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Development of sustainable packaging using orange peels

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Wassim & Dina

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List of abbreviations

ATCC: American Type Culture Collection

BN: Nutrient Broth (Bouillon nutritif)

CFU/mL: Colony-Forming Units per Milliliter

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

EC: European Commission

EN: European Norm

EU: European Union

FTIR: Fourier Transform Infrared Spectroscopy

GAE: Gallic Acid Equivalent

GRAS: Generally Recognized As Safe

IC_{50}: Half Maximal Inhibitory Concentration

MAP: Modified Atmosphere Packaging

MBC: Minimum Bactericidal Concentration

MIC: Minimum Inhibitory Concentration

DM: Dry Matter

NB: Nutrient Broth

OD: Optical Density

OPE: Orange Peel Extract

PHA: Polyhydroxyalkanoates

PHBV: Polyhydroxybutyrate-valerate

PHV: Polyhydroxyvalerate

PLA: Polylactic Acid

PS: Polystyrene

PVA: Polyvinyl Alcohol

QE: Quercetin Equivalent

RH: Relative Humidity

ROP: Ring-Opening Polymerization

CE: Catechin Equivalent

PET: Polyethylene Terephthalate

MH: Muller Hinton agar

MEM: Minimum Essential Media

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Introduction

Introduction

Plastics have played a decisive role in the development of modern society. In 2019, global production exceeded 460 million tonnes per year, generating nearly 353 million tonnes of plastic waste annually. Of this waste, 22% was poorly managed, burned in the open air or dispersed into the environment **(Payne et al., 2019).** This accumulation not only contributes to greenhouse gas emissions, but also leads to the formation of harmful microplastics, endangering ecosystems and human health. To accelerate the transition to a more sustainable and resource-efficient circular economy, specific objectives have been put forward, including the use of bio-based, biodegradable and compostable plastics **(Chamas *et al*., 2020)**

Competitive biodegradable polymers include polyhydroxyalkanoates (PHA), poly (ε-caprolactone) (PCL) and polylactic acid (PLA), which are widely used due to their good mechanical properties (tensile strength and impact resistance), suitable viscosity and effectiveness as a gas barrier. Their areas of application are very diverse, ranging from consumer products to agriculture, electronics, automotive and textiles. However, packaging remains the main sector of use, accounting for 48% of the total bioplastics market in 2022 **(Dodange *et al*., 2024).**

Significant efforts are also being made to exploit active agents from agro-industrial waste **(Grimaldi *et al.*, 2022).** Orange peel accounts for a significant proportion of this waste. In 2020, global citrus production amounted to approximately 158.49 million tonnes, of which oranges accounted for around 82%. However, during processing and consumption, more than half of the fresh fruit's mass is discarded as waste (50-55% peel and 20-40% seeds). Composed of 80% water, orange peel is particularly rich in bioactive compounds, such as carbohydrates, vitamins and various essential phytochemicals such as pectin, cellulose, flavonoids, terpenoids, glycosides, carotenoids, limonoids, vitamin C and soluble fibre. This makes it a valuable biological resource to be exploited **(Singh *et al*., 2020).**

The incorporation of active substances into packaging expands its traditional function: it no longer merely acts as a physical barrier, but now actively contributes to food preservation. These agents gradually release inhibitory molecules onto the surface in contact with the food, targeting the areas most exposed to oxidation and microbial contamination. Active packaging thus helps to maintain food quality, reduce food waste by extending the shelf life of perishable products, and enable real-time quality control during storage or

transport, thereby responding to growing consumer demand for safe, effective and sustainable packaging solutions **(Ramakrishnan *et al*., 2024).**

This work responds to the urgent need to develop sustainable food packaging by recycling orange peel waste, an abundant agri-food by-product, by incorporating it into a biodegradable polymer matrix, in this case polylactic acid (PLA). The resulting composites will be characterised not only in terms of their mechanical properties, but also for their antibacterial and antioxidant activities, with a view to their potential application in the active packaging sector. The aim is to extend the shelf life of food while promoting the principles of the circular economy. This work establishes a link between waste recovery and materials science, offering an environmentally friendly alternative to conventional plastics for the preservation of perishable goods.

Chapter I. Literature review

Chapter I. Literature review

I.1. Generalities about food packaging

I.1.1. History

The practice of packaging food dates back to prehistoric times, when hunters would wrap their game in animal skins to protect it from the elements and make it easier to transport. During this period, other natural materials such as leaves, tree bark, hollow stones and shells were also used for this purpose. Around 6000 BC, fabrics, ceramics, baskets and pottery began to be used **(Basak Yilin Colok *et al*., 2014).** The first glass containers were used by the ancient Egyptians from around 1500 BC. Until the end of the 19th century, raw materials such as leather, wood, cork and fibres, and processed materials such as glass, metals and paper were predominant. The rise of plastic as a modern, practical material coincided with 20th-century innovations **(Urvoy *et al*., 2012).**

I.1.2. Definition

A material, composed of one or more layers, whose primary purpose is to package a food product while preserving its sanitary and organoleptic properties throughout storage and until its final use **(Marsh and Bugusu, 2007)**.

I.1.3. Types and functions of food packaging

The different types and functions of packaging are listed below. There are generally three types of packaging **(Multon and Bureau, 1998; Prendergast and Pitt, 1996)**.

- **Primary Packaging**: Direct product contact. Contaminant barrier. Optimal compatibility.
- **Secondary Packaging**: Combines multiple primary packages. Marketing platform. Enhances usability and information delivery.
- **Tertiary Packaging (Transport Packaging):** Ensures transit integrity.

The different functions of packaging are: **(Zeng, 2015).**

- **Contain:** The physical nature of the product must be taken into account (liquid, gas, solid) as well as its volume, weight, shape, etc

- **Protect and preserve:** Maintains the initial quality of the product until consumption, taking into account all factors that could alter it: shocks, heat, light, humidity or dryness, loss of carbon dioxide (for carbonated drinks
- **Transport (Logistics):** Streamlines transportation, storage, and handling, Optimizes supply chain operations
- **Marketing: i.** *Alert:* Captures attention through striking aesthetics and shelf visibility Drives purchase decisions in competitive environments. ***ii.****Information*: Provides essential product data (composition, origin, usage instructions) ensuring transparency and regulatory compliance.

I.1.4. Food and packaging interactions

Severin *et al* emphasize that multiple mass transfer mechanisms, schematically illustrated in figure 1, may occur at the food-packaging interface due to physicochemical processes. These include **(Severin*et al.*, 2011)**:

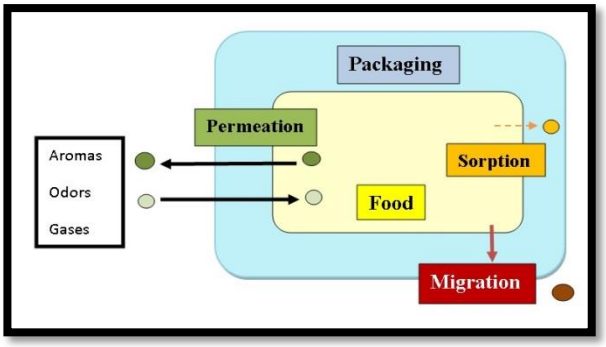

Figure 1: Food & packaging interactions

- **Migration**: Migration refers to the diffusion of packaging components into food. These may include technological additives such as monomers, oligomers, pigments, and solvents from printing inks or degradation products. Migration can cause toxicity issues as well as undesirable odors **(Boussoum M.O, 2012)**.
- **Gas permeation:** Permeation describes the phenomenon of diffusion of volatile molecules from the food and/or from the outside (gases such as O_2, CO_2, N_2, water vapor, aroma compounds) through the packaging. Permeation can thus cause aroma loss and therefore changes in the organoleptic properties of the product **(Berlinet C, 2006).**
- **Sorption**: Absorption by the packaging material of volatile foodborne molecules, particularly aromatic compounds **(Afrouzan H, 2017).**

I.1.5. Regulations and Standards on food packaging

Packaging regulations therefore focus primarily on these issues by requiring that packaging does not release constituents into food in quantities that could pose a risk to human health or cause unacceptable changes to the composition or organoleptic and toxicological characteristics of these foodstuffs.

In Algeria, the technical characteristics of packaging intended to come into direct contact with foodstuffs are governed by Decree No. 04-210, published in the Official Journal on July 28, 2004. European Regulation EC No. 1935/2004 of October 27, 2004 defines the main regulations concerning materials and objects in contact with food, as does Regulation EC No. 2023/2006, which defines good manufacturing practices for these materials and objects. Regulation (EU) No. 10/2011 of January 14, 2011, is specific to plastic materials **(Gaquerel and Costes, 2004).**

I.2. General overview of biopolymers with a focus on polylactic acid (PLA)

I.2.1. Definition

Biopolymers are organic macromolecules derived from natural sources such as plants, animals, and microorganisms, composed of repeating monomeric units. They are biodegradable, biocompatible, and serve diverse roles in nature, including structural (e.g., cellulose) and functional (e.g., DNA) applications. Widely utilized in sustainable materials for food packaging, medical implants, and drug delivery, they offer eco-friendly alternatives to synthetic polymers **(Baranwal, 2022).**

I.2.2. Classification

Biopolymers are classified into three main categories:

- **Natural biopolymers:** Directly extracted from living organisms (plants or animals), which can be divided into two families: polysaccharides (starch, cellulose, chitin) and proteins (animal or plant).
- **Biopolymers produced through bacterial fermentation,** using genetically microbial strains, such as polyhydroxyalkanoates (PHA), polyhydroxyvalerate (PHV), or polyhydroxybutyrate-valerate (PHBV) **(Biopolymersand bioplastics. (s.d.). Technical Form Agri-Industry).**

- **Synthetic biopolymers:** Synthesized through chemical polymerization of natural monomers, such as polylactic acids (PLA).

I.2.3. Advantages of bio-polymers for packaging (Rhim *et al*., 2007).

- **Environmental Sustainability:** Biopolymers reduce reliance on fossil fuels, lower greenhouse gas emissions, and degrade naturally, addressing plastic waste issues.
- **Biodegradability** They decompose into non-toxic byproducts (CO_2, water, and biomass) under industrial composting or natural conditions.
- **Versatility & Functional Properties** Engineered for diverse applications Enhanced Shelf Life in Food Packaging Biopolymers (e.g., PLA, chitosan) enable modified atmosphere packaging (MAP), reducing spoilage and extending freshness of fruits/vegetables.
- **Enhanced Shelf Life in Food Packaging** Biopolymers (e.g., PLA, chitosan) enable modified atmosphere packaging (MAP), reducing spoilage and extending freshness of fruits/vegetables

I.2.4. Polylactic acid (PLA)

a. Definition of PLA

PLA, is a bio-based thermoplastic comparable to PS and PET, it is produced through bacterial starch fermentation or chemical synthesis **(Houichi M.H, 2016).** It exists in three stereochemical forms (L-, D-, meso-lactide), with PLLA (pure L-lactide) being the most common **(Suyatma N.E, 2006).** First synthesized by Pelouze (1845), its polymerization was optimized by Carothers (1932) **(Aressy M, 2013).**

