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Thème

Innovative and bioactive food packaging advances for food preservation

Soutenu le:26/06/2024 **Devant le jury composé de:**

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Dedication

I dedicate this work TO
***ALLAH the** Almighty to whom I owe everything.*

*My dear parents" **Boualem and leila"** for their love, support and encouragement throughout my university career.*
*To my dear grandparents **"Aicha and Ahmed".***
*To my dear brothers **"Badreddine and Athmane".***

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My primary, secondry, high school and university teachers

To anyone who helped me in any way to get to this moment where I obtained my diploma.

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PARIE II : EXPERIMENTAL PART

List of abbreviation

CA: Cellulose acetate

DM: Dry matter

HPMC: HydroxyPropylMethylCellulose

Mh: Wet material

Ms: Dry material

N/A: Not available

NFC: Natural fiber composites

PBAT: Poly butylene adipate

PBS: Poly butylene succinte

PCL: Poly caprolactone.

PHA: Polyhydroxyalkanoates

PHB: Polyhydroxybutyrate

PHBV: Polyhydroxylbutyrate-co-hydroxyvalerate

PHV: polyhydroxyvalerate

PLA: Poly(lactic) acid

PHB/V: Poly(3-hydroxybutyrate)-co-(3-hydroxyvalerate)

TS: Solubility rate

WPC: Wood-polymer composites

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Introduction

One of the most significant obstacles facing the food industry is the limited shelf life of food, caused by spoilage by microorganisms as well as oxidative reactions, such as enzymatic browning, degradation and oxidative rancidity. Among the methods used to combat food spoilage, there has been an increasing focus on the use of edible films and natural biopolymer coatings as a substitute for synthetic food packaging **(Mohamed *et al.*, 2020).**

Biodegradable packaging often uses natural biopolymers like proteins, lipids and polysaccharides as a base. These materials are environmentally friendly, non-toxic and are both biodegradable and recyclable **(Dutta and Sit., 2022).** Edible films or coatings refer to thin layers of materials that are safe to consume and function as a protective barrier against different elements, such as oxygen, humidity and water vapor **(Mohamed *et al.*, 2020).**

Lately, there has been an increase in consumer demand for food products that are minimally processed and contain fewer chemicals. This has led to an increased focus on the discovery of natural substances that can serve as alternative antioxidants and antimicrobials. As a result, foods that have been preserved with natural additives have gained popularity. To prevent foods from oxidizing, active agents can be incorporated into foods, applied to the surface of foods, or included in the packaging material **(Benbettaïeb *et al.*, 2018).**

Medicinal plant extracts are an interesting ingredient for biodegradable food packaging due to their natural origin and phytochemical properties and richness in biologically active compounds such as phenolic compounds which can be used to obtain active materials to extend the duration food preservation and limit oxidation reactions **(Dutta and Sit., 2022).**

Zizyphus lotus is a plant rich in phenolic compounds, their extracts have excellent antioxidant activity and the ability to trap free radicals **(Dhibi *et al.*, 2022)** and limit lipid oxidation **(Abdoul-Azize, 2016).**

Our study is divided into two parts: In the first part, a bibliography part describing the

concepts essential to understanding our work. The second part presents the material used, the methods applied, and the results and discussion of the results obtained.

The objective of our work is the following:

- Physico-chemical caracterisation and antioxidant properties of *Zizyphuslotus*leaves.

- Development of active and biodegradable edible food biopackaging from biopolymers (polysacharide and protein) with the addition of bioactive substances *(Zizyphuslotus*extract) with antioxidant activities to extend the shelf life of foods (apples and strawberries).

Theorical part

I. Edible and biodegradable bio-packaging

I. Edible and biodegradable bio-packaging

Generally speaking, an edible film is defined as a thin layer of edible material deposited on a food as a coating or placed on or between different food constituents. The main functions of the film or edible coating are to limit the migration of water vapor, oxygen, carbon dioxide, flavors, etc., To strengthen the mechanical integrity of the food and potentially act as a carrier for ingredients or additives such as antioxidants, antimicrobials, and flavorings**(Gallo *et al.*, 1999).**

If the bio-packaging is not an integral part of the food and cannot be consumed at the same time as the food, but is made from biomolecules, it will then be qualified as a film. However, the performance of packaging based on biopolymers remains lower than that of synthetic packaging. The main barrier and mechanical properties of different biodegradable and edible packaging materials are presented in **(Table I)**.

I.1. Biopolymers

A biopolymer is a biodegradable material capable of being broken down by the action of microorganisms. Today we find biodegradable polymers from renewable sources (plant, animal and bacterial) and/or petroleum. The use of the term “bioplastic” to describe these materials creates confusion between the origin and end of life of plastic; The term “biodegradable” does not mean that the plastic comes from renewable materials, just as plastics from plant sources are not systematically biodegradable **(Krochtaand De Mulder-Johnston, 1997).**

Depending on the degradation conditions (aerobic or anaerobic) and the environment, the material decomposes into H_2O, inorganic compounds, CO_2 and/or CH_4 and new biomass **(Krochtaand De Mulder-Johnston, 1997).**

TableI: Properties of biodegradable and edible packaging.

Materials	Film preparation	Barrierproperties to		Mechanicalproperties
		Humidity	Oxygen	
Biodegradable films				
Cellophane	Aqueous suspension	Moderate	Good	Good
Cellulose Acetate	Extrusion	Moderate	Weak	Moderate
Polysacharide/PVOH	Extrusion	Weak	Good	Good
PHB/V	Extrusion	Good	Good	Moderate
PLA	Extrusion	Good	weak	Good
Edible films				
Methyl Cellulose	H_2O-EtOH	Moderate	Moderate	Moderate
HPMC	H_2O-EtOH	Moderate	Moderate	Moderate
Polysachariderich in amylose	H_2O	Weak	Moderate	Moderate
Collagen	Aqueous suspension	weak	Good	Moderate
Zein (Corn Protein)	EtOH 95%	Moderate	Moderate	Moderate
Gluten	H_2O –EtOH	Moderate	Weak	Moderate
Soy protein	H_2O	Weak	Weak	Moderate
Protein	H_2O	Weak	Weak	N / A
Whey	H_2O	Weak	Weak	N / A
Beeswax	Aqueousemulsion	Moderate	Weak	Weak
Shellac	EtOH	Moderate	Weak	Weak

(Krochta De Mulder-Johnston, 1997).

I.1.1. Classification of biopolymers

There are 3 main types of materials, called biopolymers: those of natural origin, artificial ones and their composites **(Bewa, 2006)**.

- **Materials of natural origin are those synthesized by living beings**: animals, plants and microorganisms. We find:

• The family of polysaccharides (carbohydrates) such as: polysacharide, cellulose, lignin, chitin, the family of proteins such as: gluten, protein, collagen and gelatin, and the family of lipids: oils of rapeseed, soya, sunflower.

• Polymers of bacterial origin resulting from the fermentation of sugars and polysacharide by bacteria or produced by genetically modified microorganisms. Depending on the bacteria, various polymers are obtained such as polyhydroxylalkanoate (PHA), polyhydroxybutyrate (PHB), polyhydroxyvalerate (PHV) or polyhydroxylbutyrate-co-hydroxyvalerate (PHBV).

• Biosynthetic polymers, whose monomer from biomass is obtained by fermentation. The polycondensation of these bio-monomers gives polyesters; the best known is poly(lactic) acid PLA.

- **Biodegradable materials from petroleum resources** obtained byindustrial synthesis processes. The best known are PBS (poly butylene succinate), PBAT (poly butylene adipate terephthalate), and PCL (poly caprolactone).

- **Reinforced materials**: It is possible to mix natural fibers (linen, hemp) with various biopolymers to produce "reinforced" materials allowing partial substitution of non-renewable resources. We then find natural fiber composites (NFC) and wood-polymer composites (WPC).

I.2. Characteristics of biopolymers

The main characteristics of biopolymers and those of synthetic polymers are summarized in **(Table II)**. Despite the multiple advantages that natural biopolymers have (excellent biodegradability, absence of toxic pollutants, renewability..) compared to synthetic polymers, they have poor recyclability and are still very expensive**(Bewa, 2006)**.

TableII: Comparison of the characteristics of different types of polymers.

Composition	SyntheticPolymers	Biodegradablepolymers (synthetic + naturalpolymers)	Biodegradablepolymers (Natural polymers)
Raw material	Renewable	Only a tiny part is Renewable	Renewable
Exemples	Polyethylene (PE), Polypropylene, Polystyrene, etc.	PE + Polysacharide, PE + Cellulose, etc,	Cellulose-based plastics, Polysacharide-based plastics
Biodegradability	Not at all or very Bad	only natural polymers are attacked by micro-organisms	Excellent
Photo-degradability	Addition of pro-degradants which promote low chemical attack	Addition of pro-degradants which cause chemical breakdown and allow natural polymers to be attacked by micro-organisms	May contribute to or accelerate biodegradability
Price	Very cheap for Common products	Average	Very expensive currently but improving with production capacities.
Physical and mechanicalproperties	Very good to very variable	Variable	Good and variable depending on applications

(Bewa, 2006)

The potential global demands for replacing petroleum-derived feedstocks with renewable resources in the production of valuable biodegradable polymer materials are quite significant from a social and environmental perspective **(Etienne Boulerice, 2019)**.

I.3. Polymeric biomaterials

A bioplastic is defined as a plastic material made up of at least 40% plant-based or biodegradable material. Indeed, the group of bioplastics is made up of biosourced plastics and biodegradable plastic materials, some of these materials being both at the same time. PLA is an example of this type of plastic material, which is both biosourced and biodegradable. CA is another example of a bio-based plastic material, which is however not necessarily biodegradable **(Etienne Boulerice, 2019)**.

