds to cellulose
Minerals	The date is rich in mineral elements, particularly in Na, K, and Ca, which are 0.3-0.6, 0.6-1.6, and 0.02-0.15 g/100 g of fresh mass, respectively
Vitamins	The vitamin fraction of the date is characterized by significant levels of B-group vitamins (B1, B2, B3, B5, B6, B9) and low levels of vitamin C
Energy	The energy value of the date is approximately 300 Cal/100 g.
Phenolic compound	Dates contain various phenolic compounds considered important therapeutic agents for the treatment of metabolic diseases and disorders.

2.3 Date pits

The date pit, also known as pip, stone, kernel, or seed, is the hard, oblong seed found within the date fruit. It is light brown in color and characterized by ventral grooves, a small embryo, and a hard endosperm made of cellulose deposits on the inside of the cell walls (figure 2) .

The date pit's length ranges from 12 to 36 mm, its width from 6 to 14 mm, and its weight from

0.5 to 4 g. The seeds' weight and size are determined by their maturation, variety, and growth environment (Attia A I., 2021)



Figure 2: Photography date seeds

Date pits, commonly referred to as seeds, kernels, stones, or pips; represent the residual materials generated from the processing of dates in industrial facilities. Notwithstanding the significant nutritional profile of date pits as a repository of carbohydrates, dietary fiber, protein, lipids, natural antioxidants, and bioactive polyphenols, they are frequently underexploited and predominantly regarded as waste, with date pits comprising multiple layers. (Figure 3)

- **Seed Coat (Testa):** This is the outermost layer of the date pit, offering protection to the inner embryo and endosperm. This particular layer is typically hard and exhibits resilience against various environmental factors.
- **Endosperm:** This refers to the nutrient-dense tissue situated within the seed coat that provides sustenance for the developing embryo. In fully mature seeds, it may function as the principal storage tissue for carbohydrates, lipids, and proteins.
- **Embryo:** This denotes the nascent plant encapsulated within the endosperm, containing the genetic material essential for the subsequent development of a new date palm tree when environmental conditions are optimal. Date pits are integral to the reproductive processes of date palm trees, as they encompass the genetic information essential for both germination and the subsequent development of seedlings.

Furthermore, these pits serve as a reservoir of diverse nutrients, encompassing carbohydrates, proteins, and lipids. Moreover, date pits have been employed in traditional medicinal practices as well as culinary applications across various cultures. (Al-Khalili, 2023)

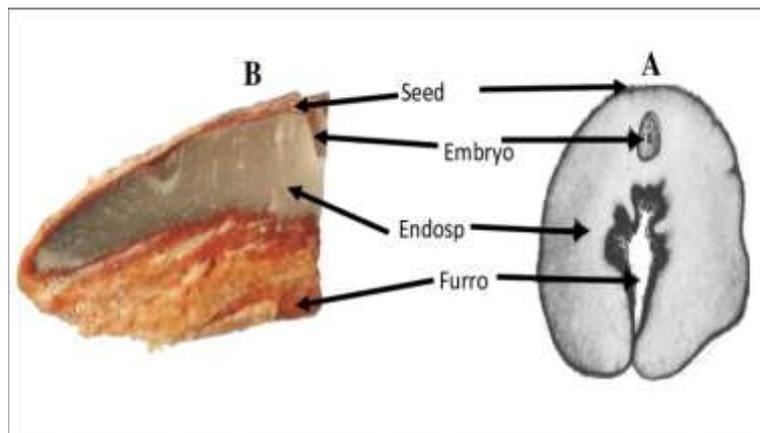


Figure 3: Composition of date pits (Al-Khalili, 2023)

2.4 Phytochemical composition of date pits

Phytochemicals have therapeutic effects when taken as prescription medications or as part of a regular diet. Bioactive substances such as carotenoids and polyphenols are common in the majority of fruits. The main components found in dates and date seeds include phenolic acids, flavonoids, sterols, and tannins, though their concentrations vary. The percentage and concentration of these components depend on several factors, including the fruit picking stage, date variety, postharvest processing, storage, soil conditions, and the dates' place of origin. In general, date seeds are a great source of phytochemical substances including as carotenoids, tannins, phytosterols, flavonoids, and phenolic acids. These substances' nutraceutical potential may be enhanced by their presence in different ratios (Idowu Anthony, 2020)

2.5 Polyphenols in date pits

The total polyphenol content of date seeds has been consistently reported to surpass that of the edible flesh, a phenomenon also observed in other fruit by-products such as grape seeds and pomegranate peels. According to Idowu Anthony (2020), the phenolic profile of date seeds is particularly diverse, comprising soluble compounds such as flavonols, hydroxybenzoates, and hydroxycinnamates. In Deglet Noor and other Algerian cultivars, specific flavonoid glycosides—including apigenin, luteolin, and quercetin—along with dactyliferic acid and its isomers, have been identified. Moreover, phenolic molecules such as kaempferol, isorhamnetin, 3-methyl-isorhamnetin, chrysoeriol, and their malonyl derivatives

have been detected, further confirming the high antioxidant capacity of date seeds. These compounds are known for their ability to neutralize free radicals, protect biomolecules from oxidative stress, and contribute to the prevention of chronic degenerative diseases.

In addition, **Mrabet Abdessalem (2022)** highlighted that tannins represent more than 50% of the phenolic fraction in date seeds, with significant variation among cultivars, ranging from 13 to 36.5 mg gallic acid equivalents/g DW. The seeds also contain about 3% DW of proanthocyanidins (condensed tannins), which are associated with strong antimicrobial, anti-inflammatory, and cardio-protective effects. The high concentration of these bioactive compounds not only enhances the nutritional and therapeutic value of date seeds but also underscores their potential as functional ingredients in food, nutraceutical, and pharmaceutical applications. Taken together, the evidence positions date seeds as a promising and underutilized source of natural antioxidants that can be valorized in the development of bioactive packaging, dietary supplements, and health-promoting products.

Material and methods

Material and Methods

1. Raw material preparation

Two date varieties, *Deglet El Ghers* and *Degla Baidha*, were procured from a local market in Bejaia, Algeria (figure 4). Date pits were manually separated from the fruit and thoroughly washed under running tap water to remove adhering pulp residues and surface contaminants. The cleaned pits were subsequently air-dried under controlled ambient conditions (45 ± 2 °C; 45–55% relative humidity). Following drying, the pits were comminuted using a high-speed laboratory blender, and the resulting material was sieved through a 500- μ m standard test sieve to obtain a uniform particle size. The powdered samples were then stored in airtight, light-protected amber glass containers at room temperature until further use in extraction procedures.