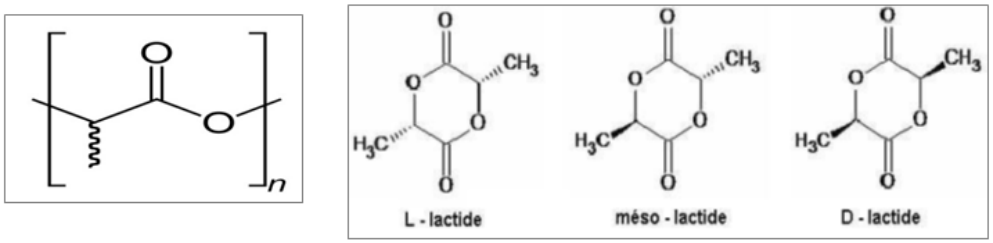

Figure3.a). Chemical structure of PLA**. b).**Different forms of acid isomers

b. Origin of PLA

Lactic acid (2-hydroxypropanoic acid), with the general formula CH_3-CHOH-COOH, containing an asymmetric carbon atom, was discovered by chemist Carl Wilhelm Scheele in 1780**(Crawford R.J, 1996),** and is produced through dextrose fermentation (primarily corn-derived), can also be synthesized from agricultural byproducts (starch, sucrose, lactose). Due to its abundance, corn dextrose remains the dominant market choice **(Linnemann B *et al*., 2003).**

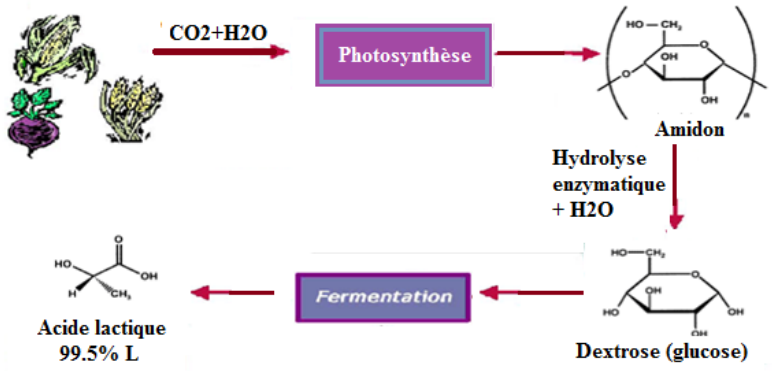

Figure 4: Production of lactic acid

c. Synthesis of PLA

Multiple processes enable the synthesis of high molecular weight PLA (Figure 5):

- ***Direct synthesis via lactic acid polycondensation***(Carothers' original method), yielding low molecular weight oligomers**(Groot and Borén T, 2010).**
- ***Azeotropic distillation-coupled polycondensation***removes water generated during the reaction, enhancing polymerization efficiency **(Kim and Woo S. I, 2002).**
- ***Ring-opening polymerization (ROP) of lactide,*** the preferred technique for obtaining high molecular weight polyesters **(Ajioka *et al*., 1998).** The predominant industrial method for PLA synthesis, notably employed by **NatureWorks**, is lactide ring-opening polymerization (ROP) due to its superior yield **(Houichi M.H, 2016).**

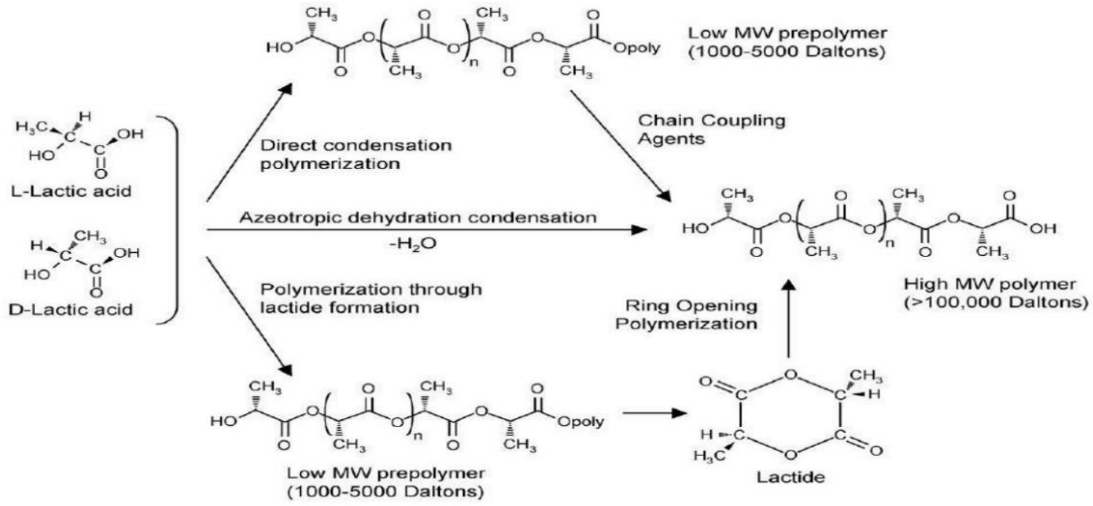

Figure 5: PLA Synthesis Pathways **(Ajioka M, 1998)**

d. PLA properties

- ***Physico-chemical properties***

PLA properties depend on the polymer's chemical structure. Its molar mass varies between 100 and 300 kg/mol. Its resistance to oils and fats and its aroma-barrier properties are excellent. It is completely soluble in chloroform and other organic solvents such as fluorine, chlorine, dioxane, furan, xylene, acetone, etc. The density of P (L-LA) is around 1.25-1.29 g/cm^3, while that of P (D- LA) is 1.27 g/cm^3(**Lindblad M.S *et al*., 2002).**

- ***Thermal properties* (Poncin-Epaillard *et al*. 2013)**

The thermal properties of PLA are shown in Table I.

Table I: Thermal properties of PLA

	PLLA	**PDLA**
Glass transition temperature	53–64°	50–57°C
The melting temperature	145–186°C	171°C
The decomposition temperature	235-255°C	255°C.
molecular weights	0.3–0.5×10^5 g/mol	0.21–5.5×10^5 g/mol

- ***Mechanical properties***

The basic mechanical properties of PLA are similar to those of polystyrene and PET. It is a hard and brittle material that requires reinforcement for widespread use **(Hao Y.P et al., 2013).** These properties depend heavily on the ratio of enantiomers (L and D) in the macromolecule. The main mechanical properties of PLA are shown in Table II **(Anderson *et al*., 2008).**

TableII: Mechanical properties of PLA **(Zhang *et al*., 2005)**

	PLLA	**PDLA**
Tensile strengths (MPa)	28–50	29 – 35
Young's modulus (GPa)	1–3	1.9 – 2.4
Flexural storage modulus (GPa)	1.4 – 3.25	1.95 - 2.35
Elastic limit elongation (%)	1.8–3.7	2.5 – 4

- ***Rheological properties***

For a given manufacturing process and application, understanding the rheological properties of PLA in its molten state is particularly interesting. These properties determine how the polymer flows during the conversion process. They are highly dependent on temperature, molar mass and shear rate. Semi-crystalline PLA has a higher shear viscosity than amorphous PLA **(M. Maiza, 2016).**

- ***Biodegradable, biocompatible and bioresorbable***

Because it is made from metabolites, PLA is a biodegradable, biocompatible and bioresorbable material, making it highly suitable for the manufacture of short- and medium-life single-use products, absorbable sutures and prostatic devices, and above all for packaging **(Si Jae Park et al., 2012)**.

I.2.5. PLA applications

Initially reserved for high-end applications (Table III) such as the biomedical field, thanks to its biocompatibility and biodegradability in the human body, and for packaging, improvements in PLA's structure have enabled this biosourced plastic to broaden its field of application **(Vink *et al*., 2003; Gupta B *et al*., 2005).**

Table III: Different PLA applications.

Sector	Key properties	Advantages	Applications
Textile	- Structural versatility. - Robust mechanical performance.	- Processing via diverse techniques (thermal bonding/carding/weaving).	- Apparel, bedding, carpets. - Innovative applications in specialized technical sectors.
Medical	- Biodegradable. - Biocompatible. - High mechanical resistance.	- Safe degradation (hydrolysis → non-toxic lactic acid). - Metabolic compatibility	- Drug delivery systems/implants/medical devices.
Packaging	- Transparent. - Biodegradable. - Compostable.	- Competitive pricing. - Outperforms polystyrene (PS). - Challenges PET on cost-performance.	- Bottles/food containers/plastic films/disposable items

I.3. Orange waste valorization

I.3.1.Generalities

Faced with increasing global food demand, driven by population growth and contemporary nutritional challenges, the valorization of agro-industrial by products into functional foods is emerging as a promising strategy. This circular approach simultaneously addresses the imperative of food security and the reduction of waste. **(Nowalid *et al.*, 2024)**

Citrus fruits, particularly oranges, lemons, grapefruits, mandarins, and tangerines, are primarily processed into juice, which is the main product of their processing industry. Depending on the variety (cultivar), these fruits contain between 45% and 58% juice **(Suri *et al.*, 2022)**. However, this production also generates significant byproducts, accounting for between 50% and 70% of the fruit's total weight. Globally, these byproducts would reach an estimated annual production of nearly 10 million tonnes **(Nieto *et al.*, 2021).** They consist predominantly of peels (60–65%), internal tissues (30–35%), and seeds (up to 10%) **(Nieto *et al.*, 2021).**

Citrus waste, particularly peels and pulp, is rich in bioactive compounds such as sugars, fiber, organic acids, amino acids, proteins, minerals, polyphenols, vitamins, lipids, and flavour compounds (with d-limonene being the predominant one). They also contain a

significant amount of pectin. These compounds are of considerable interest to various industries **(González-Molina, 2010; Sharma *et al.*, 2022).** For example, pectin is extensively used in the food industry as a gelling and stabilizing agent, while essential oils are utilized in the formulation of fragrances and flavorings.

I.3.2. Orange

It is a citrus fruit, the edible fruit of the orange tree (Citrus sinensis), an ancient hybrid resulting from a cross between the pomelo and the mandarin. It is cultivated in temperate and warm zones such as Mediterranean countries. Belonging to the Rutaceae family, it has a spherical to oval shape with a thick, rough, orange-red peel containing essential oils that have a characteristic aroma. The orange is a juicy, sweet, and invigorating fruit, rich in vitamin C. It is used to make fruit salads, jams, or to drink its juice. **(Milind et Dev, 2012)**.

I.3.3. Orange peels

This refers to the outer part of the fruit, which serves as a protective role while also constituting a significant source of phenolic compounds **(Teh *et al.*, 2014)**.

The peel (Figure 6) is composed of two distinct layers:

- **The flavedo** (or epicarp), the orange-colored outer layer, contains the oil glands responsible for producing essential oils.
- **The albedo** (or mesocarp), the white inner part, is particularly rich in pectin. **(Ladaniya, 2008).**

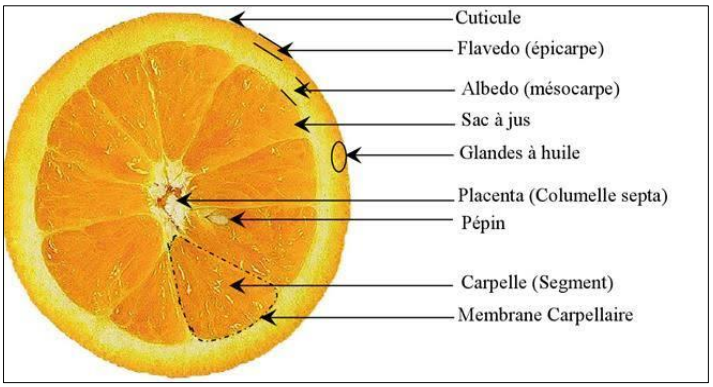

Figure 6: Cross section of an orange **(Guimaraes *et al.*, 2010)**.

I.3.4. Chemical composition of orange peels

Orange-derived byproducts, characterized by high water content (75–90% moisture), contain a wide variety of valorizable compounds including polyphenols, essential oils, carotenoids, dietary fiber, ascorbic acid, sugars, and trace elements. The peels are particularly rich in fiber both soluble (pectin) and insoluble (cellulose, hemicellulose, lignin) as well as carbohydrates (fructose, glucose, and sucrose). They also contain 4–6% protein, which can be utilized for food applications.