I.3.1.Manufacturing processes for bioplastics

The term "bioplastic" encompasses a whole family of polymers which are biosourced (for example bio-polyethylene (Bio-PE) or bio-polyethylene terephthalate (Bio-PET) from sugar cane, which may or may not have a characteristic biodegradable like polys (hydroxy-alkanoates) PHA.Among all biodegradable bioplastics, we can distinguish three main categories **(Figure 01)**:

- **Natural polymers** derived directly from natural resources such as polysaccharides (cellulose, polysacharide, chitin, alginate, etc.) and proteins
- **Biopolyesters** extracted directly from microorganisms, produced by a fermentation process, such as PHB (poly hydroxy-butyrate)
- **Polymers synthesized chemically** or biotechnologically from a biosourced monomer (e.g. poly (lactic acid) PLA) **(Nibal Hijazi, 2014)**.

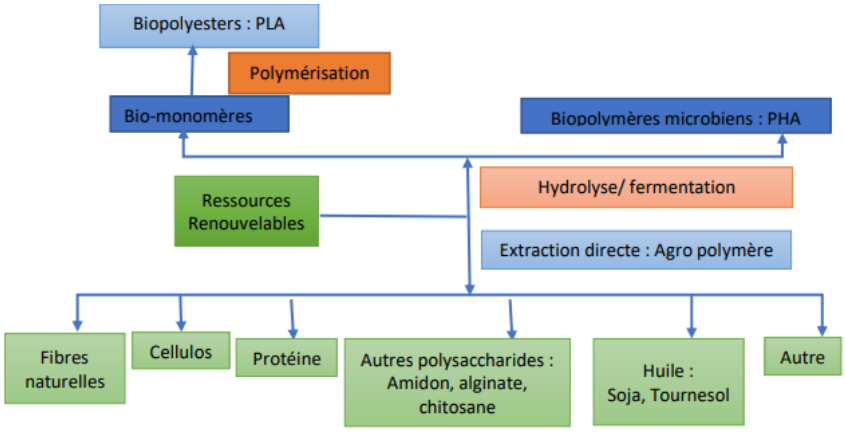

Figure 01: The routes to obtaining certain families ofbioplastics **(Nibal Hijazi, 2014)**

I.3.2. Other sources of bioplastic

✓ Corn based

Corn is made up of polysacharide at almost 71% of its dry weight. Empirically, from a bushel of corn weighing 25 kg, we can extract 15 kg of polysacharide, 1 kg of oil, 5 kg of gluten and fiber which will be used to feed livestock and 4 kg of water **(Habbazi and Sadoudi, 2019)**.

Corn has several advantages since it grows quickly, all over the world, all year round and is, moreover, economical to exploit. It dries easily and is easily stored by farmers in silos to then follow a staggered supply to processing plants until the next harvest or throughout the year if necessary **(Habbaz and Sadoudi, 2019)**.

✓ Based on protein

Among the polymers derived from renewable raw materials, proteins are suitable for plastic manufacturing for packaging purposes (films, sheets, etc.) due to their excellent gas barrier properties (O_2, CO_2), insolubility in water, and mechanical properties that are comparable to polystyrene or polyvinyl chloride (e.g., packaging made from corn gluten) **(Weber, 2000; Multimania, 2011).**

Moreover, protein-based packaging is edible if their formulation contains only edible substances. They are mainly intended for food and pharmaceutical packaging.

The proteins used in the manufacturing of plastics come from two sources: animal and vegetable. The main animal and vegetable proteins used in plastic production are presented in the **(Figure 02)**:

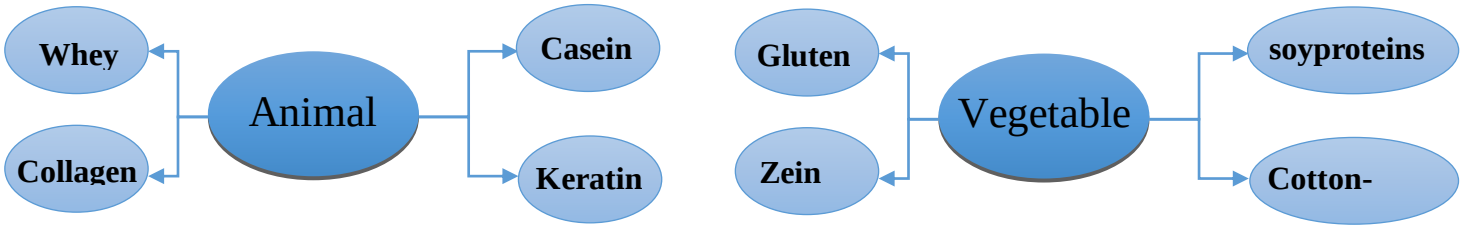

Figure 02: Different protein sources in the manufacturing of plastics**(Weber 2000; Multimania, 2011).**

Protein is a protein derived from milk. Historically, it was the first protein used for making plastics. It is easily transformable due to its random coil structure. However, the process is lengthy and costly, requiring the plasticization of protein and shaping it in a heated press, followed by hardening through cross-linking in a formaldehyde bath. The process duration can extend up to several months depending on the thickness of the object **(Woebcken, 1995; Weber, 2000)**.

Protein is used for manufacturing food-grade films because it forms transparent, flexible, and tasteless films. Additionally, it is used for bottle labeling due to its adhesive properties **(Cuq *et al*., 1998)**

I.3.3. Area of application

The use of biopolymers is hampered by competition from regular polymers, which are inexpensive and known by their users. The infrastructures allowing their valorization must also be developed and require significant investments; without their composting or recycling, biopolymers are doomed to waste **(Elyse Rémy, 2014)**. Three majorapplications are identified in relation to the properties of biopolymers: medicine, agriculture and packaging and are used in short-term applications

a- Food packaging

In the context of food packaging as it shown in **(Figure 03)**, the use of biosourced materials requires taking into account various constraints linked in particular to contact with food. “In the case of contact with water or in the event of a variation in the humidity of the ambient air, we are quickly limited, because the biopolymers that we use do not necessarily have good resistance to water. water, indicates philippeevon. We may then have to add synthetic bioplastics, most often from plants

Figure 3: Examples of commercial applications of PLA as food packaging: **(Jeancarlo Renzo Rocca, 2014).**

b- Bags

The biodegradable bag **(Figure 04)** limits the use of polymers, degrades completely and is non-toxic during composting. As disadvantages, the energy cost of this bag is significant and it is less resistant and less durable compared to a conventional plastic bag.

Figure 4: Bag labeledbiodegradable **(Eugene NdemaNsombo, 2020)**

I.4. Advantages and disadvantages

Besides many significant advantages of biodegradable plastics, there are still several disadvantages that should be noted **(Table III)**

Table III: advantages and disadvantagesof biodegradable plastics.

Advantages(Elyse Rémy, 2014)	**Disadvantages(Van Le, 2020)**
• The best benefit of biodegradable plastics is to reduce permanent waste suitable for recycling alongside organic waste. • It is clear that bio-based plastics have a much lower carbon footprint. • The advantage of energy efficiency, the energy 21os twill be lower to manufacture the plastics compared to petro plastic • Biodegradable plastics come from biomass which potentially ensures carbon neutrality and gas emissions.	• The problem of recycling:Biodegradable plastics cause pollution of soil, water and land. Although biodegradable polymers are broken down into smaller particles, there remains the potential to harm the environment • Biodegradable plastics are similarto conventional plastics, so clear labeling, disposal and recycling instructions are required. The reason is that these biodegradable polymers can contaminate conventional plastics during recycling processes.

(Elyse Rémy, 2014;Van Le, 2020) .

II. General information about Zizyphus lotus

II. General information about *Zizyphuslotus*

II.1. *Zizyphus Lotus*

Zizyphus Lotus (*Z. Lotus*), also known as lotus jujube, belongs to the angiosperm family Rhamnaceae**(Abdoul-Azize, 2016).** This family comprises 900 species within 58 genera **(Punt *et al.*, 2003).**

II.1.1.Botanical classification

According to **Ghedira (2013),** the botanical classification of *Zizyphus lotus* is as follows **(Table IV)**:

Table IV: Botanical classification of the species Zizyphus lotus**.**

Kingdom	Plantae
Subphylum	Magnoliophyta(Phanérogames)
Sob-Subphylum	Magnoliophytina(Angiospermes)
Class	Magnoliopsida(Dicotylédones)
Sub-class	Rosidae
Order	Rhamnales
Family	Rhamnaceae
Tribe	Zizyphae
Genus	Zizyphus
Species	*Zizyphuslotus(L.).*

(Ghedira, 2013).

II.1.2.Chemical composition

Phytochemical studies conducted on *Zizyphus lotus* show the presence of primary and secondary metabolites **(Table V) (Catoir *et al.*, 1999).**

II.1.2.1. Primary metabolites

The pulp of *Zizyphus lotus L*. is highly nutritious, composed of 12.8 to 13.6% carbohydrates including: 5.6% sucrose, 2.1% fructose, 1.5% glucose, and 1% polysacharide. Pectin extracted from the pulp contains D-Galactose, 2,3, 6 Tri-o-acetyl, which gives it anti-diarrheal properties and helps lower plasma cholesterol levels. The pulp of *Z. lotus* contains (4.84 g/100 g) of fibers and (1.18 g/100 g) of proteins, and the following amino acids: asparagine, arginine, aspartic acid, glutamic acid, glycine, serine, and threonine **(Abdeddaim, 2016).**

Z. lotus could be considered as a source of many vitamins for human consumption. The pulp of *Z. lotus* is rich in vitamin C and vitamin A**.** It also contains minerals such as calcium, magnesium, and potassium. Similar quantities of magnesium and calcium have been found in the pulp of *Z. lotus*, while higher contents of these three minerals are present in the seeds of *Z. lotus***.** The almonds of "*Zizyphus lotus L*." are very rich in sulfur proteins **(Abdeddaim, 2016).**

II.1.2.2. Secondary metabolites

Z. lotus contains biologically active molecules such as polyphenols (flavonoids, tannins), triterpenes, anthraquinones, alkaloids, and saponins **(Djemai, 2009).**

In the leaves, the total phenolic compounds content is 664 mg/100 g, with flavonoids ranging from 130 to 199 mg/100 g, and a high content of saponins (340 mg/100 g) **(Table V).** In the fruit, total phenols are the major compounds, ranging from 297 to 4078.2 mg/100 g DW; and flavonoids and tannins are present in moderate quantities, respectively 122 and 33 mg/100 g. The pulp of *Z. lotus* contains moderate amounts of polyphenols (325 mg/100 g) **(Abdoul-Azize, 2016).**

The seeds of *Z. lotus* contain small amounts of polyphenols (14.68 mg/100 g), while the content of polyphenols in the root bark of *Z. lotus* is 2009 mg/100 g along with a high content of saponins (219 mg/100 g), high flavonoids (120 mg/100 g), and a large amount of proanthocyanidins (156 mg/100 g) compared to other molecules such as cyclopeptidic alkaloids, ranging from 1.4 to 23.95 mg/100 g **(Abdoul-Azize, 2016).**

In summary, the aerial parts (leaves and fruits) of *Z. lotus* are the most important source of polyphenols and flavonoids (3630–8144 mg/100 g).