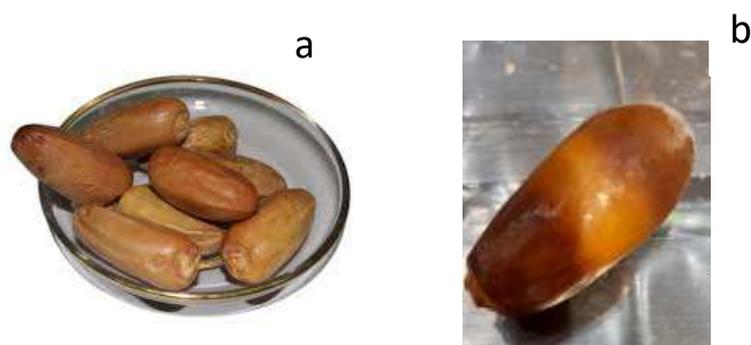


Figure 4: Photography of Degla Baidha (a) and Deglet El Ghers (b)

2. Preparation of date pits extract

Date pits extract from Deglet El Ghars and Degla Beidha were prepared by microwave assisted extraction method previously optimized by **Zemmoura et al. (2014)**. Briefly, a microwave oven (ER-SGS34MY, Toshiba, Malaysia) with dimensions of 519 × 406 × 314 mm (W × D × H) was employed. The device was equipped with a computerized control system allowing linear adjustment of both microwave power and irradiation time. To ensure a consistent sample volume, the system was adjusted so that vapors generated during extraction condensed back into the sample.

For the extraction, 1 g of the sample was placed in a round-bottom flask containing 30 mL of solvent. The type of solvent was determined based on a preliminary screening (Table S1), in

which three solvents were tested, and the optimal concentration was subsequently selected. The flask was placed in the microwave oven and connected to a condenser. After extraction, (figure 5) the mixture was filtered through Whatman No. 1 filter paper. The supernatant was collected in a volumetric flask and stored at 40 °C until further analysis.

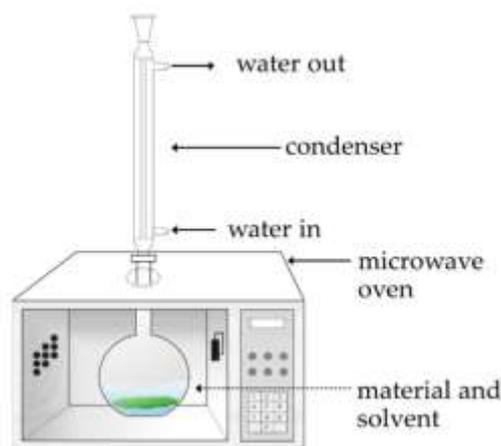


Figure 5: Schematic presentation of microwave assisted extraction equipment

3. Physico-chemical analyses of date pits

The physicochemical properties of date pits were determined according to the method described by Laaroussi et al. (2023).

3.1 Moisture and Dry Matter Content

Moisture content was determined by drying 3 g of date pit powder in an oven for 24 h at 105 °C. The moisture content was calculated using the following equation:

$$\text{Moisture (\%)} = \frac{W_i - W_d}{W_i} \times 100 \quad \text{Eq. 1}$$

where W_i represents the initial weight of the sample and W_d the dry weight after oven drying.

The dry matter content was obtained using the following formula:

$$\text{The dry matter (\%)} = 100 - \text{Moisture (\%)} \quad \text{Eq. 2}$$

3.2. Ash and Organic Matter Content

The ash content was determined by incinerating the sample in a muffle furnace at 500 °C for 3 h. The percentage of ash was calculated as:

$$\text{Ash content (\%)} = \frac{W_a}{W_s} \times 100 \quad \text{Eq. 3}$$

where W_a represents the weight of ash and W_s the initial weight of the date pit powder sample.

The organic matter content was then obtained by difference, according to the following equation:

$$\text{Organic matter content} = 100 - \text{Ash content} \quad \text{Eq. 4}$$

4. Determination of phyto-chemical properties of date pits

4.1 Determination of total phenolic compounds

The total phenolic compounds (TPC) were evaluated using the Folin–Ciocalteu colorimetric method as described by **Ouatmani et al. (2022)**, with slight modifications. Briefly, 200 μL of the sample extract was mixed with 1 mL of Folin–Ciocalteu reagent (previously diluted tenfold). After an incubation period of 5 min, 1 mL of sodium carbonate solution (6%, w/v) was added. The mixture was left to stand at room temperature for 30 min. Absorbance was then recorded at 750 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan). The phenolic content of the extract was quantified by comparison with a calibration curve prepared from gallic acid ($y = 5.8386x - 0.0134$; linearity range: 20–100 $\mu\text{g/mL}$; $R^2 > 0.999$).

Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry weight (DW) powder.

4.2 Determination of total flavonoids

The determination of flavonoid content was carried out following the procedure reported by **Belmokhtar. (2025)**, with minor adjustments. In brief, 1 mL of the extract was combined with 0.3 mL of sodium nitrite solution (5%, w/v). After standing for 5 min, 0.3 mL of aluminum chloride solution (10%, w/v) was added. Subsequently, 2 mL of sodium hydroxide solution (4%, w/v) were introduced after 6 min of reaction. The absorbance of the resulting mixture was recorded at 510 nm using a UV–Vis spectrophotometer, and the flavonoid content was expressed as milligrams of quercetin equivalents (QE) per 100gram of dry weight (DW).

4.3 Determination of proanthocyanidins

The quantification of proanthocyanidins was carried out using the HCl/butanol assay, following the procedure reported by **Chen. (2014)**. Briefly, 500 μL of date pits extracts was combined with 2 mL of the HCl/butanol reagent. The mixture was incubated in a water bath at 95 °C for 1 hour, after which the absorbance was measured at 530 nm. Proanthocyanidin levels were expressed as milligrams of procyanidin equivalents per one hundred gram of date pit dry matter (mg QE/100g DM).