Essential oils, present in the peels and seeds, are concentrated in the deep layers of the flavedo and are released during juice extraction. They contain fatty acids such as oleic, linoleic, linolenic, palmitic, and stearic acids. The pulp also contains glycerol, phytosterols, and phytosterol esters.

Notably, orange peels contain higher levels of bioactive compounds than the edible portion, particularly polyphenols including phenolic acids such as hydroxybenzoic acid and hydroxycinnamic acid **(Cirrincione *et al.*, 2024)**.

Table IV: Global chemical composition of orange peels (g/100g MS) **(M'hiri, 2015)**

Chemical composition	Concentrations
Water	60-75 %
Lipids	1,66
Proteins	1.79
Carbohydrates	15.01
Minerals	3.45
Fibers	41.64
Carotenoids	0.04
Total phenols	19.62
Vitamin C	1,15
Essentiel oils	0.6

I.3.5. Orange waste production and their applications

Citrus peels, often regarded as waste, represent a valuable source of bioactive compounds with numerous applications in the food industry particularly for flavor creation. In recent years, the utilization of citrus peels for flavor development has gained momentum, emerging as a sustainable yet economical solution to enrich food products with unique and complex flavor profiles **(Baker *et al.*, 2021)**.

Natural orange peel extracts serve a critical function in the pharmaceutical industry, particularly in formulating medications, soaps, fragrances, and other cosmetic products **(Lohrasbi *et al*., 2010).** Pectin derived from orange peel is also used to produce various pharmaceutical suspensions **(Piriyaprasarth etSriamornsak, 2011)**, Pectin derived from orange peel is also used to produce various pharmaceutical suspensions including detoxification drugs and anti-diarrheal medications. It further contributes to reducing cholesterol levels and exhibits anticancer properties **(Fernandez-Lopez *etal.*, 2004).**

In culinary applications, orange peel is used to prepare products such as candied peel, ice creams, and liqueurs both sweet and bitter varieties **(Bousbia, 2011).** Thanks to its thickening, gelling, and stabilizing properties, the pectin present in orange peel is extensively utilized in the food processing industry for diverse formulations. The natural combination of orange peel fibers and their bioactive molecules (such as vitamin C and phenolic compounds) confers multiple functional properties ideal for producing dietetic foods **(Fernandez-Lopez *et al*., 2004).**

Traditionally, orange peel has also been used in medicine to manage a range of ailments, including constipation, cramps, colic, bronchitis, diarrhea, stress, depression, hypertension, and anxiety **(Milind et Dev, 2012).**

I.3.6. Economic and environmental importance

Global population growth and worsening hunger indicators pose major challenges to food security. An innovative response to this challenge involves utilizing agri-food industry byproducts such as citrus processing waste to develop functional foods. This approach not only reduces waste but also provides nutritional solutions to address growing malnutrition **(Nowalid *et al*., 2024).**

Among these byproducts, orange peels and residues are particularly valuable due to their richness in pectin a natural dietary fiber with multiple biological properties. Citrus-derived pectin exhibits hypoglycemic and cholesterol-lowering effects, thereby contributing to the prevention of metabolic and cardiovascular diseases. It also beneficially modulates gut microbiota and the immune system while acting as a natural prebiotic **(Sharma *et al*., 2022).**

From an industrial perspective, pectin is highly sought after for its gelling, thickening, and stabilizing properties. It is used in a wide variety of products: jams, jellies,

dairy products, ice creams, salad dressings, and even cosmetic creams. Compared to other sources (cereals, soy), citrus-derived pectin exhibits superior solubility, greater water-holding capacity, and higher viscosity enhancing food texture and stability **(Sharma *et al*., 2022).**

Moreover, uncontrolled disposal of this waste (through landfilling or incineration) causes environmental issues, such as air pollution from microbial load, and economic losses due to squandering a valorizable resource. From a regulatory standpoint, citrus peels are classified as Generally Recognized As Safe (GRAS) for food use particularly in processed products like breakfast cereals, yogurts, and biscuits **(Ademosun, 2024).**

Chapter II. Materials and methods

Chapter II. Materials and methods

II.1. Materials

II.1.1. Poly lactic acid (PLA)

The poly lactic acid (PLA) matrix used is supplied in granules (figure 7) , under the reference Ingeo Biopolymer 2003D supplied by Nature Works, specifically designed for use in fresh food packaging and food serviceware applications.Its main characteristics are summarized in Table V (**Basak Yilin Colok, 2014).**

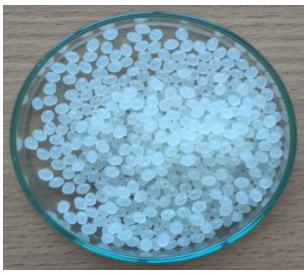

Figure 7: poly (lactic acid) polymer

Table V: Primary physico-chemical characteristics PLA

Properties	**Values (Ingeo 2003D)**
Specific Gravity (density)	1.24
Melt Temperature (°C)	145 - 160
Melt flow index(g/10 min (210°C, 2.16kg)	6
Glass Transition Temp, (Tg) (°C)	55.0 - 60.0
Tensile Strength (MPa)	53
Tensile Modulus, (GPa)	3.5
Tensile Elongation, %	6.0

II.1.2. Orange peel

Fresh orange peels (Citrus sinensis) freshly harvested at a local natural juice factory in the Bejaia region were rinsed with tap water, then distilled water, cut into small pieces, and dried in an oven at 40°C.

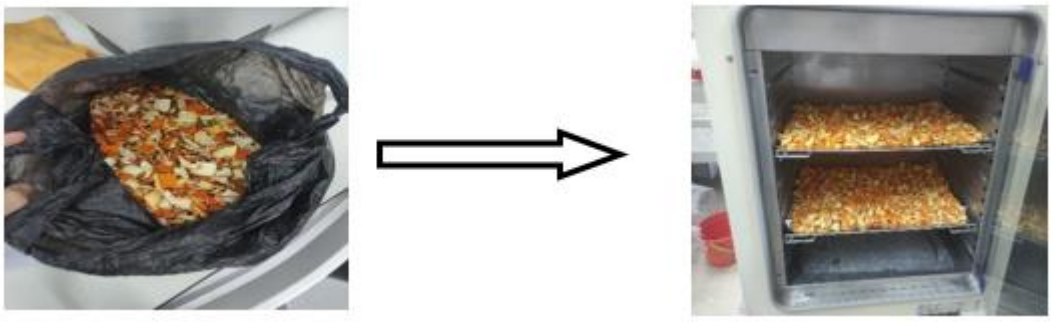

Figure 8: Photography of harvesting and drying process

They were then ground using a *Retsch ZN 200* grinder to obtain a powder with a particle size of 2 mm. The powder then stored away from light in hermetically sealed bags under controlled ambient conditions (25°C ± 2°C, 40–50% relative humidity)to minimize degradation of the bioactive compounds and microbial contamination. This powder will be used to extract the bioactive product (orange extract), which will serve as a filler in the PLA matrix.

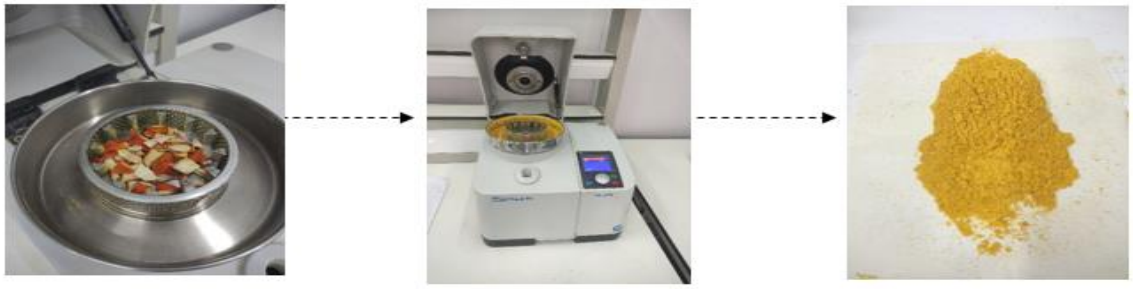

Figure 9: Photography of grinding process

II.1.3. Chemical reagents

- The solvents used in this study are of analytical grade and are supplied by BIOCHEM-Chemopharma, Canada. Chloroform is used to dissolve PLA, ethanol is used as an extraction solvent to extract bioactive compounds from orange peel, and methanol is used to dissolve the orange extract.The main characteristics of each are listed in Table VI.
- The $DPPH^{\blacksquare}$, **(**2,2-diphenyl-1-picrylhydrazyl is a stable purple free radical in solution, which can be reduced by a compound with antiradical properties into DPPH-H diphenylpicrylhydrazine, which is yellow in color **(Molyneux, 2004).**

Table VI: Main characteristics of each solvent used

	Chloroform	**Ethanol**	**Methanol**
Chemical formula	$CHCl_3$	C_2H_6O	CH_3OH
Molecular weight (g/mol)	119.38	46.07	32.04
Densityat 25°C (g/cm³)	1.478	0.789	0.791
Purity (%)	99	96	99.9

II.1.4. Bacterial strains

Two bacterial strains were used, one Gram-positive *Staphylococcus aureus* (ATCC6538) and the other Gram-negative *Escherichia coli* (ATCC25922). These strains were provided by the food microbiology laboratory at the University of Bejaia.

II.2. Experimental Methods

II.2.1. Extraction of phenolic compounds

Phenolic compounds were extracted via ethanol (70%) using Romani *et al*.'s protocol: 50 g of orange peel powder was macerated in 500 mL ethanol for 24 h under agitation. After 24 h of settling, the supernatant was collected and oven-dried at 40°C to constant weight (**Roamni *et al.*, 2006).**

Figure 10: Photography of extraction process.

II.2.1.1. Determination of total phenolic content

The method is based on the reduction of the Folin-Ciocalteu reagent (yellow) by polyphenols, forming a blue complex **(Kähkönen *et al*., 1999).** A volume of 200 µL of extract (100 µg/mL) and 1 mL of 10% Folin-Ciocalteu reagent were added to a centrifuge tube, followed by 800 µL of 7.5% sodium carbonate solution. The mixture was incubated for 30 minutes at room temperature in the dark. Absorbance was measured at 760 nm using

a blank (prepared without the extract). Total phenolic content was calculated as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g) using a gallic acid calibration curve. **(Cicco *et al*., 2009).** Three replicates (tubes) were analyzed, and one tube was used for the blank measurement.

II.2.1.2. Determination of flavonoids content

This method is based on the ability of flavonoids to chelate metal ions (e.g., aluminum), forming a yellow-colored complex. A 2 mL aliquot of the extract was mixed with 1 mL of aluminum chloride solution (prepared by dissolving 133 mg of $AlCl_3$ and 400 mg of sodium acetate in distilled water). Absorbance was measured at 430 nm. Flavonoid concentration was expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g), using a quercetin calibration curve **(Maksimovic *et al.,* 2005**). Three replicates (tubes) were analysed, and one tube was used for the blank measurement.

II.2.1.3. Determination of condensed tannins (proanthocyanidins)

This method relies on the interaction of proanthocyanidins (flavanol-3) with vanillin in an acidic medium, resulting in a red-colored complex. A 1 mL aliquot of the extract was mixed with 3 mL of 4% methanolic vanillin solution and 1 mL of concentrated sulfuric acid (H_2SO_4). After incubation for 20 minutes at room temperature, absorbance was measured at 500 nm. The condensed tannin content was expressed as milligrams of catechin equivalent per gram of dry extract (mg CE/g), using a catechin calibration curve. **(Sun *et al*., 1998).** Three replicates (tubes) were analysed, and one tube was used for the blank measurement.

