UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE CIENCIAS QUÍMICAS

TESIS DOCTORAL

Desarrollo de envases basados en polímeros biodegradables con actividad antimicrobiana

Development of biodegradable polymer-based packaging with antimicrobial activity

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Ignacio Mena Prado

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Alexandra Muñoz Bonilla
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“An idea is like a virus. Resilient.
Highly contagious.
And the smallest seed of an idea can grow.
It can grow to define or destroy you.”

- Dom Cobb, Inception.

“If you want a better answer, ask a better question”

- Dr. Gregory House

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Abstract.

Plastics currently constitute the most widely used family of materials worldwide. Their low production costs, suitable physicochemical properties, great versatility, and broad availability have ensured their fundamental role in various industrial sectors for decades. However, the life cycle of these materials entails significant environmental impacts, mainly because most are derived from non-renewable fossil resources, such as petroleum, and exhibit a considerable carbon footprint that contributes to climate change. In addition, low recycling rates lead to the accumulation of plastic waste in landfills for long periods or their incineration, thereby increasing pollutant emissions.

Within this framework, food packaging acquires particular relevance, as it represents one of the main applications of plastics and accounts for approximately 40% of total consumption. This situation poses a dual challenge for the food industry. On the one hand, the massive generation of plastic waste from packaging represents a serious threat to ecosystems, due to its persistence in the environment and the difficulties associated with its management. On the other hand, packaging plays an essential role in food preservation and safety, ensuring that products reach consumers in optimal conditions of quality and safety and reducing food waste.

Considering this scenario, the concept of active packaging gains importance, understood as packaging that, beyond containing food, incorporates compounds capable of interacting with it or with the surrounding environment in order to extend its shelf life and enhance its safety. Nevertheless, the development of such technologies requires materials that are not only functional but also environmentally sustainable.

In this regard, bioplastics emerge as a promising alternative, characterized by their renewable origin, biodegradability, or both, while maintaining performance comparable to conventional plastics. This thesis is based on the premise that replacing fossil-based plastics with bio-based polymers originate from renewable raw materials and/or compostable materials, designed to exert antimicrobial activity against foodborne pathogens and prolong the shelf life of

packaged foods, represents a key strategy to mitigate environmental impact and improve food safety in the packaging sector.

This work focuses on the development of active antimicrobial packaging systems, based on compostable polymeric matrices, in particular polylactic acid (PLA) and Ecovio® (a commercial blend of PLA and poly (butylene adipate-*co*-terephthalate), PBAT), combined with food-grade plasticizers and bioactive additives approved by regulatory bodies such as the FDA and EFSA. Novel bioplasticizers, such as esters derived from citric acid (CITREM, E472c) and acetic acid (ACETEM, E472a), were evaluated, along with antimicrobial and antioxidant agents extracted from food industry by-products, including sodium hexametaphosphate particles (E452i), sodium stearoyl lactylate (SL, E481), and chitin particles (CP). These bioactive compounds were incorporated into the polymer matrices using different processing techniques, including compression moulding, calendering, and forcespinning, with the aim of optimizing antimicrobial activity against microorganisms, primarily Gram-positive bacteria such as *Staphylococcus aureus* and *Listeria innocua*.

Furthermore, the influence of these additives on the mechanical, thermal, and barrier properties of the polymers were assessed, as well as the potential migration of compounds into food, ensuring that they remained within the limits established by current legislation and that the biopolymers retained properties such as industrial compostability.

The results demonstrate that the synergistic combination of compostable biopolymers with antimicrobial additives enables the formulation of functional packaging materials capable of extending shelf life, reducing food waste, and replacing conventional polymers in food-contact applications. This work contributes to the advancement of the field of sustainable materials for active packaging, offering a viable and scalable alternative that combines technological performance, food safety, and environmental sustainability.

Resumen.

Los plásticos constituyen actualmente la familia de materiales más utilizada a nivel global. Sus bajos costes de producción, sus adecuadas propiedades fisicoquímicas, su gran versatilidad y su amplia disponibilidad han asegurado su papel fundamental en diversos sectores industriales durante décadas. Sin embargo, el ciclo de vida de estos materiales conlleva importantes impactos ambientales, principalmente porque la mayoría se obtiene a partir de recursos fósiles no renovables, como el petróleo, y presentan una huella de carbono significativa que contribuye al cambio climático. Además, las bajas tasas de reciclaje provocan que los residuos plásticos se acumulen en vertederos durante largos períodos o sean incinerados, incrementando así las emisiones contaminantes.

En este marco, el envasado de alimentos adquiere especial relevancia, ya que constituye una de las principales aplicaciones del plástico y representa aproximadamente el 40% de su consumo total. Este hecho plantea un doble desafío para la industria alimentaria. Por un lado, la generación masiva de residuos plásticos derivados de los envases representa una seria amenaza para los ecosistemas, debido a su persistencia en el medio ambiente y a las dificultades asociadas a su gestión. Por otro lado, el envasado desempeña un papel esencial en la conservación y seguridad de los alimentos, garantizando que los productos lleguen al consumidor en condiciones óptimas de calidad e inocuidad, y reduciendo el desperdicio de alimentos.

Ante este panorama, cobra importancia el concepto de envasado activo, entendido como aquel que, más allá de contener el alimento, incorpora compuestos capaces de interactuar con él o con el entorno para prolongar su vida útil y mejorar su seguridad. No obstante, el desarrollo de este tipo de tecnologías requiere materiales que, además de funcionales, sean ambientalmente sostenibles.

En este sentido, los bioplásticos se perfilan como una alternativa prometedora, al estar caracterizados por su origen renovable, su biodegradabilidad o ambas condiciones, manteniendo al mismo tiempo prestaciones similares a los plásticos convencionales. La presente tesis parte de la premisa de que reemplazar los

plásticos fósiles por materiales biodegradables y/o compostables, diseñados, además, para proporcionar una actividad antimicrobiana frente a patógenos alimentarios y prolongar la vida útil de los alimentos envasados, representa una estrategia clave para mitigar el impacto ambiental y mejorar la seguridad alimentaria en el sector del envasado.

El trabajo se centra en el desarrollo de sistemas de envasado activos antimicrobianos, basados en matrices poliméricas compostables, en particular ácido poliláctico (PLA) y Ecovio® (mezcla comercial de PLA y poli (butilen adipato-*co*-tereftalato), PBAT), combinadas con plastificantes y aditivos bioactivos de grado alimentario, aprobados por organismos regulatorios como la FDA y la EFSA. Se evaluaron nuevos bioplastificantes, como ésteres derivados de ácidos cítrico (CITREM, E472c) y acético (ACETEM, E472a), junto con agentes antimicrobianos y antioxidantes extraídos de subproductos de la industria alimentaria, tales como partículas de hexametafosfato de sodio (E452i), estearoil lactilato de sodio (SL, E481) y partículas de quitina (CP). Estos compuestos bioactivos se incorporaron a las matrices poliméricas mediante diferentes técnicas de procesado, incluyendo moldeo por compresión, calandrado y forcespinning, buscando optimizar la actividad antimicrobiana frente a microorganismos, principalmente bacterias Gram-positivas como *Staphylococcus aureus* y *Listeria innocua*.

Asimismo, se evaluó la influencia de estos aditivos en las propiedades mecánicas, térmicas y de barrera de los polímeros, así como la posible migración de compuestos hacia los alimentos, garantizando que se mantuviera dentro de los límites establecidos por la legislación vigente y que los biopolímeros conservaran características como la compostabilidad industrial.

Los resultados evidencian que la combinación sinérgica de biopolímeros compostables con aditivos alimentarios antimicrobianos posibilita la formulación de materiales de envasado funcionales capaces de extender la vida útil, reducir el desperdicio alimentario y sustituir polímeros convencionales en aplicaciones de contacto con alimentos. Este trabajo contribuye al avance del campo de los materiales sostenibles para envasado activo, ofreciendo una alternativa viable y escalable que combina desempeño tecnológico, seguridad alimentaria y sostenibilidad ambiental.

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List of abbreviations and nomenclature.

Polymers.

Bio- PE: Bio-based polyethylene

Bio-PP: Bio-based polypropylene

Bio-PET: Bio-based polyethylene terephthalate

EC: Ethyl cellulose

Ecovio®: PLA/PBAT blend

HDPE: High-density polyethylene

LDPE: Low-density polyethylene

PA: Polyamide

PBAT: Poly (butylene adipate-*co*-terephthalate)

PBS: Polybutylene succinate

PC: Polycarbonate

PCL: Polycaprolactone

PE: Polyethylene

PET: Poly (ethylene terephthalate)

PHA: Poly (hydroxyalkanoates)

PHB: Polyhydroxybutyrate

PHBV: Poly (hydroxybutyrate valerate)

PLA: Poly (lactic acid)

PLLA: Poly (L-lactic acid)

PP: Polypropylene

PS: Polystyrene

PVA: Polyvinyl alcohol

PVC: Poly (vinyl chloride)

TPS: Thermoplastic starch

Plasticizers.

ACETEM: Acetic acid esters of mono-and diglycerides

ACO: Acetylated castor oil

ASLP: Agave sisalana leaves

ATBC: Acetyl citrate tributyl

ATEC: Acetyl triethyl citrate

BCO: Benzoyl castor oil

BPA: 2,2-bis(4-hydroxyphenyl) or bisphenol A

CITREM: Citric acid esters of mono-and diglycerides

DBP: Dibutyl phthalate

DEHP: Di(2-ethylhexyl) phthalate

DOTP: Dioctylterephthalate

EACO: Epoxy acetylated castor oil

EBCO: Epoxy benzoyl castor oil

ECO: Epoxy castor oil

Eos: Essential oils

ESFO: Epoxidized Safflower oil

ESO: Epoxidized soybean oil

FAEs: Fatty acid esters

GTA: Glycerol triacetate

OLA: Oligomers from lactic acid

OLA (MB): Masterbach of oligomers from lactic acid

OTS: Oligo(trimethylene sebacate)

PEG: Polyethylene glycol

SFO: Safflower oil

TEC: Triethyl citrate

TBC: Tributyl citrate

TDI: Toluene diisocyanate

Additives.

CP: Chitin particles

E452i: Grinded sodium hexametaphosphate microparticles

SL: Sodium stearoyl lactylate (designated as E481)

Techniques.

ATR: Attenuated Total Reflection

DSC: Differential Scanning Calorimetry

DTG/DTGA: First derivative of the TGA curves

FE-SEM: Field Emission Scanning Electron Microscopy

FTIR: Fourier Transform Infrared spectroscopy

GC-MS: Gas chromatography–mass spectrometry

MH: Microhardness

OTR: Oxygen Transmission Rate

SEM: Scanning Electron Microscopy

TGA: Thermogravimetric Analysis

WAXS: Wide-Angle X-ray Scattering

WVTR: Water Vapour Transmission Rate

XRD: X-ray Diffraction

Parameters:

Antioxidant.

A_m: Absorbance value of the sample solution

A_0: Absorbance value of the solution without the sample

CTE: Trolox equivalent coefficient

RSA: Reduction scavenging activity

TEAC: Trolox equivalent antioxidant capacity

DSC.

ΔH_{cc}: Cold crystallization enthalpy

ΔH_m: Melting enthalpy

ΔH_m^0: Melting enthalpy for a 100% crystalline

T_g: Glass transition temperature

T_m: Melting temperature

T_{cc}: Crystallization temperature

Wf: Weight fraction

X_c: Crystallinity degree

Hemolysis.

A_S: measured absorbance of the sample,

A_{nc}: absorbance of the negative control

A_{pc}: absorbance of the positive control.

Mechanical properties.

ε: Elongation at break

E: Young modulus

Rm: Maximum tensile strength

Permeability.

KP_{WV}: water vapour permeability, normalized for the thickness

KP_{O2}: oxygen permeability, normalized for the thickness

Log WVP: Logarithm water vapour permeability

Log O_2P: Logarithm oxygen vapour permeability

TGA.

T_{d5}: Temperature at 5% of mass lose

T_{d10}: Temperature at 10% of mass lose

T_{d50}: Temperature at 50% of mass lose

T_{dmax}: Temperature at the maximum degradation rate

Bacterial strains.

CFU: Colony-forming units

E. coli: Escherichia coli

L. acidophilus: Lactobacillus acidophilus

S. aureus: Staphilococcus aureus

L. innocua: Listeria innocua

L. sakei: Lactobacillus sakei

%RCFU: reduction percentage of bacterial CFU

Others.

ASTM: American Society for Testing and Materials

ATCC: American Type Culture Collection

CAGR: Compound annual growth rate

CLSI: Clinical Laboratory Standards Institute

DCM: Dichloromethane

DNA: Deoxyribonucleic Acid

DPPH: 2,2-diphenyl-1-picrylhydrazyl free radical

EFSA: European Food Safety Authority

EU: European Union

FDA: Food and Drugs Administration

FPR: Fertilising Products Regulation

GRAS: Generally Recognized As Safe

LCA: Life cycle assessments

MIC: Minimum inhibitory concentration

NADH: Nicotinamide adenine dinucleotide

PBS: Phosphate-buffered saline

PPWR: Packaging and Packaging Waste Regulation

RBCs: Fresh human red blood cells

RFID: Radio frequency identification

RH: Relative Humidity

SML: Specific migration limit

TSA: Tryptic soy agar

UV: Ultra-violet

WFD: EU Waste Framework Directive

CHAPTER 1

State of the art: Literature review.

General introduction.

Globalization has brought new challenges to the food industry, increasing both customer expectations and the requirements for products in the global market [1]. Plastic bags and packages have proliferated around the world [2]. Packaging plays a critical role in addressing these demands. Almost everyone deals with foods packaged in plastic containers on a daily basis [3]. Advancements in travel, transportation, and information systems have enabled people to obtain foods from distant parts of the world. As food suppliers and sellers have expanded their markets, consumer demand for diverse food options has grown [4]. Today, having access to a wide variety of global foods is seen as a standard convenience rather than a luxury [5], [6].

Food packaging plays a vital role in ensuring the safety, quality, and appeal of food products throughout their lifecycle from production to consumption. Effective packaging safeguards food from the loss of essential nutrients, functional properties, colour, aroma, and taste, while maintaining the visual appeal expected by consumers. To achieve this, packaging must serve as a barrier against environmental factors, particularly water vapour, oxygen, and microorganisms, which can significantly affect the shelf life and safety of food [7], [8] (**Figure C1. A0. 1.**). In addition to protection, packaging must allow for the convenient transport of food products, helping to preserve their integrity during storage, shipping, and handling [9], [10]. Modern packaging also fulfils an informational role, clearly communicating important details to consumers such as nutritional content, ingredients, expiry dates, and proper disposal or recycling instructions [11], moreover traceability systems help to improve safety and control food diseases [12], [13]. This transparency builds consumer trust and supports more sustainable consumption practices [14].

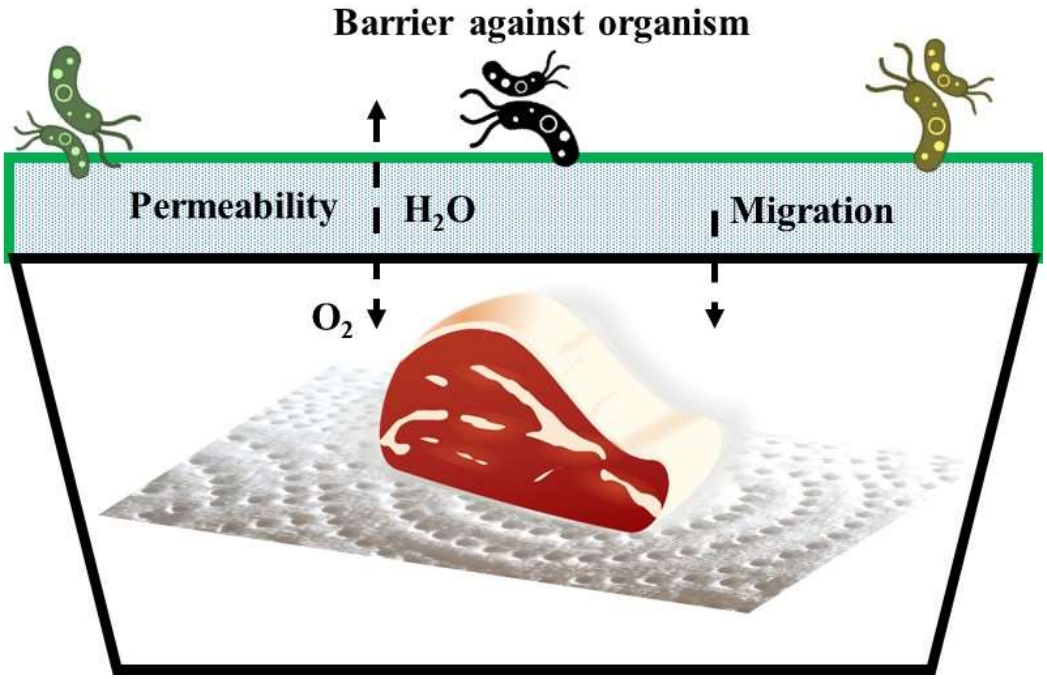

Figure C1. A0. 1. Schematic image of migration and permeability in packaging systems.

Packaging efficiency is the product of the combination of design and material selection, among the different materials employed in food packaging [11], the main properties attributed to polymers are their low price, durability and strength. Moreover, other properties such barrier properties against vapour or oxygen are fundamental to maintain the freshness, and quality of the products, extending the shelf life of the product and reducing food waste; easy processability and formability, which lead to the production of products with unlimited sizes and shape; optical properties, thermosealability and the ability to be produced in great scale [15]–[18].

Additionally, their reduced weight and ease of shaping contribute to lower CO_2 emissions during transport, and their energy-efficient manufacturing makes polymers an economically and environmentally attractive choice [19]. Polymers can also be tailored to interact with food, helping to extend shelf life by maintaining specific atmospheres inside the packaging or acting against food pathogens. However, these interactions must be carefully studied, as substance migration

between packaging and food can occur, raising safety and toxicity concerns [20], [21].

Plastic from the packaging industry accounts for ~40% of European total production [22]–[24] making it the largest area of plastic demand (**Figure C1. A0. 2**). Indeed, packaging contributes significantly in landfill waste because it is widely used and typically has a short life, particularly in the food and consumer products industry. Moreover, plastic degradation due to heat, mechanical forces or chemical processes can lead into their fragmentation into microplastics potentially contaminating landfills, oceans and natural environments [25]–[28].

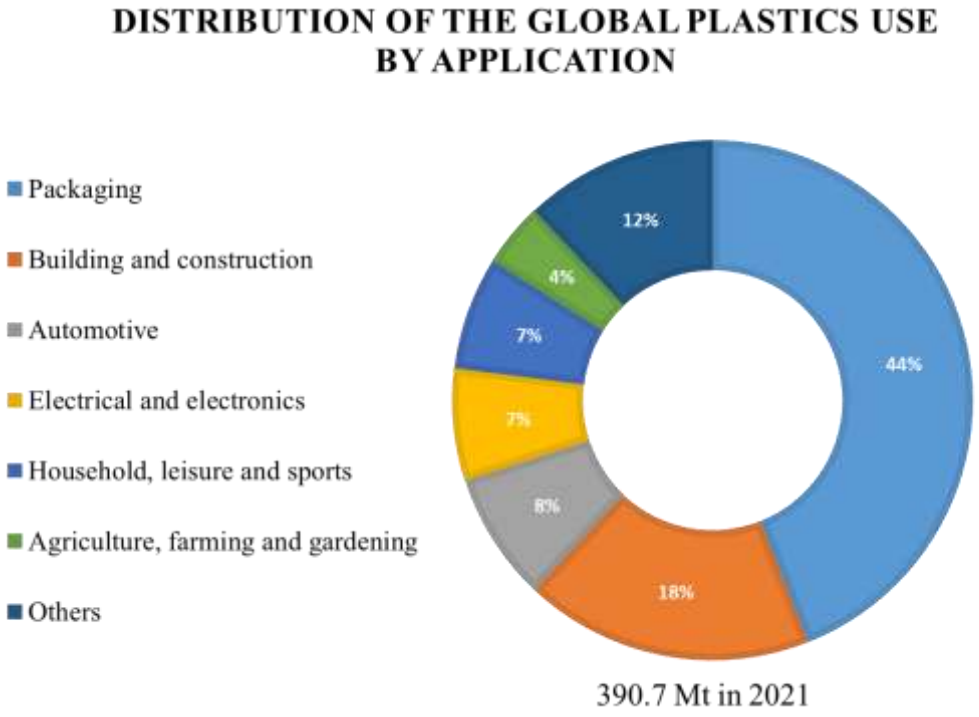

Figure C1. A0. 2. Overview of plastics employed in different applications based on Plastic Europe-THE FACTS 2022 (Europe) (https://plasticseurope.org/knowledge-hub/plastics-the-fast-facts-2022-2/).

1. Shelf life of the product and packaging systems.

Approximately 33% of global food production is estimated to be lost or wasted and therefore, the design of effective packaging would contribute to increase the shelf life of the products and substantially prevent food losses and waste. Shelf life refers to the period during which a food product meets safety, nutritional, regulatory, and

sensory standards under specific storage conditions. It is divided into primary shelf life (while the food is still packaged) and secondary shelf life (after the package is opened). The main focus of shelf life is to ensure the food remains acceptable to consumers before and after consumption [29].

The use of appropriate packaging materials and methods to reduce food losses and ensure product safety has driven the development of new packaging technologies that offer higher quality, safer, and healthier foods [30], [31]. Food stored under packaging conditions undergoes changes over time, affecting both its quality and the surrounding environment. Technologies such as intelligent packaging (**Figure C1. A0. 3.**) designed to monitor the condition of the food packaged use biosensors (such us natural dries) or hardware components like time-temperature indicators, gas sensors, freshness or ripeness indicators, and radio frequency identification (RFID) systems. These materials are used to detect the presence of a key compound or a group of compounds that indicate changes in pH, gas composition, temperature or spoilage compounds. Besides, active packaging technology involves embedding additives in the packaging material that can release or absorb substances from the food or its environment to maintain quality and extend shelf life, examples such as components that provide antioxidant or antimicrobial properties or oxygen scavengers among other materials are frequently used for that application. Furthermore, and as mentioned by Vandervoost et al. [32], smart packaging offers a comprehensive solution that both monitors changes in the product or environment (intelligent) and produces a respond to the changes (active) working synergistically [32]–[34].

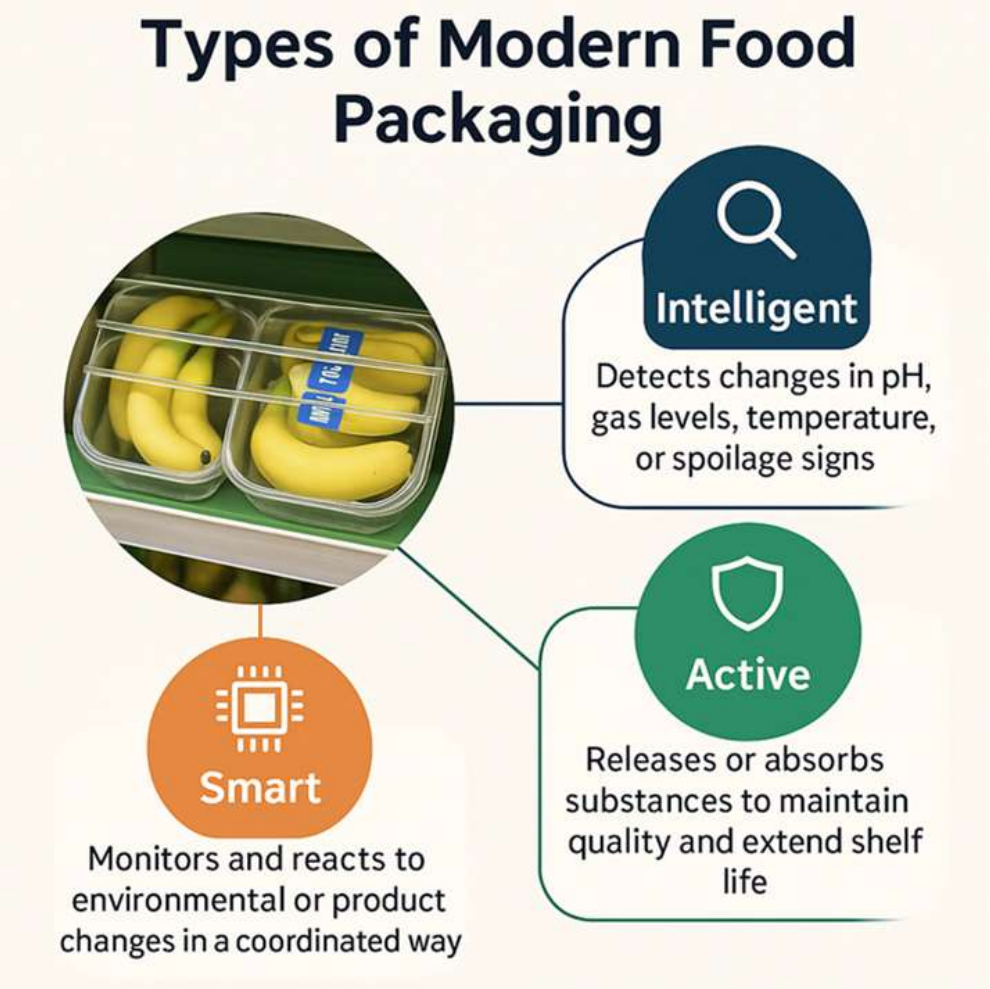

Figure C1. A0. 3. Intelligent, active and smart packaging systems.

2. Polymers used in packaging.

Polymers can be categorized based on their physical properties or molecular structure in thermoset and thermoplastics. Thermosets are a set of polymers which cannot be remoulded when the structure of the polymer chain solidifies from melting. Thermosets present strong bonds between the chains conferring resistance and durability to the material, commonly are used in construction or automobile applications. On the other side, thermoplastics are polymers in which the interactions between the chains are weaker, then, the materials are softer and can be remoulded when its exposure to heat. In fact, the easily processability of these polymers open a wide range of application for these materials, including packaging field [15].

Specifically, the food contact industry operates under very strict standards, requiring that all plastics used ensure product safety. Certifications such as Generally Recognized As Safe (GRAS) are commonly adopted for materials intended for this purpose. Among all the polymers used in packaging, many classifications can be made, although one of the most common ways to classify them is based on their origin and degradability. Therefore, conventional polymers, which have high resistance to degradation, are distinguished from biopolymers, which are those that degrade more easily.

2.1. Conventional polymers.

Conventional polymers, derived from petroleum-based resources, are widely used due to their high durability, favourable mechanical properties, and low production cost. According to Plastics Europe, petroleum-based polymers such as polyethylene terephthalate (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC), low-density polyethylene (LDPE), polypropylene (PP), and polystyrene (PS) are prominently utilized across various applications.

Conventional polymers have reigned in different applications, in particular, in food packaging application, materials as polyethylene (PE), PP, PET and PS are the primary demand from this industry [28]. However, their resistance to degradation and large-scale production contribute to significant environmental concerns, including resource depletion and the accumulation of plastic waste [17], [35]. Exists different techniques to eliminate plastics, the most common is the incineration, in which the plastic is decomposed by increasing the temperature. The main disadvantage of that method is the generation of carbon dioxide and other toxic compounds into the atmosphere. Furthermore, mechanical recycling is another option but heterogeneity and contamination of plastic waste difficulty the complete separation [22], [36]. Throughout the years, efforts have been undertaken over time on two fronts: recycling existing plastic products and developing new sustainable materials to supplant conventional polymers. The problems attributed to the accumulation of waste in the landfills and other problems associated to the plastics, such as microplastics in the oceans or leakage of toxic chemicals [24], [37], have

prompted numerous studies focused on developing new green solutions, improving the biodegradability of polymers or recyclability at the end of their life cycle [38].

2.2 Biopolymers.

The term 'biopolymer' is ambiguous; however, is primarily employed to describe polymers that can be either bio-based, biodegradable, or both (**Figure C1. A0. 4.**) [39], [40].

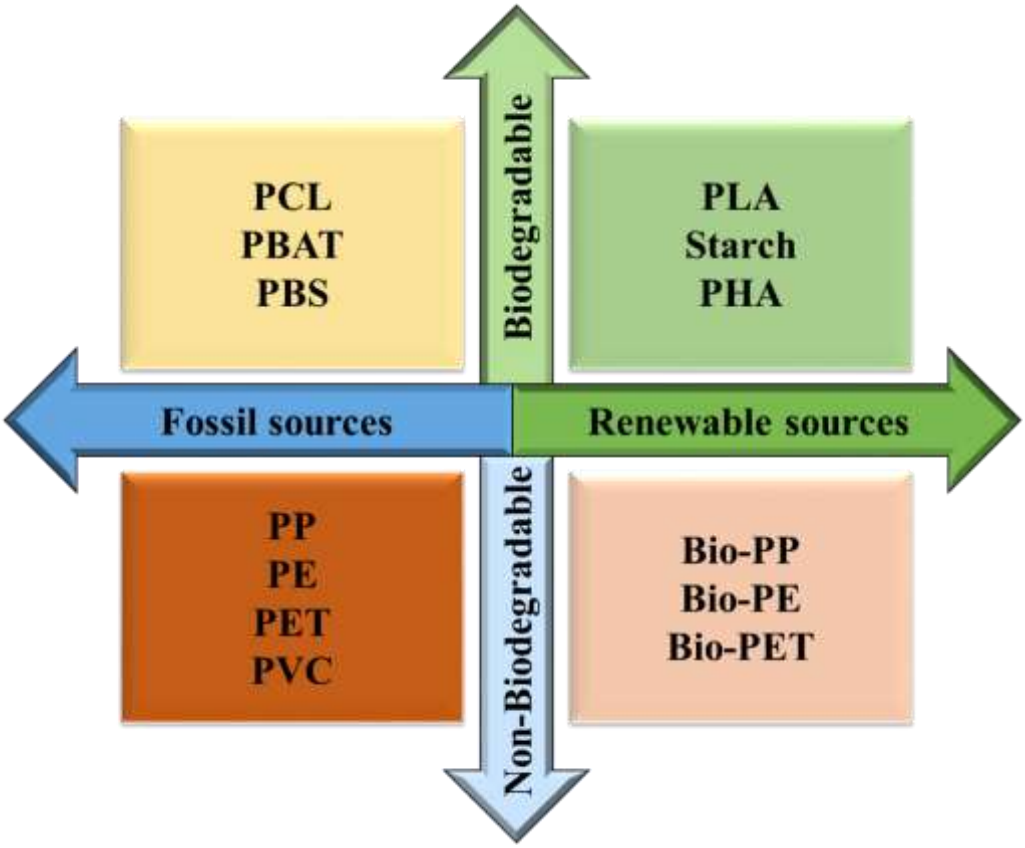

Figure C1. A0. 4. Classification of polymers based on the obtained sources and degradation process.

While biodegradability is commonly linked with bio-based materials, it is not contingent upon the polymer's origin, but rather solely on its chemical composition [17], biodegradable polymers are those that can decompose into CO_2, CH_4, H_2O, inorganic compounds, or biomass mainly through the enzymatic action of microorganisms [23], [40]. To substitute the conventional polymers, biopolymers must match their properties, also they must be more energy-efficient while

producing [41]. In the pursuit of reducing reliance on conventional plastics, manufacturers are increasingly prioritizing the large-scale production and integration of biopolymers. These materials are often compatible with existing recycling infrastructure and provide the added benefit of biodegradability at the end of their lifecycle [42]. In 2023, global bioplastics production reached 2.019 million tonnes, representing only 0.7% of the total global plastic production, which amounted to 413.8 million tonnes as reported by Plastics Europe *the Facts 2024*. According to European Bioplastics, the bioplastics market is projected to grow significantly, with a continuous annual growth rate that forecasts a production volume of 5.73 million tonnes by 2029. Notably, within this segment, biodegradable plastics are expected to experience the most substantial growth, eventually comprising the majority of biopolymer production (**Figure C1. A0. 5.**). Europe has positioned itself in the transition toward a sustainable bioeconomy, particularly in the development and integration of biopolymers. The European Union has adopted a strategic and institutionalized approach, supported by key policy frameworks like the 2018 Bioeconomy Strategy and the European Green Deal. These initiatives have established clear regulatory standards, public funding for innovation, and strong collaborations, all of which are accelerating the growth of the bioplastics and biopolymers industries. Looking ahead, Europe aims to scale up biopolymer production through investments in research, technological innovation, and circular economy integration. It also seeks to lead in setting international standards for biopolymers to prevent greenwashing and promote global trade [43]–[45].

Biopolymers are gaining attention for their environmental advantages, primarily because they are derived from renewable sources. However, despite their potential, the industry faces notable challenges. These include higher production and capital costs, limited funding, and underdeveloped technologies. Moreover, biopolymers often underperform in mechanical strength and barrier functionality compared to conventional plastics [46].

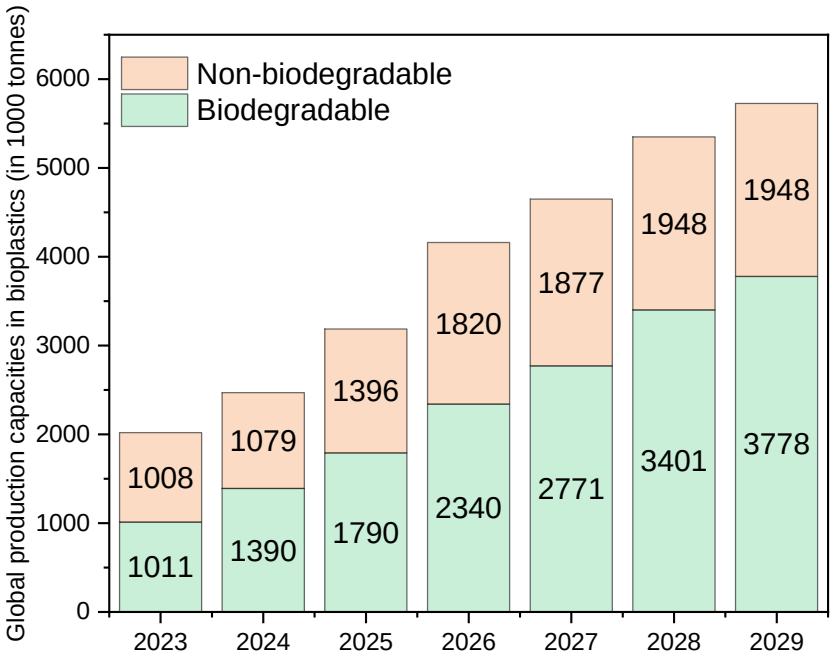

Figure C1. A0. 5. Continuous annual growth rate: global production capacities of bioplastics 2024-2029 (European Bioplastics nova-institute 2024) (https://www.european-bioplastics.org/bioplastics-market-development-update-2024/).

Biopolymers can be systematically classified based on the nature of their precursor sources **(Figure C1. A0. 6.)** [41], [47]. The first category comprises polymers directly extracted from biomass-derived products. This includes a diverse range of proteins, such as corn germ protein, casein from milk, collagen, zein, gelatin, soy protein, gluten, whey proteins, and egg-derived proteins. Additionally, lipids sourced from animal fats, animal oils, waxes, and glyceride derivatives (notably glycerol) fall within this category. Polysaccharides also represent a significant group, with examples such as starch (extracted from botanical sources like potato, avocado, and rice), cellulose (particularly from cotton), chitosan, chitin, and alginates, among others.

A second class encompasses polymers biosynthesized by microorganisms. Representative biopolymers within this group include polyhydroxyalkanoates (PHAs), bacterial cellulose, polyhydroxybutyrate (PHB), and a variety of microbial polysaccharides.

The third category consists of polymers synthesized from bio-based monomers. Notable examples include polylactic acid (PLA), polyvinyl alcohol (PVA), and synthetic polymers derived from monomers of biological origin, such as bio-based polyethylene (Bio-PE), bio-based polypropylene (Bio-PP), and bio-based polyethylene terephthalate (Bio-PET).

The final category refers to polymers synthesized from petrochemical-based monomers but recognized for their biodegradability. This group includes polymers such as polycaprolactone (PCL), poly (butylene adipate-*co*-terephthalate) (PBAT), and polybutylene succinate (PBS).

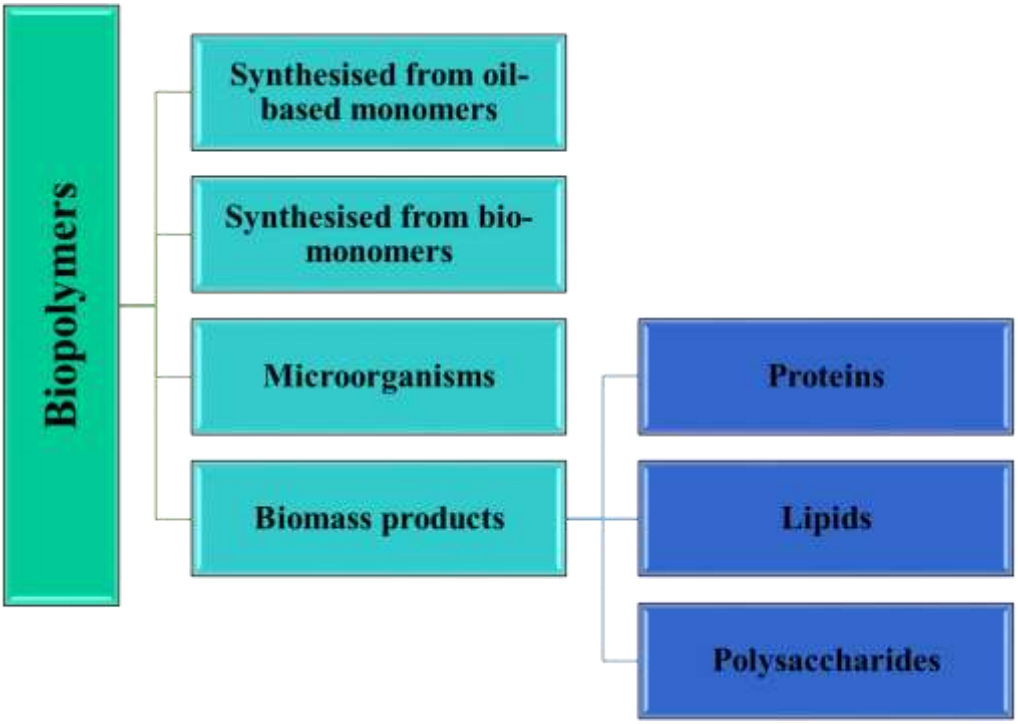

Figure C1. A0. 6. Classification of biopolymers.

Some examples of most utilized biodegradable polymers with proven potential to replace conventional non-biodegradable petrochemical-based polymers in food packaging applications are briefly described below.

2.2.1 Polylactic acid (PLA).

The list of biopolymers employed in the food industry is headed by PLA (**Figure C1. A0. 7.**) [41], [48]. PLA is a renewable aliphatic polyester synthetized from lactic acid monomers of renewable sugar feedstocks like corn, via microbial

fermentation of the sugars (**Figure C1. A0. 8.**). Lactic acid is projected to experience a compound annual growth rate (CAGR) of approximately 8% between 2022 and 2030, driven by increasing demand in bioplastics and other sustainable applications. There is a variety of methods to produce PLA but the most common is a two steps process which involves a polycondensation of lactic acid followed by ring opening. Lactic acid exists in two optically active isomers: L-lactic acid and D-lactic acid. The ratio between these isomers influences several properties of polylactic acid (PLA). As a result, the stereochemical structure of PLA is determined by various factors during polymerization, including the composition of lactic acid in the feedstock, the reaction temperature, and the type of catalyst used [48]–[52].

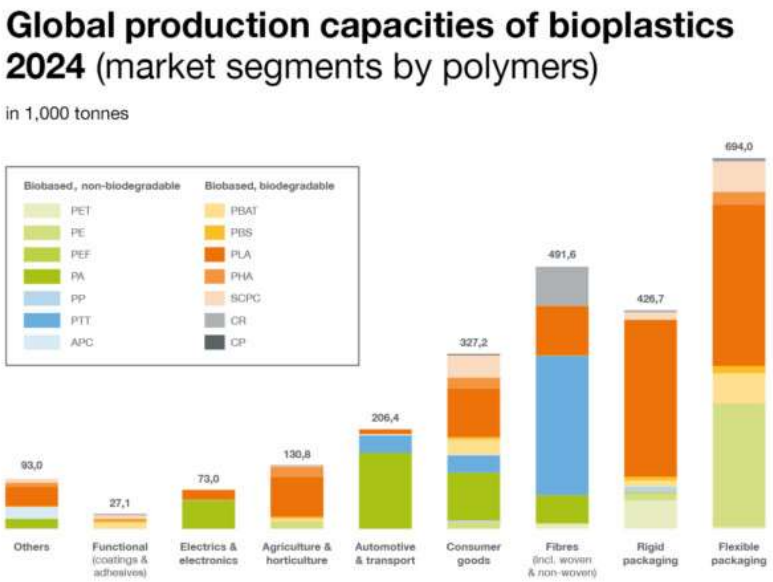

Figure C1. A0. 7. Global bioplastics production capacities by market segment and polymer type in 2024 (European Bioplastics nova-institute 2024) (https://www.european-bioplastics.org/bioplastics-market-development-update-2024/).

PLA is a thermoplastic polymer known for its high mechanical strength, transparency, good barrier properties, water resistance, thermal stability, and excellent processability. It also offers good resistance to oils, fats, and UV radiation. Additionally, PLA is biodegradable and recyclable. The lactic acid monomer used

to produce PLA is recognized as safe, and its migration within the polymer is negligible [49], [51], [53]. The main limitation of PLA is its lack of flexibility. To address this issue, plasticized PLA and PLA-based composite materials have been developed. Blending PLA with other polymers that exhibit good ductility can help to balance the overall properties of the composite, resulting in a more versatile and attractive material. To preserve key characteristics such as biodegradability, PLA is often blended with other biodegradable polymers that enhance ductility, such as PCL (polycaprolactone) or PBAT (polybutylene adipate-co-terephthalate) [54], [55].

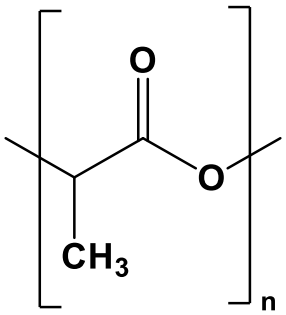

Figure C1. A0. 8. Chemical structure of PLA.

2.2.2 Poly (butylene adipate-*co*-terephthalate) (PBAT).

PBAT is a synthetic copolymer composed of both aromatic and aliphatic polyester segments, produced through copolymerization of 1,4 butanediol, adipic acid, and terephthalic acid (**Figure C1. A0. 9.**). Its properties are affected by the molecular weight of the copolymer and the relation between the number of adipic acid and terephthalic acid units. PBAT exhibits excellent flexibility, comparable to that of low-density polyethylene (LDPE) films. However, its mechanical strength and barrier performance are relatively limited. PBAT is demonstrated to decompose at 60 ºC under industrial compost, where the rate of decomposition depends on the number of terephthalic acid units and aromatic sequences [56]–[58].

PLA/PBAT materials are attractive because these blends revealed to maintain biodegradability while increase the processability and flexibility of PLA [59], [60]. Nonetheless, the ability of PBAT to enhance the toughness of PLA is restricted, primarily due to their incompatibility. This incompatibility leads to poor interfacial bonding, minimal chain interdiffusion, and entanglement at the interface. As a

result, their blends typically exhibit a coarse phase-separated morphology, with relatively large dispersed droplets often in the micrometer range [61]–[63]. However, miscibility between PLA and PBAT can be achieved by carefully controlling parameters such as molecular weight, the PLA/PBAT ratio, and the blending temperature. This compatibilization is only feasible in the melt state, since PBAT presents low glass transition temperature (T_g) which causes to behave like a rubber when storage at elevated temperatures, potentially reintroducing phase separation and limiting miscibility [64].

Nevertheless, PLA-PBAT blends have attracted significant attention due to the good mechanical resistance of PLA combined with the ductility and toughness of PBAT that led to a promising material for packaging applications. Approaches on additives such as compatibilizers, plasticizers or fillers have been studied to improve immiscibility of both phases in order to improve barrier properties and overall performance of the blend [60], [61], [65]–[69]. A notable example of commercial blend is Ecovio® developed by BASF, which is a biodegradable blend composed of PLA and PBAT produced for food contact applications [70], [71].

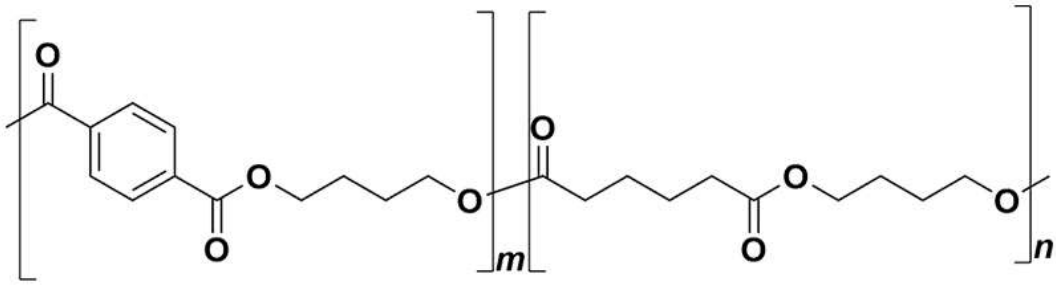

Figure C1. A0. 9. Chemical structure of PBAT.

3. Additives in packaging materials.

As the packaging industry has evolved, the demands on packaging conditions and specific properties have become increasingly stringent. Therefore, it is no longer sufficient to use a single polymer; blends of various materials and/or the addition of additives are necessary not only to favour the processability but also to improve mechanical strength, durability and tailor and enhance the properties of the polymer packaging ensuring food preservation.

An additive is defined as a substance added in small amount to a polymer matrix to enhance the processing, performance and functionality of the final material. Additives are classified based on their function rather than their chemical composition. They can act as plasticisers, impact modifiers, antioxidants, antimicrobial agents, or be served in advanced technologies such as nanocomposites. Some additives improve machinery productivity (e.g., lubricants or nucleating agents), while others enhance the aesthetic (e.g., optical brighteners, blowing agents) or functional (e.g., flame retardants, UV stabilisers) properties of the finished product [72], [73]. In particular, active packaging additives are designed to interact with the food or its surrounding environment to improve shelf life. Unlike direct food additives, integrating active agents into the packaging can be more efficient, especially since spoilage often begins on the surface of foods. By localizing their effect near the food surface, active packaging can maintain higher functional performance with lower concentrations of active substances [74].These systems fall into two main types: scavengers, which remove unwanted substances like oxygen, moisture, carbon dioxide, ethylene, or odours; and emitters, which release functional agents such as antimicrobials, ethanol, antioxidants, or flavours into the package. In addition, when selecting some additives, other requirements such as non-toxicity and low migration must be controlled. The migration of additives can not only compromise food quality but also pose health risks to consumers. Indeed, additives intended for food contact applications must be approved or regulated for instances such as Food and Drugs Administration (FDA) or European Food Safety Authority (EFSA) to ensure their safe use in direct or indirect contact with food [75], [76]. In the case of biodegradable/biobased packaging, it is essential that the additives themselves are also biodegradable and/or biobased in order to preserve the environmental benefit of the final material. For this purpose, increasing interest has been focused on additives derived from natural precursors such as cellulose, chitin, essential oils, vegetable oils, or starch, due to their compatibility with compostable packaging systems [77].

Certainly, when it comes to biopolymers, their intrinsic properties are often inferior to those of conventional polymers. For this reason, the incorporation of additives in biopolymers is even more crucial [73]. Among these, plasticizers are typically the most employed in polymer. The main role of plasticizers is to enhance the flexibility

and increase the processability of the polymer. Plasticizers generally are low molecular weight substances added in relatively high concentrations. In the context of preserving biodegradability, plasticizers used in biopolymers ideally should be bio-based and biodegradable [75], [78]. In the following chapter, the current state of the art on bio-based plasticisers derived from natural macromolecules will be reviewed.

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Article 1: Natural macromolecules used as bioplasticizer in polymer materials for food contact applications.

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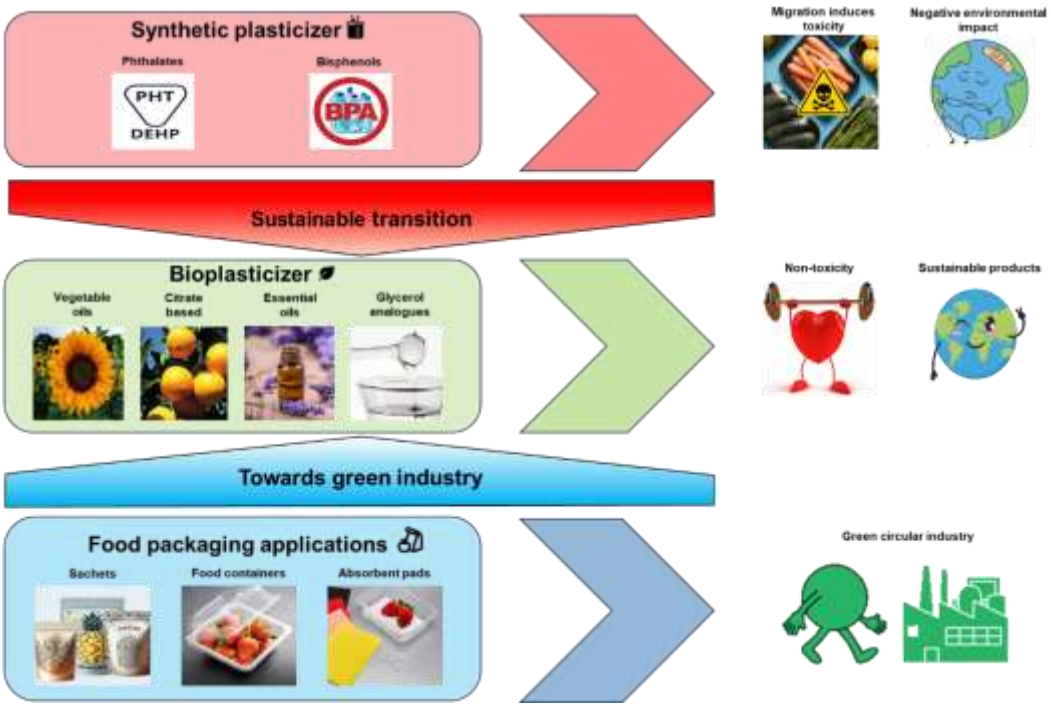

Abstract.

Synthetic plasticizers are essential additives widely used in polymer-based products such as food packaging, toys, and medical devices, etc. Despite their functional properties, they present significant environmental and human health risks due to their toxicity and migration behavior. Growing awareness of these adverse effects has driven interest in the development of bio-based plasticizers derived from natural resources. In recent years, natural plasticizers have emerged as promising alternatives, offering advantages such as low toxicity, biodegradability, and reduced migration. Their application is particularly relevant in the food packaging industry, where the transition towards sustainable materials has gained great interest, with the growing use of biopolymers such as starch, poly (lactic acid) (PLA), chitosan, and gelatin. Most of these biopolymers exhibit limitations in barrier, mechanical, and processing properties, requiring the addition of plasticizers to improve their performance. Natural plasticizers provide a synergistic solution by enhancing the properties of biopolymers while maintaining the sustainability and low toxicity of the resulting materials. Furthermore, their compatibility with biopolymers and reduced migration makes them highly suitable for industrial applications. This review explores the advancements in natural plasticizers, their integration into biopolymer systems, and the challenges associated with their industrial-scale adoption for food packaging applications.

Keywords: bioplasticizers, migration, biodegradable, food contact materials, food packaging.

1. Introduction.

Plastics dominate as the primary material in food packaging due to their excellent protective properties, lightweight, and cost-effectiveness. Their versatility, durability, and capacity to act as barriers against moisture and oxygen make them essential for preserving food freshness, extending shelf life, and minimizing food waste [1], [2]. However, the growing use of plastics and poor waste management practices have raised serious ecological concerns [3]. One of the key problems is the migration of plastics additives and their potential to contaminate the environment, the soil, water and air [4]. These additives can migrate not only to packaged food but also can be released during recycling process or at landfills. Among others, the plasticizers are the most common chemical substance added to the plastics and, therefore, are the most critical [5]. Plasticizers are low molecular weight, non-volatile compounds incorporated into polymers to enhance flexibility, ductility, and processability [6]. Their role in modifying mechanical and thermal properties has made them indispensable in applications such as food packaging [7], medical devices [8], and in other high-performance industries. As demand for plastics grows reaching an estimated 360 million tons annually so too does the reliance on plasticizers to meet specific performance requirements [9]-[11]. The increasing use of biopolymers such as thermoplastic starch (TPS) [12] and poly (lactic acid) (PLA) [13] has further underscored the importance of plasticizers because biopolymers often suffer from mechanical drawbacks, including brittleness and poor processing performance. Incorporating plasticizers is critical to improving their properties and enabling their use in food contact applications [2], [7], [14], [15].

However, recent studies have identified significant challenges related to plasticizer use, particularly in food packaging. Migration of low molecular weight plasticizers from polymeric materials into food has raised health and safety concerns due to their potential toxicity [16]. These issues have prompted a shift in the plasticizer market toward the development of alternatives that combine high performance with reduced migration and toxicity [17].

To address these challenges, innovations in the field are focused on the design of plasticizers that not only enhance polymer properties but also minimize health risks. Natural macromolecules with plasticizing effects have shown promising potential

in achieving these objectives, offering a balance between functionality and safety [18]-[20]. Moreover, the development of bio-based plasticizers complements the shift toward biopolymes, ensuring that the materials are both high-performing and sustainable. Derived from sustainable resources, they offer reduced toxicity while aligning with the broader drive toward environmentally friendly materials.

In recent decades, bioplasticizers have emerged as a compelling area of research. Numerous studies in literature have focused on both the synthesis and application of bioplasticizers, along with reviews of bio-based plasticizers used in various polymers. For instance, Omar et al. [21] and Piah et al. [22] have evaluated the effects of bio-based plasticizers on PLA, particularly, concerning their impact on mechanical properties.

Further investigations by Han et al. [23] have explored the compatibility, migration resistance, and thermal stability of cardanol- and isosorbide-based plasticizers of PLA, poly (vinyl chloride) (PVC), and TPS. Additionally, Jia et al. [24] have assessed the potential of bioplasticizers as replacements of *o*-phthalates for PVC formulations.

Recent research efforts have also focused on synthesizing new molecules derived from natural sources. Yufeng et al. [25], for example, have developed novel derivatives from castor oil, which show potential as effective plasticizers. Likewise, the impact of soybean oil-based plasticizers on polymer properties has investigated [26].

This review highlights the recent advancements in using natural macromolecules as plasticizers, focusing on their potential applications and benefits when incorporated into polymer matrices for food contact materials. It evaluates the improvements on material properties, the new functionalities introduced, and how these natural alternatives address the limitations of conventional petroleum-based plasticizers. By combining sustainability with enhanced performance, these developments pave the way for more efficient and eco-friendly materials in the packaging industry.

2. Polymers used in packaging.

2.1. Conventional polymers.

There are many common polymers utilized in the packaging industry, including polyethylene (PE) and polypropylene (PP), polystyrene (PS), poly (ethylene terephthalate) (PET) and PVC [27]. Such conventional plastics are obtained from petroleum-based polymers, present high durability, good mechanical properties and low cost. However, their resistance to degradation and massive production lead to environmental problems such as resource depletion or massive waste accumulation [1], [28].

2.2 Biopolymers.

The problems attributed to the accumulation of waste in landfills and other problems associated with plastics, such as micro- and nanoplastics in the oceans or leakage of toxic chemicals [9], [29], [30], have led to several studies to develop new green products as well as to improve their biodegradability or recyclability at end-life cycle [31]. The term 'bioplastics' or biopolymers is ambiguous; however, is primarily employed to describe polymers that can be either bio-based, biodegradable, or both [32], [33]. While biodegradability is commonly linked with bio-based materials, it is not reliant upon the polymer's origin, but rather solely on its chemical composition [1], biodegradable polymers are those that can decompose into CO_2, CH_4, H_2O, inorganic compounds, or biomass mainly through the enzymatic action of microorganisms [29], [33]. To substitute the conventional polymer, biopolymers must match the properties of the conventional polymers, but also, they must be more energy-efficient while producing.

The food contact industry has high standards, and the polymers used for this purpose must ensure the product safety, including recognition as the Generally Recognized As Safe (GRAS). Biopolymers are highlighted due to their eco-friendly and non-toxic nature; however, they present various drawbacks. The main one is related to mechanical and barrier properties when compared with conventional polymers, thus, the use of plasticizers is even more critical. The list of biopolymers employed in the food industry is headed by PLA, which is a highly promising material due to the mechanical resistance, which can be compared with polymers such as PE, presents good thermal stability and barrier properties, and can be easily

processed by conventional techniques like extrusion or injection. Starch is another promising material, due to its low price associated with its abundance as it can be extracted from different products as avocado, potato, rice, etc. [12]. Poly (hydroxy alkanoates) (PHA) are very interesting polyesters derived from algae produced by cyanobacteria. The potential of algae-based materials is because they are not cultivated in land, present short time of harvest and no competition with human food [28], [34], [35]. Finally, other natural products have been used in food packaging industry, such as egg protein [36], pectin [37] and casein [38], among others. On the other hand, biodegradable polymers derived from oil-based monomers, such as poly (butylene adipate-*co*-terephthalate (PBAT) and polycaprolactone (PCL), are also attracting interest in food packaging due to their outstanding properties. Nevertheless, they are blended with polymers such as PLA to improve the mechanical resistances and barrier properties [39], [40].

3. Plasticizers.

Commonly, plasticizers are low molecular weight resins or liquids that interact with the amorphous regions of the polymer, improving the mobility of the polymeric chains [5], [11], [41]. The interaction between the polymer and the plasticizer is known as plasticization effect, and several hypothesis have been studied about it, which are primarily based on the compatibility and solubility of the polymer [42].

3.1. Theory of plasticization.

Despite plasticizers have been used since 1800s to modify polymer characteristics [6], plasticization effects on polymers were not extensively studied until the 20th century. Different theories have been proposed since then, including the lubricity theory, viscosity theory or gel theory, among others. Also, mathematics models based on the free volume were employed to understand the plasticization effect on polymers [43]. The general idea about the plasticization effect is that the plasticizer molecules diffuse into the matrix lubricating the backbone polymer and reducing the polymer-polymer interactions [42]. The new formed 3D system reduces the resistance of the material, increasing the flexibility of the chains [43], also adding a higher number of side chains to the polymer, increasing the free volume and leading to an easier rotation and movement of the polymer [44], [45].

Then, the primary role of plasticizers is to increase the flexibility of the polymer chains, by lowering the second-order transition temperature. The reduction in this transition, known as glass transition, increases the processability of the polymers [5], [41]. The interactions with the plasticizer reduce the polymer-polymer chain secondary bonding, increasing the mobility of the macromolecules [46]. The majority of plastics are prepared by "hot compounding techniques", adding plasticizer reduces the melt viscosity and increases the filler incorporation and dispersion, lower processing temperatures and better flow properties, reducing the mixing time and the pressure of the extrusion [18], [46].

Moreover, the incorporation of plasticizer alters the properties of the polymers in several ways. It reduces intrinsic properties such as hardness, viscosity, Young's modulus, tensile strength, density, and electrostatic charge while increasing elongation at break, flexibility, and dielectric constant [6]. Furthermore, other characteristics like crystallinity, opacity, and fire resistance among others may also be affected depending on the specific nature of the plasticizer [47]. In conclusion, choosing an appropriate polymer-plasticizer system depends on factors such as the compatibility of its components, the plasticizer's efficiency, the easiness of processing, and the targeted thermal, electrical, and mechanical properties of the final material.

The plasticizers can be classified by their affinity with the polymer. Internal plasticizers are macromolecules with high affinity with the polymer leading to a chemical modification in the monomer or polymer chain, whereas external plasticizers present lower affinity with the polymer and can be lost by evaporation, migration or extraction[41], [45], [46]. Moreover, plasticizers can be divided into primary or secondary plasticizers [41], where primary plasticizers are the main component, meanwhile secondary are used to improve the primary [46].

3.2. Evolution of plasticizer market.

The first use of plasticizers was found in the literature around the 19th century, with the use of castor and camphor oils. Later, the discovery of triphenyl phosphate started a new era of plasticizers based on ester molecules. Some examples are tricresyl phosphate, which is still in use nowadays [48], or tributyl phosphate that was eventually replaced by other plasticizers [24]. In 1920, the discovery of

phthalic acid esters meant a huge step in the development of plasticized materials, being the largest class of plasticizers in the 21st century. Some examples are dibutyl phthalate (DBP), used for poly (vinyl acetate) dispersions or di(2-ethylhexyl) phthalate (DEHP) [46], responsible for over 50% of worldwide phthalate production, which is remaining as the most widely used PVC plasticizer [49], [50]. The wide range of polymers in different fields has led to the development of new plasticizers based mainly on esters, approximately 90% of the plasticizer market. From this group, 80% correspond to the use of *o*-phthalic acid esters. Other plasticizers used are fatty acid esters, benzoates, tartrates, or esters of adipic, azelaic, and sebacic acids [51]. As a result, the polymer market increased with the appearance of plasticizers, opening to a wide range of applications where polymers substitute other products due to requirements or specific properties of products [46]. Moreover, this increase in the market of plasticizers has caused regulations about their use, legislation and health issues of the plasticizers, related to toxicity problems from the migration of plasticizers. For those reasons, plasticizers development over the last 50 years must ensure safety in food, health or other industrial applications [6], [46].

In the search for new safer plasticizers, with minimal migration and low toxicity, natural-based plasticizers have gained significant relevance in the last years. The Europe bioplasticizers market is projected to undergo a compound annual growth rate (CAGR) of 8.2% from 2024 to 2031. In 2021, the market volume in Europe increased to 260.62 kilotons, reflecting a 13% growth between 2020 and 2023 [52], [53].

3.3. Regulations and limitations related to the migration and toxicity of plasticizers.

Despite the nature and toxicity of the plasticizers, migration is the major drawback of the plasticizer. During the product life, the properties of the material must remain unaffected, and migration of the plasticizer leads to an undesired change in the properties of the polymer. Also, the food product migration of the plasticizers might affect the aroma, smell, and taste of the packaged foods. In addition, depending on the nature of the plasticizer and the amount of plasticizer leached, toxicity problems can also appear [54], [55]. Migration is primarily a diffusion process where a chemical substance moves from a region of higher concentration to another with lower concentration. The main factors influencing the migration of these molecules

include the attractive forces between the molecules, the packaging materials and the food constituents, and the physical-chemical properties of them; temperature, storage time and surface-volume ratio between the packaging and food product; and the size, molecular weight and volatility of the plasticizers [56], [57]. Adsorption is another diffusion process that occurs in the food/package interface where the mass is transferred [54], [58].

For food packaging applications, the migration of the substances is simulated in different food simulants, depending on the characteristics of the food product to be in contact with. The conditions are set by the contact time and contact temperature; the test must be carried out in the worst foreseeable conditions of the product under examination. Specifically for packaging applications in Europe, the conditions and simulants of the test are reported by the EU Commission Regulation No 10/2011 EU [59].

Problems related to the migration and toxicity of the plasticizers have conducted to the creation of directives and regulations concerning materials in food contact application, for the evaluation of the substance released/migrated from the material, limits of migration were also set up by EU Commission Regulation No10/2011 [59]. In this regulation, the specific migration limit of materials and the overall migration limit of the substances are controlled. The overall migration limit of 60 mg per kg of food was established for contact with food, while for some substances a specific migration limit (SML) was established by EU Commission Regulation No10/2011 [59].

Different studies have been performed on plasticizers to evaluate their toxicity, as can migrate from the polymeric products, being one of the mayor problems, the potential bioaccumulation of these substances in the body, which can cause health consequences [17]. For instance, phthalates exposure is reported to cause metabolic disturbances, reproductive disorders, inflammatory conditions, among other diseases [8], [60]. In food contact materials, the migration of phthalate esters in PP, PVC, tin cans and glass containers was studied by Alp and Yerlikaya.Indeed, migration of phthalate was found in PVC, PP and tin cans. DEHP was found in most of the samples in high levels, only glass containers without metal closures of PVC were reported to not suffer migration. Moreover, the amount of phthalate esters in food is correlated with the contact time of the food to the packaging

materials [61]. H. Fang et al. studied the level of phthalate migration from commercial propylene food containers into food-alike aqueous solutions. The migration of DEHP and DBP increased with extended heating time and were in acid conditions (pH =3) at maximum, presenting great health risk [62]. Bisphenol A or 2,2 bis(4-hydroxyphenyl) (BPA) is another plasticizer normally used in polycarbonates (PC) based polymers. Some products made from PC as baby bottles or water bottles contain BPA, which is reported to affect the organism by altering the structure of estrogen and segregation of hormones, and is an endocrine disruptor [63]. In the literature, there are several studies related to the migration of BPA when is used in different materials as tin cans, bottles, food, etc. [64], [65].

The European Union has tackled the issue of plastic food packaging in its Plastic Strategy and Circular Economy Action Plan. It presents the transition towards a circular economy as a comprehensive solution to the plastic crisis [66]. The use of recycled polymers in the food industry is a challenge due to the safety standards of that industry, one of the fundamental aspects to tackle is the possibility of polymer migration and toxicity. The use of recycled plastic in packaging must take in consideration several aspects: cross-contamination from other packages, misbehaviours of the consumers, processing materials with different compositions and the polymer degradation or stability related to the number of recycling processes [54]. Furthermore, the migration of oligomers from plastics is also a problem due to the difficulty of identifying oligomer species. The reason is that different oligomers possess the same molecular mass, and from the legislation point of view oligomers are not evaluated differently from other substances in the EU regulations [67]. Masmoudi et al. studied the overall migration of PET recycled in food contact. The values of PET recycled remained below the normative limit (15 mg/kg) (EN13130-23, 2005) [59], [68]. Also, Castro-López at al. reported an augment in the number of oligomers in PET after 4 cycles of extrusion [69]. Velasquez et al. studied the effect of high impact PS to manufacture yogurt cups, and the results obtained showed that after reprocessing the PS, the migration values increased, exceeding the overall migration limits in simulant B and D1 [70].

