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Doctoral Tesis

RECYCLING OF BIOPOLYMERS AND THE FORMATION OF NON-INTENTIONALLY ADDED SUBSTANCES: AN APPROACH FOR FOOD CONTACT

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RECYCLING OF BIOPOLYMERS AND THE FORMATION OF NON-INTENTIONALLY ADDED SUBSTANCES: AN APPROACH FOR FOOD CONTACT

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Glossary of Terms

ALPB: Analytical linearized Poisson-Boltzmann

ANVISA: National Heath Surveillance Agency

APPI: Atmospheric pressure photoionization

ASTM: American Society for Testing and Materials

CAC: Critical aggregation concentration

CDI: Carbodiimide

CFR: Code of Federal Regulations

DCM: Dichloromethane

DoE: Design of Experiments

DRS: Deposit Return System

EC: European Commission

EF: Efficiency factor

EFSA: European Food Safety Authority

ERP: Extended Producer Responsibility

ESI: Electrospray ionization

FCMs: Food contact materials

FDA: Food and Drugs Administration

GC: Gas chromatography

GC-MS: Gas chromatography coupled with mass spectrometry

GML: Global migration limit

HS: Headspace

HS-SPME-GC-MS: Headspace solid-phase microextraction with gas chromatography coupled with mass spectrometry

HS-SPME-GC-O-MS: Headspace solid-phase microextraction with gas chromatography-olfactometry coupled with mass spectrometry

IAS: Intentionally Added Substances

IT-TOF: Ion-trap tandem time-of-flight

LC-MS: Liquid chromatography coupled with mass spectrometry

LDPE: Low-density polyethylene

LI: Liquid injection

MALDI: Matrix-assisted laser desorption/ionization

MF: Molecular formula

MFI: Melt flow index

MMI: Molar mass index

MS: Mass spectrometry

NaOH: Sodium Hydroxy

NEIMS: Neutral chemical electron impact mass spectrum

NIAS: Non-intentionally Added Substances

NIST: National Institute of Standards and Technology

PA6: Polyamide 6

PBAT: Poly (butylene adipate-co- terephthalate)

PC: Polycarbonate

PCR: Post-consumer resins

PET: Poly (ethylene terephthalate)

PLA: Poly (lactic acid)

PP: Polypropylene

PPE: Personal Protective Equipment

PU: Polyurethane

PVC: Poly (vinyl chloride)

QCEIMS: Quantum chemical electron impact mass spectrum

Q-TOF: Quadrupole tandem time-of-flight

QTOF: Quadrupole time-of-flight

RI: Retention time index

RSM: Response surfaces methodology

SIM: Selected ion monitoring

SLE: Solid-liquid extraction

SMD: Solvation model based on density

SML: Specific migration limit

SPME: Solid-phase microextraction

SPME-GC-MS: Solid-phase microextraction with gas chromatography coupled to mass spectrometry

SUPP: Single-use plastic product

UPLC-QTOF-MS^{E}: Ultra-performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer-elevated energy

VOCs: Volatile organic compounds

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Presentation

The doctoral thesis presented here is entitled **"Recycling of Biopolymers and the Formation of Non-Intentionally Added Substances: An Approach for Food Contact"**. This work was carried out at the two institutions collaborating in the academic co-tutorship agreement, which are the Federal University of São Carlos, São Carlos – Brazil, in the Department of Chemistry, where the Research Group on Recycling and Modification of Polymers (GPRMPOL), headed by Prof. Dr. Sandra A. Cruz, is located, and the University of Zaragoza – Zaragoza, Spain, in the Group of Analytical Research (GUIA), headed by Prof. Dr. Cristina Nerín De La Puerta, in the Department of Analytical Chemistry. The GUIA group is part of the Aragon Engineering Research Institute (I3A).

This thesis focuses on the evaluation of the mechanical recycling process of the biopolymer poly (lactic acid) in the formation of non-intentionally added substances (NIAS) and how each stage of the recycling process can influence the formation of these substances that present both a risk to consumer health and alterations to the material's properties. In this sense, each stage of the recycling cycle was evaluated in order to minimize the impact on the formation of NIAS, such as the study of the washing process, the formation, and determination of volatile substances with odoriferous characteristics, the efficiency of the decontamination process via evaluation of the challenge test proposed by FDA and, the determination and quantification of oligomers and the influence of the carbodiimide additive in minimizing the degradation process and migration of these compounds.

For each stage, analytical methods were used to identify the volatile and non-volatile compounds present in the samples, as well as techniques such as thermal and rheological analysis, as well as computational methods to increase the experimental and theoretical, respectively, basis for the formation and migration of NIAS. Finally, this thesis was conceived and written as a compendium of articles, resulting in six sections in which the four articles will form part of the chapters described below.

The thesis consists of six sections:

Section I provides a general introduction of the impact of bio-based polymers on the food contact packaging market, highlighting the importance and challenges of recycling these materials from renewable and/or biodegradable sources. Additionally, the section discusses changes in current lesions, besides non-target and target methods for compound identification, utilizing various analytical tools and mass spectral libraries.

Section II presents the general objective of the thesis as well as specific objectives in each experimental work.

Section III is the experimental part, which comprises four chapters. Each chapter entails a chapter-specific abstract, introduction, materials and methods, results and discussions, and conclusions. These chapters can be organized into four subsections:

Developing of a recycling methodology for biobased polymers and evaluation through physicochemical property analysis

- **Chapter 1:** Optimization of Washing Parameters to Minimize the Degradation of Poly (lactic acid) Using Design of Experiments: A contribution to the Recycling Chain

Non-target screening of volatile compounds from recycled PLA

- **Chapter 2:** Volatile Compounds and Off-odors Analysis of Recycled PLA for Packaging Applications: An Essential Factor for Ensuring Food Safety and Quality

Application of the FDA Contamination Protocol and evaluation of recycling cycle effectiveness in the decontamination process

- **Chapter 3:** Biobased Polymer Recycling: A Comprehensive Dive into the Recycling Process of PLA and Its Decontamination Efficacy

The influence of a bifunctional additive on reducing the migration of non-volatile compounds from recycled PLA

- **Chapter 4:** How Carbodiimide Modulates Oligomer Migration and Molar Mass in Recycled Poly (Lactic Acid): A Study Using UPLC-QTOF-MS^E

Section IV, V and **VI** deal with general conclusions obtained, publications and the bibliography.

Summary

The increasing demand for sustainable solutions in the packaging industry has driven the adoption of biodegradable polymers such as poly (lactic acid) (PLA). However, the efficient recycling of post-consumer PLA remains a challenge due to contamination from food residues, cleaning products, automotive substances, and improper disposal. This study addresses the optimization of the PLA recycling process, focusing on the effects of washing, the formation of recycled material for food contact applications. The influence of washing parameters on PLA degradation was evaluated using the Design of Experiments (DoE) methodology. Variables such as sodium hydroxide concentration, temperature, washing time, and surfactant concentration were analyzed. Rheological studies, based on the Cox-Merz rule, revealed that material degradation can be minimized through precise adjustment to these parameters. Specifically, surfactant concentration exhibited a less aggressive effect, even when combined with high temperatures and prolonged times. Conversely, washing time was identified as the most critical factor, particularly when paired with high temperature and sodium hydroxide concentration. The results suggest that adapting washing parameters according to the contamination level of the precursor material enables a more efficient and sustainable recycling process.

In parallel, the study examined the formation of Non-Intentionally Added Substances (NIAS) and the presence of contaminants during the recycling process, a critical aspect for meeting international food standards. PLA samples were intentionally contaminated in the laboratory, washed, and mechanically recycled under simulated industrial conditions. Using Headspace Solid-Phase Microextraction with Gas-chromatography coupled Mass spectrometry (HS-SPME-GC-MS) and olfactometric analysis (HS-SPME-GC-O-MS) methods, 34 volatiles compounds were identified, including NIAS such as benzaldehyde, benzyl alcohol, and dimethyl-1,4-dioxane-1,5-dione. Additionally, 14 odor-active compounds were detected and classified into four main groups: toasted, floral, green, and chemical. The study demonstrated a correlation between the recycling stages and NIAS formation, highlighting the importance of rigorous control to ensure compliance with safety standards. The efficiency of the recycling process in contaminant removal was also evaluated following FDA guidelines. PLA samples were contaminated with a standard cocktail composed of benzophenone, tetracosane, heptane, chloroform, and toluene. After washing and mechanical recycling steps, migration tests were

conducted using two food simulants under various time and temperature conditions. Analysis using SPME-GC-MS and direct injection (DI-GC-MS) showed a significant reduction in contaminants after the complete recycling cycle, with decreases ranging from 73% to 80% for substances such as tetracosane, heptane, and toluene. In comparison, washing or mechanical recycling alone showed lower removal efficiency. Factors such as the molecular volume of contaminants, type of simulant, temperature, and the interactions between PLA and contaminants directly influenced the results. Theoretical calculations of molecular interactions supported these observations, proving a detailed understanding of the underlying mechanisms.

Another focus of the study was mitigating molar mass loss and oligomer formation during the recycling cycle, common challenges for materials intended for direct food contact. To address these issues, carbodiimide (CDI) was added, using co-rotating twin-screw extruders. Results showed that CDI promoted an increase in PLA molar mass and reduced oligomer formation. Moisture played a key role, as carbodiimide reacts both with water molecules, preventing hydrolytic degradation, and with PLA chains, leading to increased molar mass. Oligomer migration tests were performed using three food simulants (ethanol 10%, acetic acid 3%, and ethanol 95%) and analyzed through ultra-high performance liquid chromatography coupled with mass spectrometry (UPLC-QTOF-MS^E). Sixteen types of oligomers were identified, with linear oligomers detected in all simulants and cyclic oligomers predominantly found in the ethanol 95% simulant. Theoretical calculations of electronic structure confirmed the observed interaction and migration mechanisms, offering insights into the stability and behavior of the recycled material. The addition of CDI significantly reduced the total oligomer concentration, improving the physicochemical properties of PLA and ensuring its suitability for food contact applications.

In conclusion, the study demonstrated that optimizing washing parameters, controlling contaminants and NIAS, and using additives such as carbodiimide are effective strategies for improving PLA recycling. These approaches not only ensure the quality and safety of recycled material but also align with circular economy principles, contributing to the reduction of the environmental impact associated with biodegradable plastics.

Resumen

La creciente demanda de soluciones sostenibles en la industria del envasado ha impulsado la adopción de polímeros biodegradables como el poli (ácido láctico) (PLA). No obstante, el reciclaje eficiente del PLA posconsumo sigue siendo un desafío debido a la contaminación causada por residuos alimentarios, productos de limpieza, automotrices y eliminación inadecuada. Este estudio aborda la optimización del proceso de reciclado de PLA, centrándose en los efectos del lavado, la formación de sustancias añadidas no intencionalmente (NIAS) y el uso de aditivos para garantizar la seguridad del material reciclado en aplicaciones de contacto con alimentos. Se evaluó la influencia de los parámetros de lavado en la degradación del PLA mediante la metodología de Diseño de Experimentos (DoE). Variables como la concentración de hidróxido sódico, la temperatura, el tiempo de lavado y lavado y la concentración de tensioactivos fueron analizadas. Los estudios reológicos, basados en la regla de Cox-Merz, revelaron que la degradación del material puede minimizarse mediante un ajuste preciso de estos parámetros. En particular, la concentración de tensioactivo mostró un efecto menos agresivo, incluso en combinación como el factor más crítico, especialmente cuando se combinó con altas temperaturas y concentraciones de hidróxido sódico. Los resultados sugieren que adaptar los parámetros de lavado según el nivel de contaminación del material permite un reciclado más eficiente y sostenible.

En paralelo, se examinó la formación de NIAS y la presencia de contaminantes durante el proceso de reciclado, un aspecto crucial para cumplir con las normas internacionales de seguridad alimentaria. Las muestras de PLA fueron intencionalmente contaminadas en el laboratorio, sometidas a lavado y reciclado mecánico, simulando condiciones industriales. Mediante la utilización de la microextracción en fase sólida del espacio de cabeza con espectrometría de masas acoplada a cromatografía de gases (HS-SPME-GC-MS) y análisis olfatométricos (HS-SPME-GC-O-MS), se identificaron 33 compuestos volátiles, incluidos NIAS como benzaldehído, alcohol bencílico y dimetil-1,4-dioxano-1,5-diona; Además, se detectaron 14 compuestos olorosos, clasificados en cuatro grupos principales: tostado, floral, verde y químico. El estudio mostró una correlación entre las etapas del reciclado y la formación de NIAS, subrayando la necesidad de un control riguroso para garantizar la seguridad del material reciclado. Asimismo, se evaluó la eficacia del proceso de reciclado en la eliminación de contaminantes, conforme a las

directrices de la FDA. Las muestras de PLA fueron contaminadas con un coctel de referencia compuesto por benzofenona, tetracosano, heptano, cloroformo y tolueno. Tras las etapas de lavado y reciclado mecánico, se realizaron pruebas de migración con dos simulantes alimentarios a distintas temperaturas y condiciones de tiempo. Los análisis mediante SPME-GC-MS y análisis directas (DI-GC-MS) mostraron reducciones significativas de los contaminantes después del ciclo completo de reciclado, con disminuciones del 73% al 80% para sustancias como tetracosano, heptano y tolueno. En comparación, las etapas de lavado o reciclado mecánico por separado presentaron menores eficiencias de eliminación. Factores como el volumen molecular de los contaminantes, el tipo de simulante, la temperatura y las interacciones entre el PLA y los contaminantes, influyeron en los resultados. Los cálculos teóricos de las interacciones moleculares respaldaron estas observaciones, aportando una comprensión detallada de los mecanismos implicados.

Además, se abordó la problemática de la pérdida de masa molar y la formación de oligómeros durante el reciclado, desafíos comunes en materiales reciclados destinados al contacto con alimentos. Para mitigar estos efectos, se utilizó carbodiimida (CDI), un aditivo bifuncional, como agente antihidrólisis y extensor de cadena. El reciclado mecánico se realizó en muestras de PLA húmido y seco con diferentes concentraciones de CDI, utilizando extrusora de doble tornillo co-rotatorio. Los resultados mostraron que la adición de CDI aumentó la masa molar del PLA y redujo la formación de oligómeros. La humedad desempeñó un papel clave, ya que la CDI reaccionó tanto con moléculas de agua, previniendo la degradación hidrolítica, como con las cadenas de PLA, generando un incremento en la masa molar. Las pruebas de migración de oligómeros se realizaron utilizando tres simulantes alimentarios (etanol al 10%, ácido acético al 3% y etanol al 95%) y se analizaron mediante cromatografía líquida de ultra-alta resolución acoplada a espectrometría de masas (UPLC-QTOF-MS^E). Se identificaron 16 tipos de oligómeros, predominando los oligómeros lineares en todos los simulantes y los cíclicos en el simulante de etanol al 95%. Los cálculos teóricos de estructura electrónica confirmaron las interacciones observadas, proporcionando información sobre los mecanismos de migración y estabilidad del material reciclado. La incorporación de CDI permitió una reducción significativa de la concentración total de oligómeros, mejorando las propiedades fisicoquímicas del PLA y asegurando su idoneidad para aplicaciones en contacto con alimentos.

En conclusión, el estudio demostró que la optimización de los parámetros de lavado, el control de contaminantes y NIAS, y el uso de aditivos como la CDI son estrategias eficaces para mejorar el reciclado del PLA. Estos enfoques no solo garantizan la calidad y seguridad del material reciclado, sino que también promueven practicas alineadas con los principios de la economía circular, contribuyendo a la reducción del impacto ambiental de los plásticos biodegradables.

Resumo

A crescente demanda por soluções sustentáveis na indústria de embalagens tem impulsionado a adoção de polímeros biodegradáveis, como o poli(ácido lático) (PLA). No entanto, a reciclagem eficiente do PLA pós-consumo continua sendo um desafio devido à contaminação causada por resíduos alimentares, produtos de limpeza, resíduos automotivos e descarte inadequado. Este estudo aborda a otimização do processo de reciclagem do PLA, como foco nos efeitos da lavagem, na formação de substancias adicionadas não intencionalmente (NIAS) e no uso de aditivos para garantir a segurança do material reciclado em aplicações de contato com alimentos. Avaliou-se a influência dos parâmetros de lavagem na degradação do PLA por meio da metodologia de Design de Experimentos (DoE). Variáveis como a concentração de hidróxido de sódio, a temperatura, o tempo de lavagem e a concentração de tensoativos foram analisadas. Estudos reológicos, baseados na regra de Cox-Merz, revelaram que a degradação do material pode ser minimizada mediante o ajuste preciso desses parâmetros. Em particular, a concentração de tensoativo apresentou efeito menos agressivo, mesmo em combinação com outros fatores, sendo considerada a variável mais crítica, especialmente quando associada a altas temperaturas e concentrações de hidróxido de sódio. Os resultados sugerem que adaptar os parâmetros de lavagem de acordo com o nível de contaminação do material possibilita uma reciclagem mais eficiente e sustentável.

Paralelamente, examinou-se a formação de NIAS e a presença de contaminantes durante o processo de reciclagem – um aspecto crucial para o cumprimento das normas internacionais de segurança alimentar. Amostras de PLA foram intencionalmente contaminadas em laboratório, submetidas à lavagem e à reciclagem mecânica, simulando condições industriais. Utilizando a técnica de microextração de fase solida com espaço de cabeça acoplada à cromatografia gasosa com espectrometria de massas (HS-SPME-GC-MS) e análises olfatométricas (HS-SPME-GC-O-MS) foram identificados 33 compostos voláteis, incluindo NIAS como benzaldeído, álcool benzílico e dimetil-1,4-dioxano-1,5-diona. Além disso, foram detectados 14 compostos odoríferos, classificados em quatro grupos principais: tostado, floral, verde e químico. O estudo demonstrou uma correlação entre as etapas de reciclagem e a formação de NIAS, reforçando a necessidade de controle rigoroso para garantir a segurança do material reciclado. Também foi avaliado a eficiência

do processo de reciclagem na eliminação de contaminantes, em conformidade com as diretrizes da FDA. As amostras de PLA foram contaminadas com um coquetel de referência composto por benzofenona, tetracosano, heptano, clorofórmio e tolueno. Após as etapas de lavagem e reciclagem mecânica, realizaram-se testes de migração com dois simulantes alimentícios em diferentes temperaturas e tempos de exposição. As análises por SPME-GC-MS e DI-GC-MS mostraram reduções significativas dos contaminantes após o ciclo completo de reciclagem, com diminuições de 73% a 80% para substâncias como tetracosano, heptano e tolueno. Em comparação, as etapas de lavagem ou reciclagem mecânica isoladas apresentaram menor eficiência de remoção. Fatores como o volume molecular dos contaminantes, o tipo de simulante, a temperatura e as interações entre o PLA e os contaminantes influenciaram os resultados. Cálculos teóricos de interações moleculares sustentaram essas observações, fornecendo uma compreensão detalhada dos mecanismos envolvidos.

Além disso, abordou-se a questão da perda de massa molar e da formação de oligômeros durante a reciclagem, desafios comuns em materiais reciclados destinados ao contato com alimentos. Para mitigar esses efeitos, utilizou-se a carbodiimida (CDI), um aditivo bifuncional, como agente anti-hidrólise e extensor de cadeia. A reciclagem mecânica foi realizada em amostras de PLA úmido e seco com diferentes concentrações de CDI, utilizando extrusora dupla rosca co-rotacional. Os resultados mostraram que a adição de CDI aumentou a massa molar do PLA e reduziu a formação de oligômeros. A umidade desempenhou papel essencial, pois a CDI reagiu tanto com moléculas de água – prevenindo a degradação por hidrolise – quanto com as cadeias de PLA, promovendo o aumento da massa molar. Testes de migração de oligômeros foram realizados com três simulantes alimentares (etanol 10%, ácido acético 3% e etanol 95%) e analisados por cromatografia liquida de ultra-alta eficiência acoplada à espectrometria de massas (UPLC-QTOF-MSE). Foram identificados 16 tipos de oligômeros, predominando os oligômeros lineares em todos os simulantes e os cíclicos no simulante de etanol a 95%. Cálculos teóricos de estrutura eletrônica confirmaram as interações observadas, fornecendo informações sobre os mecanismos de migração e estabilidade do material reciclado. A incorporação de CDI permitiu uma redução significativa da concentração total de oligômeros, melhorando as propriedades físico-químicas do PLA e assegurando sua adequação para aplicações em contato com alimentos. Em conclusão, o estudo demonstrou que a otimização dos parâmetros de lavagem, o controle de contaminantes e

NIAS, bem como o suo de aditivos como a CDI, são estratégias eficazes para melhorar a reciclagem do PLA. Esses enfoques não apenas garantem a qualidade e a segurança do material reciclado, mas também promovem práticas alinhadas com os princípios da economia circular, contribuindo para a redução do impacto ambiental dos plásticos biodegradáveis.

Section I: Introduction

Introduction

1. Plastic Production and Pollution

As new markets develop and search for high-performance and low-cost materials, polymers have increased their presence in the manufacturing industry and the consumer's life. Its applicability extends to various market segments, including the automotive sector, textiles, and packaging for food contact (Alam et al., 2018; Associação Brasileira da Indústria do Plástico, 2018). Associated with the exponential growth in the consumption of polymers in the generation of waste, although plastic has slowly conquered its space as a material of great importance to society, its disposal is still a worldwide problem. Moreover, its low degradability and the high volume of its waste occupy vast spaces for a very long time, which directly impacts the useful life of landfills, especially the environment (Alam et al., 2018; Shams et al., 2021).

According to the published data by the Credit Suisse Research Institute, in the research entitled Plastic Pollution: Pathways to Net Zero, plastic consumption has reached 450 million tons per year worldwide, of which 350 million tons become waste and residue**,** 46% of which is sent to landfills and 22% is improperly managed (Purcell, 2023). These numbers give us the idea that 70% of the plastic waste generated annually in the world is disposed of inappropriately, ending up in open waste dumps, rivers, seas, and oceans. In addition, according to the Global Plastics Outlook Database, 40% of this waste comes from the plastic packaging sector (Purcell, 2023).

In this sense, it is estimated that 90% of the plastic resins used in the production of packaging come from non-renewable sources such as oil refining, making it clear that this industry is extremely dependent on a finite and non-renewable source of raw materials, as well as being harmful to the environment (Purcell, 2023). In addition, the pandemic scenario caused by the COVID-19 pandemic has increased the use of single-use plastic materials and consequently exacerbated the problem to the environment and marine life (Sarkodie and Owusu, 2021).

As reported in the literature, during the period of COVID-19, there has been a significant increase in the use of plastics to produce single-use materials such as masks, gloves, hospital products, and personal protective equipment (PPE) (Leal Filho et al.,

2021). In addition, another sector that increased its consumption of packaging during this period was online shopping, which included takeaway services and food delivery (Ganguly and Chakraborty, 2024). Numerically, according to the World Health Organization, PPE production increased by 40% during COVID-19 pandemic, resulting in the generation of 129 billion masks and 65 million gloves (assuming each human uses one disposable mask per day) (Parashar and Hait, 2021). In addition, online shopping, take-away services, and food delivery have become essential during the pandemic period, causing a 5.5% increase in the consumption of single-use materials (Parashar and Hait, 2021).

As described by Ganguly *et al*. in their work on the management of plastic during and after the Covid-19 pandemic, there has been an undeniable increase in the use of thermoplastic resins from non-renewable sources in the exponential production of PPEs and single-use materials, since these thermoplastic resins have extremely important physical and chemical characteristics for the manufacture of plastic materials (Ganguly and Chakraborty, 2024). The resins that have seen an exponential increase in production are (i) Low-density polyethylene (LDPE), flexible and resistant to moisture used in the production of gloves (ii) Polyurethane (PU) is a durable and flexible resin with a high ability to protective barrier, (iii) Polycarbonate (PC), with a high impact resistance and high temperatures, (iv) Polypropylene (PP), resin is lightweight with good chemical resistance and is suitable for making items like masks and single-use materials to food contact, and (v) Poly (vinyl chloride) (PVC) is often used in medical applications with good chemical resistance and durability (Ganguly and Chakraborty, 2024).

The use of plastic in various products, including PPEs and other disposable materials, has played a key role in protecting health workers and minimizing contact between people during the COVID-19 pandemic. However, during and after this period, concerns have been raised about the exponential increase in single-use plastics and their improper disposal has become an eminent risk not only to public health but also to the environment, accumulating on seashores, lakes, sewers, landfills, and other public and private sites without public supervision (Ganguly and Chakraborty, 2024; Parashar and Hait, 2021).

In addition, plastic waste is said to be persistent and irreversible in environmental conditions and can progressively fragment into smaller particles known as macro, meso,

and microplastics, as described by Li *et al*. in their study on global plastic recycling during and after COVID-19, influencing increased environmental deterioration, risk to human health, marine, and wildlife (Li et al., 2023). Figure I-1 summarizes the production and improper disposal of plastic materials in the oceans and the direct and potential risk of human exposure to post-consumer waste during the pandemic period in 2020.

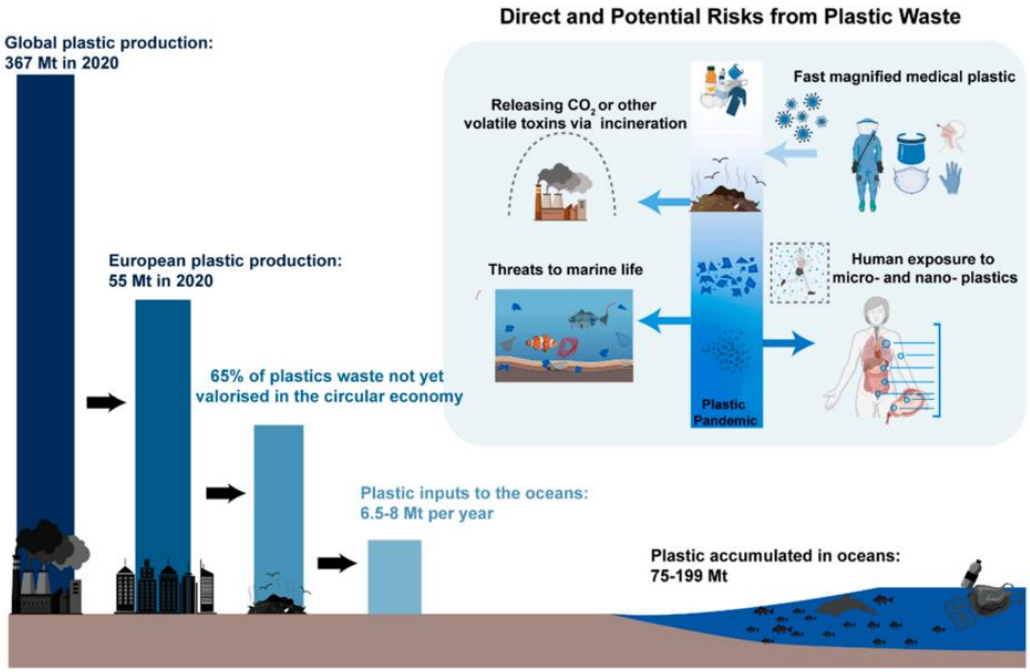


Figure I-1: Production and improper disposal of plastic materials in the oceans and the direct and potential risk of human exposure to post-consumer waste during the pandemic period in 2020 (https://plasticseurope.org/knowledge-hub/the-circular-economy-for-plastics-a-european-overview-2/, accessed on 12th September 2024).

In this sense, new government policies and interventions have been implemented to tackle the worsening scenario of environmental pollution caused by the improper disposal of post-consumer plastic materials.

2. Environmental protection measures for plastic pollution

To minimize the environmental problems caused by improper disposal of plastic products, many countries have adopted laws and actions via government, private businesses, charitable organizations, and interest groups in response to plastic pollution.

In this way, a range of regulations and policies to combat environmental degradation have been created, and often, the policies of one country are not adopted by another, causing a gap in the integration of policy information.

A recent study by the Global Plastics Policy Centre entitled "A global review of plastic policies to support improved decision making and public accountability" evaluated 100 policies implemented by governments, businesses, and civil society worldwide to tackle plastic pollution (March et al., 2022). In this study, which evaluated the policies implemented from 2018 to 2022, March and co-workers observed that most national policies have been implemented in a fragmented way, focusing on individual items or groups of plastic items such as bags, straws, and cups (March et al., 2022). In addition, this new analytical framework methodology found that there were two distinct areas in the policy landscape: 1) those aimed at plastic consumption (such as taxes and bans) and 2) those aimed at end-of-life (such as recycling) (March et al., 2022).

As mentioned earlier, regulations at the national level fluctuate in both their scope and focus, and, as such, it becomes difficult to apply prohibitive measures and taxes in low- and middle-income countries. As such, recycling regulations tend to exist as part of strategic interventions. In light of the study reviewed and conducted by the Global Plastic Policy Centre (March et al., 2022). A summary has been drawn up for each type of policy presented:

- **Bans on plastic bags**: Bans on single-use plastic bags restrictions on their importation, distribution, sale, or use. These bans primarily target shopping bags and have been implemented since the early 2000s. Currently, 127 countries have enacted some form of legislation regulating plastic bags. The scope of these regulations, however, varies significantly between countries. While some nations have imposed complete bans on non-compostable plastic bags, others have introduced partial restrictions based on bag biodegradability.
- **Bans on single-use plastic products:** Single-use plastic product (SUPP) bans target a range of plastic items designed for one-time use and disposal, such as disposable utensils, plastic bags, and commercial packaging. These bans may extend to the manufacture, import, supply, commercial distribution, and use of such items, either in full or in specific areas. Additionally, the bans can be applied to particular SUPP sectors, such as food packaging or polystyrene containers.

- **Taxes on plastic bags:** Many countries have introduced taxes on plastic as an economic tool to influence behavior by imposing charges or fees. These taxes can target manufacturers, importers, distributors, retailers, or even consumers. While initially focused on plastic bags, these taxes are now being extended to a broad range of plastic products.
- **Producer accountability:** Producer responsibility includes Extended Producer Responsibility (EPR) and Deposit Return System (DRS), both requiring companies to act and contribute financially. A key aspect of EPR policies is that the responsibility for a product's environmental impact at the end of its life lies with the original producer and seller, who must provide and cover the costs of collection waste, enhance recyclability and reusability, reduce raw materials consumption, and decrease product size. The costs of these developments are usually passed onto the consumer.
- **Recycling regulations:** Recycling policies are typically part of broader waste management strategies, and for a long time, no distinction was made between chemical and mechanical recycling. These policies focus on the selective collection and reprocessing of plastic, often with targets as a percentage of plastic packaging to be recycled. A major challenge for the recycling sector is establishing effective systems for hard-to-recycled plastics, as most recycling centers are focused on PET bottles. Furthermore, successful recycling policies are often paired with Extended Producer Responsibility (EPR), where a central non-profit organization handles plastic waste collection, sorting, and recycling. These policies can be highly effective when combined with deposit return schemes, although they only apply to PET bottles.

While these approaches offer benefits, they also involve certain risks and costs. However, new strategies explored and implemented under the 2030 Agenda demonstrate that using biodegradable polymers and mechanical recycling can positively impact environmental preservation and reduce greenhouse gas emissions.