Table V: Composition of secondary metabolites in *Zizyphus lotus.*

Organ	**Chemical composition**	**References**
Leaves	Flavonoids, Tannins, Saponins, Alkaloids, Jujuboside B3, Glycosides of jujubogenin, Jujubasaponin IV	**(Abdoul-Azize, 2016), (Djemai, 2009)**
Fruit	Flavonoids, Tannins, Saponins, Alkaloids	**(Borgi *et al.*, 2007(b))**
Root bark	Flavonoids, Tannins, Cyclopeptidic alkaloids, Saponins of the dammarane type	**(Borgi *et al.*, 2007(a))** **(Abdoul-Azize, 2016)**

II.1.3. Biological activities

Several studies have demonstrated biological activities of *Z. lotus,* some of which are mentioned in the table below **(Table VI).**

Table VI: biological Activities of *Z. lotus*

Biological activity	**References**
Antioxidanta ctivity	**(Abdoul-Azize, 2016 ;Djemai, 2009)**
Antimicrobial activities	**(Borgi *et al.*,2007)**
Anti-ulcerogenic activities	**(Borgi *et al.*,2007(a))**; **(Abdoul-Azize, 2016)**
Anti-inflammatory and analgesic activities	**(Borgi *et* Chouchane,2009)**; **(Borgi *et al.*, 2008; Borgi *et al.*, 2007).**
Antiulcerogenic activities	**(Borgi *et al.*2008)**
Antispasmodic activities	**(Borgi*et*chouchane,2009)**
Hypoglycemic activity	**(Abdoul-Azize,2016; Benammer *et al.*,2014)**

II.1.4. Dietary uses of *Zizyphus lotus*

The nutritional value of *Z. lotus* mainly relies on its rich composition of vitamin E, vitamin C, fibers, fatty acids, amino acids, calcium, and magnesium**.** The fruits of *Zizyphus lotus* are dried and processed into flour to make pancakes with a very pleasant taste. Vegetable oils are widely consumed in our diet. They contribute to the flavor, taste, and texture of foods. Accordingly, it has been reported that *Z. lotus* oil is of high quality due to its content of unsaturated fatty acids and other bioactive compounds **(Abdoul-Azize, 2016).**

In India, jujube is used for making desserts consumed in winter, and honey extracted from its flowers is of good nutritional quality with a pleasant taste. In China, jujube fruits are used for wine production **(Saadoudi, 2019)**

Material and methodes

II. Material and methods

In this sectionanoverview of material and methodesused in this study will be detailed

I.1. Preparation of plant material

The plant material used in our study consists of leaves from *Zizyphus lotus*, purchased from an herbalist in the Bouira province.

➢ Grinding and sieving

The dry leaves of the plant are ground into a very fine powder using an electric grinder. Once ground, the resulting powder is sifted through a sieve 200ùm and stored in a tightly sealed glass bottle in a dry place until use **(Figure 05).**

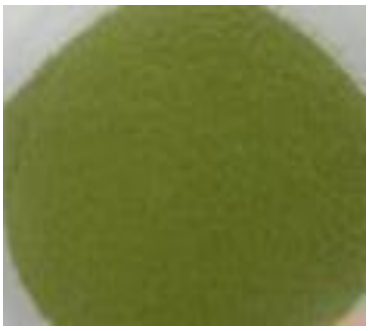

Figure 05: Photograph of *Zizyphus lotus* leaf powders **(Original, 2024).**

I.2. Physico-chemical characterization of *Zizyphus lotus*

I.2.1. Moisture content

The water content was determined by drying the sample in an oven at a temperature of 105°C until a constant weight was achieved **(Audigie *et al.*, 1980).** Brievely,the empty capsules are dried in an oven at 105°C for 15 minutes, then placed in a desiccator to cool down and subsequently weighed. A mass of 2 g of *Zizyphus lotus* powder is placed into the capsules and dried in an oven at 105°C. After 24 hours of drying, the samples are transferred to a desiccator and weighed. This process is repeated every 24 hours until a constant weight is achieved. The results were determined using the following equation:

$$H(\%)= (M1-M2)/P.100$$

Let:

- **H%:**Moisture content.
- **M1:** Mass in grams of the capsule + sample before drying.
- **M2:** Mass in grams of the whole after drying.
- **P:** Mass in grams of the test sample.

The dry substance content is determined using the following equation:

$$\mathbf{MS(\%) = 100\text{-}H\ (\%)}$$

I.2.2. PH determination

A quantity of 2 g of sample is placed in a beaker containing 100 ml of distilled water. The mixture is heated for 30 minutes and then filtered. The pH of the filtrate is measured after cooling using a pH meter, following the NFV05-108 technique **(Afnor, 1982).**

I.2.3. Ash content

The *Zizyphus lotus* powder is incinerated in a muffle furnace at 550°C until a constant weight of white ash is formed **(Laurent,1991).**02 grams of powder are placed in a capsule (M1), then put into a muffle furnace at 550°C for 5 hours until a gray, light, or whitish color appears. Aftercooling, the capsule isweighed (M2). The results are presented using the following equation

$$\mathbf{OM\% = (M_1 - M_2)/p \times 100}$$

- **OM%:** Organic matter.
- **M_1:** Mass in grams of the capsule containing the test sample before incineration.
- **M_2:** Mass in grams of the capsule with the ashes.
- **P:** Mass in grams of the test sample.

The ash amount is determined as follows:

$$\mathbf{Ash\ \%=100\text{-}MO\%.}$$

I.2.4. Extraction and quantification of phenolic compounds

I.2.4.1. Extraction

The aim of the extraction process involves the segregation of specific molecules of interest from the plant matrix. This step is crucial as it determines the quality and quantity of the extracted compounds, which in turn affects the subsequent steps such as analysis, isolation, and identification of bioactive components **(Abcha, 2020).**

To extract the active principles from the plant under study, we employed the following method:

The maceration process involves allowing the plant material powder to remain in contact with a solvent for an extended period. This allows for the extraction of active components at room temperature, which is advantageous for preserving heat-sensitive substances **(Bouchouka, 2016)**.

The maceration extraction method was carried as follow:*Zizyphus lotus* leaf powder was macerated in 160 ml of ethanol. After agitation for 2 hours at room temperature, the plant extract was filtered using filter paper. The filtrate was then rid of ethanol by evaporation at 40°C in a ventilated oven. The resulting dry extract was stored in a dry, light-protected place until use.

I.2.4.2. Total phenolic content

The total polyphenol content was determined by the Folin–Ciocalteu method described by **(Singleton and Rossi., 1965)** and reported by **(Skerget *et al*., 2005).** The folin–Ciocalteu reagent consists of a mixture of phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PMO12O40) with a yellow color. When polyphenols undergo oxidation, they reduce the Folin-Ciocalteu reagent to form a blue complex consisting of tungsten and molybdenum oxides. The intensity of the color is directly proportional to the levels of oxidized phenolic compounds **(Lapornik *et al*., 2005).**

Brievely500 µl of extract is mixed with 2.5 ml of Folin-Ciocalteu reagent and then

added to 2 ml of sodium carbonate (7.5%). After 5 minutes of incubation in a water bath at 50°C, the absorbance is measured at 760 nm. The content of phenolic compounds is expressed in mg of gallic acid equivalent per gram of dry weight of sample, referring to a calibration curve obtained with gallic acid used as a standard.

I.2.4.3. Flavonoid content

The amount of flavonoids contained in the extract of *Zizyphus lotus* leaves was determined using the colorimetric method of **(Lamaison and Carnat, 1990).** This method relies on the ability of flavonoids to form a complex with aluminum chloride (AlCl3). Flavonoids have a free hydroxyl group (OH) at position 5, which is capable of forming a colored complex with aluminum chloride. This reaction produces a yellowish color with maximum absorption at 430 nm **(Saadoudi, 2019).**1 ml of extract was mixed with 1 ml of methanolic solution of aluminum chloride hydrate (AlCl3 and incubated at room temperature in the dark for 15 minutes. After incubation, the absorbancewasmeasuredat 430 nm.

The flavonoid content is determined by reference to a calibration curve obtained with Quercetin used as a standard. The results are expressed in mg of Quercetin equivalent per gram of dry matter (mg EqQ/g DM).

I.3. Antioxidant activity of *Zizyphus lotus* extracts

The antioxidant activity of the studied extract is evaluated using two tests: the scavenging effect of the DPPH radical and the ferric reducing power.

I.3.1. DPPH radical scavenging activity

The anti-radical activity of phenolic extract using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as described by **(Brand-Williams, 1995)**, et al. (is based on the ability of antioxidants to scavenge the DPPH radical. The DPPH radical is reduced to non-radical diphenylpicrylhydrazine by accepting a hydrogen atom. The higher the loss of violet color, the stronger the hydrogen donor is considered as an antioxidant **(Figure 06)**.