New European strategies towards a green transition established a European Strategy plan for Plastics where the packaging should be reusable, recyclable or compostable (European Commission 2018). Moreover, directives as the Directive 2018/852

made to increase the recyclability of plastic to 70% by 2030, leading to an opportunity for new materials to overcome the problems and replace the conventional plastics [71].

3.4. Bioplasticizers.

The evolving plasticizer market, coupled with stricter regulations on leaching, is driving a shift toward sustainable and natural macromolecules [72], especially in food contact application it must be ensured product safety and minimize the release of substances into food. In cases of migration, migrated substances must not be hazardous. Increasing regulatory requirements and limitations have driven the development of non-toxic plasticizers with minimal migration properties. The ideal "green" plasticizer should be nontoxic, including its metabolites, possess good miscibility with the polymer, maintain efficiency comparable to conventional plasticizers, exhibit high resistance to leaching, and be relatively low in cost [17], [18]. Furthermore, the growing use of biobased and biodegradable polymers in packaging has increased the demand for producing “completely biodegradable products.” Such bioplasticizers are principally natural macromolecules derived or synthesized from agro-resources through biorefinery processes from agriculture and biomass, as well as agro-biological waste, which recycled and repurposed [35]. Such starting biobased components include starch and cellulose obtained from cereals and tubers, citric acid derived for citric fruits, adipic acid from beets, vegetable oils derived from sunflower, soybean and castor plants, among others.

Thus, bioplasticizers are typically branched structures synthesized from building blocks as citrate based, fatty acids or vegetable oils, among others [24]. Vegetable oils-based plasticizers are from the point of view of the industry one of the most promising raw materials to create bioplasticizers due to the large variety of compounds and the favourable scaling process. These products can be transformed by different techniques or used directly. Another important block is the citrate-based materials, containing citric acid, which is a well-known antioxidant and antimicrobial molecule. The production of its derivatives such as citrate esters is becoming a promising process. Citrate esters provide plasticization to different materials, but the problem is the large-scale production needed for the industry. Other products such as low molecular weight biopolyesters can be employed as plasticizers due to the increase in the free volume that is produced when are added

to the polymer matrix. Most of the bioplasticizers present esters or other polar groups in their structures that can interact with the polymer matrix through hydrogen bonding, reducing interactions between polymer chains thus, enhancing plasticising effect. Likewise, bioplasticizers usually contain aliphatic chains, which facilitate the mobility of the molecules.

3.4.1. Classification of bioplasticizers.

3.4.1.1. Vegetable oils-based plasticizers.

Vegetable oils, derived from plants, are chemically composed of various macromolecules, including triacylglycerols and esters of glycerol, and fatty acids as main components (**Figure C1. A1. 1.**). These oils are attractive raw materials for many industrial applications due to their renewable nature, biodegradability, environmental friendliness, easy availability, and large-scale production at competitive costs. Vegetable oils have two chemical characteristics that make them potentially effective plasticizers: (1) their fatty chains can intersperse and intercalate between polymer chains, increasing intermolecular spacing and enhancing mobility, and (2) the ester groups can interact with polymer chains (e.g., hydrogen bonds, van der Waals interactions), promoting compatibility [18].

One of the natural vegetable oils most used in food applications is castor oil, which is predominantly produced in Asia and Africa obtained by pressing the seeds of the *Ricinus communis* plant belonging to the *Euphorbiaceae* family [75]. It is notable for containing ricinoleic acid [76], [77]. Sunflower oil is another vegetable oil frequently used. This non-volatile oil is obtained by pressing from the seeds of the sunflower (*Helianthus annuus*) and is mainly composed of triglycerides typically derived from linoleic acid and oleic acid. Raw and fried sunflower oil are reported to be a good substitute to glycerol in PLA/TPS blends improving the thermal stability while maintaining good mechanical properties. Furthermore, plasticized TPS with sunflower oil exhibited lower polydispersity, better lubricity and properties against impact during the life cycle assessment [78]. Other interesting oils to obtain new plasticizers are soybean oil [19], [79]-[81], tung oil also known as China wood oil [82], nutshell oil (cardanol) [83], [84], among others [74].

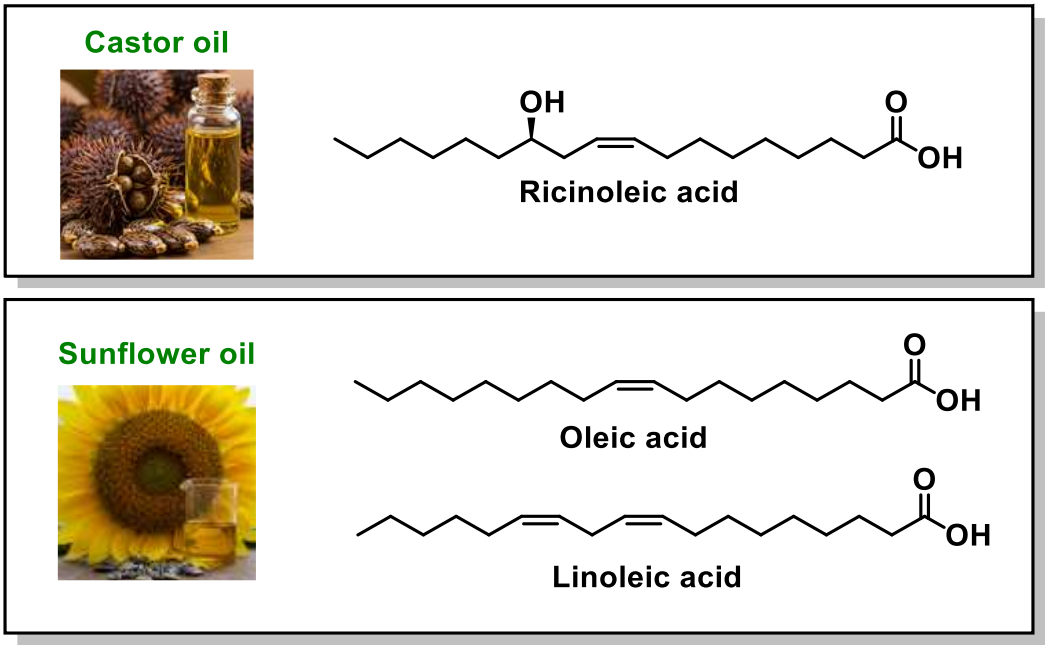

Figure C1. A1. 1. Main fatty acid components of castor and sunflower oils, commonly used as bioplasticizers.

3.4.1.2. Epoxidized, acetylated or esterified vegetable oils.

The interest in vegetable oils for food contact products has increased in the last years, and as result, many modifications of triglycerides have been investigated to enhance their plasticisation properties. Triglyceride structures feature two main reactive sites: the ester groups and the double bonds, that allow the preparation of acetylated oils [18], epoxidized oils [74] or esterified vegetable oils [85] extensively used in recent years in different polymers for food contact purpose [73]. For instance, castor oil has three reactive points, the hydroxyl group, carbon-carbon double bond, and carboxylic acid [86], enabling them to undergo reactions like epoxidation, sulfonation, halogenation, esterification, hydrolysis, and others to produce different types of plasticizers [87].

Epoxidation of vegetable oils is a chemical reaction in which the unsaturated fatty acids of vegetable oils are transformed into epoxy fatty acids (**Figure C1. A1. 2.**) [35], [74], [88]. To produce the epoxidation of vegetable oils different processes can be performed only varying the catalyst used [18], [73]. Epoxidized soybean oil methyl ester has demonstrated to be an effective replacement of DEHP in PVC, demonstrating good elongation at break up to 350%, higher resistance to migration

in all solvents and enhancing the thermal properties when added in a ratio of 1:2 to PVC [79]. In PLA, increasing the concentration of epoxidized soybean oil methyl ester produces an augment in the elongation at break (up to 800%), melt flow index and oxygen transmission until 40 wt%, nonetheless toughness and water vapour transmission values are higher at 10 wt%. Epoxidized soybean oil methyl ester showed to surpass the limit of migration (10 mg/dm^2) in 50% and 90% of ethanol in water. Films containing less than 20 wt% do not exceed the limit, and in a solid food simulant (TENAX®) only the use of up to 5 wt% stands in the limit [80]. Tung oil derived epoxidized dicarboxylic acid dimethyl ester increases the elongation at break of PVC film up to 19% showing good resistance to migration [82]; while epoxidized sunflower oil incorporated into PVC up to 20 proportions with DEHP showed to enhance the thermal stability and maintain the flexibility while reducing the amount of DEHP in PVC [89].

In PLA, epoxidized sunflower oil was compared to epoxidized soybean oil. In both cases increasing the content of plasticizers reduces the tensile stress while increases the elongation at break, achieving the maximum with 20 wt%. According to the thermal and mechanical properties the level of oxirane of epoxidized vegetable oil affects the plasticisation effect due to the formation of more hydrogen bonds with hydroxyl groups of PLA as shown by infrared spectroscopy [90]. Epoxidized linseed oil was employed in PLA/hazelnut shell flour to overcome the fragility of adding hazelnut shell flour. The results showed that increasing the amount of epoxidized linseed oil augments the mechanical and flexural properties of the material [91]. Moreover, epoxidized karanja oil was used on PLA, and it was observed by microscopy that at a concentration of 5 wt%, saturation of the matrix occurs. Anyway, for this value an improvement of the elongation at break up to 77% was observed, additionally epoxidized karanja oil showed to speed the degradation of PLA [92]. Also, safflower oil (SFO) and epoxidized safflower oil (ESFO) were employed in PLA, the addition of SFO and ESFO as plasticizers effectively enhanced PLA properties, with ESFO showing stronger interactions and better thermal stability. ESFO-PLA films exhibited improved mechanical properties, water resistance, and no surface voids [93].

Regarding plasticizers based on castor oil, the hydroxyl group on each ricinoleate chain can be used to modify the fatty chain. Plasticizers for ethyl cellulose (EC)

based on castor oil can be obtained via esterification of castor oil fatty acid with furfuryl alcohol. The results showed that up to 15 wt% of plasticizer the elongation at break increases up to 20% likely as DBP, it presents a smooth microstructure as observed by microscopy and shows a reduction in the water vapor permeability values. In addition, low migration in gasoline and petroleum was observed showing its potential as plasticizer. However, it should be also tested in food simulants [83]. Epoxy acylated ricinoleic acid binary alcohol ether ester was synthesized by different routes via esterification, acylation or epoxidation of ricinoleic acid of the castor oil and the efficiency in PVC was tested. The plasticizer obtained by synthesis of epoxy acetylate ricinoleic acid ethylene glycol butyl ether ester showed excellent performance at break and was able to decrease the mixing process energy of PVC, indicating the best properties as plasticizer [77]. Other modifications of castor oils as epoxy castor oil (ECO), acetylated castor oil (ACO), epoxy acetylated castor oil (EACO), benzoyl castor oil (BCO) and epoxy benzoyl castor oil (EBCO) were studied as plasticizers for PVC and compared to commercial dioctyl terephthalate (DOTP) and epoxidized soybean oil (ESO). PVC reinforced with EACO showed to have higher tensile strength and elongation at break than plasticized with DOTP and ESO. In addition, it presented better volatility, solvent extraction and migration stability than these plasticizers in different solvents [87]. Besides, compounds based on cardanol, i.e. cardanol esters, were synthesized and epoxidated to obtain valuable plasticizers. The obtained plasticizers showed to decrease the glass transition temperature (T_g) value of PVC and have lower volatility than other plasticizers used on PVC as di(2-ethylhexyl) phthalate (DOP). Furthermore, the leaching of the cardanol based plasticizer is lower than DOP in oil and displays excellent leaching resistance in water even at high contents (50% weight) [83].

Vegetable oil-based plasticizers improve the mechanical properties of PVC and enhance the thermal stability and flexibility of bio-based polymers, reducing reliance on phthalates. They are suitable for food packaging applications such as films, wraps, sealable bags, and dry food pouches. Additionally, their antioxidant and antimicrobial properties can help extend the shelf life of packaged products.

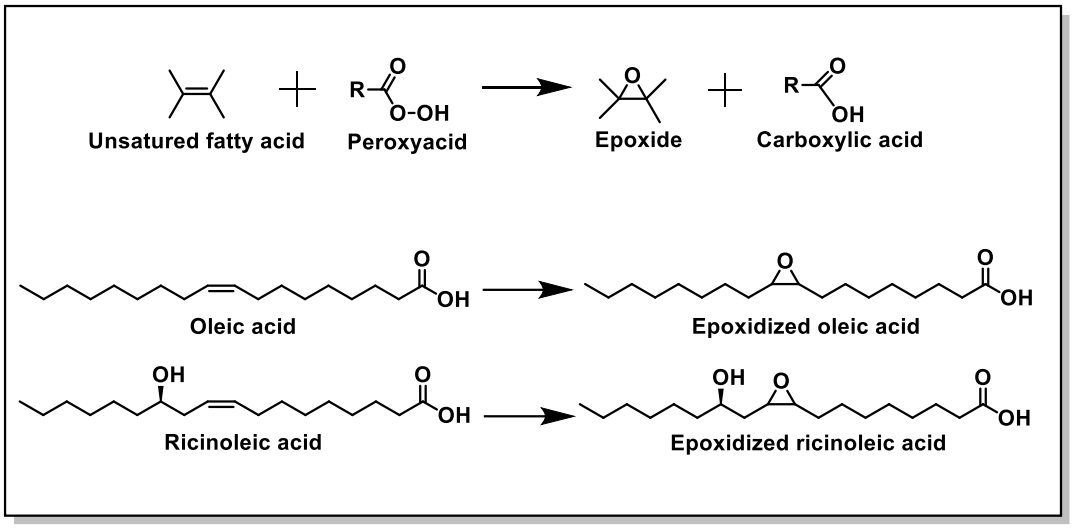

Figure C1. A1. 2. Modification reactions of fatty acids to produce epoxidized plasticizers.

3.4.1.3. Glycerol and its derivatives.

Glycerol is a polyol, composed of three hydroxyl groups, obtained through hydrolysis and transesterification of animal fat and vegetable oil when used to produce biodiesel, being the main sub-product obtained in the process [94], [95]. Glycerol can be dissolved in water [41], has good temperature tolerance, is polar and is not volatile [96]. Glycerol and glycerol derivatives are widely employed in food contact materials, due to its low cost and its good plasticization effect, especially in starch [95], [97], [98]. The three hydroxyl functional groups of glycerol are essential for interacting with hydrophilic molecules (**Figure C1. A1. 3.**). Research has shown that plasticization happens by breaking the intramolecular and intermolecular hydrogen bonds within the polymer chains. The small molecular size of glycerol allows it to occupy the spaces between polymer chains, thereby, increasing intermolecular spacing and weakening polymer-polymer interactions, while promoting polymer-glycerol interactions [100]. Nonetheless, apart from starch, glycerol has been used in other polymers as chitosan. In chitosan, glycerol is added to increase the flexibility of the polymer film by solvent casting [102], [103]. Glycerol can be functionalized by different routes [100], and its derivatives are typically employed in PLA. Glycerol functionalized with alkyl chains achieves in PLA elongation at break values of 435% by solvent casting or 257% by melt

mixing. In addition, these derivatives provided good resistance to migration when the alkyl chain is larger and non-toxicity in vitro with mammalian cell when its functionalized with six or lower carbons [104]. Glycerol triacetate (GTA) gave to PLA a value of elongation up to 418%, increased the water vapor transmission, and reduced the UV barrier properties compared with pure PLA [105]. Also, glycerol ester has been evaluated as plasticizer for PLA/PBAT blown filming, the addition of this plasticizer increased the melt flow index up to 67 g/10 min, being so high this value, meaning the incompatibility between PLA and glycerol ester for this processing method [106]. Glycerol and mixtures of glycerol were studied as plasticizers in potato flour films. The plasticizers lead to a variation in the properties of the films, especially an increase in the elongation at break from 27% to 34% when glycerol/sorbitol mixture was used, while ethylene glycol plasticizer showed the higher value of tensile strength and lower value of water vapor permeability but exhibited the lowest values of elongation at break. Polyethylene glycol (PEG200) showed higher moisture content and the film was opaquer than others. The highest water solubility and lower contact angle was obtained for polyethylene glycol (PEG400). Finally, propylene glycol exhibited higher contact angle and lower water solubility and moisture contact among all the films [97]. The effect of mixing glycerol with sorbitol in banana flour films was also reported. This mixture introduces a long-term physical stability due to the UV-visible light prevention and the heat sealability, while improves the mechanical properties of the films [98]. In another study, crude glycerol from cooking oil was evaluated as plasticizer for chitosan/starch/poly (vinyl alcohol) (PVA) blends [107].

Glycerol and its derivatives offer sustainable, flexible, and functional food packaging, making them ideal for edible coatings, dry food pouches, snack bags, and food wraps. Some also increase water vapour transmission (GTA) or water solubility (PEG derivatives), which can be useful for specific applications.

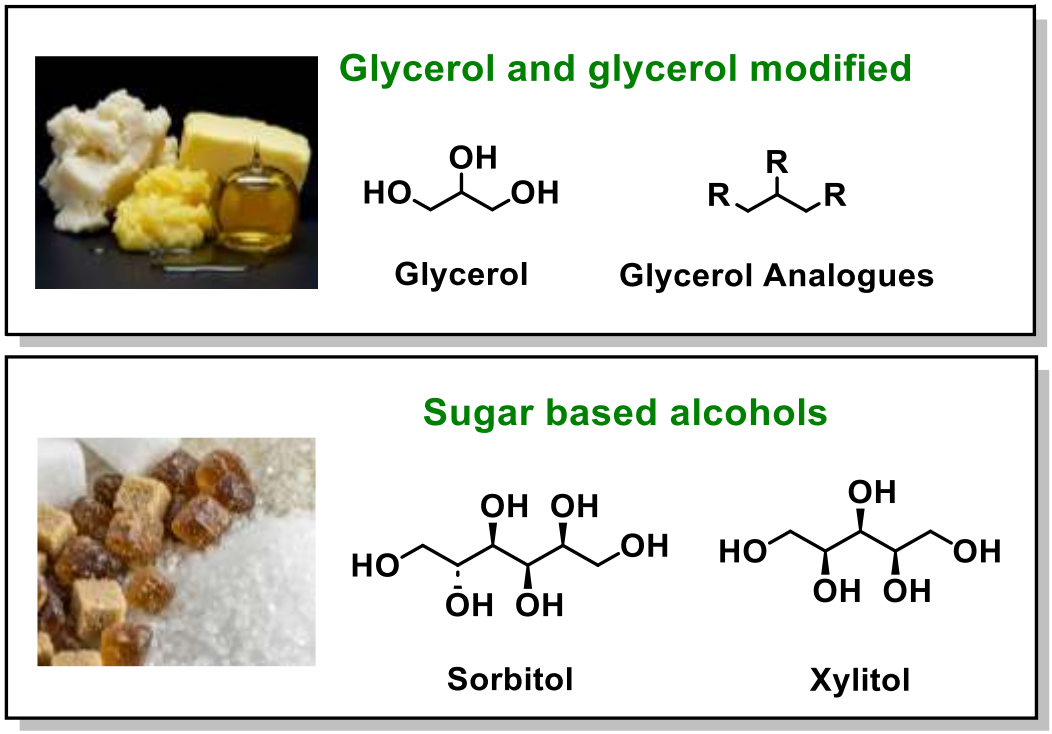

Figure C1. A1. 3. Chemical structures of main polyols used as plasticizers.

3.4.1.4. Sugar based alcohols.

Other polyols employed in food contact such as xylitol or sorbitol (see also **Figure C1. A1. 3.**) have demonstrated to produce a plasticization effect in polymers such as starch [97], PLA [108] and chitosan [41]. These plasticizers provide an increase in the elongation at break of the material, nonetheless the migration of these substances is higher compared with glycerol and must be taken in account [96].

The application of these plasticizers is mainly focused on improving the flexibility of polymers, making them suitable for flexible films in food packaging, but caution is needed regarding substance migration.

3.4.1.5. Citrate based plasticizers.

Citrate based plasticizers are based on the citric acid molecule, which has three carboxyl groups and one hydroxyl group. Citrates are synthesized by reacting citric acid with three alcohol molecules, followed by an acetylation of the free hydroxyl group with acetic acid or acetic anhydride, even if a catalyst is not needed for the esterification process it enhances the reaction (**Figure C1. A1. 4.)** [51]. There is no risk on human exposure to citrate-based plasticizers but showed to migrate to food

during packaging [109], [110]. Citrate-based plasticizers have been studied as replacement for phthalates in PVC. Triethyl citrate (TEC), acetyl triethyl citrate (ATEC), tributyl citrate (TBC) and acetyl citrate tributyl (ATBC) have been evaluated as plasticizers for PVC, all of them increase the mechanical properties of PVC achieving, for instance, a value of elongation at break of 650% with 50% of ATBC. Moreover, acetylation of TBC showed to reduce the toxicity of TBC and the binding ability of citrate protein [112]. In PLA and PLA blends, citrate-based plasticizers have been extensively employed. For example, ATBC has been used as plasticizer for PLA and PLA/PBAT blend (Ecovio®) by extrusion and compression moulding, in which elongation at break of PLA and Ecovio® samples increased from pure PLA from 4.6% to 7.0% and in Ecovio® from 2.9 to 6.2% with 15% of ATBC [113]. ATBC was also incorporated in PLA/PBS blends producing an increase in the elongation at break [114]. The migration of the plasticizers was studied and it was demonstrated that the addition of $CaCO_3$ particles and chitin nanofibrils influences and controls the migration of the plasticizer from the matrix [115]. ATBC has been also added in lignin-polyurethane/PCL, where ATBC showed the best miscibility among other tested bioplasticizers, able to decreasing the T_g and the thermal stability when 10% of ATBC was incorporated but increasing the tensile strength to 21.6 MPa and elongation at break up to 235% with only 0.5-1% of ATBC content, remaining a smooth surface and a biodegradable material in 48 days [116]. Next, TEC has been studied in PLA by solvent casting, where an increase in the elongation at break up to 390% was observed [105]. Also, the addition of TEC increases the water vapor transmission and reduces the UV barrier property. In PLA and PLA/PBAT blends obtained by extrusion, increasing proportions of TEC proportionally augments the values of elongation at break for PLA and PLA/PBAT to 200% and 300%, respectively. Nevertheless, the addition of the compatibilizer toluene diisocyanate (TDI) increases the elongation at break to 330% when more than 20 proportions of TEC are added. In this case, the structure of the film is different, showing higher compatibility and higher crystallinity. Nonetheless, the addition of TDI decreases the thermal stability [117]. In another example, TBC was employed on PLA membranes containing chitosan and alizarin, 1,2-dihydroxyanthraquinone, to enhance the flexibility, also providing higher transparency [118].

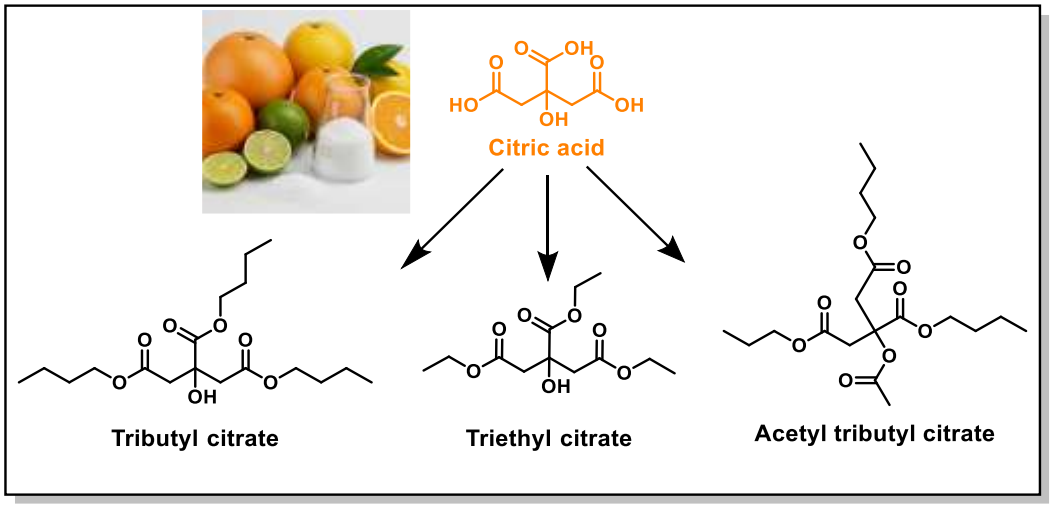

Figure C1. A1. 4. Chemical structures of main citrated based molecules derived from citric acid and used as plasticizers.

Besides, acetic acid or citric acid have been reacted with acylglycerols edibles oils with a mixture of mono and diglycerides to produce new plasticizers. In these reactions, the glycerol backbone reacts with acetic or citric acids via acetylation or esterification reaction where the molecules are covalently linked (**Figure C1. A1. 5.**) [18], [85]. As a result of the acetylation of glycerides with acetic acid, acetic acid esters of mono and diglycerides (ACETEM) were synthetized. ACETEM is a product that has been employed in PVC as plasticizer to replace DEHP [119]. Recently, ACETEM has been proved in PLA, adding new functionalities to the PLA [120]. For instance, ACETEM SOFT-NSAFE miscible with PLA provided a small increase in the elongation at break and a reduction in the T_g but more important, was able to give moderate antioxidant activity and antimicrobial properties against *Staphylococcus aureus* (*S. aureus*) and *Listeria innocua* (*L. innocua*) leading to a reduction of 80% of the colony formed units (CFU). When this biobased plasticizer is employed in PLA/PBAT blends (Ecovio®), ACETEM SOFT-NSAFE also presents good miscibility, and although the mechanical properties of the film remained low, high antioxidant and antimicrobial activity against *S. aureus* were provided. Migration study of the plasticizer from PLA and PLA/PBAT films in 50% ethanol showed to be lower than the specific limit of 10 mg/dm^2. Other interesting products are citric acid esters of mono- and diglycerides,

also known as CITREMs (**Figure C1. A1. 5.**) [120]. CITREMs are normally employed in the food industry as emulsifiers for fatty foods as chocolate or butter. Nonetheless, these products have been found as a valuable plasticizer for PLA. Among the different CITREMs studied for PLA and Ecovio®, CITREM-LR10 Extra Kosher (CITREM-LR) showed to be the most effective in plasticizing the PLA, increasing the elongation at break up to 42%. In PLA/PBAT blends the results were not so good, leading to phase separation and not improving the mechanical properties. Nevertheless, CITREM-LR provides good antioxidant activity and interesting antimicrobial properties against *S. aureus* bacteria. Another interesting CITREM is CITREM-SP70 MB (CITREM-SP), which produces a slight increase in the elongation at break up to 8%, has antioxidant activity and moderate antibacterial activity against *S. aureus* and *L. innocua* in PLA films [121].

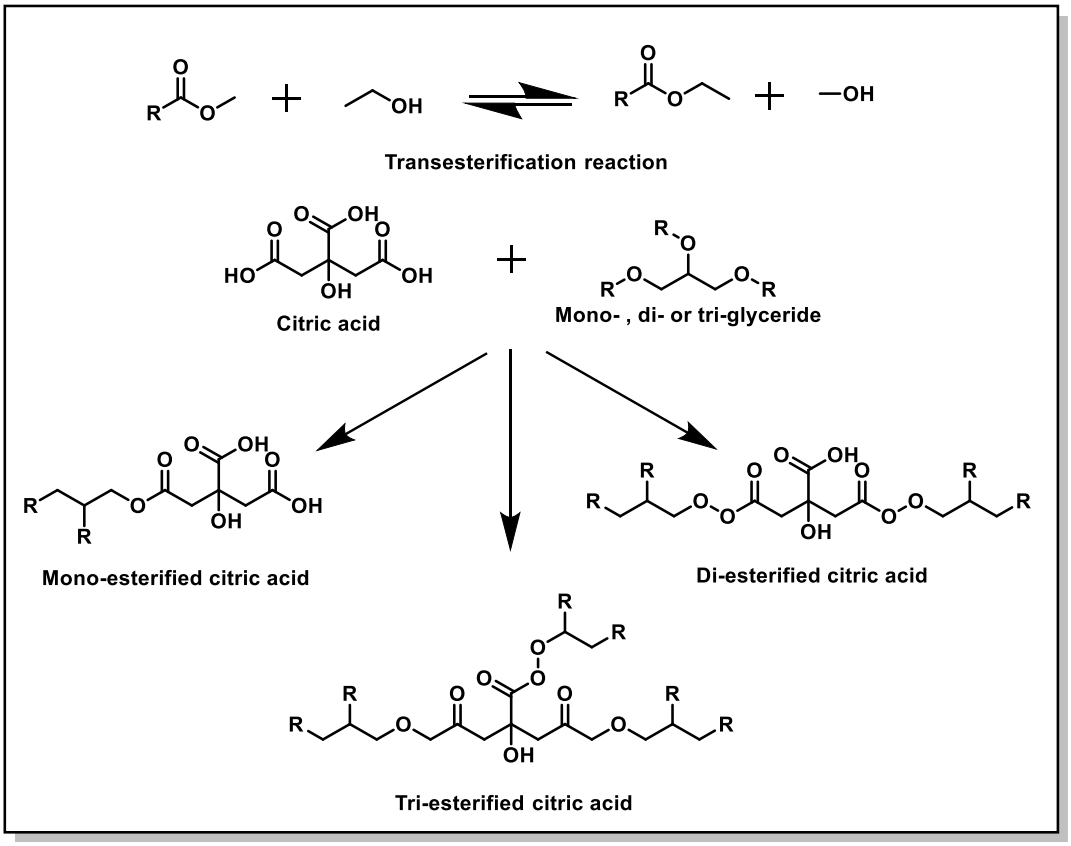

Figure C1. A1. 5. Synthesis of citric acid esters of mono- and diglycerides, CITREMs.

Food packaging applications of citrate-based plasticizers primarily focus on increasing the flexibility and thermal stability of the package, making them ideal for sealable bags and flexible packaging materials. Moreover, some citrate derivatives (ACETEM and CITREM) have demonstrated additional properties, such as antioxidant and antimicrobial effects, which can help to extend the shelf life of packaged products.

3.4.1.6. Essential oils.

Essential oils (EOs) are extracted from aromatic plants (buds, flower, leaf, leaves of the plants) and typically are liquid, uncolored and volatile, composed by natural complex compounds with a strong aroma. EOs play an important role in plants, they serve as a protective shield to the plant being antimicrobial and scare away herbivores or insects. Moreover, EOs attract insects to pollinate the plant. Around 3000 EOs have been discovered but only 300 are commercially used [122]. There are different extraction methods to obtain EOs, the most known are steam extraction and hydrodistillation [123]. EOs contain a wide series of secondary metabolites, these metabolites inhibit or reduce the growth of bacteria, yeasts and molds and give antioxidant properties to preserve the food [124], [125]. The chemical composition of EOs are characterized by having two or three major compounds in a range from 20 to 70% and other compounds in small amounts [122].

EOs normally are employed as additives to give antimicrobial or antioxidant properties to the materials [122], [126], nonetheless, they could be used as potential plasticizers. For instance, clove essential oil has been used as a plasticizer for PLA/PEG samples. The addition of clove oil in a concentration of 15 wt% and 30 wt% was reported to increase the elongation at break (80% and 115%, respectively) together with a reduction in T_g value. Films containing clove essential oil exhibited antimicrobial activity against Gram-positive *S. aureus* and Gram-negative *Escherichia coli* (*E. coli*) and a higher value of transparency [127]. Also, pine essential oil revealed to be a good plasticizer, reducing T_g value and increasing the elongation at break of PLA/PBAT blends by extrusion [128].

Essential oils are the most notorious products obtained from plants, but other molecules can be extracted and used as plasticizers. Extraction of bioplasticizers from *Agave sisalana* (*A. sisalana*) leaf involves acid hydrolysis, enzymatic hydrolysis, and mechanical process. *A. sisalana* is the source of different precursors

that are used for the synthesis of steroids. The product extracted from *A. sisalana* leaves (ASLP) incorporated into PLA increased the elongation at break of pure polymer from 22% to 40% with a content of 5% of ASLP being the amorphous nature and tiny particle size of ASLP the reason for the increase the free volume [129]. *Pedalium murex* plant is also interesting due to the variety of compounds that can be extracted from it, including alkaloids, proteins, coumaric acids, phenols, and carbohydrates, which can be potentially used as plasticizers. *Pedalium murex* plasticizer has proved to have high thermal stability and has been used in PLA. As a result, with 1 wt% content, a small increase in the elongation at break of PLA was observed from 22.4% to 25.6% [129].

Essential oils primarily increase elongation at break, while additional functionalities (thermal stability, antioxidant or antimicrobial properties) can be provided due to the specific properties of the essential oils. These products are mainly used in flexible films and smart packaging designed to extend the shelf life of packaged products.

3.4.1.7. Other plasticizers.

Low molecular weight polyols and polymers or oligomers have been employed to plasticize polymers. Those molecules increase the free volume inside the polymeric matrix and can serve as lubricants to improve the mobility of the chain. The addition of low molecular weight polymers to brittle polymers leads to a transformation in the behavior of the material reducing the storage modulus lower than the loss modulus (G´´>G´) acting as a liquid-like material [130]. That behavior is observed for PEG, which is used as plasticizer in polymers such as PLA [97], [130], [131] [132], [133] and PCL [130], [134]. It is worth noting that low molecular weight PEG macromolecules have a large number of hydroxyl groups per mole compared with high molecular weight polymers, increasing the interactions with the polymer chains. Although this plasticizer is not a bio-based plasticizer, is biodegradable, and interesting in food packaging applications. Poly (propyleneglycol diglygidyl ether) has already been used as a reactive plasticizer for PLA/PBAT and PLA/PBS blends, improving the processability and flexibility due to not only the plasticization effect but also to an improvement of compatibility between the two polymers [135].

Oligoesters are included in these low molecular weight materials that can be employed as plasticizers, for instance, oligomers from lactic acid (OLA) have been

widely used as plasticizers [136] (**Figure C1. A1. 6.**). Short branched star-shaped amorphous PDLLA exhibited melt viscosity decrease and increase in the shear-thinning behaviour, leading to a better processability of PLA [137]. OLA was employed to improve the stretchability of electrospun mats and facilitate the processing of PLA/poly (hydroxybutyrate valerate) (PHBV) [138]. Besides, OLA was incorporated in PLA/PHB blends by extrusion together with carvacol to reduce its overall migration in 10% ethanol. The increase in the free volume in the polymeric matrix results in a higher release of the active agent carvacol, improving the antimicrobial properties of the films against *S. aureus* and *E. coli* [139]. Furthermore, the combination of PEG with a masterbatch of resin containing OLA (MB) has been studied in PHB/PLA blends; the results demonstrated that by varying the range of MB and PEG, it can be obtained either flexible materials (40:60) or more rigid materials for food tray (60:40). In this study, different processing methods as extrusion, compression molding and thermoforming were also investigated [132]. Other oligoesters are reported in the literature to be used in PLA, an oligoester based on two bio-based monomers, sebacid acid and trimethylene glycol named oligo (trimethylene sebacate) (OTS) were synthetized. The addition of OTS to PLA increases the crystallinity even with only 1% of OTS and the elongation at break achieves values over 260% with contents of 5 wt% to 10 wt%. Moreover, storage modulus at the temperatures, 20 °C and 0 °C, decreases with the content increase of OTS, while at 60 °C this value increases with 5 wt% and 10 wt% [140].

Other molecules used as bioplasticizers include isosorbide diester, which was postulated as a good plasticizer of PLA and PBS. When isosorbide diester was added at 15% to the PLA/PBS blend, the elongation at break achieved a value of 240%. Moreover, the wettability is reduced with the addition of isosorbide diester [114]. Also, trybutyrin reduces the T_g of PBS and PLA while increases the crystallinity of PLA. When was used alone in PLA it produces a slight increase in the elongation at break without loss in tensile strength and Young modulus; however, in PBS an overall mechanical properties reduction was appreciated. Nonetheless, in PLA30/PBS70 with 20% of tributyrin, the elongation at break achieves 190% [141]. Also, adipate acid ester was studied among other plasticizers to plasticize PLA/PBAT blends by blown film, and it was observed that it improves

the chain mobility as well as the compatibility and interfacial adhesion between PLA and PBAT phase. The results showed an increase in the tear resistance from 4.63 to 8.67 N/mm in the machine direction and from 13.19 to 16.16 N/mm in the transverse direction [104]. These plasticizers improve flexibility, processability, and compatibility in biodegradable polymers, making them ideal for flexible films, food wraps, and compostable packaging. Some plasticizers also enhance mechanical strength and thermal stability (adipate acid esters and tributyrin), and antimicrobial properties (OLA with carvacol), helping to extend food shelf life.

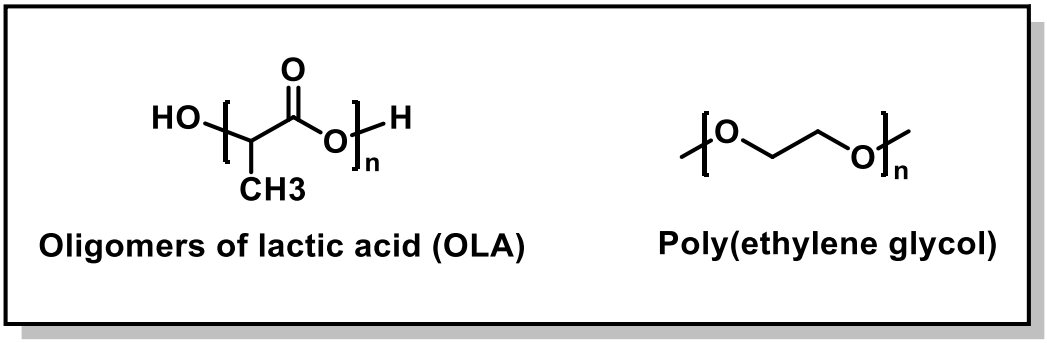

Figure C1. A1. 6. Chemical structure of oligomers of lactic acid (OLA) and poly (ethylene glycol).

4. Future aspects and safety aspects.

The transition from conventional plastics to biodegradable and biobased options presents a significant opportunity for the plasticizer industry, as these compounds are critical in improving the mechanical and functional properties of bioplastics, enabling them to compete effectively in the market. Among all the benefits attributed to bioplasticizers, their lower initial migration rates compared to conventional phthalates stand out as a desired property in many fields, especially in food packaging. This characteristic is crucial in ensuring long-term stability and reducing the risk of plasticizer leaching. Other key advantages include the superior thermal stability of bioplasticizers, which allows them to perform effectively under the elevated temperatures typical in the plastic industry, such as those involved in hot-press techniques. The ability to resist degradation during these processes ensures consistent performance and durability in various applications. Additionally, bioplasticizers like citrate-based compounds and modified vegetable oils offer

functional properties such as antioxidant and antimicrobial activity, further broadening their applicability in food-contact materials.

Despite their potential, several critical aspects remain underexplored, being challenges to their widespread implementation. One significant issue is plasticizer accumulation over time. While bioplasticizers are considered safer than their conventional counterparts, limited research exists on their long-term behavior, particularly their potential to accumulate in specific environments or systems. This accumulation could impact the performance of the polymer matrix, alter its properties, or involve unforeseen risks to human health and the environment. Addressing this knowledge gap is essential to fully understanding the lifecycle of bioplasticizers and ensuring their safety in food contact applications [17]. The lack of comprehensive information regarding the migration dynamics and accumulation of bioplasticizers further complicates their regulatory acceptance. Most studies focus on initial migration rates, but long-term studies examining their interactions with different food matrices and environmental conditions are scarce. Developing standardized methods to evaluate these aspects will be crucial for building confidence in bioplasticizers as sustainable and safe alternatives.

Another area requiring attention is the optimization of manufacturing methods, which can significantly influence the dispersion and compatibility of the plasticizer within the polymer, affecting the final mechanical and barrier properties. As is noticeable, most of the research studies found in the literature related to the effect of plasticizers in polymeric materials are typically carried out in samples obtained by casting followed by extrusion. These casting techniques are, in contrast, rarely used in industry, particularly in the food packaging industry. The preparation and processing techniques significantly affect the microstructure, structure, morphology, and properties of the final plasticized materials. Therefore, further studies are necessary to investigate the effects of new bioplasticizers in greater depth. Innovations in these methods could improve the performance of bioplasticized materials.

From an industrial perspective, scalability and cost efficiency remain significant obstacles. Although vegetable oil-based plasticizers and citrate derivatives are promising due to their abundant raw material supply, large-scale production processes often face technical challenges, such as maintaining consistency in

product quality or achieving economically viable yields. Advanced technologies like enzymatic synthesis or bioengineering could offer solutions to these limitations. Moreover, the unique properties of subgroups such as essential oils, sugar-based alcohols, and glycerol derivatives, including their ability to improve flexibility, elongation, and thermal stability, demonstrate their potential to create diverse and high-performing bioplasticizers. Unlocking the full potential of these advancements will require targeted research and investment in innovative manufacturing practices. Furthermore, the environmental impact of bioplasticizer production itself must be considered. While these compounds are derived from renewable sources, the processes involved in extracting, purifying, or chemically modifying natural macromolecules may still generate waste or consume significant energy. Conducting life-cycle assessments (LCA) of bioplasticizer production could provide insights into their true environmental footprint and identify areas for improvement.

5. Conclusions.

The safety of food about leachable plasticizers like phthalates can be addressed and minimized through various strategies. Recently, the development of natural-based plasticizers (bioplasticizers) has attracted growing interest in academic and industrial research, primarily due to their reduced toxicity and environmental impact.

Bioplasticizers have become a viable alternative to substitute conventional synthetic plasticizers. Their properties such as low toxicity and good compatibility with several polymers and their sustainability have driven this transition. Bioplasticizers derived from different natural resources such as vegetable oils, glycerols, sugar-based alcohols, citrates, essential oils, and other bio-based molecules have been studied for their effects on polymers like PVC and biopolymers such as PLA, PCL, and PBAT. These materials have been evaluated for their potential applications in the food packaging industry, demonstrating promising performance and environmental benefits.

Furthermore, the limited data on issues related to the accumulation of bioplasticizers highlights the need to understand the life-cycle of bioplasticizers and

ensure their safety in food contact applications. Most current studies focus on initial migration rates, emphasizing the need for further research into long-term migration.

As consumer awareness and regulatory pressure continue to grow, there is a clear need to adopt sustainable plasticizers derived from renewable sources. Although bioplastics currently make up a small percentage of the total plastics market, their demand is increasing rapidly, driven by this need for environmentally friendly solutions. To meet this demand, future research should focus on:

- Developing bioplasticizers with minimal migration and long-term stability.
- Investigating the effects of plasticizer accumulation over time.
- Innovative methods to optimize the incorporation of bioplasticizers into polymers.
- Conducting comprehensive studies on the interaction between bioplasticizers and food matrices under varying conditions.

By addressing these challenges, the industry can promote the full potential of bioplasticizers, making them safer and more efficient, and point them out as truly sustainable solutions in polymer materials for food contact applications. Ultimately, the successful integration of bioplasticizers into biodegradable systems will represent a critical step toward a healthier and more environmentally friendly future.

Acknowledgments.

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CHAPTER 2

Hypothesis and objectives.

Hypothesis and general objectives.

The research programme of this thesis is focused on the potential and feasibility to fabricate composites materials based on biopolymers (PLA or Ecovio®) and food approved additives including antimicrobial particles, bioplasticizers, humectifier and natural particles, that can be employed as new active and biodegradable packaging systems, giving new functionalities to extend the self-life of the packaged food such and improving the mechanical properties while maintaining the thermal and biodegradability properties. Approved food additives are totally safe, and then, the problems associated to undesirable migration from the packaging to the packaged foods or to the environment at the end of the life cycle would be limited. In addition, the packaging based on biobased and/or biodegradable polymers containing such additives would typically maintain their biodegradability. Another benefits of using the additives within the packaging instead of directly in the food is that the sensorial properties of the food would not be modified.

In this Thesis, various biocomposites polymeric materials were developed employing different processing techniques. Then, the following aspects have been taken in account to evaluate the resulting composites: I) active properties such as antimicrobial and/or antioxidant properties, II) structural, thermal and mechanical properties, III) migration of the additives and IV) biodegradability of the composites.

Specific objectives.

Chapter 3: Development and Characterization of Antimicrobial PLA/Ecovio®–E452i Composite Films.

This chapter focuses on the development of antimicrobial composite films based on PLA, Ecovio®, and the food additive E452i (sodium phosphate microparticles), using melt extrusion and compression moulding. The aim is to explore their potential for active food packaging by evaluating their antimicrobial performance and biodegradability, among other properties.

Article 2: Evaluation of poly (lactic acid) and Ecovio® based biocomposites loaded with antimicrobial sodium phosphate microparticles.

- Study the antimicrobial activity of E452i microparticles against foodborne pathogens including *Staphylococcus aureus* and *Listeria innocua* (Gram-positive strains), and *Escherichia coli* (Gram-negative strain), by determining the Minimum Inhibitory Concentration (MIC). Additionally, evaluate the hemotoxicity of E452i particles using human blood cells.
- Produce antimicrobial-loaded composite films via melt extrusion followed by compression moulding.
- Evaluate the mechanical performance through tensile testing, while analyse thermal behaviour using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Examine the microstructure of the composite films via scanning electron microscopy (SEM) and confocal Raman microscopy.
- Evaluate the antimicrobial performance of the biocomposites containing the E452i particles by contact against *Staphylococcus aureus* and *Listeria innocua* following the ASTM E2149-20 standard method.
- Additionally, asses the disintegrability of the composite films under simulated industrial composting conditions at laboratory scale according to the ISO 20200 standard.

Chapter 4: Evaluation of CITREM and ACETEM as Functional Plasticizers in PLA and Ecovio® Biopolymers.

The main objectives of this chapter are to investigate the chemical composition, thermal stability, and antioxidant properties of selected fatty acid esters, CITREM (E472c) and ACETEM (E472a), and their potential as functional plasticizers in PLA and Ecovio® biobased polymeric materials. Additionally, to tailor permeability and antimicrobial activity sodium stearoyl lactylate (SL) and chitin particles (CP) were tested as additives.

Article 3: Plasticizing PLA with Biobased Fatty Esters: Comprehensive Study on Film Properties.

- Identify the composition of the main compounds in the fatty acid esters (CITREM and ACETEM) using Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography–Mass Spectrometry (GC-MS). Subsequently, characterize their thermal properties by TGA and DSC to assess their thermal degradation behaviour. Besides, investigate their antioxidant properties using the colorimetric Blois assay, employing the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).
- Prepare plasticized films based on PLA with a variety of CITREM and ACETEM additives by melt extrusion followed by compression moulding.
- Analyse their morphology employing both SEM and confocal Raman microscopy. Investigate their mechanical properties through tensile testing, while evaluate thermal behaviour using DSC and TGA and investigate the crystallinity using X-ray diffraction.
- Evaluate the antimicrobial properties of the prepared functional and plasticized composites against *Staphylococcus aureus* and *Listeria innocua* using the ASTM E2149-20 standard method. Additionally, determine the antioxidant properties of the plasticized films using the colorimetric DPPH assay according to the Blois method.

Article 4: Enhancing functional properties of compostable materials with biobased plasticizers for potential food packaging applications.

- Prepare plasticized films based on Ecovio® and CITREM and ACETEM additives by melt extrusion followed by compression moulding.
- Analyse the morphology of the films using both SEM and confocal Raman microscopy. Investigate their mechanical properties through tensile testing, and evaluate their thermal behaviour using DSC and TGA.
- Evaluate the barrier properties of the films against oxygen and water vapour according to ASTM standard methods D3985 and F1249. Study the migration of the plasticizer from the polymer matrix into food simulants following the recommendations of the European Union and the U.S. Food and Drug Administration (FDA).
- Assess the antimicrobial activity of the plasticized films against *Staphylococcus aureus* using the ASTM E2149-20 standard method. Determine the antioxidant properties of the plasticized films using the DPPH colorimetric assay according

to the Blois method. Evaluate the disintegrability of the composite films under simulated industrial composting conditions at laboratory scale, following the ISO 20200 standard.

Article 5: Calendering Processing of PLA/PBAT Biodegradable and Functional Films Incorporating Natural Food Additives.

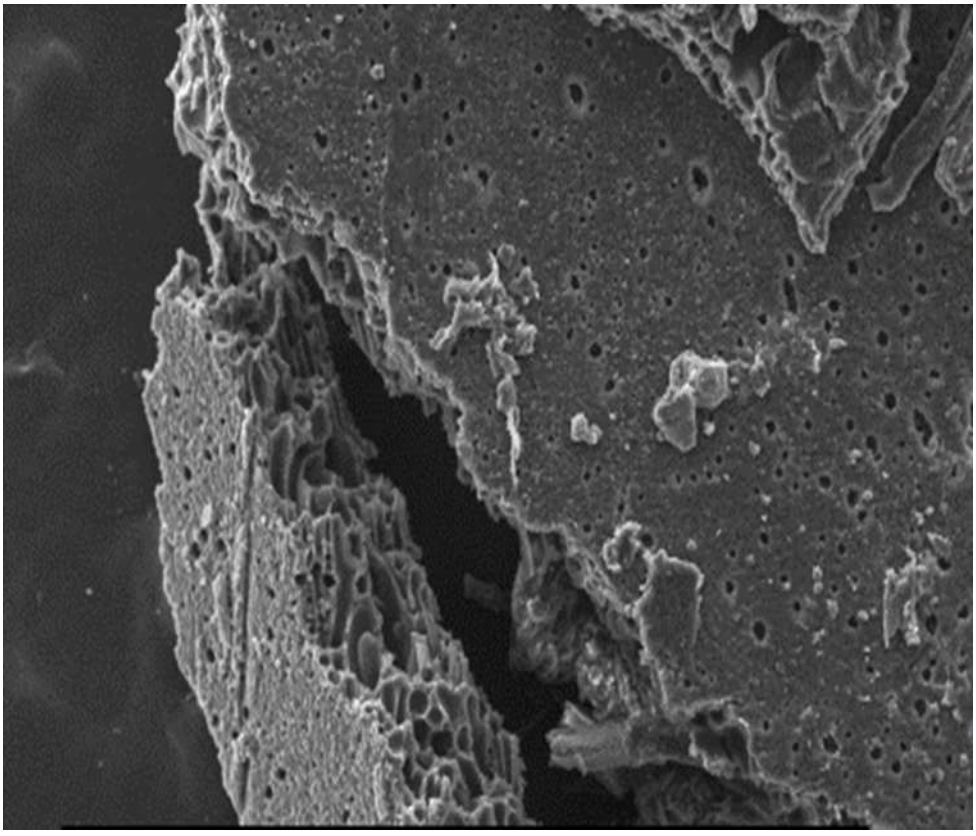
- Prepare plasticized films based on Ecovio® and either CP or SL via melt extrusion followed by calendaring. Characterize the morphology of the films using Field Emission Scanning Electron Microscopy (FE-SEM) and confocal Raman microscopy. Examine the mechanical performance through tensile testing. Study the thermal properties using DSC and TGA. Calculate the crystallinity using Wide Angle X-ray Scattering (WAXS).
- Mesure gas and water vapour permeability according to ASTM standards D3985 and F1249 to determine the barrier functionality of the films. Additionally, investigate plasticizer migration into food simulants is in accordance with current European Union and U.S. FDA guidelines.
- Asses the antimicrobial potential of the bioplasticizers and additives embedded in the polymer matrix is tested against *S. aureus*, *Listeria innocua*, and *Escherichia coli* using the ASTM E2149-20 standard. Furthermore, study the antioxidant capacity of the films is assessed via the DPPH colorimetric assay based on the Blois method. Finally, evaluate the disintegration behaviour of the composites under simulated industrial composting at the laboratory scale, following ISO 20200 specifications.

Chapter 5: Development and Characterization of PLA Fibers Plasticized with CITREM and ACETEM via Forcespinning.

The objectives of this chapter aims to develop plasticized PLA fibers via rotatory force jet spinning (forcespinning) incorporating fatty acid esters CITREM (E472c) and ACETEM (E472a), for food packaging applications.

Article 6: Bio-Based Citrate Plasticizers for PLA Fibers: Unlocking Antioxidant and Antimicrobial Potential

- Determine how the concentration of the PLA/dichloromethane solutions affects the rheological properties and evaluate their impact in the resulting fibers by investigating changes in microstructure, fiber diameter and beads formation using SEM. Subsequently, select an optimal concentration to produce PLA/ plasticizer/dichloromethane solution and re-evaluate the influence of rheological on the morphological parameters.
- Study the effect of the different plasticizers in the thermal properties of the PLA based fibers via DSC and TGA and estimate the crystallinity using X-ray diffraction.
- Evaluate the active properties of the fibers by measuring the antimicrobial potential against bacterial strains gram-negative bacteria like *Salmonella* and *Escherichia coli* and gram-positive bacteria, including *Listeria innocua*, *Staphylococcus aureus*, *Lactobacillus sakei*, and *Lactobacillus acidophilus.* Assess the antioxidant capacity of the fibers using the DPPH colorimetric assay based on the Blois method.



CHAPTER 3

Development and Characterization of Antimicrobial PLA/Ecovio®–E452i Composite Film.

Article 2: Evaluation of poly (lactic acid) and ECOVIO® based biocomposites loaded with antimicrobial sodium phosphate microparticles.

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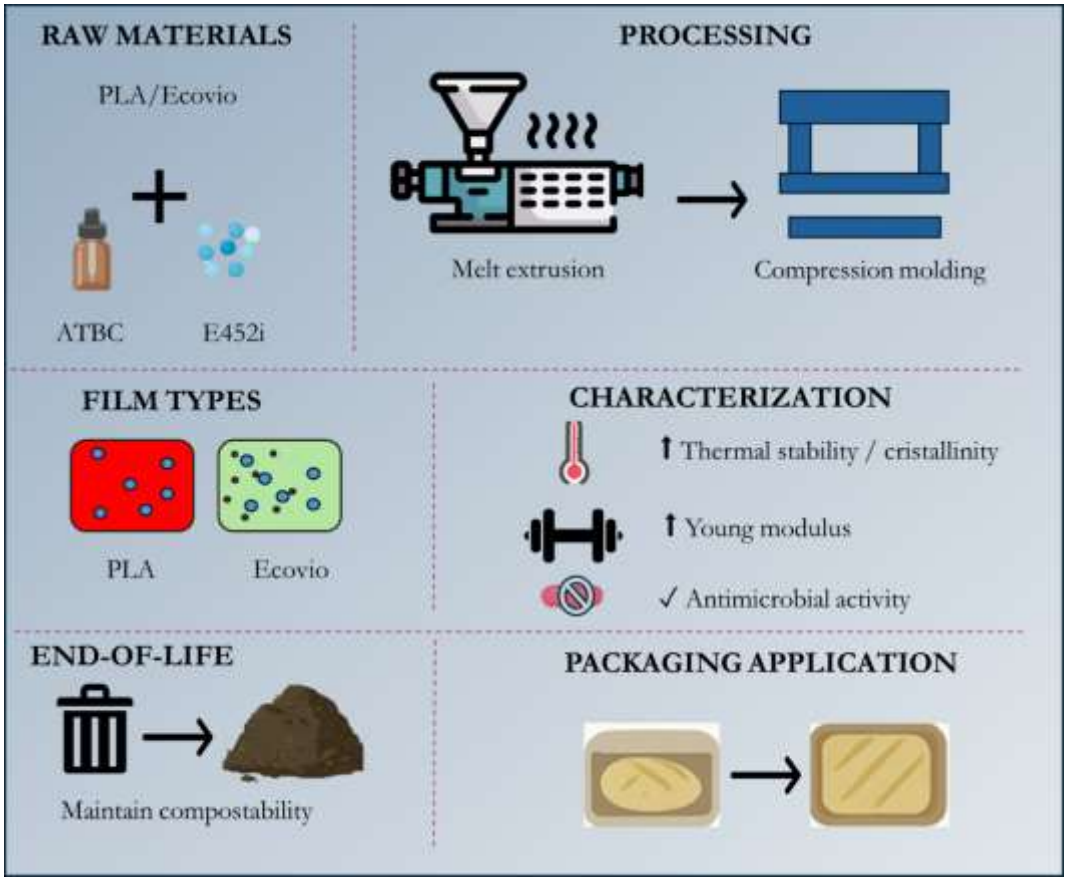

Abstract.

Herein, biobased composite materials based on poly (lactic acid) (PLA) and poly (butylene adipate-*co*-terephthalate) (PBAT) as matrices, sodium hexametaphosphate microparticles (E452i, food additive microparticles, 1 and 5 wt.%) as antimicrobial filler and acetyl tributyl citrate (ATBC, 15 wt%) as plasticizer, were developed for potential food packaging applications. Two set of composite films were obtained by melt-extrusion and compression molding, i) based on PLA matrix and ii) based on Ecovio® matrix (PLA/PBAT blend). Thermal characterization by thermogravimetric analysis and differential scanning calorimetry demonstrated that the incorporation of E452i particles improved thermal stability and crystallinity, while the mechanical test showed an increase in the Young's Modulus. E452i particles also provide antimicrobial properties to the films against food-borne bacteria *Listeria innocua* and *Staphylococcus aureus*, with bacterial reduction percentages higher than 50% in films with 5 wt% of particles. The films also preserved their disintegradability as demonstrated by an exhaustive characterization of the films under industrial composting conditions. Therefore, the results obtained in this work reveal the potential of these biocomposites as appropriated materials for antibacterial and compostable food packaging films.

Keywords: PLA, PBAT, composites, antimicrobial, compost.

1. Introduction.

Nowadays, the design of more effective packaging has become essential for the advancement of food industry, due to the globalization of the food supply chain, the projected population growth, and the escalating concerns regarding sustainability. The primary objective of traditional packaging is to enhance the shelf life of packaged food while ensuring food safety. It accomplishes these functions by providing protection against physical damage, acting as a barrier against microorganisms, and preserving the sensory properties of the food [1]-[3]. Indeed, an effective packaging also contributes to the sustainability of the food industry as can substantially reduce food losses and waste. Approximately 33% of global food production is estimated to be lost or wasted, resulting in millions of metric tons of CO_2 equivalent emissions [4]. Despite the numerous benefits of packaging, its extensive utilization leads to significant environmental consequences, with a huge amount of plastic waste ending up in landfills, oceans, and in the natural environment [5], [6]. It is estimated that around 40% of produced plastics is destined to packaging and the majority are non-biodegradable materials derived from fossil-fuels such as polyethylene terephthalate (PET), polystyrene (PS), polyethylene (PE), polypropylene (PP) and polyamide (PA) [7].

In recent years, there is an interest in reducing the amount of plastic used in the food industry. The increasingly directives to reduce the plastics of one use and the impact of plastic waste in the environment, have pushed to the industry to develop new biobased and biodegradable materials with the aims of replacing conventional plastics. Bioplastics are an example of these emerged materials that have aroused as a solution as they come from a renewable resource, can be biodegradable in landfill and/or compostable and require lower energy to be produced, resulting in lower amount of greenhouse gases emission than conventional plastics [7]. Several biobased and/or biodegradable polymers including poly (lactic acid) (PLA), poly (caprolactone) (PCL) and poly (butylene adipate-*co*-terephthalate) (PBAT) are already used in the food industry. One of the main drawbacks of bioplastics compared to conventional plastics is their relatively high cost. However, nowadays, as a result of the increasingly directives from countries and organizations along with the implementation of new taxes on conventional plastics, the cost disparity is getting reduced. In general, bioplastics also present lower mechanical properties

than conventional polymers derived from petroleum hydrocarbons. Among the biopolymers, PLA is one of the most promising biodegradable bioplastics to substitute the petrochemical plastics for packaging application. PLA possesses several properties that make it an excellent option for food packaging applications including notable processability, water resistance, acceptable barrier properties to flavours and aromas, temperature stability, GRAS (Generally Recognized as Safe) status and mechanical performance similar to traditional polymers used in packaging as PET or PE [8], [9]. PBAT is another promising biodegradable polymer for packaging applications and has been extensively blended with PLA to achieve improved properties; for instance, Ecovio® is a commercial compound to be used for packaging applications based on PLA/PBAT blend [10].

Typically, modern packaging combines different type of materials (composites) to improve the properties of the raw material or add news properties. Therefore, it is possible to reinforce polymers or enhance mechanical behaviour by the incorporation of plasticizers and fillers as well as including additional antimicrobial or antioxidant properties with the purpose to extend the shelf-life of the product [3], [11]–[13]. The use of nanoparticles for the reinforcement of plastics has been shown to be very effective; however, the utilization of nanoparticles in food contact packaging has been limited due to uncertainties regarding their safe application. It is, therefore, necessary to develop new methodologies to provide functionalities to food contact packaging while maintaining the safety requirements applicable to this type of products.

In this work, new composite materials based on PLA and Ecovio® were developed by incorporation of acetyl tributyl citrate (ATBC) as plasticizer and grinded sodium hexametaphosphate microparticles (E452i) as filler to provide antimicrobial properties. ATBC is considered one of the most efficient plasticizers for PLA due to is biobased origin, biodegradability, biocompatibility and safety for food contact [14]. The plasticizer augments the toughness and ductility of polymers by increasing the mobility of the long chains [15], however, inherently reduces the strength of the materials. To reverse this reduction in the strength, fillers are typically included in the formulation. In this work, different amounts grinded microparticles of the food additive sodium hexametaphosphate (E452i) were incorporated with the aims of improving mechanical and thermal properties, also

providing antimicrobial activity to the potential packaging applications. The sodium hexametaphosphate is typically used in food industry as emulsifier, and in recent years has been also tested as antimicrobial agent in dental applications [16]. However, its use as filler in the preparation of polymer composites has not been explored until now.

2. Experimental part.

2.1. Materials.

In this work, we develop compostable active systems based on biodegradable polymers:

PLA (ErcrosBIO® LL 652 poly (lactic acid)) purchased from Ercros (Barcelona, Spain). Technical data: melt flow index: 11 g/10 min; density: 1.25 g/cm^3; melting temperature: 180 ºC.

Ecovio® (Fblend C2224) obtained from BASF (Ludwigshafen, Germany), which is composed approximately by 45 wt% of PLA and 55 wt% of Ecoflex® (mainly poly (butylene adipate-*co*-terephthalate), PBAT). Technical data: melt flow index: <2.5 g/10 min, density: 1.24-1.26 g/cm^3; melting temperatures: 110-120 and 140-155 ºC.

These polymers were mixed with acetyl tributyl citrate (ATBC) (M_n = 402 g/mol, 98%, Sigma-Aldrich, Saint Louis, MO, USA) used as plasticizer and grinded inorganic microparticles of sodium hexametaphosphate (E452i)[17] with a diameter between 8.5-44.3 µm provided by ENCAPSULAE S.A. The microparticles were ground in a fast wolfram carbide ring mill Siebtechnik Tema S.A. (Madrid, Spain), applying 30 seconds to a load of 50 g of material.

For hemotoxicity and antibacterial tests: phosphate buffered saline powder (pH 7.4) and sodium chloride solution (NaCl, BioXtra) were acquired from Merck (San Luis, MO, USA) whereas BBL Mueller-Hinton broth was purchased from Becton, Dickinson and Company (Madrid, Spain). Columbia agar (5% sheep blood) plates were obtained from Fisher Scientific (Madrid, Spain). American Type Culture Collection (ATCC): *Listeria innocua* (*L. innocua*, ATCC 33090), *Staphylococcus*

aureus (*S. aureus*, ATCC 29213) and *Escherichia coli* (*E. coli*, ATCC 25922) used as bacterial strains, were purchased from Oxoid (Hampshire, UK).

2.2. Composite and film preparation.

Polymer/ATBC/E452i composites were obtained by melt extrusion in a micro-extruder equipped with a co-rotation twin-screw (HAAKE Minilab II Thermo Scientific, Karlsruhe, Germany), which compounds small sample amounts of 7 cm^3. The processing was carried out with a screw speed of 100 rpm at 180 ºC during 3 minutes after the entire feeding. Particles were mixed with polymers and then, fed into the extruder. Subsequently, the plasticizer was directly fed into the extruder. Prior to processing, microparticles and polymers were dried in an oven at 40 ºC for 24 hours. Afterwards, 5×5 cm films were obtained from extruded materials by compression molding using a CollinP200P press (COLLIN Lab & Pilot Solutions, Maitenbeth Germany). The extruded samples were pre-heated at 190 ºC under 1 bar pressure during 2 min, then, 100 bar pressure was applied at 190 ºC during 3 min followed by a cooling step at 100 bar. The thickness of the films of about 200 µm was obtained by averaging 10 random measurements in different part of each film using a digital micrometer (Permascope MP0, Fischer Instruments, Sindelfingen, Germany).

2.3 Biological assays.

The toxicity of the E452i microparticles were measured by hemotoxicity assay as previously described [18] from initial concentration of microparticles of 10,000 µgmL^{-1} and by determining the percentage of hemolysis. Briefly, microparticle solution was prepared in PBS at a concentration of 40,000 µg mL^{-1}. Then, 100 µL of this solution were placed in the first column of 96-well round-bottom microplate, whereas in the rest of the wells 50 µL of PBS were added. Subsequently, 150 µL of fresh human red blood cells (RBCs) solution (5% v/v) in PBS was added to each well. Then, 1:2 serial dilutions were made across the plate. Likewise, PBS was used as negative control for 0% hemolysis and 1% v/v Triton X-114 solution in PBS was employed as positive control for complete hemolysis. The microplate was incubated for 1 h at 37 ºC followed by centrifugation at 1000 rpm for 10 min. The resulting supernatants were transferred to a new 96-well microplate and the absorbance at 520 nm was measured in a microplate reader (Synergy HTX Multi-Mode Reader

spectrophotometer, Bio-Tek, Madrid, Spain). The percentage of hemolysis was calculated according to the following equation:

$$Hemolysis\ (\%) = \frac{A_S - A_{nc}}{A_{pc} - A_{nc}} \times 100 \quad (1)$$

where A_S is the measured absorbance of the sample, A_{nc} is the absorbance of the negative control and A_{pc} is the absorbance of the positive control.