II.2.1.4. Evaluation of the antioxidant activity: DPPH radical scavenging

The antioxidant capacity of OPE is determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) according to the following protocol: 500 µL of the sample was mixed with 1 mL of DPPH solution in methanol (0.1 mM). After 30 minutes of incubation in the dark at room temperature, the absorbance is recorded at 517 nm using a *BIOTECH ENGINEERING UV-9200 UK* spectrophotometer. The percentage inhibition of the DPPH• radical is calculated using the following formula: **(Balasundram *et al*., 2007).**

$$\textbf{DPPh radical scvenging } (\%) = [\frac{(\textbf{AC} - \textbf{AS}}{\textbf{AC}}] \times \textbf{100}$$

Where:**AC:** Absorbance of the control (methanol + DPPH). **AS:** Absorbance of the sample (extract + DPPH).

II.2.1.5. Anti-bacterial activity

a. Bacterial strains

The antibacterial activity of the ethanolic extract of orange peel was tested on two Gram-negative and Gram-positive strains, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6314.

b. Revival of strains

The bacterial strains were cultivated on Petri dishes containing nutrient agar (NA) followed by incubation for 24 hours at 37°C in order to obtain a young and fresh culture.

c. Bacterial strains preparation (inoculum)

Several colonies of the target strain were taken from the previously incubated boxes, then these colonies were diluted in a test tube containing 4 ml of sterile physiological water. After homogenisation by vortexing, the absorbance of the prepared bacterial suspension was read at a wavelength of 620 nm by a UV spectrophotometer, giving an OD of [0.08 to 0.10], which corresponds to 1-2 × 10^8 CFU/ml **(Yala *et al.*, 2016).**

d. Micro-dilution test on liquid medium

Serial dilutions were performed to obtain orange extract concentrations (1000, 750, 500, and 250 µg/mL). In each well, 100 µL of Nutrient Broth (NB) was mixed with 50 µL of the extract. A blank, positive control, and dose control were prepared in parallel. Finally, 50 µL of bacterial suspension was added to all wells except the blank. A second microplate was prepared under identical conditions to assess bacterial biofilm growth inhibition, with the exception of the positive control which contained 50 µL of the biofilm-dissolving reagent. The prepared microplates were incubated at 37°C for 24 h. Absorbance was read using a microplate reader (BioTeck Instruments, Inc, USA) at 630 nm **(Kowalska *et al.*, 2021).**

The percentages of bacterial growth inhibition were calculated according to the following equation:

$$\% \textbf{inibition} = \frac{(\textbf{Abs positive witness} - \textbf{Abs white})}{\textbf{Abs test} - \textbf{Abs dose control})} \textbf{x100}$$

Where:

Abs positive control: 150 µL BN + 50 µL bacterial suspension

Abs blank: 200µL BN

Abs dose control: 150µL BN+ 50 µL extract (dissolved in physiological water)

Abs test: 150µL extract (dissolved in BN) + 50 µL bacterial suspension

- The IC_{50}were calculated based on the results obtained.

II.2.2. Films preparation

II.2.2.1. Formulations

Various formulations based on virgin PLA and PLA/orange peel extract (OPE) at different loading rates (10, 20, and 30%) were prepared, coded F0, F10, F20, and F30, respectively. The compositions are shown in Table VII.

Table VII: Mass compositions of the different PLA/OPE formulations

	Percentage %		**Mass in (g)**	
Formulations	PLA	OPE	PLA	OPE
F0	100	0	5.0	0
F10	90	10	4.5	0.5
F20	80	20	4.0	1.0
F30	70	30	3.5	1.5

II.2.2.2. Manufacture of solution films

The PLA/OPE based biofilms were fabricated using a solution casting method described previously **(Basak Yilin Colok, 2014).** 5g of PLA are weighed and placed in a beaker. 100 ml of chloroform are added to it. The mixture is placed under continuous stirring at room temperaturestirring until completely dissolved (approximately 30 min), the dissolution continues for 3 hours. Then, the mixture is poured into Petri dishes (20 ml in each Petri dishes to ensure films of the same thickness), and left to dry for 24 hours at room temperature. Finally, the films are collected and stored in hermetically sealed bags.

The same procedure was used to prepare PLA/OPE films with the addition of 10%, 20%, and 30% orange extract. The PLA solution was prepared by dissolving the PLA matrix separately in 100 ml of chloroform at room temperature, stirring until completely dissolved

(approximately 30 minutes). At the same time, the same operation was performed for the orange extract, but in 20 ml of methanol and stirring until completely dissolved. Once dissolved, the two solutions were mixed and left to stir for another 3 hours, then homogenized in an ultrasonic bath for 10 minutes. Finally, the mixture was poured into Petri dishes (approximately 20 ml per dish) and left to dry for 24 hours at room temperature. The films were then collected and stored in bags.

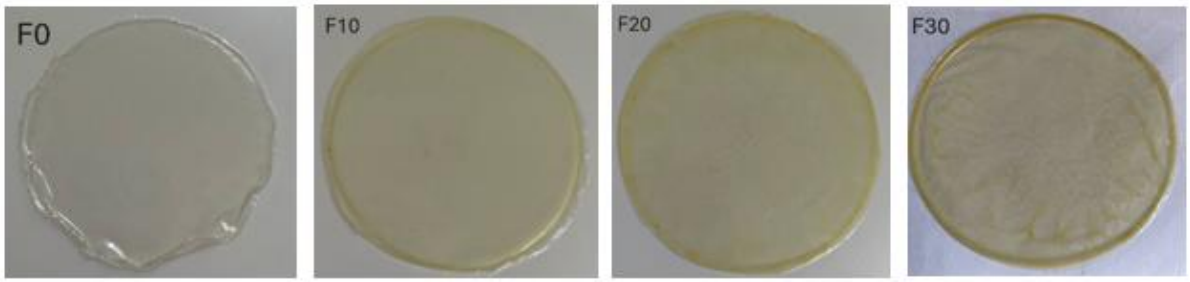

Figure 11: Photography of prepared films

II.2.2.3. Characterisation by Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy is a rapid structural analysis technique that identifies chemical functional groups in materials by measuring their absorption of infrared light. The FTIR spectra of the different samples were recorded in absorbance mode using a ***Thermo Fisher Scientific's Nicolet iS5*** infrared spectrometer. The analysis was performed on PLA/OPE films. The scanning range was between 400 and 4000 cm^{-1} with 32 scans and a resolution of 4 cm^{-1}.

II.2.2.4. Water absorption test

Squares measuring 2 cm on each side were dried in an oven for 24 hours at 40°C, cooled in a desiccator, and immediately weighed (m0) on a precision analytical balance accurate to 0.0001 g. In accordance with ***ASTM D570***, the samples were immersed in a container of distilled water at a temperature of 23°C. After 24 hours, a sample was taken, first removing the surface water with absorbent paper, then weighing the samples again (m). The change in mass (Δm (%)) is given by the following formula: Three tests were performed for each formulation. **(Q.Wang *et al.*, 2023; M. Azka *et al.*, 2024)**. The absorption rate was determined by:

$$\mathbf{\Delta m}(\%) = \frac{(\mathbf{m} - \mathbf{m_0})}{\mathbf{m_0}} \times \mathbf{100}$$

II.2.2.5. Tensile test

Tensile testing was performed using standardized dumbbell-shaped specimens to evaluate fundamental mechanical properties under constant crosshead speed (2 mm/min) until fracture. Stress-strain curves generated via a (***JINAN WDW-50 ,China***) universal testing machine (Advanced Polymer Materials Laboratory university of A.Mira Bejaia) quantified ultimate tensile strength (σ) elongation at break (ε) and Young's modulus (*E*). Three replicates per material formulation ensured statistical reliability. These parameters are related to each other according to the following equations:

Where:

$$\boldsymbol{\sigma\,(\mathrm{mPa}) = \frac{F}{S}} \quad / \quad \boldsymbol{\varepsilon(\%) = \frac{L - L_0}{L_0} * 100} \quad / \quad \boldsymbol{E(MPa) = \frac{\sigma}{\varepsilon}}$$

- S: Cross-sectional area of the specimen (mm^2) - F: Applied tensile force (N)
- L: Final length of the specimen (mm) - L_0: Initial length of the specimen (mm)

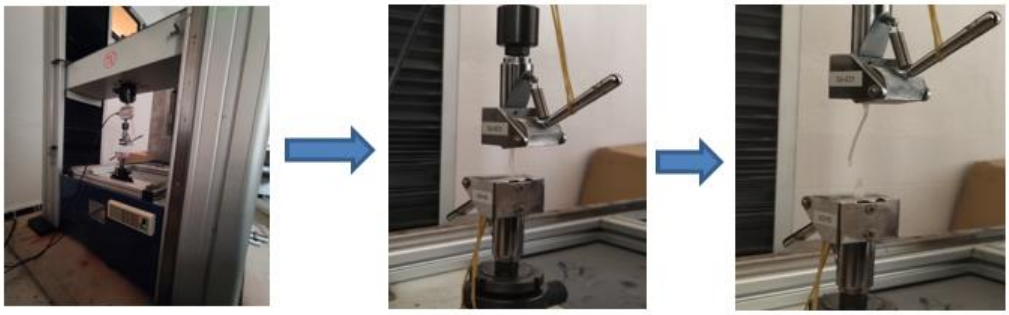

Figure 12: Tensile test on films process

II.2.2.6. Contact angle test

Contact angle measurement quantifies the wettability of a liquid on solid surfaces by analysing the angle formed at the solid-liquid-vapour interface. This angle reflects the thermodynamic equilibrium between adhesive (solid-liquid) and cohesive (liquid-liquid) forces. For PLA films modified with orange peel extract (OPE), this technique evaluates the components of surface energy (dispersive, polar, acid-base) **(Georgiev *et al.*, 2024).**

The contact angle test was performed using an optical goniometer equipped with a high-resolution camera, manufactured by ***KRÜSS*** (Drop Shape Analyser, Germany). After calibrating the camera and setting the chamber to 25 °C ± 0.5 °C and 45% RH ± 3% (to

prevent evaporation), a drop of distilled water (2 µL) was deposited using a micro-dispensing syringe onto the surface of each film (F0, F10, F20 and F30) laid flat on a glass slide with an automated dispensing speed of 0.5 µL/s) and the distance between the needle and the surface was 1 mm. Three measurements were taken on different areas of the same sample, and the average value was recorded.**(Taib *et al.*, 2019).**

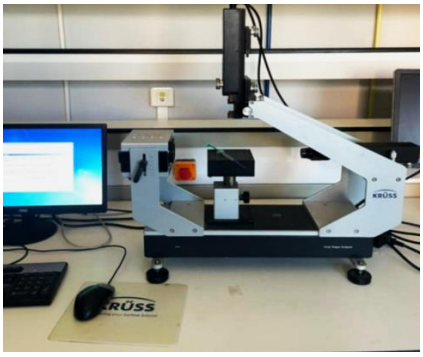

Figure 13: Photography of optical goniometer (KRÜSS Drop Shape Analyzer)

II.2.2.7. Evaluation of the antioxidant activity

The antioxidant capacity of PLA/OPE films is also determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) according to the protocol mentioned in section II.2.5. **(Balasundram *et al.*, 2007).**

II.2.2.8. Anti-bacterial activity

The antibacterial activity of PLA/OPE films was tested on two Gram-negative and Gram-positive strains, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6314, according to the protocol mentioned in section II.2.7.

Chapter III. Results and discussion

Chapter III. Results and discussion

III.1. Characterisation of Orange peel extract

III.1. 1. Phytochemical screening

Polyphenols are organic molecules of heterogeneous structure produced by plants during their secondary metabolism **(Abbas *et al*., 2017; Panche *et al*., 2016).** Extraction of polyphenols can be performed from different parts of a plant. To achieve this, samples are often dried and ground into a fine powder to maximize contact between the extraction solvent and plant particles for optimal extraction **(Vongsak *et al*., 2013; Iloki-Assanga *et al*., 2015).**

The total polyphenol, flavonoid, and condensed tannin content of the orange peel ethanol extract (OPE) was determined using two standard curves (Annex 1). The results obtained are presented in table VIII.