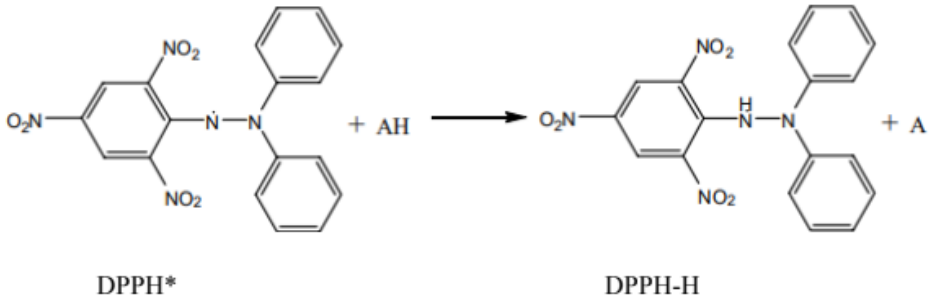

Figure 06: Reduction of the DPPH free radical **(Boutellaa, 2019).**

The concentration of crude extract inhibiting 50% of the DPPH (IC50) is determined using a series of dilutions of the extract. For this purpose, 50µl of each dilution are added to 1.95ml of freshly prepared methanolic DPPH solution. After incubating for 30 minutes in the dark at room temperature, absorbances are measured at 515 nm.The estimation of the antioxidant activity of the extract using the DPPH method is expressed as a percentage of inhibition according to the following equation:

Percentage of inhibition = [(Abs $_{control}$– Abs $_{sample}$) / Abs $_{control}$]x 100

Abs $_{control}$: Absorbance of the control.

Abs $_{sample}$: Absorbance in the presence of phenolic extract.

I.3.2. Ferric reducing power

The reducing power of the extract from the leaves of the plant under study is determined according to the method of Oyaizu (1986). It is based on the reduction of ferric iron (Fe^{+3}) to ferrous iron (Fe^{+2}) by potassium ferricyanide ($K_3Fe(CN)_6$) in the presence of a chromogenic agent **(Figure 07).**

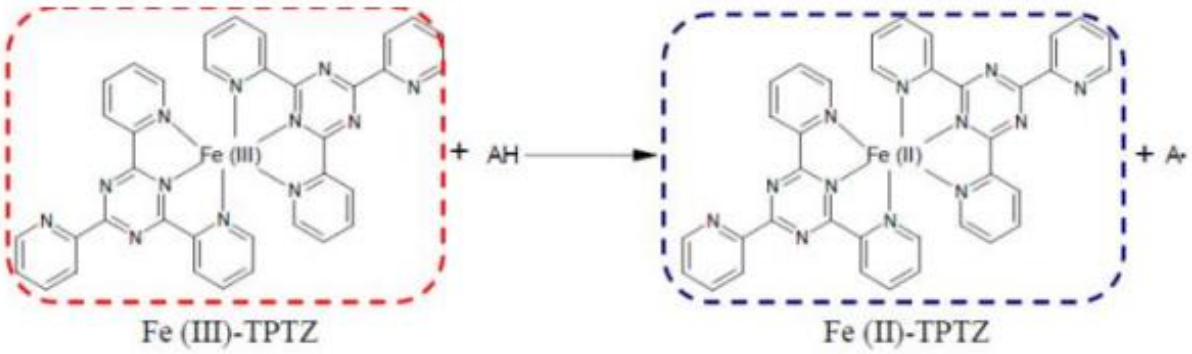

Figure 07: reduction of trivalent iron (Fe^{3+}) to divalent iron (Fe^{2+}) **(Agouazi, 2021).**

100µl of extract at different concentrations is mixed with 250µl of a 0.2 M phosphate buffer solution (pH 6.6) and 250µl of a 1% aqueous solution of potassium ferricyanide $K_3Fe(CN)_6$. After incubating the mixture in a water bath for 20 minutes at a temperature of 50°C, 850 µl of distilled water, 170 µl of an aqueous solution of $FeCl_3$, and 250µl of a 10% aqueous solution of trichloroacetic acid (TCA) are added. The absorbance is then read at 700 nm.

I.4. Biofilmspreparation

Two kindsof biofilms were studied: a polysacharides-based biofilm and a biofilm based on protein. For the preparation of these biofilms,

I.4.1. polysaccharides-based biofilms

Into a beaker, polysaccharides and plasticizer were mixed with distilled water and thenstirredon a hot plate until it becomes translucent, the mixture wascooledatroom temperature.

I.4.2.Protein-based biofilm

Distilled water were added toprotein powderand then mixed with a cross-linking agent. The solution was filtered, and then this mixture was stirred and heated under continuous agitation, then cooledatroom temperature.

I.5.Free radical scavenging assay of the biofilms

The process involves measuring the ability of the prepared biofilms to scavenge DPPH free radicals using the method described by **(Brand *et al.*, 1995)** with some modifications. The results of the antioxidant activity of the biofilms are expressed as a percentage of inhibition according to the following equation:

Percentage of inhibition = $\frac{(Abscontrol - Abssample)}{Abscontrol} \times 100$.

- **Abs $_{control}$:** Absorbance of the control.
- **Abs $_{sample}$:** Absorbance in the presence of phenolic extracts and/orpolysaccharide.

I.6. Biodegradability test

Soil burial tests were conducted to evaluate the biodegradability of polysacharide and protein based biofilms. 09 biofilm samples consisting of polysacharide based and 03 protein based formulations were prepared for testing. These samples were then buried in soil located in suitable test containers. The soil used was representative of a typical garden soil, ensuring a natural environment conducive to microbial activity. The test containers were placed in a controlled environment to maintain consistency throughout the testing period **(Figure 08).**

To ensure optimal conditions for biodegradation, regular maintenance and monitoring measures were taken. The soil was moistened almost daily or whenever it appeared dry to ensure that the moisture content was sufficient to support microbial activity. In addition, the soil was stirred regularly to ensure adequate aeration and prevent anaerobic conditions.

I.7. Swelling test

To evaluate the swelling behavior of polysaccharide-based biofilms, a series of tests were performed on nine samples. The aim was to observe changes in weight, color, texture, resistance and other physical properties of the biofilms at specific time intervals when immersed in different solutions. Three different tests were performed on the biofilms: one in distilled water, the other one in an acid solution and the last one in basic solution.

Before the start of the tests, each sample was carefully weighed to determine the baseline value. The biofilms were then immersed in the respective solutions under controlled conditions. Observations and measurements This enabled a comprehensive analysis of the swelling behavior in the short and long term.

I.8. Fourier transform infrared spectroscopy (FTIR)

In the chemistry department at the Université of Bouira, an infrared spectroscopy test was conducted to analyze the properties of various biofilms. The experiment involved 18 polysaccharide-based biofilm samples and 3 protein-based biofilm samples. The procedure was designed to evaluate the chemical stability and structural integrity of the biofilms under different immersion conditions.

All the polysaccharide-based biofilms and protein-based biofilms already prepared were analyzed to obtaine the FTIR spectrum,

- **Immersion process**

The immersion process was conducted at room temperature. The samples in the water immersion group were fully submerged in distilled water, while the samples in the acid immersion group were fully submerged in a diluted acid solution. The immersion duration was consistent across all samples to ensure uniform exposure conditions.

- **Infraredspectroscopyanalysis**

Following the immersion period, the samples were removed, dried, and subjected to infrared (IR) spectroscopy analysis.

This analysis was conducted using a Fourier-transform infrared (FTIR) spectrometer, which recorded the IR spectra of each sample. The spectrometer captured data on the molecular vibrations and chemical bonds present in the biofilms, allowing for a detailed analysis of their chemical stability and structural integrity

I.9. Application of packaging in the preservation of fresh apple slices

The development of oxidation in fresh apple slices packaged with biofilms prepared with extracts of *Zizyphus lotus and* polysaccharide is monitored by exposing them to fresh air at room temperature for several days, under normal lighting conditions. The experiment aims to observe changes and degradation in the color of the apple slices over time, providing insights into the effectiveness of the biofilm packaging in preserving the fruit's quality.

➢ Preparation and packaging of the apple slices

each containing apple slices is prepared. The dishes are divided into 3 groups based on the preservation time of the apple slices (0, 3, and 5 days) **(Figure 09 and 10)**

Figure 08:A fresh green apple **(Original, 2024).**

- ❖ Slices of apples are covered with biofilm containing only *Zizyphus lotus* extract.
- ❖ **S**lices of apples are covered with biofilm containing *Zizyphus lotus* extract + polysaccharide (PS).
- ❖ **S**lices of apples are covered with thefilmcontainingonly the PS.
- ❖ **S**lice of apple is covered with a control film without the extract and without a PS.
- ❖ **S**lice of apple is not covered at all.

I.10. Application of film-forming solutions as bio-packaging for strawberries

I.10.1. Preparation of solutions

The film-forming solutions used for the development of biofilms (based on protein)

I.10.2. Coating of strawberries

Fresh strawberries of the same size, undamaged and unspoiled, are washed with water containing a few drops of vinegar, then weighed and dipped in a film-forming solution with and without phenolic extract (3 strawberries per solution).

I.10.3. Effect of coating on appearance of strawberries

After the storage period, the visual appearance (color, overall appearance, texture and weight lost) of each group of strawberries was examined

II. Results and discussion

II.1. Physico-chemical characterization of *Ziziphus lotus*

The physico-chemical parameters that were measured include: water content, pH, and ash content.

II.1.1.Water content

To express the phenolic compound contents relative to dry weight, the water content of the leaves of *Ziziphus lotus* was determined.

Table VII: Water content and dry matter of *Ziziphus lotus*

Parameter	*Zizyphus lotus*
Water content(%)	**11± 1**
Dry matter(%)	**89**

The results obtained show that the leaves of *Ziziphus lotus* have a moisture content of 11 ± 1%. From these values, the percentage of dry matter was determined to be 89%. It is challenging to compare the results of water content in dried plant material with those in the literature due to variations in drying techniques, conditions, and storage duration. Water content can vary significantly between different materials based on these factors.