The antibacterial activity of the E452i microparticles was studied by determining their minimum inhibitory concentration (MIC) following a standard broth dilution method according to the Clinical Laboratory Standards Institute (CLSI) [19]. The test was performed against the Gram-positive bacterial strains *S. aureus* and *L. innocua.* The measurements were made at least in triplicate.

The antimicrobial activity of the films was also assessed against *S. aureus* and *L. innocua* using the E2149-20 standard method from the American Society for Testing and Materials (ASTM) [20]. This standardized method is widely recognized for evaluating the antimicrobial activity of material surfaces. For the test, polymeric films were cut into square pieces of ~100 mg weight, placed in 10 mL of bacterial suspension (10^3 CFU mL^{-1}) and incubated for 24 h at 37 ºC. The experiments were made at least in triplicate.

2.4. Disintegrability under composting conditions.

The aerobic disintegrability test of the composite films under simulated industrial composting conditions was performed at laboratory scale level following the ISO 20200 standard (58 ºC, 50% of humidity) [21]. For each formulation, several composite films of 15 mm × 15 mm size and 0.2 mm in thickness were placed in direct contact with compost medium using textile meshes. The compost was composed of 55 wt% of water and 45 wt% of solid (10% of compost (Compo®, Spain), 30% rabbit food, 10% starch, 5% sugar, 4% corn oil, 1% urea, 40% sawdust). Samples of each formulation were withdrawn periodically, and then cleaned and dried at 37 °C under vacuum for 24 h. The degree of disintegrability at different days of composting was determined by normalizing the sample weight to the initial weight.

2.5. Characterization techniques.

2.5.1. Thermogravimetric analysis (TGA).

Thermogravimetric analysis was conducted to evaluate the thermal stability of the obtained biocomposites. The analysis was performed using a TGA Q500 thermal analyzer (TA Instruments, New Castle, DE, USA) under air atmosphere (50 cm^3/min) with a heating rate of 10 ºC/min, ranging from approximately 30 ºC to 800 ºC. The weight-temperature curves were used to calculate the temperatures corresponding to 10% (T_{d10}) and 50% (T_{d50}) weight loss. Additionally, the first derivative of the TGA curves (DTG) was employed to determine the temperatures at the maximum degradation rate (T_{dmax}) for each step of degradation.

2.5.2. Differential scanning calorimetry (DSC).

The glass transition temperature (T_g), crystallization temperature (T_c), melting temperature (T_m) and crystallinity degree (X_c) of the different biocomposites were estimated using a TA Q2000 instrument (TA Instruments, New Castle, DE, USA) under dry nitrogen (50 cm^3/min). The samples were sealed in aluminium pans and equilibrated at -80°C. In the first heating run, the samples were heated to 200 °C at 10 °C/min to remove the thermal history. Subsequently, a cooling scan from 200 °C to -80 °C was performed, followed by a second heating scan from -80 °C to 200 °C at 10 °C/min. The following equation was used to determine the crystallinity, X_c, of the samples:

$$Xc(\%) = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0} \cdot \frac{1}{W_f} \cdot 100 \quad (2)$$

where ΔH_{cc} is the cold crystallization enthalpy, ΔH_m, is the melting enthalpy, ΔH_m^0 is the melting enthalpy for a 100% crystalline and W_f is the weight fraction of PLA in the sample. Values of 93.6 J/g and 114 J/g were used as ΔH_m^0 for PLA [22] and PBAT [23], respectively. Three measurements were performed for each film.

2.5.3. Mechanical Testing.

Dumbbell-shaped samples with dimensions according to UNE-ISO 37 (2013) and a thickness of around 0.2 mm were used for stress-strain measurements. Tensile tests were carried out at room temperature using a DX2000 QTest Elite MTS dynamometer (MTS Systems, Eden Prairie, MN, USA), with a 100 N load cell and at a stretching rate of 10 mm/min. Ultimate tensile strength, Young's modulus and

elongation at break values were determined from the stress-strain obtained curves. At least five specimens were tested for each sample.

2.5.4. Fourier transform infrared spectroscopy (FTIR).

The FTIR spectra of the films obtained from various incubation times under composting conditions were measured using a Perkin Elmer Spectrum Two instrument (Perkin Elmer, Waltham, MA, USA), which was equipped with an attenuated total reflection (ATR) module.

2.5.5. Confocal Raman microscopy.

The distribution of the different components on the films was analyzed using confocal Raman microscopy on a CRM-Alpha 300 RA microscope (WITec, Ulm, Germany) equipped with Nd:YAG laser (maximum power output of 50 mW at 532 nm). Optical resolution of this confocal microscope is, approximately, 250 nm and 700 nm in the longitudinal and transversal directions, respectively. Raman spectra were recorded at room temperature. The reflected laser light at each point was collected using a multimode optical fibre of 25 μm in diameter by a very sensitive CCD detector. Measurements were performed using objectives of 100x with a numerical aperture (NA) of 0.95. Raman images of sample surface (XY Raman images) were measured in an area of 175 μm x 175 μm with 0.15 s of integration time (total of 14400 Raman spectra per Raman image) and Raman depth images (XZ Raman images) were performed in an area of 60 μm x 10 um with 0.3 s of integration time (total of 2400 Raman spectra per Raman image). The acquired Raman spectra were analysed by using Witec Project 2.02 program and Witec Control Plus Software.

2.5.6. Scanning electron microscopy (SEM).

Scanning electron microscopy was employed to study the morphology of the films recovered after incubation in compost and previously coated with gold using a scanning electron microscope Philips XL30 (Phillips, Eindhoven, The Netherlands) with an acceleration voltage of 25 kV.

2.6. Statistical analysis.

Statistical analysis of mechanical and antimicrobial properties data was performed using Origin 2021 (Origin Lab Corporation, Northampton, MA, USA) via a one-

way analysis of variance (one-way ANOVA) with a significance level of P = 0.05 and Tukey's test.

3. Results and discussion.

3.1. Antibacterial activity of E452i microparticles.

The antibacterial activity of E452i microparticles was initially tested by determining the MIC values against three bacterial strains causing foodborne diseases, *S. aureus*, *E. coli* and *L. innocua* [24]. *S. aureus* produces enterotoxins which cause gastrointestinal illness [25]; similarly, listeria and *E. coli* are other bacterial strains linked to food poisoning, which are specially serious for immunocompromised individuals and pregnant women [26], [27]. The MIC values of E452i microparticles were found to be 250 μg mL^{-1} against *S. aureus* and as low as 16 μg mL^{-1} against *L. innocua*, whereas a MIC value higher than 1000 μg mL^{-1} was found against Gram-negative *E. coli*. Once the antibacterial efficiency against Gram-positive bacteria was proved, the particles were tested for their hemotoxicity against human red blood cells. It was determined that they were not hemotoxic with values lower than 5% of hemolysis observed at the highest tested concentration of 10,000 μg mL^{-1}, which is > 40×MIC and 625×MIC of the particles, for *S. aureus* and *L. innocua*, respectively.

3.2. Preparation of biocomposites films based on PLA and Ecovio® and morphological analysis.

These E452i microparticles were then employed as antimicrobial fillers in biocomposites films based on PLA and Ecovio®, using ATBC as biodegradable/biobased plasticizer approved for food contact materials and as food additive. **Figure C3. A2. 1.** shows the SEM image of E452i microparticles with sizes ranging between 8 and 45 μm.

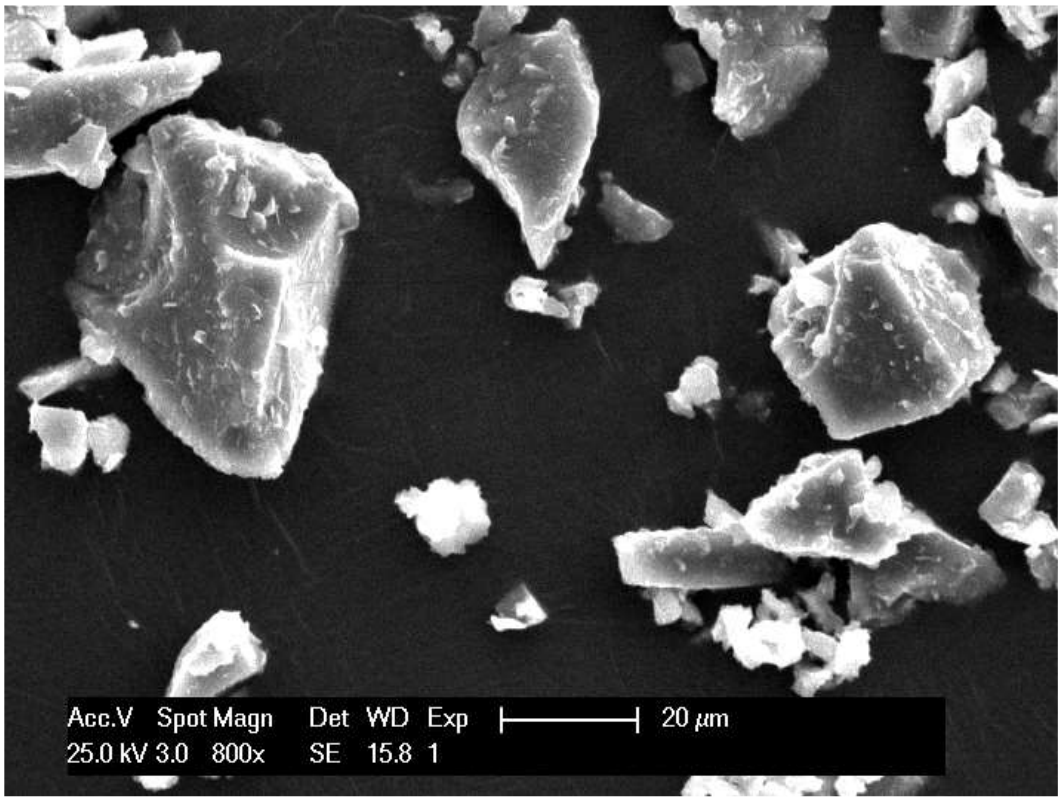

Figure C3. A2. 1. SEM image of E452i microparticles.

Composite films (thickness of about 200 µm) were prepared by melt extrusion followed by compression molding. **Table C3. A2. 1.** shows the composition of the composite samples, where PLAx and ECOx are the composites based on PLA and Ecovio®, respectively, and x is the wt% of microparticles added in the formulation. The compositions of the samples were selected based on previous works of composite materials with antimicrobial properties published by our group [10], [28], [29]. Neat PLA (nPLA) and Ecovio® (nECO) were also processed for comparison purposes.

Table C3. A2. 1. Composition of the prepared composite blends.

Sample	Polymer	wt% Polymer	wt% ATBC	wt% E452i
nPLA	PLA	100	0	0
PLA0	PLA	85	15	0
PLA1	PLA	84	15	1
PLA5	PLA	80	15	5
nECO	Ecovio®	100	0	0
ECO0	Ecovio®	85	15	0
ECO1	Ecovio®	84	15	1
ECO5	Ecovio®	80	15	5

The resulting films were analyzed by Raman confocal microscopy to evaluate the distribution of the different components of composites and their phase morphology. **Figure C3. A2. 2.** shows Raman images (XY and XZ) of PLA1 and ECO1 and the corresponding Raman spectra of each component. In the PLA based film, it is appreciated the presence of E452i particles (signalled in green colour) in the PLA matrix (signalled in red colour). Interestingly, the outermost surface of the inorganic microparticles embedded in the polymer displays a different spectrum (signalled in yellow colour) suggesting that the microparticle surface has suffered a chemical modification probably due to the hygroscopic character of the particles. These new Raman bands between 900-1150 could be associated to PO_4^{3-} anions [30]. In the Ecovio® based film, it is clearly observed the phase-separated microstructure with two main regions, PLA-rich quasi-spherical microdomains (signalled in red colour) homogeneously dispersed on PBAT-rich continuous phase matrix (signalled in blue colour), together with the presence of E452i microparticles. Similarly, the inorganic microparticles present a core-shell structure.

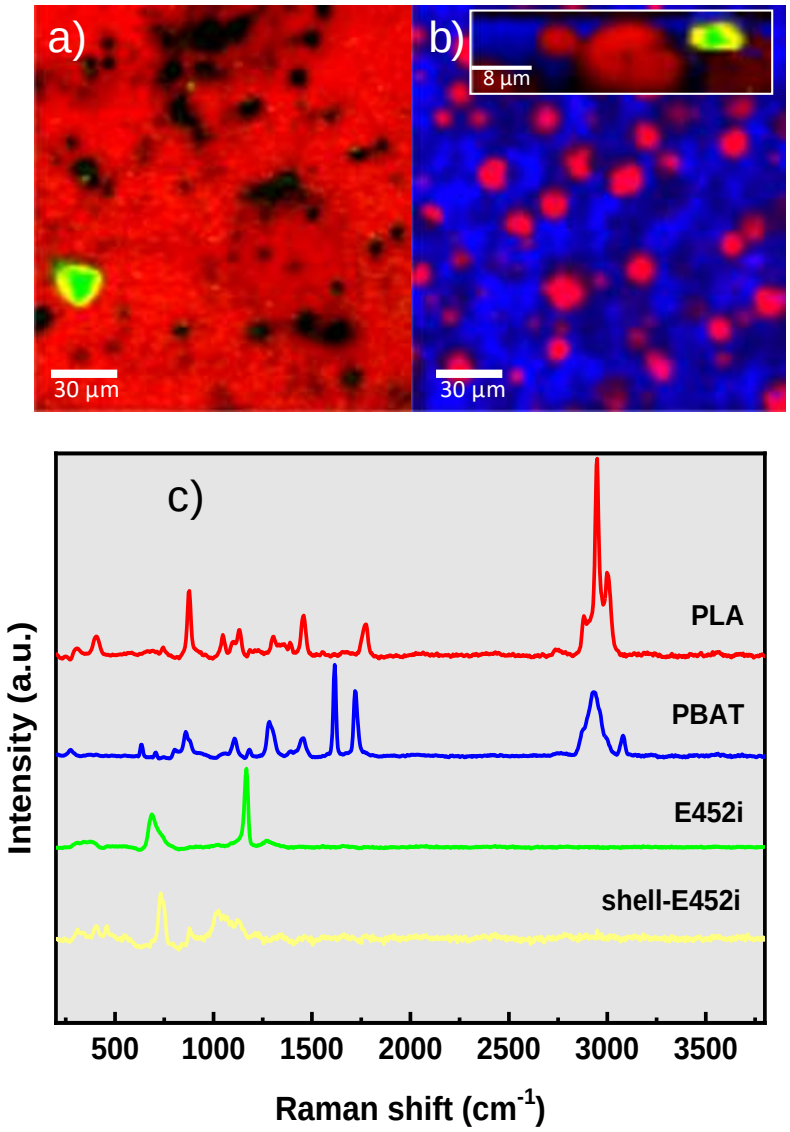

Figure C3. A2. 2. a) and b) Raman image (XY) of PLA1 and ECO1 (inset of Figure C3. A2. 2.b XZ Raman image), respectively. Experiments performed using a 100× objective, 10 mW and 0.6 s of integration time. c) Average Raman spectra of the PLA, PBAT, E452i particles and of the outer layer found in the inorganic microparticles.

3.3. Thermal and mechanical properties of the biocomposites.

Then, the thermal stability and crystallinity degree of the prepared composite films were analyzed to assess their suitability for future applications. Thermal stability of the prepared composites was first studied by TGA. The thermograms of PLAx and ECOx films were displayed in **Figure C3. A2. 3a.** and **Figure C3. A2. 3b.**, respectively and the thermal parameters summarized in **Table C3. A2. 2.**

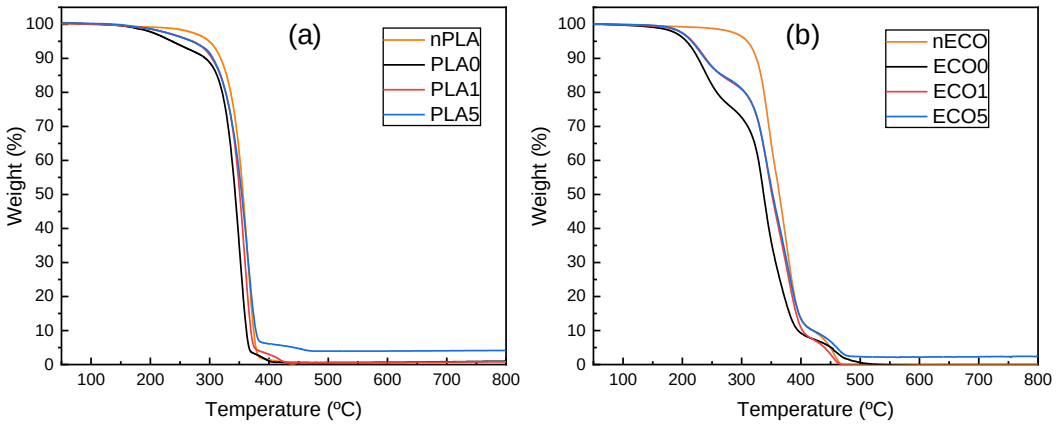

Figure C3. A2. 3. TGA curves of biocomposites films of a) PLAx and b) ECOx under air atmosphere.

Table C3. A2. 2. TGA characterization of PLAx and ECOx based biocomposites.

Sample	T_{d10} (ºC)	T_{d50} (ºC)	T_{dmax}^1 (ºC)	T_{dmax}^2 (ºC)	T_{dmax}^3 (ºC)	T_{dmax}^4 (ºC)
nPLA	318	356	-	364	-	-
PLA0	293	343	226	351		-
PLA1	304	351	227	359	-	-
PLA5	306	354	227	364	-	-
nECO	325	364	-	345	377	456
ECO0	227	337	237	337	372	462
ECO1	238	351	235	344	377	454
ECO5	238	352	232	344	377	462

In the PLAx series, a first degradation step attributed to ATBC plasticizer was showed before the main degradation of PLA matrix. As expected, the thermal stability decreases with the incorporation of the plasticizer. Remarkably, the

addition of E452i microparticles slightly increases thermal stability of the composites, in which T_{d10}, T_{d50} and T_{dmax} values proportionally augment with content of microparticles. The thermal stabilization effect of fillers incorporated in polymer matrix can be ascribed to a thermal barrier effect previously described [31]–[33]. The presence of the filler restricts the polymer chain and hinders the mass transport of degradation products. It is clearly observed that the incorporation of phosphate microparticles into the polymeric films also enhances the thermal stability of ECOx films; however, is lower than neat nECO. In this case, in addition to the first degradation process associated to ATBC, three main weight loss steps were observed under air atmosphere assigned to the PLA/PBAT blend, a step due to PLA phase (T_{dmax}^2 = 337-344 °C), and other two steps at higher temperatures corresponding to the decomposition of PBAT (T_{dmax}^3 = 372-377 °C and T_{dmax}^4 = 454-462 °C) [10].

Subsequently, thermal and crystallization performance of the prepared composites were investigated by DSC analysis. **Table C3. A2. 3.** summarizes thermal characteristics obtained from the DSC thermograms (**Figure C3. A2. 4.**), the glass transition temperatures (T_g), cold crystallization temperatures (T_{cc}), melting temperatures (T_m), and the degrees of crystallinity (X_c) of the films related to the first heating scan.

Table C3. A2. 3. Thermal parameters obtained from the first heating DSC curves of PLAx and ECOx composite films.

Sample	T_g^{PBAT} (ºC)	T_g^{PLA} (ºC)	T_{cc}^1 (ºC)	T_{cc}^2 (ºC)	T_m^{PBAT} (ºC)	T_m^{PLA} (ºC)	X_c^{PBAT} (%)	X_c^{PLA} (%)
nPLA	-	65	103	161	-	174	-	8
PLA0	-	43	88	149	-	169	-	13
PLA1	-	50	88	151	-	169	-	21
PLA5	-	52	89	152	-	171	-	25
nECO	-35	63	105	-	126	146/ 154	1	0.5
ECO0	-46	46	81	-	111	149	4	18
ECO1	-41	47	82	-	116	147	4	21
ECO5	-42	51	82	-	121	148	2	19
ECO5	-42	51	82	-	121	148	2	19

-: not applicable

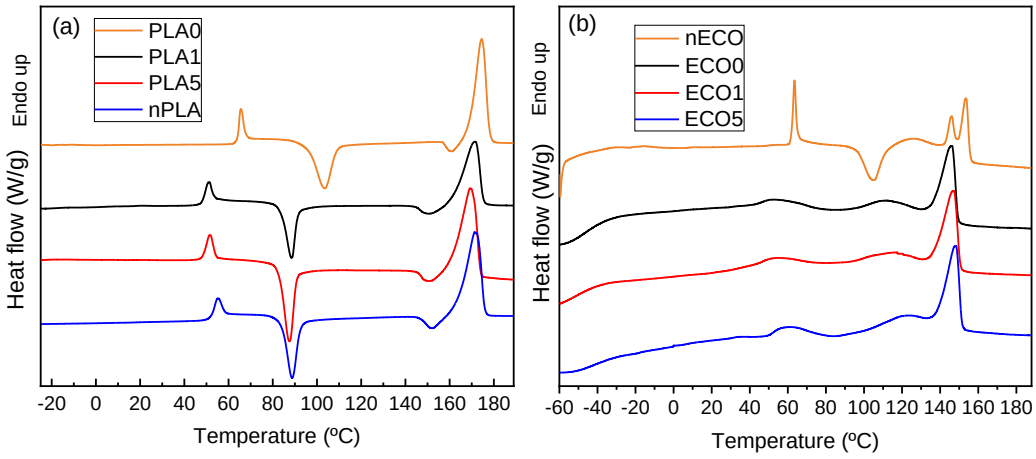

Figure C3. A2. 4. DSC thermograms, first heating curves, of a) PLAx and b) ECOx composite films.

It is clearly observed that all the composites films showed low T_g values due to the presence of ATBC plasticizers, which increases the mobility of the macromolecular chains [34]. Concretely, the addition of ATBC to the PLA based films decreases the T_g to values between 43-53 ºC. On the first heating of DSC curves (**Figure C3. A2. 4a.**), physical ageing is also appreciated in all samples. This occurrence is commonly observed in PLA samples during their storage due to the non-equilibrium state of the polymer below the T_g, associated to the high processing rate and rapid cooling during the compression molding. Remarkably, the T_g value increases with the content of inorganic microparticles in the composites, as the particles lower the mobility of the polymeric chains. Then, a cold crystallization transition is appreciated at temperatures of around 88-89 ºC; which is also low due to the presence of plasticizers [35]. A second crystallization process is appreciated at c.a. 150 ºC associated to the transformation of PLA mesophase into a thermally stable phase (α-crystals) [36]. Finally, the melting process takes place at around 170 ºC. As expected, the PLAx composites develop low crystallinity as PLA has very slow crystallization rates and the compression molding conditions used to fabricate films employed very high cooling rate which does not favour the development of crystallinity. Nevertheless, the incorporation of E452i microparticles slightly increases the degree of crystallinity, which could mean a mild nucleating effect of the particles during cooling from the melt.

In ECOx composites, the T_gs of both blend polymeric components, PLA and PBAT, are clearly observed (**Figure C3. A2. 4a.**), revealing the immiscibility of the two components [37]. Likewise, the addition of E452i microparticles provokes an augment of both T_gs. Besides, two melting points are appreciated at ~112-121 ºC and 148-149 ºC corresponding to PBAT and PLA phases, respectively. The melting region of PBAT and cold crystallization of PLA overlap in the same temperature range, then, the calculation of the degree of crystallization should be considered with caution because we could not consider the cold crystallization of PLA in the calculation of the PLA crystallinity as this transition is overlapped with the melting of PBAT. Subsequently, the mechanical properties of the composites were evaluated by tensile testing. **Table C3. A2. 4.** summarizes the results obtained. In both sets of composites and as expected, it is appreciated that the addition of ATBC

plasticizer increases the elongation at break and reduces the Young's modulus and maximum stress.

Table C3. A2. 4. Mechanical properties of the PLAx and ECOx composites. Mean values ± standard deviation. For each system, values having the different letters are contemplated significantly different for Tukey test (significance level of $P \leq 0.05$).

Sample	Young's Modulus (MPa)	Maximum stress (MPa)	Elongation at break (%)
nPLA	2000 ± 300^{a}	56 ± 5^{a}	4.6 ± 1.1^{a}
PLA0	1550 ± 180^{b}	40 ± 5^{b}	5.4 ± 1.1^{a}
PLA1	1630 ± 110^{b}	42 ± 2^{b}	5.9 ± 1.2^{a}
PLA5	1740 ± 140^{ab}	41 ± 2^{b}	7.4 ± 0.8^{b}
nECO	900 ± 70^{a}	13.9 ± 1.8^{a}	2.9 ± 0.5^{a}
ECO0	130 ± 40^{b}	1.3 ± 0.3^{b}	3.0 ± 1.0^{a}
ECO1	250 ± 40^{b}	2.8 ± 0.6^{b}	4.1 ± 1.2^{a}
ECO5	620 ± 130^{c}	7.9 ± 1.9^{c}	6.2 ± 1.3^{b}

In PLAx composite samples, it is clearly observed that the addition of inorganic microparticles slightly increases the Young's modulus, which can be associated to the higher crystallinity of composite samples and also to the reinforcement properties that the E452i microparticles provide to the PLA matrix. The ultimate tensile strength remained somewhat similar, whereas the elongation at break experiments a slight augment with the incorporation of 5% of microparticles, suggesting a relatively good adhesion between particles and polymer. Similar

behavior was observed in ECOx samples, the Young's modulus, maximum stress and elongation at break increase with the content of particles, which demonstrate that the inorganic microparticles acts as reinforcement.

3.4. Antibacterial properties of the biocomposites films

Once it was proved the improvement of the thermal and mechanical properties of the films due to addition of the phosphate based microparticles, the antimicrobial properties of the films were evaluated in order to assess if the added particles are able to provide antimicrobial activity to the polymeric films. **Figure C3. A5. 3.** depicts the antibacterial activity of the tested composite films, expressed as percentage of bacterial reduction (%) obtained by direct contact against *L. innocua* and *S. aureus* bacterial strains.

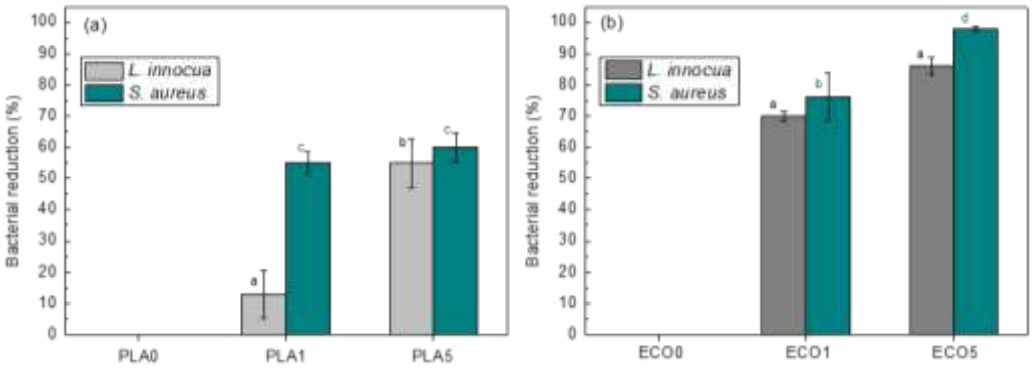

Figure C3. A2. 5. Percentage of bacterial reduction for *L. innocua* and *S. aureus* inoculated with a) PLAx composites and b) ECOx composites. Values having the same letter are not significantly different for Tukey test (significance level of $P \leq 0.05$).

All films containing E452i microparticles demonstrated to be active against both bacterial species and its effectivity increases with the content of particles. The antibacterial activity is slightly higher against *L. innocua* than that against *S. aureus*. The ECOx samples exhibit higher activity than the films based on PLA, which could be associated to the higher flexibility and segmental mobility of PBAT chains in the blend allowing a better diffusion of the antibacterial compounds to the surface. Therefore, the increase in antimicrobial efficacy with the content of microparticles is associated with a higher availability of inorganic compounds allowing them to reach the surface of the film more readily. The inorganic microparticles are discretely distributed in the polymeric matrix and are preferably

inside the film. The interaction of these particles with the moisture on the surface is produced by their high moisture avidity and takes place through the water permeability of these polar polymers, which is relatively high [38]–[40].

3.5. Disintegrability in compost of PLA and Ecovio® based biocomposites.

Next, the behavior of the prepared biocomposites under composting conditions was evaluated and thus, the potential as sustainable materials, as nowadays, this is one of the most desirable properties for food packaging applications. The films were incubated under industrial composting conditions at laboratory scale-level. Polyesters like PLA and PBAT generally undergo degradation through a mechanism involving both enzymatic and non-enzymatic hydrolysis. The rate of biodegradation of these polyesters is influenced by various factors, including environmental conditions, but also the characteristics of the polymers and the presence of additives or fillers. Photographs of biocomposite films incubated at different periods in compost are shown in **Figure C3. A2. 6.**

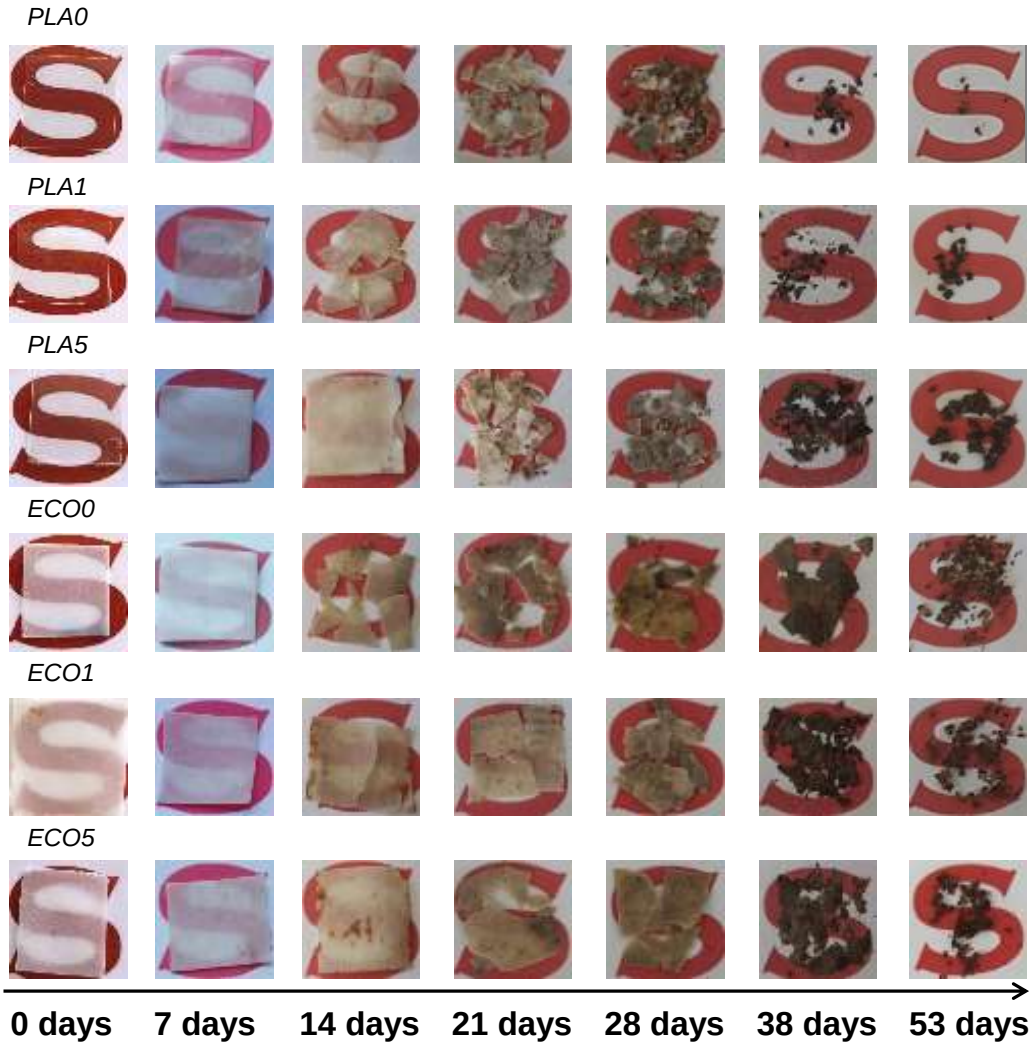

Figure C3. A2. 6. Visual appearance of disintegration under composting conditions of PLAx and ECOx biocomposite films.

After one week of composting, the colour of all the films slightly changes to yellowish and becomes opaque mainly due to the absorption of water and the initial of the hydrolytic degradation process, which produces low molecular weight compounds [41]. Then, the films were gradually degraded with the increase in composting time. The colour of the films progressively turns from yellowish to dark brown, and films become brittle and break into fragments. These alterations are attributed to both, degradation processes and the presence of sawdust. From the photographs it seems that the PLAx films disintegrate faster than the ECOx films as previously observed in literature due to the slow degradation of PBAT [10], [42]. Besides, from the visual appearance of the films, the addition of E452i

microparticles appears to slightly delay biodisintegration. **Figure C3. A2. 7.** also shows the disintegrability of films but in terms of percentage of weight loss as a function of incubation time.

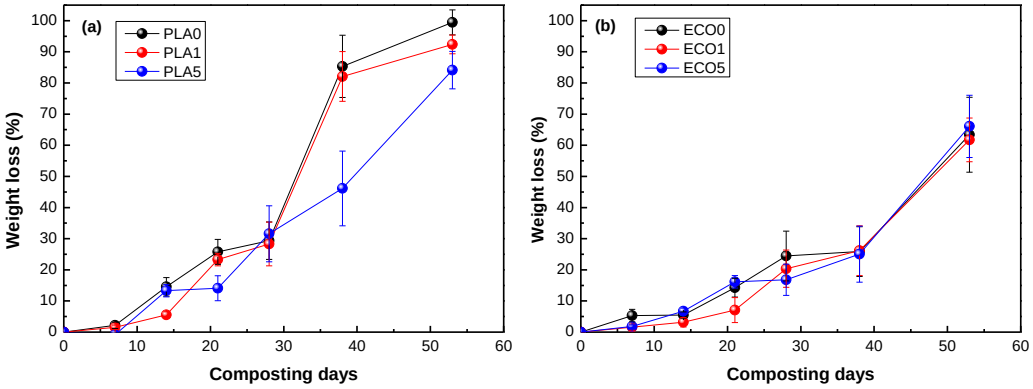

Figure C3. A2. 7. Disintegrability percentage values of PLAx and ECOx composites at different stages of incubation in composting conditions.

In the graph, it can be seen that the differences in disintegration rate are rather small and not significant between neat films and biocomposites containing microparticles. Only the PLA5 sample, with the highest content of particles seems to degrade a bit slowly. However, the weight loss results from day 38 have to be taken with caution because only small fragments of films were recovered, mostly smaller than 2 mm in size (according to the ISO 20200 standard, pieces smaller than 2 mm should be discarded). Then, at this point it is difficult to separate composite fragments from compost particles. The effect of particles addition over bioplastics disintegrability in compost has no clear conclusion, as depends on many factors such as presence of acids or bases, distribution, barrier properties, nature and crystallinity of the polymeric matrix. The degraded films under composting were also analysed by SEM and compared with the original films before composting (**Figure C3. A2. 8.**). Initially, the surface of PLA films is relatively smooth whereas the Ecovio® films show a typical phase separated microstructure as demonstrated above by Raman microscopy. Upon composting, microorganisms and biofilms are visible at the surface of all films and the roughness increases with incubation time as a result of surface erosion. Remarkably, holes are visible in PLA based composites containing the E452i microparticles, due to the loss of the inorganic

microparticles. The ECOx composites films after degradation showed a highly porous structure as a consequence of the favored degradation and the loss of the PLA phase.

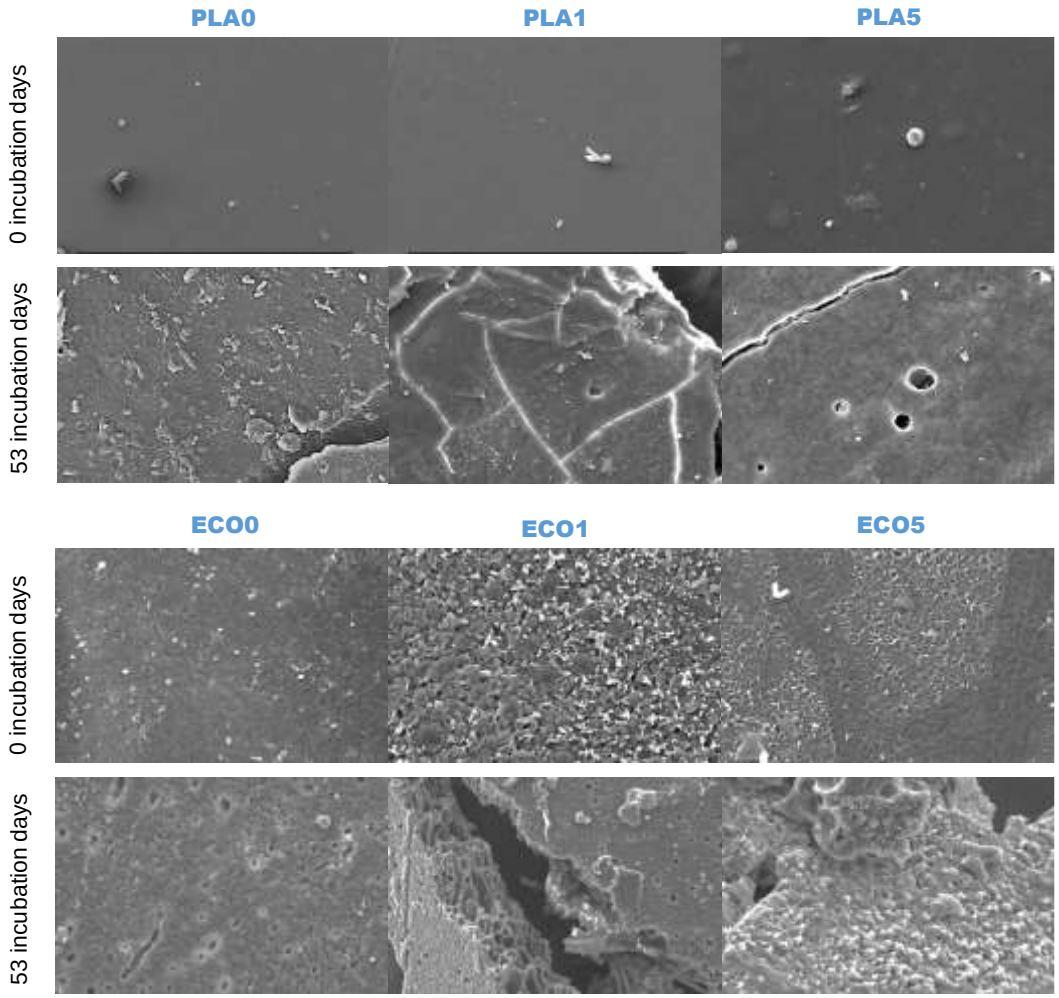

Figure C3. A2. 8. SEM images of films before and after 53 days under composting conditions. The SEM image of PLA0 was taken after 38 days of composting because no rest of film was found after 53 days.

Next, further investigations on the disintegration process were subsequently carried out by chemical analysis of the disintegrated samples. **Figure C3. A2. 9. a-c)** display the FTIR spectra of the PLAx films before and after compost incubation up to 53 days. All initial films, PLA0, PLA1 and PLA5, at 0 days of composting showed the typical stretching of carbonyl group at 1745 cm^{-1} and bands at 1453 cm^{-1} associated to the CH_3 antisymmetric bending vibration and this at 1181 cm^{-1} corresponding to the C–O–C stretching vibration. After degradation, the absorption

spectra of the films did not present significant changes with respect to the original material, because the hydrolytic degradation process of ester bonds produces low molecular weight molecules with absorption bands similar to the characteristic bands of PLA [43]. Nevertheless, during degradation it is clearly observed, a drop in the intensities of some absorption bands and the appearance of new absorption bands, in particular associated to carbonyl groups, which are shifted towards higher values, suggesting that the degradation of PLA is principally due to hydrolysis of ester linkage [44], [45]. Also, an absorption band at ~1720 cm^{-1} corresponding to C=O stretching in the crystalline phase of PLA starts to be more obvious as the degradation process progresses [46], because the degradation occurs firstly in the amorphous region. During the process, it is observed the appearance of another new absorption band at 1635 cm^{-1}, which could be associated to carboxylate ions (R-COO−), whose formation is due to the activity of microorganisms [47]. Thereafter, at 53 days under composting the main peaks cannot be further detected.

In ECOx films, **Figure C3. A2. 9. d-f)**, all films present almost the same spectrum after degradation as original materials, with no substantial differences. This observation has been previously described in other systems based on PLA/PBAT blends; although some absorption bands became weaker, for instance, the absorption band at 720 cm^{-1} due to the external bending vibration of the (–C–H) group on the benzene ring of PBAT [48]. Before degradations, the spectra of ECOx composites reproduce the respective PLA and PBAT absorption bands, although the positions of some bands slightly shift due to the interaction between both polymers, in particular the carbonyl stretching vibration at 1730 and 1710 cm^{-1} for PLA and PBAT, respectively. Remarkably, the relative intensity between these two absorption bands changes during degradation, the absorption band associated to PBAT increases in intensity with respect to the absorption band related to PLA, which means that the degradation of the PLA phase occurs at a faster rate than that of PBAT [10]. No clear differences were observed in the disintegration rate between samples with different content of E452i microparticles.

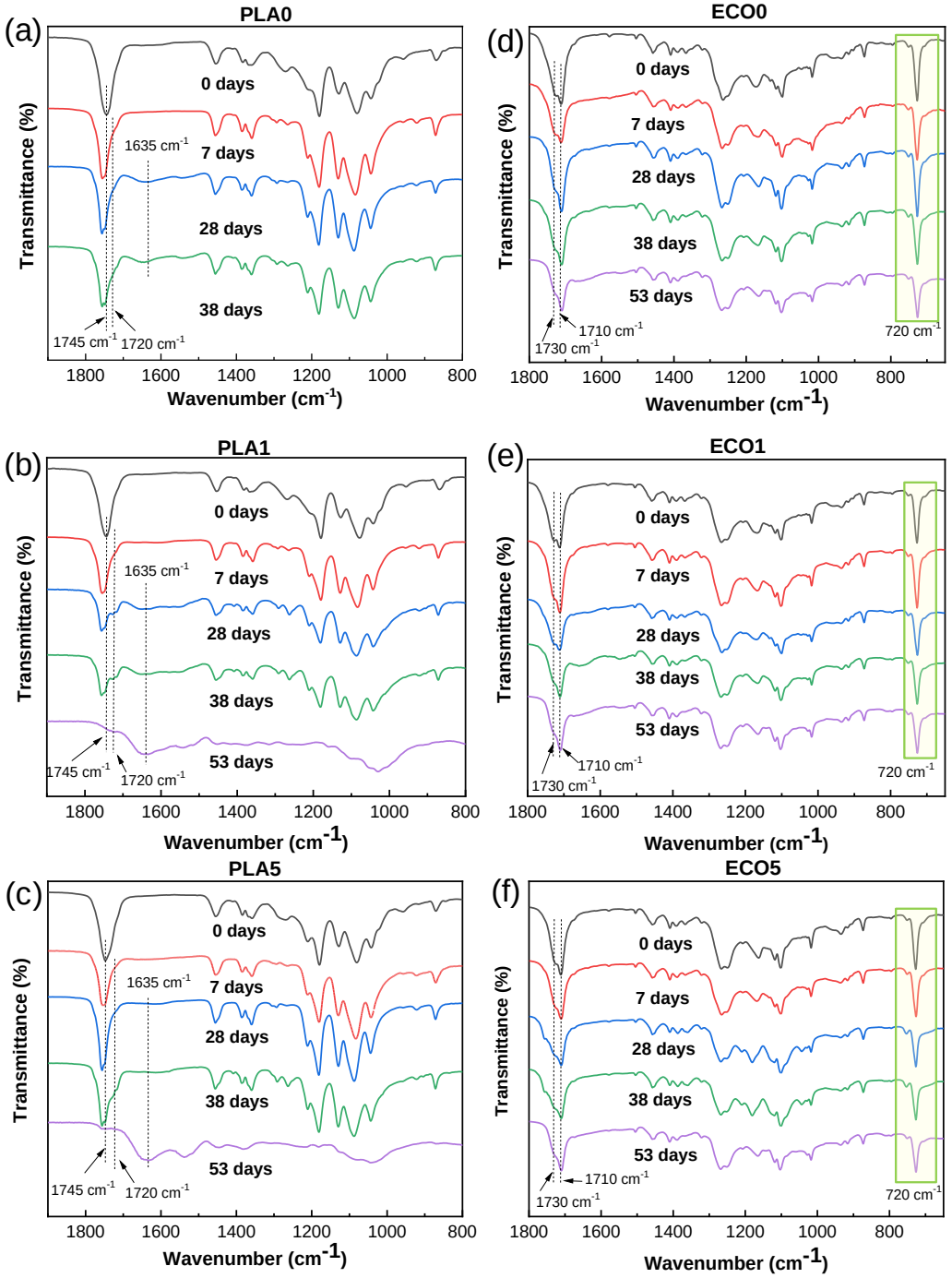

Figure C3. A2. 9. A region of FTIR spectra of PLAx (a-c) and ECOx (d-f) composites films before and after different stages of disintegration in composting conditions.

The films under degradation were also analyzed by confocal Raman microscopy, which can be a powerful analytical tool to investigate the biodegradation process of bioplastics. **Figure C3. A2. 10.** displays the average Raman spectra collected on the surface of the PLAx and ECOx composites taken after different stages of composting.

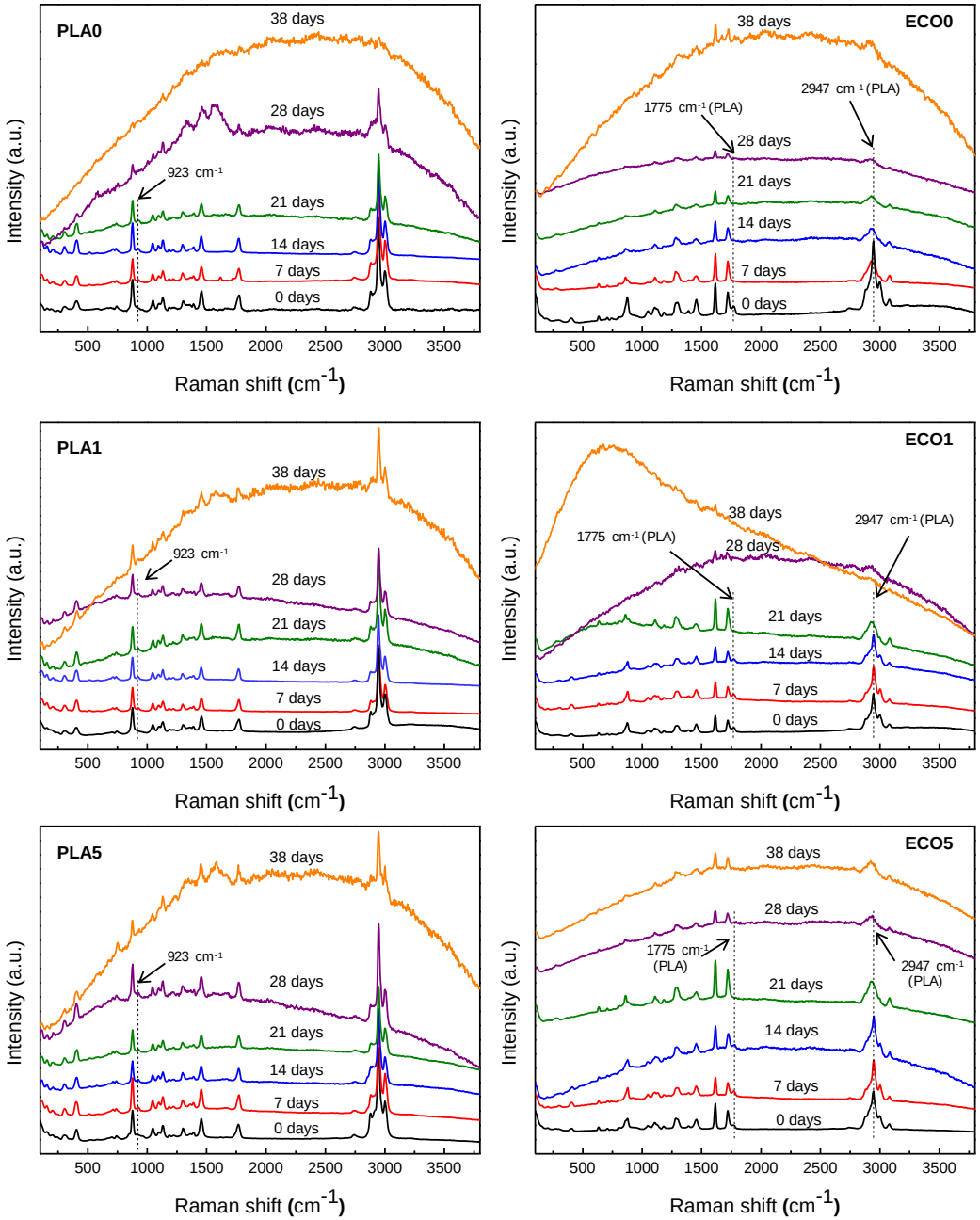

Figure C3. A2. 10. Raman spectra of PLAx and ECOx films before and after different stages of disintegration in composting conditions.

It is clearly observed an increase in fluorescence intensity with the incubation time, which can be associated to the augment of opacity and coloration of films. However, as in the FTIR spectra, no significant changes can be appreciated in the Raman bands during degradation with respect to the spectra of the pristine materials. In PLA based composites, the main Raman bands appeared at 1775 cm^{-1}, which can be attributed to the stretching vibration of the carbonyl bond, at 1453 cm^{-1}, attributed to the asymmetric deformation vibration of methyl and at 874 cm^{-1}, corresponding to the stretching vibration of the C–COO bond. In degraded PLAx samples, it is appreciated more clearly the Raman band at 923 cm^{-1}, assigned to crystalline phase [49], because the preferential degradation of the amorphous phase resulted in an increase in crystallinity. In ECOx films, the main Raman bands are associated to the PBAT phase, at 1718 cm^{-1} ascribed to the carbonyl stretching mode; at 1614 cm^{-1} the stretching vibration of the -C=C- in benzene ring while the Raman band at 1449 can be assigned to the methylene group in plane bending mode. Also, the Raman bands at 1775 cm^{-1}, due to -C=O stretching vibration, and at 2947 cm^{-1} due to symmetrical stretching vibrations of the C–H bond, of PLA is visible at short degradation time, meaning that this Raman band decreases in intensity faster than the Raman bands associated to the PBAT, which also confirm that the PLA degrades faster than the PBAT. Raman images of ECO0 film taken at different time of incubation are showed in **Figure C3. A2. 11.** It is clearly observed the evolution of the PLA domains, that tend to decrease in number and to form larger void regions (signalled in black colour in the Raman images) and then, are less visible because PLA is more easily degradable than PBAT.

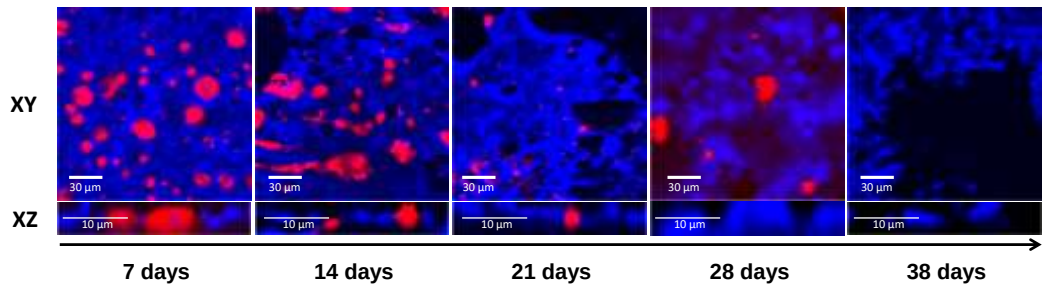

Figure C3. A2. 11. XY and XZ Raman images of ECO0 films at different incubation time in compost conditions.

In some films, it is also visible in the Raman images other compounds which are rather observed in the average Raman spectra, and can be associated to the present of biofilm. As an example, **Figure C3. A2. 12** shows the Raman images of the ECO5 film after 38 days of incubation in compost. The Raman spectrum can be assigned to carbohydrates, proteins, lipids and nucleic acids [50], [51]. The Raman band at around 1600-1670 cm^{-1} can be assigned to the amide I band of proteins, the Raman band at around 1575 cm^{-1} might be due to the δ (NH) deformation vibration and the ν (CN) stretching vibration; the Raman bands located at around 1440-1460 cm^{-1} can be attributed to CH_2 and CH_3 deformation vibration modes in lipids, proteins and carbohydrates. Polysaccharides also exhibit Raman bands at 1127 cm^{-1} and 1100 cm^{-1} (combination of symmetric and asymmetric stretching vibrations of the C–O–C glycosidic linkage).

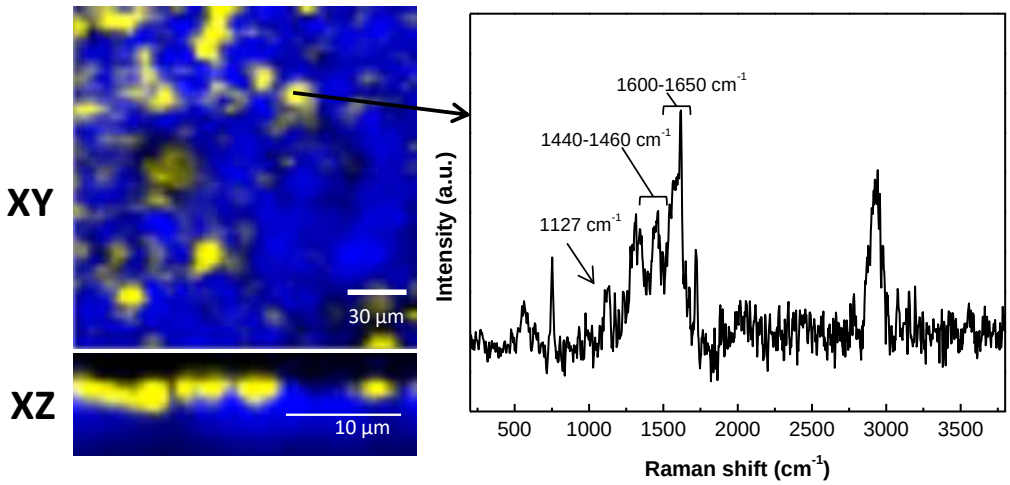

Figure C3. A2. 12. XY and XZ Raman images of ECO5 films after 38 days of composting and average Raman spectrum of yellow signalled regions associated to the present of biofilm.

Then, the films obtained at different incubation times (7, 21 and 38 days) were analyzed by DSC measurements (**Figure C3. A2. 13.**) to study modifications on the crystallinity and on the thermal behaviour of the films as a result of degradation. **Table C3. A2. 5.** summarizes the thermal parameters obtained for each sample, T_g, T_m and degree of crystallinity.

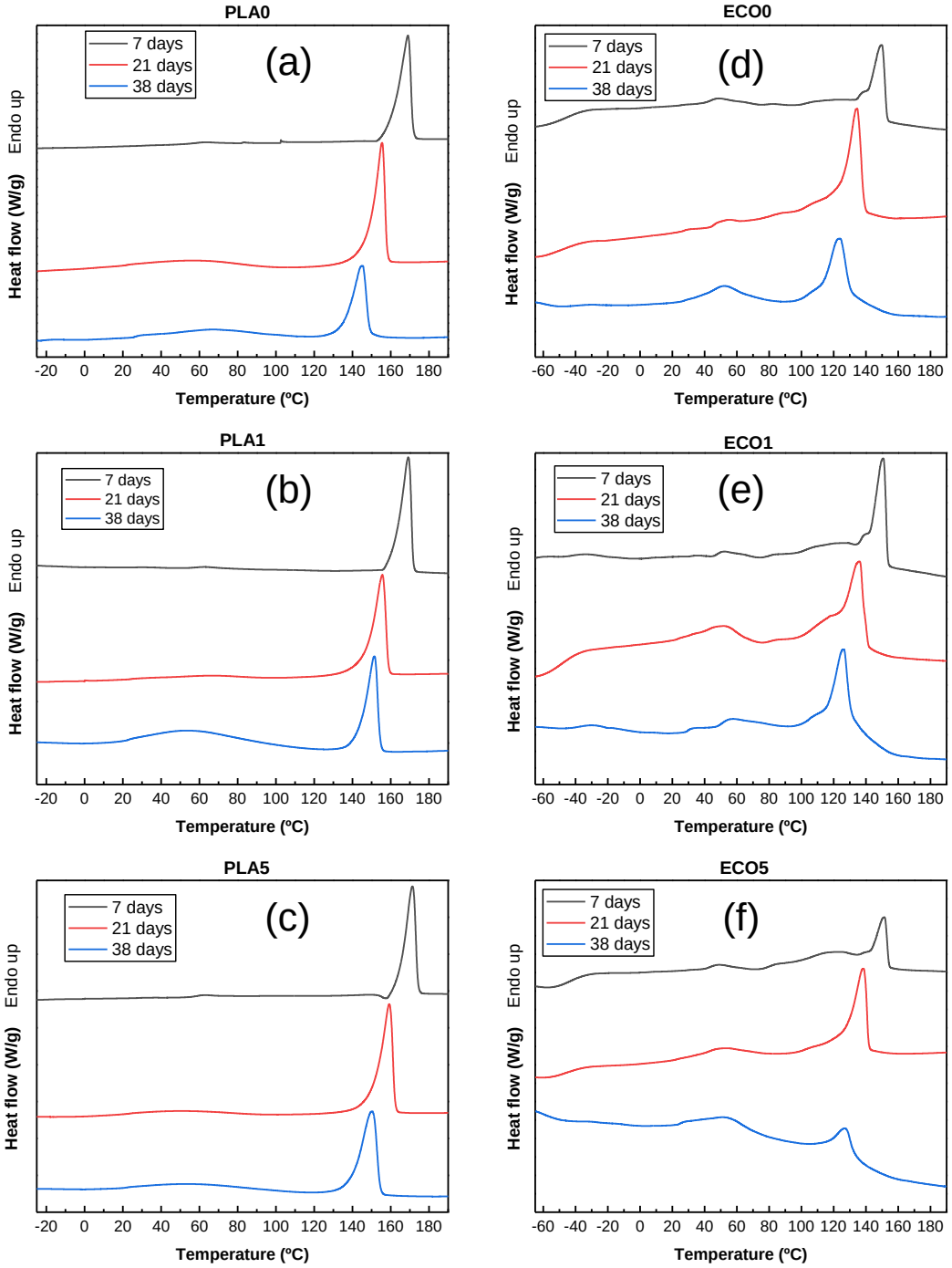

Figure C3. A2. 13. DSC graphs of the first heating of PLAx films (a-c) and ECOx films (d-f) after 7, 21 and 38 days of composting.

Table C3. A2. 5. Thermal parameters of PLAx and ECOx composite films after 7, 21 and 38 days under composting conditions. The glass transition temperatures (T_g) were obtained from the second heating run whereas melting temperatures (T_m) and crystallization degrees (X_c) were obtained from the first heating run. Mean values ± standard deviation. For each sample, values having the different letters are contemplated significantly different for Tukey test (significance level of $P \leq 0.05$).

Sample	T_g PBAT (ºC)	T_g PLA (ºC)	T_m PLA (ºC)	X_c PLA (%)
PLA0 7 days	-	45	168	62
PLA0 21 days	-	49	156	85
PLA0 38 days	-	52	145	88
PLA1 7 days	-	50	169	61
PLA1 21 days	-	48	156	80
PLA1 38 days	-	52	151	96
PLA5 7 days	-	53	171	61
PLA5 21 days	-	50	159	88
PLA5 38 days	-	49	150	93
ECO0 7 days	-38	41	150	n.a.

ECO0 21 days	-37	30	135	n.a.
ECO0 38 days	n.a.	n.a	124	n.a.
ECO1 7 days	-37	45	150	n.a.
ECO1 21 days	-42	42	135	n.a.
ECO1 38 days	n.a.	n.a	126	n.a.
ECO5 7 days	-40	46	151	n.a.
ECO5 21 days	-36	37	138	n.a.
ECO5 38 days	n.a.	n.a.	127	n.a.

-: not applicablen.a.: no analyzed since T_gs are not resolved, whereas X_c: no analyzed due to the overlapping of the peaks.

As a general trend, it can be observed that for all the compositions of PLA based composites, the disappearance of cold crystallization process after 7 days on incubation, together with a clear reduction of the melting temperature [52], a notorious widening of the peak and increment in the melting heat during composting. These changes are associated to the hydrolysis process, leading to the cleavage of polymer chains, particularly in the polymeric amorphous phase. This process significantly increases the crystallinity of the polymer matrix. Additionally, lower molecular weight chains have a higher tendency to crystallize, further contributing to the overall increase in crystallinity. The increase in mobility of polymer chains enables a secondary crystallization of the amorphous region during the composting at 58 ºC, which provokes an increase of the melting heat and the

formation of less perfect crystalline structures that melt at lower temperatures and in a more heterogeneous manner, also appearing another melting transition at low temperature (at around 55-65 ºC for PLA based films). All these processes corroborate the significant increase of crystallinity degree due to the annealing recrystallization at composting condition and to bacteria degradation of the polymeric amorphous phase fraction, which explains the strong embrittlement and fragmentation of composted films. The impact of composting on T_g values is intricate, as the mobility of chains can be influenced by multiple factors that have opposing effects. Shorter chains due to the hydrolysis process have greater mobility and then lower T_g [53]. However, the plasticizer migration and/or degradation together with the increase of the crystallinity degree reduce mobility and therefore, lead to an augment of T_g values. The T_gs were analyzed on the second heating curves as cannot be clearly detected on the first heating. Similarly, in the ECOx composites, the melting temperature of PLA decreases during composting. Multiple endothermic peaks are also observed at lower temperatures that can be ascribed to the melting of PBAT or PLA phases. In these cases, the estimation of the degree of crystallinity cannot be performed accurately due to the overlapping of different transitions. Likewise, the T_gs values of PBAT and PLA phases determined at the second heating DSC curves change during the composting. It is important to remark that these values have to be taken with caution since although the samples are cleaned maybe some unavoidable compounds can alter the thermogram and therefore, the values.

4. Conclusions.

This work highlights the use of the sodium hexametaphosphate microparticles (E452i) as antimicrobial filler in developing bioplastic composites materials for potential food packaging applications. The obtained composites films based on PLA and Ecovio® blend with ATBC as plasticizer and 1 and 5 wt.% of E452i particles exhibit antibacterial activity against food borne bacteria (*S. aureus* and *Listeria*). Besides, the incorporation of the phosphate particles improves the thermal stability and increases the crystallinity of the films, which also has an influence on the mechanical properties leading to an increase of the Young modulus. Related to the disintegration of the samples under composting conditions, the E452i particles

seem to have no significant influence in terms of kinetic and degradation process, then, maintaining the compost disintegradability character of the bioplastics. Therefore, this study has shown the potential of the E452i particles used as bioactive filler in biocomposite films for packaging applications.

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CHAPTER 4

Evaluation of CITREM and ACETEM as Functional Plasticizers in PLA and Ecovio® Biopolymer.

Article 3: Plasticizing PLA with Biobased Fatty Esters: Comprehensive Study on Film Properties.

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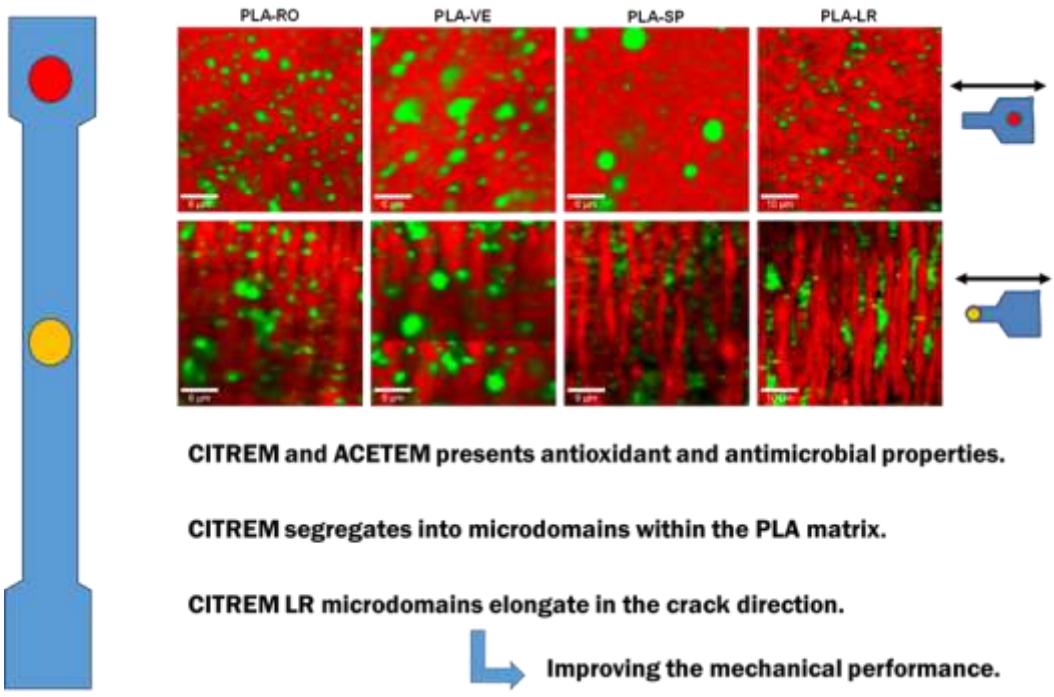
Keywords: bioplasticizers, PLA, Raman Spectroscopy, citric acid ester, acetic acid ester, antioxidant, antimicrobial



Abstract.