Table VIII: Total phenol, flavonoid and condensed tannin contents

Total phenols (mg EAG/ g MS)	Flavonoids (mg EQ/g MS)	Condensed tanins (mg EC/g MS)
281,76± 2,36	34,75 ± 1,08	359,98±3,41

Each value represents the mean of three trials ± standard deviation

The results (Table VIII) revealed that the OPE extract contains significant levels of total phenols (281.76 ± 2.36 mg GAE/g DW), flavonoids (34.75 ± 1.08 mg QE/g DW), and condensed tannins (359.98 ± 3.41 mg CE/g DW), confirming the potential of citrus residues as a source of phenolic compounds. This finding aligns with what **Ben Hsouna *et al*., (2021)** reported for other citrus peels.

The quantification results demonstrated that orange byproduct is considered as a significant source of phenolic compounds. Indeed, orange peels contain several classes of phenolic compounds particularly phenolic acids and flavonoids such as hesperidin and neohesperidin which contribute to the overall antioxidant activity of peel extracts **(Charunivedha *et al.*, 2024).**

III.1. 2. Evaluation of anti-oxidant activity

The antioxidant capacity of the OPE extract at different concentrations was evaluated using the DPPH radical scavenging assay. The results of the DPPH scavenging effect are presented in table IX and figure 14.

Table IX: Scavenger effect of DPPH radical from ethanolic orange peel extract

DPPH % Inhibition	IC50 (µg/ml)
91,11± 1,95	165,12± 0,02

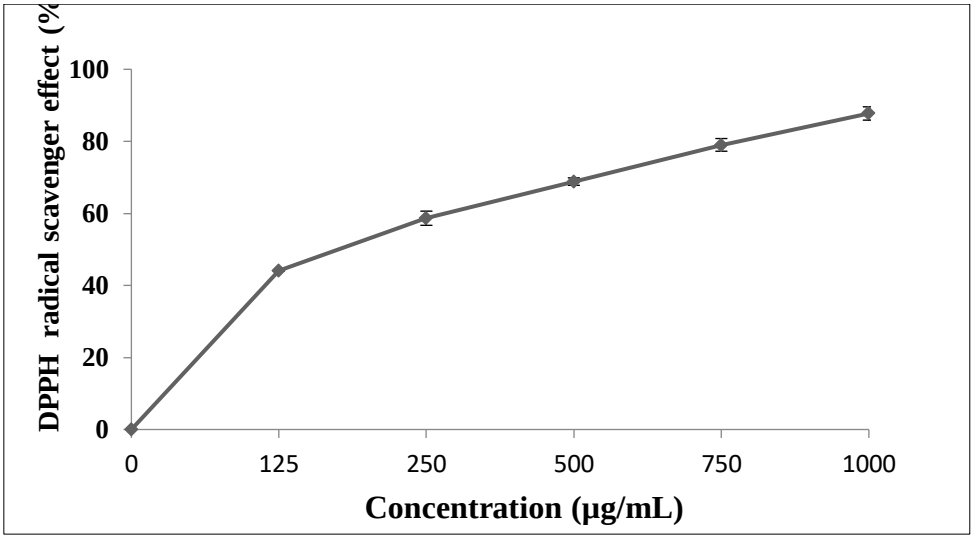

Figure 14: DPPH• free radical neutralizing activity as a function of OPE extract concentration

According to the data in Figure 14, it is observed that as the concentration of the extract increases, the free radical inhibition percentage rises, thereby enhancing antiradical activity. A maximum inhibition rate of 91.11% (Table IX) was recorded at a concentration of 1 mg/mL.

To express the results in terms of IC50, the IC50 value is defined as the amount of antioxidant required to decrease the initial DPPH• concentration by 50% **(Scherer and Godoy, 2009)**. The IC50obtained for the OPE extract was 165.12 µg/mL (Table IX).

Based on the obtained results, it is evident that the orange peel extract exhibited potent antioxidant effects in a dose-dependent manner. This may be linked to its high polyphenol content, which can serve as electron donors. Consequently, we demonstrated that phenolic

compounds are generally the primary contributors to the antioxidant potential of orange peel, consistent with other research establishing a strong correlation between phenolic compounds and antioxidant effects **(Barrales *et al.*, 2018; Saleem *et al.*, 2024).**

III.1.3. Evaluation of anti-bacterial activity

Facing the challenge of increasing bacterial resistance to synthetic antibiotics, numerous studies have explored alternative antibacterial properties. In our work, we evaluated the antibacterial efficacy of orange peel extract against two bacterial strains: *E. coli* and *S. aureus*. This efficacy assessment was conducted in liquid medium at varying extract concentrations by measuring the bacterial growth inhibition rate (Figure 15) and IC50 (Table 9).

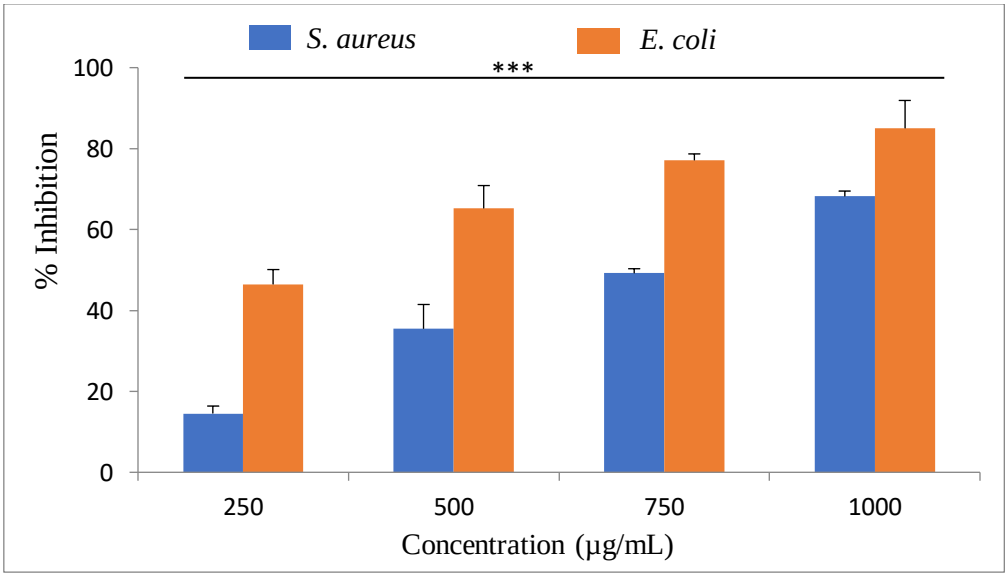

Figure 15: Evaluation of the antibacterial activity of orange peel extract against bacterial strains at different concentrations

-All values are expressed as the mean of three trials with ± standard deviation. Differences between means were statistically tested by one-way ANOVA test, followed by Tukey's test ($p < 0.05$).

The statistical study revealed the existence of a significant difference between the inhibition rates depending on the concentration of extract and the strain tested ($p<0.05$). The results demonstrated that all tested concentrations (250–1000 µg/mL) exhibited efficacy against the studied microorganisms (Figure 15). The highest antibacterial effect of 86.49%

was recorded against *E. coli* at 1 mg/mL. The lowest antibacterial effect was achieved with the OPE extract at 250 µg/mL against *S. aureus*.

Table X: Orange peel extract IC50 values in mg/mL

	E. coli	***S. aureus***
IC50	0.55 ± 0.05	1.12± 0.02

According to the results in Table X, the best IC50 (0.55 mg/ml) recorded is that of the OPE extract against *E. coli.* Furthermore, the OPE extract marked an IC50 of 1.12 mg/ml against *S. aureus.*

Our results indicate that all strains tested were susceptible to the studied extract, with *E. coli* being the most susceptible strain. These findings align with the work of **Shetty *et al.*, (2015)** and **Saleem *et al.*, (2020**), who reported that orange peel extracts significantly inhibit bacterial growth against a broad range of microorganism's particularly Gram-negative bacteria.

III.2. Characterisation of PLA/OPE films

III.2.1. Spectroscopic analysis (FTIR)

Figure 16 shows the FTIR spectra of the orange extract, pure PLA film, and PLA films loaded with 10% and 30% OPE. The assignments of chemical functional groups for each absorption band appearing in the FTIR spectra of the cellulose fiber are listed in table XI.

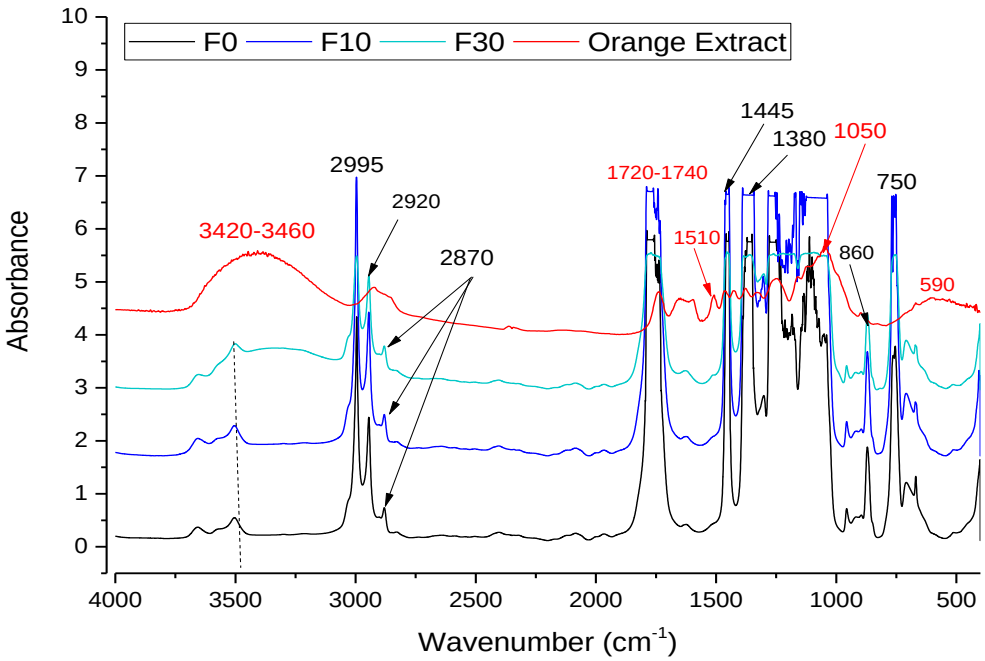

Figure 16: FTIR spectras of PLA films and orange peel extract

Orange peel is primarily composed of cellulose, hemicellulose, lignin, and to a lesser extent pectins and other organic compounds such as sugars. Analysis of the orange extract spectrum reveals distinct absorption bands that provide valuable insights into its structural composition, consistent with literature findings.

- ✓ A broad, intense band between 3420-3460 cm^{-1} denotes a strong presence of hydroxyl groups (-OH), resulting from the characteristic stretching vibration of hydroxyls in carbohydrates, cellulose, and hemicellulose **(Haya *et al.*, 2019; Zayed *et al.*, 2021; Basak Yilin Colok, 2014).**
- ✓ The basic structure of lignocellulosic material can be observed through the 2922 cm-1 band due to the symmetric and asymmetric stretching of C-H.
- ✓ The signal at 1720-1740 cm^{-1} can be attributed to carbonyl groups (**Musa *et al.*, 2022**).,

The peak at 1510 cm-1 is due to C-C stretching attributable to aromatic compounds **(Gaquerel and Costes, 2004; Handout IR, UDel, 2013).**

- ✓ Around 1445 cm-1 corresponds to the deformation vibrations, due to the aliphatic chains (CH2 and CH3) **(Lindblad *et al.*, 2002).**
- ✓ The band at 1050 cm-1 can be explained by the presence of C-O or C-O-C stretching vibrations **(Hao *et al.*, 2013).**

- The band at 590 cm-1 is due to the out-of-plane O-H hydroxyl stretching vibrations of the polysaccharides.