4.4 Determination of antioxidant activity

The antioxidant activity of date pits extract (*Deglet El Ghers* and *Degla Baidha*) was evaluated by DPPH method. The ability of the extract to scavenge DPPH free radicals was determined according to the method of **Harkat-Madouri et al. (2006)**. A volume of 100 μL of the sample was mixed with 2.9 mL of DPPH solution in methanol (6×10^{-5} M). After incubation in the dark at room temperature for 30 minutes, the absorbance was measured at 515 nm. The IC_{50} value was determined, and the scavenging activity percentage was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad \text{Eq. 5}$$

A_{cont} = absorbance of the control after 30 min et A_{s} = absorbance of the sample

5. Optimization of the preparation of biodegradable active packaging

A three-level Box–Behnken design with three replicates at the central points was employed for the optimization study using JMP software (Version 16, SAS Institute Inc., Cary, NC, USA). Fifteen packaging formulations were prepared to evaluate the individual and interactive effects of three independent variables: X1—date pits powder (g), X2—agar (mL), and X3—glycerol (mL), on the two properties of the active packaging; tensile strength (Y1—TS (MPa)) and antioxidant activity (Y2—AA (%)).

During the optimization of the coating formulation, the response variables were modeled as a function of the independent factors using a second-order polynomial equation (Eq. 6).

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 \quad \text{Eq. 6}$$

Where β_o , β_i , β_{ii} and β_{ij} represent the regression coefficients for intercept, linear, quadratic, and interaction terms respectively. X_i and X_j are independent variables, and Y represents the dependent variable (response).

5.1 Preparation of active packaging films

Active packaging films were prepared using agar-agar, glycerol, date pit powder (residual from MAE extraction), and MAE date pit extract. Distilled water was first heated to 80 ± 2 °C, after which agar-agar and date pit powder were gradually incorporated under continuous stirring and maintained at this temperature for 10 min. The solution was then cooled to 50 ± 2 °C, and glycerol was added, followed by stirring for 5 min. After further cooling to 30 ± 2 °C, the MAE date pit extract was introduced and homogenized for 2 min to ensure macroscopic uniformity. The final solution was cast into Petri dishes, allowed to gel at room temperature (25 °C) for approximately 30 min, and subsequently air-dried uncovered for 24 h at 25 °C.

5.2 Determination of Tensile Strength (TS)

The tensile strength (TS) of the biofilms was measured using a universal testing machine (Shimadzu AGS-X, Japan) according to the ASTM standard method D882-02 (ASTM, 2002), with slight modifications. Film samples were cut into rectangular strips (2 cm × 5 cm) and conditioned at 25 ± 2 °C and $50 \pm 5\%$ relative humidity for 48 h prior to analysis. Each strip was mounted between the two pneumatic grips of the machine, with an initial gauge length of 30 mm. The test was performed at a crosshead speed of 50 mm/min until the film broke. The maximum force (F , N) at break was recorded, and TS (MPa) was calculated as follows:

$$TS = \frac{F}{A} \text{ Eq. 7}$$

where F is the maximum force at break (N) and A is the initial cross-sectional area of the film (thickness × width, mm²). Film thickness was determined at five random positions using a digital micrometer (± 0.001 mm accuracy), and the average value was used for calculations. Each measurement was performed in triplicate, and mean values were report

5.3 Determination of antioxidant activity of active packaging film

The antioxidant activity of the biofilms was determined using the DPPH radical scavenging assay, following the method of **Rhimi et al. (2023)** with slight modifications. Biofilm samples (≈ 50 mg) were cut into small pieces and extracted with 5.0 mL of methanol by sonication for 30 min at room temperature. The extracts were centrifuged at 4000 rpm for 10 min, and the

supernatants were filtered through a 0.45 µm membrane. When necessary, appropriate dilutions were prepared. The DPPH radical scavenging activity of the packaging extracts was then measured according to the procedure previously described in this study. The results were expressed as a percentage of radical inhibition.

6. Microbiological studies on biodegradable active packaging

The objective of the experimental work was to assess the in vitro antimicrobial activity of innovative packaging films enriched with date pit powder and/or extract. The antimicrobial potential was evaluated against a panel of representative microorganisms, including Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella enterica* ATCC 14028), a Gram-positive bacterium (*Staphylococcus aureus*), and a unicellular fungal species (*Candida albicans* ATCC 10231). These strains were selected because they represent key foodborne pathogens and spoilage organisms of major concern in food safety and preservation. *E. coli* and *S. enterica* are frequently associated with gastrointestinal infections, *P. aeruginosa* is known for its spoilage potential in perishable foods, *S. aureus* is a well-documented toxin-producing bacterium, and *C. albicans* serves as a model yeast-like fungus that can colonize food surfaces. Assessing the inhibitory effects of bioactive films against this diverse panel provides relevant insights into their potential application as active packaging materials for enhancing food quality and safety.

6.1 Microbial Strains and Culture Conditions

Pure cultures of the following microorganisms were used in the study: Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella enterica* (ATCC 14028); the Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923); and the yeast *Candida albicans* (ATCC 10231). All strains were reactivated by streaking onto selective media Mueller Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for *Candida albicans*. Bacterial cultures were incubated at 37°C for 24 hours, while the yeast was incubated at 30°C for 24 to 48 hours to obtain fresh and active cultures. (CLSI., 2018)



Figure 6: Representative pure cultures of Gram-positive and Gram-negative bacterial strains on agar plates.

6.2 Preparation of microbial inoculum

The inoculum was prepared using the direct colony suspension method in accordance with CLSI guidelines (2018). In brief, 3 to 5 well-isolated colonies of each microorganism were picked using a sterile loop and suspended in 9 mL of sterile physiological saline (0.9% w/v NaCl). The mixture was then vortexed vigorously for 15 to 30 seconds to obtain a uniform, clump-free suspension. The optical density (OD) of the bacterial suspension was measured at 625 nm with a spectrophotometer and adjusted using sterile saline to an OD of 0.08–0.1, corresponding to a 0.5 McFarland standard, which is approximately 1.5×10^8 colony-forming units per milliliter (CFU/mL). The same standardization procedure was applied to the yeast suspension (McFarland, 1907).