ACETEM (E472a) and CITREM (E472c) are fatty acid esters used as additives in the food industry to improve quality, stability and sensory properties of food products due to their emulsifying, stabilizing properties, and antioxidant and antimicrobial activities. Herein, we have explored their use as active plasticizers in one of the most used biobased polymers, polylactic acid (PLA). Initially, different CITREMs (LR10, SP70, RO and VEG) and ACETEM (SOFT-NSAFE), with a variety compositions and physical states at room temperature, were characterized. The fatty acid esters studied demonstrate good thermal stability and moderate to good antioxidant properties. Subsequently, PLA films containing 10% of the tested fatty acid esters were prepared by melt extrusion and posterior compression molding. The obtained films were analyzed by different characterization techniques to evaluate their role as active plasticizers. Confocal Raman microscopy showed that SOFT-NSAFE is homogeneously distributed in the PLA films, whereas CITREMs form microdomains due to their immiscibility with PLA. The incorporation of these plasticizers decreases the tensile strength, Young's modulus and glass transition temperature. However, only CITREM-LR10 is able to significantly enhance the elongation at break of PLA up to 42%, due to the elongation and orientation of the microdomains along the cracks formed during the tensile test. Additionally, their incorporation provides antioxidant properties to the PLA films, being CITREM LR10, SP70 and SOFT-NSAFE that impart higher activity. In terms of antimicrobial activity, CITREM-LR10 showed effectiveness against *S. aureus*, while SOFT-NSAFE was active against *L. innocua* bacteria. These results open the possibility to use such CITREM and ACETEM food additives as plasticizers in films for a variety of applications such as active food packaging.

1. Introduction.

Biobased polyesters have attracted significant attention due to their biodegradability and biocompatibility, offering clear advantages for both consumers and the environment [1]. Among these, polylactic acid (PLA) stands out as a highly promising alternative to petrochemical polymers, primarily due to its biodegradable nature. PLA is derived from lactic acid, obtained through the fermentation of renewable raw materials, and it readily undergoes biodegradation [2], [3]. Recognized as a highly promising and extensively used biodegradable polymer, PLA finds application in food packaging [4]. Its favorable properties include excellent processability, high molecular weight, resistance to water solubility, effective barrier properties against flavors and aromas, thermal stability, and mechanical performance comparable to other polymers commonly used in the food packaging industry, such as poly (ethylene terephthalate) (PET) or polyethylene (PE) [5], [6]. Furthermore, PLA has received Generally Recognized as Safe (GRAS) status from the U.S. Food and Drug Administration (FDA) [7], [8]. Nonetheless, its widespread adoption has been hindered by significant drawbacks, including high costs and intrinsic characteristics such as hardness and brittleness [9], particularly in packaging applications [10].

To improve the main drawbacks of biobased polymers, plasticizers are widely used to increase the flexibility or workability [11]. Plasticizers are typically low molecular weight molecules, and the addition of these materials increases the flexibility and mobility of the polymer chains by lowering the second order transition temperature, also known as glass transition temperature [5], [6]. The interaction occurs in the amorphous region of the polymer, thus a variation in the properties is appreciated. Commonly, characteristics as deformation at break, toughness or flexibility increase with plasticizer addition, contrarily properties as tensile strength, hardness and melt viscosity decrease when plasticizer is added [14], [15].

Plasticization of polymers opens a wide range of new applications and a varied range of products. This ability to provide or improve functionalities to the material offers numerous options for the global market.

In the last few years, the worldwide production of plasticizers has achieved a value of around 5 million tons per year. In 1920 phthalic acid esters were established as plasticizers and are still on use nowadays [12]. Nonetheless, the mayor controversy about these plasticizers and, other generally used, is related to the toxicity caused by their migration, which has driven the development of new commercial non-toxic plasticizers, such as fatty acid esters, benzoates, tartrates and chlorinated hydrocarbons. For instance, esters of adipic, azelaic and sebacic acids are being used to create new advanced materials [15], [16]. Bio-based plasticizers derived from biological sources, have gained interest due to their low toxicity and minimal migration [2], [12], [17], [18]. Plasticizers such as epoxidized triglyceride vegetable oils from soybean oil, linseed oil, castor oil, sunflower oil, and fatty acid esters (FAEs) are included in this group [18]-[20]. Due to their availability, biodegradability, and low toxicity, vegetable oils offer a promising pathway for producing bioplasticizers. Primarily composed of triglycerides and triglycerols, which consist of glycerol and various fatty acids, vegetable oils possess distinct features. Triglycerides notably contain two active sites: the ester group and double bond, which can undergo reactions such as esterification, epoxidation and acetylation, to form more compatible plasticizers or even react with polymer chains [16].

Plasticizers can also serve as functional agents, imparting new properties to materials. Specifically, in the food packaging industry, plasticizers with antioxidant or antimicrobial properties can help to preserve the quality and freshness of packaged products by protecting them against oxidation or microbial contamination. However, only a few examples of bioactive plasticizers are found in the literature [10], [11].

The aim to obtain eco-friendly plasticizers have been carried out to develop new families of materials derived from building blocks as vegetable oils and tributyl citrate, among others. Moreover, molecules derived from citric acid, which are branched structures containing three carboxylic functional groups and hydroxyl groups; or acetic acid, containing carboxylic group, could serve to synthesize valuable plasticizers. In food applications, particularly when focusing on emulsifiers, the free hydroxyl groups of mono- and diglycerides of edible fatty acids can be esterified with short-chain food acids, such as acetic, citric, lactic, or tartaric

acids. This process results in the formation of biobased esters from these different acids [22]. Citric and acetic acids are abundant renewable resources mainly produced via fermentation of biological waste. These compounds can be modified by a esterification, acetylation or butyrylation, to obtain different plasticizers as triethyl citrate (TEC) , tributyl citrate (TBC), acetyl tributyl citrate (ATBC), among others [23]. Besides, citrate-based compounds have been investigated for their antimicrobial and antioxidant properties [24] and some of them has been also evaluated as plasticizers [25], including tributyl citrate (TBC) and acetyl tributyl citrate (ATBC) [26].

In this study, we evaluated the use of bio-based fatty acid esters (typically employed like food additives) as novel bioactive plasticizers with antimicrobial and antioxidant activities in PLA materials for food packaging applications. Concretely, citric acid esters of mono- and diglycerides, also known as citroglycerides or CITREMs (designated as E472c) and acetic acid esters of mono-and diglycerides of fatty acids, also known as ACETEM (designated as E472a), are widely used emulsifiers and thickeners in the food industry [2], also presenting antioxidative properties [22], [27]. CITREMs are, essentially, blends of diverse glycerides produced through the reaction of citric acid or its anhydride with acylglycerols (containing edible oils, combinations of diacylglycerides and/or monoacylglycerides). Throughout the reaction, citric acid naturally engages with more than one glycerol backbone, leading to variations in the composition of CITREM based on factors like reaction time, temperature, and the ratio between raw materials. Also, ACETEMs are based on distilled monoglycerides and products with a high percentage of acetylation of free hydroxyl groups available [28]. Importantly, CITREM is recognized as a food substance with very low toxicity, and its presence in foods is not hazardous to human health. Furthermore, it holds the designation of being "generally recognized as safe" (GRAS) [29].

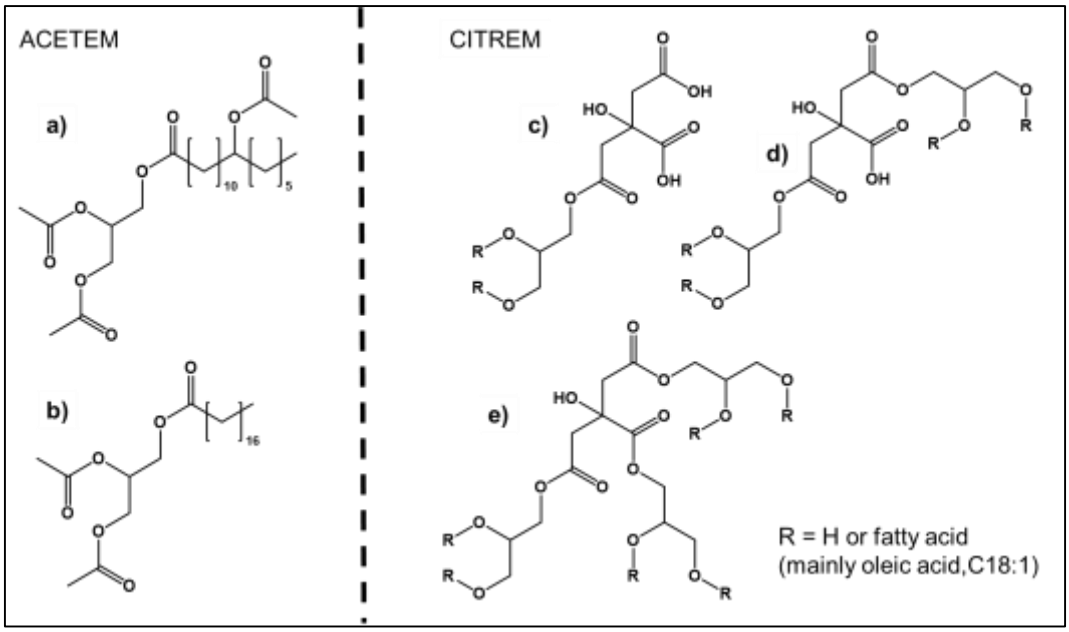


Figure C3. A3. 1. Chemical structure of the main components the tested plasticicizers of ACETEM based on (a) 12-hydroxystearic acid and (b) stearic acid and CITREM based on (c) mono-esterified citric acid, (d) di-esterified citric acid) and (e) tri-esterifies citric acid.

Accordingly, the objective of this work was to evaluate different citric and acetic acid esters products (CITREM and ACETEM) as active bioplasticizers in films of PLA. The mechanical and thermal properties were extensively investigated by different techniques. Additionally, the distribution of the plasticizer on the polymer matrix was also analyzed. Finally, the antimicrobial and antioxidant properties of these films were studied.

2. Experimental part.

2.1 Materials.

Poly (lactic acid) (PLA) pellets (ErcrosBio® LL652; density: 1.25 g/cm^3; melt flow indexing: 11 g/10 min) were obtained from Ercros (Barcelona, Spain), whereas the fatty acid esters were complimentary supplied by International Flavors & Fragrances Inc. (see **Table C3. A3. 1.** for more specifications):

GRINDSTED® ACETEM SOFT-NSAFE (SOFT): an acetic acid ester of monoglycerides made from fully hydrogenated castor oil.

GRINDSTED® CITREM LR 10 EXTRA KOSHER (LR10): is a citric acid ester of mono-diglyceride (E 472c) made from edible, refined sunflower oil. This product also contains alpha-tocopherol (E 307) max. 200 ppm and ascorbyl palmitate (E 304) max. 200 ppm.

GRINDSTED® CITREM RO SG: is a neutralized citric acid ester of monoglyceride made from hardened palm oil. This product also contains potassium carbonate (E501).

GRINDSTED® CITREM SP 70 MB: is a citric acid ester of mono-diglyceride made from edible, refined sunflower and palm-based oil. This product also contains alpha-tocopherol (E 307) max. 300 ppm and ascorbyl palmitate (E 304) max. 300 ppm.

GRINDSTED® CITREM 12 VEG-SG: is a partially neutralized citric acid ester of mono-diglyceride made from edible, fully hydrogenated palm-based oil.

Table C3. A3. 1. The state of the materials at room temperature (20 ºC) and acid value of the plasticizers.

Plasticizer	State at 25 ºC	Acid value	Oil precursor
CITREM-RO	Powder	15-32	Palm
CITREM-VE	Powder	10-25	Palm
SOFT-NSAFE	Liquid	Max. 3	Castor
CITREM-LR10	Viscous	20-40	Sunflower
CITREM-SP70	Paste	10-20	Sunflower/palm

2.2. Films preparation.

Initially, PLA pellets and plasticizers were dried at 40 ºC for 24 h in an oven and, before extrusion, both components (PLA and plasticizers) were premixed. Then,

the mixtures were fed into a micro-extruder equipped with a co-rotation twin-screw (HAAKE Minilab II Thermo Scientific, Karlsruhe, Germany) and a capacity of 7 cm^3, operating at a screw speed of 100 rpm and a temperature of 180 ºC for 3 min. From the extruded materials, films of 5 cm × 5 cm dimensions and approximately 200 μm of thickness were prepared by compression molding using a CollinP200P press (COLLIN Lab & Pilot Solutions, Maitenbeth Germany). A pre-heating treatment at 190 ºC under 1 bar pressure was performed for 2 min, followed by a compression process applying 100 bar for 3 min at 190 ºC. And finally, a cooling step at 100 bars was done.

2.3. Characterization techniques.

2.3.1 Fourier transform infrared spectroscopy (FTIR).

FTIR spectra of the plasticizers were recorded with a Perkin Elmer Spectrum Two instrument equipped with an attenuated total reflection (ATR) module.

2.3.2. Gas chromatography-mass spectrometry (GC-MS).

The main components of each plasticizer were analyzed by GC-MS. Briefly, 2 mg of plasticizers were dissolved in 1 mL of dichloromethane then, the solution was injected in an AGILENT8860 GC gas chromatograph equipped with an Agilent mass spectrometry detector model 5977B (GC-MS). The injection was carried out in split mode with a split ratio of 20:1 at 260 ºC. The separation of the compounds was performed on a HP-5MS capillary column (30 m length × 250 μm internal diameter and 0.25 μm d_f), using helium with a flow rate of 1 mL/min. Compound identification was achieved by matching with the NIST 20 MS library.

2.3.3. Thermogravimetric analysis (TGA).

Thermogravimetric analysis was carried out to study the thermal stability of the fatty acid esters and the different blends. Approximately 5 mg of the samples were tested in a TGA Q500 thermal analyzer (TA Instruments) at a heating rate of 10 ºC min^{-1} from room temperature (~30 ºC) to 800 ºC, under air atmosphere (50 cm^3 min^{-1}). From the mass-temperature curves of the samples, the temperatures at 10% (T_{d10}) and 50% (T_{d50}) of mass loss were calculated, whereas, the temperatures at the maximum degradation rate (T_{dmax}) of the samples were determined from the first derivative of the TGA curves (DTG).

2.3.4. Differential scanning calorimetry (DSC).

The glass transition temperature (T_g), cold crystallization temperature (T_{cc}), melting temperature (T_m) and crystallinity degree (X_c) of the different films were determined using a TA Q2000 instrument (TA Instruments) under dry nitrogen (50 cm^3min^{-1}). To characterize plasticizers, solid fatty acid esters were sealed in aluminum pan, while liquid and viscous samples were sealed in hermetic aluminum pan. The first scan was performed from -60 °C to 180 °C at 10 ºC min^{-1}. Subsequently, a rapid cooling scan from 180 °C to -60 °C was performed, followed by a third heating scan from -60 °C to 180 °C at 10 °C/min. Additionally, the PLA samples were sealed in an aluminum pan and equilibrated at 65 °C, then, the samples were rapidly cooled to 0 °C. The reason to do a first heating up to 65 ºC is to avoid the presence of physical aging. This phenomenon is common in PLA samples with T_gs relatively close to room temperature. A second scan was performed from 0 °C to 180 °C at 10 ºC min^{-1}. Subsequently, a rapid cooling scan from 180 °C to 0 °C was performed, followed by a third heating scan from 0 °C to 180 °C at 10 ºC min^{-1}. Value of 93.6 J/g was used as melting enthalpy for a 100% crystalline, ΔH_m^0, for PLA [30], and the following equation was used to determine the crystallinity, X_c, of the samples:

$$X_c(\%) = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0} \cdot \frac{1}{W_f} \cdot 100 \quad (1)$$

where ΔH_{cc} is the cold crystallization enthalpy, ΔH_m, is the melting enthalpy and W_f is the weight fraction of PLA in the sample.

2.3.5. Strain-stress tests.

Films were punched with a cutter into dumbbell-shaped samples with dimensions according to UNE-ISO 37 (2013) and a thickness of around 200 µm. These samples were used for stress-strain measurements. Tensile measurements were carried out using a DX2000 QTest Elite MTS dynamometer (MTS Systems, Eden Prairie, MN, USA), working at a stretching rate of 10 mm min^{-1} with a 100 N load cell at room temperature. Ultimate tensile strength, Young's modulus, and elongation at break values were calculated from the stress-strain curves obtained from the samples. At least five specimens were tested for each material.

2.3.6. Microhardness.

Microindentation measurements were conducted at 23 ºC using a Vickers indenter attached to a Leitz microhardness tester. A contact load of 0.4809 N was applied for a duration of 25 seconds. Microhardness (MH) values, expressed in MPa, were determined using Equation 2:

$$MH(MPa) = \frac{2P}{d\ sin^2 68°} \quad (2)$$

where P (in N) represents the contact load and d (in mm) denotes the diagonal length of the projected indentation area. Diagonals were measured in reflected light mode within 30 seconds of load removal, utilizing a digital eyepiece equipped with a Leitz computer-counter-printer (RZA-DO).

2.3.7. Confocal Raman microscopy.

The films were also analyzed using confocal Raman microscopy on a CRM-Alpha 300 RA microscope (WITec, Ulm, Germany) equipped with Nd:YAG laser (maximum power output of 50 mW at 532 nm) and an optical resolution of approximately, 250 nm in the longitudinal and 700 nm in the transversal direction. The reflected laser light at each point was collected using a multimode optical fiber of 25 μm in diameter equipped with a very sensitive CCD detector. Measurements were performed using objectives of 100× with a numerical aperture (NA) of 0.95. Raman depth images (XZ Raman images) were measured in an area of 75 μm × 25 μm with 0.30 s of integration time (total of 2700 Raman spectra per Raman image) and Raman of the surface (XY Raman images) were performed in an area of 25μm × 25 μm with 0.3 s of integration time (total of 10000 Raman spectra per Raman image). The acquired Raman spectra were analyzed by using Witec Project 2.02 program and Witec Control Plus Software.

2.3.8. X-ray diffraction (XRD).

X-ray diffraction patterns of fatty acid esters and prepared films were obtained using a Bruker D8 Advance diffractometer provided with a PSD Vantec detector (from Bruker, Madison, Wisconsin) at room temperature in the reflection mode fitted with a CuK_α radiation (λ=0.15418 nm) operated at 40 kV. Samples were scanned between 2º and 30º (2θ) with a scanning step of 0.02º, and a collection time of 10 s per step.

2.3.9. Contact angle.

The relaxation of the contact angle of a water droplet on films was assessed using the sessile drop method in a KSV Theta goniometer. A 3 µL drop of water was placed over the film with an initial contact angle (t = 0), drop angle was measured within less than 5 seconds and estimated based on the image of the droplet. The values were obtained by averaging five measurements per sample.

2.4. Antioxidant activity.

The antioxidant properties of plasticizers and films were assessed using the colorimetric Blois assay, employing the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Tested samples (4 mg of plasticizers or 100 mg of plasticized films) were placed in contact with 2 mL of DPPH methanol solution (24 mg of DPPH in 100 mL of methanol). The reaction was initiated, and at various time points, 100 µL of each mixture was transferred to a multi-well plate and the absorbance was measured at 527 nm using a BioTek® SYNERGY HTX multi-mode reader spectrophotometer. The solution, after each measurement, was returned to the original vials to maintain consistent concentration and volume. Measurements were taken at intervals over 24 hours. Then, the antioxidant activity of the samples was expressed as the percentage reduction of the DPPH free radical, which is determined by the ratio of the absorbance value of the sample solution (A_m) to the absorbance value of the solution without the sample (A_0). The measurements were performed at least in triplicate.

2.5. Biological Assay.

The antibacterial properties of the obtained films were tested against *Staphylococcus aureus* (*S. aureus*, ATCC 29213*) and Listeria innocua* (*L. innocua*, ATCC 33090) using the E2149-20 standard method from the American Society for Testing and Materials (ASTM) for determining the antimicrobial activity of samples under dynamic contact conditions [31]. In this analysis, bacterial suspension was prepared in PBS at a concentration of around 10^6 colony-forming units (CFU) mL^{-1}. Then, 100 mg of each plasticized films were placed in sterile Falcon tubes containing 1 mL of the bacterial suspension inoculum and 9 mL of PBS to obtain a working concentration of around 10^5 CFU mL^{-1}. Blank experiments with only the inoculum, viz. in the absence of films, were also performed. Subsequently, suspensions were incubated for 24 h at 37 ºC with shaking at 120

rpm. The number of bacteria remaining in each sample was determined by the plate counting method. The test was performed in a 96-well plate in which 100 µL of the suspension were added on the first column and 90 µL of PBS to the rest of the wells. Serial dilutions of 10 µL from the previous column were made from columns #1 to #4 Subsequently, 50 µL of each dilution were placed on 5% sheep blood Columbia agar plates and incubated for 24 h at 37 ºC. The measurements were made at least in triplicate.

3. Results and discussion.

3.1. Characterization of the plasticizers.

3.1.1 Study of the composition of plasticizers by FTIR and gas chromatography.

The FTIR spectra obtained from all the tested plasticizers derived from the fatty acids are similar as seen in **Figure C3. A3. 2.** The stretching vibrations corresponding to CH, CH_2 and CH_3 groups around 2918-2854 cm^{-1} from the long chains of fatty acids [32], [33] and the characteristic peaks related to the stretching vibration of the C=O bond of the ester group at 1734 cm^{-1} and C-OH stretching at 1105 cm^{-1} attributed to the citric acid [34], are visible in all the spectra. CITREM-LR10 and CITREM-SP70 showed a band at 3472 cm^{-1} related to the O-H bond, which could be associated with the alpha–tocopherol [35]. Plasticizers SOFT-NSAFE and CITREM-LR10 exhibit both, the peaks at 3300 and 3230 cm^{-1} due to OH stretching vibration of free hydroxyl groups; whereas, in the SOFT-NSAFE, additionally appeared a band at 1219 cm^{-1} corresponding to the C-C-O of the ester [36] and a band around 607 cm^{-1} associated to the O-C=O deformation and C=O out of plane wag.

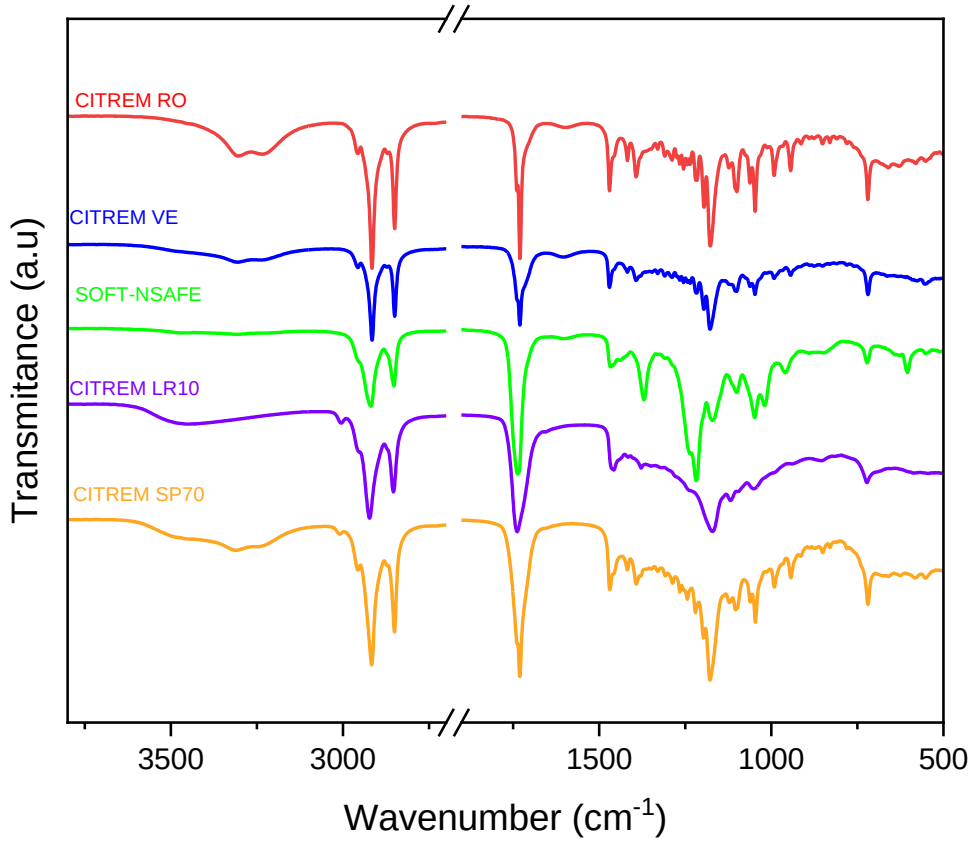


Figure C3. A3. 2. FTIR spectra of the different tested fatty acid esters.

To evaluate the main compounds of the fatty acid esters, GC-MS was used. As shown in **Table C3. A3. 2.** there are some molecules in common for all CITREMs and SOFT-NSAFE, but also some differences can be observed between them. It is worthy to note that, the two major components of SOFT-NSAFE are only present in CITREM-LR10, whereas alpha tocopherol (a well-known antioxidant) is only found in CITREM-LR10 and CITREM-SP70. Such differences would affect the bioactive properties of the fatty acid esters and thus, of the final films.

Table C3. A3. 2. Main components of CITREM and SOFT-SAFE plasticizers obtained by GC-MS.

RETENTION TIME (MIN)	COMPONENT	CITREM-RO	CITREM-VE	CITREM-LR10	CITREM-SP70	SOFT-SAFE
17.25	n-Hexadecanoic acid	X	X		X	
19.43	Octadecanoic acid	X	X		X	
20.72	Glycidyl palmitate	X	X		X	
21.86	Cyclohexane, 1,3,5-triphenyl-	X	X			
22.40	9-Octadecenoic acid (Z)-, oxiranylmethyl ester			X	X	
22.66	Glycidol stearate	X	X			
22.75	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	X	X		X	
23.74	Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester	X	X			
24.39	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester				X	
24.58	Hydrocinnamic acid, o-[(1,2,3,4-tetrahydro-2-naphthyl)methyl]-			X		
25.25	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester			X	X	
26.09	Octadecanoic acid, 2,3-bis(acetyloxy)propyl ester			X		X
26.47	Diethyl n-hexadecylmalonate			X	X	
27.87	Alpha-tocopherol			X	X	
28.13	3-((12-Acetoxyoctadecanoyl)oxy)propane-1,2-diyl diacetate			X		X
33.67	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	X	X		X	
34.77	rac 1-Oleoyl-2-palmitoylglycerol			X		
34.91	Not identified (PPM=578)	X	X			
35.89	9-Octadecenoic acid (Z)-, 2-hydroxy-1,3-propanediyl ester			X		
36.12	Not identified (PPM=606)	X	X			

3.1.2 Thermal properties.

Then, thermal properties of the proposed fatty acid esters were characterized by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The fatty acid esters were examined at various temperatures of significance: the mass loss (m_{180}) at the melt extrusion temperature (T=180 ºC) to assess material thermal degradation during the extrusion process, as well as temperatures corresponding to 10% (T_{d10}) and 50% (T_{d50}) mass loss. Additionally, the temperature associated with the peak degradation rate (T_{dmax}) was determined from the derivative of the TGA curve (DTGA). Thermal parameters of the fatty acid esters are collected in **Table C3. A3. 3.** The obtained results showed that at melt extrusion temperature (T=180 ºC) no evidence of degradation is observed in the TGA curves since the mass loss is very small. Therefore, all fatty acid esters remain stable at processing temperatures.

Table C3. A3. 3. Thermal characteristics of fatty acid esters by DSC and TGA.

	TGA				DSC	
Plasticizer	**m_{180} (%)**	**T_{d10} (ºC)**	**T_{d50} (ºC)**	**T_{dmax} (ºC)**	**dT/dm (%/ºC)**	**T_m (ºC)**
CITREM-RO	98	233	326	333	0.013	62
CITREM-VE	98	238	320	324	0.007	63
SOFT-NSAFE	99	228	264	282	0.01	1
CITREM-LR10	97	236	313	287	0.005	5/11
CITREM-SP70	98	239	329	297	0.006	15/47

Furthermore, DSC measurements were used to investigate the thermal transitions of the fatty acid esters (**Table C3. A3. 3.**). Melting temperatures (T_m) were evaluated on the first scan and were visible for all the materials. CITREM-RO and CITREM-VE exhibit relatively high melting temperatures at 62 ºC and 63 ºC, respectively; while CITREM-LR10 and CITREM-SP70 exhibit two melting peaks at lower temperatures, -5 ºC/11 ºC for CITREM-LR10 and 15 ºC/47 ºC for CITREM-SP70. Finally, SOFT-NSAFE presented only one melting peak at 1 ºC.

The melting point of these materials can explain the different states they exhibit at room temperature (see **Table C3. A3. 1.**).

3.1.3 Antioxidant properties.

In addition, the antioxidant action was studied in a DPPH-methanol solution for 24 hours. As can be seen in **Figure C3. A3. 3.**, all the proposed fatty acid esters exhibit antioxidant activity, capturing the free radicals of DPPH-solution in a reduction process. The CITREM-SP70 presented higher values of reduction capacities around 93.6 ± 0.8%, followed by CITREM-LR10 with a value around 62.3 ± 1.8% at 24 hours. These products contain alpha-tocopherol (E307: 300 and 200 ppm, respectively) and ascorbyl palmitate (E304i, 300 and 200 ppm, respectively), which are known as antioxidant additives [17], [18], [37]. On the other hand, CITREM-VE and CITREM-RO present values of the reduction capacity of 56.4 ± 3.3% and 48.9 ± 2.1%, respectively. These compounds are neutralized citric acid ester of mono-diglyceride from different palm-based oils with a minimum content of 13% of citric acid (E472c), which is also known to act as an antioxidant additive. Finally, SOFT-NSAFE, which is an acetic acid ester of monoglycerides showed the lowest value around 18.9 ± 3.1%. Nonetheless, differences in the results between the fatty acid esters could be explained not only by the variation in their compositions, but also by the differences in the surface area in contact with the DPPH-methanol solution due to the different physical states of these compounds at room temperature, since the surface area in contact of powder is different from a non-miscible liquid.

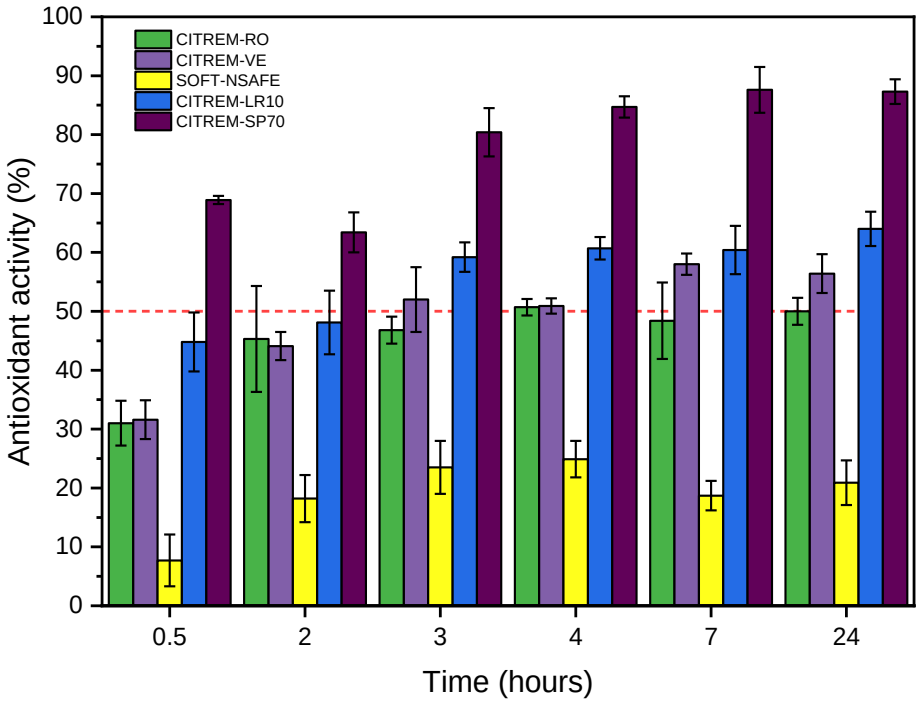


Figure C3. A3. 3. Antioxidant properties of the different plasticizers at different contact time with the DPPH solution.

3.2. Preparation of plasticized films based on PLA and morphological analysis.

Then, fatty acid esters were employed as antioxidant and potential antimicrobial compounds on PLA based films. Composite films (with a thickness of about 200 µm) were prepared by a melt extrusion process followed by compression molding. **Table C3. A3. 4.** shows the composition of the samples, where PLA-xx referred to the bioplasticizer used at a concentration of 10%.

Table C3. A3. 4. Composition of the prepared composite blends.

Sample	Biopolymer	Plasticizer
PLA	PLA 100%	-
PLA-RO	PLA 90%	CITREM RO 10%
PLA-VE	PLA 90%	CITREM VE 10%
PLA-SS	PLA 90%	SOFT-NSAFE 10%
PLA-LR	PLA 90%	CITREM LR10 10%
PLA-SP	PLA 90%	CITREM SP70 10%

Initially, confocal Raman microscopy was used to study the distribution of the plasticizers within the PLA films. The spectra of distinct components of films are displayed in left part of **Figure C3. A3. 4.** As previously reported, SOFT-NSAFE seems to be miscible with the PLA, a homogeneous distribution is observed (see right part of **Figure C3. A3. 4.**, PLA-SS). In contrast, CITREMs exhibited a heterogeneous distribution, by the formation of microdomains of plasticizer. The segregation occurs due to the lower miscibility of these fatty acid esters in the matrix PLA. In the right part of **Figure C3. A3. 4.**, XZ images for PLA-VE and PLA-LR, as examples, are shown. It can be seen a continuous phase of PLA (corresponding to red signal) and the segregated bioplasticizer microdomains (corresponding to green signal) with different sizes, depending of the type of CITREM.

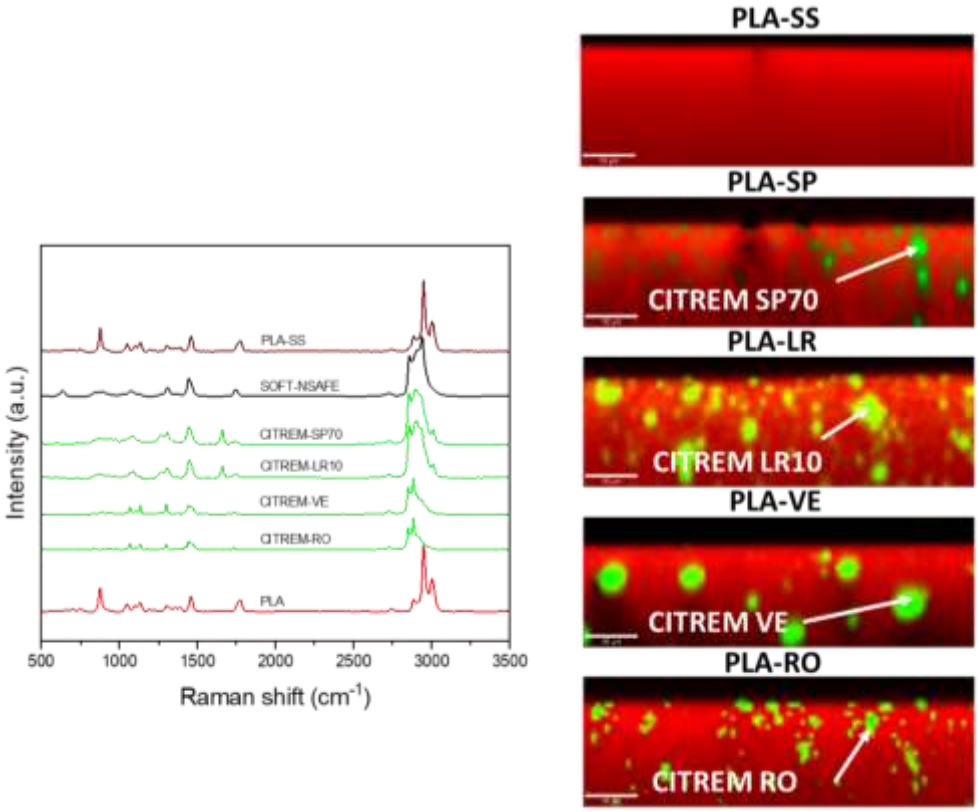


Figure C3. A3. 4. Raman spectra of PLA, CITREM-VE, CITREM-LR10, SOFT-N-SAFE and PLA-SS (left). XZ Raman images of PLA-VE, PLA-LR and PLA-SS (right). Scale bar of 10 µm.

XY Raman images of the plasticized films with CITREMs are also represented in **Figure C3. A3. 5.**, showing the creation of microdomains of the CITREMs within the PLA matrix. It is clearly observed than in most of the films, CITREMs are homogeneously distributed along the PLA matrix; however, in the case of PLA-VE, the plasticizer is heterogeneously distributed in bigger domains.

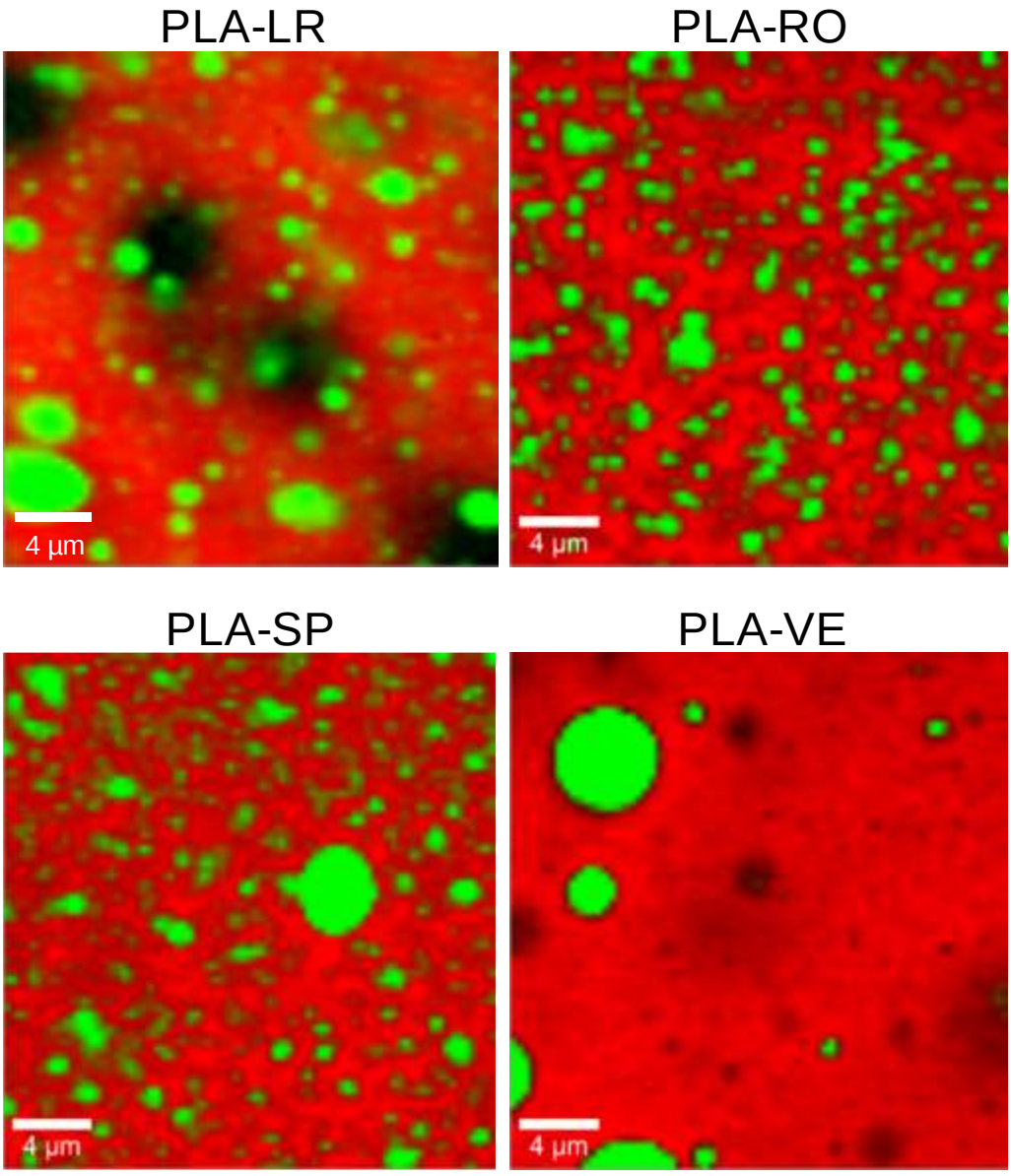


Figure C3. A3. 5. XY Raman images (25 µm × 25 µm) of PLA-LR, PLA-RO, PLA-SP and PLA-VE.

3.3. Wettability of films.

The present of fatty acid esters at the surface of the films would affect the wettability of the films. Therefore, the behavior of the materials was studied by static water contact angle measurements (**Table C3. A3. 5.**). It is clearly seen, that the incorporation of CITREMs and SOFT-NSAFE plasticizers decreases the contact angle value of neat PLA [38], [39], due to the hydrophilic nature of the tested plasticizers. There are no significant differences between plasticized films

except for PLA-VE films, possibly due to the heterogeneus microstructures and the formation of big microdomains.

Table C3. A3. 5. Results obtained from the contact angle technique. For each system, values with the same letter are not significantly different according to Tukey test (significance level of $P \leq 0.05$).

Film	Contact angle (º)
PLA	58.6 ± 3.7^{a}
PLA-RO	42.7 ± 7.3^{b}
PLA-VE	45.5 ± 6.5^{c}
PLA-SS	40.9 ± 4.9^{b}
PLA-LR	41.5 ± 2.7^{b}
PLA-SP	40.8 ± 6.2^{b}

3.4. Thermal properties.

Once morphology of the films was evaluated, the thermal analyses of the films were carried out. Thermogravimetric analysis was used to study thermal stability of the prepared films. Thermal parameters obtained from the thermograms of the samples are summarized in **Table C3. A3. 6.**

Table C3. A3. 6. TGA characterization of plasticized PLA films.

Sample	T_{d10} (ºC)	T_{d50} (ºC)	T^1_{dmax} (ºC)	dT/dm² (%/ ºC)	T^2_{dmax} (ºC)	dT/dm² (%/ ºC)
PLA	327	357	362	0.021	-	-
PLA-RO	237	280	250	0.943	286	1.593
PLA-VE	264	308	312	1.259	-	-
PLA-SS	299	354	361	0.020	-	-
PLA-LR	323	356	362	0.209	-	-
PLA-SP	268	311	300	0.002	-	-

In general, the addition of the fatty acid esters tends to reduce the thermal stability of the PLA [40], as it is clearly observed at T_{d10} of the plasticized samples compared with PLA. In PLA-RO the lowering of the thermal stability is notable. As mentioned before, first step of degradation of CITREM-RO is at 233 ºC (see **Table C3. A3. 3.**), which correlates with T_{d10} in this first step, occurring the loss of the plasticizer, and therefore, the degradation products of the plasticizer affect the plasticized PLA and originate that degradation starts earlier. Thermal stability of the films in the first step of degradation is considerably lower compared with neat PLA films, which T_{d10} is at 327 ºC. Meanwhile, in the second step of degradation at $T_{d50,}$ samples PLA-RO, PLA-VE and PLA-SP exhibit lower temperatures than pure PLA, thus, the behavior supports the idea that plasticizer degradation products accelerate the degradation of PLA. On the other hand, PLA-SS and PLA-LR showed a slight decrease of T_{d10} temperature but remains the thermal properties at T_{d50} of neat PLA. Therefore, it can be considered that the thermal degradation of plasticized PLA with 10% of SOFT-NSAFE or CITREM-LR10 does not differ from that of pure PLA.

Then, to further investigate the thermal and crystallization behavior of the plasticized films, DSC analysis was used. **Table C3. A3. 7.** summarizes the values of the thermal transition temperatures obtained from the first heating scan.

Table C3. A3. 7. Thermal parameters obtained from the first heating DSC curves PLA based films.

Sample	T_g (ºC)	T_{cc} (ºC)	T_m (ºC)	X_c (%)
PLA	59	104	174	0.3
PLA-RO	Ov	82/152	168	0.6
PLA-VE	Ov	90/154	177	1.0
PLA-SS	43	79	171	13.8
PLA-LR	53	90	172	10.5
PLA-SP	53	87/154	170	6.5

Ov- Overlap of the melting point of plasticizer with the PLA T_g.

Typically, the addition of the plasticizers causes a reduction in the value T_g of neat PLA, the decrease in this parameter is an indicator of good plasticization efficiency, increasing the mobility of the polymer chains [41]. PLA-SS showed the highest reduction, decreasing the value of T_g to 43 ºC. This reduction of the T_g could be more pronounced due to the miscibility of SOFT-NSAFE in the PLA matrix. Contrary, the incorporation of CITREMs produces a less pronounced reduction in the T_g values, as the interaction between the molecular chains and the plasticizers is lower, as demonstrated by their lower miscibility in the PLA matrix, segregating in microdomains. Additionally, the T_{cc} values decrease with the incorporation of the plasticizers, as previously commented plasticizers enhance the chain mobility and, as a result, PLA crystallizes more easily at lower temperatures [42]. The cold crystallization transition is appreciated to be lower for PLA-SS at around 79 ºC, followed by PLA-RO at temperatures around 82 ºC, meanwhile, the higher values are for PLA-SP, PLA-LR and PLA-VE at temperatures around 87 ºC, 90 ºC, and 90 ºC, respectively. All these values are considerably lower compared to neat PLA film found at 104 ºC. A secondary crystallization process is observed at approximately 150 ºC, which is attributed to the recrystallization of PLA α' crystals into more stable α-crystals [43]. Subsequently, the melting process occurs above

170 ºC. The PLA film primarily remains amorphous due to its low crystallization kinetics; however, the plasticizers appear to interact with the macromolecular chains, increasing their mobility and stimulating PLA crystallization [44]-[46]. As can be seen in **Table C3. A3. 7.**, the T_m values of the tested plasticizers slightly decrease, whereas a moderate increase in crystallinity is observed for the PLA-SS and PLA-LR films.

3.5. XRD analysis.

The low crystallinity degree of the prepared films was also confirmed by XRD. Diffraction traces of neat and plasticized PLA films are shown in **Figure C3. A3. 6.** In neat PLA, an amorphous halo is clearly appreciated as a signal of low crystallinity [47],[48]. This halo is also observed in the rest of the plasticized samples. Additionally, PLA-RO and PLA-VE films showed a crystalline peak at 21.6º, which can be associated to the CITREM-RO and CITREM-VE, which present melting temperatures around 62-63 ºC. **Figure C3. A3. 6b.** shows the XRD pattern of these CITREM plasticizers confirming their crystalline structure. The rest of the plasticizers are melted at room temperature and non-appreciated crystalline peaks appear in the diffraction patterns, although a little crystallinity was observed by DSC.

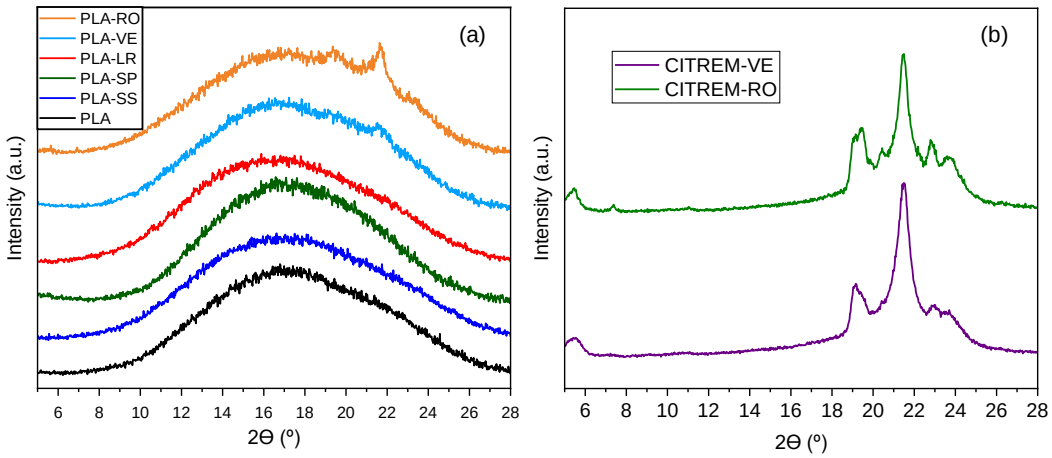


Figure C3. A3. 6. XRD patterns of a) neat PLA and plasticized-PLA films and b) CITREM-RO and CITREM-VE plasticizers.

3.6. Mechanical properties.

The mechanical properties of the films were evaluated by tensile testing and microhardness. Microhardness (MH) experiments are related to the mechanical behavior response of the surface when a pressure is done, and it can be defined as a measure of the resistance to a permanent deformation or damage. The values of the MH are represented in **Figure C3. A3. 7.**

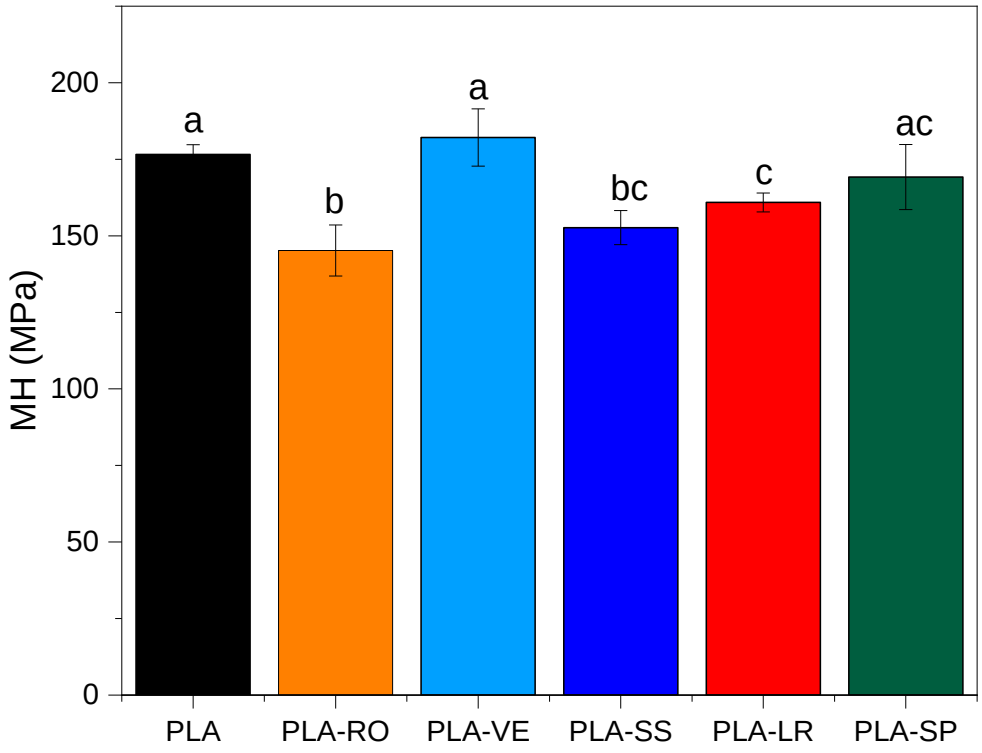


Figure C3. A3. 7. Mechanical values of microhardness measured by permanent deformation experiments.

The results showed the incorporation of the plasticizers produces a slight decrease of the hardness value in comparison with neat PLA, showing higher flexibility of the material, which could be a good indicator of plasticization and increase in the mobility of the chains [49].

Subsequently, other mechanical properties, such as Young modulus (E), maximum strength (Rm) and elongation at break (Ɛ), were evaluated by the stress-strain curves obtained from the tensile test. The corresponding values are collected in **Table C3. A3. 8.** Neat PLA film exhibits high Young's modulus and tensile strength but low

elongation at break, displaying a glass-like brittle behavior at room temperature. The inclusion of the tested plasticizers into PLA produced a notable decrease in the tensile strength, while a slight reduction of Young's modulus was observed. The reduction of the tensile strength and young modulus can be linked to a decline in the intermolecular forces between PLA polymer chains; thereby increasing their mobility and resulting in enhanced flexibility. Concerning the elongation at break, neat PLA showed a value of 4.6 ± 1.1%, and the samples PLA-RO, PLA-VE, PLA-SP, and PLA-SS did not present a significant improvement in the material behavior. In contrast, the incorporation of CITREM-LR10 produces a considerable increase in the elongation at break up to 42.5%.

Table C3. A3. 8. Mechanical properties of neat and plasticized PLA films. Mean values ± standard deviation. For each system, values with the same letter are not significantly different according to Tukey test (significance level of $P \leq 0.05$).

Sample	Elongation at break (%)	Tensile strength (MPa)	Young modulus (MPa)
PLA	4.6 ± 1.1^{a}	56.3 ± 5.2^{a}	2192.8 ± 123.6^{a}
PLA-RO	4.3 ± 0.9^{a}	28.3 ± 5.8^{b}	1901.1 ± 218.4^{a}
PLA-VE	4.9 ± 0.9^{a}	38.8 ± 3.6^{c}	1683.0 ± 187.2^{a}
PLA-SS	6.3 ± 3.0^{a}	30.5 ± 3.5^{b}	1872.6 ± 94.9^{a}
PLA-LR	42.5 ± 12.2^{b}	27.7 ± 4.8^{b}	1635.9 ± 190.8^{a}
PLA-SP	7.1 ± 2.1^{a}	28.3 ± 3.6^{b}	1775.5 ± 299.4^{a}

The increase in the elongation at break in PLA-LR may be due to the presence of CITREM-LR10 microdomains, which are homogeneously distributed throughout the PLA matrix could behave as stress concentrators under the tensile stress [50]–[52]. To further investigate this phenomenon, the morphology of such microdomains were again analyzed by Raman confocal microscopy after the tensile test. **Figure C3. A3. 8.** displays Raman images (XY) of the tested samples, which were taken near the fracture. Interestingly, cracks perpendicular to tensile direction are clearly observed in all the samples. Whereas in PLA-RO and PLA-VE and PLA-

SP films, the microdomains maintained their morphology, in PLA-LR the microdomains gets elongated in the crack direction, thus perpendicular to the tensile direction, which could retard the propagation of the cracks. The differences in the behavior of the plasticizers during the tensile test could be ascribed to the size distribution of the microdomains, to the interactions between both phases (CITREM and PLA) and the state of the plasticizer at room temperature. In this case, CITREM-LR10 forms a more homogenous distribution of microdomains, which are in viscous state at room temperature and then, can be oriented along the cracks' direction. This behavior provides important advantages as it can improve the toughness of PLA with only a slight diminution in the glass transition temperatures [53].

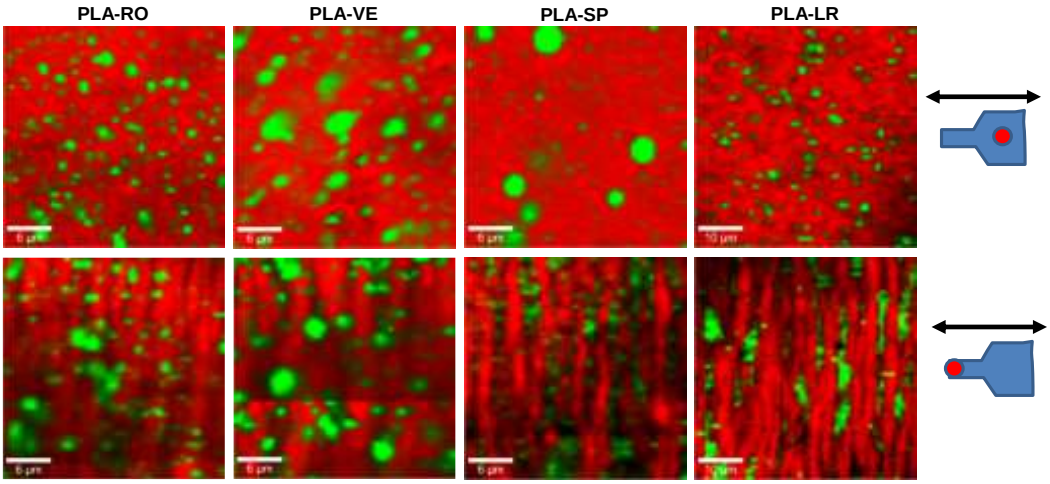


Figure C3. A3. 8. XY Raman image of the films after tensile test at different positions. Upper images represent the grabbed zone of the sample by the device, meanwhile lower represent the strained zone close to the failure point of the sample.

3.7. Antioxidant and antibacterial capacity of the films.

Once thermal and mechanical characterization of the films demonstrated the effects of the plasticizers on their properties, the antioxidant and antibacterial activity of the films were further studied. **Figure C3. A3. 9.** shows the results of the antioxidant capacity of the PLA based materials. As expected, PLA-LR and PLA-SP, after 24 h hours in contact with the DPPH solutions, exhibited the best activity achieving values of 37.2 ± 2.1% and 33.1 ± 1.9%, respectively. These plasticizers contain alpha-tocopherol in their composition, a well-known antioxidant compound. Besides the chemical composition of the plasticizers, the flexibility and

segmental mobility induced by the plasticization would increase the accessibility of the antioxidant compounds.

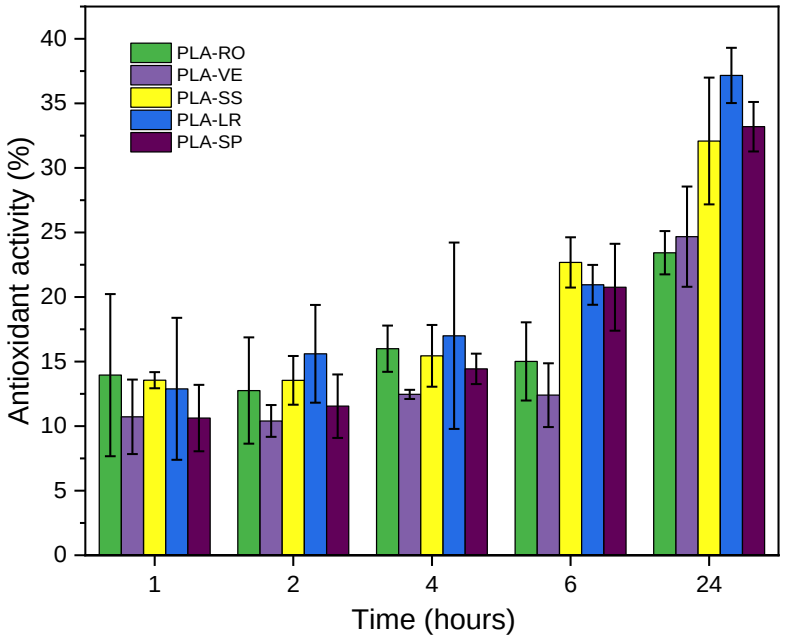


Figure C3. A3. 9. Antioxidant properties of the different plasticized PLA films at different contact times with the DPPH solution.

Next, the antimicrobial properties of the films containing the plasticizers were evaluated using a film contact method against food pathogens *S. aureus* and *L. innocua* bacteria [54]-[56]. **Table C3. A3. 9.** presents the antibacterial activity results, expressed as the log-reduction from an initial inoculum concentration of log CFU/mL: 5.3 for *S. aureus* and 5.2 for *L. innocua* and after 24 hours of contact with the films. The results indicate that PLA-RO and PLA-VE exhibit negligible activity against both bacterial strains, whereas PLA-LR, PLA-SS and PLA-SP are able to significantly decrease bacterial colonies. PLA-LR is the most effective films against *S. aureus*, nearly eliminating the bacterial colonies, and have relatively good efficiency against *L. innocua*, whereas PLA-SS films is more effective against *L. innocua*. On the other hand, PLA-SP films only shows moderate reduction against both tested strains. The antimicrobial properties of the plasticizers (SOFT-NSAFE, CITREM LR10 and CITREM SP70) that provide such activity to the films can be attributed to the germicidal activity of citric and acetic acid due to its ability to lower the local pH environment and intracellular pH. This acidity can result in

damage to proteins, enzymes, DNA, and membranes, as well as suppress nicotinamide adenine dinucleotide (NADH) oxidation. Ultimately, these effects culminate in bacterial death [24], [57]. Additionally, other active components (see **Table C3. A3. 9.**) could also contribute to the film´s antibacterial activity. The plasticizers studied, derived from different oils such as palm, sunflower and castor oils, have also been shown to possess antimicrobial properties [58].

Table C3. A3. 9. Antibacterial activity of the plasticized PLA films based expressed as the reduction percentage of bacterial CFU ($\%R_{CFU}$) counted between the inoculum and after 24h of contact with the films.

Film	*S. aureus* Log-reduction	*L. innocua* Log-reduction
PLA-RO	-	0.16
PLA-VE	-	0.15
PLA-SS	0.5	3.3
PLA-LR	3.7	2.0
PLA-SP	0.7	0.93

4. Conclusions.

A variety of food additives based on citric (CITREM) and acetic (ACETEM) acids esters of mono- and diglycerides of fatty acids were evaluated as plasticizers for PLA films. Whereas SOFT-NSAFE seems to be miscible with the PLA matrix and a homogeneous distribution is observed, CITREM plasticizers, which are citric acid ester derivatives, have heterogeneous distribution by the formation of microdomains of plasticizer. Nevertheless, all the plasticizers reduce thermal stability, glass transition temperature, tensile strength, and increase the hydrophilicity of PLA. However, only CITREM-LR10 increases elongation at break. Confocal Raman microscopy of films after the tensile test showed that CITREM-LR10 can be oriented along the cracks produced during the tensile test.

Besides, SOFT-NSAFE, CITREM-LR10 and CITREM-SP70 provide antioxidant activity to the films. Finally, films containing SOFT-NSAFE and CITREM-LR10 exhibited good antibacterial activity against *L.innocua* bacteria, and/or *S. aureus* bacteria. Such activity could be due to the carboxylic acid groups of the plasticizers and also to the presence of other active components. In summary, these food additives can be used as PLA film plasticizers, imparting functional characteristics to the materials and widening their applications, mainly in the food packaging industry.

Acknowledgments.

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Article 4: Enhancing Functional Properties of Compostable Materials with Biobased Plasticizers: A Technological and Spectroscopic Assessment for Food Packaging Applications.

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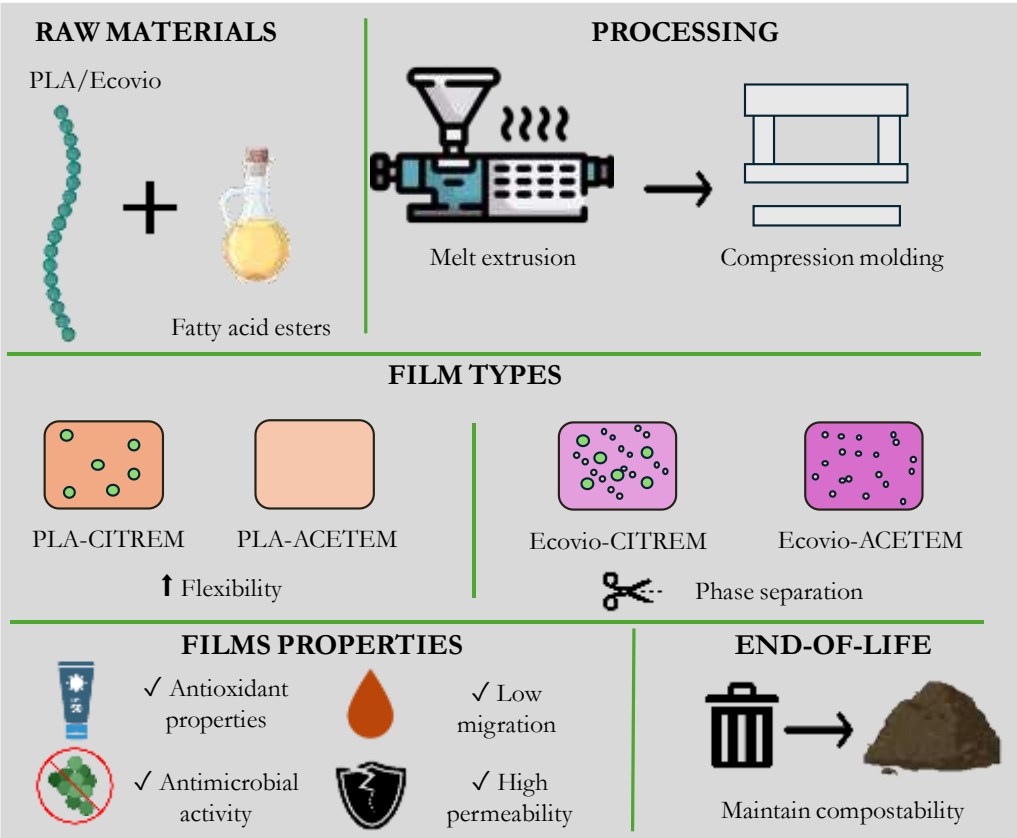

Abstract.