II.1.2. pH

The pH indicates the neutral, acidic, or alkaline nature of a solution. It is one of the parameters that determine the suitability of foods for preservation and constitutes one of the main factors affecting the growth of microorganisms. A pH range of 3 to 6 is favorable for the development of fungi **(Doukani *and* Tabak, 2015).**

The results obtained show that the analyzed powder of *Zizyphus lotus* leaves is slightly acidic with a pH value of 5.82 ± 0.045. This value is close to that found by **(MohdJailani *et al.*, 2020)** in their study on *Ziziphus mauritiana* leaves, where they found a pH of 5.47 ± 0.01.

II.1.3. Ash content

Ash content refers to the total amount of mineral salts present in products.The results obtained for the ash content of *Zizyphus lotus* are presented in **(Table 08).**

According to the results, *Zizyphus lotus* leaves contain 7.58 ± 0.025% ash content. These values are comparable to those found in other varieties of the same genus.

TableVIII: Ash Content of *Zizyphus lotus.*

Plant	**Ash Content %**
Zizyphuslotus	**7.53%**

For example, **(Renu *et al*., 2019),** in their study on leaves of *Ziziphus jujuba Lam* of the Badara variety, showed an ash content of **7.53%,** which is similar to the ash content found in our study.

II.1.4.Phenolic content

The quantification of polyphenols in plant extracts is considered the first step in assessing the antioxidant capacity of these extracts **(Saidi, 2019).**

In this context, our extracts were quantitatively characterized by spectrophotometry to determine their content of total phenols and flavonoids. The concentrations of total phenols and flavonoids were determined by reference to calibration curves using two standards: gallic acid and quercetin

The concentrations are expressed in (mg GAE /g of DW sample) for total phenols, and in (mg QE /g of DW sample) for flavonoids.

II.1.4.1Total phenolic content

The results of the total phenolic content assay from *Zizyphus lotus* extract obtained are represented in the following table.

TableVIII: Total Phenolic Content.

Plant	Phenol concentration (mg GAE/g of DM)
Zizyphuslotus	**58.7**

The analysis of the results obtained in **(TableVIII)** shows that the extracts of *Zizyphuslotus* have interesting total phenolic contents. The highest values were detected for *Zizyphuslotus* with a content of 58.7 mg GAE/g dry weight. **(Bekkar *et al.*, 2020)** found polyphenol concentrations of 233.5 ± 0.16 mg GAE/g dry weight, 233.5 ± 0.43 mg GAE/g DW for the dry matter extract and the Watery extract of *Z.lotus*, respectively. These concentrations are higher than those found in our study.

The results obtained in our study are lower than those reported by **(El-Khateeb *et al.*, 2013**) for another variety of the same genus (*Ziziphus spina-christi*), who noted a content of 147.47 mg GAE/g of total polyphenols.

The total phenolic content recorded by **(Dhibi *et al.*, 2022)** for leaf extracts of Zizyphus lotus is 468.57 ± 56 mg GAE/g DW, which is higher compared to the contents found in our study. These results are consistent with studies indicating that Zizyphus lotus leaf extracts have a high total polyphenol content.

II.1.4.2.Flavonoids content

Table 10 illustrates the flavonoid content of our extract.

According to **Table 10,** it appears that the extracts have recorded a high flavonoid content, with the extract of *Z. lotus* showing a content of 91.13 mg EQ/g of dry matter.

The results obtained in the present study are close to those recorded **(Khouchlaa *et al.*, 2017)** on the leaves of *Zizyphus lotus*, which also noted a flavonoid content of 104.12 mg EQ/g of dry sample.

Table X: Flavonoids content of extract.

Plant	**Flavonoid content mg EQ/g DM**
Zizyphus lotus	**91.13**

The extracts of *Zizyphus lotus* appear richer in flavonoids compared to the results obtained **(El-Khateeb *et al.*, 2013)** in the ethanolic extract of *Zizyphus spina-christi* leaves (16.35 mg EQ/g).

The flavonoid content of the *Z. lotus* extracts is similar to that obtained by **(Dhibi *et al*., 2022),** who found a content of 96 ± 1.25 mg EQ/g of dry matter in the leafextracts of *Zizyphus lotus.*

The results of the present study demonstrated the presence of total phenols and flavonoids in all tested extracts. Based on these results, we can confirm the richness of *Z. lotus* in total phenols and flavonoids. The results indicate that *Zizphus lotus* is richer in total phenols than in flavonoids. These findings are in agreement with those obtained by **(Dhibi *et al*.,2022)** during their comparative study on the richness in phenolic compounds and the antioxidant activity of *Zizyphus lotus*. **(Borgie and Chouchane., 2009**) and **(Abdoul-Azize, 2016)** confirm our results, stating that *Zizyphus lotus* contains biologically active molecules such as polyphenols and flavonoids in high quantities.

The variations in the content of phenolic compounds (total phenols and flavonoids) in the plant extracts studied compared to those found by other authors could be due to the origin of the plants and environmental conditions **(Ebrahimi *et al*., 2008),** storage conditions, the degree of ripeness **(Ebrahimi, 2008; Khouchlaa *et al*., 2017),** and other factors that can also influence the content of these bioactive compounds such as environmental and climatic conditions **(Abdoul-Azize, 2016).**

II.2. Evaluation of the antioxidant activity of *Zizyphus lotus* extracts

In the present study, the applied methods are the free radical scavenging method (DPPH) and the ferric reducing antioxidant power method (FRAP).

II.2.1. Evaluation of the DPPH radical scavenging activity

The DPPH free radical scavenging test was used to evaluate the antioxidant activity of the *Zizyphus lotus* leaf extract.

The IC50 inhibitory concentration is inversely proportional to the antioxidant capacity of the test compound, and it represents the amount of antioxidant required to reduce the free radical concentration by 50%. The smaller the IC 50 value, the higher the antioxidant activity of the compound. The IC 50 values of the extracts are shown in **(Table XI)**.

Table XI: The IC50 results of *Z.lotus* leaf extracts obtained by maceration are presented in the table:

Plant	IC50 mg/mL
Zizyphuslotus	**0,519**

The results obtained in our study are in agreement with the results found on *Z. lotus* leaves with an IC 50 of 0.70 mg/ml and higher than those recorded**(Bekkar *et al.*, 2020)** who found a lower IC50 (0.146 mg/Ml), and lower than those recorded by **(Letaief *et al.*, 2021)** who found an IC50 of 0.37 mg/ml.

Z.lotus extracts showed strong free radical scavenging activity compared to the extracts obtained by **(Dhibi *et al.*, 2020)** who found an IC50 of 1.28 ± 0.13 mg/ml.

According to the results, we see that the *Z.lotus* extract has a strong capacity for trapping free radicals.

II.2.2. Ferric reducing power (FRAP)

The reducing power of the extract was estimated by an effective concentration (IC50) of 50%, corresponding to an absorbance equal to 0.5. The IC50 concentrations are shown in **(Table XII).**

Table XII: Reducing power IC50 of extracts from the leaves of *Zizyphus lotus.*

plant	Ferric reducing power IC50mg/mL
Zizyphuslotus	**0,22**

After analyzing the results of quantitative evolution of phenolic compounds and the antioxidant activity of our extract by means of two tests (FRAP) and (DPPH), we see that the leaves of *Zyziphus lotus* present interesting polyphenol contents, and strong free radical scavenging activity of DPPH and strong iron reducing capacity. This is confirmed by the very frequent use of *Zizyphus lotus* leaves in traditional medicine.

II.3.Biodegradable active packaging

II.3.1. Characterization of biofilms

- **Polysacharide-based biofilm:** the biofilms obtained are transparent with a smooth, uniform elastic surface, easy to unmold from the petrie dish after drying. We lunched 9 different essais with various concentrations of the extract and the polysachharides so we can find the perfect biofilm.

In our study, 9 different polysacharide-based biofilms have been reported, with different physical properties. These variations arose from the differing percentages of ingredients used in their formulation. Some biofilms appeared blurry, indicating less homogeneity or suboptimal ingredient ratios. Other ones that contain less percentage of the polysaccharid were notably fragile, suggesting insufficient cross-linking or inadequate plasticizer content. Meanwhile, some biofilms exhibited greater elasticity, which may be attributed to higher plasticizer levels or better polymer network formation.

The most successful biofilm was the one that was transparent, resistant, and see-through, resembling real plastic. This optimal biofilm likely had the ideal balance of polysacharide, plasticizer, and other components, resulting in superior mechanical and optical properties. The transparency indicates a homogeneous mixture with minimal phase separation, while the resistance and see-through qualities suggest a robust and well-formed polymer network. These findings underscore the importance of precise ingredient ratios in developing high-quality, biodegradable polysacharide-based biofilm.

- **Protein-based biofilm:** The biofilms obtained are transparent **(Figure 09)** witha smooth, uniform elastic surface, easy to unmold from the petrie dish after drying. We lunched 9 different essais at first with different concentration of the extract and the polysachharides then we arrived to 3 essais, so we can find the perfect biofilm.

We lunched multipe essays untill we setteled with the last and the final essay, we used:

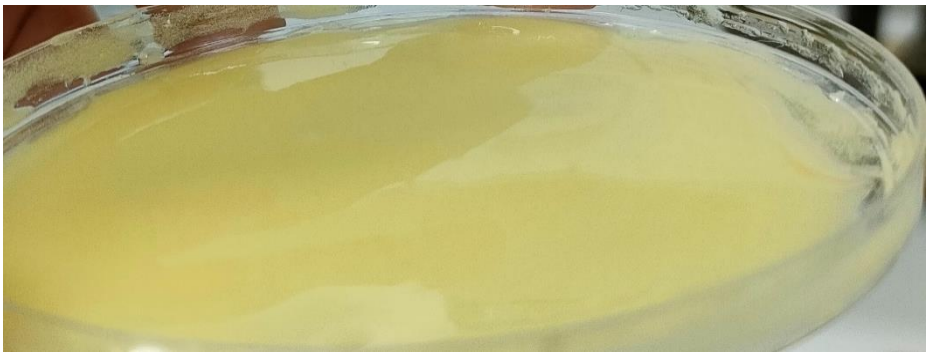

Figure09 : 10% of the linking ingredient **(Original, 2024).**

In our experiments with protein-based biofilms, we explored the impact of varying the percentage of linking ingredients on their properties. We observed distinct changes in the biofilms' characteristics with different percentages of the linking ingredient.