As mentioned above, since the launch of the 2030 Agenda, many governments have implemented measures and strategies to mitigate the environmental impact of improper plastic packaging disposal. In this context, the European Commission and Brazil's National Commission for plastic packaging production emphasize recycled plastic materials and biodegradable or compostable raw materials (Gobierno de España, 2018; Lima and Ghani, 2020; Secretaria-Geral do Governo Federal, 2024). In alignment with the Sustainable Development Goals (SDG) outlined in the UN's 2030

Agenda, political leaders anticipate positive environmental, economic, and social impacts.

3. Bio-based polymers and recycled materials for food contact

3.1 Biopolymers and bio-based polymers for food packaging materials

Biopolymers and bio-based polymers are derived from renewable resources such as corn, cellulose, and sugarcane. These naturally sourced polymers are often classified as biodegradable and/or compostable materials. According to the ASTM D6400-19 standard, a polymer must achieve a conversion rate of at least 90% of its initial biomass carbon into carbon dioxide (CO_2) under controlled conditions within 180 days to be classified as biodegradable (American society for testing and materials, 2019).

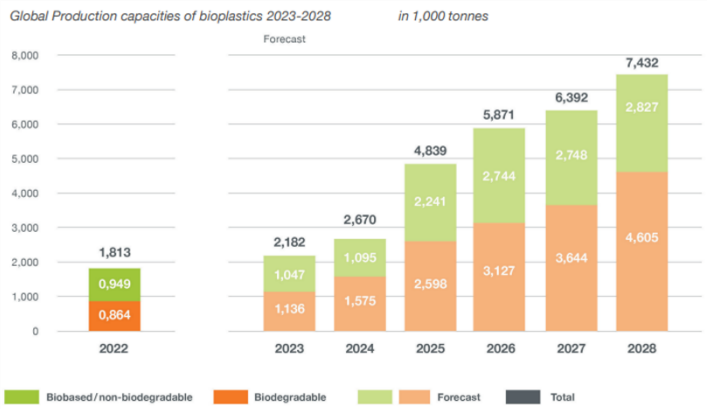
Biodegradable polymers are emerging as a viable alternative for partially replacing packaging made from non-renewable synthetic sources. The aim is to mitigate the environmental liabilities caused by these materials and contribute to a more effective circular economy since their biodegradation results in the conversion into simpler, mineralized compounds that can be redistributed through elementary cycles, such as carbon, nitrogen, and sulfur (Stoica et al., 2020). In this sense, Poly (lactic acid), better known as PLA, is one of the aliphatic polyesters that has seen the greatest market growth in recent years due to its good properties, such as processability, in specific conditions of biodegradability, and biocompatibility, thus presenting great potential in replacing petroleum-based raw materials for packaging applications (Moshood et al., 2022).

The use of bioplastic-based packaging offers significant advantages in terms of environmental impact throughout the product's life cycle, as highlighted in some studies (European Bioplastics, 2018; Lima et al., 2021). PLA is particularly notable for packaging production due to its processability, which is like conventional thermoplastics like poly (ethylene terephthalate) (PET) and polypropylene (PP). Additionally, it has a lower energy consumption, using up to 55% less energy compared to packaging made from petroleum-based polymers (Dopico-García et al., 2013; Franchetti and Marconato, 2006a; Kabir et al., 2020).

A study by the European Bioplastics Association described the increase in global production of bioplastics, both non-biodegradable and biodegradable bio-based, reaching a production of 2.18 million tons in 2023 and estimated to grow to 7.43 million tons by 2028 (European Bioplastics, 2023). This growth, as reported by the association, comes from increased demand to produce plastic materials and the environmental appeal associated with new regulations for reducing environmental impact.

The packaging sector is one of the largest consumers of bioplastics and has seen a significant increase in its usage for material production, accounting for 43% of total bioplastic consumption. Figure I-2 shows the (a) global production capacity of bioplastics with an estimate of growth up to 2028 and, the (b) fastest-growing segments for the production of bioplastics-based materials (European Bioplastics, 2023). The global production of bioplastics has been steadily increasing, gaining significant attention and focus, with biodegradable bioplastics expected to see continued growth. Among these, PLA stands out as the biopolymer with the highest projected growth compared to other bio-based polymers (European Bioplastics, 2023).

a)



b)

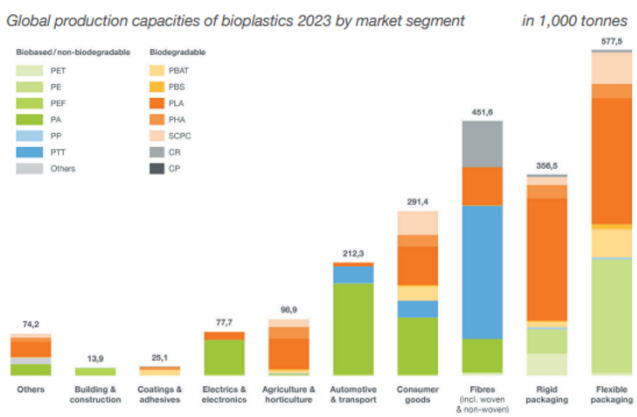


Figure I-2: (a) Global production capacities of bioplastics and (b) global production capacities of bioplastics by market segment. (https://docs.european-bioplastics.org/publications/market_data/2023/EUBP_Market_Data_Report_2023.pdf, accessed on 14th October 2024).

3.2 Recycling materials to produce food contact packaging

As reported in the literature, some types of recycling processes are described in four categories: primary, secondary, tertiary, and quaternary. Each recycling process is important for the processing industry, especially for maintaining and establishing a circular plastic system (Clark and Shaver, 2024).

Primary and secondary recycling are the most straightforward processes for reusing plastic materials. Both involve mechanical recycling, which employs specific temperatures and shear rates, ultimately producing a material ready for reshaping or pre-shaping. The distinction between these two types of recycling lies in the origin of the material. In primary recycling, the material consists of pre- or post-industrial waste directly fed into the production line. This material is generally named as "scraps" and it does not have the consideration of recycled material. It doesn't require any legal authorization. In contrast, secondary recycling involves materials that have completed their use or consumption cycle, having been manufactured, utilized for product storage, and subsequently discarded by the consumers (Clark and Shaver, 2024; Cruz et al., 2011). It is usually identified as "postconsumer material".

Tertiary recycling, also known as chemical recycling, involves converting waste into valuable raw materials through depolymerization, resulting in monomers and fuels that can be reintegrated into the production cycle. Furthermore, within tertiary recycling, a process that is currently being explored is enzymatic recycling. This process is a part of biocatalytic recycling processes and is a more environmentally friendly alternative to conventional processes. In addition, these processes have offered definitive solutions to the problem of plastic waste (Clark and Shaver, 2024).

Quaternary recycling focuses on energy recovery through processes like pyrolysis and controlled combustion of plastic waste (Clark and Shaver, 2024). Figure I-3 provides a clear summary of the significance of various recycling technologies applied at different stages of material production within a circular economy. These technologies generate diverse inputs that can address various industrial needs, as described by Clark and Shaves (Clark and Shaver, 2024).

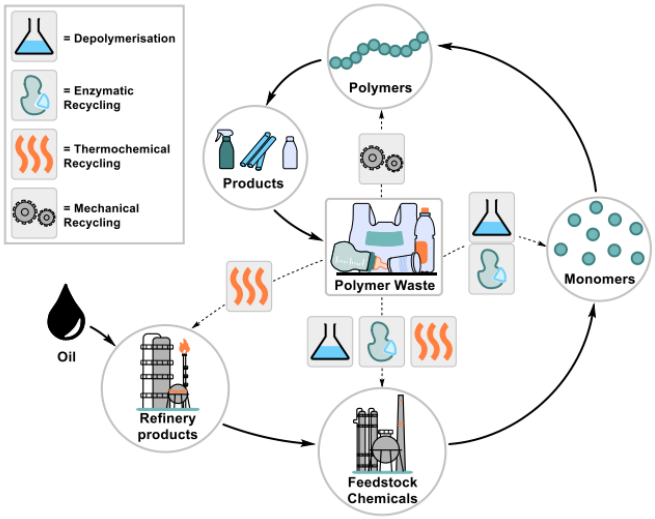


Figure I-3: Different recycling technologies offer different entry points into a plastics circular economy (Clark and Shaver, 2024).

As mentioned earlier, different recycling technologies for plastic materials play a crucial role in reintegrating waste into the consumption chain. Among these, mechanical recycling stands out as one of the most used methods to reduce the environmental impact of post-consumer plastic waste. PET, widely used in the production of plastic bottles for liquid storage, was one of the first polymers to utilize this recycling technology, giving rise to the post-consumer resins (PCR) class (Triantafyllou et al., 2002).

PCR resins have been a milestone in the recycling industry, increasing investments in technologies, generating capital, and adding value to recycled products, which have been incorporated into various industrial sectors. However, only PET PCR is considered as food grade for the production of materials intended for direct food contact (FCMs). This is due to its recycling and decontamination process, which, as reported in the literature, ensures its suitability for such applications (Associação Brasileira da Indústria do Plástico, 2018; Cruz et al., 2011). Although PET PCR is currently the only recycled material approved by regulatory agencies for being used in food-contact packaging, numerous studies are investigating the potential of recycling other thermoplastic resins, derived from both non-renewable and renewable sources, for packaging production (Research, 2020; Sadler, 1995; Thiounn and Smith, 2020; Triantafyllou et al., 2002). In addition, other recycling and decontamination systems are being studied to obtain recycled material free and/or with concentrations of contaminants and impurities that meet the standards and ensure the quality of the packaging and food

safety, prioritizing the health of the consumers (Aymerich et al., 2008; Palkopoulou et al., 2016).

3.3 Challenges and technological advances in the recycling of bio-based materials for FCMs

As described earlier, the adoption of bio-based polymers in packaging has grown significantly due to global efforts to reduce environmental impact, as they are from renewable sources, biodegradable, and offer processing advantages comparable to petroleum-based plastics (Stoica et al., 2020). Bio-based packaging materials are classified into three generations: first-generation includes synthetic polymers; second-generation blends LDPE with up to 75% gelatinized starch and hydrophilic copolymers for improved compostability. The third generation is made of 100% bio-based polymer and classified according to its processing, properties, and applications as can be seen in Figure I-4 (Asgher et al., 2020; Donkor et al., 2023).

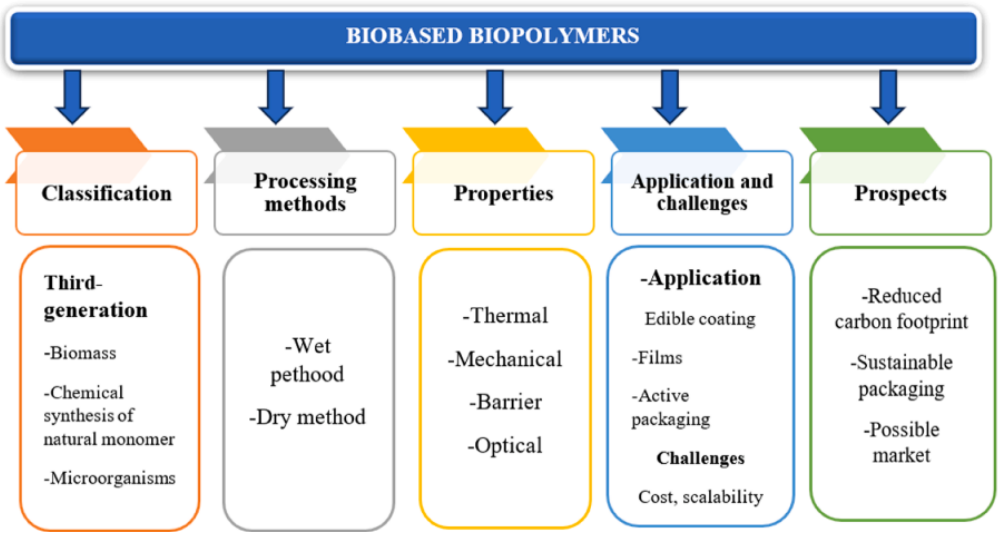


Figure I-4: Schematic representation of the scope of the overview presented (Donkor et al., 2023).

The recycling of bio-based polymers is a less explored topic, as their biodegradability and compostability are key features that contribute to reducing environmental impact. However, challenges such as high production costs, limited manufacturing facilities, loss of value in post-consumer materials, and improper disposal

highlight the importance of recycling as a strategy to reintegrate these materials into the production and consumption cycle. At first glance, the recycling of bio-based polymers may seem antagonistic due to their properties described above. However, some studies have explored the recyclability of these materials as a way of minimizing environmental impacts, developing raw materials, and applying them to the production cycle (Nathalia S.Q.S. Amorin et al., 2014; Badia et al., 2012; La Mantia et al., 2002; Scaffaro et al., 2011).

Amorin *et al.* studied the effects of subsequent extrusion cycles on PLA's thermodegradation and thermostabilizing, simulating mechanical recycling under different temperature profiles. They assessed the efficiency of thermal stabilizers, including primary and secondary antioxidant additives, and found that PLA undergoes significant degradation during reprocessing. The compatibility between additives and the polymer improved thermostability, although high temperatures and shear rates adversely affected the material's properties (Nathalia S.Q.S. Amorin et al., 2014).

Badia *et al.* studied the effects of multiple reprocessing cycles on PLA's thermal properties and thermostability. Their findings showed that repeated reprocessing, simulating industrial recycling, significantly altered the material's structure and morphology, leading to notable changes in its thermal and mechanical properties (Badia et al., 2012).

A study by Gonçalves *et al.* demonstrated that PLA recyclability is a viable alternative. The researchers subjected the material to five extrusion cycles under two conditions: dry and undried. They analyzed parameters such as molar mass, acidity, thermal properties, and color. The results indicated that PLA experienced a loss of molar mass and developed an intense yellowish color during recycling, strongly suggesting a degradative process caused by hydrolysis for intra and end-chain, as outlined in the intra and end-chain degradation mechanism in Figure I-5. Nevertheless, changes in the material's mechanical properties were less pronounced, confirming that this bio-based polymer can endure multiple recycling processes (Garcia Gonçalves et al., 2020).

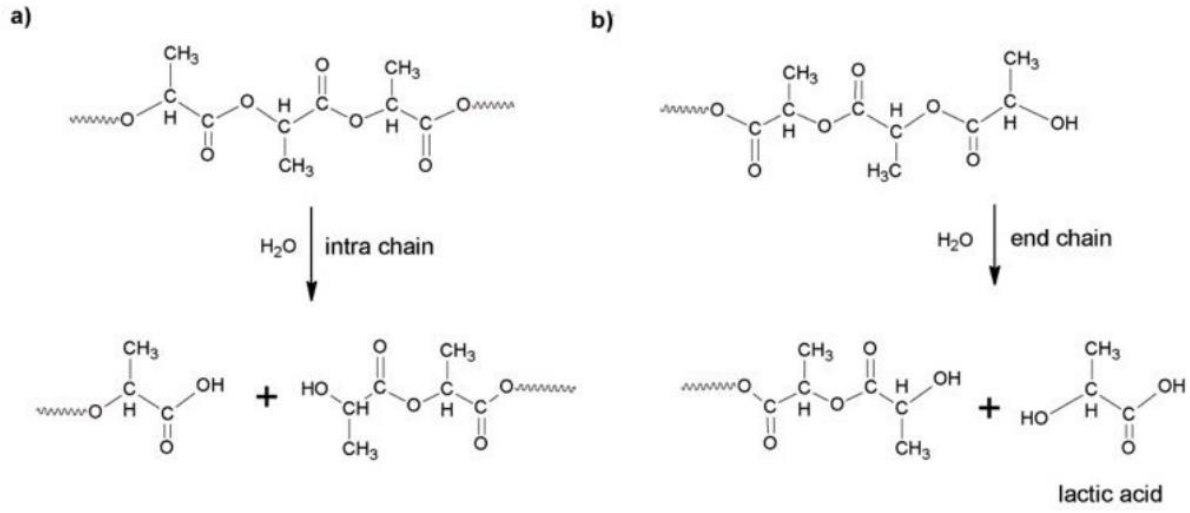


Figure I-5: Schematic representation of PLA hydrolysis in acidic conditions a) intra-chain cleavage and b) end-chain cleavage (Garcia Gonçalves et al., 2020).

As highlighted in the studies before, multiple recycling cycles can significantly affect PLA's mechanical and thermal properties. To address these changes, the incorporation of property-modifying compounds, commonly known as chemical additives, offers a promising approach to enhance the material's properties after recycling. Scaffaro *et al*. investigated the effects of incorporating small impact-modifying additives during multiple PLA reprocessing cycles. They found that these additives positively influenced the material's properties, although multiple recycling still caused significant changes in the resin's thermal and mechanical characteristics (Scaffaro et al., 2011).

Additionally, research has also focused on the recyclability of other bio-based and/or biodegradable polymers, such as cellulose and PBAT (Coltro et al., 2021; Farias et al., 2024; Moreira et al., 2024), as well as the development of biodegradable and recycled polymer blends and composites for the packaging sector (Dharini et al., 2022; Farias et al., 2024; Freitas et al., 2017; Melcova et al., 2019). These advancements make the field increasingly promising, with significant social, economic, and environmental benefits.

However, a significant challenge in reprocessing bio-based polymer is thermal degradation, shear stress, and parallel reactions like hydrolysis, which alter the material's structure, affect its properties, and lead to the formation of degradation compounds. To address these issues, the use of additives – already widely applied in the processing of

non-renewable materials – plays a crucial role in improving the processing and recycling of bio-based materials.

3.4 Use of additives in the remediation of degradative processes during recycling

The use of additives in polymer recycling, including biodegradable polymers, has proven to be an effective approach to mitigate degradative processes such as thermal degradation, shear stress, and parallel reactions like hydrolysis. Additives such as thermal stabilizers, antioxidants, and impact modifiers play a crucial role in preserving material structure, enhancing properties, and reducing the formation of degradation compounds during reprocessing cycles (Lee et al., 2018; Melcova et al., 2019; Sanches-Silva et al., 2014; Silva Freitas et al., 2020). Consequently, their application not only improves the technical feasibility of recycling but also promotes sustainability by extending the lifespan of bio-based materials.

For instance, additives like Irganox and Irgafos provide antioxidant properties, while phthalates are commonly used as plasticizers, among others, to enhance the performance and functionality of materials (Hahladakis et al., 2018). However, food safety regulatory agencies warn about the excessive use of additives in plastic packaging due to their toxicological potential when they migrate into food (Assessment et al., 2014; Hahladakis et al., 2018). In this context, the use of bifunctional additives has gained considerable attention in the packaging industry, as they reduce the need for multiple additives while providing the same range of functionalities in a single solution. Among these, carbodiimides have gained prominence for their versatility and potential applications.

Carbodiimides (CDI) are characterized by their imide functional group (N=C=N), which exhibits bifunctionality. They can react with water in the system, serving as anti-hydrolysis agents and with carboxylic and end groups of polymer chains, facilitating chain extension. Thus, the dual action of CDI mitigates degradation processes, minimizing polymer chain fragmentation and reducing the formation of oligomers and monomers (Khorana, 1953; Williams and Ibrahim, 1981).

Freitas *et al.* studied the effect of CDI on enhancing the molar mass of recycled PET. Their findings demonstrated that CDI acts as an effective chain extender by reacting with terminal groups, enabling PET to regain its original properties and making it suitable for reuse in packaging applications (cradle-to-cradle) (Freitas et al., 2021). Another study by Kunishima *et al*. investigated the incorporation of a commercial carbodiimide called Stabaxol into glass fiber-reinforced polyamide 6,6 (PA 66). The study revealed an increase in the functional groups in CDI, such as -N=C=N-, as highlighted in Figure I-6, which are highly reactive and can form bonds with carboxylic and amide groups, contributing to the improved properties (Kunishima et al., 2020).

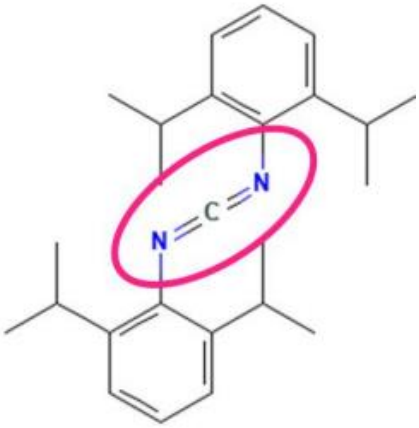


Figure I-6: Chemical structure of the Stabaxol (commercial CDI) (Kunishima et al., 2020).

The properties of CDIs have also been explored in bio-based polymers and their blends, as demonstrated by Khankrua *et al*., who studied the use of polycarbodiimide as a chain extender in PLA/PA6 blends (Khankrua et al., 2014). Their research examined the impact of CDI on the material's thermal and mechanical properties, underscoring the significance of this class of bifunctional organic additives for reprocessing and the production of biodegradable and compostable packaging.

A study by Baccarin on recycling post-industrial polyamide 6 (PA6) fabric demonstrated that adding CDI during reprocessing increased the molar mass by 10% for dry samples and 18% for wet samples. This highlights the effectiveness of carbodiimide as a bifunctional additive. Additionally, Baccarin proposed a reaction mechanism between

recycled PA6 and CDI, acting as a chain extender, as illustrated in Figure I-7 (Solferini Baccarin, 2023).

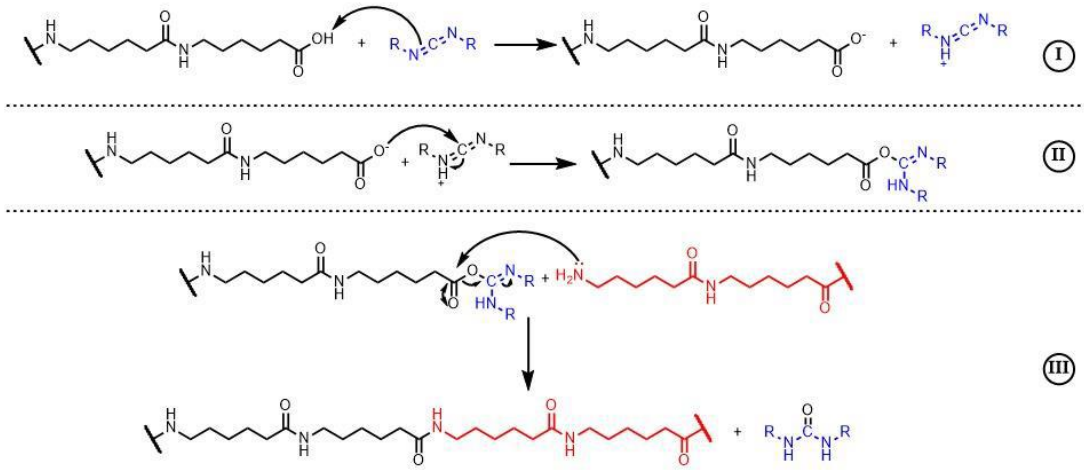


Figure I-7: Proposed chain extender mechanism between CDI and PA6 (Solferini Baccarin, 2023).

In the mechanism presented in Figure I-7 the chain extension reaction may occur in three stages, as illustrated in Figure 7. In the first stage, CDI abstracts a hydrogen atom from the carboxylic group at the end of the PA6 chain, deprotonating it and imparting a nucleophile that reacts with the carbon atom of the CDI's imide group, forming a new bond that becomes more reactive. In the second stage, the O^- of the deprotonated carboxylic acid attacks of the -N=C=N- group, forming a reactive intermediate. Finally, in the third stage, this bond is broken by a reaction with an amine chain end from another PA6 molecule, resulting in an extended polymer chain, depicted in the final stage (Solferini Baccarin, 2023).

In conclusion, the work highlights the anti-hydrolysis and chain-extending potential of CDIs, positioning them as a promising organic additive for the recycling industry. This is particularly relevant for bio-based polymers, as many of these materials possess reactive end groups that can form new bonds, enhancing their recyclability and overall material properties.

3.5 Potential contaminants in recycled plastics

As described earlier, additives are commonly used to enhance or confer specific properties to the final materials, such as antioxidant protection and UV stabilization, among others. These compounds are classified as intentionally added substances (IAS). However, there is another class of compounds described as non-intentionally added substances (NIAS), and both NIAS and IAS can be found in virgin and post-consumer recycled packaging (Birgit, 2018; Eaves and Consultant Regulatory, 2024; Nerín and Wrona, 2018).

As described in the literature, substances that have been added non-intentionally (NAIS), such as unintentionally added oligomers, incompletely reacted monomers, degradation of the additives, and polymer chain and process impurities, are classified as NIAS (Misko and Approaches, 2019). Many studies on NIAS have been conducted to identify and catalog these substances, both to assess their toxicological risks and to trace their origins (Portesi et al., 2019; Vera et al., 2022, 2018a; Wrona and Nerín, 2020; Wrona and Nerin, 2019). The goal is to minimize the migration of these compounds from food contact materials (FCMs) into food, where they could be ingested by consumers.

In January 2018, the Food Packaging Forum published a dossier on NIAS (Birgit, 2018). In this document, Geueke *et al.* define NIAS and categorize them based on their origin and classification, integrating not only the definition of NIAS but also current legislation and methods of identification of this new class of compounds. Nerin and Wrona highlight the importance of classifying NIAS based on their origin, whether they stem from degradation products, impurities, or other sources, as illustrated in Figure I-8 (Nerín and Wrona, 2018).

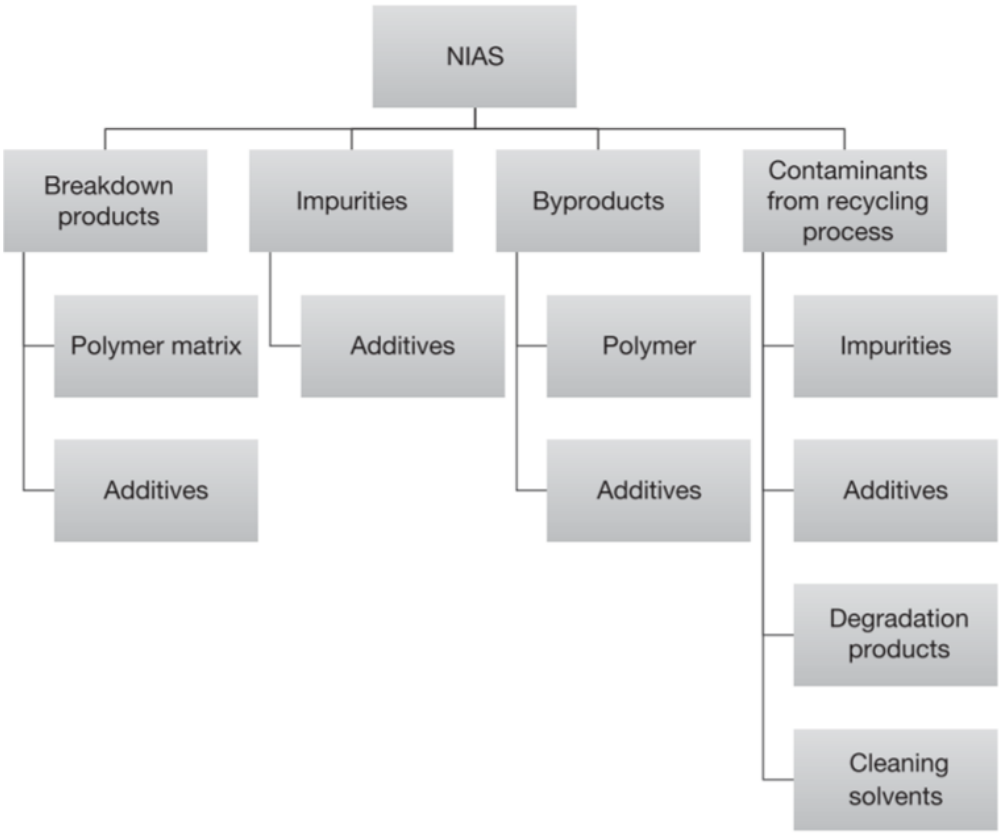


Figure I-8: Flowchart of categories and sources of NIAS (Nerín and Wrona, 2018).

For recycled food contact materials, it is crucial to monitor and assess the purity of the recycled raw materials used in producing packaging derived from post-consumer FCMs. These materials are often used or discarded, which increases the risk of contamination and degradation (Castle, 1994; Horodytska et al., 2020; Komolprasert and Bailey, 2008). Another aspect to consider regarding post-consumer FCMs is their potential reuse for storing cleaning products, automotive care products, or pesticides. This increases the risk of contamination and affects the material's purity for subsequent reintegration into the recycled production cycle (Song et al., 2019; Vera et al., 2019, 2018b). Therefore, the recycling process begins with the separation of these materials and their subsequent decontamination using processes approved by regulatory agencies, aiming to reduce the potential for contamination and degradation (Cef, 2014; Oldring et al., 2023).

3.6 Legislation and guidelines for food packaging recycled materials

As mentioned above, regulations for FCMs are crucial to ensuring consumer safety and enabling effective monitoring of their production. Consequently, different regulations are applied worldwide, varying by continent, country, economy, and region. In Europe, the overarching regulation (EC) No 1935/2004, in Section I, establishes the general principles for all materials intended for food contact (Commission Regulation (EU) No 10/2011, 2011). Commission Regulation (EU) No 10/2011, now consolidated with 16 amendments, specifically addresses plastic FCMs. In the United States, food contact materials are regulated alongside food within the Code of Federal Regulations (CFR), where additives in plastic FCMs are indirectly classified as food additives (Commission Regulation (EU) No 10/2011, 2011).

In Brazil, Article 8 of Law 9782/99 assigns the National Health Surveillance Agency (ANVISA) responsible for regulating, controlling, and inspecting products and services that pose a potential risk to public health (Câmara dos Deputados, 1999). This includes food packaging and the facilities involved in its production. Packaging regulations cover materials that come into direct contact with food and are intended to contain them throughout their production and delivery to the consumer. In addition, packaging regulations are harmonized within Mercosur, and therefore, any change to these regulations requires discussion and consensus within that framework. For packaging regulations, Mercosur uses as reference regulations on packaging and food contact materials from the European Community, the US Food and Drugs Administration, and the German Institute for Risk Assessment (BfR) (Beneventi et al., 2020).

According to RDC 843/2024 and IN 281/2024, packaging in general is exempt from the requirement to register with ANVISA *(Agência Nacional de Vigilância Sanitária -ANVISA, 2024)*. Companies are only required to submit a notice of commencement of manufacture to the health surveillance agency in their region, following the established procedures in the RDC. However, this does not exempt them from adhering to the applicable technical regulations. It is crucial to highlight that resins and precursor materials, or packaging made from post-consumer recycled PET intended for food contact, must be reported to ANVISA before marketing.

ANVISA clarifies in item 9 of Resolution 105/99, Ordinance SVS/MS 987/1998, and RDC 20/08 that only food-grade recycled PET (PET-PCR) is permitted to produce

packaging intended for direct contact with food (Ministério da Saúde -MS, 1999). Any other type of resin that has not undergone the required decontamination process is prohibited. However, these recycled resins may be used for the outer layer of the packaging. At the same time, the inner lust consists of virgin material, designed in a multi-layer format to minimize the risk of contaminant migration.

The FDA initially published the *"Points to Consider for the Use of Recycled Plastics in Food Packaging: Chemistry Considerations guideline* in 1992, focusing on the use of recycled plastics in food contact applications. This guideline recommends a "challenge test" to evaluate the decontamination efficiency of recycling processes, employing artificial contaminants to assess risks through a probabilistic approach. Updated in 2006 as *Guidance for Industry: Use of Recycled Plastics in Food Packaging (Chemistry Considerations)* and again in 2021, this guidance, though not legally binding, provides a framework for FDA evaluations of recycling processes (U.S. Department of Health and Human Services Food and Drug Administration, 2021). If a process meets the criteria for input evaluation, decontamination efficiency, and suitability for intended use, the FDA may issue a "no objection letter" to confirm compliance.