The demand of non-toxic and biobased plasticizers is substantially growing, particularly in biodegradable thermoplastics-based packaging applications. Herein, a derivative of citric acid (CITREM-LR10), usually used as food additive, was evaluated for the first time as plasticizer in PLA and Ecovio® biopolymers. Films containing 10% (w/w) of CITREM-LR10 were prepared and compared with films plasticized with another biobased plasticizer, SOFT-NSAFE, derived from acetic acid. The incorporation of both plasticizers provokes a significant reduction of the glass transition, however, only CITREM-LR10 was able to augment the toughness of PLA films. A further evaluation of the films by confocal Raman microscopy showed the segregation of the CITREM-LR10 in microdomains, which could explain the enhanced mechanical properties, behaving as stress concentrators. In addition, CITREM-LR10 provides antimicrobial activity against *S. aureus* and antioxidant properties, and almost negligible diffusion in food simulated solution. Composting studies showed that the plasticizers do not have effect on the disintegration rate of the films. Despite these outstanding properties, the water vapour and oxygen barrier properties of the films worsen with its incorporation, therefore, the inclusion of fillers in the material together with the plasticizers would be necessary to improve such properties for food packaging applications.

1.Introduction.

Polylactic acid (PLA) stands out as a highly promising and extensively employed biodegradable polymer for food packaging applications. Its favourable properties comprise excellent processability, high molecular weight, resistance to water solubility, effective barrier properties to flavours and aromas, thermal stability, and mechanical performance comparable to that of other polymers utilized in the food packaging industry, like poly (ethylene terephthalate) (PET) or polyethylene (PE). Besides, PLA is Generally Recognized as Safe (GRAS) by FDA [1], [2]. Poly (butylene adipate-co-terephthalate (PBAT) has also emerged as another promising biodegradable polymer suitable for packaging applications. It has been widely combined with PLA to enhance its properties such as its brittleness and low flexibility. An example is Ecovio®, a commercially available compound that relies on a blend of PLA and PBAT, specifically designed for packaging applications [3]. The incorporation of plasticizers into PLA is another common practice to improve its inherent limitations [4]. Plasticizers are typically low molecular weight resins and liquids that interact with the chains of the main polymer, increasing the flexibility and mobility of the polymer chains by lowering the second order transition temperature resulting in an easily deformable mass [5], [6]. Plasticizers are added to the amorphous regions of polymers, leaving the structure and size of any crystalline segments unaffected, but leading to a variation in properties. Certain characteristics, including deformation at break, toughness, or flexibility, typically show an increase with the addition of plasticizer, while modulus, tensile strength, hardness, and melt viscosity generally decrease.

The extensive range of plastic products and their diverse applications have prompted the development of advanced plasticizers to meet stringent quality and specification standards. The present market provides a multitude of options, each with distinct attributes that can be tailored to specific applications, addressing critical material requirements. These include fatty acid esters, benzoates, tartrates, chlorinated hydrocarbons, as well as esters derived from adipic, azelaic, and sebacic acids. However, the utilization of plasticizers has come under scrutiny due to potential toxicity issues linked to the migration of the products. Over the past fifty years, legal regulations and concerns about health and safety have driven the creation of a broad array of commercially available non-toxic plasticizers. In

particular, nowadays there is a growing interest in the production of bio-based plasticizers with low toxicity, minimal migration, especially for the increasing demand of bioplastics [5], [7]–[9].

The selection of plasticizers for modifying the properties of biopolymers in food packaging is constrained by both technical and legislative considerations. Specifically, Regulation EU 10/2011 and consolidated versions outlines regulations concerning plastic materials and articles intended for contact with food products. These regulations establish standards and requirements that plastics must meet to ensure the safety and suitability of food packaging [7].Then, the emergence of novel non-toxic plasticizers (bio-plasticizers) represents an inevitable trend in development of the food packaging towards more sustainable and health-conscious alternatives [8]. Among them, vegetable oil-based plasticizers stand out as a category characterized by their non-toxic nature and environmentally friendly attributes such as glycerol, commonly used for PLA based materials.

Plasticizers could also serve as a functional agent, imparting new properties to the materials. Concretely, in the food packaging industry, plasticizers with antioxidant or antimicrobial properties could help preserving the quality and freshness of the packaged product by protecting it against oxidation or microbial contamination. However, only few examples of bioactive plasticizers are found in literature [10], [11]. Typically, antimicrobial/antioxidant particles or compounds are incorporated into the materials in addition to the plasticizers.

In this study, we evaluated the use of bio-based food additives as novel bioactive plasticizers with antimicrobial and antioxidant activity in PLA and Ecovio® materials for food packaging applications. Concretely, citric acid esters of mono- and diglycerides, also known as citroglycerides or CITREMs, serve as widely utilized emulsifiers and thickeners (designated as E472c) in the food industry [9], also presenting antioxidative properties [12]. CITREMs are, essentially, blends of diverse glycerides produced through the reaction of citric acid or its anhydride with acylglycerols (comprising edible oils, combinations of diacylglycerides and/or monoacylglycerides). Throughout the reaction, citric acid naturally engages with more than one glycerol backbone, leading to variations in the composition of CITREM based on factors like reaction time, temperature, and the ratio between raw materials. Importantly, CITREM is recognized as a food substance with very low toxicity, and its presence in foods is not hazardous to human health.

Furthermore, it holds the designation of being "generally recognized as safe" (GRAS) [13]. Besides, citrate-based compounds have been investigated for their antimicrobial and antioxidant properties [14] and some of them has been also evaluated as plasticizers [15], including tributyl citrate (TBC) and acetyl tributyl citrate (ATBC) [16].

Then, the objective of this work was to use this CITREM product as a bioactive plasticizer in films of PLA and Ecovio® in comparison with other biobased and safe plasticizer, SOFT-NSAFE which is an acetic acid ester of monoglycerides made from fully hydrogenated castor oil and has been previously evaluated as alternative of phthalate plasticizers in PVC [17]. Films containing such biobased plasticizers materials were prepared to extensively investigate their effect on the mechanical and thermal properties. The barrier properties against water vapour and oxygen, the possible migration of the plasticizer, the ability to degrade in compost conditions as well as the evaluation of the antimicrobial and antioxidant activity of these films were also studied.

2. Experimental.

2.1 Materials.

Poly (lactic acid) (PLA) pellets (ErcrosBio® LL652; density: 1.25 g/cm^3; melt flow indexing: 11 g/10 min) were supplied by Ercros (Barcelona, Spain). Ecovio® pellets (Fblend C2224 density: 1.24-1.26 g/cm^3; melt flow index: <2.5 g/10 min) were acquired from BASF (Ludwigshafen, Germany).

GRINDSTE*D*® SOFT-NSAFE (CAS number 736150-63-3), an acetic acid ester of monoglycerides made from fully hydrogenated castor oil, was obtained from Danisco (**Figure C3. A4. 1.**).

GRINDSTE*D*® CITREM LR 10 EXTRA KOSHER (CAS number 97593-31-2), a citric acid ester of mono-diglyceride (E 472c) made from edible, refined sunflower oil, was obtained from Danisco (**Figure C3. A4. 1**). This product also contains alpha-tocopherol (E 307) max. 200 ppm and ascorbyl palmitate (E 304) max. 200 ppm.

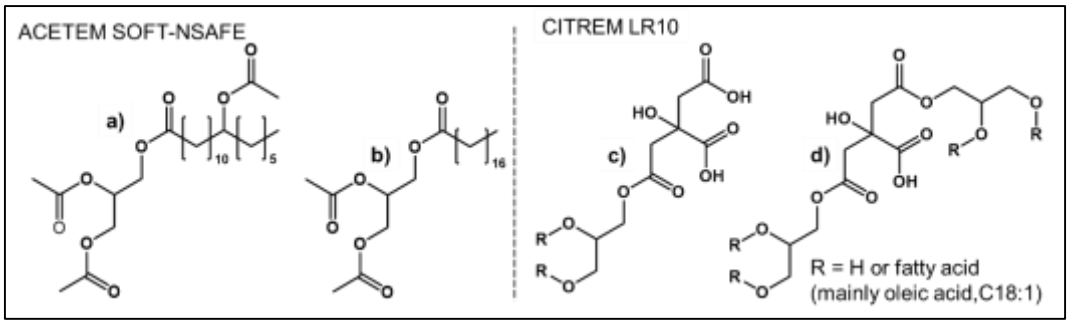

Figure C3. A4. 1. Chemical structure of the main components of SOFT-NSAFE (fully acetylated glycerol monoester based on (a) 12-hydroxystearic acid and (b) stearic acid) and of CITREM LR10 ((c) mono-esterified citric acid and (d) di-esterified citric acid).

2.2. Films preparation.

Firstly, PLA and Ecovio® pellets underwent a 24-hour drying process at 40 ºC in an oven. Subsequently, a micro-extruder with a co-rotation twin-screw (HAAKE Minilab II Thermo Scientific) and a capacity of 7 cm^3 was employed for the production of plasticized materials based on PLA and Ecovio®. The blends (polymer matrices and plasticizers) were introduced into the extruder and the processing were performed at a screw speed of 100 rpm and a temperature of 180 ºC for 3 min. Then, the extruded samples were compression moulded using a CollinP200P press (COLLIN Lab & Pilot Solutions) following several steps: 1 bar pressure for 2 min: at 190 ºC and; 100 bar during 3 min at 190 ºC; and a cooling step at 100 bars. The thickness of the films was determined utilizing a digital micrometer (Permascope MP0, Fischer Instruments), with values representing the average of 10 random measurements from various parts of each film.

2.3. Antioxidant activity.

The antioxidant properties of plasticizers and films, were evaluated employing the Blois method, using the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH, Merck). For this test, a DPPH solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol (Scharlab). For the analysis of the plasticizers, 4 mg of each plasticizers and 2 mL of DPPH solution were placed in contact in a vial; while for the film studies, 100 mg of each film sample and 2 mL of the DPPH solution were added to a vial, initiating the reaction. At different time periods, 100 µL of each mixture were withdrawn and transferred to a multi-well plate. The absorbance in

each well was measured using a BioTek® SYNERGY HTX multi-mode reader spectrophotometer at a wavelength of 527 nm. After recording the measurements, the solutions from the multi-well plate were returned to the original vials to maintain consistent concentration and volume. The absorbance of the samples was analysed at intervals of 1, 2, 3, 4, 5, 7, and 24 h.

Results were expressed as the percentage reduction of the DPPH free radical, representing the material antioxidant capacity. This is determined by the ratio of the absorbance value of the sample solution (A_m) to the absorbance value of the solution without the sample (A_0). The measurements were performed at least in triplicate.

2.4. Biological Assay.

The antibacterial properties of the obtained films were tested using the E2149-20 standard method from the American Society for Testing and Materials (ASTM), a standardized method to evaluate the antimicrobial activity of material surfaces [18]. *Staphylococcus aureus* ATCC 29213 (ATCC: American Type Culture Collection) was used as bacterial strain. In this analysis, bacterial suspension was prepared in PBS at a concentration of around 10^4 CFU mL^{-1}. Then, 100 mg of each biopolymeric films were placed in sterile Falcon tubes containing 1 mL of the bacterial suspension inoculum and 9 mL of PBS to obtain a working concentration of around 10^3 CFU mL^{-1}. Blank experiments with only the inoculum in the absence of film were also performed. Subsequently, suspensions were incubated at 120 rpm for 24 h at 37 ºC with shaking. The number of bacteria remaining in each sample was determined by the plate count method. The test was performed in a 96-well plate in which 100 µL of the suspension were added on the first column and 90 µL of PBS to the rest of the wells. Serial dilutions of 10 µL from the previous column was made from columns #1 to #4 Subsequently, 50 µL of each dilution was placed on 5% sheep blood Columbia agar plates and incubated for 24 h at 37 ºC. The measurements were made at least in triplicate.

2.5. Study of Migration in Food Simulant.

The possible migration of the plasticizer into the food when used in food packaging was investigated by a migration test performed at 20 ºC using 50% (v/v) ethanol as alcoholic food simulant D1 following the recommendation of the European Union and FDA [19]. For this assay, 100 mg of each film sample was immersed in 6 mL of 50% (v/v) ethanol aqueous solution and subjected to agitation for 10 days at 20

ºC, in order to simulate refrigerated conditions of foods with lipophilic character. Films were then extracted, dried, and weighed, while the liquid in which the films were immersed was slowly evaporated. Once the vials were completely dried, the residues remaining in the vials were dissolved in dichloromethane and analysed using an Agilent 8860 GC gas chromatograph equipped with an Agilent mass spectrometry detector model 5977B (GC-MS). The separation of the compounds was performed on a HP-5MS capillary column (30 m length × 250 μm internal diameter and 0.25 μm film thickness). The carrier gas used was helium with a flow rate of 1 mL/min. A volume of 1 μL of the obtained extract was injected in split mode with a split ratio of 20:1 at 260 °C.

2.6. Disintegrability of films under composting conditions.

The disintegration of the films under composting conditions in a laboratory-scale test was determined following the ISO 20200 standard [20]. The solid residue for composting was prepared by manually mixing of the required components: 55 wt% of water and 45 wt% of solid waste (10% of compost (Compo®, Spain), 30% rabbit food, 10% starch, 5% sugar, 4% corn oil, 1% urea, 40% sawdust). Several films of 25 mm × 25 mm size and 200 μm in thickness, for each formulation, were placed in textile mesh bags. Then, the meshes containing the square films were buried in the composting reactor. Samples of each formulation were extracted at different incubation times to carry out the analysis and weight control. Prior to these analyses, the samples were gently cleaned to eliminate the adhered compost residues and then dried at 37 °C under vacuum for 24 h. The degree of disintegrability was calculated by normalizing the sample weight, at different days of composting, to the initial weight.

2.7. Characterization techniques.

2.7.1. Thermogravimetric analysis (TGA).

Thermogravimetric analysis was carried out to study the thermal stability of the different blends, ~5 mg of the samples were tested in a TGA Q500 thermal analyser (TA Instruments) at a heating rate of 10 ºC/min from room temperature (~30 ºC) to 800 ºC, under air atmosphere (50 cm^3 min^{-1}). From the mass-temperature curves of the samples the temperatures at 10% (T_{d10}) and 50% (T_{d50}) of mass loss were calculated, whereas the temperatures at the maximum degradation rate (T_{dmax}) of the samples were determined from the first derivative of the TGA curves (DTG).

2.7.2. Differential scanning calorimetry (DSC).

TA Q2000 differential scanning calorimeter was used to evaluate the thermal transition of the films. Nitrogen purge gas (50 cm^3 min^{-1}) was applied for the whole experiments. The samples were heated from -60 °C to 65 °C at 10 °C/min, then a rapid cooling scan from 65 °C to -60 °C was performed. A second heating scan from -60 °C to 180 °C at 10 °C/min was carried out following to another rapid cooling. Finally, a third heating scan from -60 °C to 180 °C was performed. From the DSC curves, the glass transition temperature (T_g), cold crystallization temperature (T_{cc}) melting temperature (T_m) were determined. The crystallinity degree (X_c) was calculated using melting enthalpy values for a 100% crystalline, ΔH_m^0, of 93.6 J/g and 114 J/g, for PLA [21] and PBAT [22].

2.7.3. Mechanical Testing.

Tensile testing was conducted utilizing a DX2000 QTest Elite MTS dynamometer (MTS Systems) at room temperature, operating at a stretching rate of 10 mm/min with a 100 N load cell with dumbbell-shaped samples with dimensions according to UNE-ISO 37:2013 and a thickness of around 200 µm. Stress-strain measurements were used to calculated ultimate tensile strength, Young´s modulus and elongation at break values. Each material underwent testing with a minimum of five specimens.

2.7.4. Fourier Transform infrared spectroscopy (FTIR).

FTIR spectroscopy were performed using a Perkin Elmer Spectrum Two instrument equipped with an attenuated total reflection (ATR) module on samples recovered from composting.

2.7.5. Confocal Raman microscopy.

The films were also analysed using confocal Raman microscopy on a CRM-Alpha 300 RA microscope (WITec) equipped with Nd:YAG laser (maximum power output of 50 mW at 532 nm). Measurements were performed using objectives of 100× with a numerical aperture (NA) of 0.95. XZ Raman images were obtained in a representative area of 75 µm × 25 µm with 0.30 s of integration time whereas Raman images of the surface (XY) were performed in an area of 174 µm × 174 µm with 0.3 s of integration time.

2.7.6. Scanning electron microscopy (SEM).

Films recovered at different incubation times under composting were also studied in a scanning electron microscope Philips XL30 with an acceleration voltage of 25 kV. The samples were coated with gold prior to scanning.

2.7.7 Permeability test.

Oxygen permeability and water vapor measurements on circular films (2.01 cm^2) were performed by an isostatic permeabilimeter (mod. Multiperm, PERMTECH S.r.l., Pieve Fosciana, Italy) according to ASTM standard methods (D-3985 and F-1249, respectively) [23]. The water vapor transmission rate (WVTR) was evaluated at 23 °C, and 65% of relative humidity (RH), and oxygen permeability measurements were monitored at 23 °C under 50% RH. Two specimens were tested for each material.

2.8 Statistical analysis.

One-way analysis of variance (one-way ANOVA) followed by post-hoc Turkey's test was carried out to determine significant differences at $P \leq 0.05$ among different groups.

3. Results and discussion.

3.1. Study of the different plasticizers.

To evaluate the properties of the plasticizers, an antioxidant activity test was first carried out. The antioxidant action of both compounds, CITREM LR and SOFT-NSAFE was studied in the methanol-DPPH solution for 28 h, and the results are shown in **Figure C3. A4. 2.** The CITREM LR10, presents higher values of reduction capacities in comparison with the SOFT-NSAFE, achieving after 28 h a value around 70%, respect to 20%.

The main components of CITREM LR10 are derivatives of citric acid, citric acid esters of mono- and diglycerides that are well-known antioxidant products (E472c). In addition, CITREM LR10 contains alpha-tocopherol (E307, 200 ppm) and ascorbyl palmitate (E304i, 200 ppm) in the formulation, which are antioxidant additives [7] [24].

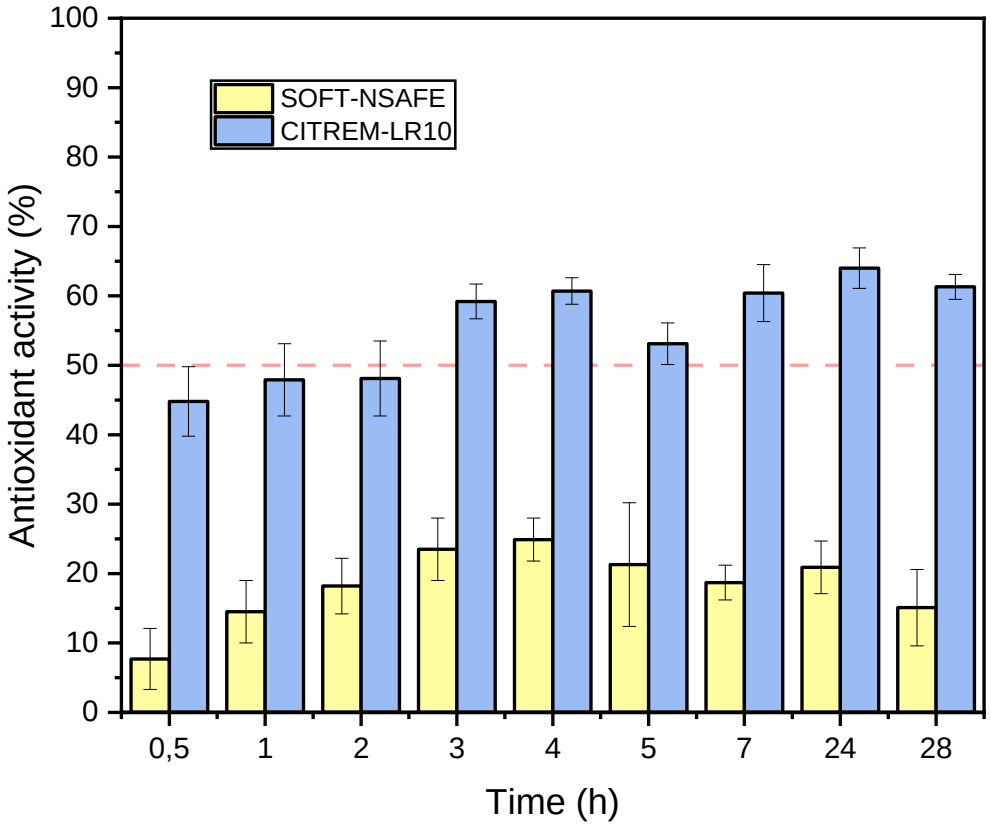

Figure C3. A4. 2. Antioxidant activities of the plasticizers (SOFT-NSAFE and CITREM-LR10) at different immersion times in the DPPH solution.

The proposed plasticizers were further characterized by thermogravimetric analysis (TGA) to assess their thermal stabilities during the processing conditions in the melt-extrusion and compression molding. **Table C3. A4. 1.** summarizes the thermal characteristics of these two plasticizers, including T_{d5}, T_{d10}, T_{d50} and T_{dmax}. The values indicate that the plasticizers could be employed at relative low processing temperature, below 200 ºC. Nevertheless, biopolymers such as PLA and PBAT are generally processed between 170-200 ºC depending on the type of polymer.

Table C3. A4. 1. Thermal characteristics of SOFT-NSAFE and CITREM-LR10 determined by TGA including the temperatures at 5% (T_{d5}), 10% (T_{d10}) and 50% (T_{d50}) of weight loss and the temperatures at the maximum degradation rate (T_{dmax}).

Plasticizer	T_{d5} (ºC)	T_{d10} (ºC)	T_{d50} (ºC)	T_{dmax} (ºC)
SOFT-NSAFE	218	228	264	272
CITREM-LR10	213	236	313	295

3.2. Preparation of plasticized films based on PLA and Ecovio® and morphological analysis.

SOFT-NSAFE and CITREM-LR10 compounds were then employed as antioxidant and potential antimicrobial plasticizers in films based on PLA and Ecovio®. These plasticized films (with a thickness of about 200 µm) were prepared by melt extrusion followed by compression molding. **Table C3. A4. 2.** shows the composition of the samples, where PLAxx and ECOxx are the films based on PLA and Ecovio®, respectively, and xx referred to the plasticizer used (SS for SOFT-NSAFE or LR for CITREM-LR10) at a concentration of 10%.

Table C3. A4. 2. Composition of the prepared samples.

Sample	Biopolymer	Plasticizer
PLA	PLA 100%	-
PLASS	PLA 90%	SOFT-NSAFE 10%
PLALR	PLA 90%	CITREM-LR10 10%
ECO	Ecovio® 100%	-
ECOSS	Ecovio® 90%	SOFT-NSAFE 10%
ECOLR	Ecovio® 90%	CITREM-LR10 10%

Next, SEM was employed to characterize the morphology of the film surface. **Figure C3. A4. 3.** shows that the surface of neat PLA and PLASS were relatively homogeneous and smooth while the PLALR film displayed a rough surface

morphology, which could be associated to a phase segregation process of the plasticizers.

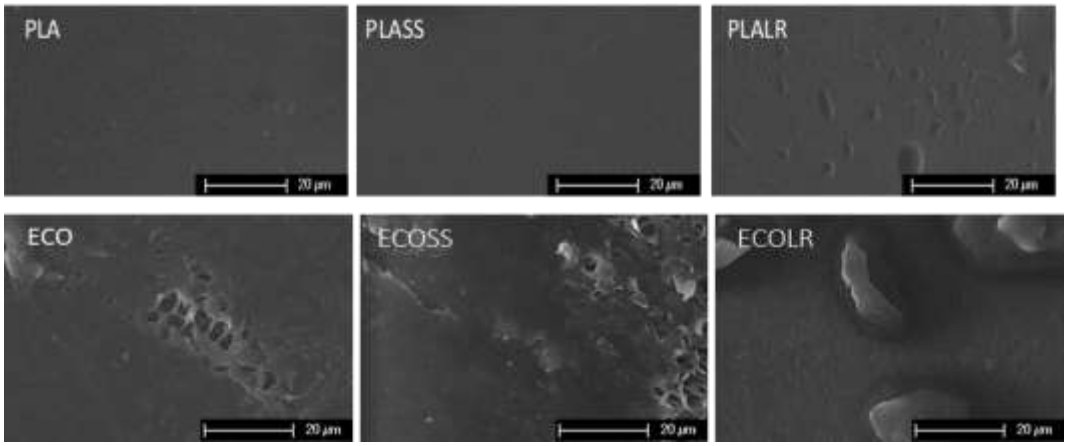

Figure C3. A4. 3. SEM images ×1000 magnification of PLA and Ecovio® based materials.

In effect, when scanning the PLALR film using confocal Raman microscopy (**Figure C3. A4. 4.**) we found two distinctly different spectra corresponding to different domains, one containing mostly PLA components (spectrum corresponding to PLA) and one containing mostly CITREM-LR10 (spectrum corresponding to CITREM-LR10). The segregation of the plasticizers in microdomains (signalled in green colour) was observed in both at the surface and inside the films. In contrast, in PLASS only one spectrum was observed in which the signals of PLA and SOFT-NSAFE components are observed, as indicative of a good miscibility between both.

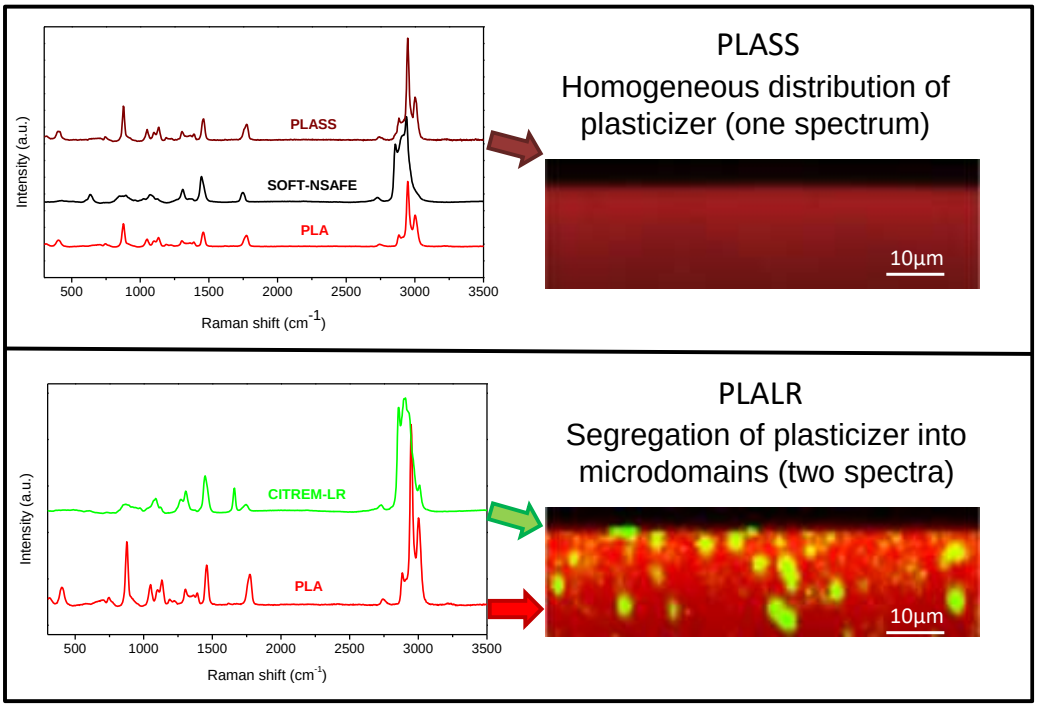

Figure C3. A4. 4. Raman spectra of PLA, CITREM-LR10, SOFT-N-SAFE and PLASS (left). XZ Raman images of PLASS and PLALR, PLA matrix was signalled in red colour whereas CITREM-LR10 was signalled in green colour.

In Ecovio® based films, the phase separation structure between PLA and PBAT could be seen by SEM (**Figure C3. A4. 3.**) and confocal Raman microscopy (**Figure C3. A4. 4.**). In the Ecovio® based films, it is clearly observed the phase-separated microstructure with two main regions, PLA-rich quasi-spherical microdomains (signalled in blue colour) and the continuous phase matrix of PBAT (signalled in pink colour). Additionally, in the ECOLR films the segregation of CITREM-LR10 (signalled in green colour) is appreciated, whereas in ECOSS the plasticizer could not be detected, probably due its homogeneous distribution along the film.

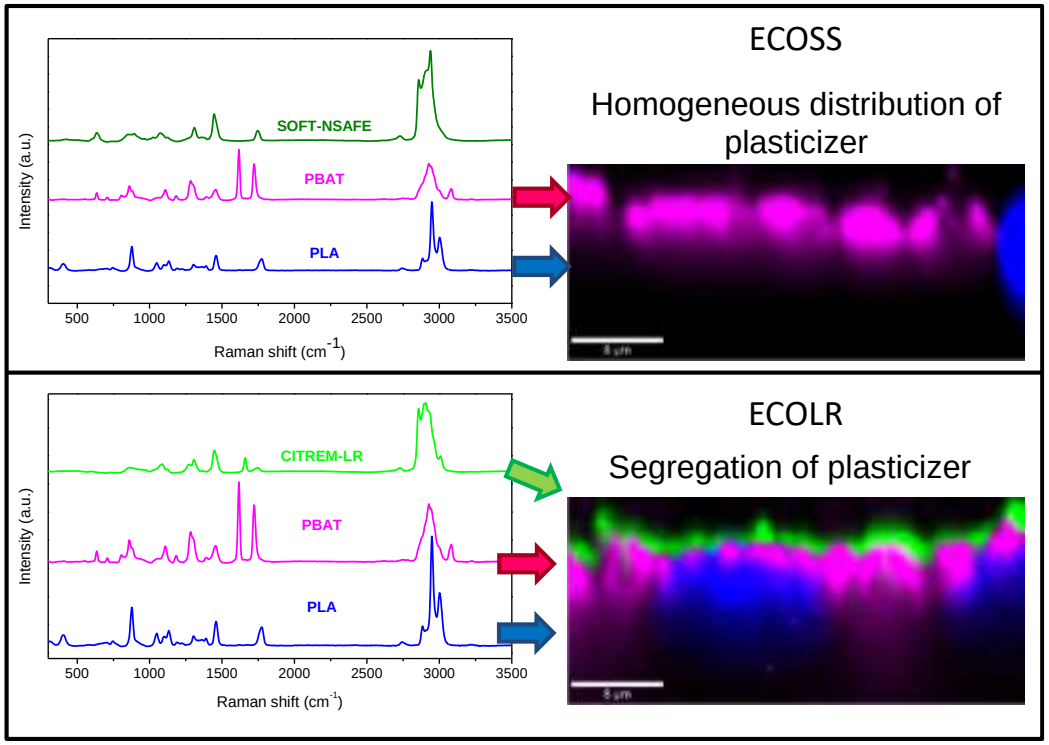

Figure C3. A4. 5. Raman spectra of PLA, PBAT CITREM-LR10 and SOFT-NSAFE (left). XZ Raman images of ECOSS and ECOLR.

3.3. Thermal, mechanical and gas permeability properties of the films.

Thermal analyses of the prepared films were carried out in terms of thermal stability, thermal transition and crystallinity, as these parameters are crucial for further applications. Thermal stability of the prepared films was first studied by TGA. The thermograms of films based on PLA and Ecovio® were displayed in **Figure C3. A4. S1a.** and **S1b.** (see supplementary information), respectively, and the thermal parameters summarized in **Table C3. A4. 3.**

Table C3. A4. 3. TGA characterization of PLA and Ecovio® based films.

Sample	T_{d10} (ºC)	T_{d50} (ºC)	T^1_{dmax} (ºC)	T^2_{dmax} (ºC)	T^3_{dmax} (ºC)
PLA	327	357	362	-	-
PLASS	299	354	361	-	-
PLALR	323	356	362	-	-
ECO	325	364	348	373	456
ECOSS	309	359	350	372	450
ECOLR	318	362	351	377	455

In both series of films, the addition of the plasticizers reduces the T_{d10} values, in particular the case of the SOFT-NSAFE compound. This reduction of the thermal stability is associated to the partial degradation of the plasticizers before the degradation of the polymers, as seen in **Table C3. A4. 1**. Thus, plasticized films are less thermally resistant than the neat PLA films at temperatures below approximately 320 °C.

Subsequently, DSC analysis was used to investigate the thermal and the crystallization behaviour of the prepared films. **Table C3. A4. 4.** summarizes thermal characteristics obtained from the DSC thermograms (**Figure C3. A4. S2.**) of the films related to the first heating scan up to 180ºC. The reason to equilibrate heating up to 65 ºC is to avoid the presence of physical ageing. This phenomenon is common in PLA samples with T_gs relatively close to room temperature.

Table C3. A4. 4. Thermal parameters obtained from the first heating DSC curves PLA and Ecovio® based films, glass transition temperature (T_g), cold crystallization temperature (T_{cc}), melting temperature (T_m) and crystallinity degree (X_c).

Sample	T_g^{PBAT} (ºC)	T_g^{PLA} (ºC)	T_{cc}^{PLA} (ºC)	T_m^{PLA} ((ºC) (ºC)	T_m^{PBAT} (ºC)	X_c^{PLA} (%)	X_c^{PBAt} (%)
PLA	-	59	104	174	-	0.3	-
PLASS	-	42	79	171	-	14.0	-
PLALR	-	53	89	172	-	10.9	-
ECO	-32	57	111	147/154	129	1.8	1.6
ECOSS	-45	42	84	139/150	120	4.4	2.7
ECOLR	-36	54	99	143/152	122	3.7	4.2

In the PLA based samples, it is clearly observed a significant reduction of the T_g with the addition of the proposed plasticizer as an indication of a good plasticization efficiency resulting in an PLA polymer chain mobility [25]. Particularly, the addition of SOFT-NSAFE to the PLA based films decreases the T_g to 42 ºC. This reduction of the T_g is less pronounced in the PLALR due to the little miscibility between PLA and CITREM-LR10, although it seems that some molecular interactions existed between PLA and CITREM-LR10. Likewise, the T_{cc} values decreases with the incorporation of plasticizers which is consistent with the enhanced chain mobility that makes PLA crystallizes easer at lower temperature [26]. The cold crystallization transition is appreciated in PLASS at temperatures of around 79 ºC, meanwhile in PLALR this temperature is observed around 89 ºC, which is considerably lower in comparison with neat PLA film found at 104 ºC. A second crystallization process is detected at c.a. 150 ºC attributed to the recrystallization of PLA α' crystals into a more stable α-crystals [27], [28], and then, melting process takes place above 170 ºC. This T_m also decreases slightly with the incorporation of plasticizers while the degree of crystallinity increases. The

PLA film remains mainly amorphous due to the its low crystallization kinetics; however, the plasticizers seem to interact with the macromolecular chains increasing their mobility and accelerating the crystallization of PLA [29]–[31].

In Ecovio® based films, the T_g 's of both polymeric components of the blend, PLA and PBAT, are clearly observed (see **Figure C3. A4. S2b.** in Supplementary information), revealing the immiscibility of the two components [32]. Likewise, the addition of SOFT-NSAFE and CITREM-LR10 compounds provokes a decrease of the T_g and T_{cc} values due to the increased mobility of the polymeric chains. Besides, two melting processes are appreciated at around 120 ºC and in the region between 140-160 ºC corresponding to PBAT and PLA phases, respectively. The wide melting peak of PBAT is because is not able to crystallize to a significant degree as is a random copolymer. Nevertheless, the melting point of PBAT is influenced by the addition of the plasticizers, slightly decreasing. The melting region of PBAT and cold crystallization of PLA overlap in the same temperature range, then, the calculation of the degree of crystallization should be considered with caution. On the other hand, the PLA presents two melting peaks, which is typically associated to melting re-crystallization and re-melting process [33]–[36]. As can be seen, the double melting behaviour of PLA was also affected by the incorporation of the tested plasticizers as indicative of the changes that they provoke in the size and perfection of the crystals.

Subsequently, the mechanical properties of the plasticized and unplasticized films were evaluated by tensile testing. **Table C3. A4. 5.** summarizes the results obtained.

Neat PLA film has high Young modulus and tensile strength but low elongation at break, presenting a glass-like brittle behaviour at room temperature. The incorporation of the tested plasticizers clearly reduces the tensile strength and Young modulus which can be associated to the decrease of the intermolecular forces between PLA polymer chains that enhances their mobility, causing an increase in flexibility. Concerning the elongation at break, the addition SOFT-NSAFE only provokes a little improvement from 4.6% to 6.3% for PLASS, whereas the CITREM-LR10 produces a considerable increase in the elongation at break up to 42.5%, despite the partial phase separation observed between PLA and plasticizers. In this case, it seems that CITREM-LR10 microdomains could act as stress concentrators under the tensile stress and were elongated parallel to crack

direction. This allows PLA matrix to deform more easily, occurring a shear-induced plastic deformation [37]–[39]. This behaviour has been previously investigated and open a wide range of possibilities for the development of toughened PLA materials as can improve some mechanical properties without a significant reduction in the glass transition temperatures.

Table C3. A4. 5. Mechanical properties of the PLA and Ecovio® based films. Mean values ± standard deviation. For each system, values having the same letter are not significant different for Tukey test (significance level of $P \leq 0.05$).

Sample	Elongation at break (%)	Tensile strength (MPa)	Young modulus (MPa)	Nº Samples tested
PLA	4.6 ± 1.1^{a}	56.3 ± 5.2^{a}	2192.8 ± 123.6^{a}	7
PLASS	6.3 ± 3.0^{a}	30.5 ± 3.5^{b}	1872.6 ± 94.9^{a}	8
PLALR	42.5 ± 12.2^{b}	27.7 ± 4.8^{b}	1635.9 ± 190.8^{a}	9
ECO	2.6 ± 0.8^{a}	13.6 ± 1.5^{a}	954.8 ± 50.9^{a}	7
ECOSS	3.2 ± 2.0^{a}	1.38 ± 0.4^{b}	124.4 ± 52.9^{b}	7
ECOLR	2.3 ± 1.0^{a}	1.6 ± 0.9^{b}	261.8 ± 67.0^{c}	7

On the other hand, for Ecovio® based samples, the films exhibit a relatively low tensile strength and modulus due to the presence of soft elastomeric PBAT phase and to the low crystallinity degree, as observed by DSC. As expected, the incorporation of plasticizers decreases such values. However, the elongation at break does not improve significantly. The poor mechanical properties for Ecovio® based sample could be ascribed to the immiscibility of the polymers that produces phase separation as can be seen by SEM and confocal Raman microscopy. In many studies, PLA/PBAT blends showed improved elongation at break, however the miscibility of the phases is a crucial factor, and the incorporation of compatibilizers are required. The processing method also affects the miscibility of the polymers, typically blow melting is used to fabricate PLA/PBAT films with greater elongation properties, whereas compression molding produces brittle films [16], [32], [34], [40].

Another crucial property to assess in biobased food packaging films is water vapor and oxygen permeability. **Figure C3. A4. 6.** shows the effect of the addition of plasticizers on the water vapour (KP_{WV}) and oxygen (KP_{O2}) permeabilities, normalized for the thickness. As can be seen both KP_{WV} and KP_{O2} increase in the plasticized films, as the free volume of the polymers augments leading to increasing the diffusion and permeation rate [41], [42]. This increase in the water vapour permeability, is much higher in films plasticized with CITREM-LR10, which could be explained by the higher hydrophilicity and hygroscopic nature in comparison with the SOFT-NSAFE plasticizer [43]. In addition, the low miscibility between PLA and CITREM-LR10 could create favourite ways of water and oxygen diffusion and thus would increase the permeability of films, making them suitable for packaging high-respiring foods such as vegetables and certain types of cheese. Otherwise, in these films the incorporation of fillers with high-barrier properties is fully required for O_2 and water vapour sensitive foods.

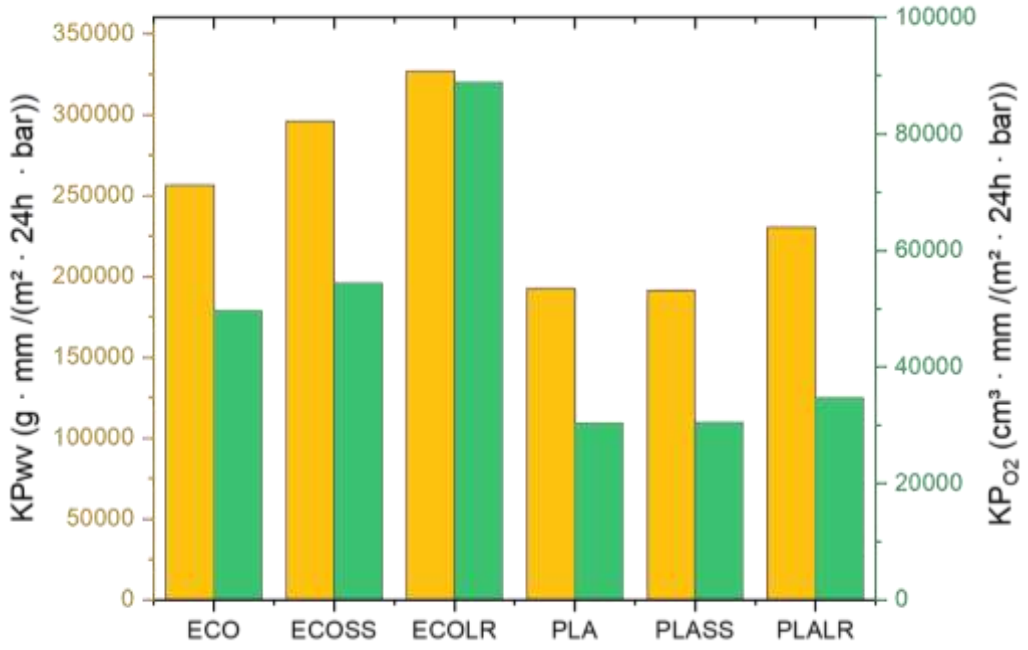

Figure C3. A4. 6. Water vapour and oxygen coefficient of permeabilities of PLA and Ecovio® based films monitored at 25 °C and under 65% RH for water vapor measurement and 50% RH for oxygen measurements.

3.4. Antioxidant and antibacterial activity of the different films containing functional plasticizers.

Once mechanical, thermal and gas permeability properties of the films based on PLA and Ecovio® have demonstrated the effect of the proposed plasticizers, the bioactivity of those films was further studied. **Figure C3. A4. 7.** illustrates the results of the antioxidant properties determined by 24 h after DPPH solution in contact. It is observed that the antioxidant capacity of the PLA based materials is lower than that of the Ecovio® samples. This behaviour could be associated to the higher flexibility and segmental mobility of PBAT chains in the blend, allowing a better accessibility of the antioxidant compounds. Besides, there are some differences when using CITREM-LR10 or SOFT-NSAFE as plasticizers in the films. As demonstrated below, the CITREM-LR10 presents higher activity that SOFT-NSAFE, then, and as expected, the films that contain CITREM-LR10 (PLALR and ECOLRR) exhibit higher antioxidant activity that those containing SOFT-NSAFE (PLASS and ECOSS).

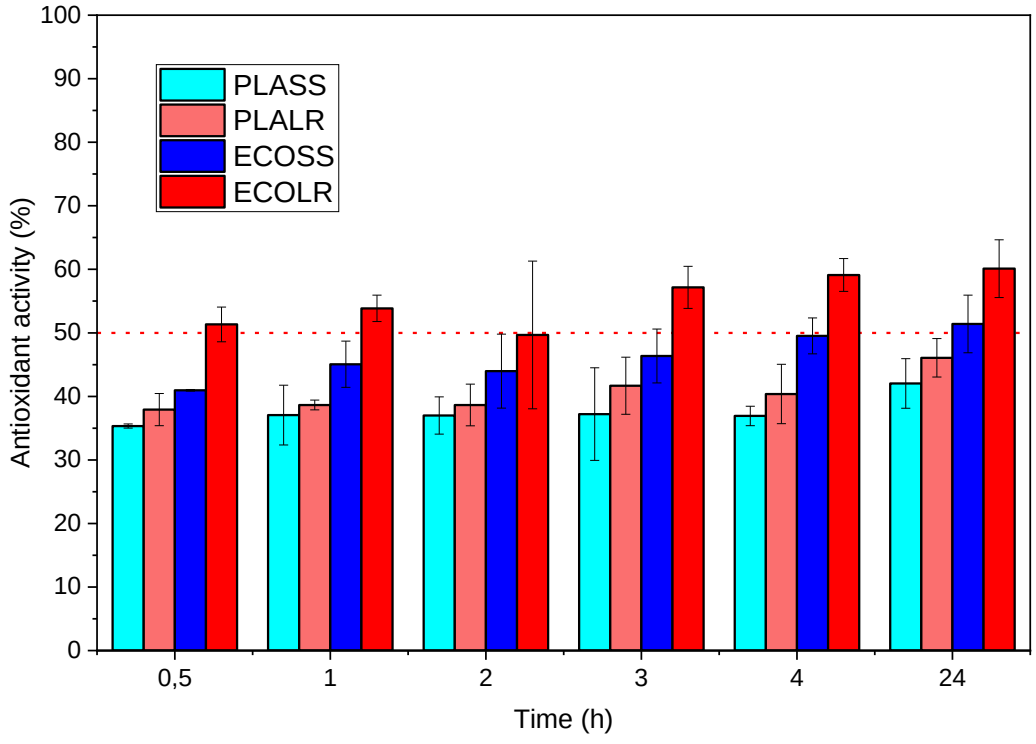

Figure C3. A4. 7. Antioxidant activity of the films containing either CITREM-LR10 (PLALR and ECOLR) or SOFT-NSAFE (PLASS and ECOSS) plasticizers obtained at different contact times with DPPH solution.

Additionally, the antibacterial activity of films containing CITREM-LR10 and SOFT-NSAFE components was evaluated using a film contact method against *S. aureus* bacteria, a typical food pathogen Gram positive bacterium, able to produce enterotoxins [44]; once formed, staphylococcal enterotoxins are extremely difficult to eliminate from foods, as they are resistant to heat, freezing and irradiation [45]. **Table C3. A4. 6.** exhibits the antibacterial activity, expressed as the difference in bacterial counts (Δlog CFU/ml) between inoculation (3.7 log CFU/mL) and after 24 h of contact with the films. It is clearly observed that the materials with CITREM-LR10 plasticizers killed 3.7 log CFU/mL bacteria in both films PLALR and ECOLR. In contrast in the PLA film with SOFT-NSAFE, the number of bacteria were not significantly reduced (0.57 log CFU/mL), only in Ecovio® based film, a relatively good reduction was observed, (1.35 log CFU/mL) associated to the better flexibility and mobility of polymeric chains that provide a better accessibility of the compounds. The results evidence that CITREM-LR10 containing citric acid and citric acid ester groups, provides good antibacterial activity to the films. It is well known the germicidal activity of citric acid associated mainly to the capacity of lowering the local pH environment and the intracellular pH that may cause damage to proteins, enzymes, DNA and membrane, or suppress the NADH oxidation, thus leading, in any case, to bacterial death [14], [46].

Table C3. A4. 6. Antibacterial activity of the plasticized films based on PLA and Ecovio®, expressed as the difference in bacterial counts (Δlog CFU/mL) between inoculum (3.7 log CFU/mL) and after 24 h of contact with the films.

Film	Δlog CFU/ml
PLASS	0.57 ± 0.03^{a}
PLALR	3.7 ± 0.0^{b}
ECOSS	1.35 ± 0.21^{c}
ECOLR	3.7 ± 0.0^{b}

3.6. Migration of the plasticizer.

The possibility of migration of plasticizers from the films is always a concern when its intended use is food packaging. Even when approved additives such as CITREM (E472c) or safe plasticizer like SOFT-NSAFE, are used, their possible migration could change the sensorial properties of the packaged food. Herein, the migration was studied using a standard food simulant, concretely Simulant D1 made with 50% ethanol/water. The films were immersed in this solution for 10 days at 20 ºC. After this period, the sample was recovered and reweighted while the simulant was evaporated to dryness in an oven, and then, weighted. As a first indication of the low migration of the plasticizers, no sensitive mass loss was found in the films after this migration test in any case. Besides, in the gravimetric analysis of the residue after simulant evaporation, any significant mass could be detected. Nevertheless, the possible residue in the vial (not detected gravimetrically) was then reconstituted in 150 µL of dichloromethane (DCM), which was used to wash the vial and extract the migrating compounds. Subsequently, the DCM solution was analysed by gas chromatography-mass spectrometry (GC–MS). A calibration was done for the main components of each plasticizer and the peaks found in the chromatographs were subsequently quantified. **Table C3. A4. 7.** summarized the results obtained from the migration study. As can be observed, the migration amount values are very small for all the tested films, lower than the specific migration value of 60 mg/kg$_{food}$ (restriction limit for the group of substances to which the additives belong, Reg.EU 10/2011 [47]).

Table C3. A4. 7. Results obtained from the migration study analysed by GC-MS.

Film	Retention time of the main component (min)	Calibration equation	R^2	Migration amount (µg/kg)
PLASS	22	y=9.5*10^7x-1.7*10^7	0.9979	1.52
PLALR	16/28	y=1.6*10^6x -1.3*10^5	0.9998	<0.60
ECOSS	22	y=9.5*10^7x-1.7*10^7	0.9979	9.81
ECOLR	16/28	y=1.6*10^6x -1.3*10^5	0.9998	1.12

3.7. Disintegrability of PLA and Ecovio® based films under composting conditions.

Figure C3. A4. 8. and **Figure C3. A4. 9.** show the visual appearance of PLA and Ecovio® films after different incubation times in composting conditions. For PLA-based samples, the films were extracted every 7 days, while 9 days for Ecovio®-based films. The disintegration of all the films under composting is evident. During the first days of incubation, the films lost transparency, become yellowish and breakable, as indicative of the hydrolytic degradation process of the polymer matrices [48], [49]. As incubation time goes by, the degradation effects become more apparent. Samples weaken and fragment into small pieces.

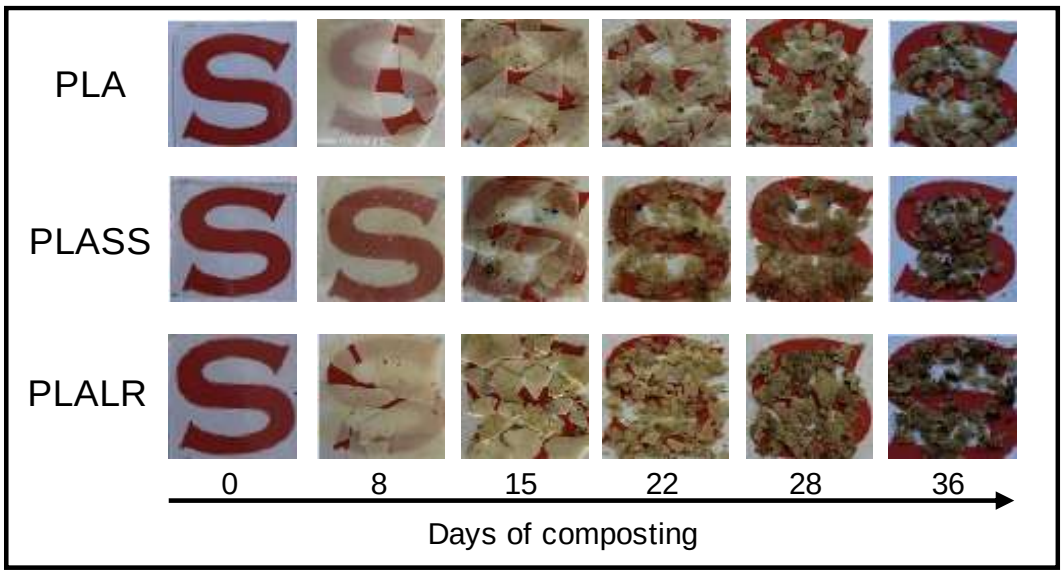

Figure C3. A4. 8. Visual appearance of disintegration of neat PLA and plasticized PLA-based films (PLASS and PLALR) under composting conditions.

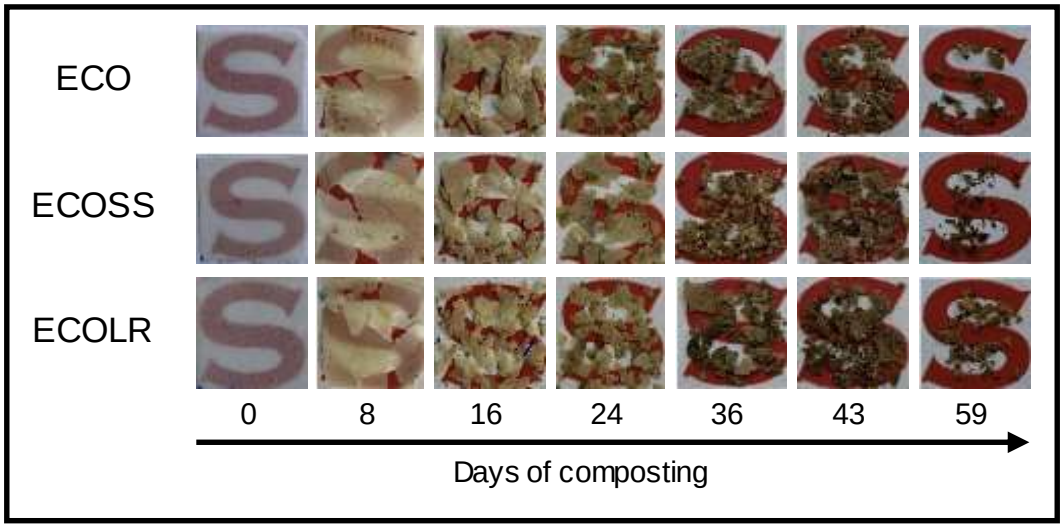

Figure C3. A4. 9. Visual appearance of disintegration of neat Ecovio® and plasticized Ecovio®-based films (ECOSS and ECOLR) under composting conditions.

Additionally, disintegrability of the films was evaluated in terms of mass loss as a function of incubation time. It is important to note that, as the composting progresses, the samples become more challenging to manipulate and recover. It becomes increasingly difficult to distinguish or remove the remaining compost attached to the films. Moreover, in accordance with the UNE-EN ISO 20200:2015 standard, fragments with dimensions smaller than 2 mm should not be taken into consideration. As reported in **Figure C3. A4. 10.**, PLA-based films showed a disintegrability value up to 90% after 40 days of incubation in composting conditions, whereas the Ecovio®-based films require longer times for degradation due to the PBAT component. At the beginning of the process the graphs show a low disintegration rate due to the decrease in the molecular weight of PLA during hydrolytic degradation without registering any important gravimetric mass loss. It is evident that the addition of plasticizers does not have a significant impact on the degradation rate, as observed.

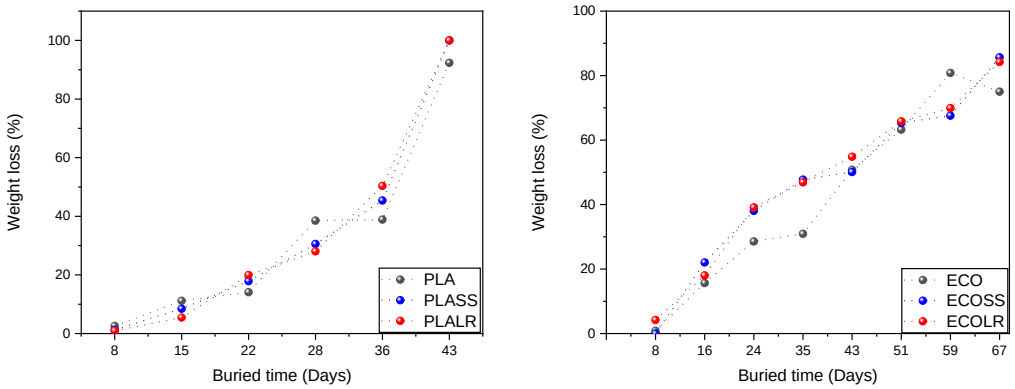

Figure C3. A4. 10. Gravimetric mass loss (%) for PLA (left) and Ecovio® (right) based films at different stages of incubation in compost.

The samples under degradation were further analysed by SEM. In the PLA series, the films exhibited smooth and homogeneous surface before the incubation (**Figure C3. A4. S3.** of Supplementary information). Then, the appearance of cracks, fractures and holes in the films surface is noticeable during degradation. On the other hand, in the Ecovio® based samples, the initial films showed the immiscibly between PLA and PBAT phases (see **Figure C3. A4. S4.** of Supplementary information). Then, the roughness increases significantly during the incubation time.

The samples extracted at different times from the disintegration test were also analysed by DSC. The calorimetric curves of composted samples are displayed in **Figure C3. A4. S5.**, while the thermal parameters are summarized in **Table C3. A4. 8.** Values were obtained from the first heating scan, except for the T_gs that were measured during the second heating scan. The most notable result was the disappearance of the cold crystallization transition (T_{cc}) of PLA in the samples incubated in compost, combined with an important increase in the crystallinity of all samples, which can be explained by a preferential degradation of the amorphous regions of the polymers [50], [51]. In the Ecovio® based samples, after this increase in crystallinity, a further reduction is clearly observed after 35-43 days, likely attributed to the degradation of residual PLA. Furthermore, the reduction in the melting temperature of the polymers is observed with increasing incubation time and attributed to the smaller size of the less perfect crystals, requiring less

temperature to melt. On the other hand, the T_g values do not have a clear tendency upon degradation, as low molecular weight chains, due to the hydrolysis, present higher mobility but the increase on crystallinity reduces such mobility.

Table C3. A4. 8. Thermal parameters of PLA and Ecovio® based films after different days of incubation under composting conditions. The glass transition temperatures (T_g) were obtained from the second heating run whereas melting temperatures (T_m) and crystallization degrees (X_c) were obtained from the first heating run.

Sample	T_g PBAT (ºC)	T_g PLA (ºC)	T_{cc} PLA (ºC)	T_{cc} PBAT (ºC)	T_m PLA (ºC)	$X_c PLA$ (%)
PLA day 0	-	59	104	-	174	0.3
PLA day 8	-	59	-	-	171	75
PLA day 15	-	55	-	-	161	89
PLA day 22	-	57	-	-	160	97
PLA day 28	-	57	-	-	159	83
PLASS day 0	-	42	79	-	171	14
PLASS day 8	-	43	-	-	160	65
PLASS day 15	-	40	-	-	160	82
PLASS day 22	-	44	-	-	159	82

PLASS day 28	-	45	-	-	151	81
PLALR day 0	-	53	89	-	172	11
PLALR day 8	-	54	-	-	168	70
PLALR day 15	-	52	-	-	161	83
PLALR day 22	-	52	-	-	157	85
PLALR day 28	-	53	-	-	155	75
ECO day 0	-32	57	111	129	147/154	2
ECO day 8	-34	46	-	101	148	56
ECO day 16	-29	51	-	102	143	86
ECO day 24	-33	47	-	102	140	48
ECO day 35	-25	50	-	106	135	38
ECO day 43	-21	47	-	105	129	8
ECO day 51	-21	30	-	108	-	-
ECOSS day 0	-45	42	84	120	139/150	4
ECOSS day 8	-21	46	-	121	151	45

ECOSS day 16	-30	39	-	99	140	64
ECOSS day 24	-35	36	-	99	138	50
ECOSS day 35	-24	40	-	105	130	n.a
ECOSS day 43	-20	33	-	107	120	5
ECOSS day 51	-20	-	-	107	-	-
ECOLR day 0	-36	54	99	122	143/152	4
ECOLR day 8	-29	49	-	120	151	34
ECOLR day 16	-34	44	-	100	142	60
ECOLR day 24	-30	45	-	101	139	49
ECOLR day 35	-21	48	-	105	134	39
ECOLR day 43	-21	-	-	107	-	-
ECOLR day 51	-26	-	-	108	-	-

-: not applicable

n.a.: no analysed since T_gs are not resolved, whereas X_c: no analysed due to the overlapping of the peaks.

Likewise, in the Ecovio® based films, the melting temperature of both phases, PLA and PBAT decreases during composting. However, the curves showed broad endothermic peaks that can overlap different transitions and, therefore, the

estimation of the degree of crystallinity cannot be performed accurately. Also, the T_gs values of PBAT and PLA phases, determined at the second heating DSC curves, suffer modifications during the composting.

Next, the disintegrated films were studied by FTIR and Raman spectroscopies, which can provide insights into the changes occurring in the chemical structure after composting. Firstly, **Figure C3. A4. 11.** displays the FTIR spectra of the samples. FTIR spectra of the disintegrated PLA films did not exhibit significant changes compared to those of the original material before incubation. This is because the hydrolytic degradation process produces oligomers or monomers with almost similar chemical structure that the PLA and thus similar bands associated to bonds characteristic of PLA [16], [52]. For instance, it is observed at 1745 cm^{-1} associated to the stretching of the carboxyl group –C=O, at 1453 cm^{-1} corresponding to the CH_3 antisymmetric bending vibration, and at 1181 cm^{-1} due to the C-O-C stretching vibration. Nevertheless, small changes can be seen during degradation process. The most appreciable change is a new band at 1635 cm^{-1} which could be attributed to carboxylate ions (R-COO−). The formation of these ions is attributed to the activity of microorganisms [53]. Also, an increase in the band associated to OH groups (broad band at around 3320 cm^{-1}) is visible in the spectra of films under degradation [54], [55].

Similarly, in Ecovio® based films (**Figure C3. A4. 11.**, right spectra), all films exhibit nearly identical spectra after degradation as the original materials, with no substantial differences, aligning with observations in other systems based on PLA/PBAT blends. Although some absorption bands weaken, such as the absorption band at 720 cm^{-1} attributed to the external bending vibration of the (–C–H) group on the benzene ring of PBAT. The carbonyl stretching vibrations of PLA and PBAT are reported at approximately at 1730 cm^{-1} and 1710 cm^{-1} for PLA and PBAT, respectively. In the figure, it can be seen both signals as a broad peak with a shoulder at higher wavenumbers, this shoulder attributed to the carbonyl group of PLA decreases during composting, which indicates that the degradation of the PLA phase occurs faster than the process PBAT [3], [16].

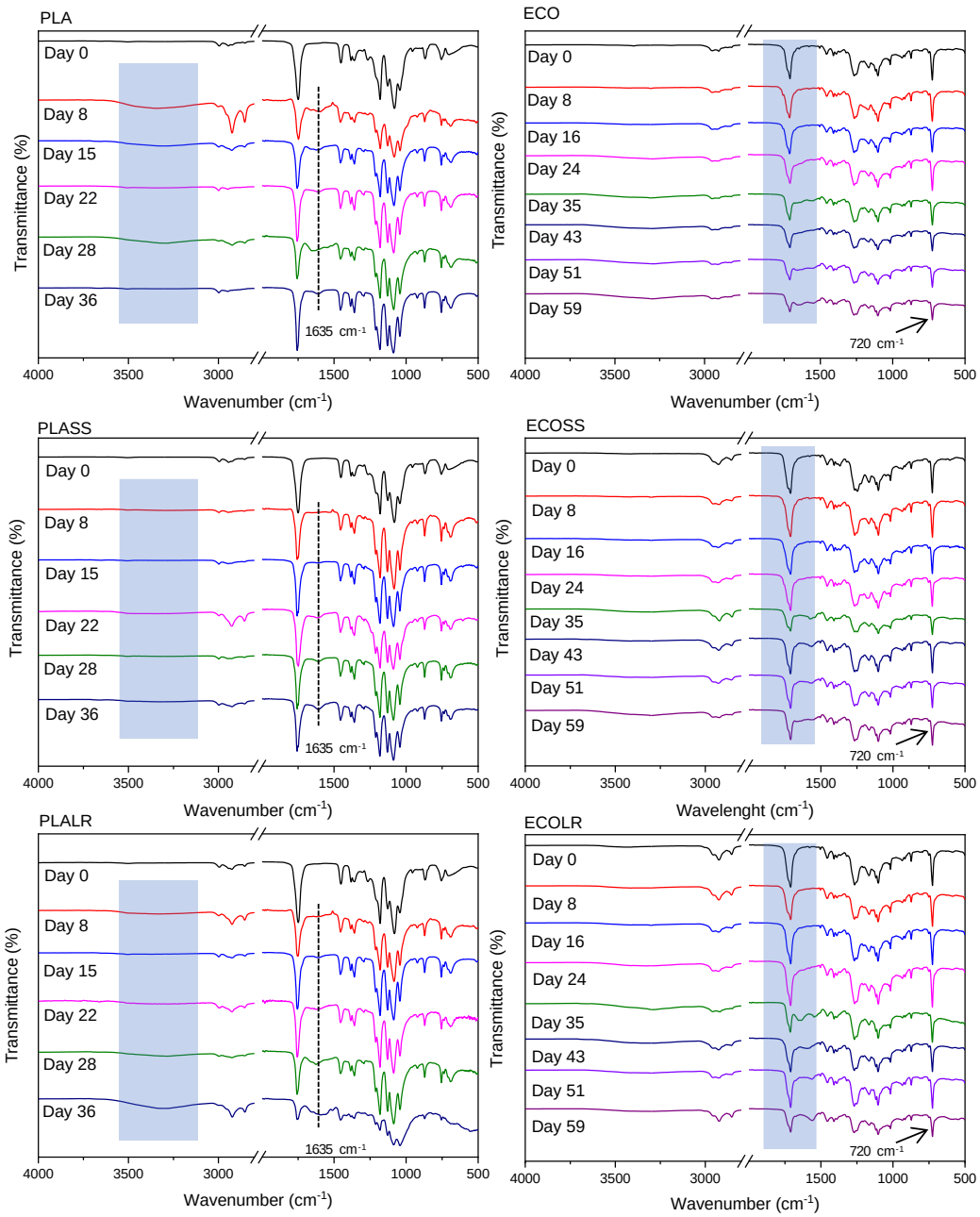

Figure C3. A4. 11. FTIR spectra of PLA (left) and Ecovio® (right) based films before and after different stages of disintegration under composting conditions.