6.3 Antimicrobial Test Using Disk Diffusion on Agar

Agar plates (Mueller-Hinton Agar for bacteria and Sabouraud Dextrose Agar for yeast) were inoculated by evenly streaking the surface in three directions with a sterile cotton swab dipped in the standardized microbial suspension, ensuring a uniform lawn of growth. Using sterile forceps (flamed and cooled), film sample disks were aseptically placed onto the inoculated agar surface and gently pressed to ensure proper contact. The test films included: C (Composite), containing both date pit extract and powder; SP (Sans Poudre), containing extract only (without powder); SE (Sans Extrait), containing powder only (without extract); and B (Blanc), a plain control film without any active components.



Figure 7 : "Experimental Setup of the Disk Diffusion Assay for Evaluating the Antibacterial Potential of Bioactive Film Samples".

6.4 Antibacterial and antifungal activities of films against pathogenic bacterial and fungal strains

The plates were allowed to diffuse at room temperature for 10–15 minutes before incubation in an inverted position. Bacterial plates were incubated at 37 °C for 24 hours, while the yeast plates were incubated at 30 °C for 48 hours. Following incubation, the zones of inhibition (ZOI)—defined as the clear areas around the disks where microbial growth was prevented—were examined. The diameter of each ZOI, including the disk diameter, was measured precisely using a digital caliper. All assays were performed in triplicate to ensure reproducibility. The mean zone diameter for each film type and microorganism was calculated and reported in millimeters.



Figure 8: Assessment of Antibacterial Activity by Disk Diffusion Method with Measured Zones of Inhibition

Results and discussion

Results and discussion

1. Physico-chemical analysis

The physicochemical properties of date pits from two Algerian cultivars, Deglet El Ghers and Degla Baïdha, were evaluated and are summarized in **Table 3**. Deglet El Ghers exhibited a moisture content of $6.92 \pm 0.01\%$, slightly higher than Degla Baïdha ($5.69 \pm 0.05\%$), whereas Degla Baïdha had a higher dry matter content ($94.31 \pm 0.05\%$) compared to Deglet El Ghers ($93.08 \pm 0.01\%$). Conversely, ash content was higher in Degla Baïdha ($1.45 \pm 0.02\%$) than in Deglet El Ghers ($1.09 \pm 0.01\%$), while the organic matter content was slightly higher in Deglet El Ghers ($98.91 \pm 0.01\%$) relative to Degla Baïdha ($98.55 \pm 0.02\%$).

These values are consistent with previously reported data for date seeds. Moisture content in date pits typically ranges from 4% to 10%, dry matter content from 90% to 96%, and ash content from 1% to 2.6%, depending on cultivar, geographic origin, and harvesting conditions (**Al-Khalili et al., 2023; Attia et al., 2021**). The observed differences between the two cultivars may be attributed to varietal genetic factors, environmental conditions during growth, and post-harvest handling.

From a technological perspective, the low moisture content and high dry matter of both cultivars make them suitable for milling into stable powders for incorporation into bioactive films (**Khwaldia et al., 2022**). The relatively high organic matter content suggests a significant presence of polysaccharides, proteins, and other bioactive compounds, which can contribute to the functional properties of films (**Subhash et al., 2024; Chaira et al., 2007**).

The slight variations between Deglet El Ghers and Degla Baïdha could influence the mechanical and barrier properties of films. For instance, Degla Baïdha, with higher dry matter and ash content, may produce films with enhanced rigidity and structural stability, whereas Deglet El Ghers, with higher organic matter content, may offer better flexibility and bioactive compound retention (**Al-Khalili et al., 2023**).

Physico-chemical test	Composition of date pits on %	
	<i>Deglet El Ghers</i>	<i>Degla Baidha.</i>
Moisture content	6.92±0.01 ^a	5.69±0.05 ^b
Dry matter content	93.08±0.01 ^b	94.31±0.05 ^a
ash content	1.09±0.01 ^b	1.45±0.02 ^a
organic matter content	98.91±0.01 ^a	98.55±0.02 ^b

Table 3: Physico-chemical composition of date pits from Deglet El Ghers and Degla Baidha cultivars (% dry weight)". Values are expressed as mean ± standard deviation (n = 3). Different superscript letters within the same row indicate significant differences between means (p < 0.05), as determined using Student's t-test.

2. Phytochemical and antioxidant properties of date pits

The phytochemical profile of date pits revealed slight but meaningful differences between the two studied cultivars. Deglet El Ghers exhibited marginally higher total phenolic content (15.03 mg GAE/g DW) than Degla Baidha (14.84 mg GAE/g DW), while its flavonoid concentration (51.39 mg QE/100 g DW) was also superior (**Table 3**). In contrast, Degla Baidha contained significantly higher levels of proanthocyanidins (85.13 mg CE/g DW) compared to Deglet El Ghers (71.14 mg CE/g DW). Interestingly, antioxidant activity measured by the DPPH assay (**Table 4**) demonstrated that Deglet El Ghers had the lowest IC₅₀ value (6.91 µg/mL), indicating stronger radical scavenging capacity than Degla Baidha (9.76 µg/mL) and even surpassing Trolox (8.56 µg/mL) as a reference standard. These findings suggest that differences in qualitative composition, rather than total phenolic content alone, may account for the superior antioxidant potential of Deglet El Ghers. Comparable ranges of phenolic compounds and antioxidant capacities have been reported in other date pit varieties, with total phenolic contents typically ranging from 11 to 30 mg GAE/g DW depending on cultivar and extraction method (**Amin et al., 2023 ;Shi et al., 2023**). The relatively high proanthocyanidin levels observed in

Degla Baidha also fall within values described for polyphenol-rich date seeds (Amin et al., 2023). From an application perspective, these results highlight the potential of both cultivars as sources of natural antioxidants for active packaging. Extracts are particularly attractive for enhancing antioxidant functionality due to their concentrated bioactive content, whereas powders can contribute additional mechanical reinforcement and barrier properties when incorporated into biopolymer films. Previous studies have demonstrated that date pit extracts and powders can effectively improve the antioxidant activity, mechanical properties, and storage stability of biopolymer-based films, confirming their value as sustainable additives for active packaging systems (Khwaldia et al., 2021; Thakwani et al., 2023; Al-Khalili et al., 2024).

Table 4. Phytochemical composition and antioxidant activity of date pits from two cultivars (Deglet El Ghers and Degla Baidha).