The FDA guidance advises cooperatives and recycling companies to employ materials with diverse chemical and physical properties to simulate potential consumer misuse effectively. Specifically, it suggests that surrogate contaminants should represent substances commonly encountered by consumers, encompassing a volatile polar organic compound, a volatile non-polar, a polar non-volatile organic compound, a non-polar non-volatile organic compound, and a heavy metal salt (with exceptions for PET) (U.S. Department of Health and Human Services Food and Drug Administration, 2021). The FDA provides examples of these substances and states that using one surrogate per category is adequate to formulate the contamination cocktail for testing purposes.

- **Volatile Polar:** chloroform, chlorobenzene, 1,1,1-trichloroethene, diethyl ketone.
- **Volatile Non-Polar:** toluene.
- **Heavy Metal:** copper (II)-2-ethylhexanoate.
- **Non-Volatile Polar:** benzophenone, methyl salicylate.
- **Non-Volatile Non-Polar:** tetracosane, lindane, methyl stearate, phenyl cyclohexane, 1-phenyldecane, 2,4,6-trichloroanisole.

The European Commission follows a similar principle, requiring cooperatives and recyclers that intend to produce food-contact recycled plastics to submit a detailed dossier of their recycling processes to the European Food Safety Authority (EFSA), in line with EFSA's directive (2011) (Beneventi et al., 2020). EFSA then evaluates the recycling process and issues an opinion on the dossier, after which the European Commission decides on the final authorization. Specific regulations for recycled plastic in food-contact applications are still absent in Brazil and other countries. However, with new environmental preservation measures and the 2030 Agenda goals, these nations will likely adopt comparable guidelines, aligning with global trends in sustainable development.

4. Analytical methods for determining compounds present in FCMs

4.1 Target and non-target screening analysis

Target analyses are widely employed to detect specific compounds or classes of compounds, known as targets. In the context of FCMs, these analyses focus on identifying known intentionally added substances (IAS) that these materials may contain, such as antioxidants (e.g., Irganox and Irgafos) in polyolefins, phthalates in PVC, bisphenols in polycarbonate, and other chemical additives (Wrona and Nerín, 2020). Many studies have been conducted to develop analytical methods for the determination of target compounds, both volatile and non-volatile, ensuring precise identification and quantification (Nerín et al., 2022; Nerin et al., 1992; Nerín and Wrona, 2018; Vera et al., 2018).

The most widely used analytical methods for determining compounds are chromatographic tools, either gas chromatography and liquid chromatography, and their coupling accessories and detectors. Each analytical method is developed and optimized based on the specific characteristics of the target compounds. For example, gas chromatography coupled with mass spectrometry (GC-MS) is commonly used for identifying volatile and semivolatile compounds, while liquid chromatography with a fluorescence detector (LC-FLD) or UV or diode array detector have been developed and applied for detecting phthalates and UV stabilizers (Kupiec, 2004; Otoukesh et al., 2020; Pezo et al., 2008; Wise et al., 1993). Additionally, liquid chromatography coupled with mass spectrometry (LC-MS-MS) is frequently employed for the determination of

aromatic amines, bisphenols and other complex compounds (Pezo et al., 2012) as target substances, usually IAS. However, for identification purposes, high resolution mass spectrometry (HRMS) is required, usually coupled to UPLC. Orbitrap or QTOF can be used as HRMS detectors and the use of specific software, in-house libraries and chemical databases are essential for the identification of chemical structure of the compounds (Song et al., 2022; Su et al., 2020).

Furthermore, the use of selected ion monitoring (SIM) in chromatographic techniques enhances analytical sensitivity. This approach focuses on specific ions of interest, providing targeted detection and improved precision compared to the broader scanning mode typically applied in mass spectrometry. Target compounds are quantified using reference standards corresponding to each identified compound, as their structures are already known. This approach enhances the accuracy and reliability of the analysis (Nerín et al., 2003).

However, as mentioned above, non-intentionally added substances (NIAS) require comprehensive screening analytical methodologies. These compounds are often not cataloged in databases and lack reference standards for confirmation. Consequently, non-target screening analysis is the most widely used approach for their detection and identification and without a doubt, it is the most difficult task in this work.

NIAS is found in almost any type of FCMs and, as noted earlier, can be categorized as impurities, degradation products of the polymer matrix, or IAS, secondary reaction products, and contaminants from external sources (particularly in recycled materials) (Assessment et al., 2014), including material processing. Advances in research have led to the identification and quantification of a series of NIAS, such as the degradation products of the Irgafos 168 additive, among others. However, target analysis is limited in its ability to predict the structure of more complex compounds that may be present in plastic materials, emphasizing the need for broader and more detailed analytical approaches.

Non-target analysis involves the structural elucidation of the compounds being analyzed. As highlighted in literature, mass spectrometry is the most widely used technique for identifying and determining the structures of these compounds (Ibarra et al., 2019; Xu et al., 2023). However, this technique typically requires integration with a separation system, such as gas chromatography (GC-MS) or liquid chromatography (LC-

MS), to enhance its effectiveness and accuracy. The fragment ions of a molecule and their relative intensities, as observed in the chromatograms generated during analysis, provide valuable insights into the structural and substructural characteristics of the compounds. This information enables analysts to deduce the structure of the detected compounds with greater accuracy (Kato and Conte-Junior, 2021; Ramos et al., 2019).

However, challenges remain in elucidating these substances, as the chemical products used in the production and processing of FMCs, are often fully disclosed. This lack of information complicates sample preparation and the optimization of instrumental conditions, hindering the development of standardized analytical methods (Kato and Conte-Junior, 2021). Additionally, the diverse structures and properties of these chemicals necessitate tailored studies and analytical methodologies to ensure their effective detection. In this context, there is currently no universal non-target analysis method suitable for all situations. In practice, different laboratories and research groups often develop and rely on their own preferred methodologies to address the specific requirements of their analyses.

4.2 Analyzing the chemical composition of plastic FCMs

The use of advanced analytical methods for characterizing IAS and NIAS ensures the quality and safety of both virgin and recycled polymers in FCM production. Comprehensive studies and continuous advancements in analytical techniques are crucial to assessing the risks of hazardous toxic chemicals in food-contact packaging that could endanger human health. The choice of appropriate techniques depends on the sample type, whether involving direct polymer analysis or migration studies, to ensure the material's integrity and compliance with safety standards. Figure I-9 shows a scheme of sample treatment and how to guide them to the most appropriate analytical methods, which were developed and applied by Nerín and collaborators to demonstrate the challenges of identifying NIAS in FCMs (Nerin et al., 2013, Nerin et al., 2022).

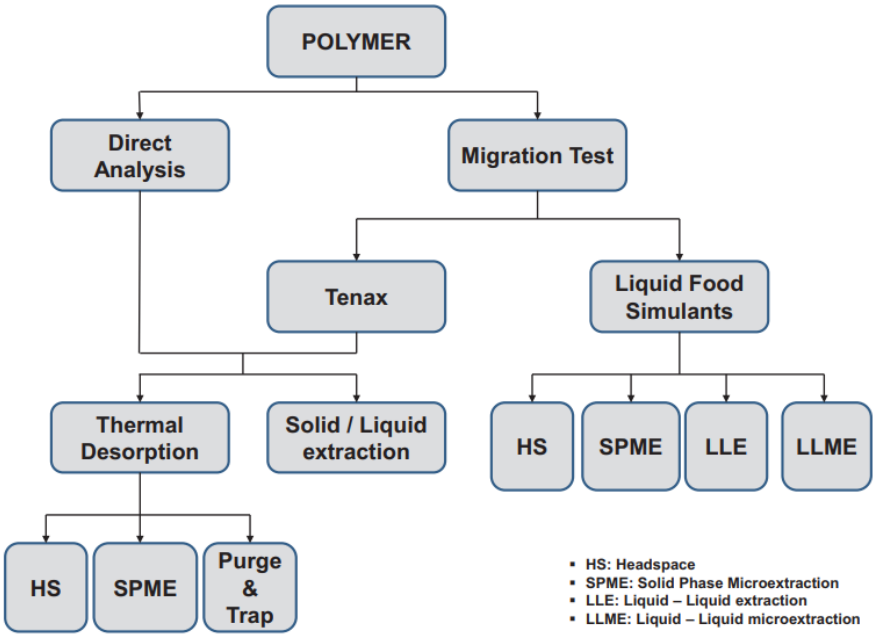


Figure I-9: General scheme for sample treatment procedures (Nerin et al., 2013).

4.2.1 Direct analysis

Samples prepared for direct analysis can take the form of pellets, flakes, small pieces, or powders. Smaller sample sizes are advantageous as they offer a higher surface/volume ratio, enhancing the efficiency of compound extraction and ensuring greater release of the compounds present in the material (Wrona and Nerín, 2020). However, for more rigid plastics, techniques like cryogenic grinding should be considered to achieve proper sample preparation and adequate material articulation for direct analysis.

After the samples have been prepared, the direct analysis process can begin. First, the samples are heated to a temperature that avoids thermal decomposition, as the generation of new compounds could interfere with the analysis. Heating the sample causes the compounds to desorb, allowing them to be captured by techniques such as headspace (HS), solid-phase microextraction (SPME), or purge-trap (Souza Silva et al., 2017). These captured compounds are then transferred to analytical instruments for separation, such as chromatographs, and detection, such as mass spectrometers.

The advantage of thermal desorption is that it does not require complex sample preparation methods, as described by Nerin *et. al* (Wrona and Nerín, 2020). However,

its primary limitation is its focus on volatile compounds, making it less effective for desorbing semi-volatile and non-volatile substances from the polymer matrix. Consequently, the gas chromatography (GC) technique, even with its accessories, is restricted in its ability to determine these less volatile compounds (Kupiec, 2004).

Many studies in the literature employ the GC-MS technique for the determination of volatile and semivolatile compounds (Chen et al., 2020; Clemente et al., 2016; Vera et al., 2023; Yang et al., 2012). For example, Paiva et *al*. and Vera et *al* 2014 utilized the SPME-GC-O-MS method to identify odoriferous volatile compounds in recycled PP packaging (Paiva et al., 2021a; Vera et al., 2012). Additionally, other approaches, such as HDC-GC-FID, have been used by Fabris et al. for determining chemical substances in PET packaging and their application to post-consumer materials (Fabris et al., 2010). These methods exemplify the versatility of chromatographic techniques in addressing the analytical challenges associated with the determination of volatile compounds in different plastic packaging materials (Osorio et al., 2019; Salafranca et al., 2000; Vera et al., 2018).

There are additional extraction techniques available for sample preparation and direct analysis, particularly to preserve the integrity of the polymer, such as solid-liquid extraction (Rodríguez-Ramos et al., 2024). Each can also be used to obtain non-volatile compounds, microwave-assisted extraction (Gomez et al., 2020). Accelerated solvent extraction (Li et al., 2012), ultrasound-assisted (Priego-Capote and de Castro, 2007) extraction and total dissolution can also be used in some cases of sample preparation.

4.2.2 Migration test

As established by regulatory bodies such as the EU Regulatory Commission, migration tests are conducted using either real food or food simulants (Commission Regulation (EC), 2008). These tests are particularly noted for their lower level of rigor compared to other types of extraction analyses. The solvents employed in these tests are less aggressive, which minimizes the risk of sample degradation during analysis. This approach ensures that the results better reflect real-world conditions, as they do not intend to apply an exhaustive extraction but to mimic the behaviour of the material in contact with food, thus providing more accurate assessments of material safety.

Migration tests provide a realistic evaluation of compounds present in packaging that may migrate to food simulants. These tests enable the identification and quantification of compounds in compliance with regulatory standards, such as the global migration limit (GML) and specific migration limit (SML) (Commission Regulation (EU) No 10/2011, 2011). However, the technique has certain limitations. Compounds detectable through other extraction methods may not necessarily appear in food simulants during migration tests. This limitation is due to the physicochemical interactions between the simulant and the compounds, which are influenced by factors such as polarity (Nerín et al., 2022). Additionally, certain compounds may only appear during migration tests, as food simulants encompass a broader polarity spectrum than specific extraction solvents. Furthermore, these compounds may transform depending on the type of simulant used in the analysis, as has been observed in studies where migration tests identified compounds that had undergone hydrolysis during the testing process (Sara Ubeda et al., 2019).

A methodology often employed to enhance sensitivity in compound identification involves the initial application of an extraction technique, such as SLE, to screen compounds of interest using a non-target approach. Following this, migration assays are conducted to quantify these identified compounds. This integration of methodologies improves the analysis of potential contaminants that may pose risks to human health, streamlines the structural elucidation process, and focuses efforts on compounds that are truly relevant to the study objectives (Wrona and Nerín, 2020). The advantage is that, in this case, the concentration of potential migrants is much higher than in migration tests and this fact facilitates the identification. However, most of the extracted compounds will never migrate. Then, the initial extraction requires a lot of analytical effort that will not be useful later.

The conditions for food simulants and migration tests are specified in the regulations above mentioned. Once the migration tests are completed, the simulants are analyzed using suitable analytical techniques, with liquid (LC) and gas chromatography (GC) being the most employed methods. Additionally, for GC, extraction techniques such as HS and SPME are widely utilized to concentrate the compounds, thereby enhancing the sensitivity of the analysis, as can be seen in Figure I-9 (Nerin et al., 2013).

5. Identification of compounds by mass spectrometry

5.1 Volatile compounds

As detailed in the literature, volatile organic compounds (VOCs) are defined as organic substances with a boiling point of 250 °C or lower at a pressure of 101.3 kPa. Gas chromatography coupled to mass spectrometry (GC-MS) is the most employed analytical method to determine these VOCs, owing to its precision and effectiveness in identifying and quantifying these compounds (Nerin et al., 2013; Wrona and Nerín, 2020).

As described by Nerín et al., the GC-MS technique enables the identification of VOCs through the use of commercially available mass spectra libraries. These libraries contain MS spectra obtained via electron impact and analyzed with a quadrupole mass analyzer. While variations may arise when using other mass analyzers, such as ion-trap systems, the libraries remain the primary resource for verifying and identifying VOCs, ensuring reliable characterization of these compounds (Nerin et al., 2013). Currently, the most recent low-resolution commercial libraries available for VOCs identification, include Wiley Registry 12th Edition and NIST (National Institute of Standards and Technology) library, which features over a million nominal spectra. In addition, to these extensive commercial options, there are publicly accessible libraries, such as the one compiled by the RIKEN Center for Sustainable Resource Science, offering a smaller but valuable range of compounds for research and analysis.

Recent advances in combining gas chromatograph (GC) with quadrupole time-of-flight (QTOF) mass analyzers have significantly enhanced the ability to identify NIAS (Nerin et al., 2013). While these compounds are often initially identified in a “tentative” manner using spectral library comparisons, true identification requires more than just a good match. To ensure robust and precise structural validation, additional information must be considered. This additional information includes retention times or Kovats indices, which are obtained from authentic standards analyzed under similar conditions. When these criteria are met, the compound can be classified as having “confident structural assignment”, providing a higher level of certainty in its identification. This approach ensures a more reliable analysis, essential for advancing the understanding and management of NIAS in various applications (Assessment et al., 2014; Eaves and Consultant Regulatory, n.d.; Nerin et al., 2013; Nerin et al., 2022).

Even with significant advances, limitations persist in the identification of emerging compounds, as many are not represented in existing spectral libraries. This gap complicates the analysis and recognition of such substances. Tools like Mass Frontier (Thermo Scientific), MS Fragmenter (ACD Laboratories), and MOLGEN-MS (Gugisch et al., 2014), which predict spectra based on structural databases such as PubChem, have shown promise, but their real-world effectiveness remains insufficiently validated.

More recent models, like quantum chemical electron impact mass spectrum (QCEIMS) (Spackman et al., 2018) and neutral chemical electron impact mass spectrum (NEIMS) (Ji et al., 2020), offer enhanced accuracy and faster spectrum prediction. However, these models are constrained by their reliance on pre-existing data, limiting their utility for identifying truly novel compounds. These challenges underscore the necessity of combining various analytical approaches. By integrating complementary methodologies, researchers can address existing gaps and achieve more reliable identification of new compounds. This multidimensional strategy is essential for advancing studies centered on the detection and analysis of NIAS, paving the way for more comprehensive and accurate assessment.

5.2 Non-volatile compounds

Whereas GC-MS is considered one of the most suitable techniques for analyzing VOCs, LC-MS stands out as the preferred method for detecting non-volatile substances. This popularity is largely due to its versatility and the variety of ionization sources available, which enable the detection of a broad range of compounds. Among these ionization sources are electrospray ionization (ESI), atmospheric pressure photoionization (APPI), matrix-assisted laser desorption/ionization (MALDI), and the EI-direct LC-MS, an indispensable tool in modern analytical chemistry, particularly for studying complex and non-volatile compounds (Su et al., 2021).

The identification of compounds using LC-MS faces significant challenges due to the lack of comprehensive and standardized libraries for the mass spectra generated by this technique, making it a more complex and time-consuming process than GC-MS (Simal-Gándara et al., 2002). The primary difficulty arises from the variability in LC-MS spectra, influenced by factors such as instrument design, ionization source settings, mobile phase composition, buffer additives, and sample components. These parameters

are challenging to standardize across laboratories and can even fluctuate within the same instrument over time (Nerin et al., 2013; Simal-Gándara et al., 2002; Wrona and Nerín, 2020).

Unlike GC-MS, where spectra are relatively consistent, LC-MS/MS spectra for the same compound can differ substantially between instruments. Variations occur in the fragment ion profiles and their relative abundances, making the creation of universally reproducible libraries a daunting task (Bristow et al., 2002). For many years, commercial LC-MS/MS libraries were unavailable until NIST introduced its first MS/MS library in 2005, featuring 5,000 spectra from 1,943 unique compounds. Since then, these resources have grown exponentially. The latest NIST 2020 library now includes 1,32 million MS/MS spectra covering 30,000 unique compounds, offering a valuable reference for researchers (Hopley et al., 2008; Song et al., 2022; Su et al., 2021; Sun et al., 2021). Despite this progress, LC-MS/MS libraries exhibit specific characteristics that set them apart from EI-MS libraries, including the need to account for instrumental and methodological variability (Hopley et al., 2008).

As mentioned earlier, the identification of compounds using LC-MS, while challenging, has seen significant advancements in recent years, driven by technological innovations. Different types of mass spectrometers offer distinct advantages and limitations, but hybrid instruments have emerged as a powerful solution by combining the strengths of multiple technologies. Systems such as quadrupole tandem time-of-flight (Q-TOF) and ion trap TOF (IT-TOF) stand out for their ability to determine the exact masses of precursor ions and their fragments (Lacorte and Fernandez-Alba, 2006; Nerín et al., 2022; Nerin et al., 2013). This capability provides critical insights into fragmentation patterns, which are essential for accurately identifying unknown compounds. These hybrid systems have become invaluable tools in the untargeted analysis of complex samples, including foodstuffs, offering enhanced precision and reliability in compound identification.

High-resolution hybrid spectrometers, such as the LTQ-Orbitrap, have proven highly effective in identifying non-targeted compounds, especially in complex sample matrices (Brosnan et al., 2012; Nerín et al., 2022; Nerin et al., 2013; Osorio et al., 2022). For instance, acquiring spectra at both low and high collision energies provides complementary data – low-energy spectra reveal information about precursor ions, while high-energy spectra elucidate fragment patterns crucial for structural identification. The

choice of ionization source significantly impacts detection sensitivity. Electrospray ionization (ESI) is often the preferred option due to its high sensitivity for various compounds. While positive ionization typically offers greater efficacy, negative ionization may be more appropriate depending on the compound's chemical properties, offering flexibility in accommodating diverse analytical needs (Brosnan et al., 2012; Nerín et al., 2022; Nerin et al., 2013; Osorio et al., 2022).

Assigning a molecular formula (MF) is a crucial first step in structural elucidation, serving as the foundation for identifying unknown compounds (Su et al., 2021). This process begins with accurately determining the precursor ion and its potential adducts, such as $[M+H]^+$ or $[M-H]^-$, often derived from low-energy spectra analysis. In some instances, compounds can ionize in positive and negative modes, offering complementary information that aids in pinpointing the molecular ion. Once the molecular ion is identified, possible MFs are computed based on predefined constraints, including elemental composition, atomic counts, and precise mass tolerance. Elements such as carbon, hydrogen, nitrogen, oxygen, and sulfur are commonly encountered, while phosphorus, chlorine, fluorine, bromide, or other elements appear less frequently. To further refine the list of candidates, chemical rules – such as double bond equivalence calculations, isotopic distribution patters, and logical checks against known chemical structures – are applied. These additional criteria significantly reduce ambiguity, ensuring a more accurate and reliable MF assignment (Ji et al., 2020; Nerin et al., 2013; Su et al., 2021).

These complexities underscore the necessity for continued standardization efforts and the development of advanced tools tailored to analyze intricate compounds like NIAS. Improvements in these areas will be instrumental in overcoming current limitations and enhancing the reliability and accuracy of LC-MS-based identifications.

Section II: Objectives

Objectives

The principal objective of this thesis is to examine the effects of the recycling cycle on PLA, focusing on the formation of NIAS and its potential as a raw material for manufacturing biodegradable and/or compostable recycled plastic packaging intended for food contact. To achieve this, both experimental and theoretical approaches were employed to address the following general objectives:

- Subject PLA to the washing and mechanical recycling process to evaluate the influence of washing variables on changes in molar mass ratio and physicochemical properties.
- Assess the effects of the recycling cycle and the presence of contaminants introduced during the challenge test on the material's physicochemical and organoleptic properties.
- Investigate the presence and generation of NIAS after the PLA recycling cycle, identifying the volatile and semivolatile compounds using SPME-GC-MS and analyzing the odor profile via SPME-GC-O-MS.
- Evaluate the efficiency of the recycling process in decontaminating the material, focusing on the target compounds specified in the FDA challenge test protocol, using DI-GC-MS and SPME-GC-MS to quantify their concentrations.
- Analyze the impact of the bifunctional carbodiimide additive on physicochemical properties, its role as an anti-hydrolysis and chain extender, and its influence on oligomer migration (NIAS) using UPLC-QTOF-MS^E.

Session III: Experimental part

Experimental part

Development of a recycling methodology for biobased polymers and evaluation through physicochemical property analysis

Chapter 1: Optimization of Washing Parameters to Minimize the Degradation of Poly (lactic acid) Using Design of Experiments: A Contribution to the Recycling Chain

Non-target screening of volatile compounds from recycled PLA

Chapter 2: Volatile Compounds and Off-odors Analysis of Recycled PLA for Packaging Applications: An Essential Factor for Ensuring Food Safety and Quality

Application of the FDA Contamination Protocol and evaluation of recycling cycle effectiveness in the decontamination process

Chapter 3: Biobased Polymer Recycling: A Comprehensive Dive into the Recycling Process of PLA and Its Decontamination Efficacy

The influence of a bifunctional additive on reducing the migration of non-volatile compounds from recycled PLA

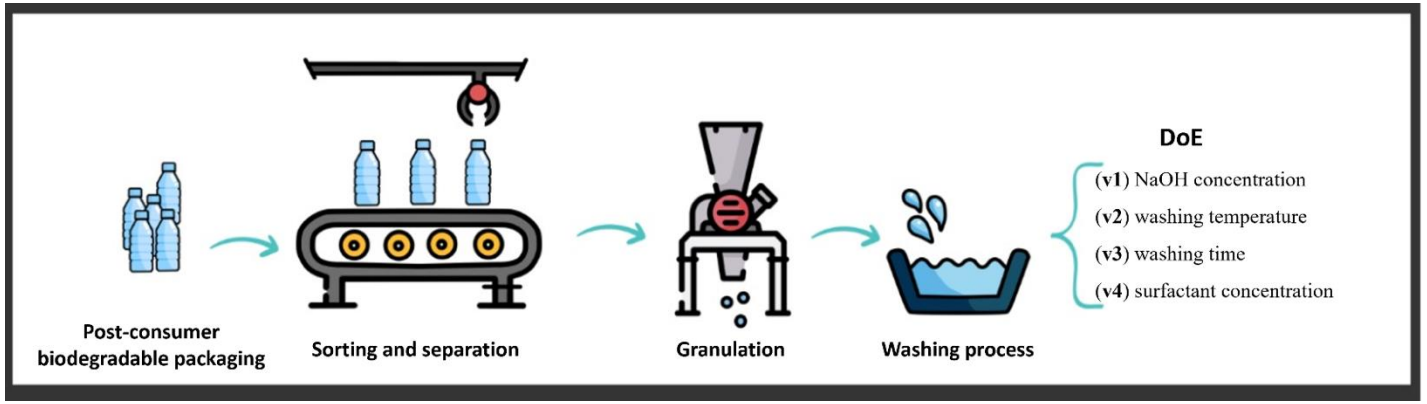
Chapter 4: How Carbodiimide Modulates Oligomer Migration and Molar Mass in Recycled Poly (Lactic Acid): A Study Using UPLC-QTOF-MS^E

Chapter 1

Optimization of Washing Parameters to Minimize the Degradation of Poly (lactic acid) Using Design of Experiments: A Contribution to the Recycling Chain

1. Abstract:

The increasing demand for sustainable packaging solutions has led to the widespread adoption of biodegradable polymers such as poly (lactic acid) (PLA) in the packaging industry. However, the efficient recycling of post-consumer PLA packaging remains challenging due to contamination from residual food and other substances. This study presents the application of Design of Experiments (DoE) in the evaluation of washing parameters, typically used in the recycling industry, in the degradation of post-consumer PLA packaging. The experiments were conducted using a factorial design to investigate the effect of key washing parameters, including sodium hydroxide concentration, washing temperature, washing time, and surfactant concentration on the degradation of PLA. The degradation was accompanied by rheology employing the Cox-Merz rule, which establishes the relationship between complex viscosity and steady state viscosity. The results indicated that a washing process with lower degradation levels could be obtained by adjusting the washing parameters. The surfactant concentration, whether isolated or in combination with variables such as time and temperature at their higher levels, resulted in a less damaging effect in terms of degradation. On the other hand, time has a great influence on the level of degradation in PLA. This variable is more detrimental when considered in conjunction with others, such as temperature and the concentration of sodium hydroxide. Thus, the application of DoE allowed the identification of the most influential factors and their interactions in the degradation of PLA during the washing step and may be adjusted according to the dirtiness of the precursor material. This study demonstrates that this is valuable in the development of sustainable recycling processes for packaging materials, contributing to a circular economy and reducing environmental impact.


Post-consumer
biodegradable packaging
Sorting and separation
Granulation
Washing process
DoE
(v1) NaOH concentration
(v2) washing temperature
(v3) washing time
(v4) surfactant concentration

2. Introduction

The use of biodegradable polymers, either from non-renewable or renewable sources, in the production of packaging for food contact and hospital products is one of the most discussed topics in the world today. The concern is primarily related to the environmental problem caused by the inadequate disposal of non-biodegradable post-consumer plastic materials (Brito et al., 2011; Kabir et al., 2020). Moreover, in the aftermath of the pandemic caused by the COVID-19 virus, there has been an exponential increase in the use of single-use disposable materials produced from conventional polymers, which has directly impacted the environment and especially marine life (Sarkodie and Owusu, 2021; Shams et al., 2021).

One of the proposals to be implemented in the new EU directives is the total elimination of the use of pristine petroleum polymers in single-use manufacturing. The directive aims to encourage the use of recycled and/or biodegradable polymers from renewable sources to mitigate environmental impacts (First Vice-president Frans and Jyrki, 2019; Shams et al., 2021).

In fact, the new proposals can positively affect the circular economy and reduce the environmental impact caused by these materials (First Vice-president Frans and Jyrki, 2019). In addition to the cost of producing plastic packaging from biodegradable polymers, another problem has emerged: the mishandling of post-consumer packaging. Although these materials are biodegradable and are obtained from renewable sources, biodegradation only occurs under specific conditions, such as those described in ASTM-D-883 (Ahmed et al., 2018).

Poly (lactic acid), PLA, is one of the most widely used hydrolytically degradable bio-based polymers from renewable sources in the packaging and pharmaceutical industries, and more recently in the production of rapidly disposable hospital products. This is due to its biocompatibility, availability of commercialization on large scale, and wide range of grades, which allows it to be used in the same manufacturing processes as conventional thermoplastic resins, such as extrusion, molding, lamination, and others (Garcia Gonçalves et al., 2020). Data from 2020 indicates that PLA represented more than 10% of the global production and commercialization of the bio-based plastics market, making it a promising candidate to meet the new directives established by regulatory agencies (Garcia Gonçalves et al., 2020).

As mentioned earlier, the European directives aim to encourage the use of recycled packaging for direct or indirect contact with food. As a result, an alternative that has been studied is the recycling of post-consumer biodegradable packaging by means of pre-established procedures for packaging made of conventional polymers such as poly (ethylene terephthalate) (PET), polypropylene (PP) and other thermoplastic resins (Franchetti and Marconato, 2006; Stoica et al., 2020). However, further motivations are taken into consideration to propose the recycling of biodegradable plastics (i) the increasing industrial demand, due to development and population growth (ii) the reduction of the consumption of non-renewable resources, (iii) cost of the bio-based polymer production, (ix) some compostable materials do not suffer total degradation, what can cause the residual accumulation and the loss of the raw material that could be reused (Aryan et al., 2021; Badia et al., 2012; Scaffaro et al., 2011; Zenkiewicz et al., 2009). However, during the mechanical recycling stages of bio-based polymers, contamination can occur with non-renewable sources such as PP and PET. It is therefore very important that the sorting stage is carried out thoroughly (Kuzhanthaivelu, 2018). Therefore, several studies have investigated the possibilities of recycling bio-based polymers, with particular attention to PLA (Nathalia S.Q.S. Amorin et al., 2014; Badia et al., 2012; La Mantia et al., 2002; Maga et al., 2019; Scaffaro et al., 2011; Zenkiewicz et al., 2009). It is known that this polymer thermodegrade during processing, and consequently, its recyclability is strictly related to the actual extent of thermodegradation phenomena during the recycling stages (Nathalia S.Q.S. Amorin et al., 2014; Rojas-González and Carrero-Mantilla, 2015).

Amorin *et al*. (Nathalia S. Q. S. Amorin et al., 2014) and Zhao *et al*. (Zhao et al., 2018) subjected PLA to multiple reprocessing cycles and evaluated its impacts on the molar mass of the polymer. The results indicated that the increase in the number of cycles results in a reduction of the molar mass which directly affects the material properties. However, in the first cycles, the reduction and change in properties were minimal, indicating the feasibility of recycling.

Before the plastic material goes through the extrusion process, it first goes to a washing stage. This step is a process of extreme importance in recycling because it is at this stage that the pre-decontamination of post-consumer plastic material occurs. However, one of the problems presented in the recycling process of biodegradable polymers is their ability to suffer degradation by hydrolysis (McKeown and Jones, 2020).

In this sense, the washing step becomes critical, since basic solutions and high temperatures are commonly used in the processes that can accelerate the degradation by hydrolysis. Despite its importance, no studies were found in the literature that systematically investigate the variables, including NaOH concentration, washing temperature, washing time, and surfactant concentration of the washing process that influence the degradation of PLA. On the other hand, there are several studies in the literature on this theme applied to conventional polymers such as PET (Gere and Czigany, 2018; Louzi, 2015; Ogino and Nagatsu, 2007; Taylor and Bayer, 2010).