It is worthy to mention that no clear differences were observed between samples with the different plasticizers and we were unable to identify any peaks related to the presence of CITREM-LR10 or SOFT-NSAFE to track the disappearance of the plasticizer during the composting process.

These films were also analysed by confocal Raman microscopy. **Figure C3. A4. 12.** and **Figure C3. A4. S6.** (see Supplementary information) show the Raman

spectra of all the tested films. In all the series, the fluorescence intensity considerably increases with the days of incubation in compost, due to the increase of coloration and opacity in the films under degradation. Likewise, no important variations can be observed in the main bands of the spectra in comparison with those found in the original films before the composting process, that is, with respect to the spectra of the pristine materials. In PLA based films (**Figure C3. A4. 12.**) in addition to the main Raman bands at 1775 cm^{-1} (stretching vibration of the carbonyl bond), at 1453 cm^{-1} (symmetric deformation vibration of methyl) and at 874 cm^{-1} (stretching vibration of the C–COO), during degradation, the Raman band at 923 cm^{-1} (assigned to crystalline phase) become more evident due to the preferential degradation of the amorphous phase [49].

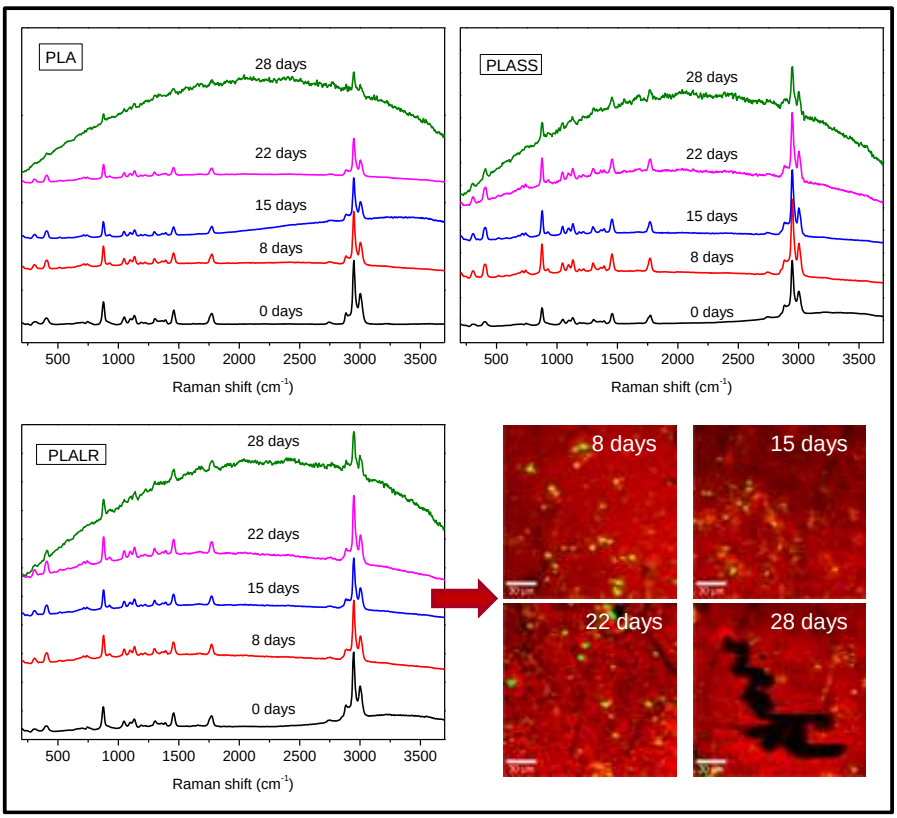

Figure C3. A4. 12. PLA based films (PLA, PLASS and PLALR) Raman spectra at different times of incubation under compost conditions. XY Raman images of the PLALR films after 8, 15, 22 and 28 days of disintegration.

Remarkably, the CITREM-LR10 microdomains visible in the Raman images remain in the films although the presence of smaller and less regions is evident,

which seems to indicate the low diffusion of the plasticizers to the soil during the degradation process.

Similarly, in Ecovio® based films (**Figure C3. A4. S6.**) a considerable augment of fluorescence is appreciated in the spectra during the degradation. In the average spectra is observed the main Raman bands associated to PLA and PBAT. For instance, at 1718 cm^{-1} (carbonyl stretching mode), at 1614 cm^{-1} (stretching vibration of the -C=C- in benzene ring) and at 1449 cm^{-1} (methylene group in plane bending mode) are ascribed to the PBAT phase; whereas the bands at 1775 cm^{-1} (-C=O stretching vibration), and at 2947 cm^{-1} (symmetrical stretching vibrations of the C–H bond) are associated to the PLA component. It is worthy to remark, that during incubation in compost, the bands associated to PLA decrease in intensity in comparison with the bands corresponding to PBAT, demonstrating the PLA degrades faster than the PBAT.

4. Conclusions.

In this article, we have extensively evaluated a citric acid derivative, not fully esterified and typically used as food emulsifier additive, as new biobased and safer plasticizer for bioplastics such as PLA and Ecovio®. CITREM-LR10 significantly increases the mechanical properties of the films in terms of toughness, almost maintaining their thermal stability. Notably, CITREM-LR10 provides outstanding antimicrobial, at least against *S. aureus* and antioxidant activities to the films, while negligible migration in food simulant solution was appreciated. Also, the plasticizer does not modify the biodisintegradability process under composting conditions. However, the barrier properties against water vapour and oxygen slightly decrease with the incorporation of plasticizers, then the addition of barrier fillers would be required for those food packaging applications that require high protection against water and oxygen.

Acknowledgments.

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Supplementary info

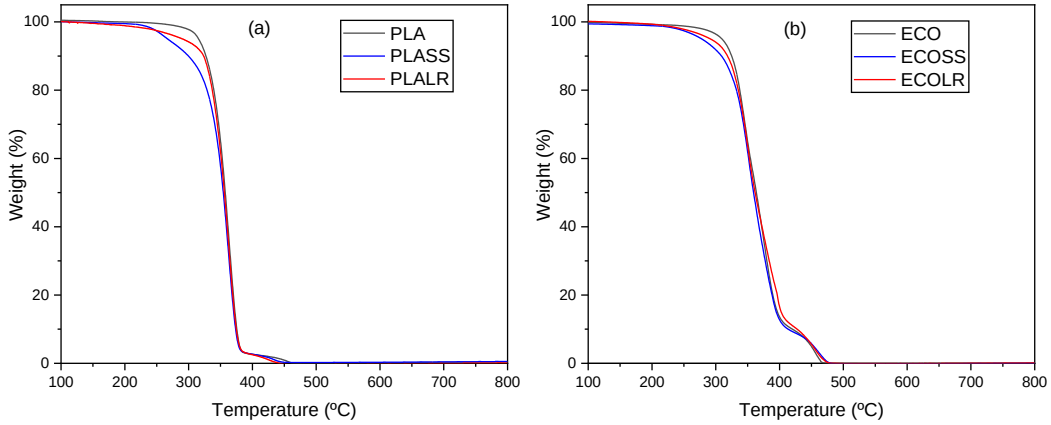

Figure C3. A4. S1. TGA curves of the obtained films based on: a) PLA and b) Ecovio® under air atmosphere.

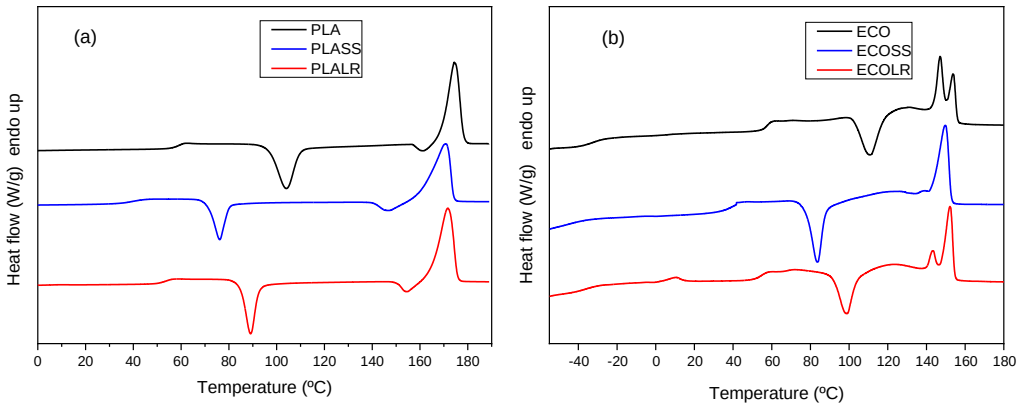

Figure C3. A4. S2. DSC thermograms, first heating curves of a) PLA and b) Ecovio® based films.

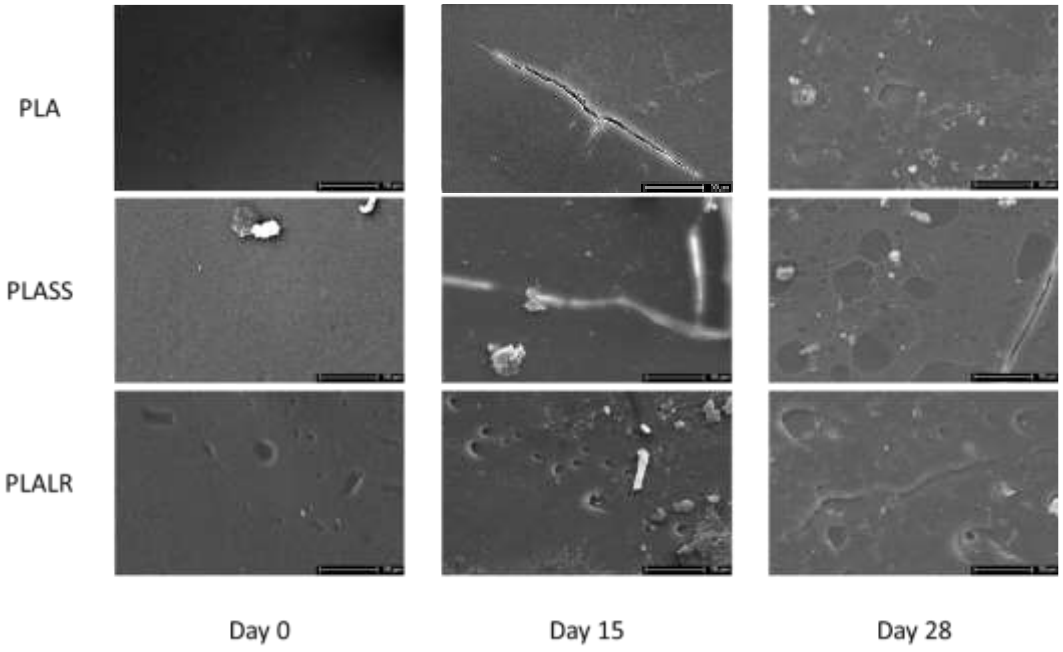

Figure C3. A4. S3. SEM images of PLA based films taken at different days of incubation.

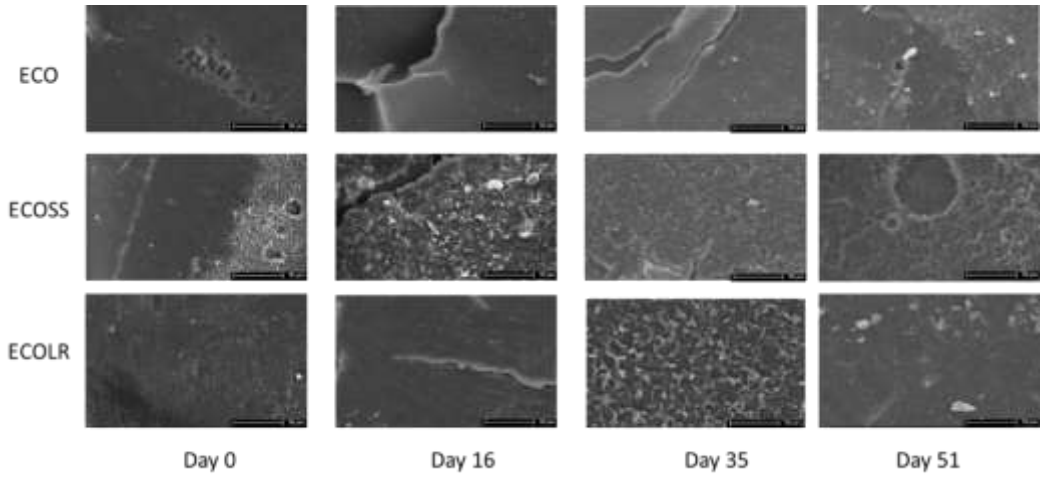

Figure C3. A4. S4. SEM images of Ecovio® based films taken at different days of incubation.

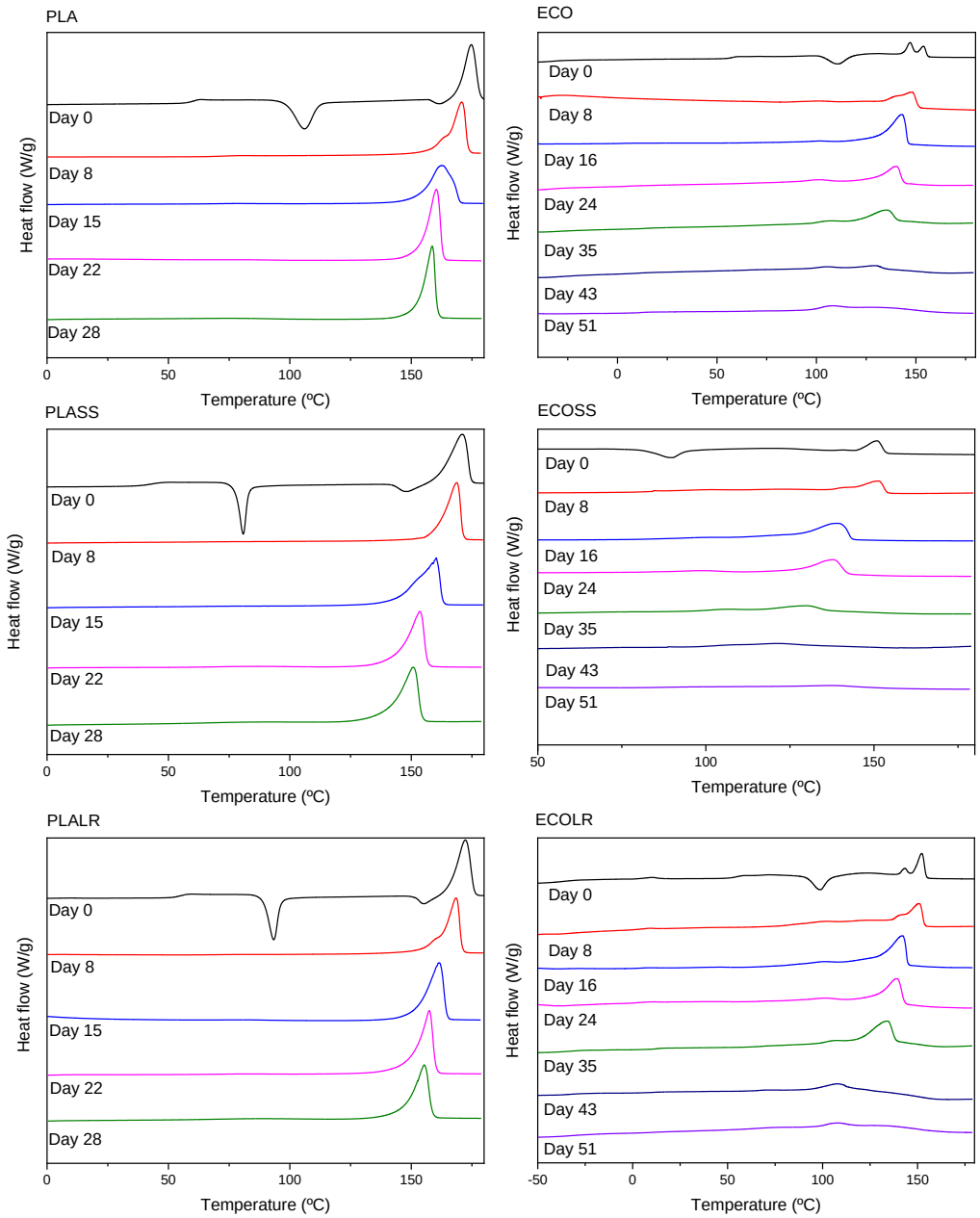

Figure C3. A4. S5. DSC graphs of the second heating of PLA and Ecovio® based films after different days of composting.

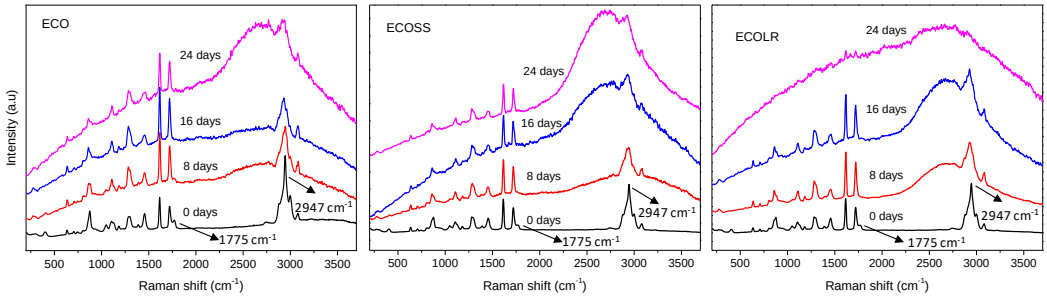

Figure C3. A4. S6. Ecovio® based films (ECO, ECOSS and ECOLR) Raman spectra at different time of incubation under compost conditions.

Article 5: Calendering Processing of PLA/PBAT Biodegradable and Functional Films Incorporating Natural Food Additives.

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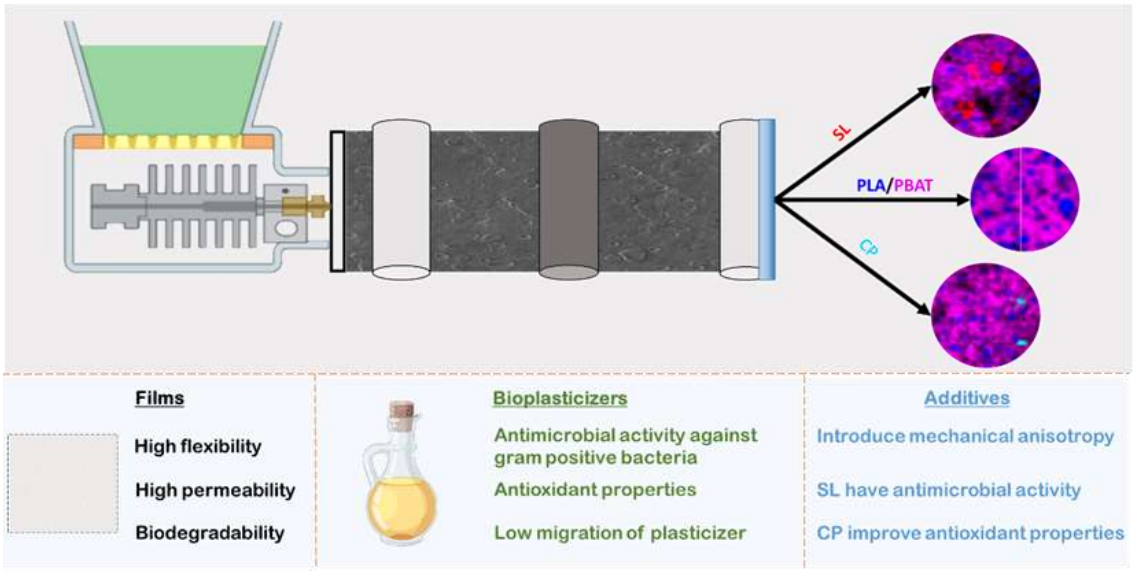


Abstract.

Herein, biodegradable and functional films based on PLA/PBAT (Ecovio®) blends were prepared by calandering processing that allows high-quality films and proper blend mixing. To provide functional properties such as antioxidant and antimicrobial activity, food additives were incorporated including sodium stearoyl lactylate (designated as E481) at 5 wt% and bioplasticizers derived from vegetable oils, namely CITREM (E472C) and ACETEM (E472A) at 10 wt%. Chitin nanocrystals were also added to enhance barrier and mechanical properties. The films of around 200 µm thick were produced resulting in uniform and high-quality materials. Scanning electron microscopy and confocal Raman microscopy demonstrated good mixing of PLA and PBAT biopolymers, as well as a homogeneous dispersion of the additives. This effective blending provides excellent mechanical performance, particularly elongation at break, with values approaching 500% for most of the prepared films. The additives had minimal effect on the crystallinity, thermal and barrier properties while imparting antioxidant and antimicrobial properties with negligible migration into food simulant media, bellow 0.08 mg/Kg in all the cases. Citroglycerides, mono-and diglycerides of fatty acids and sodium stearoyl lactylate provides Trolox equivalent antioxidant capacity (TEAC) values between 14 and 18 µmol Trolox/g, and were effective against Gram-positive *Staphylococcus aureus* and *Listeria innocua* achieving complete bacterial inhibition at the contact tested concentration (ΔLog CFU/mL: 4.54 and 3.71, respectively). Finally, the disintegration of the films under industrial composting condition was demonstrated; this issue is essential to evaluate the environmental impact of the packaging at the end of its life cycle.

1. Introduction.

General consumers along with legislation aimed at reducing plastic waste, have shifted their focus and financial support toward the development of new systems designed to address problems associated with the slow degradation of plastics, which is a significant environmental concern particularly due to their accumulation in both marine and terrestrial environments. Moreover, the production of plastics is highly controversial due to either the unsustainability of the process and the limited availability of fossil fuels [1].

New opportunities have been opened for the development of bioplastics, which can replace non-biodegradable and fossil-based plastics. Bioplastic production is expected to rise from 2.37 to 5.73 million tonnes over the period 2024-2029. This increase is driven by new and upcoming mandatories that are either being implemented or waiting to approval. These includes mandatories such as the European Packaging and Packaging Waste Regulation (PPWR, Reg. EU 2025/49) [2], which emphasizes the role of biobased plastics in achieving circular economy and climate goals. Indeed, biobased and compostable plastics offer sustainable packaging solution; the revision of the EU Waste Framework Directive (Dir. 2008/98/EC under amendment, WFD) should acknowledge the vital role of bioplastics in reducing environmental and climate impacts by promoting bio-based, recyclable, and compostable materials [3]. Similarly, the EU Fertilising Products Regulation (Reg. EU 2019/1009 FPR) recognises that biodegradable mulch films have agronomical efficiency compared to that of non-biodegradable plastics [4]; among other relevant regulations [5].

Bioplastics are a diverse family of plastics categorized into biodegradable and non-biodegradable types. Biodegradable bioplastics vary in their degradation rates and pathways, with examples including poly (lactic acid) (PLA), polyhydroxyalkanoates (PHA), cellulose, and starch. Non-biodegradable bioplastics, such as biopoly (ethylene terephthalate) (bio-PET), polyol-polyurethane, and bio-polyethylene (Bio-PE), retain the durability of traditional plastics while being derived from renewable resources. Most bioplastics are synthesized from biopolymers obtained from renewable biomass. Due to their natural origin, many exhibit compostable or biodegradable properties. However, a key limitation of biopolymers is their generally lower mechanical performance compared to conventional plastics [6]–[8].

PLA, a highly promising biopolymer synthesized from agro-industrial wastes, among others sources, is noted for its mechanical strength, which is comparable to that of PE, along with its excellent barrier properties against flavours and aromas and good processability [9]. As a result, PLA has already been employed in food packaging and food contact applications [10]. Further, to enhance the inherent properties, PLA is often reinforced with other polymers or substances. Among the various composites, a promising biodegradable copolyester suitable for packaging is poly (butylene adipate-*co*-terephthalate) (PBAT) which combined with PLA enhances the flexibility and brittleness of PLA while maintaining the disintegration in industrial composting conditions [7], [11]. Additionally, to improve the mechanical properties of PLA and its blends, bioplasticizers are widely incorporated to the formulation [12]. Examples include natural macromolecules such as citric acid esters of mono- and diglycerides, also known as citroglycerides or CITREM (designated as E472C) and acetic acid esters of mono-and diglycerides of fatty acids, also known as ACETEM (designated as E472A). Both are commonly used as emulsifiers in food products and approved by the US FDA (Food and Drugs Administration) [13]. When added to the composite blend, these bioplasticizers not only improve mechanical properties but also impart additional functionalities such as antimicrobial and antioxidant properties [11], [12].

Both materials have demonstrated the ability to plasticize PLA through compression moulding. However, their effects on improving the mechanical properties of PLA/PBAT blends were not significant. This outcome can be attributed to phase separation of both polymers occurring during compression moulding [15]. Indeed, PLA/PBAT blends processed by calendered rolls exhibit a more favourable phase distribution, resulting in higher homogeneity [16]. Thus, the processing and film formation techniques of such biopolymers also influence their performance.

Thin films are extensively employed in food packaging applications to provide flexible, lightweight and cost-effective solutions while maintain the quality and food safety of the products. Reducing the amount of plastic used in the film is a strategy that reduces plastic waste. Different techniques can be employed to produce thin films depending on the desired properties such as flexibility, barrier performance and the polymer properties. Among the different techniques to obtain thin films, processing methods such as blown filming and calendered rolls are widely em-

ployed to achieve high uniformity and smoothness of the film [17], [18] Calendering is a manufacturing technique used to obtain a thin layer by feeding a polymer throw a sequence of rolls rotating at controlled speeds, allowing precise control of sheet dimensions. Calendered films can be employed to package food products when uniformity, thickness control, high transparency, or improved mechanical properties are required [19].

PLA/PBAT blends with different polymer ratios have been studied using calendered rolls and blown film techniques to produce thin films suitable for food packaging applications. As a result, PLA/PBAT composites have shown a highly homogeneous microstructure when processed via calendering compared to films obtained through extrusion and compression moulding [16].

In this study, a commercial blend of PLA/PBAT, named Ecovio® (containing a 45/55 ratio), was reinforced with ACETEM and CITREM plasticizers processed by extrusion followed by calender rolling. To further enhance the properties of the PLA/PBAT blends, sodium stearoyl lactylate (designated as E481) (SL) and chitin particles (CP) were added. Chitin is a polysaccharide obtained from the shell of crustaceans and insects that exhibits antimicrobial properties and different functionalities in food packaging systems [20]. Particularly, chitin nanoparticles have been incorporated as a reinforcement to enhance the impermeability of the materials [21]. The addition of particles generally increases mechanical resistances by reinforcing the polymer matrix, resulting in an increase of stiffness while reducing the elongation at break [22]. Additionally, SL is commonly used in food applications to preserve quality and extend shelf life [23]. Acting as a humectant, SL helps to retain moisture in foods and is approved by the FDA as a food additive [17]. In this study, SL was incorporated to increase liquid absorption, with the idea of using these composites in applications such as absorbent pads. The resulting films were evaluated by comparing properties of the films containing different CITREM and ACETEM and either SL or chitin CP. Properties such as antimicrobial, antioxidant, microstructural, permeability, migration of substances as well as thermal and mechanical properties were analysed and discussed.

2. Materials and methods.

2.1 Materials.

Ecovio® pellets (Fblend C2224 density: 1.24-1.26 g/cm^3; melt flow index: 2.5 g/10 min) purchased from the Company BASF (Ludwigshafen, Germany).

Different compounds were employed as bioplasticizers for Ecovio® samples, as follows:

GRINDSTED® ACETEM SOFT-NSAFE (CAS number 736150–63-3), an acetic acid ester of monoglycerides (E 472a) made from fully hydrogenated castor oil, was obtained from Danisco.

GRINDSTED® CITREM LR 10 EXTRA KOSHER (CAS number 97593–31-2) (Product code 173636), a citric acid ester of mono-diglyceride (E 472c) made from edible, refined sunflower oil, was obtained from Danisco. This product also contains alpha-tocopherol (E 307) max. 200 ppm and ascorbyl palmitate (E 304) max. 200 ppm

GRINDSTED® CITREM SP70 MB (CAS number 97593–31-2) (Product code 172734), a citric acid ester of mono-diglyceride (E 472c) made from edible, refined sunflower and palm-based oil, was procured by Danisco. This product also contains α-tocopherol (E 307) max. 300 ppm and ascorbyl palmitate (E 304) max. 300 ppm.

Sodium stearoyl lactate with a purity of 97% (CAS Number: 25383-99-7) was purchased from Indagoo.

2.2. Preparation of the chitin particles.

Chitin particles were synthesized as previously reported [24]. Briefly, 20 g of chitin (from shrimp shells, Merck) were placed in a flask with 600 mL of 3 M HCl and heated at 100 °C for 90 min under magnetic stirring. The resulting product was dialyzed in water for at least three days, centrifuged several times, and lyophilized for three days. The obtained particles presented a rod-like morphology with a length of 123 ± 16 nm.

2.3. Preparation of the films.

Firstly, Ecovio® pellets were dried in an oven for 24 h at 55 ºC. Subsequently, Ecovio® pellets were mixed with the plasticizer at 10% in a beaker before being fed

into the extruder. The Ecovio® blends were prepared using a Brabender MetaStation 16 extruder equipped with a double co-rotating screw (L/D 40). The temperature profile across zones 1 to 7 was set at 170/170/170/170/160/160 °C, with a screw speed of 25 rpm to produce the pellets.

The extruded filaments were cooled in a water bath at room temperature and subsequently cut into pellets using an automatic knife cutter. The granules were then dried at 60°C before being mixed with the particles. Xplore MC 15HT Micro Extruder instrument equipped with two conical co-rotating screws was used to mix the pellets and particles at a temperature of 165 ºC and a screw speed of 40 rpm, the blend remained in the extruder for a residence time of 3 min.

For the production of thin films and to facilitate the collection of samples through the roll system, the nozzle was set to a temperature of 155 °C, along with an air system that helps cool the polymer as it exits the nozzle. Three rolls were placed 25 cm apart with the following velocity profile of 300/300/30 rpm.

2.4. Antimicrobial test.

The antibacterial properties of the obtained films were tested using the E2149-20 standard method from the American Society for Testing and Materials (ASTM), a standardized method to evaluate the antimicrobial activity of material in contact with the bacteria [19]. Food pathogens *Escherichia coli* (ATCC 25922:), *Staphylococcus aureus* (ATCC 29213), as well as *Listeria innocua* (ATCC 33090) were used as bacterial strains. In this analysis, bacterial suspension was prepared in PBS. Then, 100 mg of each biopolymeric films were placed inside sterile Falcon tubes containing 1 mL of a bacterial suspension and 9 mL of PBS. Blank experiments with only the inoculum in the absence of film were also performed. Subsequently, tubes were incubated for 24 h at 37 ºC with shaking at 120 rpm. The number of bacteria remaining in each sample was determined by the plate count method. The measurements were made at least in triplicate.

Results were expressed as the difference in logarithm between the initial colony formed units (CFU) concentration and the result after 24 h of suspension of the film inside the bacterial solution, following the next equation:

$$\Delta Log\ CFU/mL = Log\ CFU/mL\ (blank) - Log\ CFU/mL\ (after\ 24\ h) \quad (1)$$

2.5. Antioxidant assay.

The antioxidant properties of the films were evaluated employing the Blois method using the 2,2-diphenyl-1-picrylhydrazyl (DPPH, Merck) free radical. For this test, a DPPH solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol (Scharlau). For each test, 50 mg of each sample and 2 mL of the DPPH solution were added to vials, provoking the reaction. At different time periods, 100 µL of each mixture were withdrawn and transferred to a multi-well plate. The absorbance in each well was measured at 527 nm using a BioTek® SYNERGY HTX multi-mode reader spectrophotometer. After each measurement, the aliquots were returned to the original vials to maintain consistent concentration and volume. Measurements were taken at 1, 2, 3, 4, 5, 7, and 24 h.

Antioxidant capacity was determined relative to Trolox, (Thermo Scientific 97% CAS: 53188-07-1), used as the reference antioxidant. Results obtained at 24 h were expressed as Trolox equivalent antioxidant capacity (TEAC). All measurements were performed in triplicate. The following equations were employed to determine the TEAC values:

$$\text{RSA}\ (\%) = 1 - \left(\frac{\Delta\text{Absorbance of sample}}{\Delta\text{Absorbance of control}}\ x100\right) \quad (2)$$

$$\text{CTE}\ (\mu g/mL)\ =\ (\text{RSA})/\text{mcal} \quad (3)$$

$$\text{TEAC}\ (\mu mol/mg) = (C_{TE} / M_{Trolox})/ C_{sample} \quad (4)$$

In the equations, RSA is the radical scavenging activity, CTE denotes the Trolox equivalent coefficient, m_{cal} is the slope of the calibration curve, M_{Trolox} is the molecular weight of Trolox, and C_{sample} refers to the concentration of the sample in the DPPH solution.

2.6 Analysis of the migrated substances.

The potential migration of the plasticizers from the packaged into food was evaluated through a migration test performed at 20 ºC for 10 days, following the recommendation of the European Union and FDA recommendations (modified from the method of Yang [25]). Tests were carried out using standardized food simulants D1 (10% ethanol), D2 (3% acetic acid) and D4 (50% ethanol).

For this assay, 2 × 2 cm^2 squares of each film sample were immersed in 10 mL of each simulant and subjected to agitation for 10 days at 20 ºC to simulate storage conditions of foods. Films were then extracted, dried, and weighed, while the liquid in which the films were immersed was slowly evaporated in an oven. Once the vials were dried, dichloromethane was used to dissolved the remaining residues in the vials. The extracted residues were analysed using an Agilent 8860 GC gas chromatograph equipped with an Agilent mass spectrometry detector model 5977B (GC–MS). The separation of the compounds was performed on a HP-5MS capillary column (15 m length × 250 μm internal diameter and 0.10 μm film thickness). The carrier gas used was helium with a flow rate of 1 mL/min. A volume of 1 μL of the obtained extract was injected in split mode with a split ratio of 20:1. Injector temperature was 260 ºC. Compound identification was achieved by matching with the NIST 20 MS library.

2.7 Differential scanning calorimetry (DSC).

The thermal transitions of the Ecovio® based blends were analysed using a TA Q2000 differential scanning calorimeter (TA Instruments, New Castle, DE, USA). A nitrogen purge gas (50 cm^3/min) was applied throughout the experiments. The samples were initially heated from 20 °C to 65 °C at a rate of 10 °C/min to revert the effect of physical aging, followed by rapid cooling from 65 °C to −80 °C. A second heating scan was performed from −80 °C to 180 °C at 10 °C/min, followed by another rapid cooling, a third heating scan from −80 °C to 180 °C was conducted.

From the resulting DSC curves, the glass transition temperature (T_g), cold crystallization temperature (T_{cc}), and melting temperature (T_m) were determined. The degree of crystallinity (X_c) was calculated using the melting enthalpy values for 100% ($\Delta H_m°$) crystalline PLA (93.6 J/g) [26] and PBAT (114 J/g) and the weight fraction (Wf) of PLA/PBAT [27].

$$Xc(\%) = \frac{\Delta Hm - \Delta Hcc}{\Delta Hm° \; x \; Wf} x100 \qquad (5)$$

2.8 Thermogravimetric analysis (TGA).

Thermogravimetric analysis (TGA) was performed to evaluate the thermal stability of the various blends. Approximately 5 mg of each sample was analysed using a TGA Q500 thermal analyser (TA Instruments, New Castle, DE, USA). The samples were heated at a rate of 10 °C/min from room temperature (~30 °C) to 800 °C under an air

atmosphere (50 cm^3/min). From the resulting thermograms, the temperatures corresponding to 10% (T_{d10}) and 50% (T_{d50}) mass loss were determined. Additionally, the temperature at the maximum degradation rate (T_{dmax}) was identified from the first derivative of the TGA curves (DTG).

2.9 Confocal Raman microscopy.

To evaluate the miscibility and compatibility between different component of the films, a cryogenic fracture was performed, and the cross-section was analysed by confocal Raman microscopy on a CRM-Alpha 300 RA microscope (WITec, Ulm, Germany) equipped with Nd: YAG laser (maximum power output of 50 mW at 532 nm) and an optical resolution of approximately, 250 nm in the longitudinal and 700 nm in the transversal direction. The reflected laser light at each point was collected using a multimode optical fiber of 25 μm in diameter equipped with a very sensitive CCD detector. The acquired Raman spectra were analysed by using Witec Project 2.02 program and Witec Control Plus Software.

2.10 X-ray diffraction.

The diffraction patterns of Ecovio® functionalized films were analysed using wide-angle X-ray scattering (WAXS). Measurements were conducted on a SAXSpoint 5.0 system (Anton Paar), equipped with a Cu Kα microfocus source (λ = 1.5418 Å) and a two-dimensional Pilatus 1M detector (Dectris). This setup enables the investigation of preferred orientations in the formation of polymer crystals. The 2D X-ray diffractograms were radially integrated to generate 1D diffractograms using the SAXS Analysis software (Version 4.30.0.148 Release) provided by Anton Paar.

2.11 Mechanical properties.

Tensile testing was performed using a DX2000 QTest Elite MTS dynamometer (MTS Systems) at room temperature. The test was conducted at a stretching rate of 10 mm/min with a 100 N load cell. Dumbbell-shaped specimens were used, with a thickness of approximately 200 μm, a total length of 35 ± 0.5 mm, end width of 6 ± 0.5 mm, a narrow section length of 12 ± 0.5 mm, and a narrow section width of 2 ± 0.1 mm. Stress-strain measurements were used to calculate the ultimate tensile strength, Young's modulus, and elongation at break values.

The test was conducted in the horizontal (parallel to film orientation during calendering) and transversal directions (perpendicular to film orientation during

calendering). A minimum of five specimens were tested for each material and orientation to ensure reliability.

2.12 Field Emission Scanning Electron Microscopy (FE-SEM).

Surface and cross-section microstructure of the films was analysed using a SU 8000 Hitachi FE-SEM microscopy to evaluate the distribution and compatibility of PLA and PBAT phases. To observe the cross-section of the films, a cryogenic fracture was induced by immersing the samples in liquid nitrogen.

2.13 Permeability test.

The films were cut into a circular shape (2.01 cm^2) and used to analyse water vapour and oxygen permeability using an isostatic permeability analyser (Multiperm, PERMTECH S.r.l., Pieve Fosciana, Italy) following ASTM standard methods D-3985 and F-1249, respectively. The water vapour transmission rate (WVTR) was evaluated at 23 °C and 65% relative humidity (RH), while oxygen transmission rate (OTR) measurements were monitored at 23 °C under 50% RH. Two specimens were tested for each material.

2.14 Disintegration test.

The disintegration of the films under composting were incubated following the ISO20200 standard. The solid residue for composting was prepared by manually mixing with the following composition: 55 wt% water and 45 wt% of solid waste which consist of 10% compost (Compo®, Spain), 30% rabbit food, 10% starch, 5% sugar, 4% corn oil, 1% urea, and 40% sawdust. Two specimens were tested for each material.

Several films, each measuring 20 mm × 20 mm, were placed in textile mesh bags. These mesh bags containing the square films were then buried in the composting reactor at 58 °C under thermophilic conditions (pH 5–8).

Samples of each formulation were extracted at different incubation times for analysis. Before analysis, the samples were gently cleaned to remove adhered compost residues and then dried under vacuum at 60 °C for 24 h. The degree of disintegration was evaluated either visually and by Raman confocal microscopy using the conditions described in section 2.9.

2.15. Statistical analysis.

A one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was performed to identify significant differences ($P \leq 0.05$) among the groups.

3. Results and Discussion.

3.1 Preparation of functionalized Ecovio® based films.

Functionalized Ecovio® films were prepared by a melt extrusion process followed by calendering to obtain thin films (**Figure C4. A5. 1.**).

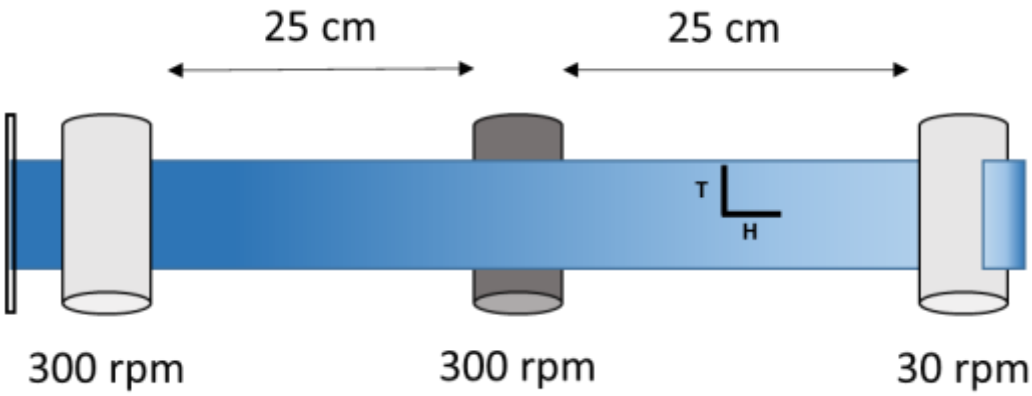


Figure C4. A5. 1. Schematic of the calendered roll system and the principal direction H and the transversal T.

Films contained 10 wt% of the bioplasticizers and 5 wt% of either CP or SL. **Table C4. A5. 1.** shows the composition of the different composites obtained using the nomenclature ECOXXYY, where XX is referred to the bioplasticizer (SOFT-NSAFE, SS; LR 10, LR; SP70 MB, SP) and YY is referred to the additives (Sodium stearoyl lactate, SL, and chitin particles, CP). Films of approximately 200 µm of thickness were obtained.

Table C4. A5. 1. Composition of the prepared samples.

Sample	Polymer	Plasticizer (10%)	Additive (5%)
ECO	PLA/PBAT	-	-
ECOSS	PLA/PBAT	SOFT-NSAFE	-
ECOSSSL	PLA/PBAT	SOFT-NSAFE	SL
ECOSSCP	PLA/PBAT	SOFT-NSAFE	CP
ECOLR	PLA/PBAT	LR 10	-
ECOLRSL	PLA/PBAT	LR 10	SL
ECOLRCP	PLA/PBAT	LR 10	CP
ECOSP	PLA/PBAT	SP70 MB	-
ECOSPSL	PLA/PBAT	SP70 MB	SL
ECOSPCP	PLA/PBAT	SP70 MB	CP

To evaluate the composition of the films, FTIR spectroscopy was performed (**Figure C4. A5. S1.**). The analysis revealed that, for the SL samples, a new absorption band appeared at 1594 cm^{-1}, which corresponds to the carbonyl (C=O) stretching vibration from R–CO_2 groups. In contrast, for the plasticized and CP samples, no significant differences were observed in the spectra.

3.2 Evaluation of the thermal properties of the films.

Thermal analysis of the prepared films was carried out to evaluate the thermal stability, crystallinity and thermal transitions, being crucial these parameters for the future applications. TGA and DTGA curves of the Ecovio® based samples were

displayed in **Figure C4. A5. S2.** and **Figure C4. A5. S3.** of the Supporting Information and the thermal parameters are summarized in **Table C4. A5. 2.**

Table C4. A5. 2.TGA and DTGA characterization for Ecovio® samples.

Sample	T_{d10} (ºC)	T_{d50} (ºC)	$T_{dmax}{}^{1}$ (ºC)	Der. weight[1] (%/ºC)	$T_{dmax}{}^{2}$ (ºC)	Der. Weight[2] (%/ºC)	$T_{dmax}{}^{3}$ (ºC)	Der. weight [3] (%/ºC)
ECO	330	371	345	0.013	385	0.012	466	0.006
ECOSS	326	379	343	0.013	385	0.013	455	0.002
ECOSSSL	282	381	285	0.003	389	0.018	438	0.016
ECOSSCP	339	378	353	0.009	389	0.015	481	0.009
ECOLR	333	375	344	0.011	383	0.012	460	0.003
ECOLRSL	289	390	287	0.003	397	0.020	441	0.016
ECOLRCP	335	378	350	0.011	388	0.014	460	0.003
ECOSP	330	375	349	0.007	388	0.014	457	0.009
ECOSPSL	281	384	284	0.003	389	0.015	436	0.018
ECOSPCP	335	382	349	0.012	392	0.014	464	0.002

A three-step degradation process was observed in the samples, first step is associated to the PLA while the second and third are associated to the PBAT [11]. The addition of plasticizer almost does not affect the value of T_{d10}, whereas the addition of additives produced a variation in T_{d10}. CP particles produced a slightly increase; contrary, SL decreases the value. On the other hand, the value of T_{d50} slightly increased with the functionalization of Ecovio®. In general, the addition of both plasticizers and CP preserved a degradation mechanism similar to that of neat Ecovio® without causing notable changes in the derivative weight loss with temperature,

while the presence of SL altered the degradation behaviour. Specifically, the derivative weight loss in the first step decreased, while it increased in the third step. As shown in **Figure C4. A5. S4.**, the degradation of SL starts at lower temperatures than $T_{dmax}{}^{1}$ of the samples, the degraded products of SL could affect degradation reactions of the biopolymers such as intermolecular transesterification reactions and depolymerisation [30].

Next, DSC was employed to examine the thermal transitions and crystallization behaviour of the prepared films. **Table C4. A5. 3.** presents the values obtained from the DSC thermograms of the Ecovio® samples during the second heating scan, conducted from -80 °C to 180 °C. The initial heating up to 65 °C was specifically performed to eliminate the effects of physical aging, a phenomenon commonly observed in PLA-based materials due to their glass transition temperatures (T_g) being relatively close to room temperature. The DSC thermograms exhibited two glass transitions, the first one attributed to PBAT at lower temperatures and the second attributed to PLA at higher temperatures. This behaviour indicates the non-miscibility between these two polymers as previously reported [16], [31].

In the Ecovio® samples with the plasticizer SOFT-NSAFE, the value of the T_g of PLA is reduced, as a result of the plasticisation effect of the bioplasticizers, resulting in an increase in the mobility of the polymer chains, while the T_g of PBAT is not modified practically with the addition of the plasticizer [32]. Besides, melting temperature (T_m) of PBAT is slightly reduced with the addition of this plasticizer. The plasticizer does not vary the crystallinity of the PLA and PBAT phase in comparison with the ECO neat film, while the addition of the SL additive increases the crystallinity of the PBAT and PLA phases. This behaviour could be explained due to the good distribution of the additive which can act as a nucleating agent increasing the crystallinity. Likewise, the addition of CP in ECOSSCP slightly increases the crystallinity of both phases. In the rest of the films with the other bioplasticizers the thermal parameters do not noticeably vary with the incorporation of any component.

Table C4. A5. 3. Thermal parameters obtained from the second heating DSC curves of Ecovio® based films: glass transition temperature (T_g), melting temperature (T_m) and degree of crystallinity (X_c).

Sample	T_g^{PBAT} (ºC)	T_g^{PLA} (ºC)	T_m^{PBAT} (ºC)	T_m^{PLA} (ºC)	X_c^{PBAT} (%)	X_c^{PLA} (%)
ECO	-32	59	123	149	6.6	6.1
ECOSS	-30	53	117	150	8.5	5.8
ECOSSSL	-32	54	116	151	10.2	6.4
ECOSSCP	-33	51	117	150	10.9	6.3
ECOLR	-32	58	123	147	6.6	7.3
ECOLRSL	-32	56	120	152	10	9.3
ECOLRCP	-32	60	125	148	3.5	5.6
ECOSP	-32	59	122	145	6.5	6.5
ECOSPSL	-31	58	122	151	7.4	9.2
ECOSPCP	-32	59	123	150	6.9	4.7

3.3 Characterization of the structural, mechanical and permeability properties of the films.

Structural and mechanical properties.

FE-SEM was employed to examine the surface morphology and the interior, after cryogenic fracturing, of the calendered samples. As shown in **Figure C4. A5. 2a.**, the surface of ECO sample appears relatively homogeneous, with the PLA and PBAT phases displaying good miscibility. FE-SEM was also used to investigate the cross-section of the composites after cryogenic fracturing. The images in **Figure C4. A5. 2b.** reveal the presence of spherical domains likely originating from one of the polymers embedded within the matrix of the other, indicating phase separation at the interlayer level [16], [29], nevertheless good homogeneity is observed.

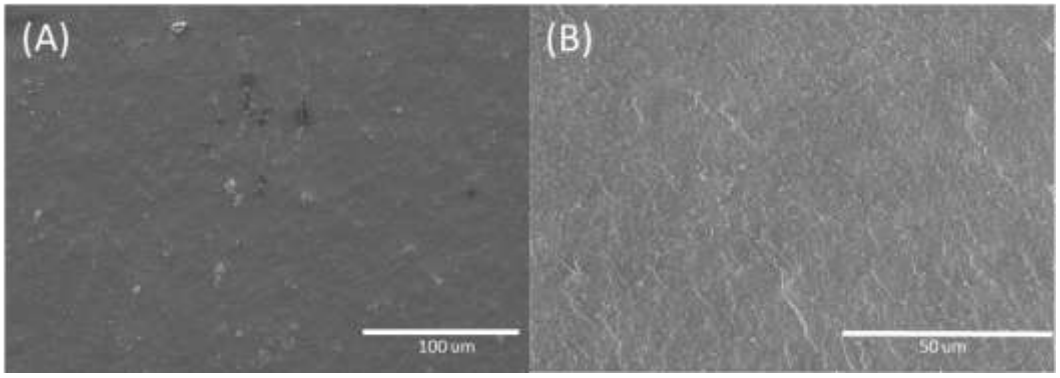


Figure C4. A5. 2. B. FE-SEM images of ECO sample showing the surface morphology (A) and cross-section (B).

Furthermore, the incorporation of SL and CP altered the cross-section morphology, introducing variations in surface roughness, as illustrated in **Figure C4. A5. 3.**

To further study the morphology, Raman confocal microscopy was employed to study the distribution of PLA and PBAT phases and to evaluate the dispersion of the additives, CP and SL in the Ecovio® based films.

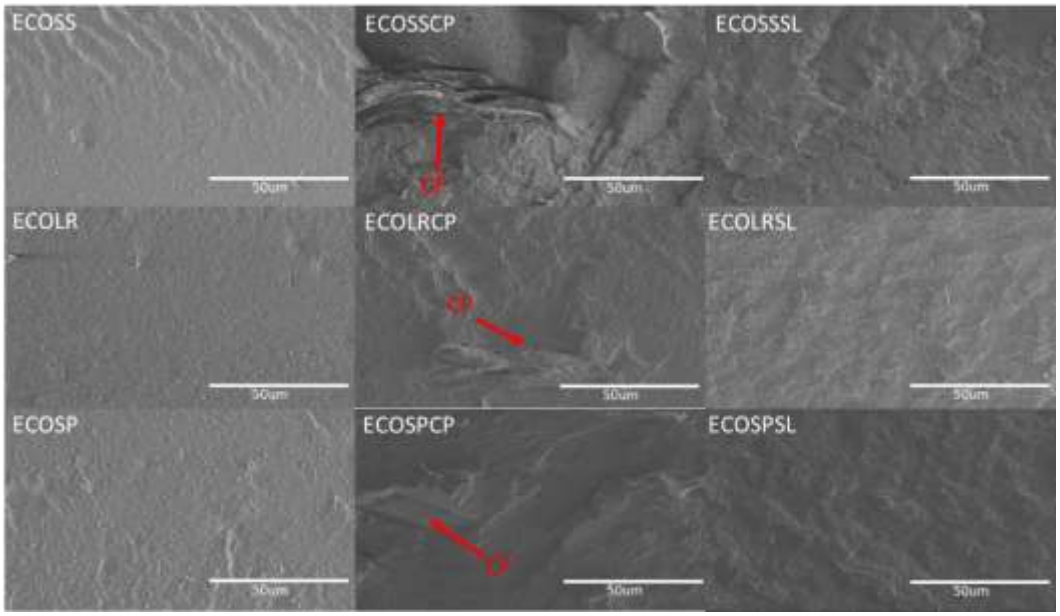


Figure C4. A5. 3. FE-SEM cross-section images with a 1000x magnification of the prepared films containing the bioplasticizers and the additives.

Initially, Raman confocal microscopy was used to scan the surface of Ecovio®-based samples (see **Figure C4. A5. S5.** of Supporting Information). Although small

regions or domains of PLA could be poorly distinguished from those of PBAT; however, the distribution and size of these domains could not be clearly observed due to the limited depth resolution of the Raman signal at the surface. To overcome this limitation, samples were cryo-fractured and analysed further.

Figure C4. A5. 4. shows as an example, Raman maps in the cross-section of the films, acquired using a 10 mW laser power and a 100× objective lens, for the samples ECOSP, ECOSPSL, and ECOSPCP. The images reveal that the PLA phase (indicated in blue) is homogeneously distributed in quasi-spherical microdomains throughout the PBAT matrix (shown in pink). These microdomains are also smaller in size than the segregated domains obtained in previous publications in which the Ecovio® based films were produced by compression moulding [15], indicating a better mixing process between both polymeric phases. Furthermore, SL (shown in red) appears to be homogeneously distributed in the interfacial regions, forming microdomains within the polymer matrix. In contrast, CP exhibited a less homogeneous distribution, forming domains of different sizes (represented in cyan). Similar results were observed for the rest of the films.

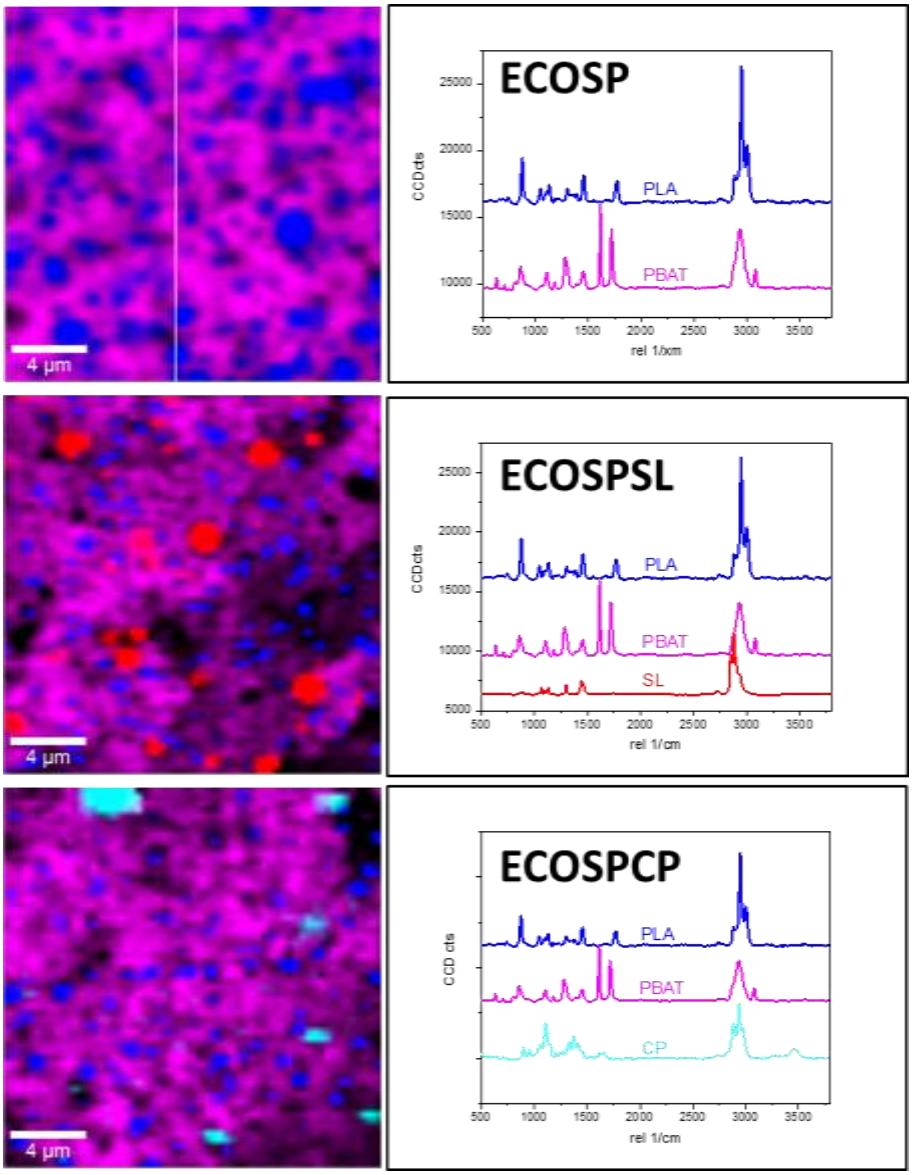


Figure C4. A5. 4. Raman spectra of ECOSP, ECOSPSL and ECOSPCP (right). XY Raman images of ECOSP, ECOSPSL and ECOSPCP matrix (left) where PBAT was signalled in pink, PLA was signalled in blue and SL and CP particles were signalled in red, and cyan, respectively.

The mechanical parameters derived from the stress-strain curves obtained during the tensile tests are summarized in **Table C4. A5. 4.** Remarkably, the elongation at break values for the calendered samples show a significant improvement compared to the functionalized Ecovio® samples produced by compression moulding recently published [15], probably due to the better mixing process, which give smaller microdomains of PLA homogeneously distributed within the PBAT matrix. In general, no notable increase in elongation at break was observed with the incorporation

of the plasticizers; nonetheless, the values of Young's modulus and ultimate stress were slightly reduced. This suggests that the used bioplasticizers do not provide additional plasticizing effects beyond those already achieved by the good blending of the two polymeric phases, PLA and PBAT. Another significant differences in calendered films in comparison with the films produced by compression moulding is the introduction of anisotropy, which affects the mechanical behaviour of films, evident in the parallel (H) and transverse (T) directions. In this case, this anisotropy is, however, not significant in ECO, ECOLR, ECOSS and ECOSP films. Only the incorporation of SL additives provokes significant differences in the mechanical properties between the two directions. The addition of SL particles produces a high anisotropy between the H and T direction resulting in higher elongation at break in the H direction and lower in the T direction. A similar trend is observed in the ultimate tensile strength and Young's modulus. As reported in the literature for oriented PLA/PBAT films containing fillers, the addition of particles produces anisotropy. This is due to molecular orientation that occurs during processing [33], [34]. Moreover, another explanation is that additives added into the polymeric matrix can agglomerate and result as stress concentrators points that produce an early failure of the composite [35]. On the other hand, the incorporation of CP particles leads to an overall deterioration of mechanical properties in both directions [36], [37] due to the big size and/or their aggregation.

Table C4. A5. 4. Mechanical properties of the functionalized films.

Sample	Ultimate Stress (MPa)	Young Modulus (MPa)	Elongation at break (%)
ECO H	24.4 ± 4.2^{a}	179.2 ± 7.8^{b}	490.2 ± 91.1^{ab}
ECO T	25.7 ± 1.6^{a}	135.2 ± 4.8^{cdef}	542.8 ± 36.2^{ab}
ECOSS H	21.3 ± 2.1^{abc}	109.7 ± 0.5^{fg}	478.1 ± 50.4^{abc}
ECOSS T	21.6 ± 1.4^{abc}	95.7 ± 7.5^{g}	530.6 ± 67.7^{ab}
ECOSSSL H	22.0 ± 0.8^{ab}	154.0 ± 10.1^{bcd}	587.3 ± 15.3^{a}
ECOSSSL T	12.5 ± 0.8^{g}	119.8 ± 8.6^{defg}	298.3 ± 32.6^{ef}
ECOSSCP H	18.1 ± 0.5^{bcde}	123.8 ± 12.6^{defg}	397.4 ± 18.4^{bcde}
ECOSSCP T	13.3 ± 1.4^{efg}	112.7 ± 2.6^{efg}	296.3 ± 60.6^{def}
ECOLR H	22.2 ± 1.4^{ab}	120.4 ± 4.5^{defg}	518.8 ± 37.6^{ab}
ECOLR T	22.6 ± 0.9^{ab}	136.3 ± 3.8^{cdefg}	515.5 ± 17.7^{ab}
ECOLRSL H	19.3 ± 0.7^{bcd}	225.0 ± 18.0^{a}	449.10 ± 70.2^{abcd}
ECOLRSL T	11.0 ± 0.8^{g}	128.4 ± 4.4^{defg}	259.8 ± 44.8^{ef}
ECOLRCP H	14.8 ± 0.7^{defg}	142.3 ± 4.0^{cdef}	282.8 ± 29.8^{ef}
ECOLRCP T	12.8 ± 1.0^{fg}	134.5 ± 5.4^{cdefg}	217.5 ± 27.5^{f}
ECOSP H	21.9 ± 0.9^{ab}	162.3 ± 4.0^{bc}	473.1 ± 64.1^{ab}
ECOSP T	21.5 ± 2.0^{abc}	138.0 ± 9.6^{cdef}	510.2 ± 67.8^{ab}
ECOSPSL H	19.9 ± 2.4^{bc}	182.6 ± 29.1^{b}	450.6 ± 8.2^{abcd}

ECOSPSL T	12.2 ± 0.7^{g}	138.8 ± 16.0^{cdef}	248.1 ± 22.6^{ef}
ECOSPCP H	17.2 ± 0.7^{cdef}	149.8 ± 4.0^{bcde}	333.8 ± 18.1^{cdef}
ECOSPCP T	14.2 ± 1.4^{efg}	138.6 ± 10.4^{cdef}	273.3 ± 34.0^{f}

Values having the same letter are not significantly different for Tukey test (significance level of $P \leq 0.05$)

To better understand the influence of the parallel and transverse stretching directions on the mechanical properties, especially in the films containing SL additives, X-ray diffraction analyses were conducted in both orientations, specifically, Wide-Angle X-ray Scattering (WAXS).

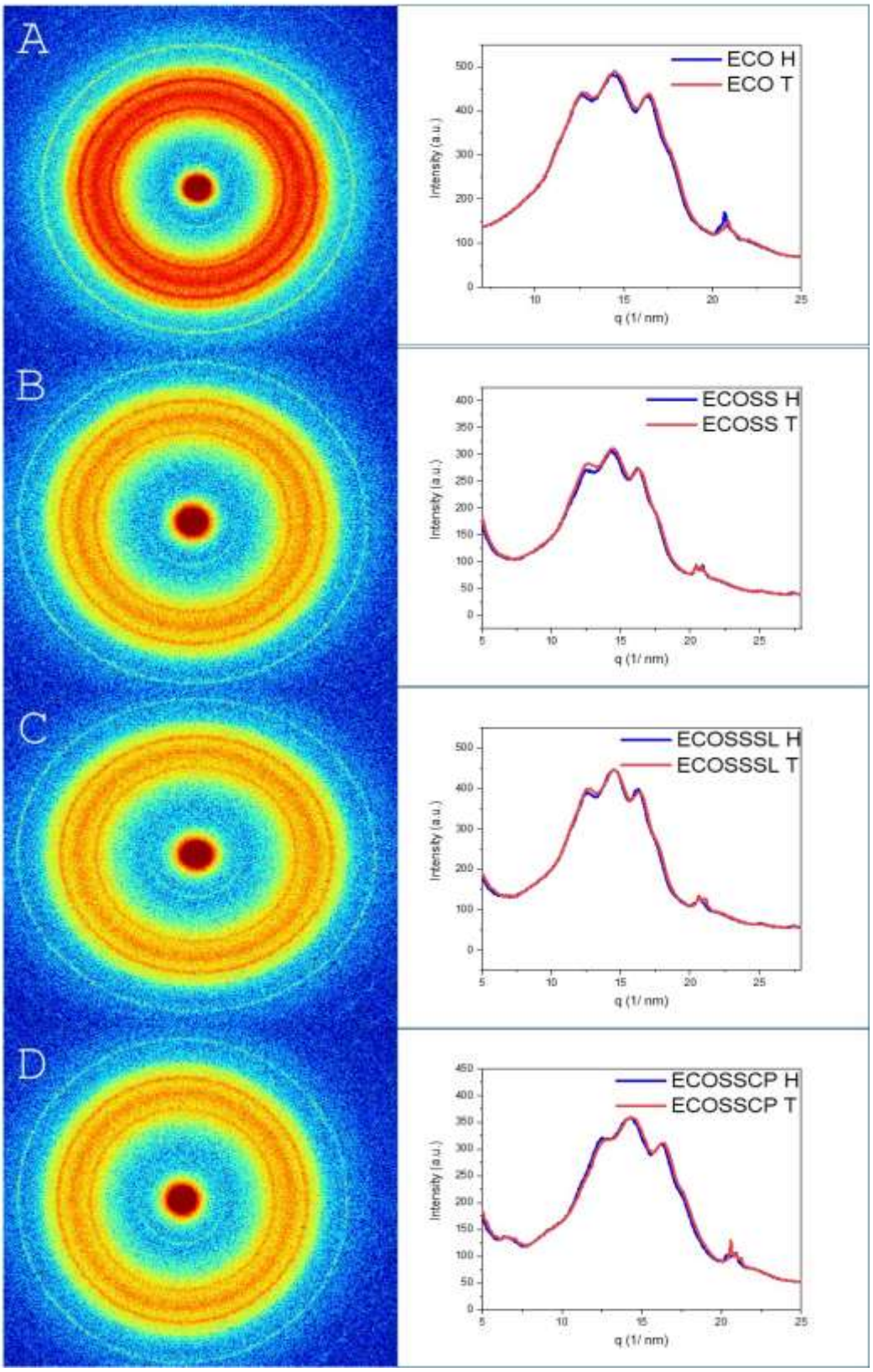
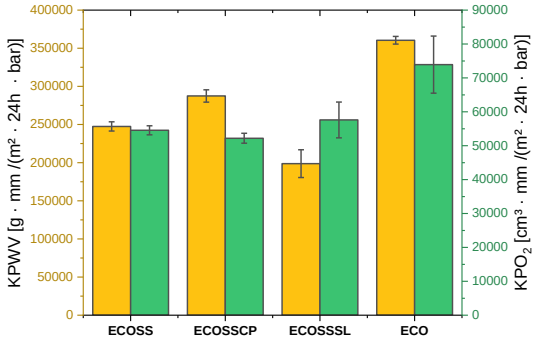


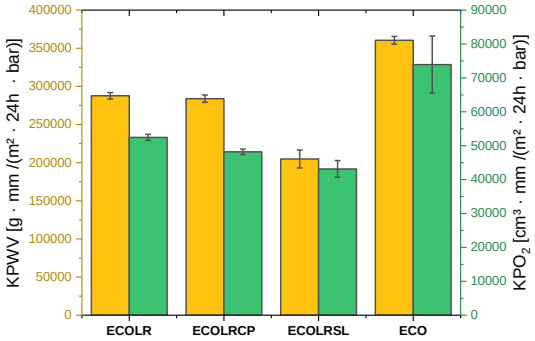
Figure C4. A5. 5. WAXS images and diffractograms in 1D of A) ECO, B) ECOSS, C) ECOSSSL, D) ECOSSCP.