	Phytochemical profile		
	<i>Deglet El Ghers</i>	<i>Degla Baidha.</i>	
Total phenolic compounds (mg GAE/g DW)	15.03±0.32 ^a	14.84±0.26 ^b	
Total flavonoids (mg QE/100g DW)	51.39±2.20 ^a	47.07±0.07 ^b	
Proanthocyanidins (mg CE/g DW)	71.14±1.78 ^b	85.13±0.67 ^a	
	Antioxidant activity		
	<i>Deglet El Ghers</i>	<i>Degla Baidha.</i>	Trolox
IC50 (µg/mL)	6.91±0.82 ^a	9.76±0.09 ^c	8.56±0.01 ^b

Values are expressed as mean ± standard deviation (n = 3). Different superscript letters within the same row indicate significant differences between means (p < 0.05), as determined using one-way analysis of variance (ANOVA) followed by Student's t-test for pairwise comparisons.

3. Design and Optimization of Biodegradable Active Packaging Materials

A Box–Behnken design consisting of 15 experimental runs (TA was employed to develop biodegradable active packaging and to establish the correlation between the input variables, namely **X1 – amount of date pit powder (g)**, **X2 – amount of agar (g)**, and **X3 – volume of**

glycerol (mL), and the output responses, Y1 – tensile strength (TS, MPa) and Y2 – antioxidant activity (AA, %). These parameters were selected based on insights from the literature review. The experimental results are summarized in Table X. The tensile strength of the biofilms ranged from **2.25 to 33.57 MPa (Mega Pascal)**, while their antioxidant activity varied between

22.17 % and 49.38 %.

Table 5: Experimental design and results for the optimization of biofilm properties

Experimental setup	Date pits powder (g)	Agar (g)	Glycerol (mL)	TS (MPa)	AA (%)
000	0.5	1	0.6	24.18	49.38
0+-	0.5	1.5	0.3	24.8	44.84
-0-	0.25	1	0.3	29.81	40.68
000	0.5	1	0.6	23.65	47.17
+ -0	0.75	0.5	0.6	9.07	45.75
0--+	0.5	0.5	0.9	2.25	46.57
000	0.5	1	0.6	22.23	48.12
0++	0.5	1.5	0.9	14.04	48.23
+0+	0.75	1	0.9	10.21	44.59
--0	0.25	0.5	0.6	33.57	35.12
0--	0.5	0.5	0.3	30.82	47.91
++0	0.75	1.5	0.6	25.35	35.12
-0+	0.25	1	0.9	15.85	37.91
+0-	0.75	1	0.3	23.88	40.05
-+0	0.25	1.5	0.6	18.21	43.75

3.1 Model fitting:

The analysis of variance (ANOVA) results for the quadratic polynomial model fitted for **Tensile Strength** performance and **antioxidant activity** are presented in the table below. The F-test suggests that the both models have a very high F-value and a very low p-value ($p < 0.05$), indicating that the models were highly significant. The p-value for lack of fit was greater than 0.05, indicating that it was not statistically significant compared with the pure error and that the models' equations were suitable for predicting TS performance and AA for any combination of variable values

Source	F Value	P value Prob > F	F Value	P value Prob > F
Model	7.75	0.0066	5.01	0.0226
X_1 -Date pit powder	0.40	0.5468	0.90	0.3752
X_2 -Agar	0.30	0.6021	0.12	0.7373
X_3 -Glycerol	40.52	0.0004	1.80	0.2211
X_1X_2	13.30	0.0082	7.78	0.0270
X_1X_3	5.79	0.0471	12.70	0.0092
X_2X_3	4.21	0.0793	0.47	0.5155
X_1^2	0.18	0.6809	20.83	0.0026
X_2^2	3.31	0.1118	0.18	0.6804
X_3^2	1.59	0.2483	0.19	0.6777
Lack of Fit	3.30	0.1395	0.84	0.5405

Table 6: Analysis of variance (ANOVA) for the fitted quadratic polynomial models for tensile strength (TS) and antioxidant activity (AA).

The model's goodness of fit was evaluated using a number of descriptive statistical analyses, such as coefficient of determination (R^2), adjusted coefficient of determination ($AdjR^2$), adequate precision (Adeq Precision), and coefficient of variation (CV). The sample variation was statistically significant at 98% for TS and 95% for AA according to the R^2 value of 0,9883 and 0.9552 respectively. Stated otherwise, a coefficient of determination in the neighborhood of 1 denotes a strong correlation between the observed and predicted data (**table 7**).

The model's significance was also satisfactory confirmed by $Adj R^2$ of both TS (0.97) and AA (0.95%). The coefficient of variation (CV) represents the level of dispersion in the data. As a general rule, a low CV value leads to better reproducibility while a high CV value (superior to 10%) indicates high variance of the mean value and the inability to develop an adequate response model. In our case coefficient of variation is inferior to 10% in both TS and AA responses.

Table 7: Descriptive parameters for ST and AA model fitting

	Tensile Strength (TS MPa)	Antioxidant Activity (AA%)
R-Squared	0.9883	0.9552
Adj R-Squared	0.97	0.95
Adeq Precision	10.16	7.45
C.V. %	8.90	7.98

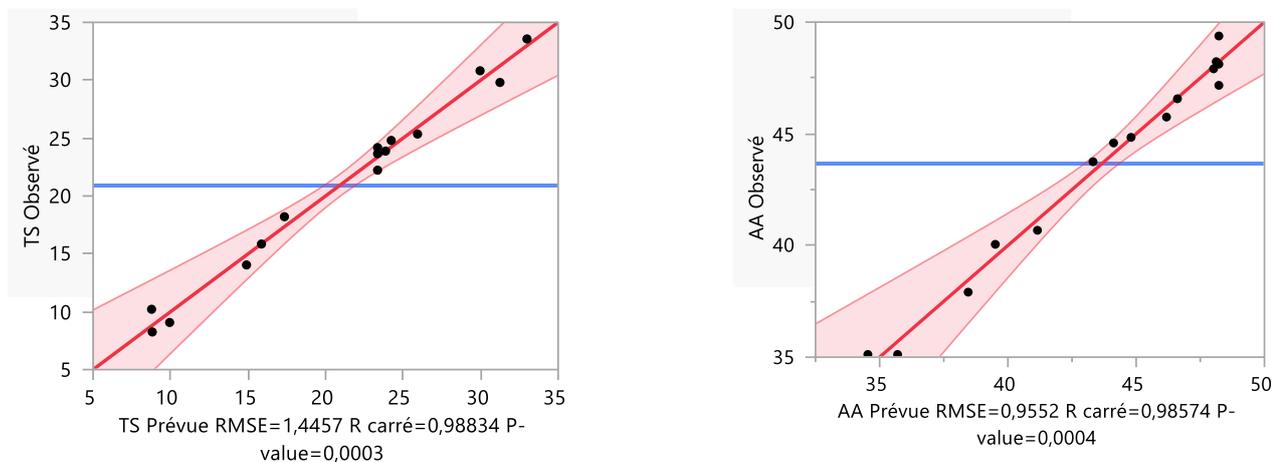


Figure 9: Observed values plotted against predicted values for TS and AA responses