Therefore, this is a pioneering study that unites two themes at the forefront of knowledge: (1) bio-based polymer recycling and (2) the effect of washing variables including NaOH concentration, washing temperature, washing time, and surfactant concentration on PLA degradation. Thus, to minimize the effects caused by the degradation by basic hydrolysis, and to maintain the existing steps employed by recycling industries, this study has as its goal the use of a Design of Experiment (DoE) to study the variables employed in the PLA washing process, which are sodium hydroxide (NaOH) concentration, washing temperature, washing time and the presence of surfactant with the resulting molar mass index (MMI) used to predict the ideal conditions to minimize the degradation process and maintain the properties of the material.

3. Materials and methods

3.1 Chemicals

The reagents used in this study were sodium hydroxide (Panreac 98%, CAS No. 1310-73-2), surfactant Triton X-100 (Êxodo science, CAS No. 9036-19-5), and distilled water.

3.2 Bio-based polymer sample

The commercia biopolymer used was poly (lactic acid), PLA Ingeo 4043D, from NatureWorks. The biopolymer presents a melt flow index (MFI) of 6 g/10 min. (ASTM D1238, 210 °C, 2.16 kg) and a density of 1.24 g/cm^3 (ASTM D792).

3.3 Washing process

To analyze the influence of the washing parameters a DoE was employed to study these variables and relate them to the increase in the PLA degradation process. After the washing process, the PLA pellets were rinsed for 5 minutes in water to remove any excess residue from the washing process and dried in a vacuum oven for 4 h at 80 °C with subsequent analysis of their molar mass using rheometer.

3.4 Design of Experiment

As described earlier, different variables were chosen based on the washing processes employed for PET and PP.

- (**v1**) NaOH concentration (%);
- (**v2**) washing temperature (ºC);
- (**v3**) washing time (min.);
- (**v4**) surfactant concentration (%).

The DoE results allow investigating of the influence of the combined variables from two levels, 1 (high) and -1 (low), as described in Table 1.1. Therefore, a full factorial design 2^4 (Experiments 1 to 16) was obtained from the combination of the four variables in their two coded levels. The response analyzed was the molar mass variation. Thus, a full of 19 experiments were developed, with three experiments being central point (CP). Moreover, for the analysis and organization of the data, the Excel program and the Octave free program were used, and to analyze the experimental parameters the ANOVA was applied to the proposed methodology.

It is important emphasized that DoE using chemometric tools is extremely important for both research laboratories and industry. As described by Pereira et al., DoE offers advantages compared to univariate procedures, such as (i) a reduced number of planned experiments (iii) saving financial resources, (iii) results with greater chemical and statistical reliability, and most importantly, (iv) the possibility of obtaining a mathematical model capable of making predictions under untested conditions that allow visual observation on response surface (Pereira and Pereira-Filho, 2018) .

Table III-1.1 Variables evaluated in the washing process at two levels.

Level coded	(v1) NaOH %*	(v2) T ºC	(v3) Time (min.)	(v4) Surfactant %*
1	2	75	15	3
-1	1	25	5	0
CP	1.5	50	10	1.5

* m/v

3.5 Determination of molar mass index (MMI)

A parallel plate rheometer (Anton Paar- MCR501) was used to measure the complex viscosity (η^*) as a function of frequency (ω). For the test, plates with a diameter of 25 mm were used at a temperature of 200 ºC in a dynamic regimen and the range of frequencies used was 0.01 to 500 rad s^{-1} at 1% as well as the gap between plates was 1.00 mm. The same methodology was employed by Garcia et al. (Garcia et al., 2013). The zero shear-rate viscosity, determined by rheometry, is associated with molar mass according to the Mark-Houwink equation (Equation 1.1).

$$\eta_0 = KM^a \qquad (1.1)$$

where M is the molar mass and K and a are constants of the polymer (Garcia et al., 2013).

When frequency sweep measurements are performed the Cox-Merz rule is usually used and establishes the relation between the complex viscosity ($\eta(\omega)$) with the steady state viscosity ($\eta(\dot{\gamma})$), represented by Equation (1.2) (Barnes, 1996).

$$\eta(\dot{\gamma}) = |\eta(\omega)|; \text{ with } \dot{\gamma} = \omega \qquad (1.2)$$

Through Equations 1.1 and 1.2, it is possible to calculate the ratio between molar masses M1/M2, where M1 is the molar mass of analysed samples, M2 is the molar mass of pristine PLA and a is 3.4.

$$\frac{\eta_{01}}{\eta_{02}} = \frac{KM_1^a}{KM_2^a} \qquad (1.3)$$

Furthermore, the ratio of the molar mass of the PLA samples can be calculated through Equation (1.4).

$$\frac{M_1}{M_2} = \sqrt[a]{\frac{\eta_{01}}{\eta_{02}}} x\ 100 \qquad (1.4)$$

when the η_{01} and the η_{02} represent the zero shear-rate viscosity of the samples and pristine PLA, respectively.

Thus, from Equation (1.4) it is possible to quantitatively evaluate the molar mass variation of the samples and correlate the influence of the studied variables of the washing process in the degradation of PLA.

4. Results and Discussion

A Table 1.2 shows the full overview of the design of experiments that resulted in the factorial design 2^4 with the 4 variables and their two levels and, MMI (M1/M2) responses. The responses were normalized and scaled in % based on the control sample (PLA) on a scale from 0-100%.

Table III-1.2 Full DoE considering four variables: (**v1**) concentration of sodium hydroxide, (**v2**) temperature, (**v3**) time, and (**v4**) concentration of surfactant.

Experiment	v(1)	v(2)	v(3)	v(4)	M1/M2(x100)
PLA	-	-	-	-	100
1	-1	1	1	-1	74
2	1	-1	1	1	78
3	-1	-1	1	-1	70
4	-1	1	-1	1	72
5(CP)	1.5	50	10	1.5	80
6(CP)	1.5	50	10	1.5	81
7	1	1	1	-1	59
8	-1	-1	1	1	71
9	-1	1	1	1	76
10(CP)	1.5	50	10	1.5	81
11	1	-1	1	-1	78

12	1	1	-1	1	79
13	1	1	-1	-1	84
14	1	1	1	1	89
15	-1	-1	-1	1	88
16	1	-1	-1	1	78
17	-1	1	-1	-1	74
18	1	-1	-1	-1	69
19	-1	-1	-1	-1	77

To obtain the results, the effects of the combinations of variables studied in the PLA washing process and the most important variables were calculated the effects using Equation (1.5). Furthermore, the effects of the significant variables were evaluated according to the response surfaces methodology (RSM).

$$Effect = \overline{Y_+} - \overline{Y_-} \qquad (1.5)$$

where $\overline{Y_+}$ and $\overline{Y_-}$ are the mean of the responses of the variables at the high and low levels, respectively.

Through the experiments described in Table 1.3 it was possible to identify four significant effects: two main effects for two individual variables (v3 and v4), and two third order interaction effects (123 and 234) as described in Table 3. It is clear from these results that the third order interaction of variables v1v2v3 (123), as well as v3 has a negative influence on the washing process while the third order interaction of variables v2v3v4 (234) and v4 have a positive influence.

Table III-1.3 Effect of a calculated variables.

Variables Interaction	Effect value
v1v2v3 (123)	-6.5
v3	-3.25
v4	5.75
v2v3v4 (234)	7.25

4.1 Influence of negative and positive effects on the PLA washing process

As described in the literature, the use of basic sodium hydroxide solutions and high temperatures is used for pre-decontamination post-consumer packaging before reprocessing (Krehula et al., 2013). However, the main concern in the PLA washing process is the possibility of degradation by basic hydrolysis catalyzed by high temperatures and excessive exposure time of the material to washing solution (von Burkersroda et al., 2002; Xu et al., 2011). McKeown *et al*. (McKeown and Jones, 2020) report that various degradation processes for PLA can occur such as alcoholysis and hydrolysis, leading to the formation of monomers and other compounds non-intentionally added to the material.

Bukersroda *et al*. (von Burkersroda et al., 2002) described that hydrolysis degradation in bio-based polymers is influenced by the water diffusion process. Furthermore, a bulk erosion process can result in higher diffusivity of water permeating the polymer chains, facilitating higher intramolecular hydrolysis, leading to a higher molar mass loss. These erosive processes are greatly influenced by the environment in which the bio-based polymer is exposed, and the important factors influencing the type of erosive process are pH and temperature which both act as catalysts for hydrolysis degradation (von Burkersroda et al., 2002).

Figure 1.1 shows the complex viscosity curves as a function of frequency for experiments 7, 14, 15, and 18, as well as pristine PLA. These experiments were chosen to exemplify the extreme cases of the lowest level of degradation (Experiments 14 and 15) and the highest level of degradation (Experiments 7 and 18), as presented in Table 1.2. Furthermore, a reduction in complex viscosity is clearer for the sample from Experiment 7, but this distinct behavior after the Newtonian plateau, there are not significant changes in the curve profile at higher frequencies.

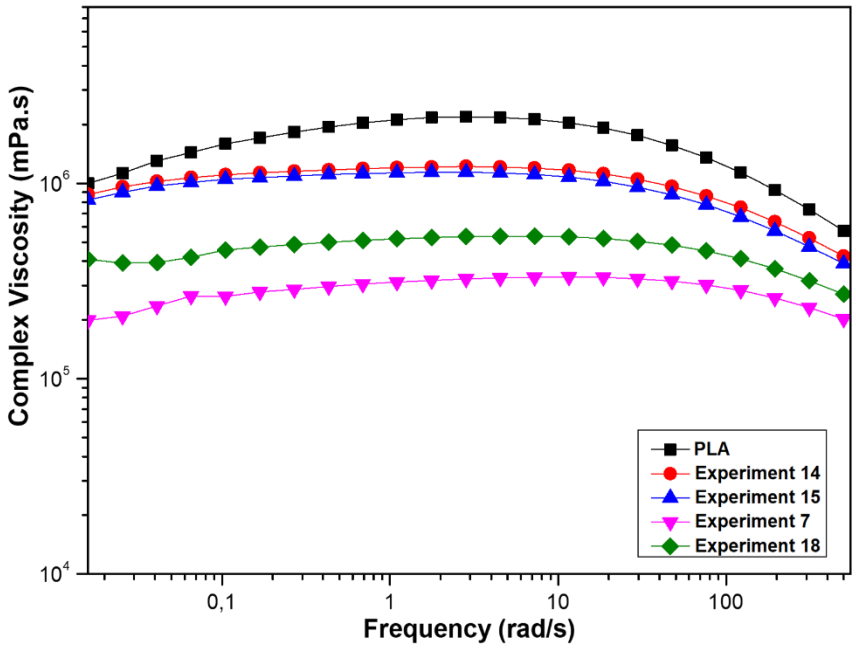


Fig. III-1.1 Complex viscosity as a function of frequency for the samples.

From the results obtained by the DoE and the calculation of the effects of the variables, using Excel tools and the free Octave program, the mathematical model represented by Equation 1.6 was obtained.

$$Response = b_0 + b_1 v_3 + b_2 v_4 + b_3 v_{123} + b_4 v_{234} \qquad (1.6)$$

Where b_0 represents the constant coefficient of the mean of the responses (MMI), b_1 and b_2 are the linear coefficients, and b_3 and b_4 are third-order coefficients to the calculated effects. In addition, v_3, v_4, v_{123}, and v_{234} represent the variables that can alternate in their levels (1 and -1) depending on the maximization or minimization of the response to be analyzed. Once this model is in place, the data will be analyzed using three-dimensional response surface curves, where the z-axis will be represented by the molar mass index, and the x- and y-axes will be represented by the first-order variables that had the most significant effect on the washing process (Table 3), i.e., the variables v_3 and v_4, respectively.

By applying Equation 6 to analyze the negative or positive influence of the variables on the washing process, Equation 1.7 was obtained.

$$Response = 76 + (-1.625)(\pm 1) + (2.875)(\pm 1) + (-3.250)(\pm 1) + (3.625)(\pm 1) \quad (1.7)$$

The system obtained is dependent on both the first-order variables and the most significant third-order interaction variables, as described above. This behavior can be exemplified by working with the variables that harm the washing process, v_3 and v_{123}, at their maximum levels (1) and the first-order variable that has a positive impact, v_4, at its lowest level (-1). In this condition, the highest level of degradation is observed in the 70-60% range, as can be seen in the RSM (Figure 1.2a). This can also be seen in Experiment 7 obtained by DoE, where v_1, v_2, and v_3 are at their maximum levels and v_4 at its lowest.

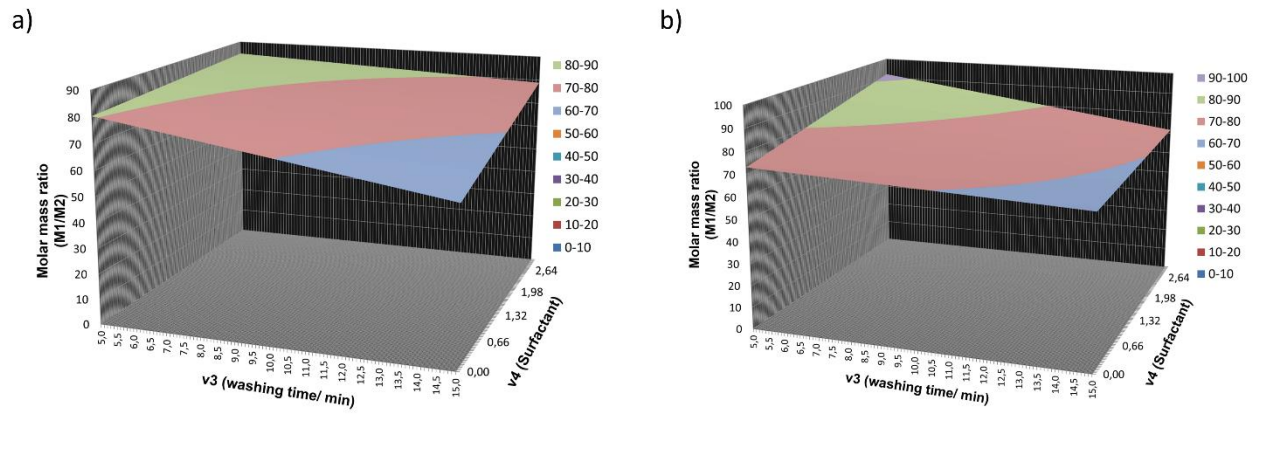


Fig. III-1.2 Response surface for the a) negative effects and the b) positive effects in the MMI.

Xu and co-workers (Xu et al., 2011) describe that the hydrolysis degradation process of PLA occurs at temperatures near and below its T_g (~57 °C). At this temperature the mobility of the polymeric chains of the amorphous phase increases, which facilitates the water diffusion process and directs the hydrolysis degradation mechanism towards the bulk. Therefore, as described above, the temperature and the basic pH of the washing solution act as a catalyst for the degradation process, resulting in the formation of compounds such as oligomers and small molecules, as can be observed in the mechanism proposed in Fig. 1.3. In addition, these degradation compounds have been identified in works such as those by Ubeda *et al*. and Gravil *et al*. who studied biodegradable and drug-release packaging and, using sensitive analytical techniques determined and quantified the presence mainly of cyclic and linear oligomers from the degradation of the PLA polymer chain (Gavril et al., 2019; Sara Ubeda et al., 2019).

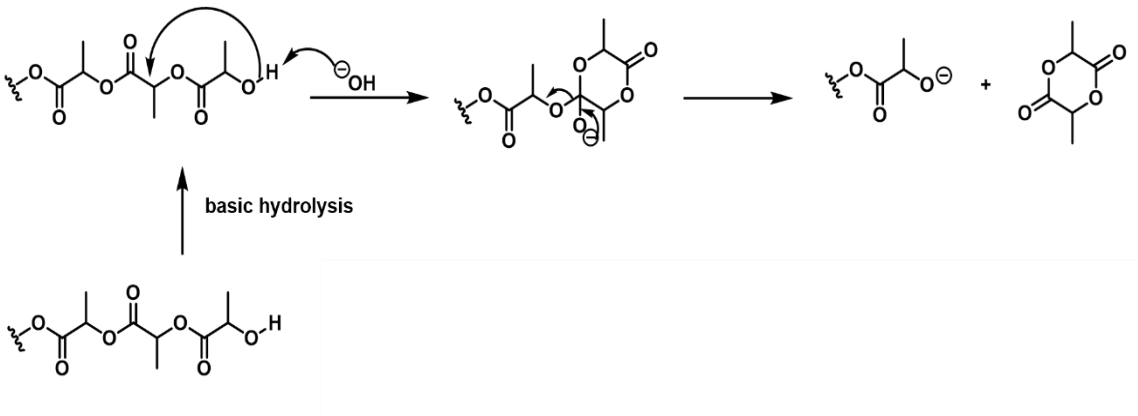


Fig. III-1.3 PLA degradation mechanisms by basic hydrolysis.

On the other hand, when we work with the first-order variable v4 at its highest level and keep the other variables at their lowest levels, the lowest level of degradation is observed, around 13%, as shown in Figure 1.2b. This result is related to one of the most widely studied topics in literature, that of the interaction of surfactants and macromolecules which has enabled their use in several industrial applications (Goddard and Ananthapadmanabhan, 1993).

The surfactant can interact with the polymer by anchoring micelle-like structures onto the polymer chains. This effect occurs when the interaction exceeds the critical aggregation concentration (CAC) (Lochhead and Huisinga, 2011) and this model of micelles attaching to polymer chains was first proposed by Saito and Sata with reference to hydrophobic polymers (Saito, 1967). However, the model is widely used to describe the chain expansion of hydrophilic polymers interacting with non-ionic surfactants (Lochhead and Huisinga, 2011). In the case of PLA, the surfactant attached to the polymer chain decreases the hydrolysis degradation process because the water diffusion is hindered by the micellar behavior of the surfactant that solvates the polymer being observed as a positive effect of the variable v_4.

5. Conclusions

In this study, the application of DoE was successfully employed to develop an optimized washing process for the recycling of biodegradable post-consumer PLA packaging. By systematically investigating the effects of various washing parameters and

their interactions, it is possible to identify the most influential factors and their optimal ranges for achieving a clean and mechanically stable recyclable PLA. The use of DoE allowed for a comprehensive understanding of the relationships between the process parameters and response variables concerning MMI. The surfactant variable appears to have a positive and high effect on the washing process, as does its association with the temperature and time variables, producing materials with the lowest levels of degradation. On the other hand, time is a critical variable in the washing process, leading to increased polymer degradation. This variable together with the concentration of NaOH and temperature also increases PLA degradation.

By optimizing the recycling process, this investigation contributes to the promotion of a circular economy and reduced environmental impacts from packaging waste. Future research may focus on further refining the washing process and expanding the application of DoE to other biodegradable polymers or recycling processes to enhance the sustainability of packaging materials and waste management practices.

Chapter 2

Volatile Compounds and Off-odors Analysis of Recycled PLA for Packaging Applications: An Essential Factor for Ensuring Food Safety and Quality

1. Abstract

Recent European guidelines support the use of recycled and biodegradable packaging for food applications. The approval of such packaging must not alter food's taste or be harmful to health. In this work, PLA pellets were subjected to a post-consumer contamination procedure, washing process, and mechanical recycling, under common conditions of the recycling industry. HS-SPME-GC-MS and HS-SPME-GC-O-MS methods were used to detect volatile compounds and off-odor profiles. 33 different volatile compounds were identified in all samples. Intentionally added and non-intentionally added substances (IAS and NIAS) were identified, including benzaldehyde, benzyl alcohol, and dimethyl-1,4-dioxane-2,5-dione. The relationship between the formation of different NIAS and the PLA recycling process steps was determined. 14 different odor compounds such as benzyl alcohol, benzaldehyde, nonanal, decanal, dodecanal, 2,3-dimethylnaphthalene and 2,4-di-tert-butylphenol were detected and classified into 4 aroma groups (Toasted, Flower, Green and Chemical). The results obtained are essential for the food safety of recycled plastic material for food contact.

2. Introduction

Biodegradable and renewable plastics represent a new alternative that can reduce the environmental impact of using plastics from non-renewable sources and their improper disposal. In addition, new European Commission guidelines aim to eliminate single-use plastic packaging made from non-renewable resources in favor of biodegradable bioplastics. However, the environmental impact of the improper disposal of this type of plastic packaging remains an extremely important factor to analyze (Commission, 2018; Sarkodie and Owusu, 2021).

Even when biodegradable polymers made from renewable resources are used in plastic packaging, the risk of improper disposal or misuse by consumers remains. It is important to note that most biodegradable packaging only degrades under certain conditions, as defined by the ASTM-D-883 standard. In other words, these materials can still cause harm to the environment (Ahmed et al., 2018). Therefore, an alternative that complies with the new guidelines is the recycling of biodegradable bioplastics, which reduces the environmental impact, increases the added value of the material, and reintroduces it into the circular economy (F. V. Frans and Jyrki, 2019).

Poly (lactic acid) (PLA) is a compostable bio-based polymer used in the manufacture of food packaging and hospital supplies, but compostability requires specific temperature and humidity levels, such as those found in industrial plants. Its ability to melt during processing and its biocompatibility render it a valuable material for recycling (Gonçalves et al., 2020). However, recycled material for direct food contact must not alter the organoleptic properties or endanger consumers health. To maintain food quality and safety, packaging must be free of odors and migratory compounds (Vera et al., 2020). The risk of these changes in sensory properties and health risks has been reported in the literature, mainly occurring in the production, contamination and recycling of plastic materials, where substances may be added intentionally or non-intentionally (IAS and NIAS) (Eaves, 2021; Geueke, 2018; Misko, 2019).

In this context, no studies were found in the literature that relate the traditional recycling process steps of polymers, examining and correlating each step with the formation of NIAS and IAS. In particular, descriptions of the odor profile of the material after each recycling cycle are lacking.

Therefore, the aim of this study was to identify the odorous compounds of PLA resulting from each step of the recycling process (contamination, washing process, and mechanical recycling). In addition, this study aimed to evaluate the presence of IAS and different NIAS that may be formed, such as NIAS from polymer chain degradation, contaminants, additive degradation, and others. The volatile and semi-volatile compounds were extracted by headspace solid-phase microextraction (HS-SPME) and identified by gas chromatography coupled to the mass spectrometer (GC-MS), while the compounds responsible for the odor were identified by gas chromatography-olfactometry coupled to the mass spectrometer (GC-O-MS). Furthermore, the correct identification of the aroma type, the retention index, and the mass spectrum of odors were key tools for a proper qualitative analysis of odorous compounds (El-Sayed Ashraf, 2003; Garg et al., 2018).

3. Material and methods

3.1 Chemicals

The following chemicals were used for the contamination process: heptane (Synth, 99%, CAS 142-82-5), chloroform (Vetec, 99.8%, CAS 67-66-3), toluene (Vetec, 99.5%, CAS 108-88-3), tetracosane (Merck, 99%, CAS 646-31-1) and benzophenone purchased in Sigma Aldrich (Madrid, Spain).

3.2 Sample

The commercial polymer used in this work was poly (lactic acid), PLA Ingeo 4043D, supplied by NatureWorks. The polymer has a melt flow index (MFI) of 6 g/10 min (ASTM D 1238).

3.3 Sample preparation

The sample preparation, described below (Table 2), was based on the (1) contamination process to simulate the worst case, (2) washing and, (3) mechanical recycling. These are steps used by the recycling industry for conventional plastic packaging and adapted for biopolymer recycling. Also, blank sample (1) without

contamination and washing was analyzed (PLA (Pristine)). All samples have been analyzed in the same way according to protocol in section 3.7 (Extraction and analysis).

3.4 Contamination protocol of poly (lactic acid) pellets

PLA pellets were subjected to contamination using the protocol procedure described by the USFDA (US Food and Drug Administration (FDA)., 2006). The protocol involved mixing surrogates to obtain a cocktail representing the worst possible post-consumer conditions for plastic food packaging, with a high risk of contamination. The same protocol was used by Paiva *et al*. (Paiva et al., 2022) to analyze the degradation compounds present in recycled polypropylene. In the first step, the PLA was immersed in the contamination cocktail for 14 days in a hermetically sealed chamber with constant agitation under a controlled temperature of 40 ºC. After this time, the pellets were submitted to the washing process. Table 2.1 describes the contaminants present in the cocktail, their concentrations, and physicochemical properties.

Table III-2.1: List of contaminants present, their concentration and physico-chemical properties in the cocktail according to FDA protocol.

Contaminants	Concentration	Properties
Benzophenone	1% (m/v)	Non-Volatile Polar
Tetracosane	1% (m/v)	Non-Volatile and Non-Polar
Heptane	78% (v/v)	Volatile and Non-Polar
Chloroform	10% (v/v)	Volatile Polar
Toluene	10% (v/v)	Volatile Non-Polar

3.5 Washing Process

The washing process used in this work was the same as that used in the recycling industry for conventional polymers from non-renewable resources as described in the literature (Franchetti and Marconato, 2006; Paiva et al., 2022). However, as described in some works, PLA is degraded by basic hydrolysis and the parameters for the washing process were studied to minimize this effect (McKeown and Jones, 2020). For this step

the following washing conditions were used; sodium hydroxide 2% (w/w), washing time 15 minutes, and 75 ºC for washing temperature.

3.6 Mechanical Recycling

Mechanical recycling of PLA was performed using the Thermo Scientific Process 11 Parallel Twin-Screw Extruder with barrel length of 40 L/D, 11 mm diameter screw. The samples went through the recycling cycle at 200 ºC, using a co-rotation speed of 400 RPM and a feeding tax 3% during the extruder. Table 2.2 presents the employed nomenclature for the steps that the samples were submitted.

Table III-2.2. Nomenclature of the PLA samples.

Sample Nº	Samples	Nomenclature #
1	PLA (Pristine)	PLAp
2	PLA contaminated	PLAc
3	PLA washed	PLAw
4	PLA recycled	PLAr
5	PLA contaminated washed	PLAcw
6	PLA contaminated washed and recycled	PLAcwr
7	PLA contaminated recycled	PLAcr

3.7 Extraction and analysis by HS-SPME-GC-O-MS

Extraction and analysis of odorous compounds were performed as follows. 0.52 g of PLA sample was added to the 20 mL SPME vial and heated at 100 ºC for 15 min. Equilibration time was 2 min and desorption time was 2 min. Odors were extracted by the headspace solid-phase microextraction (SPME) and analyzed by gas chromatography coupled to mass spectrometry together with an olfactometry port (GC-O-MS).

The fiber used for extraction of the compounds was DVB/CAR/PDMS 50/30 µm. Oliveira and Nerín, and Paiva et al. showed in their previous studies that the micropores present in this fiber facilitate the extraction of molecules with high and low molar masses (Oliveira et al., 2014; Paiva et al., 2021). Furthermore, the GC 7890N coupled to the MS detector 5077D (Agilent Technologies. Santa Clara, CA) with a sniffing port (Phaser, GL

Sciences, Germany) was used for the simultaneous analysis of volatile compounds and odor-sensitive compounds. The HP-5MS column (30 m x 25 mm x 0. 25 µm film thickness) from Agilent Technologies was used. Helium was used as carrier gas at a flow rate of 2.00 mL/min. The injector temperature was 250 ºC and the MS detector was used in scan mode (45-350 *m/z*). The oven temperature program was as follows; the initial temperature was set at 40 ºC for 5 min, with a heating rate of 10 ºC/min up to 300 ºC, then it was held for 2 min. The analytes were adsorbed in the SPME fiber. The fiber was then manually injected into the GC injection port.

3.8 Qualitative analysis of volatile and semi-volatile compounds

To analyze the volatile compounds, present in the PLA samples, we performed a combination of mass spectra and identified unknown peaks by comparing them with the NIST mass spectra library. During the olfactometric analysis, three panelists described the odors and their intensities on a scale from 1 (low intensity) to 3 (high intensity) of the compounds coming from the sniffing port installed in the GC. All panelists analyzed each sample in triplicate. After the characterization of the compounds, the retention index (RI) of the compounds detected by GC-O-MS for the PLA samples was calculated using a series of *n*-alkanes (C7-C40) injected under the same chromatographic conditions. The equation of de Van den Dool and Kratz was used (van Den Dool and Dec. Kratz, 1963). The RI and aroma characteristics of the compounds were then compared with literature and odor databases such as Flavornet, Pherobase (database pheromones), VCF online (Volatile Compounds in Food) and FlavorDB (Dongen et al., 2000; El-Sayed Ashraf, 2003; Garg et al., 2018).

4. Results and Discussion

4.1 Qualitative analysis of volatile and semi-volatile compounds

Table 2.3 shows the 33 compounds identified in all analyzed the PLA biopolymer samples. Most of the analyzed compounds originated from the degradation of the biopolymer chain and are considered NIAS of chain degradation, which are linear or branched alkanes and alkenes such as dodecane, tridecanal, octadecane and others (Misko

and Approaches, 2019). As described in the dossier entitled “Non-intentionally added substances (NIAS)”, published by the Food Packaging Forum, NIAS can originate from a number of sources including degradation of the polymer chain, generated during the recycling process, additives degradation and others (Birgit, 2018).

Furthermore, as expected, the compounds added to the contamination cocktail, and considered as IAS (chloroform, toluene, heptane, benzophenone, and tetracosane), were also identified in the samples that were submitted to this protocol. Furthermore, other NIAS were identified, and their origin cataloged as shown in Table 3. They can be attributed to the degradation of the biopolymer or to the interaction between other compounds (cocktail molecules, plasticizers, stabilizers, antioxidants additives, and UV-protectors), that may be present in the post-consumer material.

The most important of these, present in all samples, is the dimethyl-1,4-dioxane-2,5-dione (lactide), derived from the cyclic dimer of the lactic acid. Ubeda et al. also determined the presence of lactide in samples of biopolymers based on PLA and polyester, and Salazar et al. described that this dimer comes from intramolecular transesterification, unzipping depolymerization or an oxidative process (Salazar et al., 2017; S. Ubeda et al., 2019). Furthermore, this dimer is not included in the positive list of the European Regulation EU/10/2011. Therefore, the migration of this compound must be controlled (Commission Regulation (EU) No 10/2011, 2011).

Table III-2.3. Volatile compounds from biopolymer PLA samples detected by HS-SPME-GC-MS.