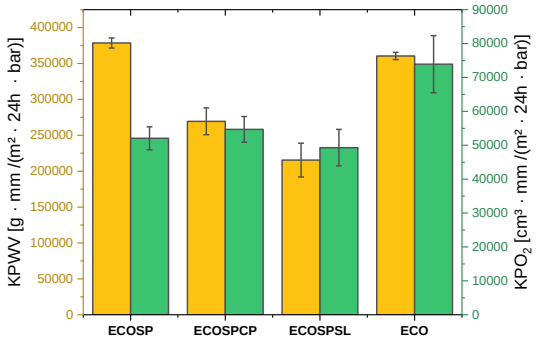
As shown in **Figure C4. A5. 5.** when comparing the crystallinity based on the halos obtained by WAXS, no significant differences are observed. This indicates that the crystallinity is similar in both the transverse and parallel directions. This result is also confirmed in **Figure C4. A5. 5.** and **Figure C4. A5. S6.**, where the WAXS diffractograms for both directions are presented and exhibited no significant differences between both directions. Nevertheless, the overall degree of crystallinity is very low. This low crystallinity is attributed to the inherently slow crystallization rate of PLA. When PLA is stretched, it primarily promotes orientation within the amorphous phase rather than increasing crystallinity [38]-[40]. This orientation of the amorphous phase might be responsible for the observed anisotropy in the mechanical properties rather than differences in the crystalline structure.

Permeability of the films.

Water vapour and oxygen permeabilities are also critical properties in assessing biobased food packaging films [41]. Permeance refers to the transmission rate of a substance through a film divided by the partial pressure difference whereas across it, while permeability is obtained by multiplying the permeance by the film thickness [42]. **Figure C4. A5. 6.** shows the water vapour (KP_{WV}) and oxygen (KP_{O2}) permeability values. Compared with the ECO sample, both KP_{WV} and KP_{O2} decreased with the addition of plasticizers, SL and CP. Nevertheless, when expressed as logarithmic values (Log WVP for water vapour and Log O_2P for oxygen), the results showed no significant change from ECO sample. The films displayed high permeability, with Log WVP values ranging from approximately 5.3 to 5.6 and Log O_2P values from 4.6 to 4.8, suggesting potential suitability for applications requiring high gas transmission, as packaging for bakery products, fruits, salads, or vegetables [42].







Sample	Log(WVP)	Log(O_2P)
ECO	5.56	4.87
ECOSS	5.39	4.74
ECOSSSL	5.30	4.76
ECOSSCP	5.46	4.72
ECOLR	5.46	4.72
ECOLRSL	5.31	4.63
ECOLRCP	5.45	4.68
ECOSP	5.58	4.72
ECOSPSL	5.33	4.69
ECOSPCP	5.43	4.74

Figure C4. A5. 6. KP_{WV} and KP_{O2} value for Ecovio® based materials and Table of the values of Log WVP and Log O_2P.

3.4 Antioxidant and antimicrobial assays.

After confirming the effects of plasticizers and particles on the thermal and mechanical properties of the films, their antioxidant and antibacterial activities were further evaluated. **Table C4. A5. 5.** presents the values of CTE and TEAC after 24 h of exposure to DPPH solutions. The functionalized Ecovio® samples exhibited antioxidant activity, in contrast to the neat Ecovio®, which showed a TEAC value of 3.20 ± 0.49 µmol Trolox/g. Functionalization with the bioplasticizers drastically increased antioxidant capacity by a factor of approximately 10, while the incorporation of CP enhances a bit more the antioxidant activity. It has been previously reported that chitin nanocrystals can provide certain antioxidant activity to polymeric films [43], although the biggest contribution is due to the bioplasticizers ACETEM and CITREM. Among the functionalized films, ECOSPCP exhibited the highest TEAC value at 18.58 ± 0.48 µmol Trolox/g, followed by ECOLRCP with

17.54 ± 0.24 µmol Trolox/g. When comparing films within the same plasticizer series, the addition of CP additive consistently enhanced the TEAC values.

Table C4. A5. 5. Antioxidant properties of the functionalized films.

Sample	CTE (µg Trolox/mL)	TEAC (µmol Trolox/g)
ECO	4.00 ± 0.61	3.20 ± 0.49[a]
ECOSS	17.79 ± 0.50	14.21 ± 0.40[b]
ECOSSSL	18.79 ± 0.65	15.02 ± 0.52[b]
ECOSSCP	19.43 ± 0.30	15.53 ± 0.24[b]
ECOLR	16.52 ± 0.35	13.20 ± 0.28[bc]
ECOLRSL	17.87 ± 0.73	14.27 ± 0.58[b]
ECOLRCP	21.95 ± 0.30	17.54 ± 0.24[d]
ECOSP	18.31 ± 0.35	14.63 ± 0.28[b]
ECOSPSL	21.00 ± 0.12	16.79 ± 0.10[bc]
ECOSPCP	23.26 ± 0.60	18.58 ± 0.48[d]

Values having the same letter are not significantly different for Tukey test (significance level of $P \leq 0.05$)

Next, the antimicrobial properties of the films containing plasticizers were evaluated using a film contact method against *Escherichia coli*, *Staphylococcus aureus* and *Listeria innocua* bacteria. Samples did not exhibit antibacterial activity against the Gram-negative *Escherichia coli*, due to the double lipid bilayer membrane that limits the penetration of antimicrobial compounds. **Table C4. A5. 6.** presents the antibacterial activity of the tested materials against the two Gram-positive strains, expressed as the log reduction in bacterial count after 24 h of contact with the films. The initial inoculum concentrations were 4.54 ± 0.01 log CFU/mL for *S. aureus* and 3.71 ± 0.04 log CFU/mL for *L. innocua*. As regards *S. aureus*, total inhibition was observed in most of the samples, except for ECOSS and ECOSSCP, which showed minimal bacterial reduction counts of 0.63 ± 0.06 log CFU/mL and 0.11 ±

0.01 log CFU/mL, respectively. These results highlight the antimicrobial activity of SL, which allowed for a total bacterial inhibition when using the ECOSSSL sample. Additionally, SL and CP in CITREM containing samples did not produce any antagonistic effects, as antimicrobial activity against *S. aureus* was maintained.

In the case of *L. innocua*, the Ecovio® samples containing CITREM SP demonstrated a complete inhibition, both with and without the addition of particles, pointing out the good antimicrobial activity of such bioplasticizer. For CITREM LR10, antimicrobial activity was evident in the ECOLR and ECOLRCP samples, with reductions of 2.30 ± 0.04 log CFU/mL and 1.76 ± 0.27 log CFU/mL, respectively, although the inhibition achieved was not complete. Notably, ECOLRSL containing SL additive showed complete inhibition. Finally, ECOSS and ECOSSCP exhibited minimal antimicrobial activity against *L. innocua*, with reductions of 0.42 ± 0.01 log CFU/mL and 0.10 ± 0.03 log CFU/mL, respectively. On the other hand, the incorporation of SL additive significantly enhanced the antimicrobial effect, resulting in total inhibition, as shown for *S. aureus*. The antimicrobial activity of the bioplasticizers CITREM LR10 and CITREM SP is presumably attributed to the germicidal effects of citric acid, which lowers the local and intracellular pH, causing damage to proteins, enzymes, DNA, and membranes, as well as suppressing NADH oxidation, ultimately leading to bacterial death [40] [41]. SL is likely able to damage bacteria through a dual mechanism: (1) its fatty acid chain disrupts bacterial membranes by integrating into the lipid bilayer, causing leakage and cell damage particularly in Gram-positive bacteria with simpler membranes [46]; and (2) its lactylate component acidifies the cytoplasm by releasing protons and lactate anions, disrupting cellular processes and consuming energy needed for growth [47]. The enhanced susceptibility of Gram-positive bacteria to SL further supports this dual mechanism, as their limited membrane defences render them more vulnerable to both membrane disruption and acid stress.

Table C4. A5. 6. Antimicrobial properties against *S. aureus* (initial inoculum 4.54 ± 0.01 Log CFU/mL) and *L. innocua* (initial inoculum 3.71 ± 0.04 Log CFU/mL).

Sample	*S. aureus* (ΔLog CFU/mL)	*L. innocua* (ΔLog CFU/mL)
ECOSS	0.63 ± 0.06[b]	0.42 ± 0.01[b]
ECOSSSL	4.54 ± 0.01[a]	3.71 ± 0.04 [a]
ECOSSCP	0.11 ± 0.01[b]	0.10 ± 0.03[c]
ECOLR	4.54 ± 0.01 [a]	2.30 ± 004[d]
ECOLRSL	4.54 ± 0.01 [a]	3.71 ± 0.04 [a]
ECOLRCP	4.54 ± 0.01 [a]	1.76 ± 0.27[e]
ECOSP	4.54 ± 0.01 [a]	3.71 ± 0.04 [a]
ECOSPSL	4.54 ± 0.01 [a]	3.71 ± 0.04 [a]
ECOSPCP	4.54 ± 0.01 [a]	3.71 ± 0.04 [a]

3.5 Migration of the plasticizers.

Migration of plasticizers from packaging films is a persistent concern, especially for applications involving direct contact with food. Even when employing authorized additives by the FDA like sodium ACETEM (E472a) or CITREM (E472c), the migration from the packaging into the food can affect properties like flavours, aromas, among other sensorial properties.

To evaluate the migration of products, The European Commission has established directives, such as Commission Regulation (EU) No 10/2011, to define the simulants used for studying the migration of substances from materials intended to come into contact with food [48]. In this study, the extent of migration was assessed using standard food simulants: Simulant A, composed of 10% ethanol in water; simulant B containing 3% acetic acid in water; and simulant D1, composed by 50% ethanol in water. Film samples were submerged in the medium for a period of 10 days at 20 °C. After the exposure period, the films were retrieved and weighed again, while

the simulant solution was subjected to complete evaporation in an oven. Across all samples, no measurable mass loss was observed in the films, suggesting minimal or negligible migration. Even if the residue was not seen by gravimetric analysis in the vial, 150 µL of DCM were employed to wash the vial and extract the possible migration products. That solution was analysed by gas chromatography–mass spectrometry (GC–MS). Prior to analysis, calibration was conducted for the key components present in each plasticizer, enabling quantification of the peaks identified in the chromatograms. In the case of samples in simulants A and B, no migration of the plasticizer was detected. However, in simulant D, some substances were detected (**Table C4. A5. 7.**). The migration value of the main components were far below to the maximum specific migration limit established at 60 mg/kg_{food} (restriction limit for the group of additives to which it belongs) [48].

Table C4. A5. 7. Results obtained from the migration study analysed by GC–MS in simulant D (50% ethanol in water).

Sample	Retention time (min)	Main component	R^2	Migration amount (mg/kg)
ECOSS	22.0	3-((12-Acetoxyoctadecanoyl)oxy)propane-1,2-diyl diacetate	0.999	0.0594
ECOSSCP	22.0	3-((12-Acetoxyoctadecanoyl)oxy)propane-1,2-diyl diacetate	0.999	0.0744
ECOSSSL	22.0	3-((12-Acetoxyoctadecanoyl)oxy)propane-1,2-diyl diacetate	0.999	0.0486
ECOLR	29.8	9-Octadecenoic acid (Z)-, 2-hydroxy-1,3-propanediyl ester	0.999	0.0132
ECOLRCP	29.8	9-Octadecenoic acid (Z)-, 2-hydroxy-1,3-propanediyl ester	0.999	0.0036
ECOLRSL	29.8	9-Octadecenoic acid (Z)-, 2-hydroxy-1,3-propanediyl ester	0.999	0.0030
ECOSP	28.7	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester	0.995	0.0594
ECOSPCP	28.7	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester	0.995	0.0072
ECOSPSL	28.7	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester	0.995	0.0072

3.6 Disintegrability of the films under composting conditions.

The end-of-life study of packaging is also essential for assessing its environmental impact. Herein, the disintegradability of the prepared films in industrial composting conditions was evaluated. **Figure C4. A5. 7.** shows the degradation of the functionalized Ecovio® samples over different incubation times under composting. Samples were collected after 20, 45, 90, and 135 days to evaluate properties such as fragility and colour changes. All films showed visible disintegration during composting. During the first 20 days of incubation, the films lost their transparency and became yellowish. By day 45, the samples darkened further and exhibited fractures, indicating the onset of hydrolytic degradation of the polymer matrix. With prolonged incubation,, the degradation effects intensified, leading to the formation of holes and fragmentation into smaller pieces [49], [50].

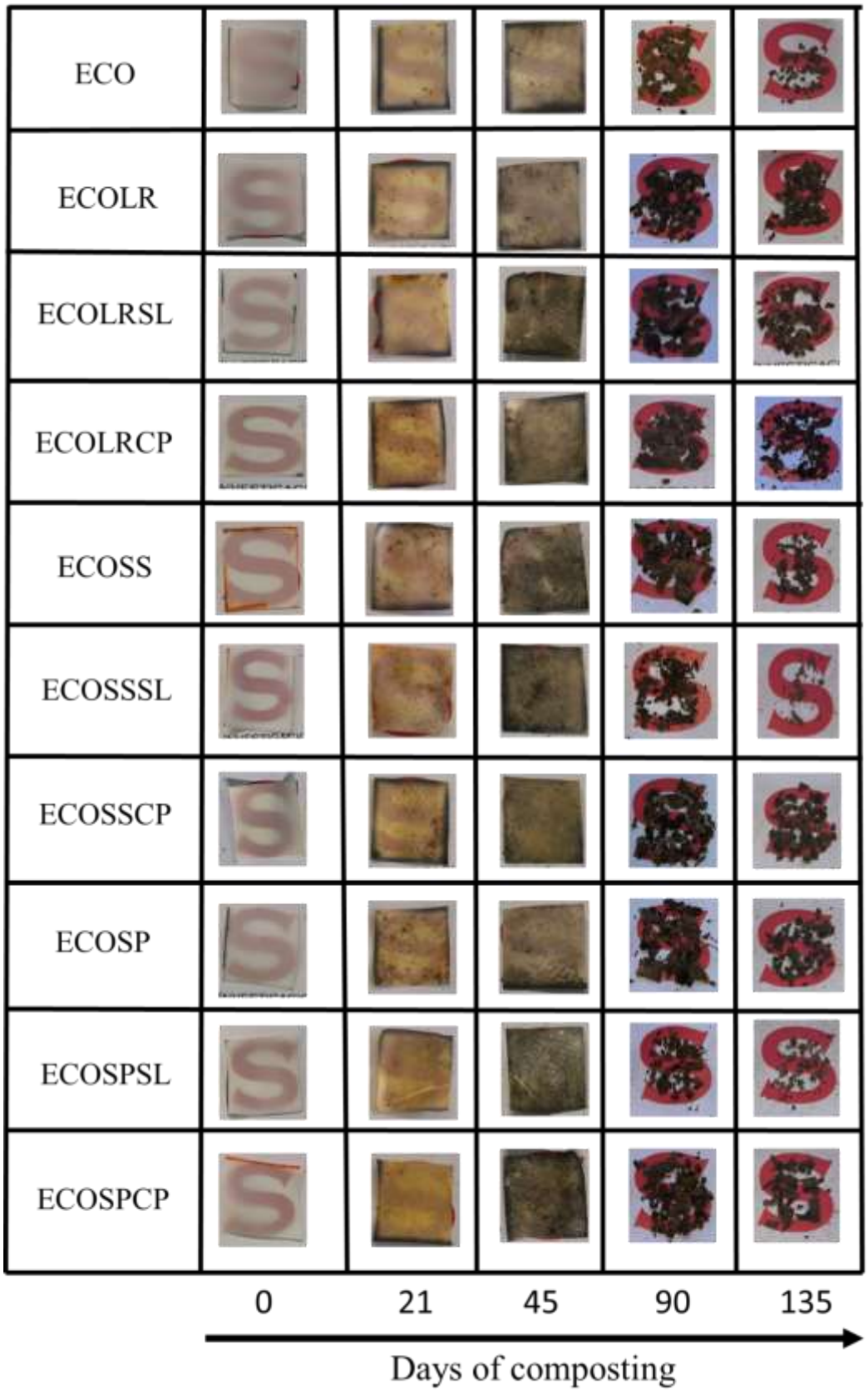


Figure C4. A5. 7. Visual appearance of the tested films at different composting times. Samples were collected at 20, 45, 90, and 135 days.

To further understand the disintegration under composting of the composite films Raman confocal micoscopy was employed to anaylise the degraded films. **Figure C4. A5. 8.** presents, as example, the Raman images obtained for ECOSP, ECOSPSL, and ECOSPCP at different composting times. In these images, it is possible to observe that both PLA and PBAT phases coexist up to 45 days of composting. However, in the image corresponding to 90 days, the PLA phase is no longer visible. This observation is consistent with previous studies, which have demonstrated that PLA undergoes faster disintegration under composting conditions compared to PBAT [11], [15], [51], [52]. Moreover, CP and SL particles were present in the matrix up to 20 days, but were no longer observed at 45 days. Similar behaviour was observed in the other samples exhibited in **Figure C4. A5. S7.** of Supporting Information.

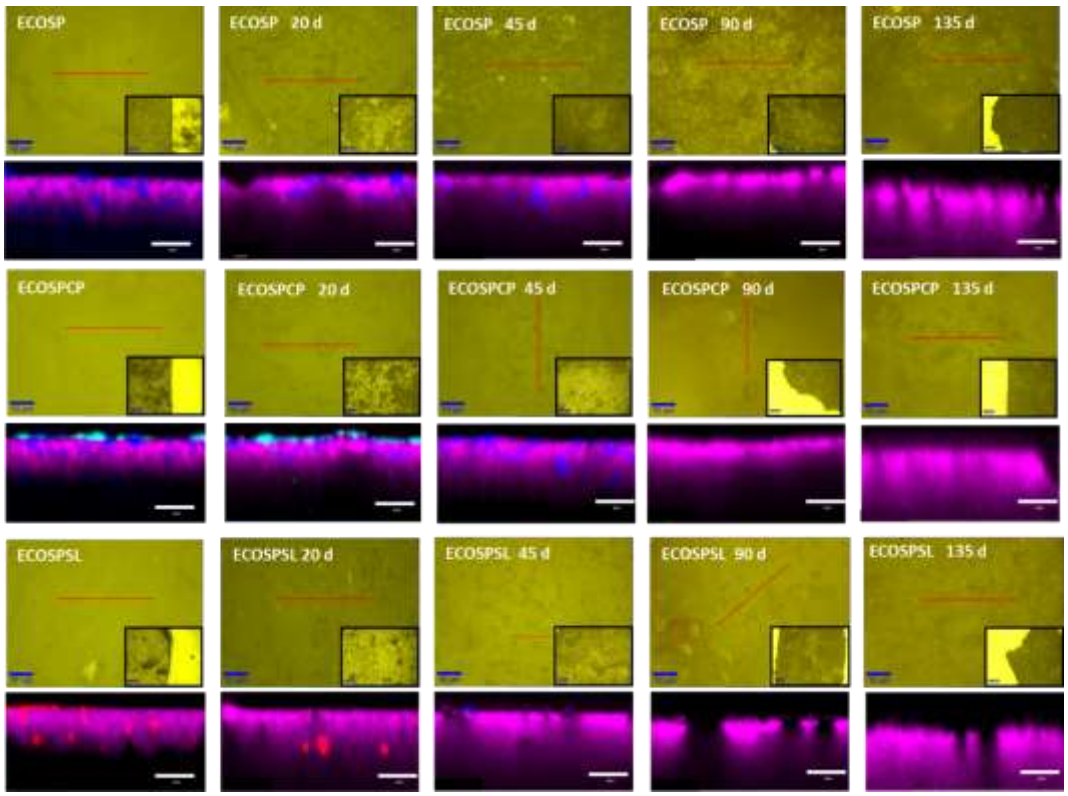


Figure C4. A5. 8. Raman images of disintegration of ECOSP, ECOSPSL and ECO-SPCP samples at different days under composting conditions. Scale bar of 8 µm.

These results allow us to propose the hypothesis that films degradation of the occurs in different stages. The first stage involves the disappearance of the particles, observed between 20 and 45 days, which likely generates small holes where fracture

begins. Secondly, the complete disintegration of the PLA phase occurred before 90 days, then, the total degradation of PBAT take longer times.

4. Conclusions.

In summary, biodegradable Ecovio® (PLA/PBAT) films incorporating food-grade additives such as CITREM, ACETEM, sodium stearoyl lactylate and chitin particles, has resulted in promising functional materials with notable antioxidant, antimicrobial, and mechanical properties for food packaging applications.

The calendering process has enabled the production of uniform, high-quality films with excellent elongation at break and good additive dispersion; however, the incorporation of SL additive induces anisotropy in the mechanical properties. Besides, the thermal and barrier properties remained largely unchanged, while the films exhibited strong antimicrobial activity against key gram-positive bacteria and demonstrated effective antioxidant capacity, with minimal migration into food simulants.

Importantly, the produced films were shown to disintegrate under industrial composting conditions, confirming their potential for sustainable end-of-life management. Overall, these findings underscore the suitability of such formulations for active and environmentally friendly food packaging applications.

Acknowledgments.

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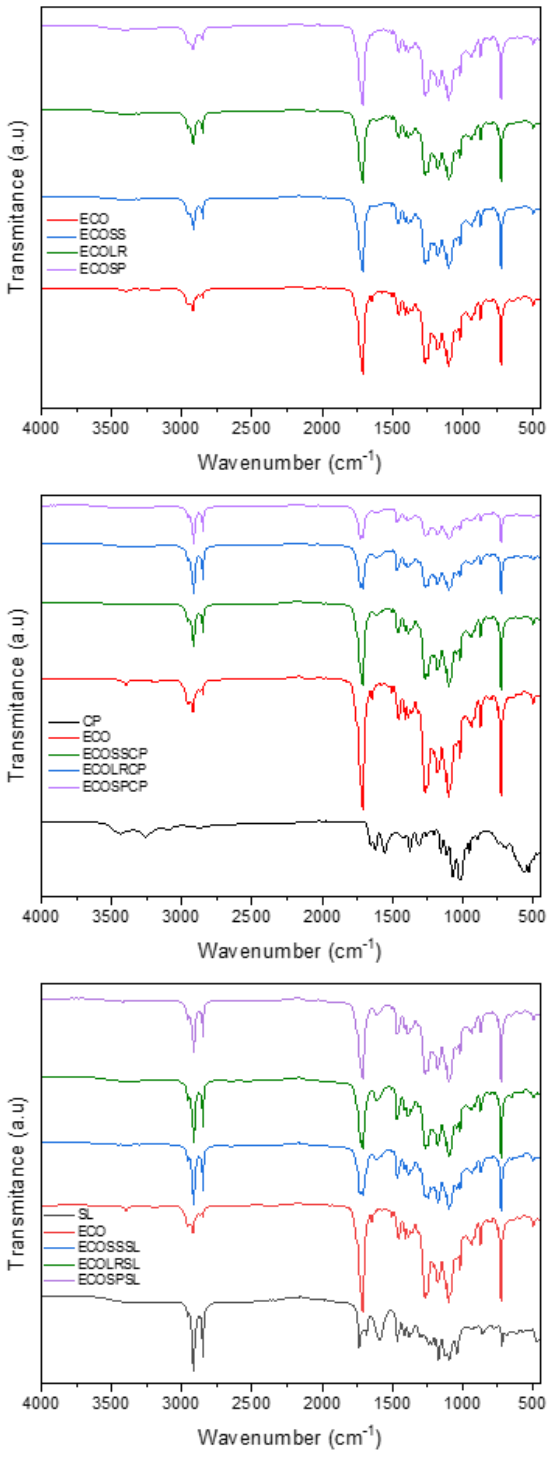
Supplementary info



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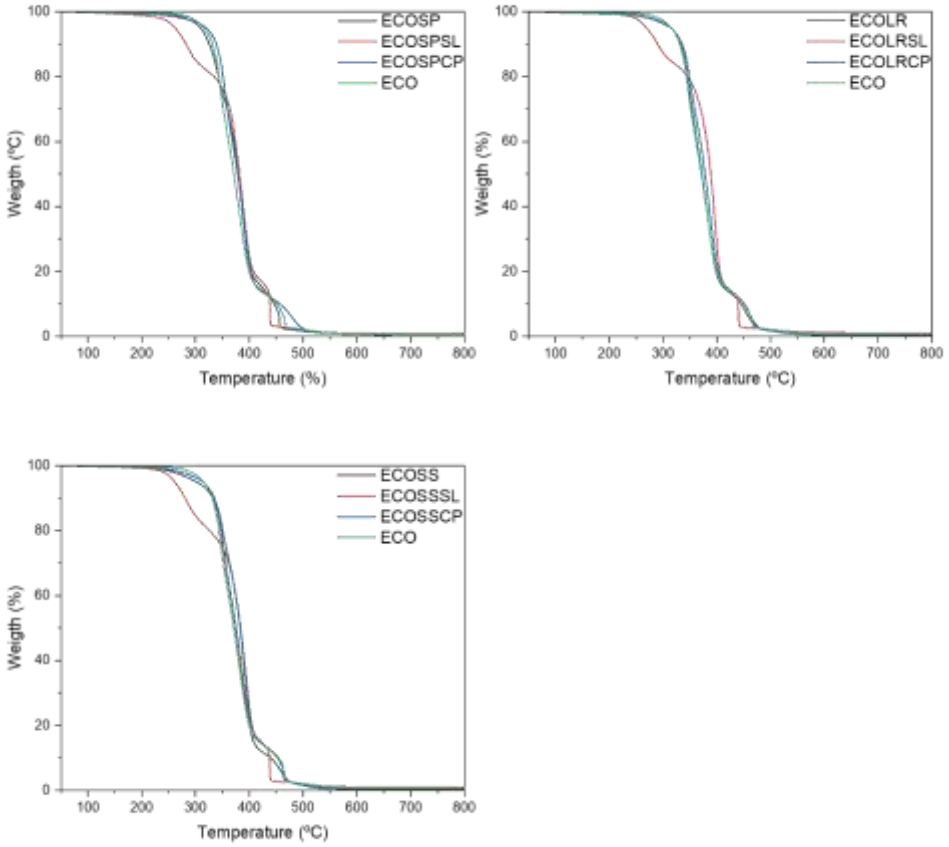

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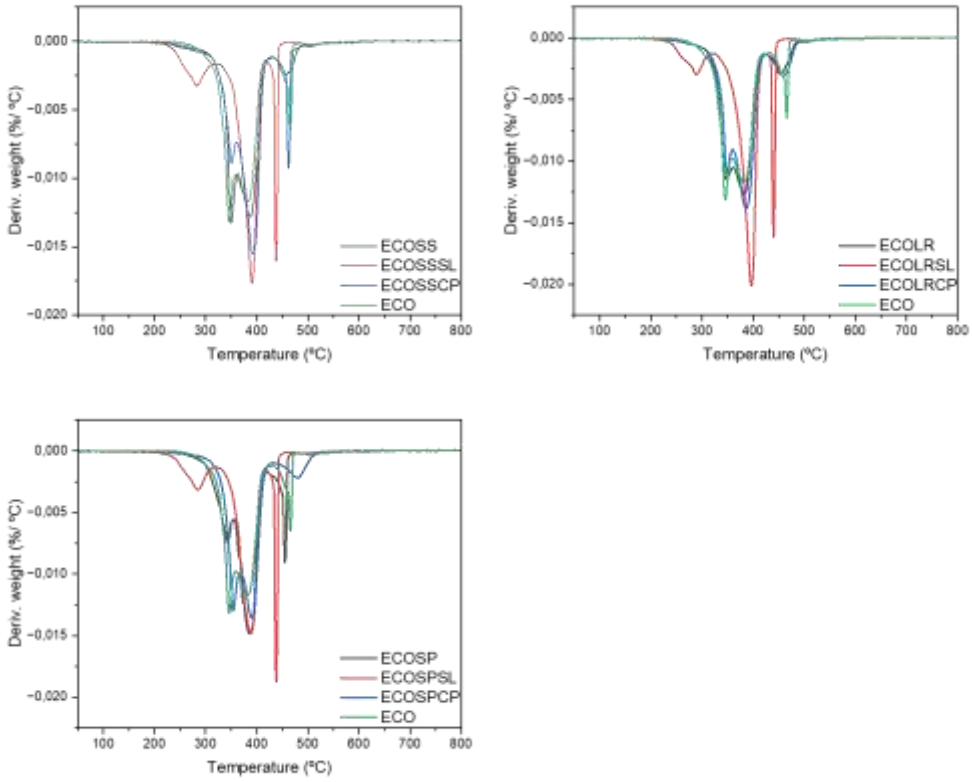

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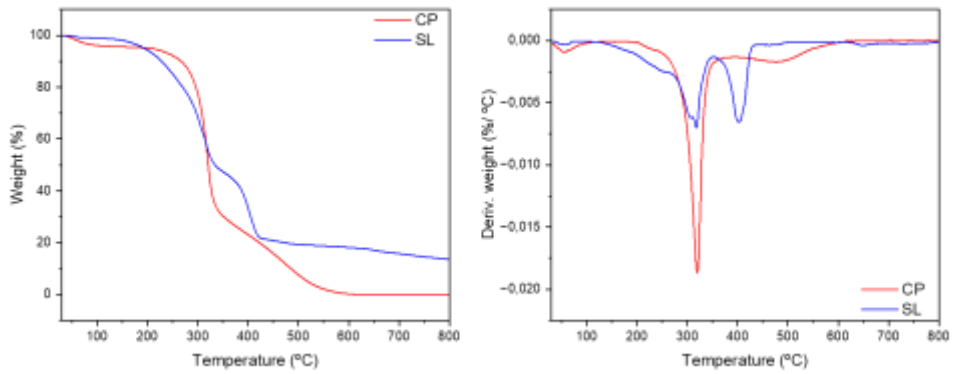

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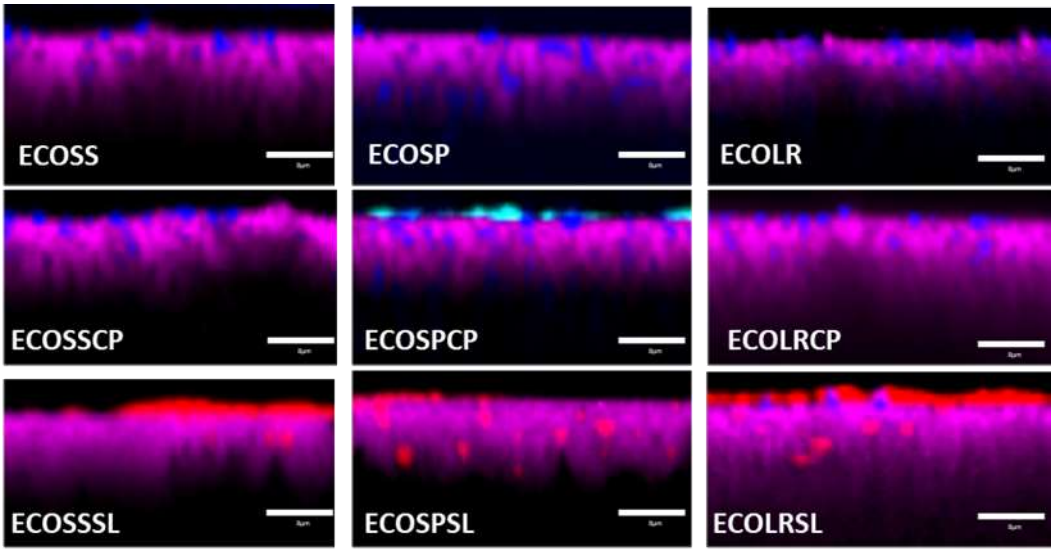


Figure C4. A5. S5. XY Raman images of Ecovio- based samples. Scale bar of 8 µm.

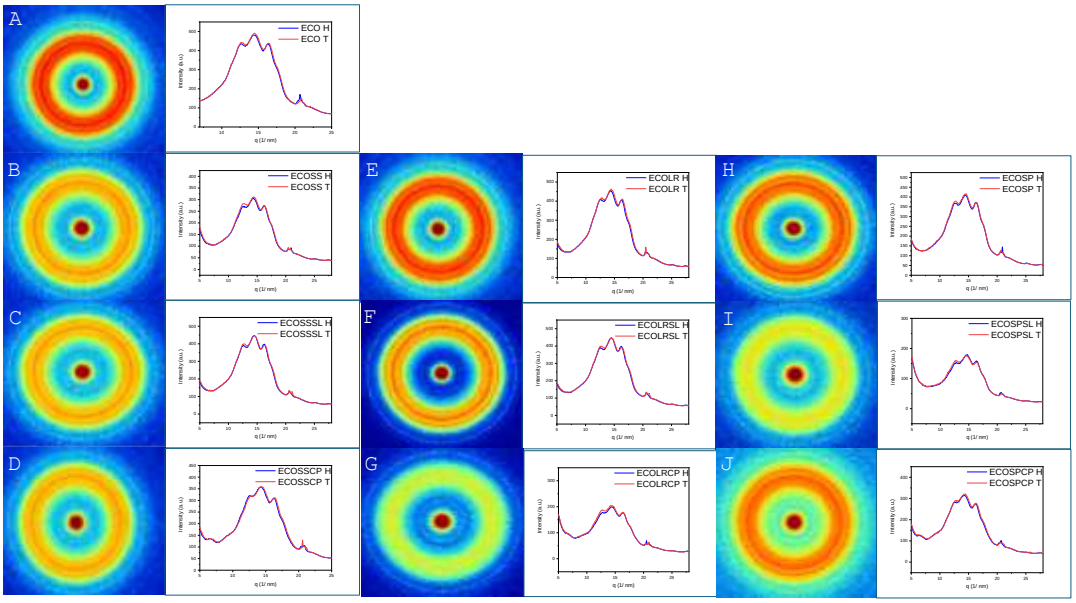
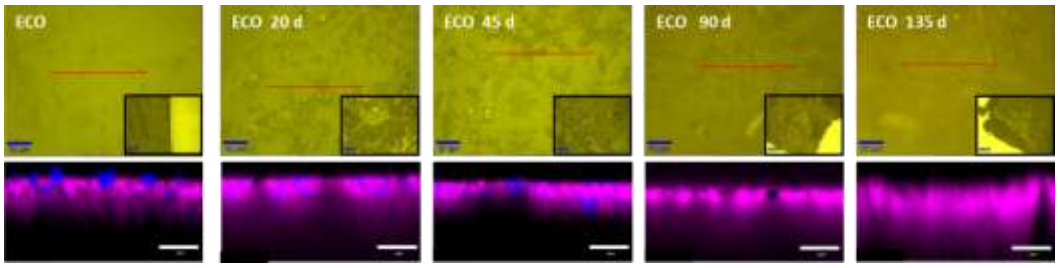
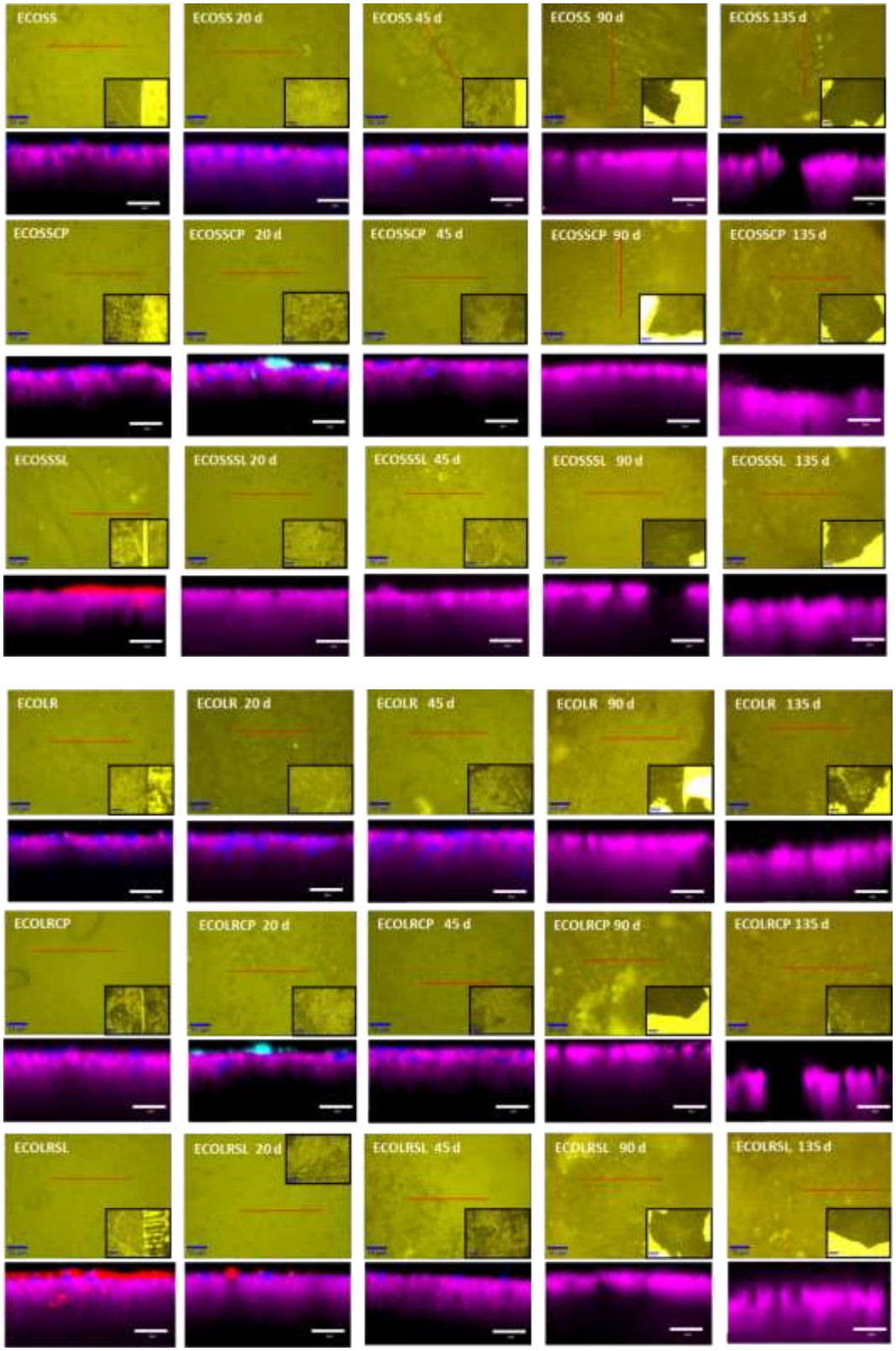


Figure C4. A5. S6. WASX images and diffractograms of A) ECO, B) ECOSS, C) ECOSSSL, D) ECOSSCP, E) ECOLR, F) ECOLRSL, G) ECOLRCP, H) ECOSP, I) ECOSPSL, J) ECOSPCP.

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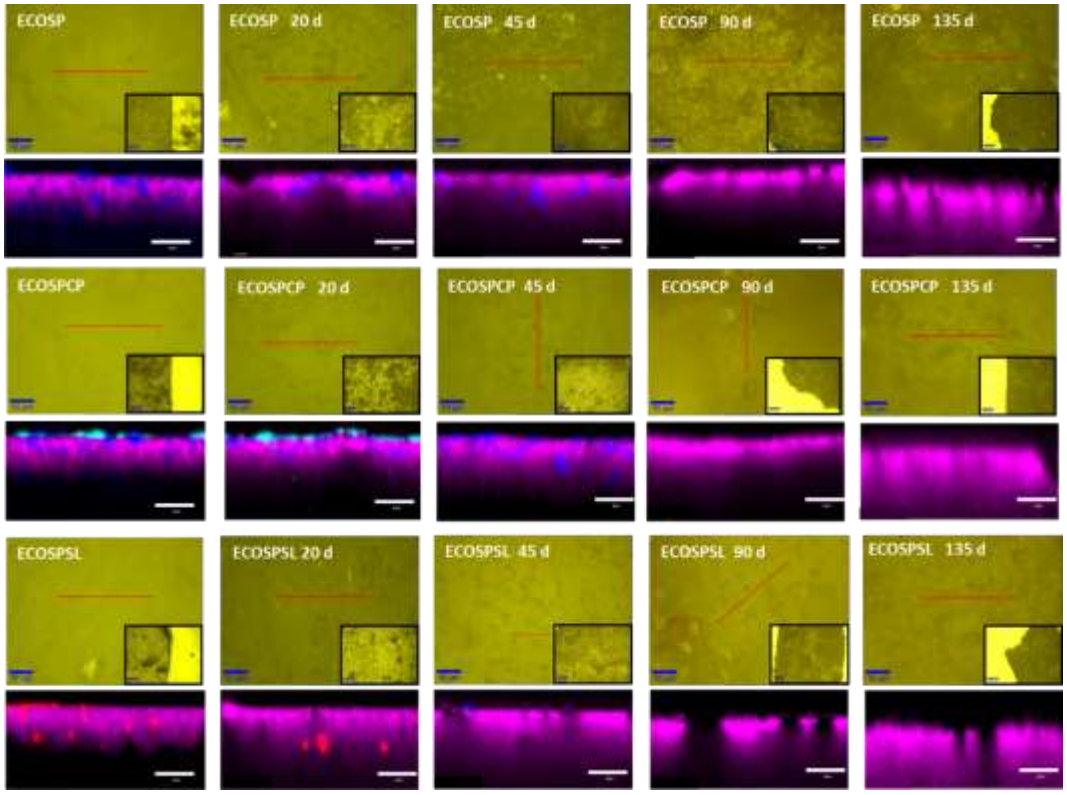
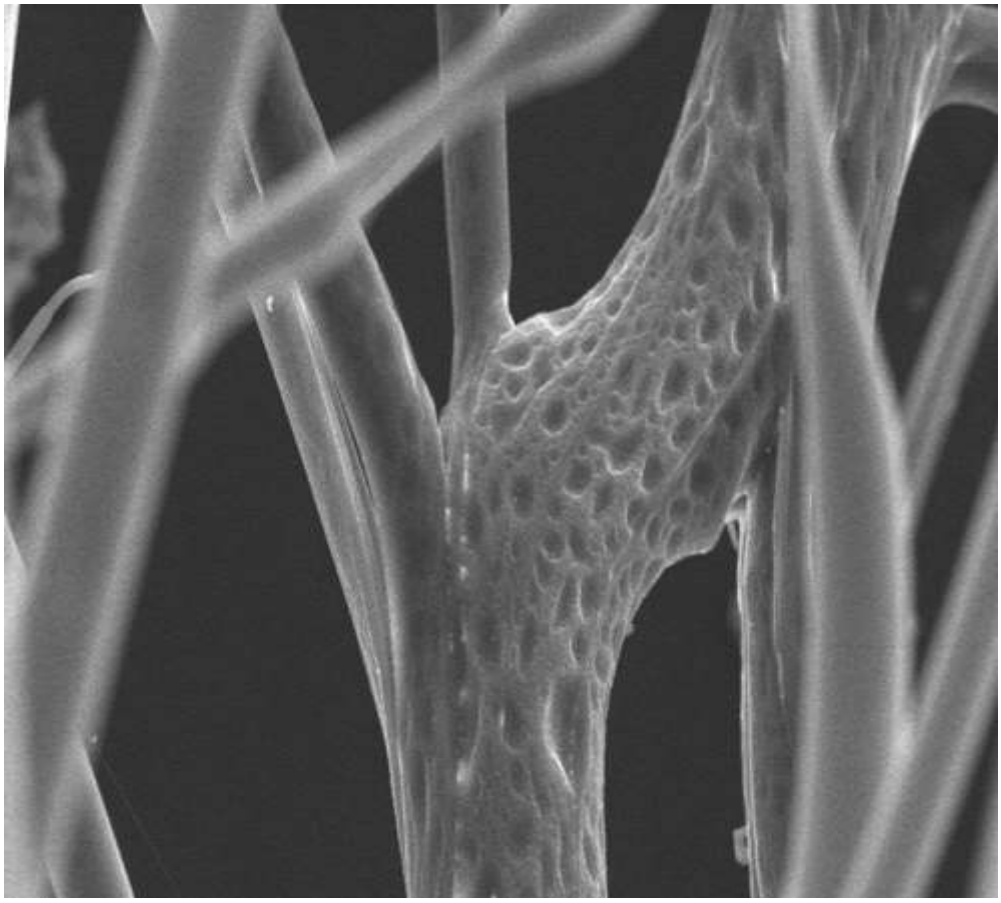


Figure C4. A5. S7. Raman images perpendicular to the surface of disintegration of Ecovio tested samples at different days under composting conditions, where PBAT was signalled in pink, PLA was signalled in blue and the SL and CP particles was signalled in red and cyan respectively. Scale bar of 8 µm.



CHAPTER 5

Development and Characterization of PLA Fibers Plasticized with CITREM and ACETEM via Forcespinning.

Article 6: Bio-Based Citrate Plasticizers for PLA Fibers: Unlocking Antioxidant and Antimicrobial Potential.

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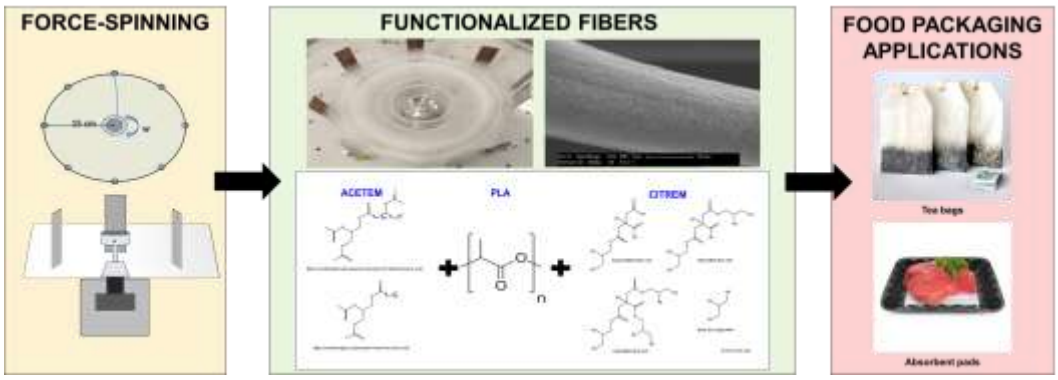
Keywords: force-spinning, PLA, fibers, bioplasticizers, antimicrobial, antioxidant



Abstract.

Active packaging systems improve food safety, preserve quality, and extend shelf life. Nano- and microfibers are ideal for such systems due to their high surface area, enabling efficient delivery of active compounds. In this study, polylactic acid (PLA) fibers were blended with antioxidant and antimicrobial bioplasticizers (CITREM and ACETEM) and produced using force-spinning. Various PLA solution concentrations were tested, with 20% PLA chosen for optimal fiber formation. Uniform fibers containing 10% bioplasticizers were analyzed via Raman confocal microscopy and scanning electron microscopy, that demonstrate the uniform formation of functional fibers. Thermogravimetric analysis showed a slight decrease in thermal stability due to plasticizer addition whereas differential scanning calorimetry revealed a plasticization effect, indicated by reduced glass transition temperature and increased crystallinity. Antimicrobial activity was assessed using standard method E2149-13a against Gram-positive and Gram-negative bacteria, while antioxidant activity was measured using the Blois method. The results indicate that the bioplasticizers impart new functionalities to PLA, including antimicrobial activity against Gram-positive bacteria and antioxidant properties. These findings support the potential of PLA fibers in food packaging applications, contributing to longer shelf life and reduced food waste.

1. Introduction.

The growing interest in nano- and micro-sized materials across various fields has driven the development of advanced fabrication technologies based on these structures. Among these materials, polymeric fibers have gained attention due to their high surface area, porosity and versatility [1], enabling applications in areas such as biomedical engineering [2], where they are mainly used in regenerative medicine/tissue engineering or drug delivery [3]; textiles, including military clothing [4]; nanosensors [5]; filtration[6] and energy storage [7]. Recently, fibers have also been employed in food packaging [8] [9]. Packaging plays a crucial role with continuous innovation driven by consumer demands, global trends, and regulatory changes, along with challenges like long-distance transportation. Food packaging faces challenges such as ensuring product safety during long-distance transportation and reducing environmental impact. The shelf life of products is closely linked to the effectiveness of the packaging material, which must protect the product during transportation. To address these challenges, active packaging systems with functional additives have been developed [10]. These active systems modify the package environment by incorporating oxygen scavengers, moisture absorbers, antioxidants, and antimicrobial or antifungal agents. Nano- or micro-fibers are particularly well-suited for these systems due to their high surface area acting as efficient carriers for active compounds.

Various techniques are available to produce polymeric fibers, with electrospinning being the most widely used [1],[9],[11]. Electrospinning is a method that utilizes electrostatic forces generated by applying a voltage between the needle and the collector, which creates an electric field. This field charges the droplets at the nozzle, stretching them to form fibers that are deposited onto the collector. By controlling the parameters of electrospinning, various fiber morphologies can be obtained[3]. However, several limitations exist: the amount of fibers produced is very low relative to the time required for production, the solvents used are typically organic solvents with specific dielectric properties, and the need for a high electric field makes the process costly, restricting scalability for industrial applications [12]. To overcome these drawbacks, alternative spinning methods have been developed. For instance, solution blow spinning is a gas-assisted technique similar to solution electrospinning. In this case, the solution is forced through the nozzle, resulting in the formation of

droplets, while the high pressure created by the gas stream stretches the droplets [3], [13]. Centrifugal spinning (Rotary jet spinning/Force spinning) has also gained attention due to its scalability and versatility. This method utilizes centrifugal force instead for stretching the polymer [11,][14]. The solution is injected into a reservoir with an orifice that rotates at high speed (> 2000 rpm) [6], [11]. The rotation of the reservoir pushes the polymer solution to the inner walls, and when the solution is ejected, the droplets stretch due to the attenuated forces produced during centrifugal ejection [1], [9], [11]. The fibrous web is created as the polymer jet elongates and the solution evaporates [6], [15]. Literature reports that fiber diameter and defects depend on various parameters such as rotational speed, orifice size of the reservoir, distance to the collector, and the concentration of the polymer and viscosity [3], [16], [17]. Compared to the electrospinning industrial equipment, centrifugal force spinning, like FibeRio's Cyclone Fiber Engine FX (FibeRio company), can produce 12000 g/h of polymer fibers with a diameter of 500 nm, while electrospinning using Nanospinner416 (Inovenso) can produce 216 g/h [18]. Furthermore, the most important requirement for the centrifugal spinning technique is the need for a solvent that evaporates during the spinning process. Additionally, temperature can be applied to facilitate solvent evaporation [18]. A wide broad of polymers can be employed by this technique, including polyamide [11], polyethylene oxide [19] and biobased polymers [1]. Among biobased polymers, polylactic acid (PLA) stands out as a highly promising alternative to petrochemical polymers, due to the biodegradability, biocompatibility and safety for food-related applications properties [20]. PLA is a material frequently used in electrospinning [21], [22]. However, the use of PLA in force spinning has not been extensively studied. PLA presents competitive mechanical properties compared to petroleum-based thermoplastics, however, its inherent brittleness and low chain flexibility limit its applications. To improve these properties, PLA can be modified with plasticizers, increasing characteristics such as deformation at break, toughness, and flexibility, whereas properties like tensile strength, hardness, and melt viscosity typically decrease [23], [24]. Particularly interesting is the use of bio-plasticizers in PLA for packaging application. Bio-plasticizers are typically structures containing carboxylic and hydroxyl groups derived from building blocks as vegetable oils or tributyl citrates, and usually exhibit additional bioactive properties. Such bioactivity, especially antioxidant and antimicrobial activity further expand PLA's potential making it suitable for active food packaging applications by protecting

products from oxidation and microbial contamination. Products as citric acid esters of mono- and diglycerides, also known as CITREM or acetic acid esters of mono-and diglycerides of fatty acids, also known as ACETEM have been employed as bioactive bio-plasticizers in PLA films [25] - [27]. This study focuses on the production of PLA-based fibers incorporating the bio-based plasticizers CITREM and ACETEM through the force-spinning technique. Optimal spinning conditions were determined through rheological and microstructural evaluations. The fibers were extensively characterized for their thermal, antioxidant, and antimicrobial properties, and plasticizer distribution was analysed using Raman confocal microscopy and scanning electron microscopy. These fibers aim to enhance the functionality and sustainability of active packaging solutions, addressing the challenges of modern food preservation.

2. Materials and methods.

2.1. Materials.

Poly (lactic acid) (PLA) pellets (ErcrosBio® LL652; density: 1.25 g/cm^3; melt flow indexing: 11 g/10 min) were supplied by Ercros (Barcelona, Spain), and dichloromethane (DCM; 99% purity) used as solvent was obtained from Sigma-Aldrich (99% purity) Different bioplasticizers, all procured from Danisco (Denmark), were employed:

GRINDSTED® ACETEM SOFT-NSAFE (ACETEM SOFT): an acetic acid ester of monoglycerides, made from fully hydrogenated castor oil.

GRINDSTED® CITREM LR 10 EXTRA KOSHER (CITREM LR-10): a citric acid ester of mono-diglyceride (E 472c) made from edible, refined sunflower oil. This product also contains alpha-tocopherol (E 307) max. 200 ppm and Ascorbyl palmitate (E 304) max. 200 ppm.

GRINDSTED® CITREM SP 70 MB (CITREM SP-70): a citric acid ester of mono-diglyceride made from edible, refined sunflower and palm-based oil. This product also contains alpha-tocopherol (E 307) max. 300 ppm and Ascorbyl palmitate (E 304) max. 300 ppm.

2.2 Preparation of PLA fiber mats.

Different concentrations of PLA in DCM were prepared to identify the optimal concentration for the spinning process: 12, 17, 20, 22, and 27 wt%. PLA pellets were

dissolved in DCM in Pyrex bottles under magnetic stirring. Subsequently, fiber mats were obtained from these solutions using a homemade force-spinning device. This device allows control over experimental parameters such as centrifugal rotation rate and various positions for the collector. The reservoir of the spinneret has a capacity of 14 mL and an orifice of 0.5 mm. The rotational speed ranges from 2000-10000, the collector is positioned at 20 cm, and the relative humidity is maintained at 30%.

PLA fiber mats with bioplasticizers preparation: PLA fiber mats containing 10% functional plasticizers (ACETEM, CITREM LR-10, and CITREM SP-70) were prepared by force-spinning at 20 wt% concentration, 8000 rpm, and a distance to the collector of 20 cm.

2.3 Characterization.

2.3.1. Scanning electron microscopy (SEM).

The diameter, porosity, and morphology of the fibers were examined using a Philips XL30 scanning electron microscope at an acceleration voltage of 25 kV. The samples were coated with gold before scanning.

2.3.2. Differential scanning calorimetry (DSC).

The glass transition temperature (T_g), crystallization temperature (T_c), melting temperature (T_m), and crystallinity (X_c) of prepared fiber mats were studied in a TA Q2000 instrument (TA Instruments) under dry nitrogen (50 cm³/min). The samples were sealed in aluminium pans and equilibrated at 0 °C, samples were heated to 190 °C at 10 °C/min to analyse the main thermal transitions. Value of 93.6 J/g was used as melting enthalpy for a 100% crystalline (ΔH_m^0) for PLA [28], and the following equation was employed to determine the crystallinity, X_c, of the samples:

$$Xc(\%) = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0} x \frac{1}{W_f} x100 \quad (1)$$

where ΔH_{cc} represents the cold crystallization enthalpy, ΔH_m denotes the melting enthalpy, and W_f refers to the weight fraction of PLA in the sample.

2.3.3. Thermogravimetric analysis (TGA).

Thermogravimetric analysis was carried out to study the thermal stability of the different samples. The tests were performed in a TGA Q500 thermal analyser (TA

Instruments) at a heating rate of 10 ºC/min from room temperature (~30 ºC) to 800 ºC, under air atmosphere (50 cm^3/min). From the weight-temperature curves of the samples, the temperatures at 10% (T_{10}) and 50% (T_{50}) of weight loss were calculated, while the temperatures at the maximum degradation rate (T_{max}) of the samples were obtained from the first derivative of the TGA curves (DTG).

2.3.4. Confocal Raman microscopy.

The fibers were also analysed using confocal Raman microscopy on a CRM-Alpha 300 RA microscope (WITec, Ulm, Germany) equipped with an Nd:YAG laser (maximum power output of 50 mW at 532 nm) and an optical resolution of approximately 250 nm in the longitudinal direction and 700 nm in the transverse direction. The reflected laser light at each point was collected using a multimode optical fiber with a diameter of 25 µm by a highly sensitive CCD detector. Measurements were performed using 100× objectives with a numerical aperture (NA) of 0.95. Raman depth images (XZ Raman images) were measured in an area of 75 µm × 25 µm with an integration time of 0.30 s (a total of 2700 Raman spectra per Raman image), while Raman surface images (XY Raman images) were taken in an area of 25 µm × 25 µm with an integration time of 0.3 s (a total of 10000 Raman spectra per Raman image). The acquired Raman spectra were analysed using the Witec Project 2.02 program and Witec Control Plus Software.

2.3.5. Rheological characterization.

A TA Instruments ARG2 rheometer was utilized to measure the sample's shear viscosity across a shear rate range of 0.001 s^{-1} to 1000 s^{-1}. A standard Peltier Concentric Cylinder temperature system, featuring a cup radius of 15 mm and configured with a DIN Rotor, was employed. The instrument was initially calibrated according to standard protocol to determine the inertia of the rotor and its geometry, ensuring that the geometry was positioned at a gap distance of 2000 µm. All measurements were carried out at 25 ºC. At each targeted shear rate, the fluid viscosity was recorded after 5 seconds of equilibration.

2.3.6. X-ray diffraction analysis

X-ray diffraction (XRD) patterns of the fibers were recorded at room temperature in reflection mode using a Bruker D8 Advance diffractometer equipped with a PSD Vantec detector (Bruker, Madison, Wisconsin). Using Copper K-alpha (CuKα)

radiation with an average wavelength of λ (Kα) = 0.15418 nm and operating at a voltage of 40 kV, the samples were mounted on an appropriate holder and scanned between 2º and 60º (2θ) with an angular increment of 0.02º, and a collection time of 10 s per step.

2.3.7. Antimicrobial assay

The antibacterial test of the obtained fiber mats was conducted using the E2149-20 standard method to evaluate the antimicrobial activity of material surfaces from the American Society for Testing and Materials (ASTM) [29]. *Staphylococcus aureus ATCC* 25923, *Listeria innocua ATCC 33090, Lactobacillus acidophilus* DSM 20079, *Lactobacillus sakei* DSM 6333, *E. coli* DSM 25923 and *Salmonella* spp. were used as bacterial strains. In these analyses, fiber mats were cut into 2 cm x 2 cm pieces and then immersed in a sterile Falcon tube containing 10 mL of an appropriate bacterial suspension in PBS buffer. Additionally, blank experiments were performed with only the inoculum in the absence of samples. Subsequently, these suspensions were incubated in shaking condition at 150 rpm at 37 ºC. After 24 h, 1 mL of each suspension was taken and serially diluted at a ratio of 1:10 v/v. The dilutions were then inoculated pour-plating in Tryptic soy agar (TSA, Merck, Darmstadt, Germany) medium and incubated for 24 h at 37 ºC. Finally, the number of bacteria in each sample was determined using the plate counting method. The measurements were made in triplicate at a minimum.

2.3.8. Antioxidant assay

The antioxidant properties of the fiber mats were evaluated employing the Blois method using the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH, Merck)[30]. For this test, a DPPH solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol (Scharlau). Briefly, 50 mg of each sample and 2 mL of the DPPH solution were added to a vial, initiating the reaction. At different time periods (1, 2, 3, 4, 5, 7, and 24 h), 100 µL of each mixture were withdrawn and transferred to a multi-well plate. Absorbance in each well was measured using a BioTek® SYNERGY HTX multi-mode reader spectrophotometer at 527 nm. After recording the measurements, the solutions were returned to the original vials to maintain a consistent concentration and volume.

Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, (>97%, Fischer-Scientific), was used as the reference antioxidant and the results obtained at 24 h were

given in Trolox equivalent antioxidant capacity (TEAC). The measurements were performed at least in triplicate. The equations 2- 4 were employed to determine the TEAC values:

$$\text{RSA}\ (\%) = \left(1 - \frac{\text{Absorbance of sample}}{Absorbance\ of\ control}\right) \text{x } 100 \quad (2)$$

$$\text{CTE}\ (\mu g/mL) = (\text{RSA})/\text{mcal} \quad (3)$$

$$\text{TEAC}\ (\mu mol/mg) = (C_{TE} / M_{Trolox})/ C_{sample} \quad (4)$$

where, RSA represents the reduction in scavenging activity, CTE denotes the Trolox equivalent coefficient, m_{cal} is the slope of the calibration curve, M_{Trolox} is the molecular weight of Trolox, and C_{sample} refers to the concentration of the sample in the DPPH solution.

2.4. Statistical analysis.

One-way analysis of variance (one-way ANOVA) followed by post-hoc Turkey's test was carried out to determine significant differences at $P \leq 0.05$ among different groups.

3. Results and discussion

3.1. Preparation of the PLA fiber mats

Initially, the experimental conditions of the force-spinning process were optimized from PLA solutions using previously established parameters studied by our group: 8000 rpm of rotational speed, 10 mL of solution, and a distance to the collector of 20 cm [31]. Different polymer concentrations (12, 17, 20, 22, and 27 wt%) were evaluated, as concentration is one of the parameters that most strongly influences fiber formation, homogeneity, bead formation, and fiber continuity [11]. Once the fibers were spun onto the collector, the mats were removed with the help of scissors and aluminum foil. After that, the material was dried in an oven at 50 ºC for 24 h to evaporate the remaining solvent in the polymer. **Table C5. A6. 1.** summarizes the composition of PLA fibers obtained at the different polymer concentrations, along with their respective quality, diameter, and the viscosity of the precursor solutions, while **Figure C5. A6. 1a.** shows SEM images of representative regions of these fiber mats. At a low PLA concentration (12 wt%), no fibers were formed due to the very low viscosity, while increasing the concentration to 17 wt% resulted in a combination of

beads and non-uniform fibers. A subsequent increase in concentration to 20 wt% lead to continuous, homogeneous, and bead-free fibers with a diameter of 19.3 ± 3.0 µm.

Table C5. A6. 1. Composition and evaluation of PLA fibers at different concentrations.

Sample	Polymer concentration (wt %)	Fibers quality	Diameter (µm)	Viscosity (Pa·s)
PLA12	12	Not possible to yarn	-	0.07
PLA17	17	Presence of beads, fibers lack uniformity	22.9 ± 7.9	0.2
PLA20	20	Uniform fibers	19.3 ± 3.0	0.42
PLA22	22	Uniform fibers	27.8 ± 7.8	0.42
PLA27	27	Beads present; fibers lack uniformity	41.2 ± 9.4	1.1

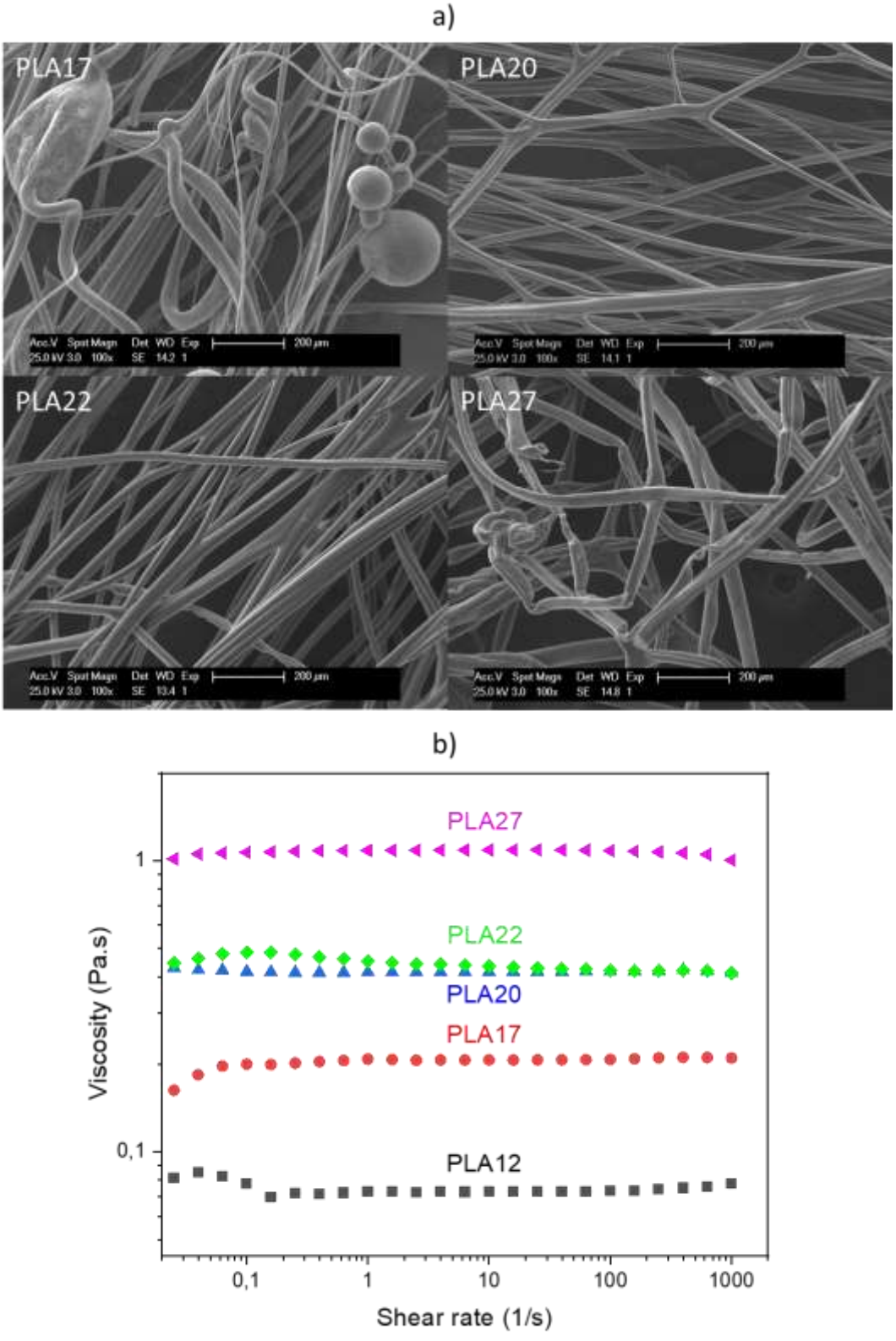


Figure C5. A6. 1. (A) SEM images of PLA fiber mats and (B) rheological curves of the precursor solutions at various polymer concentrations.