3.2 Variable significance:

The significance of each coefficient was determined using the *p-value*. This value is used as a tool to assess the significance of each coefficient and the strength of interactions between each independent variable. A *p-value* of less than 0.05 indicates that the model terms are significant. In this case, the main factor affecting TS was glycerol amount however the main factor affecting AA was amount of date pits powder. After neglecting all non-significant terms ($p > 0.05$), the fitted quadratic model for TS and AA in coded variables are given in the following equations:

$$\text{TS} = +24.49 - 9.77X_3 + 7.91X_1X_2 - 5.22X_1X_3$$

$$\text{AA} = +46.89 - 4.82X_1X_2 + 6.15X_1X_3 - 7.68X_1^2$$

3.3 Response surface plots

Response surface methodology (RSM) and three-dimensional (3D) plots are powerful tools to visualize the relationships between independent variables and response factors. They not only highlight the main effects of each parameter but also reveal possible interactions, allowing the identification of optimal conditions for desired film properties (**Weremfo et al. (2022)**).

In the present study, the 3D response surface plots illustrate the combined influence of date pit powder, agar, and glycerol concentrations on tensile strength (TS) and antioxidant activity (AA) of the biofilms.

In **figure 10**, the plot shows how varying the amounts of date pit powder and agar affects the tensile strength (TS) of the biofilms.

The results indicate that TS increased progressively with higher levels of agar, reflecting the reinforcing role of this gelling agent in the polymer matrix. Date pit powder also contributed positively to TS up to an intermediate level, after which further addition led to a slight reduction, likely due to particle aggregation and weaker interfacial interactions. The interaction between the two factors suggests that an optimal balance exists, where a moderate amount of date pit powder combined with sufficient agar enhances film strength.

Overall, the 3D response surface confirms that both variables have significant effects, with agar exerting a stronger influence. Identifying this balance is crucial for tailoring the mechanical properties of biodegradable active packaging.

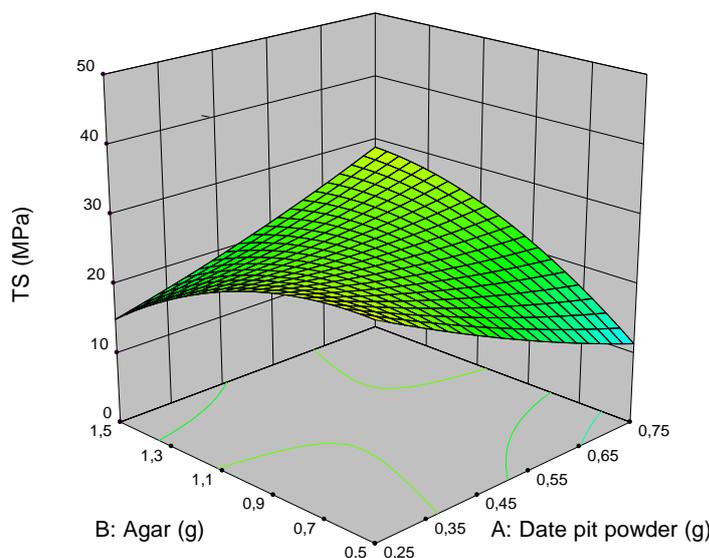


Figure10:3-D curve showing the interaction effect of Amount of date pit powder and amount of agar on tensile strength

Figure11 shows a decrease in tensile strength with higher amounts of glycerol. This result is consistent, since glycerol acts as a plasticizer that increases elongation at break and flexibility, while at the same time reducing tensile strength. Glycerol intercalates between the polymer chains of agar, weakening strong intermolecular bonds (hydrogen bonds) and enhancing chain mobility (e.g., degradation of hydrogen-bonded interactions).

For example, in films based on arrowroot starch plasticized with glycerol (15-45 %), tensile strength dropped significantly with increasing glycerol, while elongation at break increased, and FTIR analyses showed more hydrogen bonding between glycerol and the starch but reduced starch-starch intermolecular interactions (**Tarique et al.2021**).

Similarly, in films of low-methoxyl pectin, addition of glycerol (20-40 %) causes reduction in tensile strength and Young's modulus, with an increase in elongation, attributed to hydrogen bond disruption among pectin chains by the plasticizer molecules (**Jantrawut et al. 2017**)

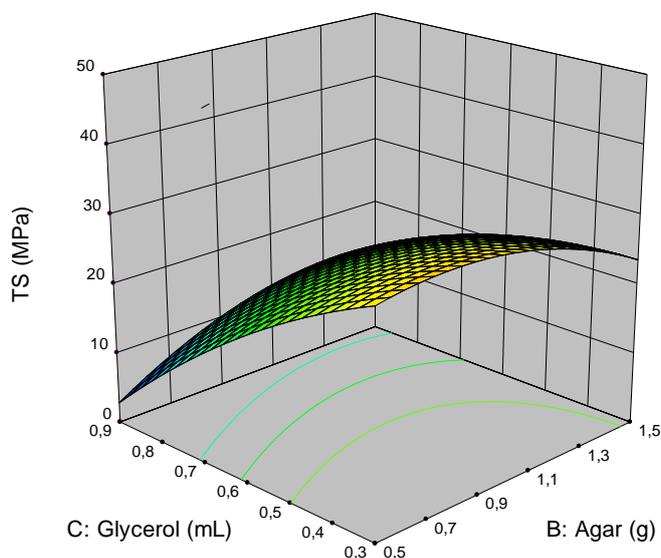


Figure 4:3-D curve showing the interaction effect of Amount of Agar and amount of agar on tensile strength

Figure 12 and 13 show that, glycerol addition led to a decrease in the antioxidant activity of the biofilms, which can be attributed to its plasticizing role. Glycerol forms hydrogen bonds with phenolic compounds, thereby reducing their availability for radical scavenging, and its hygroscopic nature may also accelerate oxidative degradation of bioactive molecules (**Gautam & Singh, 2021**). Conversely, increasing agar content enhanced the antioxidant activity, likely due to its gelling and network-forming properties that provide better stabilization and controlled release of phenolic compounds (**Arham et al., 2016; Fransiska et al., 2024**). However, a significant negative interaction between agar and date pit powder was observed. This suggests that, although agar alone supports antioxidant efficiency, excessive amounts combined with high levels of date pit powder may result in reduced activity, possibly due to aggregation, reduced dispersion of phenolics within the agar network, or competitive interactions between agar chains and bioactive compounds. Such antagonistic interactions highlight the importance of optimizing the formulation to balance both structural integrity and antioxidant functionality.