No	Compound	RI experimental	RI literature*	Sample	Process	Origin
1	Trichloromethane	585	590	2,5	contamination, washing	FDA protocol
2	Heptane	594	Not found	2,5	contamination, washing	FDA protocol
3	Acetic acid	621	625	4	recycling	Plasticizer
4	Toluene	764	773	2,4,5,6,7	contamination, washing, recycling	FDA protocol
5	Benzaldehyde	958	962	2,5,6,7	contamination, washing, recycling	NIAS from process
6	Benzyl alcohol	1036	1045	2,5,6	contamination, washing	NIAS from process
7	Nonanal	1110	1104	1	blank	NIAS from chain degradation
8	Dimethyl-1,4-dioxane-2,5-dione (Lactide)	1136	Not found	All samples	blank, contamination, washing, recycling	PLA oligomer
9	Dodecane	1200	1200	1	blank	NIAS from chain degradation
10	Decanal	1210	1206	1	blank	NIAS from chain degradation
11	Nonanoic acid	1272	1280	1	blank	Adhesive food-contact
12	Undecanal	1314	1309	7	contamination, recycling	NIAS from chain degradation
13	Cyclododecane	1395	Not found (1384**)	1	blank	NIAS from monomer degradation
14	2-Dodecanone	1399	1397	7	contamination, recycling	NIAS from process
15	Tetradecane	1400	1400	4	recycling	Chemical additive
16	Dodecanal	1417	1405	4,7	contamination, recycling	NIAS from chain degradation
17	2,3-Dimethylnaphthalene	1435	1410	1	blank	Plasticizer and additive

18	Diphenylmethane	1440	1418	6,7	contamination, washing, recycling	NIAS degradation-upcycling
19	2-Tridecanone	1501	1496	7	contamination, recycling	NIAS from chain degradation
20	2,4-Di-tert-butylphenol	1515	1512	1	blank	NIAS from degradation Irgafos 168 additive
21	Tridecanal	1519	1513	4	recycling	NIAS from chain degradation
22	Hexadecane	1600	1600	1,4,7	blank, contamination, recycling	NIAS from chain degradation
23	Benzophenone	1666	1644	All samples	blank, contamination, washing, recycling	FDA protocol and photocatalyzer
24	Heptadecane	1700	1700	4	recycling	NIAS from chain degradation
25	7-Ethyl-hexadecane	1706	1727	4,6,7	contamination, washing, recycling	NIAS from chain degradation
26	Tridecyloxirane	1723	Not found	4	recycling	Chemical additive
27	Octadecane	1800	1800	4	recycling	NIAS from chain degradation
28	N-Oxide-methadone	1942	Not found	2	contamination	Non identified
29	Ethyl 1-(8-dimethylamino-1-naphthyl)-1H-1,2,3-triazole-5-carboxylate	2109	Not found	2	contamination	Non identified
30	2,4,7-Trimethylcarbazole	2110	Not found	5	washing	Chemical additive
31	Benzene,1,1`,1``-(1-ethenyl-2-ylidene) tris	2183	Not found	2,4	Contamination, recycling	Non identified
32	Tricosane	2300	2300	2,3	contamination, washing	NIAS from chain degradation
33	Tetracosane	2400	2400	2,5,6,7	contamination, washing, recycling	FDA protocol

* *Collected from NIST webbook* (NIST (National Institue of Standards and Technology), 2023).

*** Normal alkane retention index was checked when retention index according to van den Dool and Kratz formula was not available.*

As mentioned above, Table 2.3 shows several compounds consisting of both IAS and NIAS. Additionally, some impurities may be present due to manufacturing and recycling process that the polymer has undergone.

Figure 2.1 shows a flowchart for the classification of NIAS types according to Birgit´s dossier description and the distribution of NIAS identified in the samples before and after the PLA recycling process according to their cataloging (Table 3) (Birgit, 2018). It can be observed that the highest concentration of NIAS was detected after the recycling process, representing 71% of the identified compounds (Table 3), while before the recycling cycle, they represented approximately 29%. Before the recycling cycle, 67% of the NIAS were attributed to polymer chain degradation, while 33% originated from the degradation of additives incorporated into the raw material, possibly during the processing step. For example, compounds such as 2,4-di-tert-butylphenol originate from the degradation of the additive Irgafos 168, and cyclododecane is classified as a NIAS compound resulting from monomer degradation (Birgit, 2018; Canellas et al., 2021).

The amount of NIAS generated by different processes increases following the recycling cycle and can be divided into three categories: (i) NIAS resulting from polymer chain degradation, which account for 56%; (ii) NIAS generated during the recycling process (including all three steps of the recycling cycle), which account for 26.7%; and (iii) NIAS not cataloged or not identified in databases, which account for 20%. As observed, the number of compounds resulting from the polymer chain degradation processes escalates due to factors such as concurrent reactions, temperatures above the glass transition temperature (Tg) of the material – thereby increasing chain mobility – and persistent shear stress during mechanical reprocessing, leading to polymer chain scission, as reported in the literature (Scaffaro et al., 2019). Examples of compounds that are NIAS in the recycling process include benzyl alcohol and benzaldehyde. Both compounds have been found in samples submitted to the three stages of the recycling process (contamination, washing, and mechanical recycling) and are a result of the contamination process.

The following substances, N-oxide-methadone, ethyl1-(8-dimethylamino-1-naphthyl)-1H-1,2,3-triazole-5-carboxylate, and benzene,1,1',1"-(1-ethenyl-2-ylidene)

tris, belong to the group of NIAS that have not been identified in the literature or listed in databases. This is a challenge and highlights the importance of reporting the ingredients and processes involved in the production of such materials.

As mentioned above, NIAS can occur in a variety way, such as chain degradation, additives, impurities, or parallel reactions, and it is not easy to predict or control them.

One of the problems associated with NIAS is that caused by the presence of IAS in materials, as is the case with Irgafos. Regulatory bodies classify, catalog the risk levels and allowable concentrations of IAS used to improve plastic packaging, such as the use of phthalates. Despite being classified as hazardous compounds; phthalates are still allowed to be used as additives in plastic packaging production. The FDA allows the use of 23 types of phthalates in FCM, while the European Regulation (EU) No. 10/2011 sets the migration limit for six phthalates at 0.05 to 30 mg/kg (Commission Regulation (EU) No. 10/2011, 2011; Food and Drug Administration, 2022). In the case of IAS, they are subject to relevant regulations and cataloged by regulatory authorities. However, for some IAS classified as chemical additives, there is no catalog specifying their use, as in the case of compounds tridecyloxirane and 2,4,7-trimethylcarbazole.

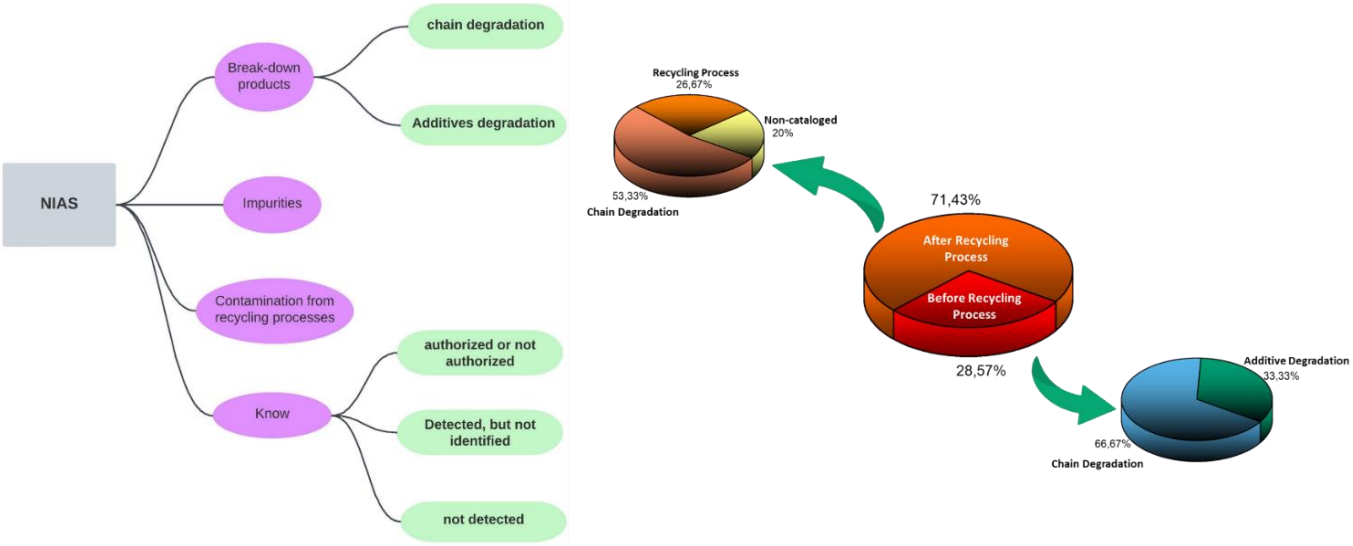


Figure III-2.1. Flowchart of NIAS classification and distribution before and after the recycling process applied to PLA biopolymer samples.

4.2 Qualitative analysis of odorous compounds in PLA sample

The HS-SPME-GC-O-MS olfactometric analysis revealed that 14 out of the 33 compounds identified in the PLA samples had odor characteristics (see Table 2.4). The odorous compounds were mostly aldehydes from the degradation of the PLA chain, which is an organic polymer composed of lactic acid molecules (McKeown and Jones, 2020). In addition, cyclic compounds, naphthalenes, and alkanes also showed odor characteristics. The panelists classified the odor type and intensity in each sample, as described in Table 3. The odors were classified into aroma groups listed and catalogued in databases such as FlavoDB and Pherobase and compared with data from the literature (El-Sayed Ashraf, 2003; Garg et al., 2018).

Therefore, the aroma groups were established and are listed in Table 2.4, correlating with the detected odors which were classified as Group Toasted (cherry, almond, burnt sugar, sharp, strong, bitter), Group Flower (berry, balsamic, rose, floral, walnut, sweet, cherry, phenolic, flower, grapefruit), Group Green (gravy, green, tallowy, fruity, gas, chlorine, floral, sweet, melon, soapy, fatty, lavender, citrus fruit), Group Green distasteful (stewed, burnt, green, floral, lemon, fatty, herbal, soapy), Group Chemical, acid (green, fat, musty, sweaty, sour), Group Oil, waxy (oil, naphthalene-like), Group Glue-like (glue-like) and Group Alkene (fruity, sweet, alkane).

Table III-2.4. Results of qualitative analysis of odorous compounds identified in different biopolymer PLA samples and their classification into aroma groups by olfactometric panelists.

No	Compound	RI experimental	RI literature	Panelists odor description	Odor literature description	Aroma Group	Intensity	Sample
1	Benzaldehyde	958	962	geranium	cherry, almond, sweet, burnt sugar, sharp, strong, bitter, almond.	Toasted	1.5	2
2	Benzyl alcohol	1036	1045	antibiotic, poultry, wastewater channel	berry, balsamic, rose, floral, walnut, sweet, cherry, phenolic,	Flower	2 1.5 1.5	2 5 6

					flower, grapefruit, sweet.			
3	Nonanal	1110	1104	carob	gravy, green, tallowy, fruity, gas, chlorine, floral, waxy, sweet, melon, soapy, fatty, lavender, citrus fruit.	Green	2,5	1
4	(3S)-cis-3,6-Dimethyl-1,4-dioxane-2,5-dione	1136	Not found	raw resin, forest, chestnuts	Not found		1.5 1.5 1 2 1.5 2	1 2 4 5 6 7
5	Decanal	1210	1206	pickled cabbage rotten	stewed, burnt, green, floral, lemon, fatty, herbal, soapy.	Green, distasteful	1.5	1
6	Nonanoic acid	1273	1272	textile store	green, fat, musty, sweaty, sour	Chemical, acid	1.5	1
7	Cyclododecane	1395	Not found (1384*)	tool shed	Not found		2	1
8	Dodecanal	1417	1405	cake, vanilla	berry, balsamic, rose, floral, walnut, sweet, cherry, phenolic, flower, grapefruit, sweet.	Flower	2,5	4
9	2,3-Dimethylnaphthalene	1435	1410	vanilla	Oil, Naphthalene-like	Oil, waxy	2	1
10	Diphenylmethane	1440	1418	car oil + chemicals	glue-like	Glue-like	1.5	6
11	2,4-Di-tert-butylphenol	1515	1512	Naphthalene, solvents	oil, naphthalene-like	Oil, waxy	2	1

12	Benzophenone	1666	1644	old buses, fuel, kerosene	berry, balsamic, rose, floral, walnut, sweet, cherry, phenolic, flower, grapefruit, sweet.	Flower	2.5 1.5 1.5 2.5 1.5 1.5	2 3 4 5 6 7
13	7-Ethylhexadecane	1706	1727	mixed pickles	fruity, sweet, alkane	Alkene	1.5	4
14	Tridecyl oxirane	1723	Not found	Celery, moderate rose	Not found		1	4

** Collected from NIST webbook* (NIST (National Institue of Standards and Technology), 2023).

*** Normal alkane retention index was checked when retention index according to van den Dool and Kratz formula was not available*

As described in the literature, compounds of the aldehyde and alcohol classes presented odors that classified them into natural odor groups, for example, benzyl alcohol in the Group Flower and nonanal in the Group Green (Garg et al., 2018). The contamination cocktail proposed by the USFDA and adopted to simulate the worst conditions that PLA packaging can be exposed to contain toluene and chloroform. Therefore, from the chlorination of toluene and the subsequent hydrolysis process can produce compounds such as benzyl alcohol and benzaldehyde can be formed as described in the literature. These two compounds are classified as NIAS processes, but they do not pose a risk to the properties of the material at low concentrations, as they are widely used in the flavoring and pharmaceutical industries and as precursors to a variety of esters and ethers (Shoukat et al., 2021).

Furthermore, as can be seen in Table 4, the odoriferous characteristic of benzyl alcohol was only detected in sample 2 (PLAc), although it was identified by GC-MS in samples 5, 6 and 7 (Table 2.3). It is possible that the recycling process could affect the way we perceive the smell and identify the odor of the compound. This could be due to subsequent stages of the process interfering with olfactometric perception, or a decrease in the concentration of the compound after recycling. Benzyl alcohol is a volatile compound and can be affected by changes in temperature.

Nonanal, decanal, and dodecanal are volatile aldehydes with a major impact on the odor profile of the analyzed PLA samples. These compounds are naturally present in many foods and therefore do not pose a risk to food safety and packaging (Osorio et al., 2019, 2019). Furthermore, the EU implementing regulation 2017/53 of December 14, 2016, has authorized the use of these compounds as additives in animal feed, and therefore they may be present in meat available to consumers (Ministerio de la Presidencia, 2003). Osorio *et al*. also determined the presence of these compounds in starch-based polymers and concluded that the odoriferous profile of the samples was determined by the odor influence of these compounds (Osorio et al., 2019). The phenolic compound 2,4-di-tert-butylphenol is described in the literature as a NIAS derived from the degradation of additives such as Irgafos 168 and Irganox 1010, two antioxidants widely used as additives in PE, PP, and in other thermoplastic packaging resins (Nerín et al., 2023). However, in the PLA sample, the presence of these additives, which could give rise to 2,4-di-tert-butylphenol, was not observed by HS-SPME-GC-O-MS, as expected, since they are not volatile compounds, but the possibility of the presence of this additive at low concentrations cannot be ruled out.

2,3-Dimethylnaphtalene is a polycyclic aromatic hydrocarbon (PAH) that is commonly found in polystyrene (PS), PP and PET. It should be highlighted that PAH is classified as an environmental pollutant and, as described by Rochman et al. (Rochman et al., 2013). PHAs are mainly found in virgin polymer pellets and may be present in plastic material after manufacture. In the case of PLA samples, this compound was identified only in the original sample (PLAp (1)), in virgin pellets and with a characteristic vanilla odor.

Benzophenone is an organic compound with many applications such as a photoinitiator in polymerization processes, protector against UV light degradation, ingredient in cosmetic products and in the flavor industry (Silano et al., 2017). Nevertheless, this compound causes a health hazard when used at high concentrations (Food Standards Agency, 2006). In addition, it is present in the formulation list of the contamination cocktail proposed by the USFDA to represent food safety risk of phenolic compounds. It has odor characteristics and is classified in the Group Flower because it is a natural compound produced by flowers as reported in FlavorDB database (Garg et al., 2018). Among the aroma compounds identified in samples and classified into groups, three compounds were not found and classified (NF*) because of a lack of information.

The radar graph shown in Figure 2.2 elucidates the impact of the aroma compounds classified and separated into odor groups, as described in Table 2.4, present in the PLA samples. As can be seen, the odor profile of the PLA samples is concentrated in 4 of the 7 groups listed as follows: Group Toasted, Group Flower, Group Green, distasteful and Group Chemical, acid.

PLA is a biopolymer from a renewable source, it contains many organic compounds that can be found in nature and has odoriferous characteristics ranging from sweet to floral profiles. However, when PLA is subjected to the recycling steps, other odors from compounds present in PLA may be added and/or intensified, either by the presence of NIAS, IAS, impurities or degradation compounds, altering the odor profile of the material.

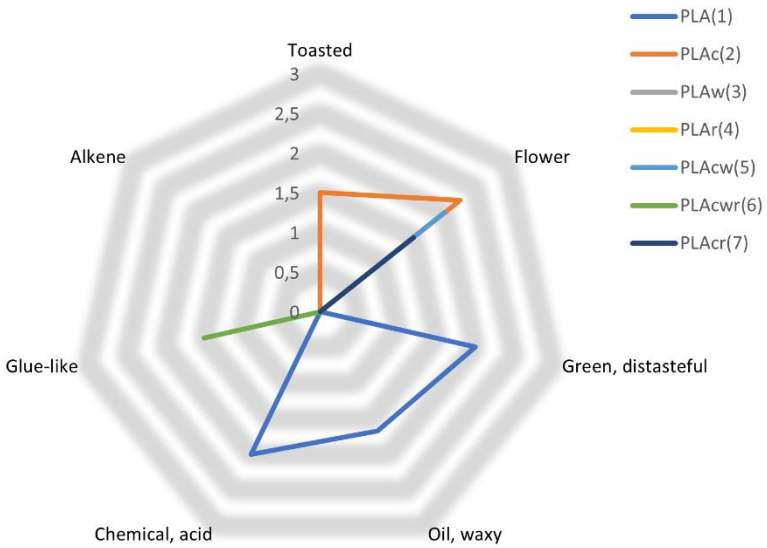


Figure III-2.2. Results of the odor profile of the different PLA biopolymer samples, represented by the radar plot comparing the odor intensity described by the panelists and the catalogue in different odor groups.

5. Conclusions

In the present study, 34 different volatile and semi-volatile compounds were identified by HS-SPME-GC-MS in the PLA pellets subjected to the post-consumer process, washing process and recycling cycle. Most of the substances detected in the analysis were the result of the biodegradation biopolymer chain, such as linear or branched alkanes and alkenes. The primary NIAS found in all samples was dimethyl-1,4-

dioxane-2,5-dione (lactide), derived from the cyclic dimer of lactic acid. It should be noted that this dimer is not included in the positive list of European Regulation EU/10/2011, and therefore the migration of this substance has to be monitored. Therefore, the relationship between the production of NIAS and its categorization with the recycling process has been established. An increase in NIAS was observed after the recycling stages, while the types before (from additives and chain degradation) and after (from the recycling process and chain, as well as non-cataloged) were determined. However, no formation of NIAS or specific degradation compounds was detected during the washing process. Analysis by HS-SPME-GC-O-MS revealed 14 different odorous compounds classified into 4 aroma groups (Toasted, Flower, Green and Chemical). Among them, compounds such as nonanal, decanal and dodecanal (volatile aldehydes), 2,3-dimethylnaphthalene (PAH) and 2,4-di-tert-butylphenol were detected. Moreover, the odorous compounds benzyl alcohol and benzaldehyde were formed during the chlorination of toluene (a component of the contamination cocktail) and subsequent hydrolysis process. The presence of off-odors can indicate the formation of harmful compounds that may can affect the sensory properties of the food, and even compromise its safety. Therefore, it is essential to identify the source of off-odors and volatile compounds in recycled PLA and take appropriate measures to prevent their formation or remove them from the material. The results obtained show that the analysis of volatile compounds and off-odors in PLA from recycling cycles is crucial to ensure the food safety and quality of biopolymers used for packaging applications.

Chapter 3

Biobased Polymer Recycling: A Comprehensive Dive into the Recycling Process of PLA and Its Decontamination Efficacy

1. Abstract

The recycling process and the presence of contaminants play an important role in alimentary security. In this research, polylactic acid (PLA) samples were contaminated using an FDA-defined framework by immersing them in a cocktail of five surrogates (benzophenone, tetracosane, heptane, chloroform, and toluene). Migration tests were then conducted on the samples after different recycling stages using two food simulants and varying conditions. To this end, it was used SPME-GC-MS and GC-MS techniques in order to evaluate the efficiency of the recycling technology. The contaminants in PLA samples decreased after being submitted to the total recycling cycle. Compounds such as tetracosane, heptane, and toluene showed decreased values between 73% and 80%, much higher than those obtained when the sample went only through washing or mechanical recycling. The theoretical molecular volume of contaminants, the type of food simulants, the temperature of the test, and the interactions between polymer and surrogates influence the input and output of contaminants. The interaction energy values estimated from electronic structure calculations prove useful for predicting and analyzing global interaction trends between contaminants and the polymer. Consistency was observed between the results obtained from the Hansen sphere analysis and the theoretical approach.


PLA
contaminated
Using a FDA contamination protocol
MECHANICAL RECYCLING
Determination of the initial concentration of contaminants
POLYMER RECYCLING Vs FOOD SAFETY
DI-SPME-GC-MS
HS-SPME-GC-MS
ANALYSIS
ANALYSIS
QUANTUM CHEMICAL CALCULATIONS

2. Introduction

Efforts to substitute traditional plastic materials with those derived from renewable resources have gained prominence due to the environmental impact caused by improper disposal of plastic products. Since the 1950s, the establishment of the polymer and plastics processing industry has contributed to improving the quality of society in areas ranging from textiles to food packaging or hospitals (Pires et al., 2022). According to Leal Filho et al. and the Plastic Atlas data, packaging accounts for 50 % of the annual production of plastic products, which has overtaken other industries during the COVID-19 pandemic due to the increase in the use of single-use plastic materials (Böll, 2019; Leal Filho et al., 2021). However, plastic packaging accumulation and improper disposal are increasing over time, and reuse opportunities and environmental protection policies are not keeping pace with production and consumption (Böll, 2019; Stiftung, 2020).

The European Commission has published new directives to minimize impact, promote a culture of environmental responsibility, and facilitate the implementation of the circular economy to reduce non-renewable resources to produce single-use packaging until their total elimination/exchange for materials from renewable sources. In addition, the new guidelines aim to promote the use of recycled raw materials and include the use of biodegradable bio-based polymers in manufacturing plastic packaging (Commission, 2018; First Vice-president Frans and Jyrki, 2019).

The use of bio-based polymers from renewable sources is an essential alternative for reducing environmental concerns, mainly because it reduces the dependence on petroleum-based materials (Stoica et al., 2020). However, the problem of accumulation and improper disposal will persist as an environmental aggravating factor since not all bio-based polymers are biodegradable. Furthermore, bio-based polymers are only classified as biodegradable and/or compostable under specific conditions as described in ASTM D6400, D5988-18, and ISO 14855 standards (American society for testing and materials, 2019; Pires et al., 2022).

On the other hand, recycled materials with the necessary approvals can also be used to manufacture plastic packaging intended to come into contact with food, as described in many studies and indicated by the European Commission and other amendments (ANVISA, 2004; Commission Regulation (EU) No 10/2011, 2011; US Food and Drugs Administration (FDA), 2021). Moreover, if the packaging is intended to

contact food, it must not pose any risk to consumers of ingesting hazardous substances transferred from the packaging to food (Beneventi et al., 2020; Vitrac and Hayert, 2005; Wrona and Nerin, 2019).

Although it may seem contradictory initially, several studies have investigated the recycling of bio-based polymers, specifically those made of PLA, due to their good recyclability and biocompatibility properties (Beltrán et al., 2021; Dhar et al., 2018; McKeown and Jones, 2020; Moreno et al., 2020; Scaffaro et al., 2011). In addition, using recycled bio-based polymers can improve the added value of the material since their production from renewable sources is frequently more expensive than petroleum-based counterparts.

One of the concerns about adopting the conventional recycling process applied to post-consumer materials from non-renewable sources, such as poly (ethylene terephthalate) (PET) and polypropylene (PP), to bio-based polymers, is the washing stage, which uses high temperatures and alkaline solutions that can catalyze the degradation process by basic hydrolysis, affecting the material's physic-chemical properties and making it impossible to recycle. However, some studies focusing on optimizing the washing parameters for recycling PLA have shown that reducing the molar mass can be controlled (Chariyachotilert et al., 2012; Paiva et al., 2024). Paiva *et al*. demonstrated in an in-depth study of these variables that using experimental design can reduce the impact on molar mass during the washing process while maintaining the properties of the bio-based polymer (Paiva et al., 2024).

Thus, this work has important relevance to the study of PLA bio-based polymer recycling, analyzing the stages of the recycling cycle, which are (i) post-consumer packaging material using the contamination protocol proposed by the FDA, (ii) washing process and, (iii) mechanical recycling. This approach is particularly valuable as it not only addresses a significant gap in the current literature — where a fully detailed recycling cycle for bio-based polymers, particularly for food contact applications, remains unexplored — but also offers practical insights directly applicable in industrial settings, especially concerning the mitigation of contaminants. By detailing each stage, this study contributes to optimizing recycling processes, reducing environmental impact, and promoting the sustainable management of bio-based polymer waste.

Understanding the migration of contaminants is crucial to ensuring the safety of recycled products, especially for applications involving food contact. Monitoring and understanding the behavior of migration of these substances allows for the development of more accurate standards and regulations. Furthermore, establishing theoretical aspects to predict and understand migration behavior enables anticipating potential risks, optimizing recycling processes and polymer formulations, thereby advancing material science and creating safer, more efficient solutions for bio-based polymers.

In this sense, the efficiency of recycling in decontaminating these materials was evaluated through migration tests in different simulants and the total dissolution of the pellets after the process. The contaminants were analyzed using solid-phase microextraction employed direct injection into the gas chromatograph coupled to mass spectrometer using the DI-SPME-GC-MS technique. The understanding of these behaviors was only elucidated through an in-depth evaluation of Hansen's solubility parameter and a theoretical methodology capable of evaluating the molecular volume of each system, as well as the interaction energy between each contaminant and the monomeric structure of PLA.

3. Materials and Methods

3.1 Chemicals

The chemicals used for the FDA contamination protocol were benzophenone (Across Organics, 99 %, CAS 119-61-9), tetracosane (Merck, 99 %, CAS 646-31-1), toluene (Vetec, 99.5 %, CAS 108-88-3), heptane (Synth, 99 %, CAS 142-82-5) and chloroform (Vetec, 99.8 %, CAS 67-66-3). Sodium hydroxide (Panreac, 98 % CAS 1310-73-2) and Triton X-100 (Exôdo science, CAS No. 9036-19-5) were used for washing. For the dissolution protocol, ethanol (EtOH) (No. CAS 64-27-5), and dichloromethane (DCM) (No. CAS 75-09-2) were purchased from Panreac (Barcelona, Spain).

Ethanol (HPLC grade No. CAS 64-27-5) and acetic acid (Sigma Aldrich, 99.8 %, CAS No. 540-84-1) were supplied by Scharlau Chemie S.A. (Sentmenat, Spain) and used to prepare food simulants. Ultrapure water was obtained from a Milli-Q Ultramatric Wasserlab GR 216071 (Barbatain, Spain).

3.2 Sample

The commercial bio-based polymer used in this work was poly (lactic acid), PLA Ingeo 4043D, with an L-lactide content of 98%, supplied by NatureWorks. The polymer had a melt flow index (MFI) of 6 g/10 min (ASTM D 1238), 210 °C, 2.16 g/cm^3 (ASTM D792). A challenge test was applied to demonstrate the efficiency of the decontamination technology under study.

3.3 Sample preparation

3.3.1 FDA contamination protocol of poly (lactic acid) pellets

The PLA pellets were subjected to a forced contamination protocol procedure (challenge test) described in the USFDA (2006) (US Food and Drug Administration (FDA), n.d.). The protocol is described as a contamination cocktail containing a mixture of surrogates representing the worst post-consumer conditions to which a plastic package can be subjected. As described previously, these contaminants encompass a diverse range of chemical substances that could potentially expose consumers to harmful compounds. The concentrations used in this study were deliberately elevated to simulate a worst-case scenario. The surrogate cocktail comprised chloroform, toluene, tetracosane, and benzophenone (US Food and Drug Administration (FDA), n.d.). Chloroform and toluene were selected as they are standard components of cleaning solvents, benzophenone was chosen to represent non-volatile polar pesticides, and tetracosane was included to simulate motor oil contamination. Although this protocol is widely used for polymers such as PET and PP, it can also be applied to all recyclable polymers that will be employed in food-contact material, including PLA simulating of plastic packaging with high recycling potential (Boutroy et al., 2006; Cruz et al., 2009; Incarnato et al., 2003; Romão et al., 2009; US Food and Drug Administration (FDA), n.d.).

In the first step, PLA was immersed in the contamination cocktail for 14 days in a hermetically sealed chamber under constant agitation at a controlled temperature of 40 °C. The surrogates used in the cocktail have physicochemical characteristics that cover a wide class of possible contaminants that can be present in post-consumer packaging. The

physicochemical characteristics of the compounds used in this step and their concentration, as determined by the FDA protocol, are listed in Table 3.1.

Table III-3.1: Concentration and properties of FDA protocoled contaminants (surrogates) used in a cocktail.

Contaminants	Concentration	Properties
Benzophenone	1 % (m/v)	Non-Volatile Polar
Tetracosane	1 % (m/v)	Non-Volatile and Non-Polar
Heptane	78 % (v/v)	Volatile and Non-Polar
Chloroform	10 % (v/v)	Volatile Polar
Toluene	10 % (v/v)	Volatile Non-Polar

Afterward, PLA pellets were subjected to washing and mechanical recycling, as described below.

3.3.2 Washing and mechanical recycling

As described earlier, the washing process is similar to that used in the plastics recycling industry for plastics made from conventional non-renewable source polymers. In this way, the parameters used in the washing process were the same as those studied by Paiva et al. (Paiva et al., 2024). To minimize the effects of degradation by hydrolysis that PLA can suffer, the following washing conditions were used: sodium hydroxide 2 % (w/w) and surfactant 3 % (w/w) as washing solution, applied for 15 minutes at 75 °C.

Mechanical recycling of PLA was performed using a Thermo Scientific Process 11 Parallel Twin-Screw Extruder with 40 L/D barrel length, 11 mm diameter screw, and standard configuration using push screws. The samples went through four recycling cycles at 200 °C, using the co-rotation speed of 400 rpm and feed rate of 3 % during the extruder. Table 3.2 describes the samples and the terminology used in this work.

Table III-3.2: Description of the PLA samples for migration and dissolution testing.

Sample	Nomenclature	Description
1	PLAc	PLA contaminated
2	PLAcw	PLA contaminated and washed
3	PLAcr	PLA contaminated and mechanically recycled
4	PLAcwr	PLA contaminated, washed, and recycled

3.3.3 Total dissolution methodology

This methodology was developed and applied by Ubeda et al. (Úbeda et al., 2017). This protocol was adequate for the determination and quantification of volatile compounds in bio-based polymers. In this protocol, 0.25 g of PLA pellets stored in 20 mL glass vials were dissolved in 3 mL dichloromethane solvent in an ultrasonic bath for 1 hour. Then, 6 mL of ethanol was added to the solution, acting as an antisolvent, and the mixture was centrifuged at 500 rpm for 15 minutes. The supernatant was removed, and the mixture was filtered through a 0.25 µm PET filter. Triplicates of each sample were prepared, and the compounds were injected into a gas chromatograph coupled to a mass spectrometer (GC-MS) by liquid injection (LI) and solid phase microextraction (SPME). Aznar and co-workers systematically studied this methodology to determine non-volatile components based on polyester and PLA in biodegradable food packaging. Its effectiveness in dissolving samples and not detecting the apparent reabsorption of the analyte after reprecipitation was demonstrated (Aznar et al., 2019).