PLA is an aliphatic polyester, so its IR spectrum is relatively simple and dominated by ester and methyl groups. The spectra of loaded PLA films exhibit spectral profiles similar to the control film (pure PLA), with identical bands and peaks listed in Table XI, though with variable intensities. In PLA/OPE composite films, the hydroxyl group peaks at 3500 cm^{-1} increase in intensity with higher extract content. This enhancement is primarily attributed to the increased concentration of (-OH) groups from polysaccharides present in the orange extract. These results are confirmed by water absorption tests and align with findings reported by **Fidalgo*et al.,* 2016; Anderson *et al*., 2008).**

Table XI: Absorption bands recorded for PLA and PLA/OPE films

Wavenumber cm^{-1}	**Type of vibration**
3500	Stretching vibration of unbonded O-H hydroxyls probably due to water absorption
2995-2920	Asymmetric stretching vibration of C-H bonds of the CH3 and -CH group
1720-1740	Vibration of the C=O carbonyl groups of the ester
1440-1460	Asymmetric deformation vibration of C-H bonds of methyl groups ($-CH_3$) (PLA) and aromatic ring (PLA/OPE)
1379	Symmetrical deformation vibration of in-plane C-H bonds in polysaccharides
860	attributed to deformation vibrations of the C–C skeleton of PLA
750	Symmetric stretching vibration of C-O-C bonds of cellulose and hemicellulose

III.2.2. Tensile test

III.2.2.1 Evolution of tensile strength

The evolution of tensile strength in PLA and PLA/OPE films as a function of orange peel extract content is illustrated in Figure 17. A decrease in tensile strength is observed in loaded composites compared to unmodified PLA. This reduction declines from 12.54 MPa (F0) to 10.09 MPa (F10), 7.31 MPa (F20), and 3.14 MPa (F30). This strength reduction is attributed to poor interfacial compatibility between the PLA matrix and phenolic compounds in the peel extract, creating instability zones in the composite material. These results are predictable and align with numerous studies**(Fidalgo, A *et al* 2016; Maiza, 2016),** who

attributed this decrease to reduced interfacial bonding strength between the extract and the matrix, which obstructs stress propagation likely due to extract agglomeration inducing heterogeneities and non-uniform stress transfer within the matrix, consequently causing film embrittlement. Similar results have also been reported by **Sambudi *et al.* (2021)**, where incorporating orange peel powder weakened PLA matrix cohesion by reducing intermolecular interactions. **Paul *et al.*, (2021)** also observed a significant decrease in mechanical strength as natural fiber concentration increased, due to non-uniform dispersion.

Improved tensile strength could be achieved by enhancing interfacial interactions through the use of compatibilizing agents, as reported in literature **(Si Jae Park *et al.* 2012).**

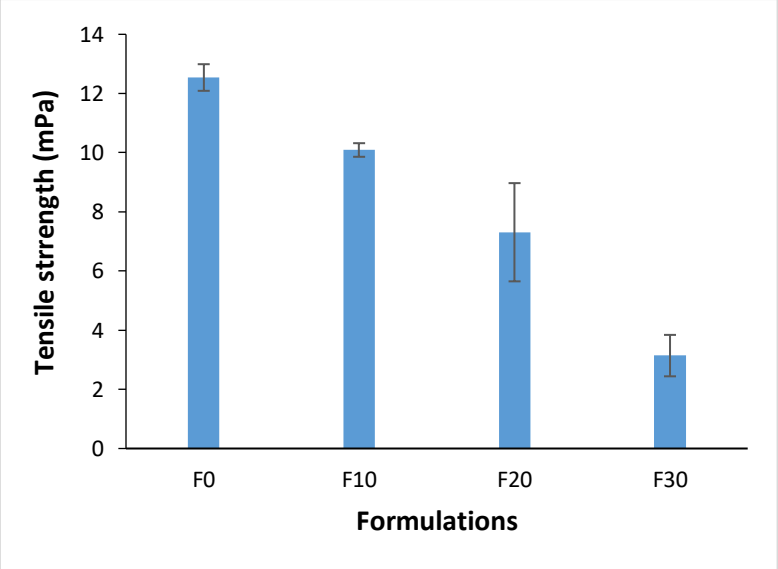

Figure 17*:* Evolution of the tensile strength of PLA and PLA/OPE films as a function of the orange extract content

III.2.2.2. Evolution of elongation at break

The evolution of elongation at break for PLA and PLA/OPE films as a function of orange extract content is illustrated in Figure 18. It is observed that incorporating orange extract into the PLA matrix reduces strain deformation. This reduction can be explained by the hydrophilic nature of the filler, primarily due to polysaccharides in the orange extract that absorb more moisture creating defects in the system and reducing interchain interactions resulting in a ductile-to-brittle transition in the material's behavior **(Maiza, 2016).**

Increasing the orange extract content yields a distinct improvement in elongation at break estimated at 14.3% and 72.42% for F20 and F30 formulations respectively but not in overall deformation compared to pure PLA. This enhancement is attributed to the plasticizing effect of specific bioactive compounds (limonene, flavonoids) contained in peel

extracts**(Si Jae Park *et al.* 2012).Lertphirun & Srikulkit (2019)** reported that natural extracts with high essential oil content could act as plasticizing agents, thereby increasing flexibility at high loading levels. According to **Zhou *et al.*, (2020)**, certain natural fillers disrupt PLA crystallinity, rendering the material more ductile at high loading levels.

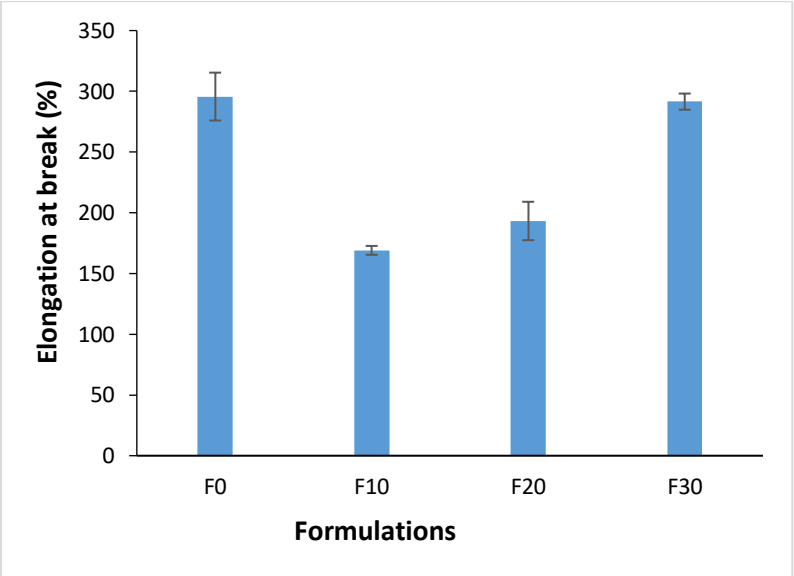

Figure 18: Evolution of the elongation at break of PLA and PLA/OPE films as a function of the orange extract content

III.2.2.3. Evolution of Young's modulus

Figure 19 illustrates the evolution of Young's modulus for PLA and PLA/OPE films as a function of orange peel extract content. The incorporation of 10% orange extract into the PLA matrix increases film stiffness from 1.3 to 2.2 GPa. This suggests that at low loading levels, orange extracts act as stiffening agents through crystalline nucleation effects and effective dispersion, promoting chemical bonding between filler and matrix that consequently enhances the modulus. This increase also aligns with findings by **Zhou *et al.*, (2020).** However, at higher loading levels (F20 and F30), the opposite effect is observed: the structure becomes overly heterogeneous, causing a loss of stiffness, as reported by **Sambudi *et al.*, (2021)**, due to discontinuities in the dispersed phase and weak interfacial interactions between PLA and the extract, which acted as a plasticizer.

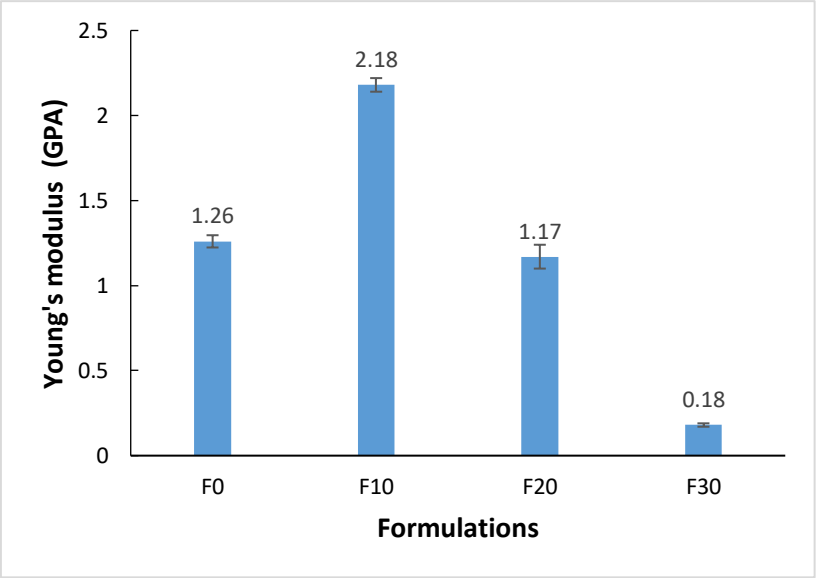

Figure 19: Evolution of the Young's modulus of PLA and PLA/OPE films as a function of the orange extract content

III.2.3. Contact angle

The micrographs taken during the contact angle analysis of our films are shown in Figure 20.

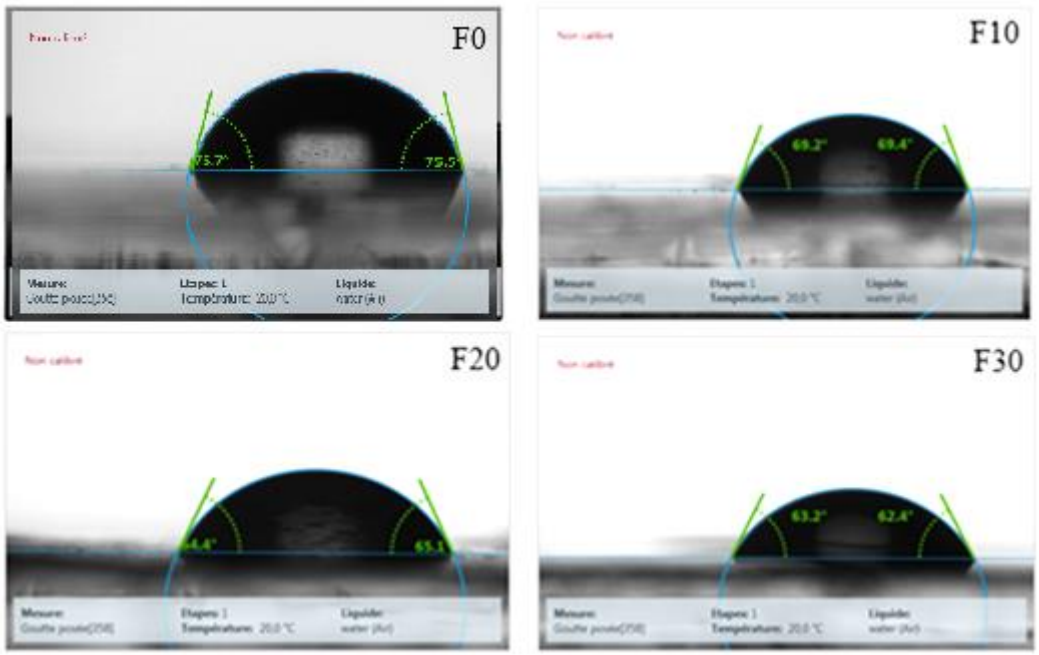

Figure 20: Micrographs showing the contact angles of the different films

Figure 20 shows the evolution of contact angles for various PLA/OPE films. The pure PLA film exhibits the highest contact angle at 75.6°, which is below 90° indicating hydrophilic (though weakly hydrophilic) behavior. This result aligns with the 70–80° range reported in literature for unmodified PLA **(As-syirazi *et al.*, 2023)**. The contact angle <90° confirms the presence of polar carbonyl groups in the polymer chain, generating attractive interactions with water molecules through hydrogen bonding **(Sivakumar, A., *et al.,* (2023)).** This relative hydrophilicity is partially explained by the presence of ester carbonyl groups (C=O) **(Shibata, M. & Inoue, Y. (2021)**. And on the other hand by the semi-crystalline nature of PLA, which features water-permeable amorphous regions unlike purely non-polar polymers such as polypropylene (PP, θ >90°) **(Mensitieri, G. *et al.*, (2020).**