When the percentage of the linking ingredient was high, the biofilms exhibited silicone-like properties, including increased thickness, altered texture, and a yellow coloration. This suggests that higher concentrations of the linking ingredient may have led to excessive cross-linking or polymerization, resulting in undesirable physical properties.

Conversely, when we decreased the percentage of the linking ingredient to 1 percent, the biofilms underwent a remarkable transformation. They became transparent, exceptionally resistant, and see-through, with a texture reminiscent of real plastic. These biofilms displayed optimal properties, indicating a delicate balance of ingredients that resulted in superior mechanical strength and optical clarity.

These findings highlight the importance of precise formulation in achieving desired biofilm properties. By adjusting the percentage of linking ingredients, we can tailor the characteristics of protein-based biofilms to meet specific application requirements, such as transparency, strength, and flexibility. Further research in optimizing formulation parameters can lead to the development of high-performance, biodegradable biofilms for various industrial and environmental applications.

II.3.2. Free radical scavenging assay of the biofilms

Next generation food packaging processes have been specifically developed to increase their functionality by adding natural bioactive compounds such as antioxidants, these approaches can help extend the shelf life of foods. It can have a positive health effect on consumers when the antioxidant potential/content of fresh produce has been improved or is saved **(Yahyaoui, 2020).**

The main characteristic of packaging is its antioxidant activity, as it protects the food product and extends the shelf life of the product **(Ali *et al*., 2023).**

The antioxidant activity of biofilms developed based on polysacharide and based on polysaccharides and polysacharide is measured by the DPPH method.

- **Polysacharide-based biofilms**

The anti-radical activity of DPPH in biofilms is expressed as a percentage of free radical trapping (%)

The results of the antioxidant activity of the biofilms by the DPPH radical scavenging method (**Table XIII**), revealed that all the biofilms indicated high DPPH radical scavenging activity, which confirmed that they have antioxidant properties.

The best DPPH radical inhibition results were recorded by biofilm number 07/09 based on *Z.lotus* extracts with 17%.

TableXIII: Results of DPPH for the strach based biofilms.

The samples	***Inhibition %***
S1 (Stratch+ Extract)	35%
S2(Stratch+ polysacharid)	28%
S3 (Stratch+ polysacharid+ extract)	**70.36 %**
S5 (Stratch+ polysacharid+ extract)	56%
S7 (Stratch+ polysacharid+ extract)	14.17%
S8 (Stratch)	21.28%
S9(Stratch+ Extract)	33.39%

After comparing the results of the antioxidant activity of biofilms **(polysacharide-based)** and biofilms **(protein-based)**, we note that both types of biofilms have significant antioxidant activity.

The percentage of DPPH inhibition of active biofilms ((based on polysacharide and biofilms (based on protein)) added with *Z.lotus* extract is higher than that previously obtained with bioactive films of chitosan added with the extract peppermint (at different concentrations)

Researchers link this antioxidant capacity to the polyphenol and flavonoid content of the extracts and suggest that the developed films could be useful in inhibiting oxidation and extending the shelf life of foods **(Muñoz-Tebar *et al*., 2023).**

- **Protein-based biofilms**

  The anti-radical activity of DPPH in biofilms is expressed as a percentage of free radical trapping (%)

The best DPPH radical inhibition results were recorded by biofilm number 03/03 based on *Z.lotus* extracts with 25 %.

When the color of the DPPH solution changes, it usually indicates a reduction in the DPPH free radical, which is indirect evidence of the presence of antioxidants. In this case, a protein-based biofilm could well have antioxidant properties.

Based on **(Meisel, H., and Bockelmann)** W Protein itself has moderate antioxidant activity, which can be significantly increased by enzymatic or chemical hydrolysis, releasing bioactive peptides with antioxidant properties. In our biofilm containing protein, NaOH, and other components, it is possible that structural changes or specific interactions enhanced the antioxidant activity of protein**(Meisel, H., &Bockelmann, W, 2020)**as it's shown **(Table XIV).**

TableXIV: Results of DPPH for the protein based biofilms.

The samples	***Inhibition %***
C1 (Protein+ Extract)	12%
C2(Protein+ polysacharid)	20%
C3 (Protein+ polysacharid+ extract)	28 %

II.3.3. Biodegradability test

Biodegradability testing was conducted for one month, and the physical condition of the biofilm was monitored regularly. Throughout the observation period, changes in the appearance of the biofilm were observed, including signs of degradation such as fragmentation, discoloration, and eventual disappearance.

By the 20th day, the biofilms had broken down so much that they were barely visible to the naked eye. Only small, scattered remnants were visible, indicating that most of the material had been absorbed from the ground. At the end of one month, the biofilms had completely disappeared, confirming their biodegradability.

II.3.4. Aspect of films after immersion in different mediums

As a results to the evaluation of the swelling behavior of our biofilms, we obtained the following parameter:

- **Weight change:** Each biofilm was re-weighed at each time interval to determine weight gain or loss due to swelling and interaction with the solution.
- **Color change:** Any color change of the biofilm was recorded to assess possible chemical interaction with the solution.
- **Texture:** The texture of the biofilm was assessed by visual inspection and careful handling, noting softening, brittleness, or other changes.
- **Durability:** The biofilms were evaluated for structural integrity and resistance to mechanical handling throughout the test.

The swelling test demonstrated that the polysacharide-based biofilms behaved differently when immersed in water and acid solutions.

- **In water**

- **Weight change:** within the first 5 minutes, the biofilms exhibited swelling, which resulted in an increase in weight due to water absorption. The weight gradually rose over time, eventually stabilizing. By 72 hours, the biofilms had restored to their original weight, showing that they absorbed and then released water with minimal breakdown **(Table XV)**.

TableXV: Changes in the weight (g) of the startch based biofilms emmerged in water

Time	*S1*	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*	*S9*
0 min	0.14	0.09	0.12	0.09	0.1	0.1	0.1	0.09	0.16
72h	0.14	0.09	0.12	0.09	0.1	0.1	0.1	0.09	0.16

- **Color change:** during the initial stages, the biofilms preserved their original color, with very mild deterioration seen after prolonged immersion, indicating negligible leaching of soluble components.

- **Texture:** when time passed, the texture became softer and more gelatinous, reaching a soft peak before gradually regaining firmness when the water was released.

- **Resistance:** initially, the biofilms had good structural integrity. They grew more fragile and less cohesive during the peak swelling period, but regained part of their former resilience as they dried and reverted to their original weight**.**

❖ In acidic solution

- **Weight change:** the biofilms showed the same pattern of swelling and weight change in the acid solution as in water. original swelling resulted in weight increase, followed by a gradual return to original weight by 72 hours, indicating that the biofilms absorbed the acidic solution and then released it with little breakdown.

Table XVI: Changes in the weight (g) of the startch based biofilms emmerged in acid.

Time	*S1*	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*	*S9*
0 min	0.06	0.08	0.13	0.06	0.1	0.1	0.1	0.09	0.18
72h	0.06	0.08	0.13	0.06	0.1	0.1	0.1	0.09	0.18

- **Color change:** the biofilms changed slightly, becoming yellowish, indicating some interaction with the acidic medium, but this was not significantly different from water immersion.

- **Texture:** compared to water, the texture became more gelatinous in the acid solution. The biofilms grew softer and more jelly-like more quickly, reflecting a distinct molecular interaction between the acid and the biofilm.

- **Resistance:** as the biofilms got more gelatinous, they showed a lower resistance to mechanical handling; however, this change wasn't significantly different from the behavior seen in water**.**

❖ In basic solution

The biofilms immersed in a basic solution dispersed after only a few minutes. This rapid dispersion suggests that the alkaline environment had a significant impact on the biofilm's structure.

- **Polysacharide-based biofilm**: alkaline conditions can cause the hydrolysis of glycosidic bonds in polysacharide, breaking it down into simpler sugars such as maltose and glucose. This hydrolytic reaction weakens the structural integrity of the biofilm, leading to its dispersion. And we know that polysacharide is generally insoluble in cold water but can swell and dissolve in alkaline solutions, contributing to the disintegration of the biofilm matrix.

- **Protein-based biofilm**: in a basic environment, proteins like protein can undergo denaturation, a process where the protein structure unravels due to the breaking of hydrogen bonds, disulfide bridges, and other interactions maintaining its tertiary structure. Denaturation disrupts the protein matrix of the biofilm, causing it to disperse.

The swelling test showed that, other from texture, polysaccharide-based biofilms respond equally in acidic and watery environments. The biofilms responded to both solutions by absorbing moisture, releasing it, and eventually gaining back their original weight. The biofilms changed texture more gradually in water, first getting softer and then becoming somewhat firmer again. The biofilms changed in texture more noticeably in the acid solution, becoming more jelly-like and gelatinous.

These results imply that polysaccharide-based biofilms are well suited for applications where temporary swelling is beneficial as they show strong resistance to dissolution in both acidic and aqueous environments. However, their mechanical characteristics and handling may be affected by the enhanced gelatinization in acidic environments.