3.3.4 Migration tests

The PLA samples were subjected to a migration test in order to determine the transference of surrogates from packaging to food simulants at the different recycling steps. The simulants used for the study were simulant C, ethanol 10% (v/v), and simulant B, acetic acid 3% (v/v). Both simulants are assigned to food with hydrophilic character. Food simulant B shall be used for those foods with a pH below 4.5 (Commission Regulation (EU) No 10/2011, 2011). Furthermore, migration tests were performed by total immersion. For this purpose, 2 x 3 cm pieces of the samples were placed in 20 mL vials, and 20 g of the simulant was added according to the rate of 6 dm^2 contact surface/kg

of simulant, established by Regulation EU/10/2011. Once the vials were filled with the simulant, they were subjected to the following migration conditions: 10 days at 40 ºC and 10 days at 60 ºC. Both test conditions are established in the legislation to simulate storage at room temperature and mild conditions not to compromise the integrity of the PLA (Commission Regulation (EU) No 10/2011, 2011).

3.4 Analysis by GC-MS

As described previously, the contamination cocktail contained volatile and semi-volatile compounds, and for a better performance in the analysis of these compounds, both SPME and liquid injection were tested. In the case of total dissolution samples, chloroform, and heptane were analyzed by SPME, and toluene, benzophenone, and tetracosane were analyzed by direct liquid injection (1 µL) since they provided better sensitivity. Migration samples were all analyzed by SPME. For the analysis of the total dissolution samples, 20 µL of the extracts were added to a 20 mL vial that was hermetically closed and analyzed by headspace SPME (HS-SPME). For the analysis of migration samples, 15 mL of the migration solutions were transferred to vials that were hermetically closed and analyzed by total immersion SPME (TI-SPME). The SPME fiber used for the analysis was a DVB/CAR/PMDS 50/30 µm. This fiber was selected based on its microporous structure, which has been reported in the literature (Osorio et al., 2019c; Paiva et al., 2021a) to increase extraction efficiency. The SPME extraction temperature and time conditions were 60°C and 15 min, respectively.

GC-MS analysis was performed in a CTC Analytics CombiPal from CTC Analytics AG (Zwingen, Switzerland) coupled to a GC 6890N gas chromatograph from Agilent (Palo Alto, CA, USA). The separation of the analytes was performed on an Agilent HP-5 MS column (30 cm x 0.25 mm, 0.25 µm layer thickness). The temperature program used started at 40°C for 5 min and increased by 10 °C min^{-1} to 300 °C hold for 1 min. The injector temperature was 250°C, and helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. An Agilent 5975 mass spectrometer was used as the MS source, and a quadrupole temperature detector was set at 230 °C and 250 °C, respectively. The SIM mode was used for acquisition with the following ion parameters: chloroform (83.00 and 118.00 m/z), heptane (57.00 and 71.00 m/z), toluene (51.00 and 91.00 m/z), benzophenone (77.00 and 105.00 m/z), and tetracosane (338.00 m/z).

Standard solutions were prepared in DCM:EtOH (1:2) (similar to the final solvent of the PLA dissolution protocol), and in both food simulants (EtOH 10% and HAc 3%) for quantitative analysis. The analytical parameters of the methods used were determined: linearity, linear range, limits of detection (LOD), and limits of quantification (LOQ). LOD and LOQ were determined by applying SPME-GC-MS to pure standards of the compounds, under the same analytical conditions as those applied.

To enhance the understanding of the data, statistical analyses were performed for each contaminant in each simulant liquid under the applied migration test conditions. A one-way ANOVA test was conducted to determine the F and P-value, which are presented in Table 3.4 in the Annex section.

3.5 Theoretical Methodology

Electronic structure calculations theoretical computational methods and the solubility model proposed by Hansen were used to discuss the polymer-solvent/non-solvent interaction (HANSEN SOLUBILITY PARAMETERS A User's Handbook Second Edition, 2007; Redelius, 2007). The structure of each system, heptane, chloroform, benzophenone, toluene, tetracosane, and PLA monomer was optimized at the GFN2-XTB method (Bannwarth et al., 2019), as implemented in the ORCA program (Neese, 2018). The L-lactic molecule was considered for modeling the PLA system because electronic structure calculations using the actual polymer would be impractical. Furthermore, at the atomic level, these calculations allow us to rationalize the main local interactions present in the macroscopic system. All structures were confirmed as a minimum in the explored potential energy surface. The GEPOL algorithm, though the solvation model based on density (SMD) at the DFT/M06-2X/def2-TZVP level of theory (Marenich et al., 2009; Pascual-ahuir et al., 1994), was used to evaluate the molecular volume of each system in the heptane solvent. Furthermore, the interaction energy (ΔE), based on the GFN2-XTB energies, between each contaminant and the PLA monomer was estimated by considering the cluster (PLA + contaminant) with the lowest energy. The non-covalently bound complexes (PLA + contaminant) with the lowest energy were found using the NCI model CREST program (Pracht et al., 2020). Finally, Figure 3.1 shows the flowcharts of the work from the PLA recycling steps to sample preparation and subsequent chromatographic and theoretical-computational analysis.

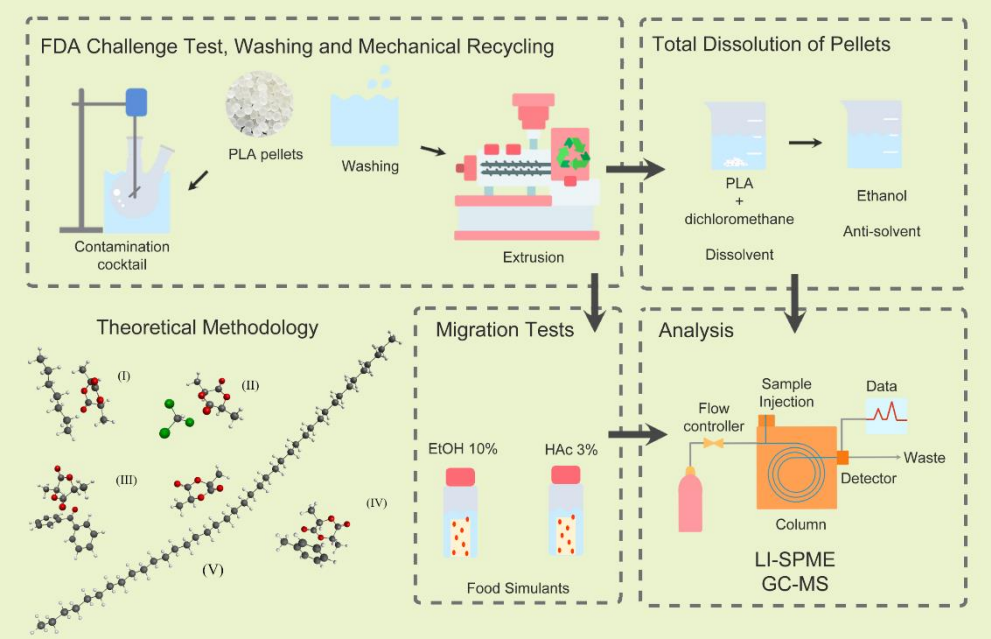


Figure III-3.1: Flowcharts of the work: FDA challenge test, washing and mechanical recycling steps, total dissolution of pellets, migration tests, analysis, and theoretical methodology.

4. Results and discussion

4.1 Influence of the PLA recycling cycle on the concentration of contaminants

a) Contaminants teor on pellets: an experimental and theoretical approach

Figure 3.2a shows the concentration of contaminants in the PLA pellets, which means the initial concentration of surrogates. The actual concentration of contaminants present in PLA was analyzed for the contaminated samples before and after the recycling cycle, being analyzed at each cycle stage. Furthermore, Figure 3.2b shows the results of calculating the Efficiency factor of the recycling process in decontaminating the samples by Equation 3.1, comparing each stage of the recycling cycle and the complete process. In addition, the analytical parameters of the FDA contaminant protocol calibration curves are provided in Annex 1 and 2 (Table 3.1 and Table 3.2). Furthermore, Table 3.3 (Annex)

shows the same results of the concentration of surrogates that migrated to the two different simulants under different migration conditions for a better interpretation of the data.

$$Ef\ (\%) = \frac{C_i - C_f}{C_i} \tag{3.1}$$

Where, C_i is the initial concentration of the contaminants present in the PLA sample, PLAc, and C_f the final concentration after the recycling steps.

After undergoing the washing stage, there was a significant decrease in the concentration of the contaminant tetracosane, plummeting from 223.30 to 46.84 mg/kg. In contrast, samples solely subjected to the mechanical recycling stage showed a lesser reduction (91.12 mg/kg), showing this step is fundamental. However, a reverse trend emerged when heptane was analyzed in the same samples. The mechanical recycling stage proved to be more effective in reducing heptane concentration, registering 26.53 mg/kg compared to 71.08 mg/kg for the washing stage, despite the initial concentration of 109.38 mg/kg for PLAc.

As can be seen in Figure 3.2b, individually, the washing (PLAcw) and mechanical recycling (PLAcr) steps have shown high efficiency in reducing the concentration of contaminants, with the mechanical recycling step being more effective than the washing step values above 60%. However, the combination of the two steps (PLAcwr) is more effective than each individual step because, as described above, the decontamination efficiency for some surrogates is more significant in one step than in the other. In general, when comparing final values with the high levels of contamination before the process, it can be seen that the recycling cycle showed great potential in the decontamination process.

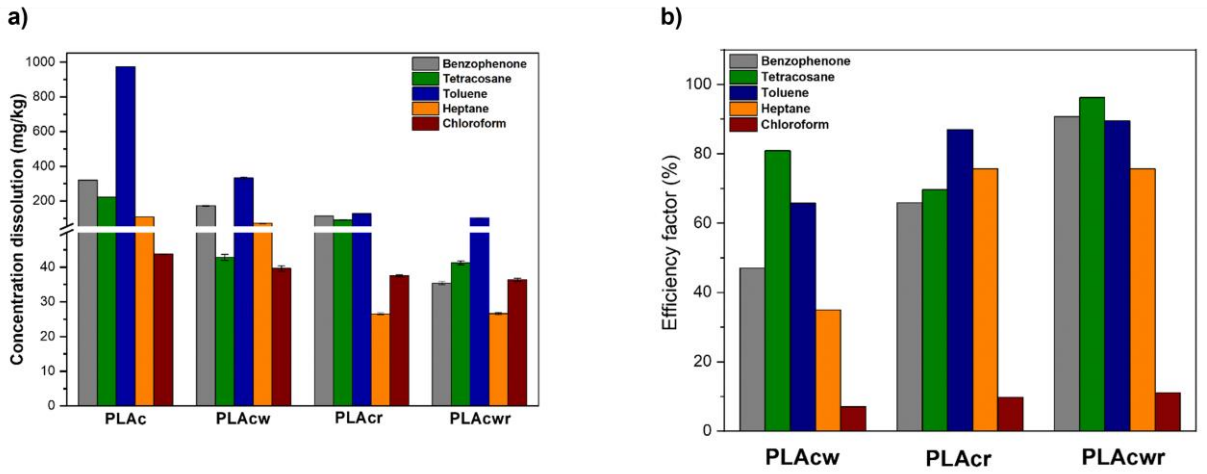


Figure III-3.2: a) Contaminants concentration for the samples and, b) efficiency factor for PLA decontamination.

The content of contaminants presents in a polymer sample after contact with the cocktail to simulate the worst case depends on thermodynamic factors. This complexity can be understood in the same way as the solubility of polymers since migration into polymers depends on phases similar to those of solubilization. In both processes, first, the solvent needs to wet the polymer. Then, the solvent/contaminants diffuse into the polymer, causing it to swell. This process may take several hours or longer for polymers of high molar mass, depending on sample size, temperature, and other factors. Thus, our discussion will focus on (1) solubility parameter aspects, which guide the initial phase and entry of contaminants into the polymer, (2) molecular volume which directly impacts diffusivity into the polymer, and (3) vapor pressure, discussed through interactions and in a liquid-vapor equilibrium, its permanence in the liquid medium to allow contamination of the polymer.

Considering that the first step is associated with the interaction between polymer and contaminants, a model used to evaluate the solubility/interaction between a polymer and a solvent or non-solvent was proposed by Hansen (HANSEN SOLUBILITY PARAMETERS A User's Handbook Second Edition, 2007; Redelius, 2007). This theoretical-experimental model defines solubility in terms of the cohesive energy density of the molecule (δ_H), which can be described from the partial contributions of three main intermolecular interactions described in Hansen's extended equation (Equation 3.2) (HANSEN SOLUBILITY PARAMETERS A User's Handbook Second Edition, 2007).

$$\delta_H = \sqrt{\delta_d + \delta_p + \delta_h} \quad (3.2)$$

Where δ_d is the dispersive interaction, δ_p is the polar interaction and δ_h is the hydrogen bonding interaction. These components are listed for the PLA and for each of the compounds present in the cocktail, Table 3.3. For PLA the components are listed in Table 3 and its solubility radio is R_0 = 8 (HANSEN SOLUBILITY PARAMETERS A User's Handbook Second Edition, 2007). In addition, Table 3.3 presents the molecular volume of each system obtained at the SMD (heptane)-DFT/M06-2X/def2-TZVP level of theory, as described above. It has been hypothesized that analyzing these parameters allows associating a higher content of migrants, since chemical affinity is the first thermodynamic barrier to be overcome and will dictate the amount of diffusion into the polymer. In addition, the molecular volume associated with thermodynamic factors may affect the process of contaminant diffusion into the polymer.

Table III-3.3: Hildebrand solubility parameters, vapor pressure and theoretical molecular volume of the compounds present in the contamination cocktail.

Compound	δ_d (MPa)$^{0.5}$	δ_p (MPa)$^{0.5}$	δ_h (MPa)$^{0.5}$	P^o (hPa)	Molecular Volume (Å^3)
PLA	18.6	6.0	9.9	-	194.3
Benzophenone	19.6	8.6	5.7	1.33	211.7
Chloroform	17.8	3.1	5.7	210	140.9
Toluene	18.0	1.4	2.0	30.88	109.6
Heptane	15.3	0	0	111	136.5
Tetracosane	-	-	-	-	707.9

δ_d= dispersive interaction; δ_p= polar interaction; δ_h= hydrogen bonding interaction and P^o = vapour pressure.

In addition, Equation 3.2 and the tabulated components allow us to quantify the polymer's solubility from the interaction distance, D, as described in Equation 3.3.

$$D = \sqrt{4\left(\delta_{d\,sol} - \delta_{d\,pol}\right)^2 + \left(\delta_{p\,sol} - \delta_{p\,pol}\right)^2 + \left(\delta_{h\,sol} - \delta_{h\,pol}\right)^2} \quad (3.3)$$

Finally, from Equation 3.3 and the solubility radius of PLA, a three-dimensional solubility sphere can be represented in Figure 3.3a (HANSEN SOLUBILITY PARAMETERS A User's Handbook Second Edition, 2007). The solubility sphere makes it possible to analyze the polymer-solvent/non-solvent interaction since the smaller this interaction distance, the greater potential for the compound to be classified as a good solvent, which means that it has a higher interaction with the polymer. It has been hypothesized that analyzing these parameters allows associating a higher content of migrants since chemical affinity is the first thermodynamic barrier to be overcome and will dictate the amount of migrants diffused into the polymer.

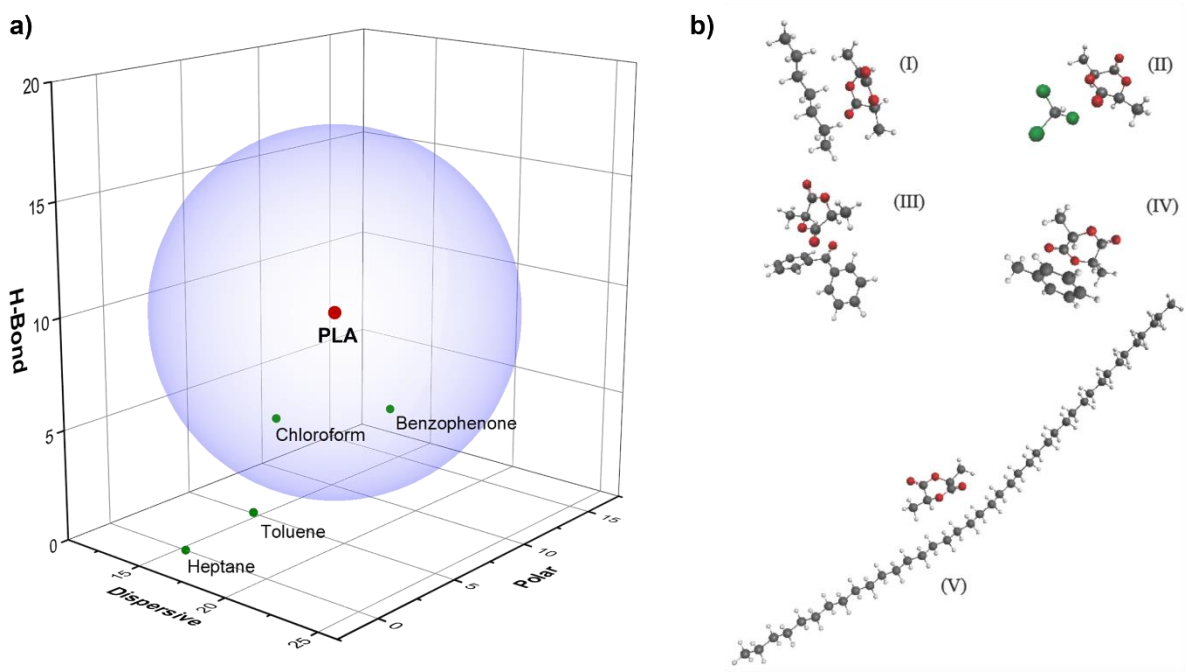


Figure III-3.3: a) Sphere of solubility of PLA and the solubility parameter components of contaminants, and b) the lowest energy aggregates formed between PLA monomer and each molecular contaminants I) heptane, II) chloroform, III) benzophenone, IV) toluene and V) tetracosane. Hydrogen = white; oxygen = red; chlorine = green.

A rudimentary examination of Figure 3.3a enables us to deduce that chloroform and benzophenone exhibit favorable solvent properties, whereas toluene and heptane can be categorized as non-solvents. Nevertheless, upon conducting a comparative analysis with Figure 3.2 for the PLAc sample, it becomes apparent that greater diffusivity into

PLA was observed with the contaminants toluene, benzophenone, tetracosane, heptane, and chloroform, in sequential order. However, the model has its limitations, and as noted, it was not possible to analyze the polymer-solvent/non-solvent interaction for tetracosane because the Hildebrand solubility parameters for this compound are not available in the literature.

Another way to discuss the interaction between the PLA monomer and each compound in the cocktail contamination is to calculate the electronic structure to obtain the interaction energy, ΔE (Equation 3.4), allowing comparisons on a perfectly equal footing.

$$\Delta E = E_{\text{cluster}} - (E_{\text{PLA}} + E_{\text{contaminant}}) \quad (3.4)$$

Where, E_{cluster} is the energy of the lowest energy cluster formed between the PLA monomer and a molecule contaminant (Figure 3.3b), E_{pla} is the energy of the PLA monomer, and $E_{\text{contaminant}}$ is the energy of a molecule contaminant. The ΔE for each system is shown in Table 3.4.

Table III-3.4: Lowest energy interaction system.

System*	ΔE (kJ mol^{-1})
Heptane/PLA	-23.04
Chloroform/PLA	-29.50
Benzophenone/PLA	-53.12
Toluene/PLA	-32.44
Tetracosane/PLA	-24.83

* *In ΔE calculations, the L-lactide unit was used to model the local structure of PLA.*

As can be observed, the system with the highest interaction energy is benzophenone/PLA, foremost due to its polar affinity, while the heptane/PLA one shows the lowest interaction energy. Therefore, estimated ΔE values based on electronic structure calculations can be an interesting tool to predict and analyze the overall interaction trend between contaminating molecules and polymers. Specifically, in this study, there is an agreement between the data obtained by the Hansen sphere and the theoretical methodology, both presented in Figure 3.3. The only exception is chloroform,

whose behavior may be partially attributed to its vapor pressure at the experiment's temperature. During the contamination process, which followed FDA protocol, the PLA was immersed in a cocktail for 14 days at 40 °C in a closed system. However, under these conditions, contaminants with higher vapor pressures could vaporize during the contamination and also in sample preparation and recycling steps. As a result, the presence of these contaminants depends on their volatility, meaning that those with higher vapor pressures are less available in the immersion process.

The Hansen interaction model, based on solubility parameters, is an effective tool for predicting the affinity between contaminants and PLA, considering dispersion, dipole, and hydrogen bonding interactions. This model allows for precise estimation of the affinity between contaminants and the polymer, aiding in the prediction of contaminant content. Applying an additional mathematical model complements this analysis, providing detailed quantitative predictions of the interactions and behaviors of contaminants in PLA. An analysis of Figure 3.2(a) for the PLAc samples suggests that both models are effective tools for assessing contamination in these systems. The contaminants present in the highest concentrations are toluene and benzophenone, which exhibit the lowest interaction energies and, consequently, the strongest affinity with PLA. Although toluene is not within the solubility sphere, it is positioned close to it. In contrast, a different pattern is observed for heptane and tetracosane, which show the lowest values in Figure 3.2(a), maintaining consistency with the predictions. As previously mentioned, various factors can influence contaminant uptake, and in the case of chloroform, chemical affinity does not appear to be the primary factor governing its migration behavior in PLA.

b) The effect of recycling processes on migration analysis in various food simulants

After analyzing the total content of contaminants present in the PLA pellets through complete dissolution, the samples underwent a migration test using two different food simulants (3% acetic acid and 10% ethanol) under various migration conditions. In addition, as outlined in the methodology section, statistical analyses were performed using the one-way ANOVA (Table 3.4 in Annex I). The results indicate a statistically significant reduction in the concentration of the individually analyzed contaminants, as the calculated F-values exceeded the critical F-values, and in over 90% of the tests, the

P-values were below 0.05. However, for tetracosane, statistical analysis could not be performed at 60 °C in the EtOH 10% simulant and under any conditions in the HAc 3% simulant, as its concentrations were below the quantification and detection limits.

Figures 4a and 4b show the results for the non-polar contaminants (heptane and toluene), while 4c and 4d show the results for the polar ones (benzophenone and, chloroform).

In this way, as observed in the dissolution results, for the non-polar surrogates (Figure 3.4a and b), a drastic decrease in their concentration was observed when the bio-based polymer was subjected to the total recycling cycle (PLAcwr), compared to the samples that went only through the washing process (PLAcw) or the mechanical recycling (PLAcr). This behavior can be seen for all migration test conditions and food simulants. Furthermore, as described in the literature, the diffusion process is related to the molar mass, which means that the lower the molar mass of a compound, the higher its diffusion process (Dole et al., 2006; Voultzatis et al., 2007). However, Paiva and co-workers reported that in the case of contaminant migration, this relationship is not limited, and other factors should be considered, mainly the physical and chemical characteristics of both the polymeric matrix and the food simulants, paying more attention to the triple interaction polymeric matrix-surrogates-food simulants. Furthermore, the entire recycling cycle still proved to be the most efficient process for decontaminating PLA.

On the other hand, when the concentration of polar contaminants was analyzed (Figure 3.4c and d), different aspects of the polymer matrix-surrogate interaction from those observed for the non-polar contaminants were observed.

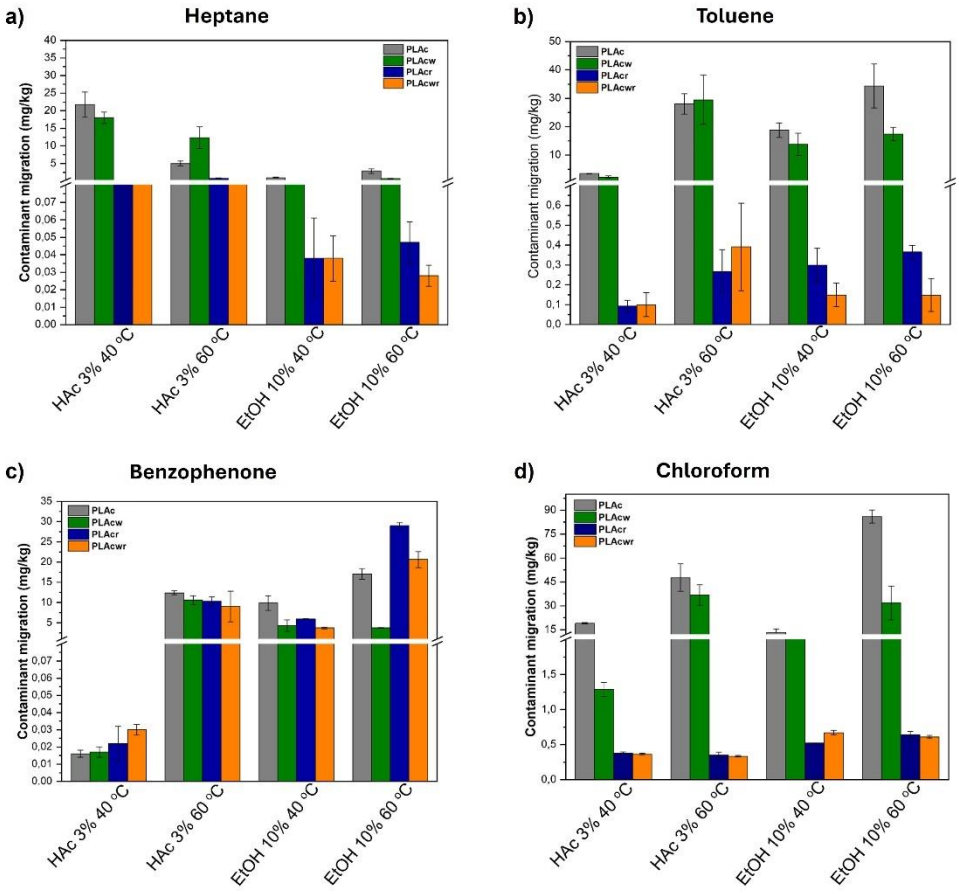


Figure III-3.4: Comparison of concentration of contaminants in different migration tests for all PLA samples and analyzed by non-polar (a and b) and polar (c and d) surrogates.

As mentioned above, PLA is a polar polymer with carbonyl and hydroxyl groups throughout its structure, which increases its interaction with polar compounds. In this way, an increase in concentration was observed for the benzophenone surrogate when comparing the contaminated sample (PLAc) with the samples that had undergone the recycling cycle. Comparing migration tests at 40 °C for HAc 3% and EtOH 10 % simulants, there was a higher concentration of benzophenone in the alcoholic simulant due to the high polarity, making the surrogate-simulant interaction greater. However, when the temperature of the tests was increased, there was an increase in concentration for both the acidic and alcoholic simulants, indicating that the polarity factor had no longer a high influence compared to temperature.

The polarity of the simulants and the temperature used for the migration tests may increase the diffusion of this contaminant since the temperature used in the test (60 °C)

was higher than the glass transition, Tg, of PLA (~57 °C). In this condition, the mobility of the polymer chains of the amorphous phase facilitates the diffusion process (McKeown and Jones, 2020; Scaffaro et al., 2011). In fact, in the dissolution, the benzophenone followed the same pattern as the non-polar compounds; all those concentrations decreased on the individual recycling steps (PLAcw and PLAcr) and the total cycle (PLAcwr). In addition, the dissolution process can result in loss of analyte due to the extraction and reprecipitation steps, which can affect the actual concentration analyzed. For chloroform, the concentration of the samples that underwent the dissolution protocol remained close in value. Furthermore, the use of DCM solvent interferes with the concentration due to the similarity between the solvent and the analyte analyzed.

On the other hand, the quantification and analysis of the recycling efficiency decontamination of tetracosane was only possible in the dissolution process, remaining always below the detection limit for the samples submitted to the migration tests. Tetracosane represents the class of non-polar and non-volatile contaminants with long chain and high molar mass, 338.65 g/mol, and therefore, its interaction with the polymer matrix is low, which justifies its concentration below the detection limit for the migration tests.

As outlined in European Regulation 10/2011, Article 11, substances with specific restrictions must not exceed their assigned Specific Migration Limit (SML). For substances without an SML, the generic migration limit of 60 mg/kg applies. In this context, the contaminants used in the FDA's challenge test included only benzophenone, which has an SML of 0.6 mg/kg. As previously discussed, its concentration increases throughout the recycling steps, exceeding the established limit. However, for the other contaminants, a decrease in concentration is observed as the samples progress through the recycling stages, reaching levels below the limits set by the legislation. Moreover, this trend is consistent across both simulants and under both migration test conditions, demonstrating the effectiveness of recycling in reducing contamination levels.

One of the concerns pointed out in some studies is the migration of intentionally and non-intentionally added substances (IAS and NIAS) during the migration tests, especially the NIAS formed by the degradation of PLA. Ubeda *et al*. (S. Ubeda et al., 2019) identified the migration of monomer dimethyle-1,4-dioxane-2,5-dione (lactide), derived from lactic acid dimer, which is not on the positive list of European Regulations

(Skjevrak et al., 2005). For these substances, the concentration must be controlled and follow the recommendation of the European Commission (< 0.01 mg/kg). However, in this study, the presence of this monomer was not a factor that affected the experimental results since the target mode analysis and system parameterization efficiently determined only the compounds present in the contamination cocktail in the FDA challenge test.

5. Conclusions

In this study, the recycling process applied to previously contaminated PLA pellets was evaluated with regard to the presence of contaminants, as well as their migration and interaction with PLA. A comparative analysis of contaminant concentrations in PLA pellets and those migrating to various food simulants was conducted, revealing the remarkable effectiveness of the recycling process in reducing contaminants, with concentrations in PLA pellets exceeding 40 mg/kg and reaching up to 800 mg/kg for certain surrogates. The research also delves into the nuanced interactions between different contaminants (both polar and non-polar) and the biopolymer at different stages of the recycling process, revealing a complex interplay influenced by factors such as molar mass, physical and chemical properties, and the specific conditions of migration tests. In addition, the study provides critical insight into the impact of recycling on the structural integrity of PLA, with microscopic images revealing changes in surface structure after recycling and migration testing. The results presented for both the dissolution and migration tests prove that each stage of the recycling cycle is important in the decontamination process. The individualized assessment of each contaminant under different conditions provided a broad overview of the contaminants' input and output. Furthermore, the dissolution protocol and the migration tests for different simulants were complementary techniques that increased reliability and explored in different ways the characteristics of both the polymer matrix-surrogate-simulant interaction and the diffusion processes related to the physicochemical properties of the material and the contaminant. Moreover, the FDA contamination protocol, originally developed for conventional polymers, was found to be applicable to biopolymers such as PLA, demonstrating a high degree of interaction between the contaminant cocktail and the biopolymer. This applicability ensures that worst-case post-consumer conditions of a package can be effectively analyzed, providing a robust framework for evaluating contamination and decontamination in biopolymers. The migration of contaminants is

influenced by the theoretical molecular volume of the contaminants, the type of food simulants, the test temperature, and the interactions between polymers and surrogates. Interaction energy values estimated from electronic structure calculations are a valuable tool for predicting and analyzing the overall interaction trends between contaminant molecules and polymers. Remarkably, there is strong consistency between the results obtained from Hansen sphere analysis and those derived from the theoretical approach.