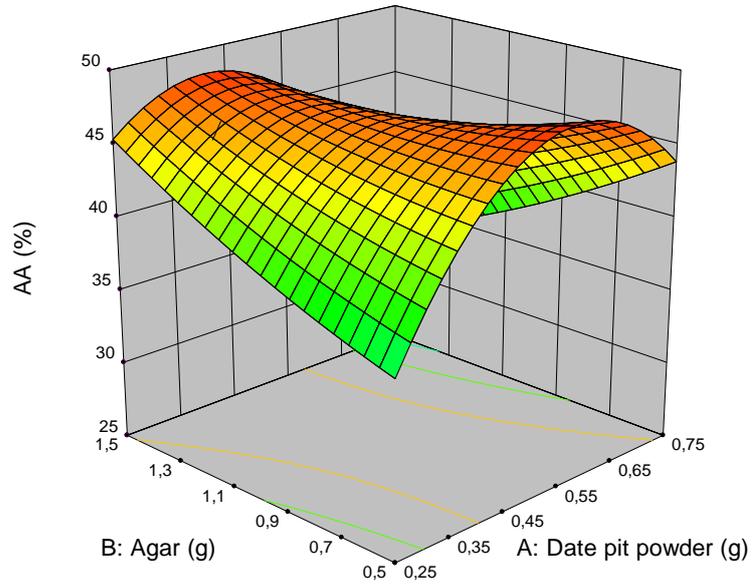


Figure 12: 3-D curve showing the interaction effect of Amount of glycerol and amount of date pit powder on activity antioxidant

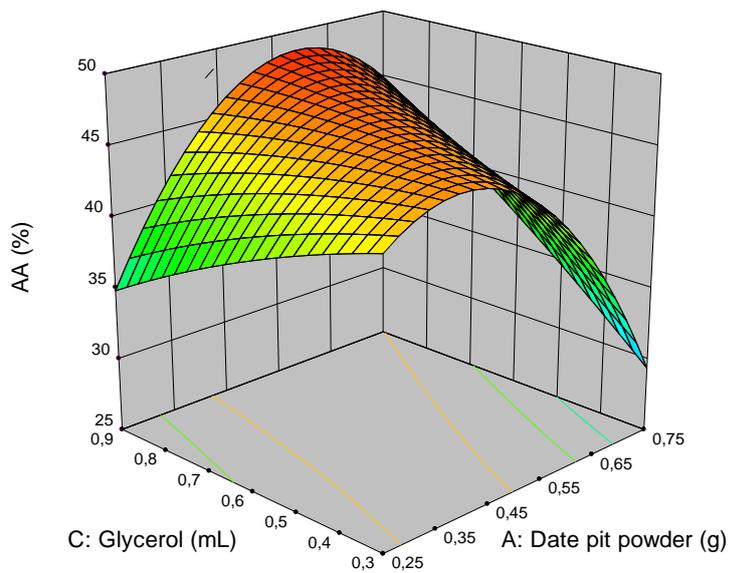


Figure 13: 3-D curve showing the interaction effect of Amount of Agar and amount of Date pit powder on antioxidant activity

3.4 Prediction of optimal conditions

The response profiles highlight how the three formulation factors influence the mechanical and antioxidant properties of the films. A moderate level of date pit powder (~0.47 g) and agar (~0.56 g) maximized antioxidant activity, whereas higher amounts reduced both tensile strength and activity. Glycerol showed a consistent negative effect, lowering film strength and bioactivity as its level increased.

The desirability analysis indicated that the most suitable balance was achieved with 0.47 g of date pit powder, 0.56 g of agar, and 0.30 mL of glycerol, corresponding to predicted values of 31.07 MPa (TS) and 47.59% (AA) (Figure 14). With a global desirability index of 0.84, this formulation provides a promising compromise between strength and functionality, making it suitable for bioactive packaging development.

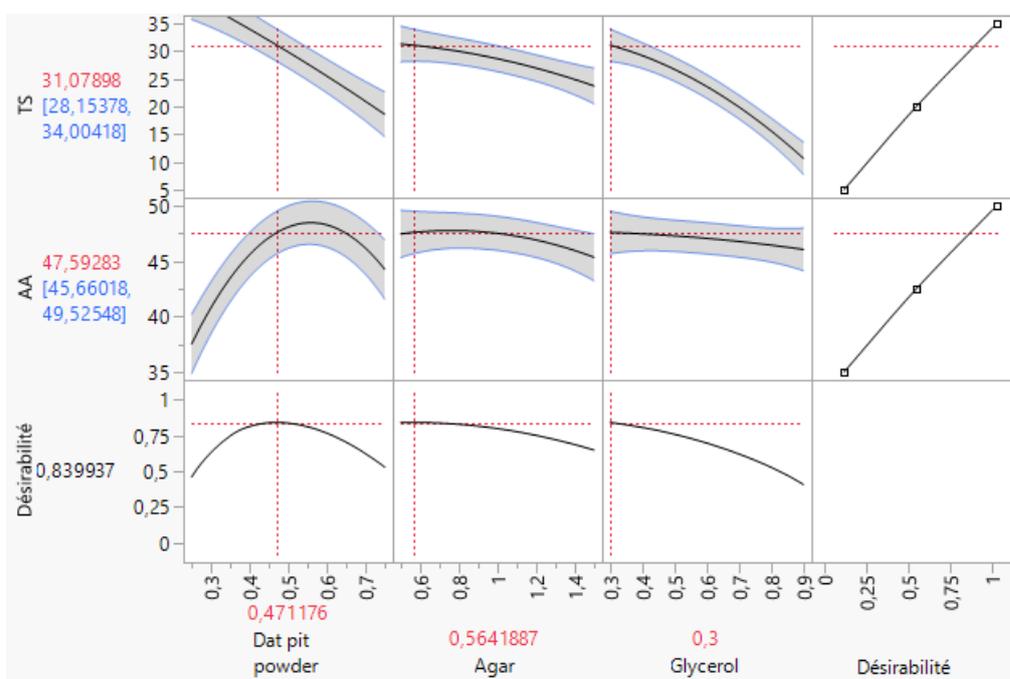


Figure 14: Effect of date pit powder, agar, and glycerol levels on film properties and overall desirability