II.3.5.FTIR analysis

II.3.5.1. Characterization of elaborated films

The different elaborated polysaccharide films are characterized by using the infrared spectrophotometer, the FTIR spectra obtained are shown in **(Figure 10) (Kripal Singh *et al.*, 2012).**

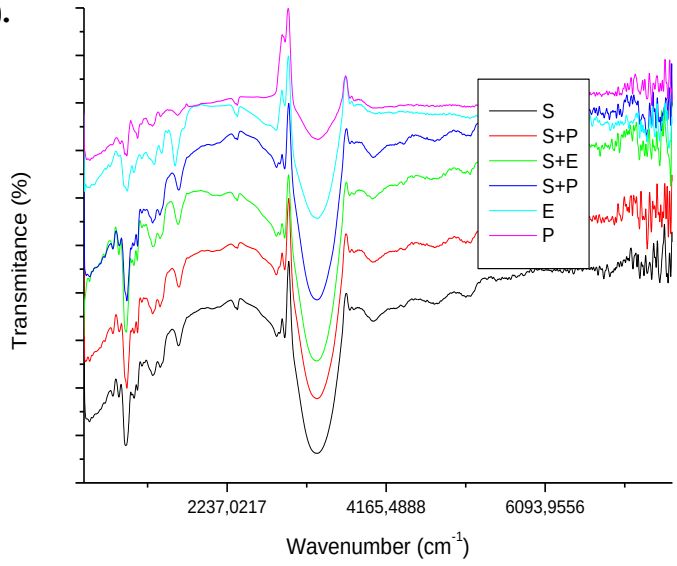

Figure 10: Infrared spectra of different elaborated polysaccharidefilms, (S) strach, (P) polysaccharide, (E) extract.

The film obtained with protein and other compounds have been characterized, and the FTIR spectra are shown in **(Figure 11)**.

The FTIR spectral analysis reveals significant structural and chemical modifications in the protein-based film upon the addition of an extract and polysaccharide.
Based on the studies of **(Ali *et al.*, 2019), t**he addition of plant extracts leads to new FTIR peaks and enhanced functional properties to the films.

By comparing these results with recent research, it is evident that the conclusions drawn from the protein-based film study are well-supported and reflect common outcomes observed in the field of biopolymer composite films. These similarities underline the potential of combining protein, polysaccharides, and extracts to develop films with enhanced mechanical, barrier, and functional properties **(Figure 11).**

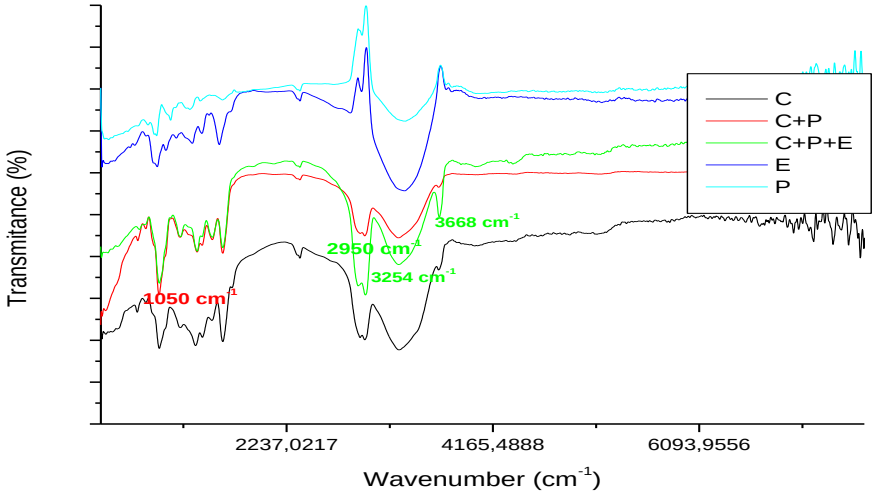

Figure 11 : Infrared spectra of protein films elaborated (C; C+P; C+P+E; E;P)

II.3.6. Application of different films as an active packaging of apple

A study was carried out applying 09 different biofilm samples to assess the effectiveness of various biofilm formulations in preventing apple slice oxidation. These biofilms were utilized to cover apple slices, and their effect on oxidation rate was measured and compared to uncovered apple slices. The major goal was to see if biofilms, particularly those loaded with plant extracts, might reduce the oxidation process and keep the color of the apple slices over time.

The biofilms studied included formulations containing and excluding plant extracts renowned for their antioxidant activity. The apple slices were observed for many days to assess color changes, which are a key sign of oxidation.

The study's findings revealed significant changes in the rate of oxidation across the various treatments:

1. **Plant-enriched biofilms:** these slices had the slowest rate of color change, indicating a considerable decrease in oxidation. The antioxidant qualities of the plant extracts in the biofilms helped to keep the apple slices fresh and colorful for a longer period of time than alternative treatments
2. **Biofilms with polysacharid:** these slices also showed a reduction in oxidation when compared to the uncovered slices, though not as much as those covered in enriched biofilm. The biofilms formed a physical barrier, limiting exposure to oxygen and slowing the oxidation process.
3. **Uncovered apple slices:** the uncovered slices had the fastest rate of oxidation, with significant browning occurring far faster than the covered slices. This outcome underlined the necessity for some type of protection to keep the apple slices fresh.

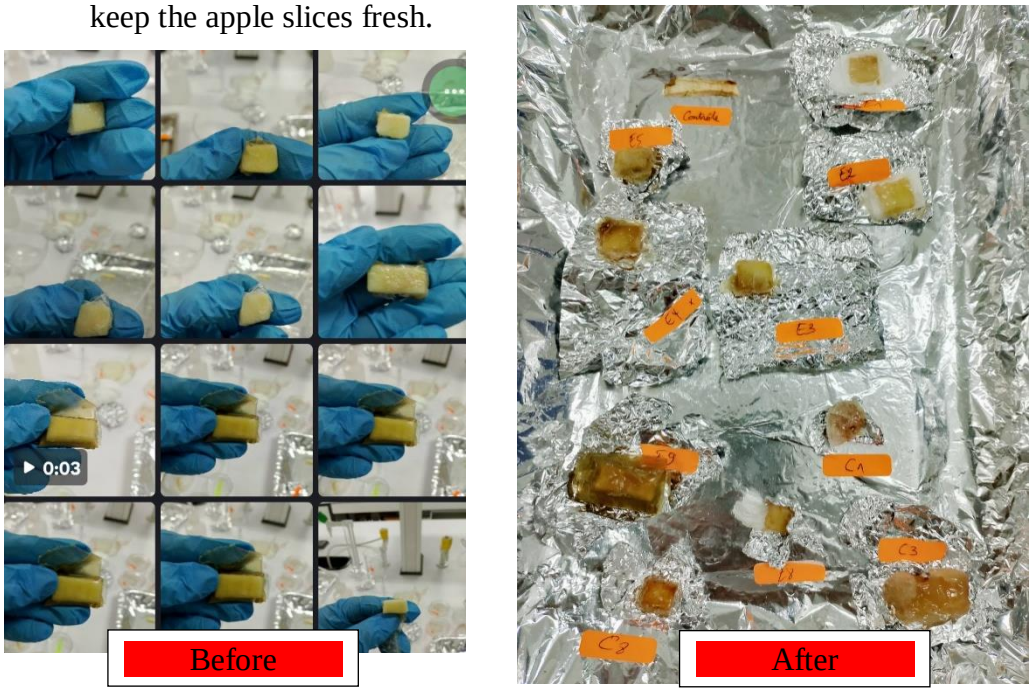

Figure 12 : Multiple slices of apples samples covered with different stratch based biofilms enriched with polysacharid and plant extract after 3days **(Original, 2024).**

The study clearly shows that biofilms **(Figure 12)**, particularly those supplemented with plant extracts, can greatly reduce the oxidation of apple slices. The antioxidant chemicals included in plant extracts are most likely responsible for this benefit, as they may neutralize free radicals and lessen oxidative stress on apple tissue.

Recent studies back up the results of this experiment. For example, **(Yildirim and Röcker., 2021)** discovered that bio-based films containing natural antioxidants can successfully inhibit oxidation in food goods. Similarly, a study by **(Nisar *et al.*, 2020)** found that adding plant extracts into edible films improves their anti-oxidation properties in fruits and vegetables.

These references support the hypothesis that biofilms supplemented with plant extracts offer higher oxidation resistance compared to simple biofilms. The biofilms' physical barrier provides some protection on its own, but the addition of antioxidants dramatically improves this impact **(Figure 12).**

II.3.7. Application of film-forming solutions as bio-packaging for strawberries

A study was done making use of three different biofilm samples to assess the effectiveness of various biofilm formulations in preserving the freshness of strawberries. These biofilms were applied to strawberries, and their effect on the color and form of the fruit was studied and compared to that of uncovered strawberries over several days. The major goal was to see if biofilms enriched or not with plant extracts could better preserve the strawberries' appearance and structural integrity.

The biofilms evaluated included those supplemented with plant extracts for preservation qualities, those without, and a control group with uncovered strawberries.

The strawberries were observed over several days to evaluate changes in color, shape, and overall form.The study's findings revealed differences in the rate of color change and shape preservation across treatments:

- **Plant extract-enriched biofilms on strawberries**

These strawberries changed color more quickly than uncovered strawberries. The strawberries, on the other hand, retained their shape and structural integrity for an extended period of time. The biofilm created a protective layer that helped the fruit maintain its shape, but it did not effectively prevent color changes.

- **Strawberries covered in biofilms without plant extracts**

Similar to the enhanced biofilms, these strawberries changed color faster than the uncovered strawberries . Nonetheless, the strawberries retained their shape and form better than the control group. The biofilm served as a physical barrier, slowing the breakdown of the fruit's structure.

- **Uncovered strawberries**

Exhibited slower color changes than covered strawberries, but lost form and structural integrity faster. Without a protective coating, these strawberries were more susceptible to dehydration and physical deterioration.

The study concludes that, while biofilms, whether enriched with plant extracts or not, may not dramatically slow strawberry color change, they do play an important role in protecting the fruit's shape and structural integrity. This preservation is most likely due to biofilms functioning as a barrier to physical damage and dehydration.

Recent research back up the results of this experiment. For example, **(Jamróz *et al.*, 2020)** discovered that, while bio-based films can slow the physical degradation of fruits, their efficiency in preventing color changes is dependent on the precise formulation and the presence of active compounds. Similarly, **(Sharma *et al.*, 2021)** found that edible coatings, while excellent at preserving the texture and hardness of fruits, may not always avoid oxidative color changes.

These references support the fact that biofilms help preserve the structural integrity of strawberries but are less successful at preventing color changes. The physical barrier formed by biofilms inhibits dehydration and physical degradation, allowing the fruit to preserve its shape and firmness.