Raising the polymer concentration to 22 wt% significantly increased the fiber diameter to 27.8 ± 7.8 µm. When the concentration further increased, to 27 wt%, the diameter increased considerably due to the increased viscosity (see **Figure C5. A6. 1b.**); the presence of beads along the fibers became apparent, and the fibers were less homogeneous. From these results, it is evident that the optimal concentration from the tested range is between 20 and 22 wt%. Indeed, we selected 20 wt% as the optimal polymer concentration because it produces a smaller fiber diameter. PLA fiber mats containing 10 wt % functional plasticizers (ACETEM, CITREM LR-10, and CITREM SP-70) were then prepared by force-spinning at 20 wt% concentration, 8000 rpm, and a distance to the collector of 2200 cm.

The compositions of the prepared fibers with the bioplasticizers, along with the diameter, surface density, and viscosity of the precursor solutions are summarized in **Table C5. A6. 2.**

Table C5. A6. 2. Composition of the different functionalized PLA fibers.

Sample	PLA (%)	Plasticizer (%)	Diameter (µm)	Viscosity (Pa·s)	Surface density (mg/cm^2)
PLAf	100	-	19.3 ± 3.0	0.41	6
PLAf-SS	90	ACETEM SOFT (10)	26.2 ± 8.0	0.34	9
PLAf-LR	90	CITREM LR-10 (10)	18.8 ± 1.5	0.45	8
PLAf-SP	90	CITREM SP-70 (10)	27.3 ± 11.6	0.65	8

The surface density of the fibers was determined by weighing each sample with dimensions of 2 × 2 cm. Additionally, photos of these squares were taken to visually analyse the differences between the materials (**Figure C5. A6. 2a.**). Next, SEM was employed to analyse the morphology and diameter of the fibers (**Figure C5. A6. 2b.**). **Table C5. A6. 2.** illustrates the variation in diameter among the samples. From these results, it is evident that PLAf-LR fibers exhibited the lowest diameters and excellent homogeneity, whereas the incorporation of the other two plasticizers, ACETEM SOFT and CITREM SP-70, results in fibers with higher diameters, greater heterogeneity, and the formation of beads, especially in the PLAf-SP containing CITREM SP-70. At higher magnifications in the SEM images, small surface porosity was apparent in the

PLAf-SS fibers, and also observed in the PLA fibers without plasticizer (PLAf). In contrast, surface roughness rather than pores was visible in the PLAf-LR and PLAf-SP fibers. The porosity in spun polymeric fibers was generally attributed to the breath figure process due to the relative humidity of the environment or the presence of water in the solvent [32],[33]. In this case, humidity was maintained at 30% for all the spinning processes, suggesting that the evaporation rate of the solvent was affected by the presence of the plasticizers, which significantly modifies the porosity of the solution.

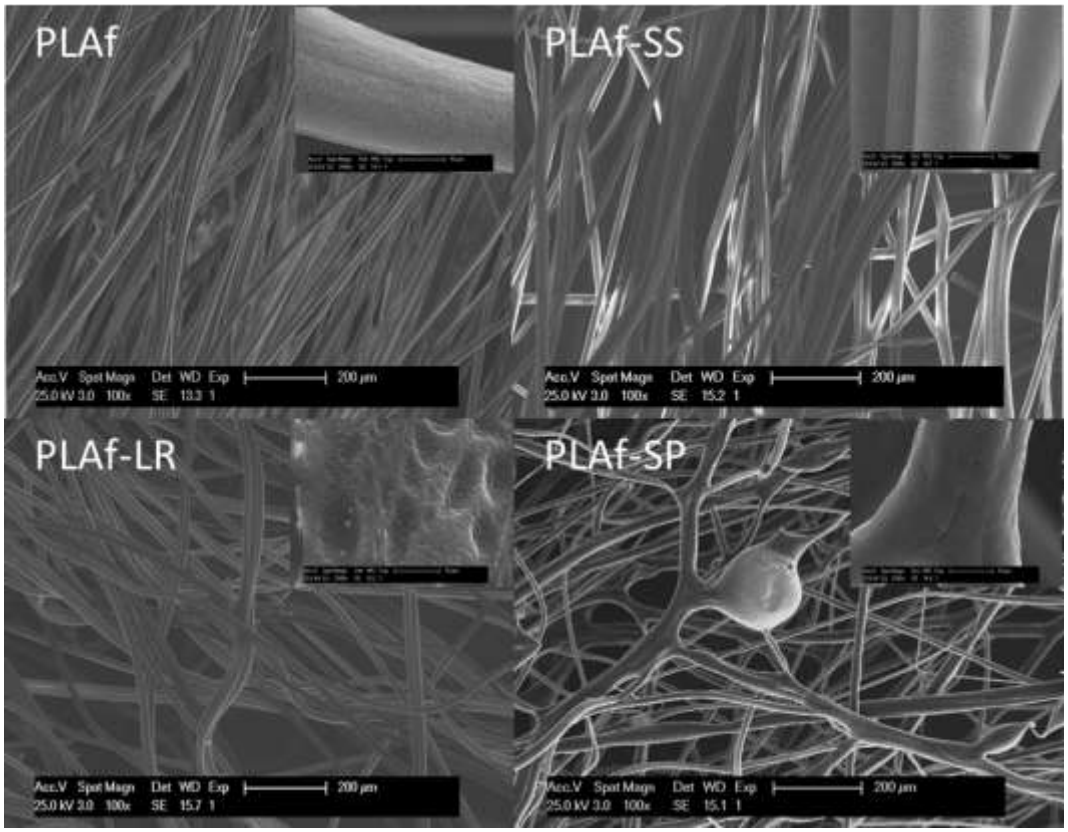


Figure C5. A6. 2. (A) Photos of the functionalized fibers (squares of 2 x 2 cm) and (B) their SEM images at different magnifications: 100x and 2500x (inset).

The incorporation of plasticizers leads to variations in the viscosity and rheological behavior of the polymeric solutions, as shown in **Table C5. A6. 2.** and **Figure C5. A6. 3.** The addition of ACETEM SOFT reduces the viscosity of PLA solutions from 0.41 Pa·s to 0.34 Pa·s. Furthermore, at low shear rates, the apparent viscosity increased as the shear rate increases. With the other CITREM plasticizers, pseudoplastic behavior was observed, followed by Newtonian behavior. Indeed, the curve obtained in

polymeric solutions with CITREM LR-10 closely resembled the curve of the PLA solutions; thus, the incorporation of this plasticizer barely modified its rheological behavior. In contrast, the addition of CITREM SP-70 caused a dramatic increase in viscosity up to 0.65 Pa·s. While ACETEM SOFT is liquid at ambient temperature (melting temperature at 1 ºC) and CITREM LR-10 has a melting temperature of 11 ºC, CITREM SP-70 exhibits a higher melting temperature at 47 ºC, being a viscous solid at ambient temperature and thereby affecting the solution's viscosity.

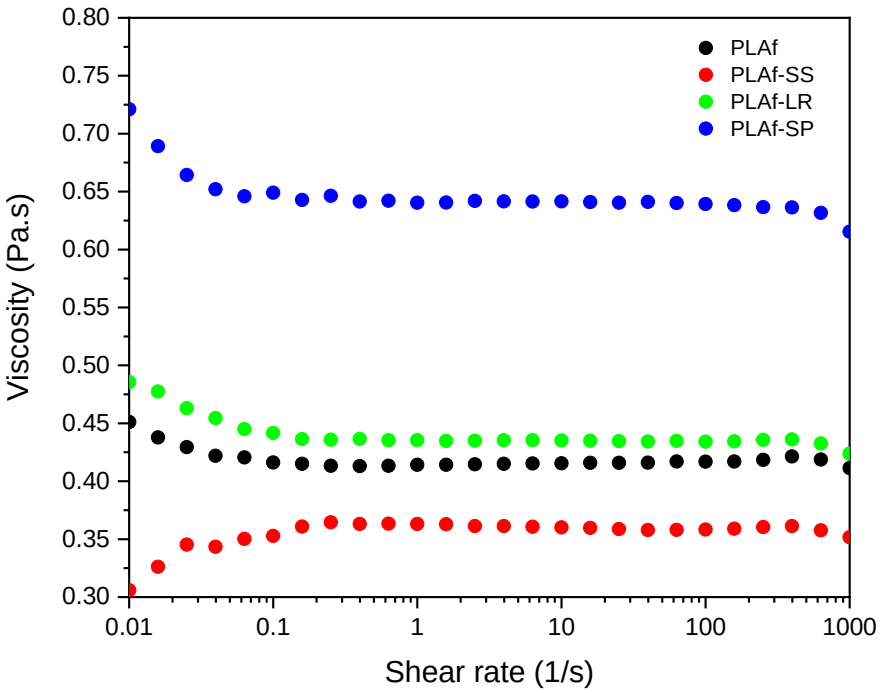


Figure C5. A6. 3. Rheological curves of PLA and PLA plasticized solutions.

3.2. Characterization of the PLA fiber mats containing the functional plasticizers.

Initially, the distribution of the tested plasticizers along the obtained PLA fibers was studied using Raman confocal microscopy. Previous studies on such CITREM plasticizers in PLA films produced via melting processes revealed clear phase separation, forming microregions, whereas ACETEM-SOFT was entirely miscible with the PLA matrix [27]. **Figure C5. A6. 4.** presents the XZ Raman images of PLAf, PLAf-SS, PLAf-LR, and PLAf-SP fiber mats, where only one phase is visible, indicating a homogeneous distribution of the plasticizers in the PLA matrix.

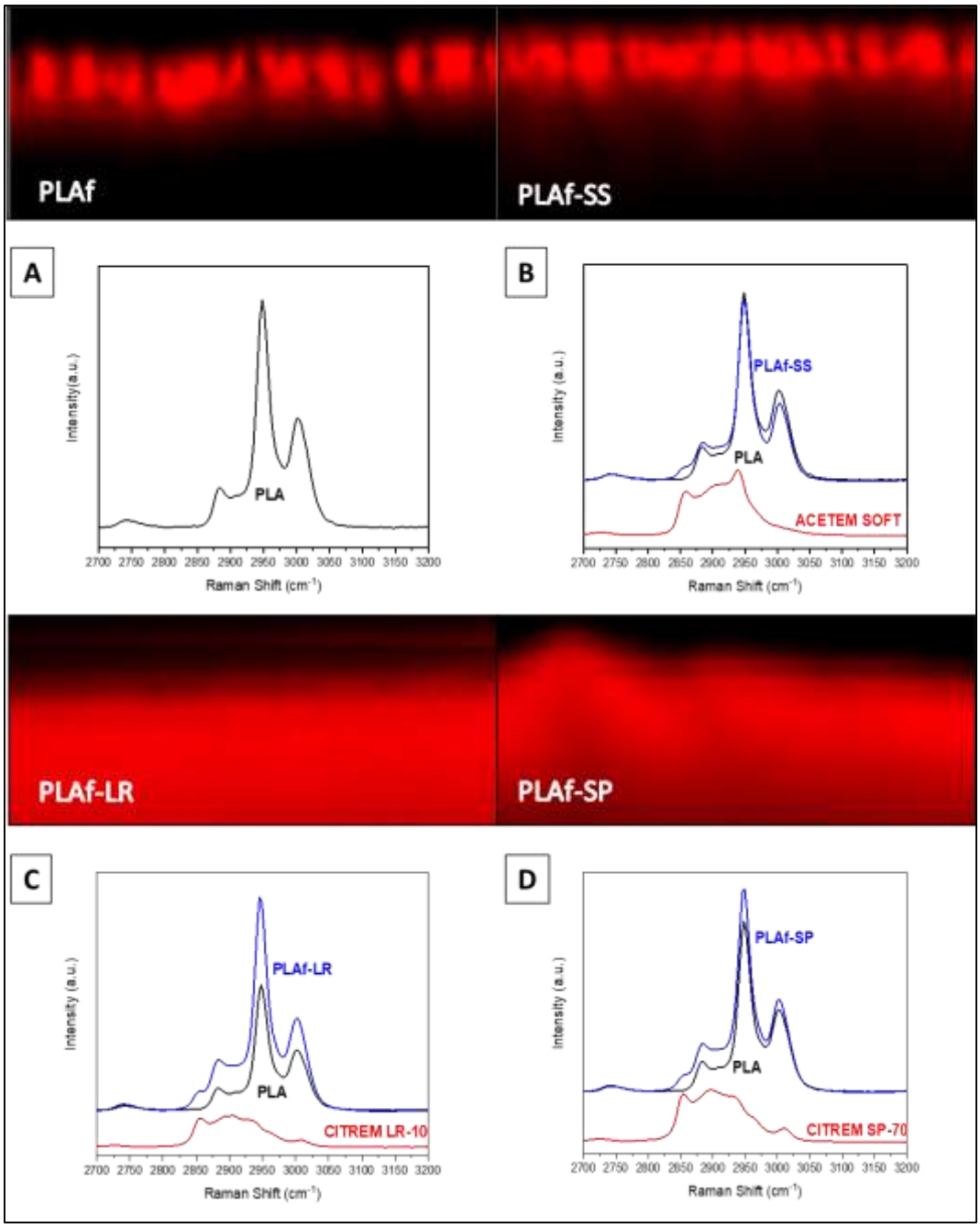


Figure C5. A6. 4. Raman images and spectra of the different plasticized PLA fibers.

In the spectra obtained at each point of the measured fibers, bands for PLA and plasticizers (at 2850 cm^{-1}) were prominently observed, demonstrating a uniform location of the plasticizers throughout the fibers. This is because, in the spinning process, the mixture of PLA and plasticizers involves the dissolution of both components in DCM, which facilitates better blending.

Next, the structural and thermal properties of the prepared fibers were analysed to assess the effect of plasticizer incorporation and their uniform distribution on those properties. XRD measurements (**Figure C5. A6. 5.**) show a clear increase in crystallization with the incorporation of plasticizers. While primarily an amorphous halo is observed in the PLAf mats, the other fibers display distinct bands corresponding to different planes. At a wavelength of 16.5º, a sharp peak associated with the (110/200) plane and a broader peak at 19º corresponding to the (203) plane were observed [34],[35].

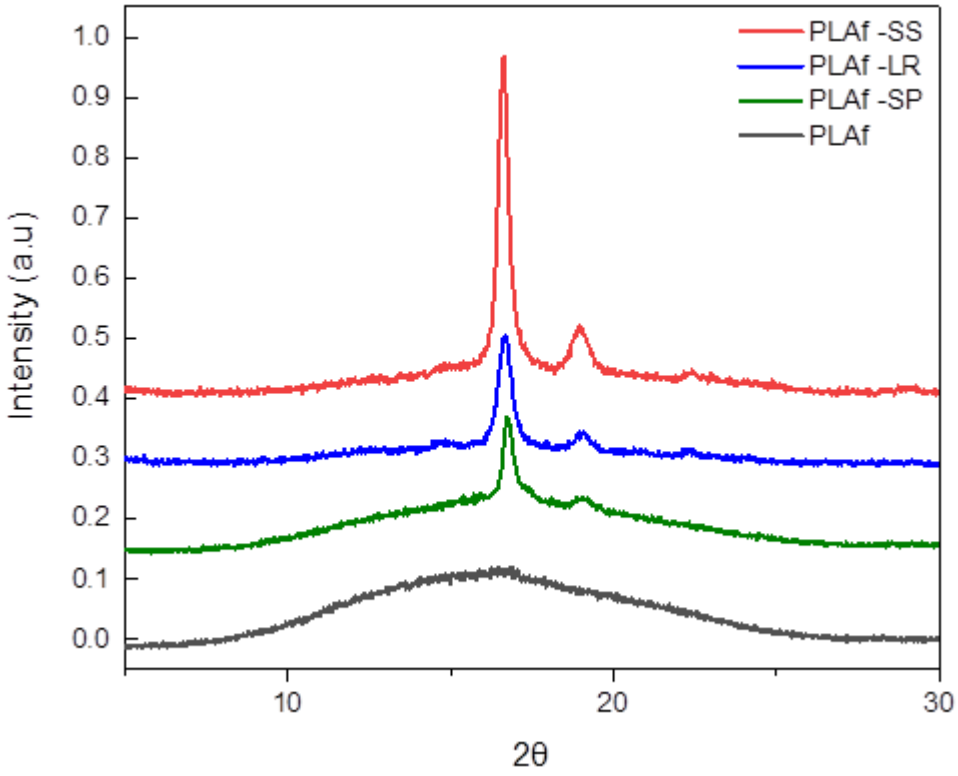


Figure C5. A6. 5. XRD patterns of functionalized PLA fibers.

The crystallinity was further studied by DSC and the obtained results are summarized in **Figure C5. A6. 6.** and **Table C5. A6. 3.** The presence of plasticizers increases the crystallinity and the cold crystallization transition is considerably reduced in enthalpy, ΔH_{cc}, and also in temperature, T_{cc}. Indeed, in the case of PLAf-SS fibers, this process is completely eliminated. The incorporation of plasticizers significantly increased the mobility of the polymeric chain facilitating the crystallization at lower temperatures, and then, the T_{cc} values decreases [36], [37].A second crystallization process is

detected at around 155 ºC, attributed to the recrystallization of PLA α' crystals into more stable α-crystals [38], [39], followed by a melting process that occurs above 170 ºC, which also slightly decreases with the addition of plasticizer. Besides the increase in crystallinity, a clear reduction in glass transition temperature is observed, resulting from the higher chain mobility. This indicates the efficiency of plasticization [40]. Particularly, the addition of ACETEM SOFT to the PLA-based fibers lowers the T_g to 51 ºC. This T_g reduction is less pronounced with the addition of CITREM LR10 and CITREM SP70.

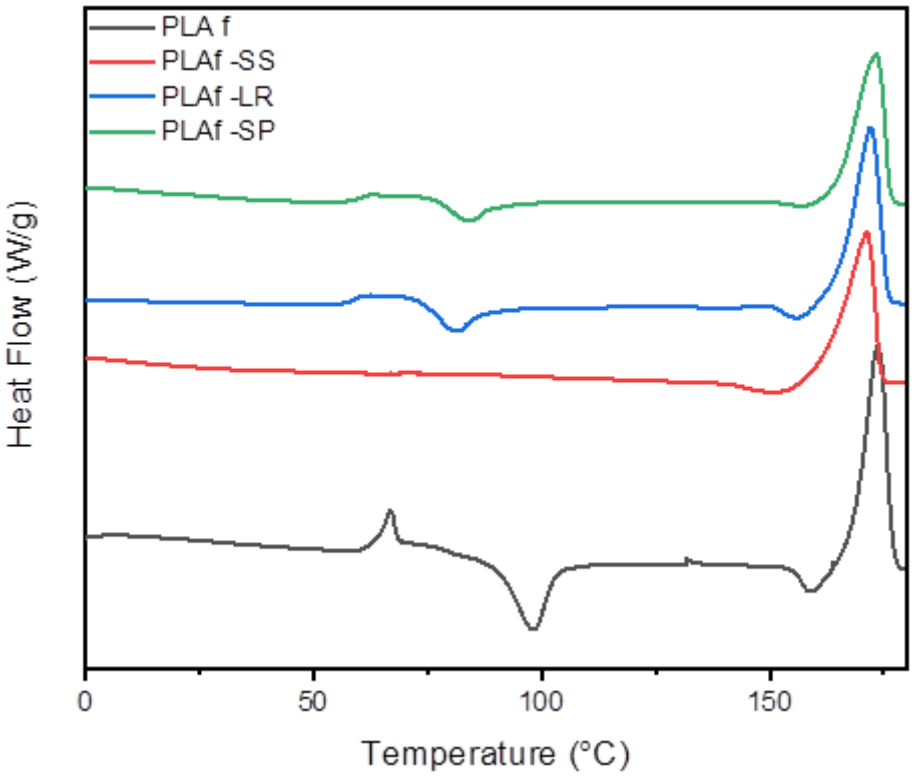


Figure C5. A6. 6. DSC curves of the functionalized PLA fibers.

Table C5. A6. 3. Thermal parameters derived from the heating DSC curves of functionalized PLA fibers include glass transition temperature (T_g), cold crystallization temperature (T_{cc}), melting temperature (T_m), melting enthalpy (ΔH_m), cold crystallization enthalpy (ΔH_{cc}), and degree of crystallinity (X_c).

Sample	T_g (° C)	T_{cc} (° C)	T_m (° C)	ΔH_m (J/g)	ΔH_{cc} (J/g)	X_c (%)
PLAf	66	91	174	51	24	32
PLAf-SS	51	-	171	47	0	56
PLAf-LR	59	75	172	51	10	49
PLAf-SP	61	77	173	49	8	47

Thermal analyses of the prepared fibers were carried out to evaluate thermal stability using TGA (**Table C5. A6. 4.**). Results indicate that the addition of CITREM-SP70 caused a significant decrease in the temperatures corresponding to 10% ($T_{d10\%}$) and 50% ($T_{d50\%}$) mass degradation. In contrast, the addition of ACETEM SOFT and CITREM-LR10 produced a slight reduction in the $T_{d10\%}$ value. Furthermore, the addition of CITREM-SP70 decreased the temperature at the maximum degradation rate (T_{dmax}). As expected, the inclusion of plasticizers reduces the thermal stability of PLA.

Table C5. A6. 4. TGA characterization of functionalized PLA fibers.

Sample	$T_{d10\%}$ (°C)	$T_{d50\%}$ (°C)	T_{dmax} (°C)
PLAf	324	354	354
PLAf-SS	313	348	357
PLAf-LR	313	353	354
PLAf-SP	283	307	308

3.3 Characterization of the bioactive properties of the functionalized PLA fiber mats.

The antimicrobial properties of the prepared PLA fibers were tested against various bacterial strains associated with the food industry, including *E. coli*, Salmonella, *Listeria*, *Staphylococcus aureus*, *Lactobacillus sakei*, and *Lactobacillus acidophilus*. Control experiments were also carried out using PLA mats without functional plasticizers, which did not exhibit any antimicrobial activity. The fibers containing the functional plasticiers showed no antimicrobial effect against Gram-negative bacteria such as *E. coli* and *Salmonella*, emphasizing the challenge of penetrating the double membrane of Gram-negative organisms. In contrast, samples demonstrated antimicrobial activity against Gram-positive bacteria, including *L. innocua*, *S. aureus*, *L. sakei*, and *L. acidophilus*. **Table C5. A6. 5.** summarizes the results, expressed as the differences in bacterial counts (Δlog CFU/mL) after 24 h of contact with the produced fibers.

Table C5. A6. 5. Antibacterial activity of the plasticized PLA fibers, expressed as the difference in bacterial counts (Δlog CFU/mL) after 24 hours of contact (initial concentration 2.9 CFU/mL for *L. innocua*, 4.6 CFU/mL for *S. aureus*, 2.7 CFU/mL for *L. sakei*, and 3.1 CFU/mL for *L. acidophilus*).

Sample	***L. innocua*** **(Δlog CFU/mL)**	***S. aureus*** **(Δlog CFU/mL)**	***L. sakei*** **(Δlog CFU/mL)**	***L. acidophilus*** **(Δlog CFU/mL)**
PLAf-SS	0.81 ± 0.03	2.40 ± 0.11	1.00 ± 0.12	1.90 ± 0.11
PLAf-LR	0.59 ± 0.04	2.93 ± 0.14	2.71 ± 0.0	1.76 ± 0.05
PLAf-SP	0.47 ± 0.02	0.20 ± 0.01	1.05 ±0.03	1.70 ± 0.01

Next, antioxidant capacity of the prepared fibers was evaluated using the Blois method with DPPH as the free radical and a contact time of 24 h, expressed as Trolox Equivalent Antioxidant Capacity (TEAC). The PLA fiber mats containing functional plasticizers exhibited certain antioxidant activity, showing values of 15.7 ± 0.1 µmol Trolox/g of fibers for PLAf-SS, 18.7 ± 0.6 µmol Trolox/g of fibers for PLAf-LR, and

23 ± 1 µmol Trolox/g of fibers for PLAf-SP. Therefore, the addition of these bioplasticizers (CITREMs and ACETEM) effectively provides both antioxidant and antimicrobial properties to PLA fibers, enhancing their suitability for active food packaging and other applications that demand protective functionality.

4. Conclusions

Force-spinning is a promising technique to obtain high amounts of nano- and micro-fibers of polymeric materials. In this research, experimental conditions of the spinning process were optimized to fabricate uniform and bead-free PLA fibers containing functional biobased plasticizers for potential application in food packaging. In particular, CITREM and ACETEM bioplasticizers were incorporated in the PLA fibers at a concentration of 10 wt%. The plasticizers were distributed along the PLA fibers in a homogeneous manner as confirmed by SEM and Raman confocal microscopy. The incorporation of the bioplasticizers enhances the crystallinity of the PLA fibers and reduces the glass transition temperature due to the plasticizing effect. The results specifically demonstrate the antioxidant properties and antimicrobial activity against Gram-positive food pathogens, including *Listeria innocua*, *Staphylococcus aureus, Lactobacillus sakei, and Lactobacillus acidophilus*. Thereby, functionalized PLA fibers hold great potential for food packaging applications, such as absorbent pads for capturing exudates in fresh products or tea bags, offering antioxidant and antimicrobial properties that can extend shelf life and reduce food loss.

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CHAPTER 6

Integrative discussion

Integrative discussion.

The following is an integrative discussion of the most relevant results obtained in the different chapters of this Doctoral Thesis, which focuses on the development of compostable polymeric materials with antimicrobial and antioxidant properties, intended for their use as active food packaging. For this purpose, two biobased reference materials were selected: polylactic acid (PLA) and Ecovio®. Polylactic acid (PLA) is widely used in the packaging sector due to its good mechanical and thermal properties, ease of processing, aroma and flavor barrier capacity, and compostability under industrial conditions. However, its main limitation is the brittleness of the matrix, which generally requires reinforcement through tougher polymers or the incorporation of additives such as plasticizers. The second material selected was Ecovio®, a commercial blend composed of 45% PLA and 55% PBAT, a copolymer with lower mechanical resistance but considerably more flexibility, widely used in the food industry to produce bags and films, providing a compostable material suitable for various applications.

The central objective of this thesis was to incorporate additives, both reinforcements and plasticizers, that would provide functional properties to the polymer matrices, focusing on compounds from natural sources or approved for food industry use. The purpose of the developed packaging was to turn them into active systems capable of prolonging the shelf life of food products through antimicrobial and antioxidant properties (**Figure C6. A7. 1.**)

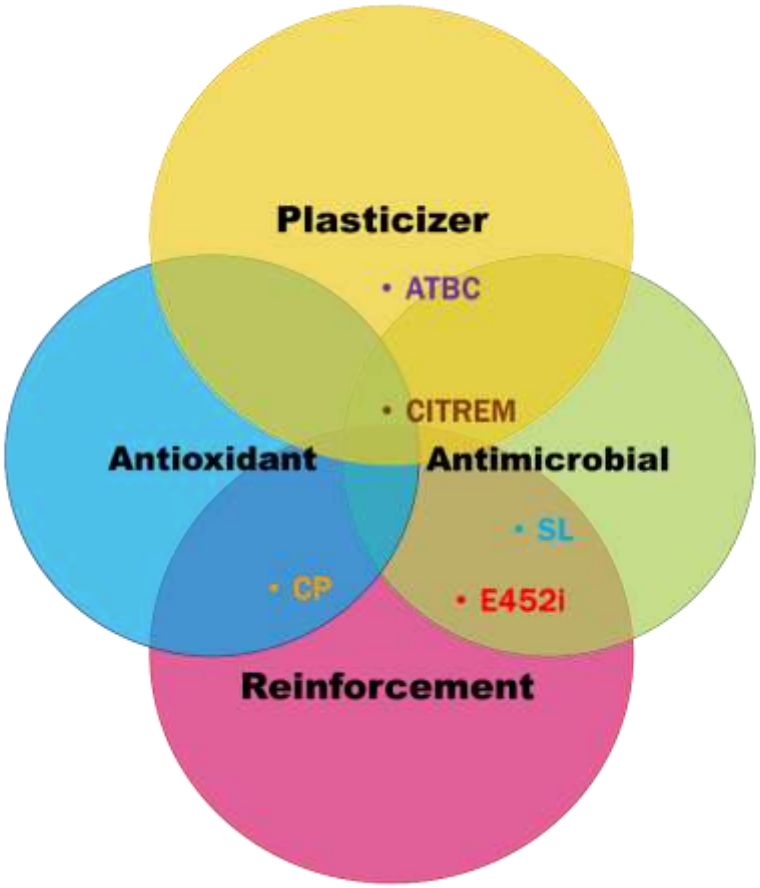

Figure C6. A7. 1. Additives evaluated in PLA and Ecovio®.

In an initial study, the potential of antimicrobial particles was evaluated, selecting sodium hexametaphosphate (E452i) particles. Hemotoxicity tests showed no hemolysis, and MIC assays against Gram-positive bacteria such as *Listeria innocua* and *Staphylococcus aureus* confirmed antimicrobial efficacy. Based on these results, polymer films were designed by combining the polymer (PLA and Ecovio®), the plasticizer ATBC at 15%, and E452i particles at concentrations of 1% and 5%. The films were obtained by extrusion in a twin-screw extruder followed by compression molding, reaching an approximate thickness of 200 μm.

Microstructural analysis by SEM and confocal Raman microscopy revealed that Ecovio® films exhibited a heterogeneous microstructure, with immiscible PLA and PBAT phases, and particles distributed non-uniformly, tending to segregate, with

even some surface chemical modifications observed. Thermal properties were evaluated by TGA, indicating that both PLA and Ecovio® have high thermal stability, slightly reduced by the addition of plasticizer, while the incorporation of inorganic reinforcements increased stability. DSC showed that ATBC reduced PLA's T_g and T_{cc} by increasing chain mobility, favoring crystallization, while E452i particles increased T_g and acted as nucleating agents. Mechanically, ATBC slightly increased PLA's elongation at break, and the presence of particles mitigated the loss of strength and Young's modulus. In contrast, Ecovio® maintained brittleness and low mechanical resistance, with no appreciable improvements. Antimicrobial activity tests according to ASTM E2149-20 confirmed efficacy against *L. innocua* and *S. aureus*, more pronounced in Ecovio®, and composting tests showed that PLA degraded almost completely in 43 days, while Ecovio® reached over 60% degradation in 59 days. Despite the properties achieved, E452i particles were unstable, non-homogeneous, and lost their properties over time. Moreover, the films particularly Ecovio® did not display the expected mechanical performance of PLA/PBAT blends due to poor phase miscibility and microdomain formation, as well as the low effectiveness of ATBC as a plasticizer. Therefore, research shifted toward the use of new plasticizers, specifically biobased compounds with bioactive properties, to achieve both bioactivity and improvements in the mechanical and physicochemical properties.

Vegetable oil derivatives, commercially known as CITREM and ACETEM, defined as mono- or diglycerides of fatty acids esterified with citric or acetic acid, respectively, were evaluated as bioplasticizers. Their composition was characterized by GC-MS and FTIR, identifying antioxidant molecules such as α-tocopherol, particularly in CITREM-LR10 and CITREM-SP70. Antioxidant capacity was confirmed using the Blois method, highlighting plasticizers containing α-tocopherol, while thermal stability verified by TGA showed no degradation up to 180 °C, thereore, its use for extrusion and molding processes of PLA and Ecovio® was possible.

PLA films with 10% of these bioplasticizers showed that ACETEM was homogeneously distributed, while CITREMs formed microdomains, indicating differential miscibility. Contact angle measurements revealed reduced surface tension, and thermally the plasticizers decreased T_g and T_{cc} while increasing

crystallinity, although XRD confirmed the absence of defined crystalline structures. Mechanically, PLA-SS, PLA-LR, and PLA-SP films showed a significant increase in elongation at break up to 42% in PLA-LR with a decrease in strength and Young's modulus. Confocal Raman microscopy revealed that CITREM microdomains could orient during fracture. The films retained antioxidant and antimicrobial activity against *L. innocua* and *S. aureus*, demonstrating active functionality.

The same plasticizers were later applied to Ecovio®, selecting CITREM-LR10 and ACETEM SOFT-NSAFE. As in PLA, ACETEM distributed homogeneously while CITREM segregated or formed microdomains, maintaining the PLA/PBAT phase separation. Thermally, stability slightly decreased, T_g was reduced, and crystallinity increased. Mechanically, strength and modulus decreased without improvement in elongation at break. Oxygen and water vapor permeability were higher than in PLA and increased with the incorporation of plasticizers, while migration to food simulants remained minimal and within European limits. Functionally, the films retained antioxidant and antimicrobial activity, even higher than that observed in PLA, and compostability was maintained. Despite these good properties, the mechanical performance of Ecovio® films remained suboptimal, as expected for PLA/PBAT blends, due to the pronounced phase separation that was not improved by ACETEM or CITREM plasticizers.

To improve microstructure, compression molding was replaced by calendering, incorporating 10% CITREM-SP70. In addition, other additives were incorporated to enhance functional and physicochemical properties: sodium stearoyl lactylate (SL) and chitin particles (CP) at 5%. This two-step processing allowed for a more uniform distribution of microdomains, greater elongation at break, and retention of active properties, although SL addition generated mechanical anisotropy and CP addition worsened mechanical behavior. Permeability remained high, suitable for packaging fresh and bakery products, while functionalization enhanced antioxidant capacity and boosted antimicrobial activity against *S. aureus* and *L. innocua*. Plasticizer migration was minimal, and under industrial composting conditions, film degradation time was only slightly increased.

Finally, PLA microfibers were explored as a new system for active packaging using force-spinning, incorporating CITREM-LR10, CITREM-SP70, and ACETEM

SOFT-NSAFE. Polymer concentration was optimized at 20%, yielding homogeneous, defect-free fibers. With the addition of plasticizers, CITREM-LR10 produced thinner and more uniform fibers, while ACETEM and CITREM-SP70 generated larger diameters and greater heterogeneity. Thermally, the fibers showed increased crystallinity and reduced T_g, with slightly lower thermal stability, more pronounced in CITREM-SP70. Functionally, the fibers exhibited antimicrobial activity against *L. innocua*, *S. aureus*, *L. sakei*, and *L. acidophilus*, as well as significant antioxidant capacity, highest in CITREM-SP70. These results demonstrate that force-spinning enables the reproducible production of PLA fibers functionalized with bioplasticizers, improving structural, thermal, and bioactive properties, with potential application in active food packaging.

Overall, these results show that both CITREM and ACETEM act as functional plasticizers in PLA and Ecovio®, imparting antimicrobial and antioxidant properties, improving deformability and microstructure of the polymers using different processing methods **Figure C6. A7. 2.**, and that the design of films and fibers enables the exploration of different active packaging strategies depending on the food application.

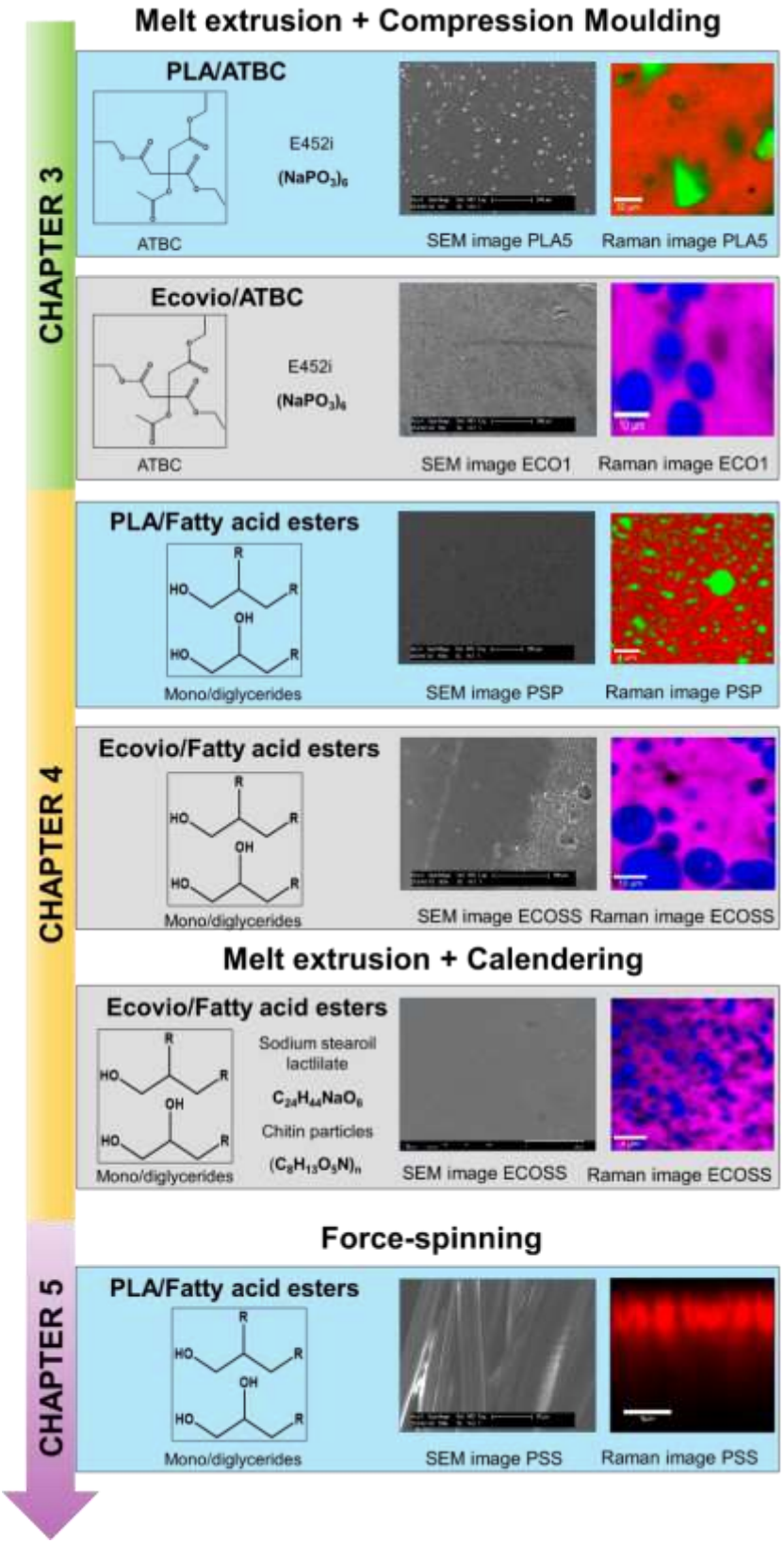

Figure C6. A7. 2. Evolution of the microstructure in PLA and Ecovio® systems modified with plasticizers and additives: a comparative analysis by SEM and Raman.

CHAPTER 7

Conclusions.

Conclusions

The development of biodegradable packaging materials represents a significant challenge and opportunity for the food packaging industry. This work aimed to contribute to that challenge by designing biodegradable films capable of replacing conventional plastics, thereby reducing environmental impact caused by persistent plastic waste and improper disposal. Throughout this thesis, various biodegradable active packaging systems were developed using polymeric matrices with compostable properties, combined with active additives sourced from the food industry to enhance functionality and extend self-life of the packaged food which would contribute to reduce waste food. Multiple fabrication techniques were employed to assess the performance of different composite materials based on PLA and Ecovio® for potential packaging applications.

In **Chapter 3**, bioactive E452i particles were incorporated into the polymer matrix plasticized with a known bioplasticizer, ATCB, through compression moulding, producing films with tailored properties. **Chapter 4** introduced novel bioplasticizers such as CITREMS (used here for the first time in polymer blends) and ACETEM (previously used but still uncommon), which were incorporated for the first time into PLA and Ecovio® films to impart antioxidant and antimicrobial properties via compression moulding and calendering. Further, SL and CP additives were evaluated in calendered films to improve film properties such as barrier properties, mechanical performance, antioxidant and antimicrobial activity. In **Chapter 5**, the potential of the forcespinning technique was explored to fabricate PLA microfibers and functionalized fibers using ACETEM and CITREM as bioplasticizers, demonstrating a promising route for producing active, biodegradable packaging materials.

Overall, this research contributes to the ongoing effort to develop sustainable, functional packaging alternatives and highlights new bioplasticizers, food additives and processing techniques that could pave the way for future innovations in the food industry.

The most relevant findings of this work are summarized in the following text:

Chapter 3.

- E452i particles were successfully incorporated as antimicrobial fillers into the polymeric films (PLA and Ecovio®). Biocomposites containing 1% and 5% of
- E452i demonstrated effective antibacterial activity against common foodborne pathogens such as Staphylococcus aureus and Listeria innocua, indicating their potential suitability for active food packaging applications. Besides, no hemotoxicity was observed for E452i particles.
- The incorporation of E452i also enhanced the thermal and mechanical properties of the films, leading to increased thermal stability, crystallinity, and Young's modulus.
- The reinforced films maintained their compostability. Despite the addition of E452i particles, the disintegration kinetics under composting conditions did not vary significantly, preserving the biodegradability of both PLA and Ecovio® systems.
- Overall, this study confirms that E452i can be effectively integrated into PLA and Ecovio® blends plasticized with ATBC, resulting in biodegradable and active packaging materials.

Chapter 4.

- In these studies, various food-grade esters derived from citric (CITREM) and acetic (ACETEM) acids were evaluated as biobased plasticizers for PLA and Ecovio® films obtained by two different processing methods: extrusion followed by compression moulding and extrusion followed by calendering. The study of the obtained films was performed with a particular focus on their mechanical, thermal, antioxidant, and antimicrobial properties.
- The interaction between the polymeric matrix and the plasticizers differs notably across the studied systems. In the case of PLA, CITREM plasticizers exhibit poor miscibility, leading to the formation of microdomains within the composite. In contrast, ACETEM demonstrates good miscibility with the PLA matrix, as evidenced by changes in the Raman spectra and the absence of microdomain

formation. For Ecovio®, no miscibility is observed with CITREM, however, microdomains are not detected within the matrix. This suggests that the CITREM plasticizer may be migrating or exuding to the surface rather than integrating into the bulk polymer. Conversely, ACETEM shows clear miscibility with the Ecovio® matrix, indicating homogeneous distribution.

- For compression-moulded films:
    - CITREM-LR10 proved to be the most effective plasticizer, increasing the elongation at break of PLA-based films to 42.5% without compromising thermal stability. In comparison, other plasticizers such as ACETEM, SOFT-NSAFE, and CITREM-SP70 also improved the elongation at break, but to a lesser extent and with a more pronounced reduction in thermal stability. Morphological analysis using confocal Raman microscopy revealed that CITREM-LR10 tends to form microdomains within the PLA matrix and aligns along cracks during mechanical deformation, suggesting a unique plasticization mechanism.
    - Nonetheless, one of the major drawbacks observed in Ecovio® films is the poor compatibility between the PLA and PBAT phases in Ecovio® when processed by compression moulding. This incompatibility extends to the interaction between PBAT and the plasticizers, primarily due to differences in hydrophobicity between the two materials. As a result, the plasticizers tend to migrate or exude to the surface, reducing their effectiveness and ultimately leading to poor mechanical properties in the resulting films
    - The antioxidant properties of the plasticizers isolated were evaluated, the results demonstrated the antioxidant capacity of the samples, fatty acid esters containing acetic and citric acid included in the polymeric films maintain their scavenging activity, for PLA the results exhibited are lower due to differences in the hydrophobicity and swelling ratio between PLA and Ecovio®.
    - Antimicrobial activity was observed for CITREM-LR10, CITREM-SP70, and ACETEM SOFT-NSAFE. When incorporated into PLA and Ecovio® matrices, CITREM-LR10 demonstrated strong antimicrobial effectiveness against *Staphylococcus aureus*. In contrast, ACETEM SOFT-NSAFE showed lower antibacterial activity, though it was more

effective in Ecovio® than in PLA. Specifically, for PLA, ACETEM SOFT-NSAFE exhibited greater activity against *Listeria innocua* than CITREM-LR10. Meanwhile, CITREM-SP70 showed moderate antimicrobial activity against both pathogens when added to the PLA matrix.

- Although the incorporation of CITREM-LR10 and ACETEM SOFT-NSAFE into PLA and Ecovio® resulted in negligible plasticizer migration into food simulant, it led to a slight reduction in barrier properties against water vapour and oxygen. However, the biodegradability of the films under composting conditions remained unaffected.

- For calendered-moulded Ecovio® films:
  - Remarkably, by modifying the processing method Ecovio® presented a better homogeneity and compatibility between PLA and PBAT as was confirmed via SEM and confocal Raman microscopy. Also, SL and CP additives exhibited good dispersion.
  - The addition of plasticizers CITREM-LR10, CITREM-SP70, and ACETEM SOFT-NSAFE helped maintain the good mechanical, thermal and barrier properties of Ecovio® films. These plasticizers also imparted notable antioxidant activity, moreover CITREM-LR10 and CITREM-SP70 demonstrated antimicrobial effectiveness against *Staphylococcus aureus* and *Listeria innocua*, enhancing the films' potential for active food packaging. Additionally, minimal migration of the plasticizers into food simulants was observed, confirming their suitability and safety for food contact applications.
  - The incorporation of the SL additive into plasticized films preserved good mechanical properties in the parallel direction but introduced anisotropy, resulting in reduced mechanical performance in the transverse direction. While the thermal properties were slightly reduced with SL addition, the barrier properties remained stable. Furthermore, SL-containing films exhibited good antimicrobial activity against *Staphylococcus aureus* and *Listeria innocua*, supporting their potential use in active packaging applications.

- The incorporation of CP negatively affected the mechanical properties of the functionalized films and did not lead to any improvement in barrier performance. However, it maintained the thermal properties and slightly enhanced the antioxidant activity of the films.
- The films disintegrated effectively under industrial composting conditions, confirming their biodegradability and suitability for sustainable end-of-life management.

Chapter 5.

- PLA forcespinning microfibers were produced with a concentration of PLA/DCM solution ranging from 12% to 27%. PLA fibers produced with a concentration of 20 % exhibited a more homogenous diameter and no defect was observed in their microstructure. That concentration of PLA/DCM was employed to produce plasticized fibers with CITREM and ACETEM.
- The plasticized fibers were produced with 10% of plasticizer, the addition of plasticizer affected the rheological properties of PLA/DCM solution which affects fiber microstructure and morphology and slightly reduces the thermal stability of PLA.
- The crystallinity of PLA fibers increased with the addition of ACETEM SOFT-NSAFE, CITREM-LR10, and CITREM-SP70, with SOFT-NSAFE inducing the highest crystallinity, followed by CITREM-LR10 and then CITREM-SP70. Same behaviour is observed in the value of T_g where SOFT-NSAFE produces the highest decrease in the value followed by CITREM-LR10 and CITREM-SP70.
- The plasticized fibers exhibited strong antioxidant capacity headed by CITREM-SP70. The antimicrobial trials of the plasticized fibers demonstrated the activity against Gram-positive food pathogens, including *Listeria innocua*, *Staphylococcus aureus, Lactobacillus sakei, and Lactobacillus acidophilus*.
- These results highlight PLA fibers' potential as active packaging materials, ideal for absorbent pads in fresh food, tea bags, and other formats requiring antioxidant and antimicrobial properties to extend shelf life and reduce food loss.

This thesis has evaluated new materials that can serve as plastic additives and are approved for use in food products by regulatory bodies such as the FDA and EFSA. The incorporation of these additives has enabled the development of active food biodegradable packaging systems with enhanced functionalities, including antimicrobial and antioxidant properties. These findings pave the way for the advancement of new functional materials for food packaging applications based on compostable biopolymers, contributing to more sustainable and high-performance packaging solutions.

Appendix: Articles First Page

Article 1: Natural Macromolecules used as bioplasticizer in polymer materials for food contact applications.

Packaging Technology and Science, 2025.

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REVIEW ARTICLE

Natural Macromolecules Used as Bioplasticizer in Polymer Materials for Food Contact Applications

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Keywords: biodegradable | bioplasticizers | food contact materials | food packaging | migration

ABSTRACT

Synthetic plasticizers are essential additives widely used in polymer-based products such as food packaging, toys and medical devices. Despite their functional properties, they present significant environmental and human health risks due to their toxicity and migration behaviour. Growing awareness of these adverse effects has driven interest in the development of bio-based plasticizers derived from natural resources. In recent years, natural plasticizers have emerged as promising alternatives, offering advantages such as low toxicity, biodegradability and reduced migration. Their application is particularly relevant in the food packaging industry, where the transition towards sustainable materials has gained great interest, with the growing use of biopolymers such as starch, poly(lactic acid) (PLA), chitosan and gelatin. Most of these biopolymers exhibit limitations in barrier, mechanical and processing properties, requiring the addition of plasticizers to improve their performance. Natural plasticizers provide a synergistic solution by enhancing the properties of biopolymers while maintaining the sustainability and low toxicity of the resulting materials. Furthermore, their compatibility with biopolymers and reduced migration makes them highly suitable for industrial applications. This review explores the advancements in natural plasticizers, their integration into biopolymer systems and the challenges associated with their industrial-scale adoption for food packaging applications.

1 | Introduction

Plastics dominate as the primary material in food packaging due to their excellent protective properties, lightweight and cost-effectiveness. Their versatility, durability and capacity to act as barriers against moisture and oxygen make them essential for preserving food freshness, extending shelf life and minimizing food waste [1, 2]. However, the growing use of plastics and poor waste management practices have raised serious ecological concerns [3]. One of the key problems is the migration of plastics additives and their potential to contaminate the environment, the soil, water and air [4]. These additives can migrate not only to packaged food but also can be released during recycling process or at landfills. Among others, the plasticizers are the most common chemical substance added to the plastics and, therefore, are the most critical [5]. Plasticizers are low molecular weight, non-volatile compounds incorporated into polymers to enhance flexibility, ductility and processability [6, 7]. Their role in modifying mechanical and thermal properties has made them indispensable in applications such as food packaging [8], medical devices [9] and in other high-performance industries. As demand for

Article 2: Evaluation of poly (lactic acid) and ECOVIO® based biocomposites loaded with antimicrobial sodium phosphate microparticles.

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Evaluation of poly(lactic acid) and ECOVIO based biocomposites loaded with antimicrobial sodium phosphate microparticles

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ABSTRACT

Herein, biobased composite materials based on poly(lactic acid) (PLA) and poly(butylene adipate-co-terephthalate) (PBAT) as matrices, sodium hexametaphosphate microparticles (E452i, food additive microparticles, 1 and 5 wt%) as antimicrobial filler and acetyl tributyl citrate (ATBC, 15 wt%) as plasticizer, were developed for potential food packaging applications. Two set of composite films were obtained by melt-extrusion and compression molding, i) based on PLA matrix and ii) based on Ecovio® matrix (PLA/PBAT blend). Thermal characterization by thermogravimetric analysis and differential scanning calorimetry demonstrated that the incorporation of E452i particles improved thermal stability and crystallinity, while the mechanical test showed an increase in the Young's modulus. E452i particles also provide antimicrobial properties to the films against food-borne bacteria *Listeria innocua* and *Staphylococcus aureus*, with bacterial reduction percentages higher than 50 % in films with 5 wt% of particles. The films also preserved their disintegradability as demonstrated by an exhaustive characterization of the films under industrial composting conditions. Therefore, the results obtained in this work reveal the potential of these biocomposites as appropriated materials for antibacterial and compostable food packaging films.

1. Introduction

Nowadays, the design of more effective packaging has become essential for the advancement of food industry, due to the globalization of the food supply chain, the projected population growth, and the escalating concerns regarding sustainability. The primary objective of traditional packaging is to enhance the shelf life of packaged food while ensuring food safety. It accomplishes these functions by providing protection against physical damage, acting as a barrier against microorganisms, and preserving the sensory properties of the food [1–3]. Indeed, an effective packaging also contributes to the sustainability of the food industry as can substantially reduce food losses and waste. Approximately 33 % of global food production is estimated to be lost or wasted, resulting in millions of metric tons of CO_2 equivalent emissions [4]. Despite the numerous benefits of packaging, its extensive utilization leads to significant environmental consequences, with a huge amount of plastic waste ending up in landfills, oceans, and in the natural environment [5,6]. It is estimated that around 40 % of produced plastics is destined to packaging and the majority are non-biodegradable materials derived from fossil-fuels such as polyethylene terephthalate (PET), polystyrene (PS), polyethylene (PE) polypropylene (PP) and polyamide (PA) [7].

In recent years, there is an interest in reducing the amount of plastic used in the food industry. The increasingly directives to reduce the plastics of one use and the impact of plastic waste in the environment, have pushed to the industry to develop new biobased and biodegradable materials with the aims of replacing conventional plastics. Bioplastics are an example of these emerged materials that have aroused as a solution as they come from a renewable resource, can be biodegradable in landfill and/or compostable and require lower energy to be produced, resulting in lower amount of greenhouse gases emission than conventional plastics [7]. Several biobased and/or biodegradable polymers including poly(lactic acid) (PLA), poly(caprolactone) (PCL) and poly (butylene adipate-co-terephthalate) (PBAT) are already used in the food industry. One of the main drawbacks of bioplastics compared to conventional plastics is their relatively high cost. However, nowadays, as a

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Article 3: Plasticizing PLA with Biobased Fatty Esters: Comprehensive Study on Film Properties.

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ORIGINAL PAPER

Plasticizing PLA with Biobased Fatty Esters: Comprehensive Study on Film Properties

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Abstract
ACETEM (E472a) and CITREM (E472c) are fatty acid esters used as additives in the food industry to improve quality, stability and sensory properties of food products due to their emulsifying, stabilizing properties, and antioxidant and antimicrobial activities. Herein, we have explored their use as active plasticizers in one of the most used biobased polymers, polylactic acid (PLA). Initially, different CITREMs (LR10, SP70, RO and VEG) and ACETEM (SOFT-NSAFE), with a variety of compositions and physical states at room temperature, were characterized. The studied fatty acid esters demonstrate good thermal stability and moderate to good antioxidant properties. Subsequently, PLA films containing 10% of the tested fatty acid esters were prepared by melt extrusion and posterior compression molding. The obtained films were analyzed by different characterization techniques to evaluate their role as active plasticizers. Raman confocal microscopy showed that SOFT-NSAFE is homogeneously distributed in the PLA films, whereas CITREMs form microdomains due to their immiscibility with PLA. The incorporation of these plasticizers decreases the tensile strength, Young's modulus and glass transition temperature. However, only CITREM-LR10 is able to significantly enhance the elongation at break of PLA up to 42%, due to the elongation and orientation of the microdomains along the cracks formed during the tensile test. Additionally, their incorporation provides antioxidant properties to the PLA films, being CITREM LR10, SP70 and SOFT-NSAFE that impart higher activity. In terms of antimicrobial activity, CITREM-LR10 showed effectiveness against *S. aureus*, while SOFT-NSAFE was active against *L. innocua* bacteria. These results open the possibility to use such CITREM and ACETEM food additives as plasticizers in films for a variety of applications such as active food packaging.

Keywords Bioplasticizers · PLA · Raman Spectroscopy · Citric acid Ester · Acetic acid Ester · Antioxidant · Antimicrobial

Introduction

Biobased polyesters have attracted significant attention due to their biodegradability and biocompatibility, offering clear advantages for both consumers and the environment [1]. Among these, polylactic acid (PLA) stands out as a highly promising alternative to petrochemical polymers, primarily due to its biodegradable nature. PLA is derived from lactic acid, obtained through the fermentation of renewable raw materials, and it readily undergoes biodegradation [2, 3]. Recognized as a highly promising and extensively used biodegradable polymer, PLA finds application in food packaging [4]. Its favorable properties include excellent processability, high molecular weight, resistance to water solubility, effective barrier properties against flavors and aromas, thermal stability, and mechanical performance comparable to other polymers commonly used in the food packaging industry, such as poly(ethylene terephthalate) (PET) or polyethylene (PE) [5, 6]. Furthermore, PLA has received Generally Recognized as Safe (GRAS) status from the U.S. Food and Drug Administration (FDA) [7, 8]. Nonetheless, its widespread adoption has been hindered by significant

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Enhancing functional properties of compostable materials with biobased plasticizers for potential food packaging applications

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ABSTRACT

The demand of non-toxic and biobased plasticizers is substantially growing, particularly in biodegradable thermoplastics-based packaging applications. Herein, a derivative of citric acid (CITREM-LR10), usually used as food additive, was evaluated for the first time as plasticizer in PLA and Ecovio® biopolymers. Films containing 10 %(w/w) of CITREM-LR10 were prepared and compared with films plasticized with another biobased compound, SOFT-NSAFE, derived from acetic acid. The incorporation of both plasticizers provokes a slight reduction of the glass transition, however, only CITREM-LR10 was able to augment the elongation at break value of PLA films. A further evaluation of the films by Raman confocal microscopy showed the segregation of the CITREM-LR10 in microdomains, which could explain the enhanced elongation at break value, behaving as stress concentrators. In addition, CITREM-LR10 provides antimicrobial activity against *S. aureus* and both plasticizers give antioxidant properties, and almost negligible diffusion in food simulated solution. Composting studies showed that the plasticizers do not have effect on the disintegration rate of the films. In spite of these outstanding properties, the water vapour and oxygen barrier properties of the films worsen with its incorporation, therefore, the inclusion of fillers in the material together with the plasticizers would be necessary to improve such properties for food packaging applications.

1. Introduction

Poly(lactic acid) (PLA) stands out as a highly promising and extensively employed biodegradable polymer for food packaging applications. Its favourable properties comprise excellent processability, high molecular weight, resistance to water solubility, effective barrier properties to flavours and aromas, thermal stability, and mechanical performance comparable to that of other polymers utilized in the food packaging industry, like poly(ethylene terephthalate) (PET) or polyethylene (PE). Besides, PLA is Generally Recognized as Safe (GRAS) by FDA [1,2]. Poly(butylene adipate-co-terephthalate) (PBAT) has also emerged as another promising biodegradable polymer suitable for packaging applications. It has been widely combined with PLA to enhance its properties such as its brittleness and low flexibility. An example is Ecovio®, a commercially available compound that relies on a blend of PLA and PBAT, specifically designed for packaging applications [3].

The incorporation of plasticizers into PLA is another common practice to improve its inherent limitations [4]. Plasticizers are typically low molecular weight resins and liquids that interact with the chains of the main polymer, increasing the flexibility and mobility of the polymer chains by lowering the second order transition temperature resulting in an easily deformable mass [5,6]. Plasticizers are added to the amorphous regions of polymers, leaving the structure and size of any crystalline segments unaffected, but leading to a variation in properties. Certain characteristics, including deformation at break, toughness, or flexibility, typically show an increase with the addition of plasticizer, while modulus, tensile strength, hardness, and melt viscosity generally decrease.

The extensive range of plastic products and their diverse applications

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Appendix 2: Conferences & Publications.

Publication of the thesis results:

I. Mena-Prado, J. J. Reinosa, M. Fernández-García, J. F. Fernández, A. Muñoz-Bonilla, and A. del Campo, "Evaluation of poly (lactic acid) and ECOVIO based biocomposites loaded with antimicrobial sodium phosphate microparticles," Int. J. Biol. Macromol., vol. 253, no. October, 2023, doi: 10.1016/j.ijbiomac.2023.127488.

I.Mena-Prado, M. Fernández-García, E. Blázquez-Blázquez, A. Muñoz-Bonilla, and A. del Campo, "Plasticizing PLA with Biobased Fatty Esters: Comprehensive Study on Film Properties," J. Polym. Environ., 2024, doi: 10.1007/s10924-024-03454-8.

I. Mena-Prado, E. Navas-Órtiz, M. Fernández-García, E. Blázquez-Blázquez, S. Limbo, M. Rollini, D. M. Martins, A. Muñoz-Bonilla, and A. del Campo, "Enhancing functional properties of compostable materials with biobased plasticizers for potential food packaging applications," Int. J. Biol. Macromol., vol. 280, no. September, 2024, doi: 10.1016/j.ijbiomac.2024.135538.

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I. Mena-Prado, M. Fernández-García, D. López, S. Limbo, M. Rollini, D. M. Martins, A. Muñoz-Bonilla, and A. del Campo, "Bio-Based Citrate Plasticizers for PLA Fibers: Unlocking Antioxidant and Antimicrobial Potential", Natural Science, Submitted.

I. Mena-Prado, M. Fernández-García, E. Blázquez-Blázquez, J.P. Fernández-Blázquez, S. Limbo, M. Rollini, D. M. Martins, A. Muñoz-Bonilla, and A. del Campo, "Calendering Processing of PLA/PBAT Biodegradable and Functional Films Incorporating Natural Food Additives", Polymer Testing, Submitted.

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Conferences:

Poster – "Materiales compostables poliméricos para envasado de alimentos". Ignacio Mena-Prado, Alexandra Muñoz-Bonilla, Adolfo del Campo. Nuevos desarrollos para una economía circular (CIRMAT 2022).

Flash presentation – "Compostable polymeric materials for active food packaging". **Ignacio Mena-Prado**, Alexandra Muñoz-Bonilla, Adolfo del Campo, Julián J. Reinosa, José F. Fernández. La XVI Reunión del Grupo Especializado de Polímeros GEP de la Real Sociedad Española de Química (RSEQ) y de la Real Sociedad Española de Física (RSEF) (GEP 2022) / El XVII Simposio Latinoamericano de Polímeros (SLAP 2022) / El XV Congreso Iberoamericano de Polímeros (CIP 2022).

Oral presentation – "Compostable polymeric materials for active food packaging". **Ignacio Mena-Prado**, Alexandra Muñoz-Bonilla, Adolfo del Campo. Seminario de Jovenes Investigadores en Polímeros (SEJIPOL2022).

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Poster – "Films antimicrobianos para envasado activo de alimentos". **Ignacio Mena-Prado**, Marta Férnandez-García, Alexandra Muñoz-Bonilla, Adolfo del Campo. PhDay I CSIC.

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Oral presentation- "Characterization of PLA and Ecovio based films plasticized with different citric acid esters of mono and diglycerides (CITREMs)". **Ignacio Mena-Prado**, E. Navas-Órtiz, M. Fernández-García, E. Blázquez-Blázquez, S. Limbo, M. Rollini, D. M. Martins, A. Muñoz-Bonilla, and A. del Campo. VIII Simposio Anual en Química Avanzada (SAQA24).

Oral presentation – "Improving pla performance with biobased plasticizers: technological assessment and raman spectroscopy analysis for food packaging". **Ignacio Mena-Prado**, Marta Férnandez-García, Alexandra Muñoz-Bonilla, Adolfo del Campo. La XVII Reunión del Grupo Especializado de Polímeros GEP de la Real Sociedad Española de Química (RSEQ) y de la Real Sociedad Española de Física (RSEF) (GEP 2024).

Oral presentation – "Sustainable plasticizers from vegetable oils". **Ignacio Mena-Prado**, Elena Navas-Órtiz, Marta Fernández-García, Enrique Blázquez-Blázquez, Sara Limbo, Manuela Rollini, Daniele Maria Martins, Alexandra Muñoz-Bonilla, and Adolfo del Campo. American Chemical Society Spring (ACS SPRING 2025).

Poster – "Desarrollo y caracterización de fibras de PLA con bioplastificantes activos para aplicaciones en envasado alimentario". **Ignacio Mena Prado**, Marta Fernández-García, Daniel López, Sara Limbo, Manuela Rollini, Daniele Maria Martins, Adolfo del Campo, Alexandra Muñoz-Bonilla. PhDay II CSIC

Oral presentation – "Development of Antimicrobial and Antioxidant PLA Fibers for Food Packaging Applications". **Ignacio Mena Prado**, Marta Fernández-García, Daniel López, Sara Limbo, Manuela Rollini, Daniele Maria Martins, Adolfo del Campo, Alexandra Muñoz-Bonilla. XII Congreso Jóvenes Investigadores en Polímeros (JIP 2025).

Oral presentation – "Sustainable Packaging Films from PLA/PBAT Blends Functionalized with Natural Additives" Ignacio Mena Prado, Marta Fernández-García, Enrique Blázquez-Blázquez. Juan Pedro Fernández-Blázquez, Sara Limbo, Manuela Rollini, Daniele Maria Martins, Adolfo del Campo. Seminario de Jovenes Investigadores en Polímeros (SEJIPOL2025).