4 Characterisation of biofilm antimicrobial activity

The *in vitro* antimicrobial efficacy of the developed bioactive films was evaluated against a panel of common foodborne pathogens, including Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*), Gram-positive bacteria (*Staphylococcus aureus*), and the yeast (*Candida albicans*), using a standard disk diffusion assay.

The results of the antimicrobial screening are summarized in Table 8. The plain agar-based control film (B), devoid of any date pit components, exhibited no inhibitory activity against any of the tested microorganisms, as evidenced by the absence of a zone of inhibition (ZOI = 0 mm).

A significant and targeted antibacterial effect was observed specifically against the Gram-negative bacterium *Pseudomonas aeruginosa*. The film incorporated with date pit powder only (SE) demonstrated a measurable ZOI of 9.0 ± 0.5 mm. A notably stronger effect was observed for the film containing the date pit extract only (SP), which produced a ZOI of 11.2 ± 0.8 mm. Most notably, the composite film (C), which synergistically combined both date pit powder and extract, yielded the largest inhibition zone of 15.5 ± 0.5 mm, indicating a clear synergistic enhancement of antimicrobial activity. Conversely, no detectable activity (ZOI = 0 mm) was observed against *E. coli*, *S. enterica*, *S. aureus*, or *C. albicans* for any film formulation. This suggests that the bioactive compounds present in the date pits (e.g., specific phenolic acids, flavonoids, or tannins) possess a selective and narrow spectrum of action, showing pronounced efficacy specifically against pseudomonads.

The potent and selective inhibition of *P. aeruginosa* is a finding of significant practical relevance. *P. aeruginosa* is a ubiquitous spoilage organism renowned for its intrinsic resistance to many antimicrobial agents and its role in the rapid deterioration of protein-rich foods, particularly meat, poultry, and fish, under refrigerated conditions (Gram, 2002). The demonstrated efficacy of the composite film (C) suggests its potential application as an active packaging material specifically designed to extend the shelf-life of such products by targeting a key spoilage bacterium.

These results conclusively confirm the successful valorization of date pit by-products into a functional packaging material with targeted and significant antimicrobial properties. The synergistic effect observed in the composite film underscores the advantage of utilizing the whole date pit valorization approach, leveraging both the fibrous matrix (powder) and the concentrated bioactive compounds (extract).

Values represent the mean diameter of the Zone of Inhibition (ZOI in mm) including the disk diameter (6 mm) \pm standard deviation (n=3). Different superscript letters (a-d) within the same column indicate significant differences between film formulations for each microorganism ($p < 0.05$).

Table 8: Antimicrobial activity of biofilm formulations against foodborne pathogens.

Film code	ZOI (mm)				
	<i>e.coli</i>	<i>S. enterica</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
B	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sp	0 ± 0	0 ± 0	0 ± 0	9 ± 0.5	0 ± 0
Se	0 ± 0	0 ± 0	0 ± 0	11.2 ± 0.8	0 ± 0
C	0 ± 0	0 ± 0	0 ± 0	15.5 ± 0.5	0 ± 0

The test films included: C (Composite), containing both date pit extract and powder; SP (Sans Poudre), containing extract only (without powder); SE (Sans Extrait), containing powder only (without extract); and B (Blanc), a plain control film without any active components.

Conclusion

Conclusion

This study successfully demonstrated the valorization of date pit (*Phoenix dactylifera L.*) waste into a functional and biodegradable active packaging material, offering an eco-friendly alternative to conventional plastics. Physicochemical characterization of Algerian cultivars confirmed their suitability for integration into biofilm matrices, with Deglet El Ghers extract displaying remarkable antioxidant activity (IC₅₀: 6.91 µg/mL), even surpassing the Trolox standard. Such findings highlight the nutritional and bioactive richness of date pits, a by-product that is often discarded, and support their transformation into high-value functional ingredients.

The development and optimization of bioactive films were achieved through Response Surface Methodology (RSM), which effectively modeled the influence of date pit powder, agar, and glycerol on key film properties. This statistical approach enabled the prediction of tensile strength and antioxidant activity, ensuring that the films combined both structural integrity and biofunctional performance. The optimal formulation, consisting of 0.47 g of date pit powder, 0.56 g of agar, and 0.30 mL of glycerol, achieved predicted values of 31.07 MPa (TS) and 47.59% (AA), with a high desirability index of 0.84. These outcomes emphasize the critical role of formulation balance in designing films that meet both mechanical and bioactive requirements.

The antimicrobial evaluation further demonstrated the practical potential of these films. The bioactive packaging displayed a targeted inhibitory effect against *Pseudomonas aeruginosa*, a major food spoilage bacterium, with a synergistic effect observed in films incorporating both powder and extract (inhibition zone: 15.5 ± 0.5 mm). This dual functionality—mechanical reinforcement and antimicrobial protection—positions date pit-based biofilms as a promising candidate for food preservation applications.

Collectively, the results confirm that date pits are not only a sustainable resource but also an effective functional component for active packaging production. Beyond their environmental and economic significance, this research establishes a reproducible methodological framework for tailoring biofilms with specific mechanical and functional properties. By integrating food safety, waste valorization, and material innovation, the study contributes to advancing a circular bioeconomy and aligns with global sustainability goals.

Future research should expand toward real-food shelf-life testing, barrier property assessments, and mechanistic investigations to better understand the release kinetics of bioactive compounds. In addition, scaling up production and evaluating industrial feasibility will be crucial steps for

transitioning this technology from laboratory to commercial application. Ultimately, the incorporation of agricultural by-products such as date pits into biodegradable packaging could help reduce reliance on petroleum-based plastics while simultaneously addressing waste management challenges in date-producing regions.

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