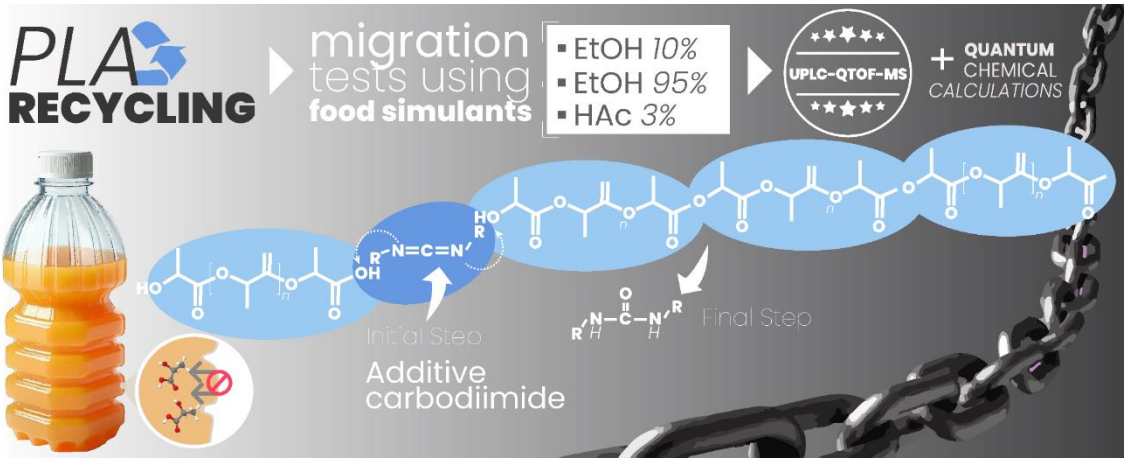
The recycling of bio-based polymers to return this product to its original application is in line with aspects of sustainability, and in this sense, the results presented shed light on the theme of food safety based on the quantification of contaminants supported by quantum chemical calculations.

Chapter 4

How carbodiimide modulates oligomer migration and molar mass in recycled poly (lactic acid): A study using UPLC-QTOF-MS^E

1. Abstract

The recycling of polymers for direct food contact faces initial challenges due to the loss of molar mass and the generation of Non-Intentionally Added Substances (NIAS), among which oligomers are prominent. In this study, carbodiimide (CDI) was employed as an anti-hydrolysis agent and chain extender to prevent oligomer formation in the recycling of poly (lactic acid) (PLA). Mechanical recycling was performed on wet and dried PLA with varying concentrations of CDI using co-rotating intermeshing twin-screw extrusion. CDI contributed to the increase in polymer molar mass and influenced the kinetics of its non-quiescent crystallization. Moisture played a key role in this process, as CDI can react either with water molecules, preventing hydrolytic degradation, or with available sites on the PLA molecule, resulting in increased molar mass and a decrease in the oligomer content. Oligomer migration tests were conducted using three food simulants: ethanol 10%, acetic acid 3%, and ethanol 95%. Ultra-performance liquid chromatography coupled with a quadrupole time-of-flight mass spectrometer was utilized for qualitative and quantitative PLA oligomer analyses. Electronic structure calculations based on the GFN-xTB Hamiltonian were employed for structural optimizations and energies to understand migration and interaction processes in the described systems. Sixteen different PLA oligomers were detected in the food simulant samples. Linear oligomers were identified in all simulants, while cyclic were detected mainly in the 95% ethanol simulant. The anti-hydrolysis and chain-extending effects of CDI in recycled PLA reduced the total oligomer concentration in both wet and dry samples, improving the physicochemical properties of PLA and ensuring food safety.


PLA
RECYCLING
migration
tests using
food simulants
▪ EtOH 10%
▪ EtOH 95%
▪ HAc 3%
UPLC-QTOF-MS
+ QUANTUM
CHEMICAL
CALCULATIONS
Initial Step
Additive
carbodiimide
Final Step

2. Introduction

New protection and remediation measures have gained prominence in global discussions due to climate change, environmental challenges, and the depletion of non-renewable resources. The United Nations' 2030 Agenda aims to promote sustainable development, alleviate poverty, protect the environment, and mitigate the impacts of climate change (Gobierno de España, 2018; Lima and Ghani, 2020). One key strategy for contributing to environmental preservation is the adoption of biodegradable, compostable, and bio-based materials for use in single-use plastic products, particularly those intended for food contact and medical applications (Ahmed et al., 2018). Poly (lactic acid) (PLA) is a biodegradable, bio-based aliphatic polyester that exhibits processing behavior similar to conventional thermoplastics derived from non-renewable sources, such as poly (ethylene terephthalate) (PET) and polypropylene (PP) (Bradley, 2010; da Silva et al., 2022; Moshood et al., 2022; Ncube et al., 2020). In addition, its biocompatibility, origin from renewable sources, and biodegradability make it a promising alternative to non-renewable materials in food packaging production.

According to the literature, bio-based polymers are classified as biodegradable and/or compostable under specific biodegradation conditions outlined by standards such as ASTM D6400, D5988-18, and ISO 14855. In this context, for example, plastic material is considered compostable if at least 90% of it is decomposed within six months in an industrial composting facility (American society for testing and materials, 2019).

While the use of bio-based polymers in single-use products can reduce dependence on non-renewable resources, it does not inherently address the environmental impact of post-consumer waste. Improper disposal of these materials continues their accumulation in the environment and landfills, contributing to contamination. One proposed solution to mitigate this issue is recycling, which adds value to post-consumer waste by reintroducing it into the production cycle.

Mechanical recycling has become one of the most used methods for reinserting these materials into the production of food contact products (Gall et al., 2020; Kaiser et al., 2018; Sadler, 1995; Thiounn and Smith, 2020). Studies on PLA recycling are proving to be significant for developing a new generation of packaging from renewable sources, as PLA exhibits processability similar to that of conventional polymers (Beltrán et al., 2021; McKeown and Jones, 2020; Moreno et al., 2020; Ncube et al., 2021; Scaffaro et

al., 2011). In addition, the Food and Drug Administration (FDA), the European Commission (EC), and the Brazilian Health Regulatory Agency (ANVISA) authorize the use of recycled materials in food-contact applications, provided that migration limits of substances within the material are adhered to, ensuring no health risk to consumers from ingestion of these substances (ANVISA, 2004; Bradley, 2010; Commission Regulation (EU) No 10/2011, 2011; U.S. Department of Health and Human Services Food and Drug Administration, 2021; US Food and Drug Administration (FDA), 2021). However, as described in the literature, one of the challenges in the mechanical recycling of PLA is the hydrolytic degradation process, which is accelerated by elevated processing temperatures and high shear rates (Maga et al., 2019). This results in a reduction in molar mass and a decline in the PLA's properties, sometimes rendering the polymer unsuitable for reuse in its original application.

Furthermore, concerning the loss of properties after recycling polymers, a new class of substances has gained attention in the context of food safety: Non-Intentionally Added Substances (NIAS). These compounds may arise from the degradation of the polymer matrix, from Intentionally Added Substances (IAS), or as a result of contamination during processing. Several studies have highlighted that NIAS was overlooked for a long time. However, since 2011, European Regulation 10/2011 has included these substances among those found in food-contact packaging that may pose risks to consumer health and compromise the integrity of the materials (Aznar et al., 2019; Birgit, 2018; Nerín et al., 2022; Sara Ubeda et al., 2019; S. Ubeda et al., 2019b; Vera et al., 2022, 2018).

To mitigate the degradative processes during the recycling of polyesters, an additive named carbodiimide (CDI) has been studied (Freitas et al., 2021; Khorana, 1953). As described in the literature, CDI is known due to its imide group (N=C=N), which can react with water and provide anti-hydrolysis action. Freitas *et al.* investigated carbodiimide's effect on increasing PET's molar mass. The results demonstrated that carbodiimide works as an efficient chain extender by reacting with end groups, allowing PET to recover its original properties and enabling its reapplication in the packaging (cradle-to-cradle). These functionalities of CDI could contribute to mitigating degradative processes by reducing polymer chain scission while increasing the molar mass of the polymer (Holcapkova et al., 2017; Kurzer and Douraghi-Zadeh, 1967; Williams and Ibrahim, 1981).

The formation of NIAS, including oligomers, in recycled materials is well documented in the literature and remains a concern regarding the overall benefits of recycling. This issue arises from the potential migration of these substances, which can contaminate food products or the surrounding environment. These concerns highlight the need for more advanced recycling processes and stringent control measures to ensure the safety and sustainability of recycled polymers, particularly in food contact applications (Alberto Lopes and Tsochatzis, 2023; Gelbke et al., 2019; Sara Ubeda et al., 2019; Úbeda et al., 2017). In this way, European Regulation 10/2011 on materials intended for contact with food establishes specific rules for their use in food packaging, particularly regarding the migration limits that listed substances must maintain. However, oligomers classified as NIAS are not cataloged/listed as authorized substances and, therefore, cannot exceed the migration limit of 0.01 mg/kg of food (Birgit, 2018; Commission Regulation (EU) No 10/2011, 2011).

Given this context, herein it was investigated the influence of carbodiimide content, used as a functional molecule, on the recycling of PLA from two perspectives: (1) the increase of PLA molar mass, allowing the material to return to its original application, and (2) the presence of oligomers, which are classified as NIAS and require their levels to be monitored. Changes in molar mass and flow-induced crystallization were evaluated through rheological measurements. Additionally, two powerful techniques were integrated to quantify cyclic and linear oligomers and predict the interactions between the oligomers and the food simulants.

Ultra-performance liquid chromatography coupled with a quadrupole time-of-flight mass spectrometry-elevated energy (UPLC-QTOF-MS^E) was used as a highly sensitive analytical technique to determine and quantify oligomers that may migrate into the food simulants. MSE is a cutting-edge technology that enables thorough, consistent profiling and characterization, standing out as one of the most powerful techniques for identifying non-volatile compounds. This approach involves a simultaneous collection of low-energy (first function) and high-energy (second function) data, with an energy ramp set between 15 eV and 30 eV. MSE enables the precise acquisition of both precursor ion masses and fragment ion masses within a single analysis run. Mass precursors and mass fragments are recorded for every detectable compound in the sample. MSE is faster than traditional mass spectrometry or tandem MS techniques, and an added benefit is the reduced need for labor-intensive sample preparation and minimal solvent usage.

Furthermore, computational methods were employed to help understand the general trend observed in the interactions between oligomer and food simulants.

Although some studies have explored the anti-hydrolysis and chain extender potential of carbodiimide in PLA, few focus on evaluating its influence on the molar mass and its crystallization kinetics (Azevedo et al., 2021; Holcapkova et al., 2017; Khankrua et al., 2014; Melcova et al., 2019). Furthermore, as far as we know, there are no reports in the literature on the role of this molecule in mitigating the migration of oligomers, currently classified as NIAS, which could contribute to improving recycling processes and enhancing food safety.

3. Experimental Methods

3.1 Chemicals

The chemicals used for the migration analysis as food simulants were ethanol (HPLC grade, CAS No. 540-84-1) supplied by Scharlau Chemie S. A. (Sentmenat, Spain), acetic acid (99.8%, CAS No. 540-84-1) from Chemie S. A. (Sentmenat, Spain), and ultrapure water sourced from a Milli-Q Ultramatric Wasserlab GR 216071 (Barbatain, Spain). Cyclic $[LA]_6$ and linear OH-$[LA]_4$-H oligomers (both with a purity greater than 90%, where LA stands for lactic acid) were isolated as described in the literature and used as standards for oligomer quantification (Aznar et al., 2016).

3.2 Sample

The commercial bio-based polymer used in this study was the PLA Ingeo™ 4043D (98% L-lactide content), supplied by NatureWorks LLC. The polymer has a melt flow index (MFI) of 6 g/10 min (ASTM D 1238) at 210 °C, with a density of 2.16 g/cm^3 (ASTM D792). A commercial carbodiimide, Carbodilite HMV-15CA, supplied by Nisshinbo Chemical Inc., was used during the PLA recycling.

3.3 Sample preparation

The recycling of PLA was performed using a co-rotating intermeshing twin-screw extrusion Thermo Scientific Process 11 Parallel Twin-Screw Extruder (D=11 mm;

L/D=40) at 200°C and 400 rpm. Subsequently, the recycled PLA was reprocessed with varying concentrations of CDI (0.5, 1.0, and 3.0 m/m %). Before incorporating CDI, the PLA samples underwent either a drying step in a vacuum oven (*dry samples*) or were reprocessed without drying (*wet samples*) to ensure the presence of moisture during the reprocessing. The drying was conducted in a vacuum oven at 80 °C for 4 hours. Afterward, the samples were thermo-pressed at 200 °C for 3 minutes at 0.2 torr to form PLA films. Table 4.1 describes the sample after the preparation.

Table III-4.1. Nomenclature employed in this study.

Samples	Nomenclature
PLA reprocessed	PLAr
Dried PLA reprocessed + 0.5 m/m% CDI	PLA$_{0.5\%d}$
Dried PLA reprocessed + 1.0 m/m % CDI	PLA$_{1\%d}$
Dried PLA reprocessed + 3.0 m/m % CDI	PLAd$_{3\%d}$
Wet PLA reprocessed + 0.5 m/m % CDI	PLA$_{0.5\%w}$
Wet PLA reprocessed + 1.0 m/m % CDI	PLA$_{1\%w}$
Wet PLA reprocessed + 3.0 m/m % CDI	PLA$_{3\%w}$

3.4 Rheological characterization

The rheological measurements were performed on an Anton Paar MCR 302 at 200°C, using 25 mm diameter plates with a gap distance of 1 mm, under an inert nitrogen atmosphere. Complex viscosity (η*) was measured via dynamic frequency sweep tests performed in the linear viscoelasticity regime at 1% strain amplitude within the angular frequency range of 0.1-500 rad/s. The time sweep measurements were also performed, and details about measurements can be found in the Supporting Information.

The non-quiescent (shear flow-induced) crystallization of PLA with varying levels of CDI was conducted on the same rheometer under an inert nitrogen atmosphere. The samples were heated to 200 °C for this analysis and then cooled to 115 °C. Once

stabilized at this temperature, a shear rate of 0.1 s^{-1} was applied while monitoring the shear stress (τ) as a function of time. The onset of crystallization under shear flow, referred to as the 'induction time,' was identified by the sudden increase in shear stress.

3.5 Migration Tests

The PLA film samples were subjected to migration experiments using food simulants (6 dm^2 of material per 1 kg of simulant) according to European Regulation 10/2011 (Commission Regulation (EU) No 10/2011, 2011).

All samples (5.4 x 1 cm) were tested in triplicate for migration using ethanol 10% (simulant A), acetic acid 3% (simulant B), and ethanol 95%, substitute simulant of D2 (vegetable oil) as it is indicated in EU 10/2011, for fatty simulant in the food category. These simulants represent hydrophilic foods capable of extracting hydrophilic compounds, foods with a pH below 4.5, and lipophilic foods, respectively. The vials were filled with 18 grams of simulant and subjected to migration testing for 10 days at 60 °C, simulating worst-case conditions that packaging might encounter. The samples were then analyzed using UPLC-QTOF-MS^E. Gravimetric control was conducted to determine the mass of the simulant used precisely to express the results as indicated in EU 10/2011 (Commission Regulation (EU) No 10/2011, 2011).

3.6 Analytical instrumental conditions

UPLC-QTOF-MS^E was carried out in an Acquity™ system using an Acquity UPLC BEH C18 column (2.1 mm x 100 mm x 1.7 µm particle size), both from Waters (Milford, MA, USA). The solvents used as mobile phases for positive mode were water and methanol with 0.1% formic acid (solvents A_1 and B_1). The column flow was 0.3 mL/min at 40 °C. Gradient elution was carried out with mobile phase A/B varying from 98/2% to 0/100% over 15 minutes, followed by a return to the initial conditions within 2 minutes. The sample injection volume was 10 µL.

The detector was an API source (atmospheric pressure ionization) with ESI (electrospray ionization) coupled to a mass spectrometer (Xevo G2) consisting of a quadrupole, a collision cell, and a time-of-flight (QTOF) detectors, all supplied by Waters (Milford, MA, USA). The electrospray probe was operated in positive (ESI+) mode and

in sensitivity analyzer mode. The mass ranged from 50 to 1200 Da, and the capillary voltage was 2.5 kV. The sampling cone voltage was also set to 30 V and 70 V for positive mode. The source temperature was 120 °C, and nitrogen was used as the desolvation gas, with a flow rate of 450 L/h at 400 °C. The cone gas flow rate was 20 L/h.

The acquisition was performed in MS^E mode to allow low and high collision energy (CE) use in the collision cell during the same run. The mass spectrum at low energy (CE 4V) provided information about the fragment ions. Mass correction was ensured by infusion of a LockSpray solution of leucine-enkephalin (2 ng/mL in water/acetonitrile with 0.1% formic acid) at a flow rate of 5 µL/min. Test mix solution of known standards were injected every 20 injections to ensure accurate mass readings. MassLynx v.4.1 (Waters, Milford, MA, USA) was used for sample analysis.

The PLA oligomers were semi-quantified using a calibration curve for cyclic oligomers with $[LA]_6$ and linear oligomers with OH-$[LA]_4$-H (Aznar et al., 2016). The instrumental limits of quantification (LOQ) were 3.6 µg/g for $[LA]_6$ and 0.45 µg/g for OH-$[LA]_4$-H, and the instrumental limits of detection (LOD) were 1.2 µg/g and 0.15 µg/g for the respective oligomers. In addition, two-factor ANOVA was applied to migration tests performed in the different simulant liquids. This method was used to evaluate whether the concentration of oligomers changed significantly with the increasing CDI content in both wet and dry samples.

3.7 Theoretical methodology

To help understand the interaction between oligomers and simulants, electronic structure calculations using the GFN-xTB (Grimme et al., 2017) approach was carried out in the ORCA program (Neese, 2018, 2012). The interaction with the simulants was considered using the implicit analytical linearized Poisson–Boltzmann (ALPB) solvation model (Sigalov et al., 2006). As the EtOH 95% medium is basically ethanol, this was the solvent defined in the ALPB model. For EtOH 10% and HAc 3%, which are mainly composed of water, the implicit solvent considered in the ALPB computations was water. Therefore, we have simulated two conditions, named EtOH or H_2O, within the theoretical data.

Finally, Figure 4.1 shows the flowcharts of the work from the PLA recycling step to sample preparation, the rheological analysis, and the determination of oligomer by UPLC-QTOF-MS^{E} and, the theoretical-computational analysis.

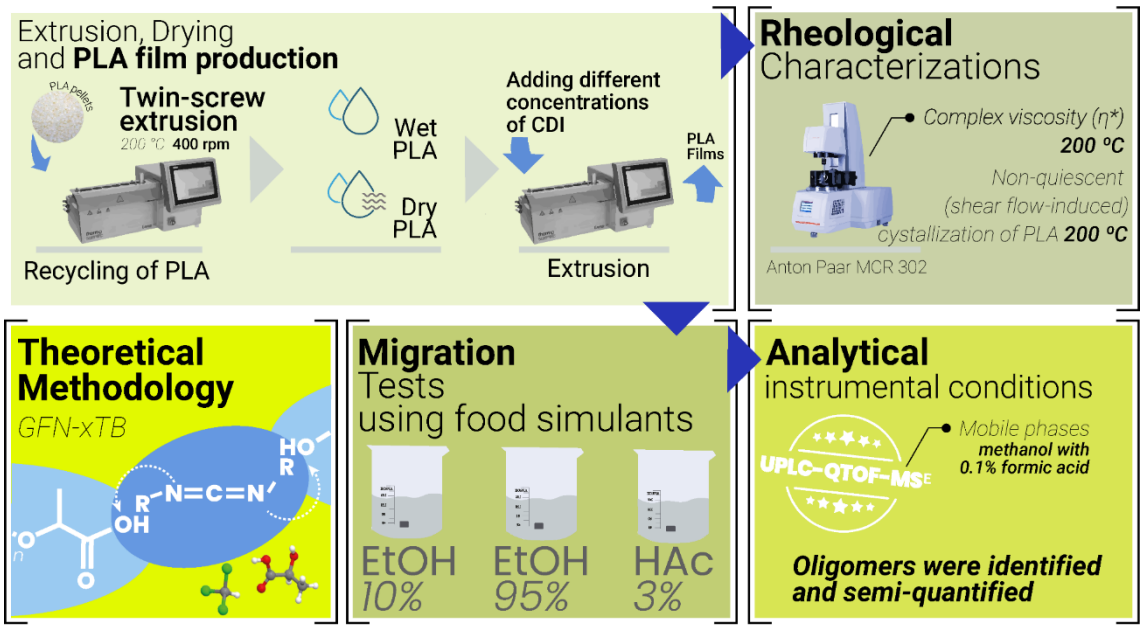


Figure III-4.1. Flowcharts of the work: samples preparation, rheological characterization, migration tests, analytical procedures, and theoretical methodology.

4. Results and Discussion

a) Effect of carbodiimide content on PLA molar mass and non-quiescent crystallization

The understanding of the molar mass increase as a function of carbodiimide content in PLA was analyzed. The study was designed to (i) assess the effect of varying carbodiimide concentrations on molar mass and (ii) determine how the presence of moisture influences this process. Figure 4.2 (A and B) presents the viscosity complex as a function of angular frequency for all samples that exhibit a clear Newtonian plateau at low frequencies, regardless of the presence of moisture. The incorporation of CDI leads to a significant increase in complex viscosity, accompanied by a slight decrease in the length of the Newtonian plateau, indicating a more pseudoplastic behavior.

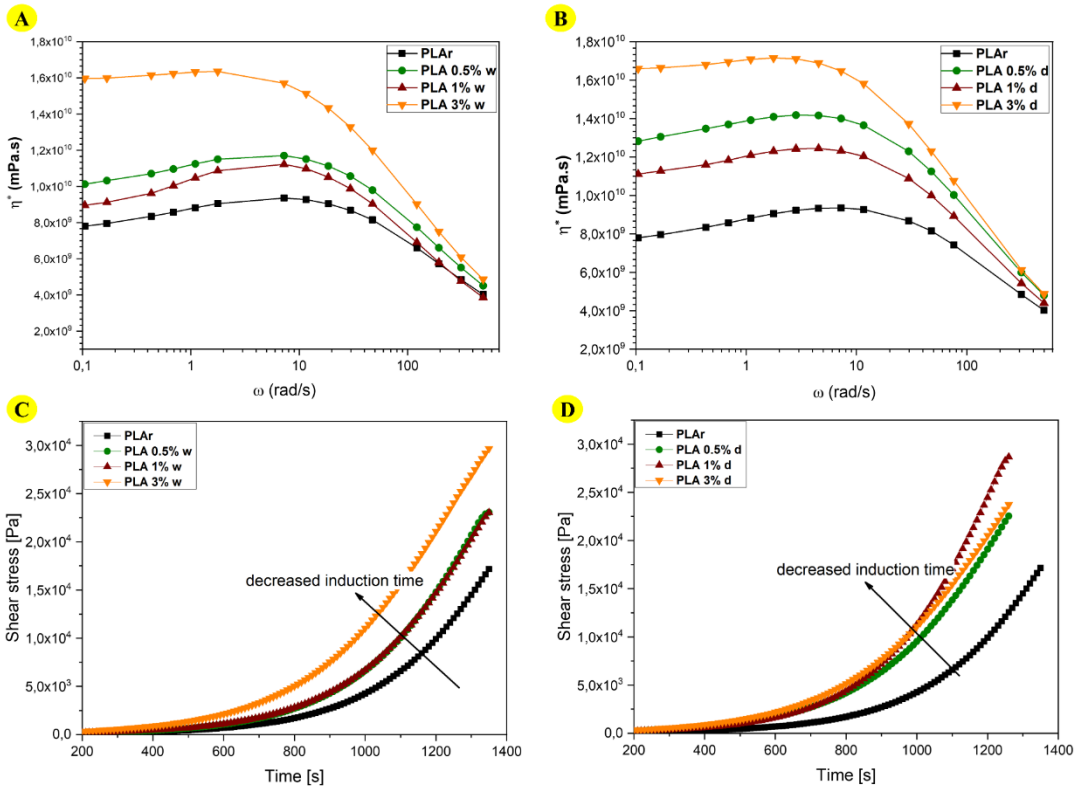


Figure III-4.2. Complex viscosity (η*) versus angular frequency at 200 °C under (A) wet and (B) dry conditions, and shear stress (τ) versus time at 115 °C under (C) wet and (D) dry conditions for PLAr and PLA with CDI concentrations of 0.5, 1.0, and 3.0 m/m %.

The time sweep measurements were performed (annex – Figure 4.1) to verify the stability of the samples. A slight linear decrease was observed until 1250 s, which means that degradation reactions may occur during the experiment resulting in chain scission (McKeown and Jones, 2020). Although this, an increase in the molar mass was observed with the addition of carbodiimide, corroborating the results of the frequency sweep.

The alteration in molar mass affects the kinetics of PLA crystallization; therefore, an investigation into non-crystallization conditions was carried out. Figure 4.2 (C and D) shows the stress (τ) as a function of time under non-quiescent conditions at a shear rate of 0.1 s^{-1}. The onset time can be determined by observing when the viscosity increases abruptly. At this point, the viscosity tends to infinity, indicating that the material begins to behave as a solid. It has been indicated that the induction time is approximately proportional to the inverse of the nucleation rate (Cruz et al., 2019). At a constant temperature, the free energy associated with the flow depends solely on the entanglement

density and the Deborah number. Since the crystallization rate is the result of the product between nucleability and transportability, it is highly influenced by the mobility and diffusivity of the polymer chains. Despite the increase in molar mass, a reduction in flow-induced crystallization time is observed with increased carbodiimide content.

Table 4.2 shows the results of the degree of crystallinity ($X_c(\%)$) for the PLA with the addition of carbodiimide, for the wet and dry samples, compared to recycled PLA. The $X_c$was obtained from Equation 1:

$$X_c(\%) = \frac{\Delta H_m}{\Delta H_m^o} x100, \quad (4.1)$$

Where, ΔH_m is the enthalpy of melting obtained by DSC analysis for each sample and ΔH_m^o is the theoretical enthalpy of melting assuming 100% crystalline PLA with a value of 93 J/g.

Table III-4.2. Enthalpy of melting and degree of crystallinity results for the PLA without and with carbodiimide addition in wet and dry samples.

Sample	ΔH_m (J/g)	$X_c(\%)$
PLAr	24.81 (±3.67)	26.67 (±3.94)
PLA0.5%w	27.51 (±1.03)	29.60 (±1.10)
PLA1%w	21.58 (±2.37)	23.20 (±2.53)
PLA3%w	20.05 (±0.75)	21.56 (±0.84)
PLA0.5%d	26.85 (±0.58)	28.87 (±0.63)
PLA1%d	20.24 (±0.50)	21.77 (±0.54)
PLA3%d	17.89 (±0.74)	19.23 (±0.79)

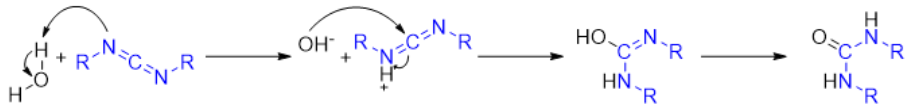
The literature widely acknowledges that, under quiescent conditions, molar mass significantly influences the rate of nuclei formation (Cruz et al., 2019; Jalali et al., 2017). Polymer chains with lower molar mass require less time for nucleus formation and subsequent crystal growth. This behavior was observed in wet and dry samples (Table 4.2), which means that the lower the molar mass, the higher the degree of crystallinity. However, the crystallization behavior of polymers is often influenced by the application of shear flow to the sample. Under real processing conditions, this effect becomes crucial, as crystallization typically occurs in a flowing environment. By examining flow-induced crystallization, it is possible to determine the induction time, revealing how shear flow

impacts crystalline structure formation. It is well established that the crystalline structures formed under shear differ from those formed under quiescent conditions. Shear flow can align polymer chains, promoting the formation of primary nuclei precursors. The crystallization efficiency is closely linked to (i) the formation of shear-induced nuclei and (ii) the relaxation time of the polymer chains. Therefore, polymers with higher molar mass present longer relaxation times and exhibit greater chain alignment, which enhances the efficiency of the crystallization process, as observed in Figure 4.2.

Figure 4.3 presents a mechanism proposal for the reaction of CDI with water molecules and the reaction of CDI with meres of PLA. It illustrates the hydrolysis mechanism between water and carbodiimide, where carbodiimide abstracts a proton from water molecules via electrophilic attack, leading to the formation of hydroxyl, which will react with carbon in the carbodiimide group (R'-N=C=N-R'). This nucleophilic attack forms a transition state and a subsequent intermediate, which undergoes proton transfer and rearrangement. Ultimately, the reaction forms a urea derivative (R-NH-C(=O)-NH-R'), a common outcome when carbodiimides are exposed to water. In this manner, carbodiimide functions as an anti-hydrolysis agent, protecting the PLA polymer chain from degradation through hydrolysis.

In addition, CDI can act as a chain extender, as illustrated in the mechanism shown in Figure 4.3, which is divided into two stages. As described by Schotman (Schotman, 1991), carboxylic acid groups can react with carbodiimides through an electrophilic attack, initiating coupling reactions. Initially, the proton from the carboxylic acid group at PLA structure is abstracted by the carbodiimide, which subsequently reacts with the nucleophilic carbon of the protonated carbodiimide intermediate (I). This is followed by the coupling of another terminal carboxyl group of PLA in the same reaction, resulting in polymer chain extension and the formation of a urea derivative (II).

Anti-hydrolysis



Chain-extending

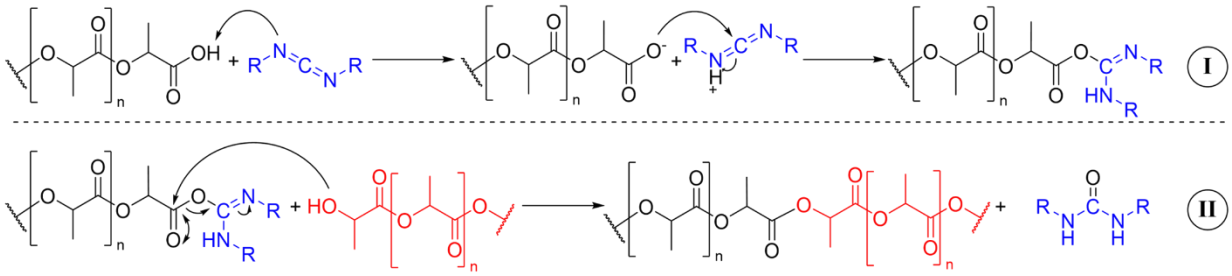


Figure III-4.3. Proposed mechanism illustrating the anti-hydrolysis and chain-extending action of CDI.

b) Identification of oligomers in recycled PLA

Table 4.3 shows the total number of candidate oligomers identified in all the PLA samples, with and without the addition of the CDI, for the three different food simulants. Sixteen (16) oligomers were identified, including those initially present in the PLA, where 69% were linear chains, while 31% were cyclic structures.

As described in the literature, many of these oligomers have been previously cataloged in some studies (Aznar et al., 2019; Canellas et al., 2022; Hoppe et al., 2016; Sara Ubeda et al., 2019; S. Ubeda et al., 2019), mainly employing and involving PLA for food contact applications. Additionally, the sodium ion adducts [MNa^+] were detected for all oligomers. Each oligomer contained the single monomer [LA], corresponding to the formula $C_3H_4O_2$, as also reported by Aznar *et al*. in their study on the migration of PLA oligomers in PLA/polyester blend employed in packaging (Aznar et al., 2019). The oligomer candidates were identified based on their retention time, molar mass with the sodium ion adduct, molecular formula, and chemical structure, as described in Table 4.3, and Figure 4.4.