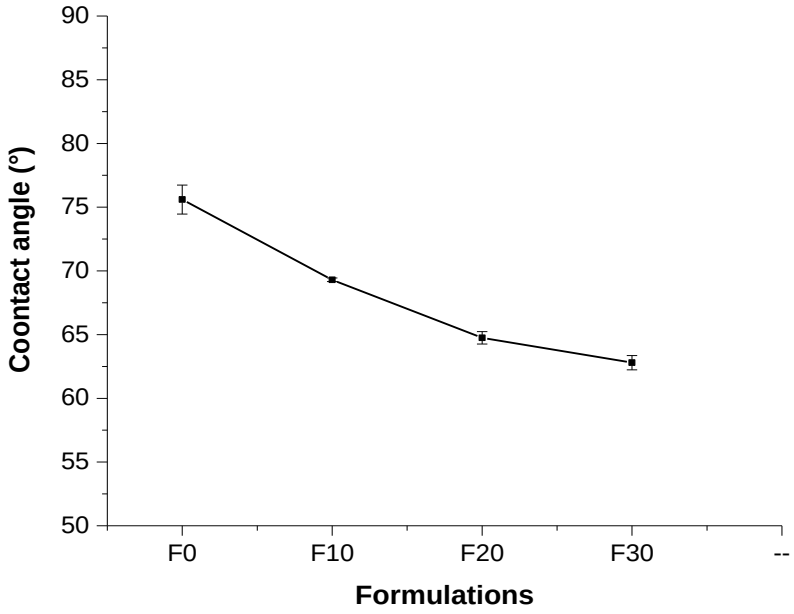

Figure 21: Contact angle of the films PLA/OPE

Conversely, the loaded films F10, F20, and F30 exhibit contact angles of 69.3°, 64.75°, and 62.8° respectively. These lower contact angles compared to F0 suggest that incorporating orange peel extract significantly modifies PLA's surface properties, enhancing its hydrophilicity **(Torres-Giner, S. *et al.,* (2023).** Droplets exhibit increased spreading, indicating a hydrophilic surface (easily wetted by water) that intensifies with higher orange peel extract content. This is entirely logical given that orange peel extract is rich in polar groups such as polyphenols, citric acids, and flavonoids enhancing the hydrophilic character of these films. This behavior aligns with similar systems reported in the literature**. (Durmus,**

***Z. et al.*, 2025, Koutoulis, A. S. *et al.*, 2024).** Other studies show that plant extracts (e.g., kenaf or rosemary) can reduce PLA's contact angle, with values ranging between 60° and 80° **(Sambudi, N. S. *et al.*, 2022).**

The close agreement between right and left angle measurements reflects surface homogeneity of the film crucial for applications requiring uniform physical properties like active packaging or medical materials. Nevertheless, the contact angle reduction corroborates the previously measured increase in water absorption.

III.2.4. Water absorption test

The results of the water absorption test are summarized in Table XII.

Table XII: Water absorption test results

Formulations	**Water absorption rate (%)**
F10	1.41 ± 0
F20	32.47 ± 1.21
F30	35.46 ± 7.25

The pure PLA matrix clearly exhibits very low water absorption approximately 1.4% over 24 hours due to its polar nature conferring hydrophobic character. While PLA is slightly polar, it is globally classified as a weakly polar polymer. This aligns with PLA's intrinsically hydrophobic nature, where low water affinity stems from its semi-crystalline polymeric structure and absence of polar functional groups **(Armentano *et al.*, 2019).** With the addition of OPE there is a strong increase, this is due to:

- The high concentration of hydroxyl groups in the orange peel extract, these groups form hydrogen bonds with water molecules. Consequently, higher extract content increases OH group concentration, resulting in significantly elevated water absorption rates **(Tawakkal *et al.*, 2020).**
- The plasticizing effect of the extract increases polymer chain mobility, creating intermolecular spaces that facilitate water diffusion **(Ferri, J. M. *et al.*, 2019).**
- Partial migration of additives into the aqueous medium generates residual micropores that amplify water molecule sorption and retention within these porous spaces **(Su *et al.*, 2021).**

III.2.5. Evaluation of bio-films anti-oxidant activity

The results of the statistical analysis shown in figure 22 demonstrate significant differences in the antiradical activity among the various biofilms.

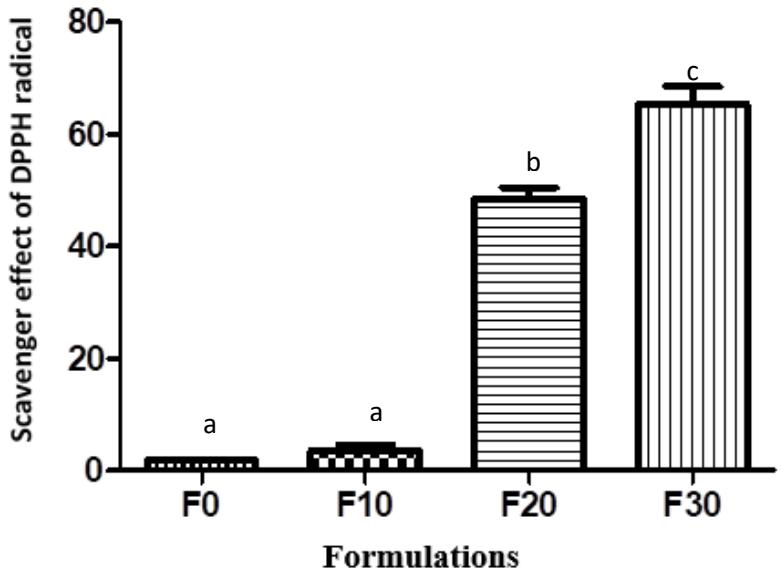

Figure 22: Scavenger effect of different films

- All values are expressed as mean ± standard deviation (SD) of three trials.
- Values designated with the same superscript letters indicate no significant difference (P > 0.05).

According to the results, there is a significant difference among the formulated biofilms, particularly biofilm F30 which exhibited the highest average DPPH radical inhibition percentage (63.84%), followed by biofilm F20 (48.5%). The weakest activity was shown by biofilms F10 and F0, which demonstrated no significant difference (P > 0.05). These observations align with several studies reporting that incorporating orange peel powder into chitosan and polyvinyl alcohol (PVA) films significantly enhances the antioxidant activity of the films. Such films display improved thermal stability and increased UV-light blocking capacity essential for food preservation **(Terzioğlu *et al.*, 2021).** Furthermore, **Yun *et al.*, (2023)** demonstrated that orange peel powder-based films exhibit antioxidant activity comparable to, if not superior than, other active films made from fruit waste (e.g., mandarin-based films), which were also reported to effectively inhibit corn oil oxidation, highlighting the importance of bioactive components in food packaging.

Orange peel-enriched food packaging films show promise for preserving various food products including fruits, vegetables, and baked goods. Their ability to release

antioxidants and inhibit microbial growth makes them particularly suitable for extending food freshness and safety (**Siripatrawan & Harte, 2010; Wang *et al.*, 2019).**

III.2.6. Evaluation of anti-bacterial activity of the bio-films

The varied results summarized in Figures 23 and 24 demonstrate the effect of the produced biofilms on bacterial growth against *E. coli* and *S. aureus*, respectively.

The results showed that all tested biofilm formulations were found to be active against both bacterial strains. Statistical analysis revealed a significant difference in inhibition rates depending on biofilm type and the tested bacterial strain.

We observed a highly significant elevation ($p < 0.001$) in inhibition rate (Figure 23) for *E. coli* treated with F30 and F20 biofilms (80.34% and 75.78%, respectively). The F10 biofilm demonstrated weaker antibacterial effects with a 32.24% inhibition rate, though still significantly higher than F0 ($p < 0.001$).

Regarding *S. aureus*, biofilms F10 and F0 exhibited similar antibacterial effects ($P > 0.05$). Furthermore, we observed enhanced antibacterial activity in F30 and F20 formulations with inhibition rates exceeding 70% (Figure 24).

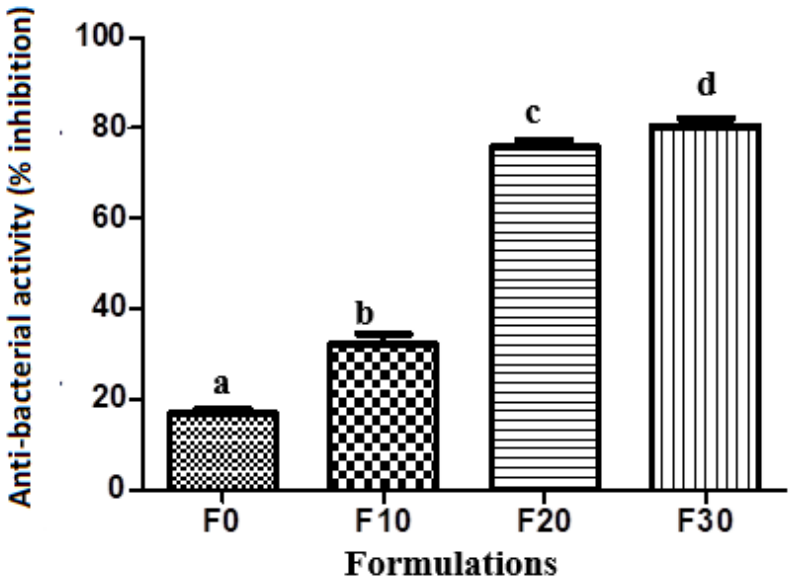

Figure 23: Anti-bacterial activity of biofilms against *E. coli*

- *All values are expressed as the mean of three trials with ± standard deviation.*
- *Values with the same letters indicate that the difference is not significant (P>0.05).*

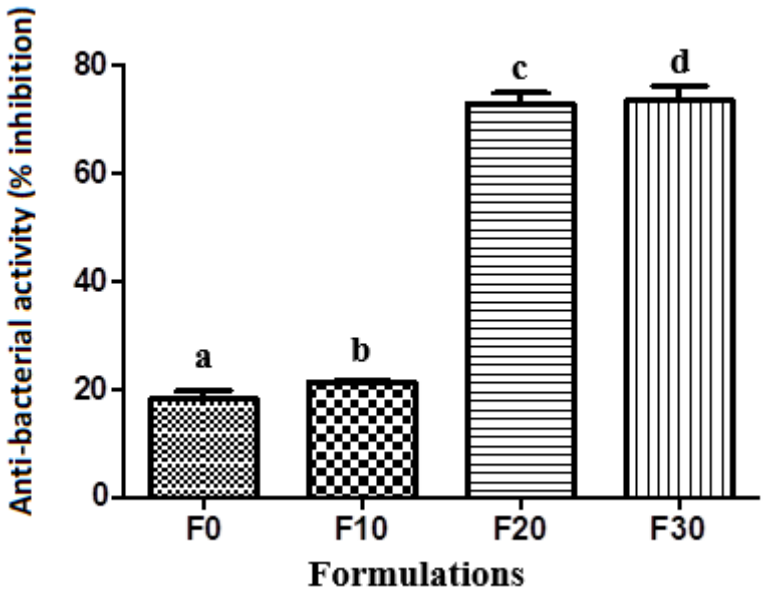

Figure 24: Anti-bacterial activity of biofilms against *S.aureus*

-All values are expressed as the mean of three trials with ± standard deviation.
-Values with the same letters indicate that the difference is not significant (P>0.05).