In this work, we found that strawberries covered with biofilms, both enriched and unenriched with plant extracts, showed faster color changes than uncovered strawberries. This behavior is remarkable and can be linked to a variety of causes including the interaction of biofilm components with strawberries.

Biofilms containing proteins such as protein may react with the sugars found in strawberries. This interaction can result in non-enzymatic browning reactions, such as the Maillard reaction, which involves amino acids (from proteins) and reducing sugars. The Maillard process causes browning and color changes in food products, giving them a darker appearance. In the presence of moisture, proteins and carbohydrates can interact more easily. The biofilm holds moisture, creating an ideal environment for these reactions to occur, hastening the browning process.

Recent studies provide additional insights into these observations, Bio-based Films and Non-enzymatic Browning: **(Akhtar *et al.*, 2020)** found that bio-based films including proteins and sugars could cause greater non-enzymatic browning when applied to fruits due to interactions between film components and fruit sugars.

Impact of Edible Coatings on Oxidation: **(Baldwin *et al.*, 2018)** discovered that, while edible coatings can reduce moisture loss and maintain texture, they can also alter the fruit's oxidative environment, resulting in increased browning due to changes in oxygen diffusion and localized humidity levels.

Conclusion

The present study focused on evaluating the properties and antioxidant activity of *Zizyphus lotus* leaves and the incorporation of plant extract into edible and biodegradable packaging applied to the preservation of apples and strawberries.

Quantitative analysis of phenolic compounds in *Zizyphus lotus* leaf extracts revealed that the plant extract is rich in total phenols (58. 7 mgGAE/g DM) and flavonoids(91.13 mg QE/g DM). The results of the antioxidant activity evaluation using FRAP and DPPH tests demonstrated that the extract possesses strong antioxidant properties, the values were (IC50: 0.22 mg /mL and IC 50: 0.519 mg/mL) respectively.

The incorporation of *Zizyphus lotus* leaf extracts into both polysacharide-based and protein-based biofilms resulted in a significant antioxidant effect, with the biofilms exhibiting excellent free radical scavenging activity as measured by the DPPH test.

The results of FTIR analysis of the elaborated films (polysacharide and protein), show that the bindings between the different compounds are physical, because there is not apparition of nouvel peaks in the different films spectra comparing to those of compound alone, we have noticed a new peak at 3668 cm^{-1} in the protein film with extract and polysaccharide due to the interactions between the three compound.
Some changes in the FTIR spectra of the different polysacharide films, after immersion in waterwere detected (espacialy in –OH binding); these are due to theire swelling capacity. The intensities of –OH bindings decrease in all the films spectra after immersion in acidic medium, which is attributed to the interaction of these bindings in acidic medium.

Furthermore, coating strawberries with protein-based solutions incorporating *Zizyphuslotus* extract minimized their weight loss over time. The biofilms provided a protective barrier that maintained the structural integrity and appearance of the strawberries, although color changes due to oxidative reactions were observed.

For apple slices, biofilms enriched with *Zizyphus lotus* extract significantly slowed down the oxidation process, preserving the color of the slices for a longer period compared to those covered with non-enriched biofilms or left uncovered. These results indicate that biofilms with plant extracts are more effective in maintaining the freshness and extending the shelf life of fruits.

The lack of means and time to fully develop this interesting subject which is part of a research project relating to the addition of *Ziziphus lotus* leaf extract to biodegradable edible packaging.

Our results have shown an excellent biodegradability of the prepared films. These packaging materials exhibited high degradability in our tests, confirming their significant potential to reduce environmental impact. Their composition and design facilitated effective decomposition under natural conditions, highlighting a promising solution for sustainable plastic waste management.

The findings of this study highlight the potential of biodegradable edible biofilms incorporated with *Zizyphus lotus* leaf extracts in food preservation. The strong antioxidant properties of the extracts contribute to reduced oxidation and enhanced shelf life of various food products. Moving forward, several perspectives can be considered to further optimize and utilize these biofilms:

- **Formulation Optimization:** Further research should focus on optimizing the formulation of biofilms to enhance their protective effects, particularly in preventing color changes in fruits. This could involve varying the concentration of plant extracts and exploring other natural antioxidants.
- **Application to Diverse Food Products:** While this study focused on apples and strawberries, future research could extend the application of these biofilms to other fruits and vegetables, as well as other perishable food products, to evaluate their effectiveness across a broader range of items.
- **Long-term Storage Studies:** Conducting long-term storage studies would provide valuable insights into the effectiveness of these biofilms over extended periods, helping to determine their practical applicability in real-world storage and transportation scenarios.

- **Commercial Viability:** Investigating the scalability and cost-effectiveness of producing these biofilms on a commercial scale would be crucial for their widespread adoption in the food industry.
- **Consumer Acceptance:** Assessing consumer acceptance of foods coated with these biofilms would help in understanding market potential and addressing any sensory or aesthetic concerns.

In conclusion, the incorporation of *Zizyphus lotus* leaf extracts into biodegradable and edible biofilms presents a promising approach to enhancing food preservation. The strong antioxidant properties of these extracts can significantly extend the shelf life of various food products, making them an attractive option for natural and sustainable food packagings.

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Abstract

Medicinal plant extracts represent a promising ingredient for biodegradable food packaging. The general objective of this research was to study the antioxidant activity of Zizyphus lotus leaf extract and its incorporation into biofilms and biodegradable edible coatings based on polysacharide and protein. These biofilms were then applied as active packaging ingredients to preserve apples and strawberries.

This research studied the antioxidant activity of Zizyphus lotus leaf extract and its incorporation into biodegradable and edible biofilms made from polysacharide and protein for preserving apples and strawberries. Zizyphus lotus leaf extracts, rich in phenols and flavonoids, showed strong antioxidant properties. Polysacharide-based biofilms with Zizyphus lotus extract achieved 90.32% anti-radical activity, while protein-based films showed 40%.

Applying these biofilms to strawberries provided stability against dehydration, increasing shelf life and preserving sensory quality. For apples, the biofilms slowed oxidation, maintaining color and structural integrity longer than non-enriched or uncovered slices.

The incorporation of Zizyphus lotus leaf extracts into biodegradable and edible biofilms presents a promising approach to enhancing food preservation.

Keywords :*Zizyphus lotus*, biofilm, edible coating, biodegradable, polysaccharides, active packaging, polyphenol, DPPH.

Résumé

Les extraits de plantes médicinales représentent un ingrédient prometteur pour les emballages alimentaires biodégradables. L'objectif général de cette recherche était d'étudier l'activité antioxydante de l'extrait de feuille de *Zizyphus lotus* et son incorporation dans des biofilms et des enrobages comestibles biodégradables à base d'amidon et de caséine. Ces biofilms ont ensuite été appliqués comme ingrédients actifs d'emballage pour conserver les pommes et les fraises.

Cette recherche a étudié l'activité antioxydante de l'extrait de feuille de *Zizyphus lotus* et son incorporation dans des biofilms biodégradables et comestibles à base d'amidon et de caséine pour la conservation des pommes et des fraises. Les extraits de feuilles de *Zizyphus lotus*, riches en phénols et en flavonoïdes, ont montré de fortes propriétés antioxydantes. Les biofilms à base d'amidon contenant l'extrait de *Zizyphus lotus* ont atteint une activité antiradicalaire de 90,32 %, tandis que les films à base de caséine en ont montré 40 %.

L'application de ces biofilms sur les fraises a assuré une stabilité contre la déshydratation, augmentant la durée de conservation et préservant la qualité sensorielle. Pour les pommes, les biofilms ont ralenti l'oxydation, conservant la couleur et l'intégrité structurelle plus longtemps que les tranches non enrichies ou découvertes.

L'incorporation d'extraits de feuilles de *Zizyphus lotus* dans des biofilms biodégradables et comestibles présente une approche prometteuse pour améliorer la conservation des aliments.

Mots clés : Zizyphus lotus, biofilm, enrobage comestible, biodégradable, polysaccharides, packaging actif, polyphénol, DPPH.

الملخص

تمثل المستخلصات النباتية الطبية عنصرا واعدا لتغليف المواد الغذائية القابلة للتحلل. كان الهدف العام من هذا البحث هو دراسة النشاط المضاد للأكسدة لمستخلص أوراق نبات اللوتس ودمجه في الأغشية الحيوية والطلاءات الصالحة للأكل القابلة للتحلل والتي تعتمد على النشا والكازين. ثم تم تطبيق هذه الأغشية الحيوية كمكونات تعبئة نشطة للحفاظ على التفاح والفراولة.

درس هذا البحث النشاط المضاد للأكسدة لمستخلص أوراق نبات اللوتس ودمجه في الأغشية الحيوية القابلة للتحلل والأكل المصنوعة من النشا والكازين لحفظ التفاح والفراولة. أظهرت مستخلصات أوراق نبات اللوتس، الغنية بالفينول والفلافونويد، خصائص قوية مضادة للأكسدة.
حققت الأغشية الحيوية القائمة على النشا مع مستخلص نبات اللوتس نشاطًا مضادًا للاكسدةبنسبة 90.32%، بينما أظهرت الأغشية القائمة على الكازين 40%.

إن تطبيق هذه الأغشية الحيوية على الفراولة يوفر ثباتًا ضد الجفاف، ويزيد من مدة الصلاحية ويحافظ على الجودة الحسية. بالنسبة للتفاح، أبطأت الأغشية الحيوية عملية الأكسدة، مما حافظ على اللون والسلامة الهيكلية لفترة أطول من الشرائح المكشوفة.

يمثل دمج مستخلصات أوراق نبات اللوتس في الأغشية الحيوية القابلة للتحلل والصالحة للأكل طريقة واعدة لتعزيز حفظ الأغذية.

الكلمات المفتاحية: زيزيفوس لوتس، الغشاء الحيوي، غلافصالح للأكل، قابل للتحلل، السكريات، التغليف النشط، البوليفينول، DPPH.