In this study, the antibacterial activity of biofilms against *E. coli* and *S. aureus* strains - particularly for the F30 and F20 formulations which proved most effective. Could be linked to their high concentrations of total phenolic compounds and flavonoids. These formulations may contain optimal concentrations of antibacterial agents or plant extracts that act synergistically to inhibit bacterial growth.

Comparing the antibacterial activity results of the OPE extract and the elaborated biofilms (specifically F30 and F20), the findings align with prior studies. The enhanced antibacterial activity observed in plant extract-based food packaging films compared to the extract alone can be explained by multiple mechanisms and factors related to film formulation and structure.

Food packaging films frequently incorporate multiple ingredients, including biopolymers like PLA which possess intrinsic antibacterial properties. When plant extracts are added to these matrices, they can interact synergistically with film components, thus enhancing the overall antimicrobial effect **(Shao *et al.*, 2022; Bartošová *et al.*, 2025).**

Conclusion

Conclusion

This study successfully addressed the crucial challenge of developing sustainable and functional food packaging by utilising agri-food by-products, particularly orange peel waste, as a bioactive filler in a polylactic acid (PLA) matrix. The main conclusions are:

Ethanolic extraction of orange peel waste yielded an extract rich in bioactive substances, with high levels of phenolic compounds, flavonoids and condensed tannins, demonstrating powerful antibacterial activity against the strains tested and DPPH radical scavenging antioxidant activity of up to 91%, with an IC50 of 165.12 µg/ml.

The incorporation of OPE into PLA matrices conferred antioxidant and antibacterial properties to the composite films, with films containing 30% OPE (F30) achieving high DPPH radical inhibition and inhibiting the growth of *E. Coli* and *S. aureus,* respectively, thanks to the synergistic effects of the PLA barrier and the bioactive materials in OPE. FTIR analysis confirmed the structure of the orange peel extract and the PLA matrix and an increase in interactions through hydrogen bonds between the filler and the matrix. Meanwhile, the tensile test revealed a decrease in tensile strength due to interfacial incompatibility, but an increase in elongation at break depending on the loading rate, indicating plasticization that induces a decrease in stiffness. The hydrophilicity of the films also increased with the loading rate, confirmed by contact angle analyses of the films, which decreased from 75.6° to 62.8° and water absorption from 1.41% to 35.46% for formulations F0 and F30, respectively.

This research demonstrates that orange peel waste can be used as a multifunctional filler for active packaging, and that the PLA/OPE films developed offer a double advantage in terms of the environment and preservation. This approach is in line with the principles of the circular economy, reducing greenhouse gas emissions and resource consumption. The antioxidant and antibacterial properties incorporated into the films mitigate the deterioration and microbial contamination of perishable goods, directly combating food waste while extending the shelf life of products.

Despite promising results, several challenges must be addressed to advance this topic. The decrease in tensile strength at high OPE loads requires interfacial optimisation strategies, such as compatibilisers (e.g. PLA grafted with maleic anhydride) or nano-reinforcements (e.g. cellulose nanocrystals), in order to balance mechanical integrity and flexibility.

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Annexes

Annexes

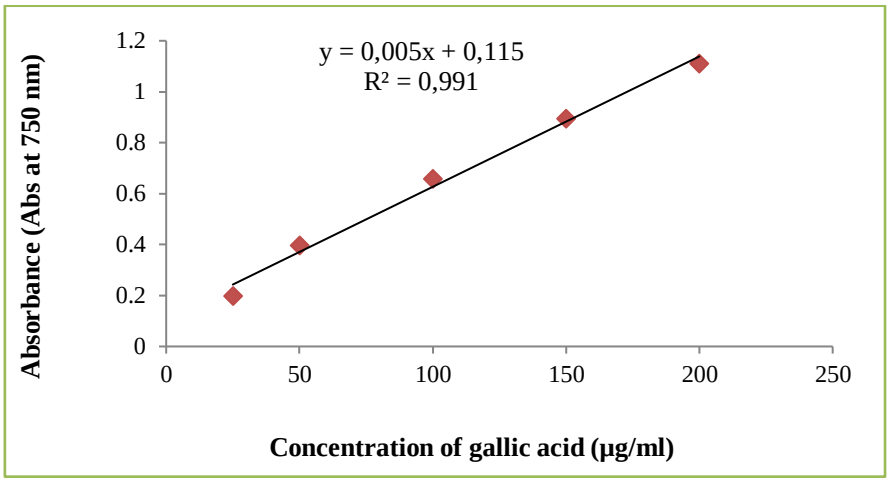

Figure 1: Calibration curve of total phenols

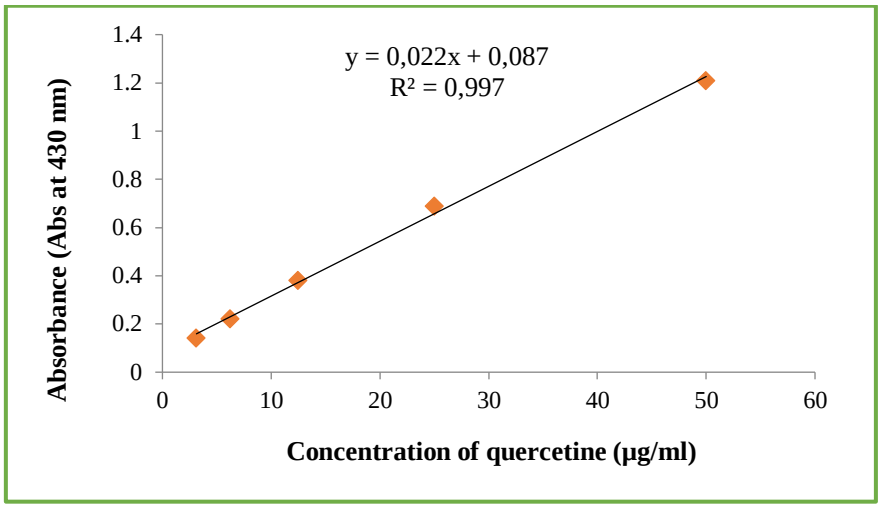

Figure 2: Calibration curve of total flavonoïds

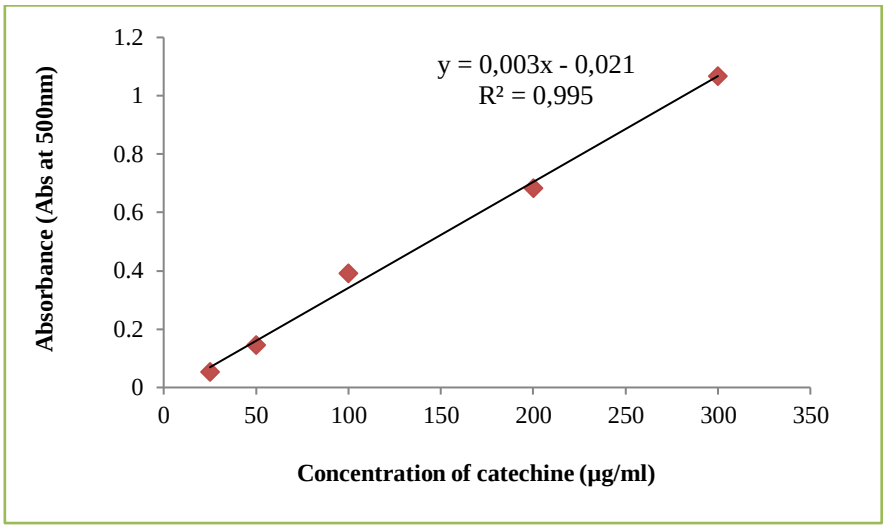

Figure 3: Calibration curve of condensed tanins

Abstract

This study addresses the urgent need for sustainable food packaging by valorizing orange peel waste an abundant agri-food by-product through its integration into polylactic acid (PLA) matrices. Orange peel extract (OPE), rich in phenolic compounds (281.76 mg GAE/g DW), flavonoids (34.75 mg QE/g DW), and condensed tannins (359.98 mg CE/g DW), was incorporated into PLA at 10%, 20%, and 30% loadings to fabricate biocomposite films. The OPE demonstrated potent bioactivity, with high antioxidant capacity (IC_{50}: 165.12 µg/mL) and significant antibacterial effects against *E. coli* (IC_{50}: 0.55 mg/mL) and *S. aureus.* Films with 30% OPE exhibited enhanced functionality, achieving 63.84% DPPH radical inhibition and >70% bacterial growth suppression. Physicochemical characterization revealed increased hydrophilicity (contact angle: 62.8°; water absorption: 35.46%) and altered mechanical properties (tensile strength reduction but improved ductility at higher loadings). FTIR confirmed intermolecular interactions between PLA and OPE polysaccharides. These results validate OPE as a multifunctional filler for active packaging, extending food shelf life while advancing circular economy principles. The research bridges waste valorization with material science, offering an eco-friendly alternative to conventional plastics for perishable food preservation.

Keywords : Polylactic acid (PLA), Orange peel extract, Biocomposite films, Antioxidant activity, Antibacterial properties, Active packaging.

Résumé

Cette étude répond au besoin urgent de mettre au point des emballages alimentaires durables en valorisant les déchets d'écorces d'orange, un sous-produit agroalimentaire abondant, grâce à leur intégration dans des matrices d'acide polylactique (PLA). L'extrait d'écorce d'orange (OPE), riche en composés phénoliques (281,76 mg GAE/g DW), en flavonoïdes (34,75 mg QE/g DW) et en tanins condensés (359,98 mg CE/g DW), a été incorporé dans le PLA à des teneurs de 10 %, 20 % et 30 % pour fabriquer des films biocomposites. L'OPE a démontré une bioactivité puissante, avec une capacité antioxydante élevée (IC_{50} : 165,12 µg/mL) et des effets antibactériens significatifs contre E. coli (IC_{50} : 0,55 mg/mL) et S. aureus. Les films contenant 30 % d'OPE ont présenté une fonctionnalité améliorée, atteignant une inhibition des radicaux DPPH de 63,84 % et une suppression de la croissance bactérienne supérieure à 70 %. La caractérisation physico-chimique a révélé une hydrophilie accrue (angle de contact : 62,8° ; absorption d'eau : 35,46 %) et des propriétés mécaniques modifiées (réduction de la résistance à la traction mais amélioration de la ductilité à des charges plus élevées). La FTIR a confirmé les interactions intermoléculaires entre le PLA et les polysaccharides OPE. Ces résultats valident l'OPE comme charge multifonctionnelle pour les emballages actifs, prolongeant la durée de conservation des aliments tout en faisant progresser les principes de l'économie circulaire. La recherche fait le lien entre la valorisation des déchets et la science des matériaux, offrant une alternative écologique aux plastiques conventionnels pour la conservation des denrées périssables.

Mots clés : Poly (acidelactique (PLA), extrait d'écorce d'orange, films biocomposites, activité antioxydante, propriétés antibactériennes, emballage actif.