The linear oligomers were identified in the EtOH 10%, and Hac 3% simulants, although at lower levels in the acid simulant. In contrast, both linear and cyclic oligomers were detected in the EtOH 95% simulant. Figure 4.4A shows the high-energy collision

spectrum for one of the linear oligomers identified in the three food simulants. This oligomer, with the structure formula CH_3-CH_2-O-$[LA]_3$-H, was identified by its mass of 285.0953 m/z, corresponding to the molecular formula $C_{11}H_{18}O_7$. Additionally, the cyclic oligomer with the structure formula OH-$[LA]_5$-H, having a mass of 383.0936 m/z, corresponding to the molecular formula $C_{15}H_{20}O_{10}$ is shown in Figure 4.4B and was identified in the EtOH 95% simulant.

As shown in Figure 4.4, the identified fragments demonstrated a high degree of reliability in confirming the structure of the proposed oligomers. However, the Lactide monomer ($C_6H_8O_4$) cannot be identified due to the difficulties in its ionization and detection by mass spectrometry, as reported in the literature (Sara Ubeda et al., 2019).

Table III-4.3. Candidate oligomers classified by retention time (t_r), sodium molar mass [MNa^+], and molecular formula (MF) for each food simulant.

Number#	t_r (s)	Mass [MNa^+]	MF	Candidate oligomer	Simulant		
					EtOH 10%	EtOH 95%	HAc 3%
1	5.417	213.0732	C8H14O5	CH_3-CH_2-O-$[LA]_2$-H			
2	5.719	329.0844	C12H18O9	OH-$[LA]_4$-H			
3	6.279	285.0953	C11H18O7	CH_3-CH_2-O-$[LA]_3$-H			
4	6.461	401.1059	C15H22O11	OH-$[LA]_5$-H			
5	6.801	473.1282	C18H26O13	OH-$[LA]_6$-H			
6	6.920	357.1167	C14H22O9	CH_3-CH_2-O-$[LA]_4$-H			
7	7.04	383.0936	C15H20O10	CYCLIC $[LA]_5$			
8	7.151	545.1475	C21H30O15	OH-$[LA]_7$-H			
9	7.342	429.1367	C17H26O11	CH_3-CH_2-O-$[LA]_5$-H			
10	7.448	617.1697	C24H34O17	OH-$[LA]_8$-H			
11	7.46	455.1160	C18H24O12	CYCLIC $[LA]_6$			

12	7.655	501.1591	C20H30O13	CH_3-CH_2-O-[LA]$_6$-H			
13	7.760	527.1376	C21H28O14	CYCLIC [LA]$_7$			
14	7.900	573.1780	C24H32O16	CYCLIC [LA]$_8$			
15	7.99	645.1978	C26H38O17	CH_3-CH_2-O-[LA]$_8$-H			
16	8.18	671.1790	C27H36O18	CYCLIC [LA]$_9$			

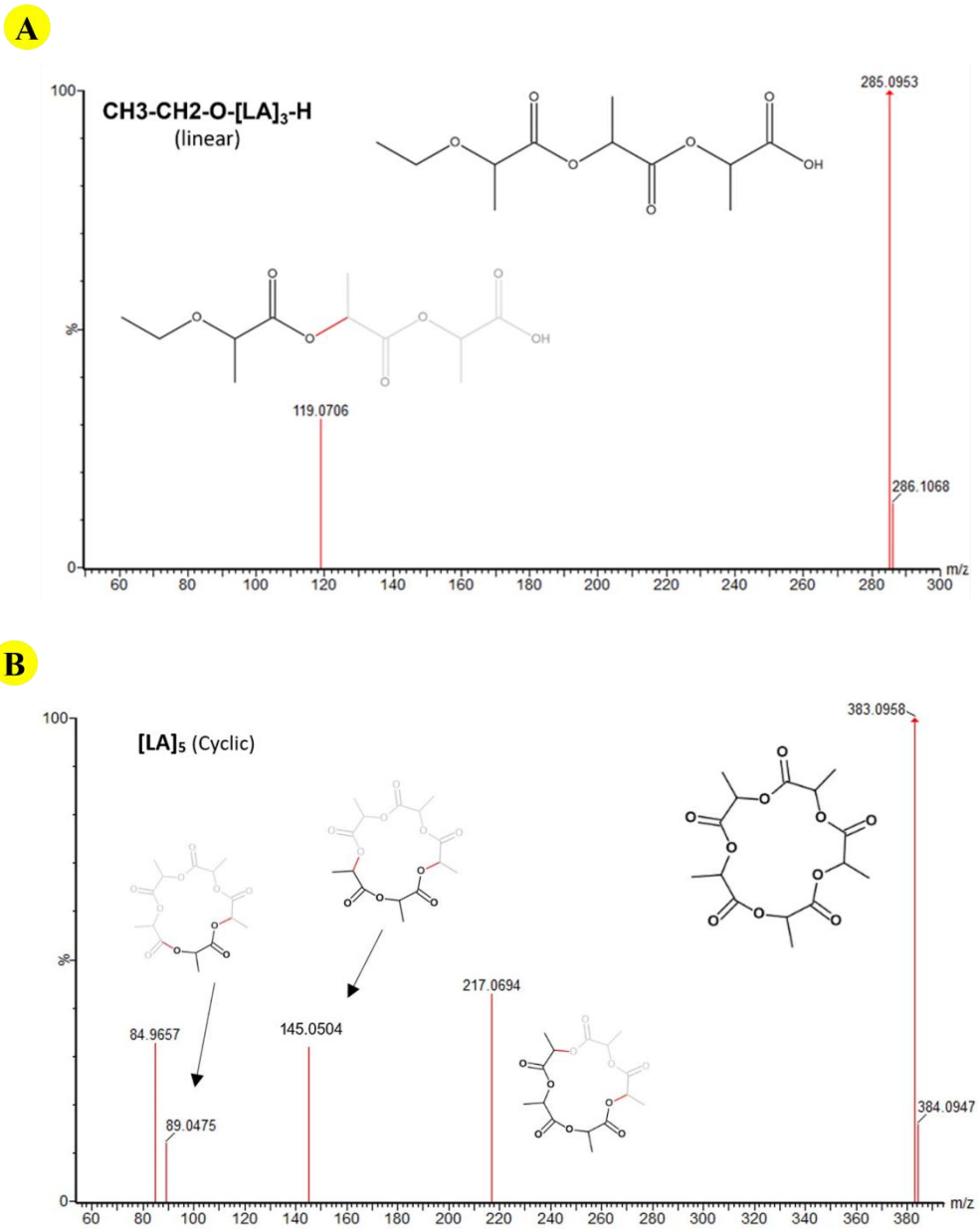
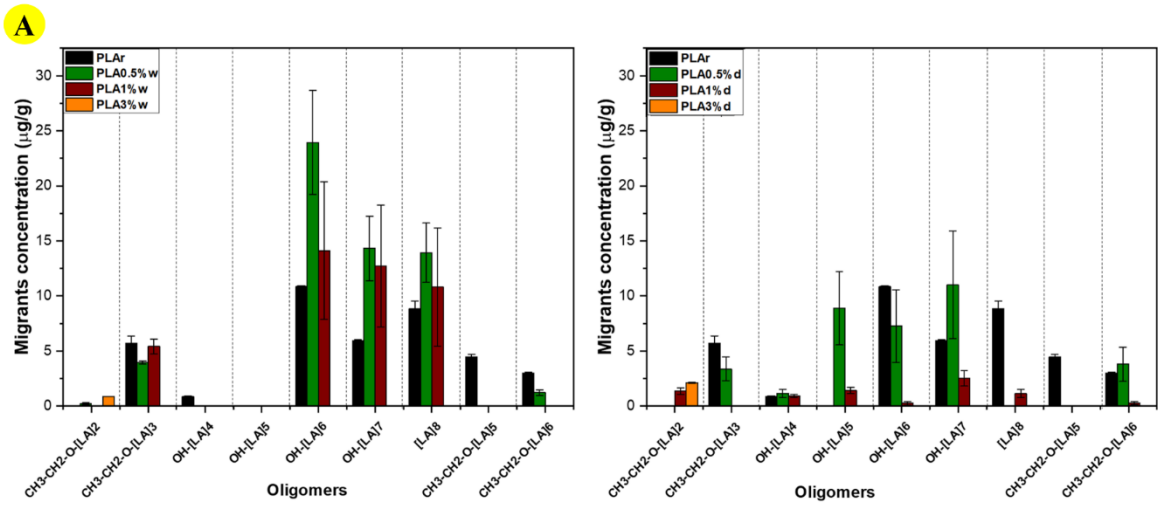


Figure III-4.4. High-energy collision and fragmentation spectra of candidate (A) linear and (B) cyclic oligomers identified in food simulants.

c) Migration of PLA oligomers and the influence of the presence of carbodiimide

Figure 4.5 presents the migration data for each identified oligomer listed in Table 3, covering both wet and dry conditions for each food simulant, EtOH 10%, HAc 3%, and EtOH 95%. To ensure consistency, comparisons of oligomer concentrations were conducted exclusively on reprocessed PLA samples, with recycled PLA as the control. In addition, the results compare samples without the CDI to those containing three different concentrations (0.5, 1.0, and 3.0 m/m %) across the various food simulants. The oligomer migration levels are expressed in µg/g, clearly comparing and illustrating how the presence and concentration of the CDI influence migration behavior in each simulant.

As described in the methodology, the samples were reprocessed under dry and wet conditions to evaluate the influence of humidity on the hydrolysis degradation process.



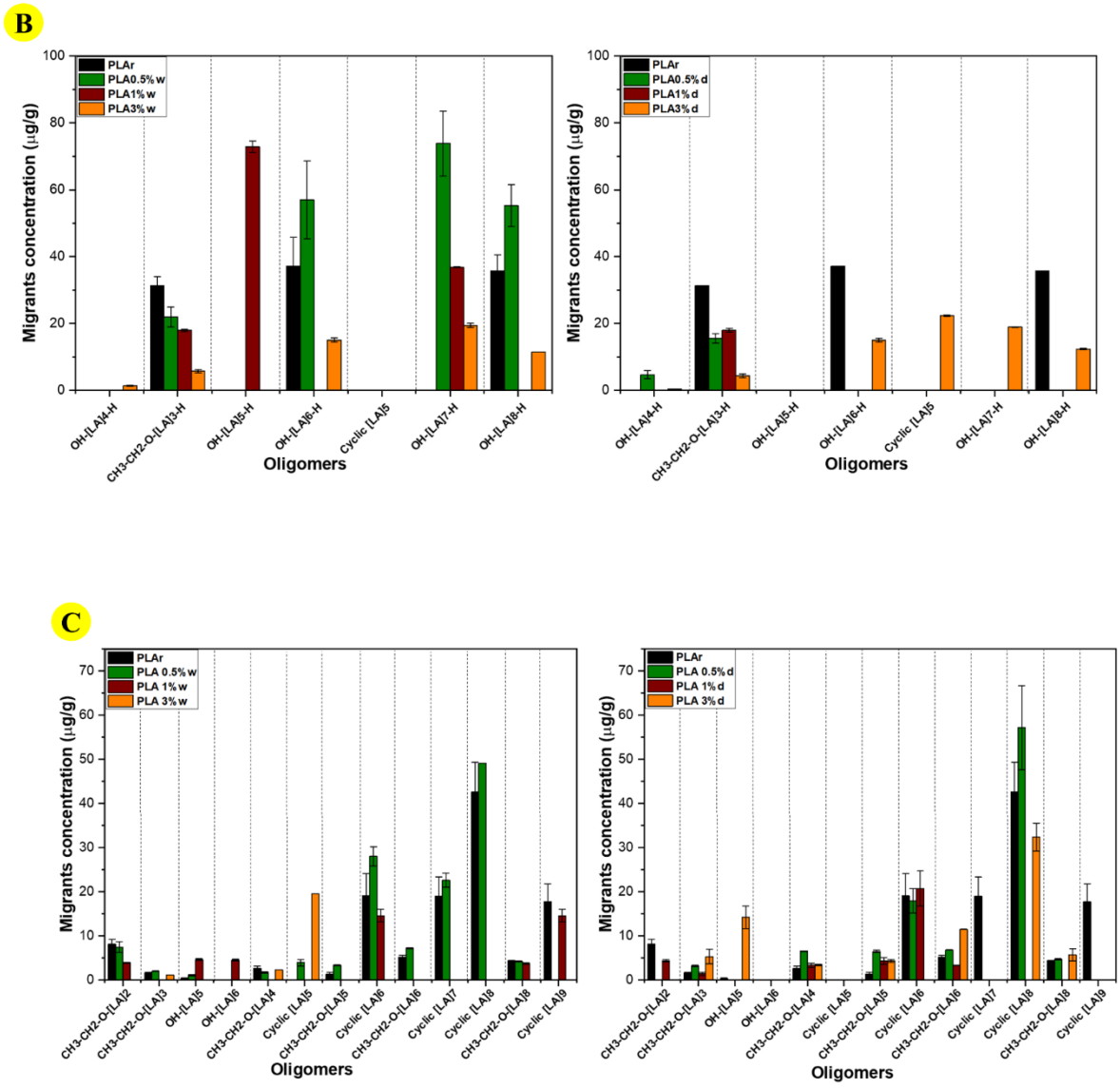


Figure III-4.5. Oligomer migration for the samples with different concentrations of CDI (0.5%, 1%, and 3%), wet and dry, and each food simulant, (A) EtOH 10%, (B) HAc 3%, and (C) EtOH 95%.

As stated, linear oligomers were identified in the EtOH 10% and HAc 3% simulants, totaling 9 and 7, respectively, as shown in Figures 4.5 (A and B). A cyclic oligomer ($[LA]_5$) was identified for HAc 3%. The concentration of oligomers in dried samples decreases compared to wet samples for both the EtOH 10% and HAc 3%. However, although the number of oligomers detected was higher for the samples exposed to the EtOH 10% simulant, their concentration was lower when compared to the HAc 3%

simulant. This behavior is observed for dry and wet samples, as shown in Figures 4.5 (A and B).

On the other hand, linear and cyclic oligomers were quantified for the EtOH 95% simulant, totaling 13 oligomers, as shown in Figure 4.5C. In this case, the difference between the dry and wet samples was less pronounced than the other simulants.

As described in the methodology section, statistical analysis using ANOVA was used to assess whether there was a significant difference in the concentration of oligomers according to the addition of the carbodiimide additive. The analysis was carried out on the wet and dry sample groups, and the calculated F-value was compared with the critical F-value (tabulated), and the P-value was also analyzed, with a 95% degree of reliability established. The data in Table 4.4 shows a significant difference between the samples, as the calculated F-value was greater than the critical F-value, with a P-value of less than 0.05 for the sample groups in all the food simulants.

Table III-4.4. ANOVA statistical analysis of wet and dry sample groups, comparing the calculated F-values, critical F-values, and P-values for PLA samples with and without the carbodiimide additive across the three food simulants.

Food Simulants	**EtOH 10%**			**EtOH 95%**			**HAc 3%**		
Sample Groups	F calculate	F critical	P-value	F calculate	F critical	P-value	F calculate	F critical	P-value
Wet	5,663	2,093	4,87e-5	2,348	1,859	0,011	2,450	2,250	0,0402
Dried	4,409	2,891	0,010	4,593	1,859	1,98e-5	2,851	2,250	0,0442

Figure 4.6 (A, B, and C) shows the total oligomer concentration as a function of CDI concentration for the three food simulants, evaluating both wet and dry samples. This compilation includes the total concentration of all oligomers that migrated to each food simulant, encompassing both cyclic and linear forms. These results enable us to assess the influence of CDI concentration on the migration of PLA oligomers present in the samples.

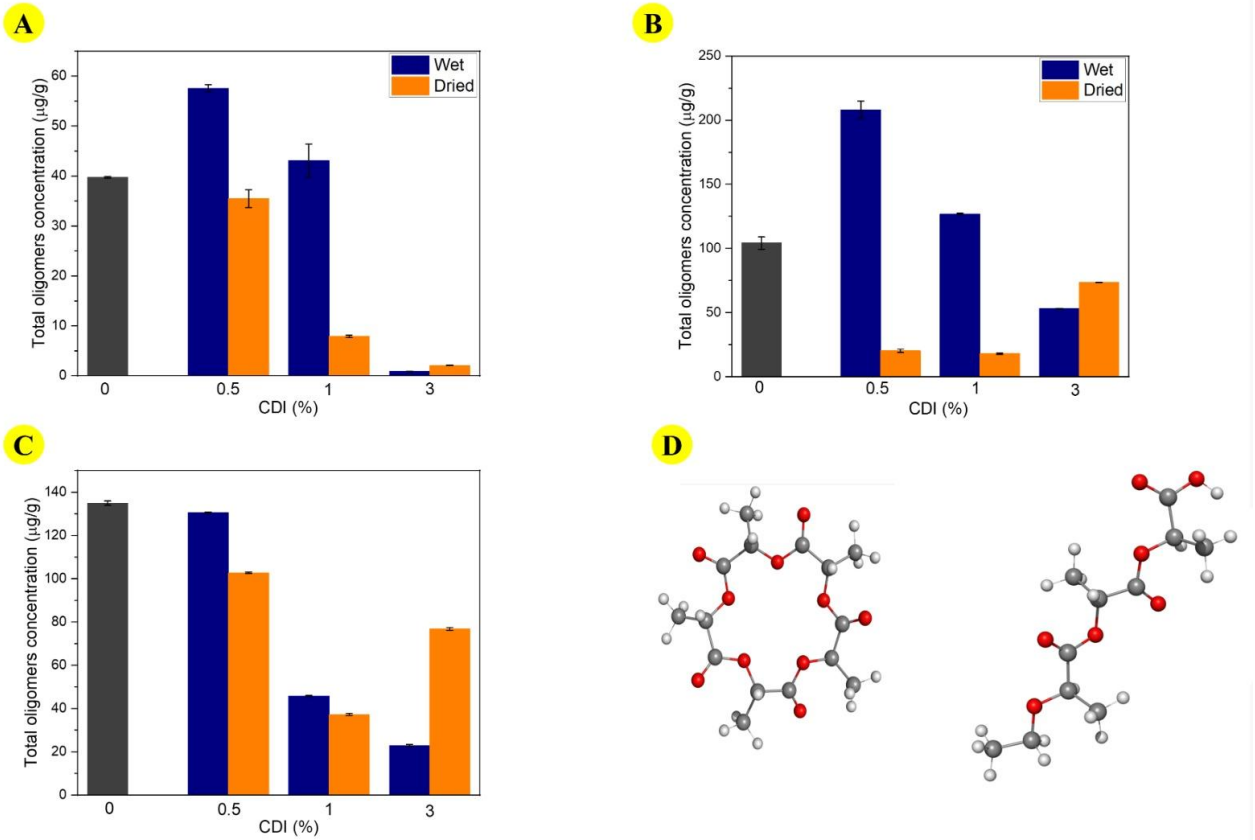


Figure III-4.6. Total oligomer concentration as a function of carbodiimide concentration for each simulant: (A) EtOH 10%, (B) HAc 3%, and (C) EtOH 95%, for dry and wet samples; and (D) model systems used in computational investigations, considering cyclic and linear oligomers.

Figure 4.6 shows that, in general, the wet samples exhibit higher concentrations of oligomers. Additionally, it was observed that for the EtOH 10% simulant, the total oligomer concentration tended to decrease as the CDI concentration increased for wet and dry samples. Samples containing 0.5 and 1.0% CDI show higher total oligomer values than the control sample. This phenomenon can be explained by the fact that the control PLA was reprocessed after undergoing the typical drying process for this type of polymer. However, for the wet material, CDI incorporation occurred without the drying step, which may have led to hydrolytic reactions, as previously described, increasing total oligomer content, especially at lower CDI concentrations. However, increasing the CDI concentration to 3.0% causes a substantial reduction in this value.

For CDI concentrations between 0.5 and 1.0 m/m%, the decrease in total oligomer concentration is more pronounced in the dry samples because the presence of water is

minimal, which hinders the process of degradation by hydrolysis and the presence of CDI also acts as an anti-hydrolysis agent, preventing further formation of oligomers that could migrate to the simulants. On the other hand, at a 3% CDI concentration, a significant reduction is observed, with virtually no difference between the two sample types.

A similar trend was observed for the samples exposed to the HAc 3% simulant, especially in the wet condition. However, there was an initial reduction in the detected values for the dry samples, followed by an increase as the CDI concentration increased. A similar behavior was noted in the samples exposed to EtOH 95%. As described in the literature (Kurzer and Douraghi-Zadeh, 1967; Schotman, 1991; Williams and Ibrahim, 1981), CDIs are recognized for their bifunctionality, acting as an anti-hydrolysis additive and a chain extender. This functional duality is evident in the individual oligomer migration results and the total oligomer analysis as a function of CDI content. In both wet and dry samples, there is a downward trend in the concentration of oligomers migrating to the simulants as the concentration of CDI increases.

However, the wet samples have a higher concentration of oligomers than the dry ones, due to hydrolytic reactions. In addition, the efficiency of CDI as a chain extender is more pronounced in the dry samples, as verified in the rheological analysis, because the lower the concentrations of water in the system, the more available CDI is to react with the end groups of the molecules present in the PLA (Schotman, 1991; Wang et al., 2024).

As described in European Regulation 10/2011, food contact materials are subject to strict rules regarding the migration of IAS and NIAS (Commission Regulation (EU) No 10/2011, 2011; Magnuson et al., 2013). The EU recognizes lactic acid as an authorized monomer for the bio-based polymer PLA with no restrictions on its migration in contact with food. However, cyclic lactide, along with linear and cyclic oligomers are not included in this positive list and are classified as NIAS. Therefore, their migration limit must not exceed 0.01 mg/kg (µg/g). As shown in Figure 4.6, some of the oligomers exceed the specific migration limits, while others meet or are below the limit stipulated by the EU.

One reason the total concentration of oligomers exceeds the specific limit is related to the conditions of the migration tests. These migration tests simulate the extreme conditions a packaging may experience, and it is near the glass transition temperature of PLA (55-60 °C). At this temperature, the amorphous chain gains mobility, facilitating the

migration of substances. Additionally, the interaction between the simulant and the PLA, as well as between the simulant and the oligomers, plays a crucial role in understanding the migration process, impacting both food safety and quality. However, the total oligomer migration shows a clear tendency to decrease with increasing CDI concentration in all the simulants, with a reduction of up to 98.5% observed for samples containing 3% CDI in the EtOH 10% simulant.

d) Understanding oligomer's migration to food simulants

As described above, the oligomers present in PLA can originate from prior processing and the recycling stages of these materials. The degradative processes, primarily hydrolysis, significantly increase oligomer formation, which can render PLA unsuitable for use in food contact packaging. In this context, understanding oligomer migration and employing strategies to mitigate this mass transfer process is crucial for effecting PLA that is suitable for direct food contact and enhancing safety.

Literature suggests that the diffusion process is strongly influenced by the molar mass of the compounds and the polymer's glass transition temperature (Ramesh and Duda, 2000). However, these are not the primary factors in the analysis of the contaminant migration process. As noted by Cruz *et al*., other factors, especially the characteristics and interactions between the systems, must also be considered when evaluating compound migration (Cruz et al., 2021). In this context, the triple interaction between the polymeric matrix, oligomers, and food simulants plays a crucial role in migration. Furthermore, the conditions under which migration tests are conducted can significantly influence diffusion processes.

Another aspect not yet addressed in the literature is the influence of carbodiimide on oligomer migration. As reported, carbodiimides possess two critical properties in the processing and recycling of polymeric materials: their anti-hydrolysis effect and their function as polymer chain-extending agent (Holcapkova et al., 2017; Khorana, 1953; Kurzer and Douraghi-Zadeh, 1967; Williams and Ibrahim, 1981). As an anti-hydrolysis additive, carbodiimide can react with water in the system (Figure 4.3), preventing the polymer chain from undergoing degradation and reducing the formation of oligomers formation and other NIAS. In this context, the additive's potential is more significant in wet PLA samples.

On the other hand, as previously mentioned, CDI can act as a chain extender by reacting with the end groups of PLA. Compared to PLA chains, oligomers' lower molar mass facilitates the diffusion process. In short polymers, movement follows Brownian diffusion, whereas in long-chain polymers, reptation governs the diffusion process, leading to significantly slower mobility (Gennes, 1983). Consequently, oligomers are expected to have higher diffusivity, which favors their reaction with CDI. As a result, the presence of CDI reduces the formation of free oligomers in the sample and inhibits their migration to the simulants. As described previously, a proposed mechanism illustrating the dual action of CDI, both as an anti-hydrolysis chain-extender agent (Figure 4.3).

e) Understanding oligomer's migration to food simulants through molecular modeling

As described in the theoretical methodology, the structure of each oligomer, cyclic (OH-[LA]$_5$-H) and linear (CH_3-CH_2-O-[LA]$_3$-H), was optimized using the GFN-xTB method, as can be seen in Figure 4.6D. The solvation energy, ΔE, which describes the affinity between the oligomer and its medium (simulant) was obtained by Equation 4.2:

$$\Delta E = E_{medium}^{oligomer} - E_{vacuum}^{oligomer} \qquad (4.2)$$

$E_{medium}^{oligomer}$ is the energy of the oligomer (cyclic or linear) in a specific solvent (ethanol or water) and $E_{vacuum}^{oligomer}$ is the energy of an oligomer in gas-phase. Therefore, to calculate ΔE, the models for the cyclic and linear (Figure 4.6D) oligomers were optimized in gas-phase and each medium. The more negative ΔE is, the greater the affinity of the oligomer with the simulant. All structures were confirmed as a minimum in the explored potential energy surface by vibrational frequencies analysis.

To understand the overall trend in why linear oligomers were identified in EtOH 10%, EtOH 95%, and HAc 3% simulants, while the cyclic oligomer was detected in EtOH 95% simulant only, the interaction between oligomer and simulants, through the solvation energy (ΔE), was estimated and collected in Table 4.4. The more negative ΔE is, the more significant the affinity of the oligomer with the simulant. Due to its favorable interactions with the solvent, about -134.3 kJ/mol in EtOH and -89.53 kJ/mol in H_2O, the linear

species appear more in any solution than the cyclic one. In the EtOH simulant, ΔE for the cyclic oligomer becomes greater, about -110.0 kJ/mol, and this species is now experimentally identified in this medium. It is worth noting that although real cyclic and linear oligomers exhibit varying repeating length distributions, incorporating all possible variations would be impractical for electronic structure calculations. Hence, the employed model provides an initial view of the systems and an upper-bound estimate for the solvation energies.

Table III-4.5. Solvation energy, ΔE (kJ/mol) for each oligomer in ethanol and water.

Oligomer	ΔE (EtOH)	ΔE (H_2O)
cyclic	-110.0	-60.6
linear	-134.3	-89.5

5. Conclusion

The anti-hydrolysis and chain extension effects of carbodiimide in PLA were achieved by incorporating it via co-rotating intermeshing twin-screw extrusion. After this, the PLA samples were subjected to migration tests in three food simulants: 10% ethanol, 3% acetic acid, and 95% ethanol (fatty simulant), and oligomers from PLA were qualitatively and quantitatively determined by UPLC-QTOF-MS^E.

The addition of carbodiimide directly increased PLA physical properties such as complex viscosity, influencing PLA molar mass and molar mass distribution and enhancing the nucleation rate of shear flow-induced PLA crystals.

Moreover, carbodiimide was beneficial for food safety, as the total migration of oligomers exhibited a clear tendency to decrease with increasing CDI concentration. This is attributed to CDI's ability to prevent the degradation of PLA chains, which reduces the formation of oligomers and other non-intentionally added substances, while also acting as a chain extender.

The theoretical methodology allows the identification of the linear species as more prevalent in both solutions compared to the cyclic one, due to its favorable interactions

with the solvent. In the ethanol simulant, the energy difference for the cyclic oligomer increases, making this species experimentally identifiable in this medium.

Finally, this study provides valuable insights into the dual role of carbodiimide in enhancing both the physical properties and safety profile of PLA for food packaging applications. By improving the polymer's resistance to hydrolysis and reducing the migration of potentially harmful oligomers, this work advances the development of safer, more sustainable bio-based materials suitable for direct contact with food.

Session IV: Conclusions

General Conclusions

The findings from this study showcase significant advancements in optimizing the recycling process for biodegradable polymer like poly (lactic acid) (PLA), focusing on ensuring their suitability for food packaging applications. By employing a Design of Experiments (DoE) approach, the research systematically analyzed key washing parameters that influence PLA degradation during recycling. This method enabled a thorough evaluation of variables such as temperature, time, surfactant concentration, and sodium hydroxide levels, establishing optimal conditions to minimize degradation while achieving effective decontamination.

One of the key insights was the critical role of surfactant concentration in mitigating degradation, especially when combined with higher temperatures and extended washing durations. Conversely, prolonged washing times were found to significantly exacerbate degradation, particularly when coupled with elevated temperatures and sodium hydroxide concentrations. These findings provide a clear framework for tailoring washing protocols to the contamination level of post-consumer PLA, enhancing the sustainability and efficiency of the recycling process.

The study also explored the complex interactions between PLA and contaminants, particularly the formation of NIAS during recycling. The identification of 34 volatile and semi-volatile compounds, mainly resulting from biopolymer chain degradation, underscored the intricate dynamics of the recycling process. Notably, the detection of dimethyl-1,4-dioxane-2,5-dione (lactide) in all samples highlighted the importance of monitoring such substances, especially given their exclusion from the positive list of European Regulation EU/10/2011. The categorization of NIAS types before and after recycling – including those originating from additives, chain degradation, and recycling induced changes – provides valuable insights for reefing decontamination strategies and ensuring the safety of recycled materials.

The investigation into odorous compounds further underscored their impact on the sensory and safety profiles of recycled PLA. Advanced analytical techniques like HS-SPME-GC-MS and HS-SPME-GC-O-MS identified 14 odorous compounds categorized as toasted, floral, green, and chemical. Compounds such as nonanal, decanal, dodecanal, and 2,4-di-tert-butylphenol were traced back to contamination processes and subsequent hydrolysis reactions. Addressing these off-odors is critical, as they can compromise both

the sensory quality and safety of food products. These findings emphasize the need for targeted measures to mitigate or remove these compounds during recycling.

The study demonstrated the recycling process's effectiveness in reducing contaminant levels, with reductions of up to 80% for substances like tetracosane, heptane, and toluene. A comprehensive recycling cycle proved more effective than isolated washing or mechanical recycling steps. The research also revealed nuanced interactions between PLA and contaminants, influenced by factors like molecular volume, food simulant type, test temperature, and polymer-contaminants dynamics. Theoretical molecular interactions analysis, including electronic structure calculations and Hansen sphere analysis, provided valuable frameworks for understanding these interactions and optimizing recycling protocols.

A particularly innovative aspect was using carbodiimide (CDI) as an anti-hydrolysis agent and chain extender in PLA recycling. Incorporating CDI through twin-screw extrusion enhanced PLA's physical properties, such as increased molar mass and viscosity, while reducing oligomer formation. Migration tests with food simulants, including ethanol and acetic acid, confirmed that CDI significantly decreased oligomer migration, improving the safety profile of recycled PLA. The dual role of CDI in preventing hydrolysis and extending polymer chains highlights its potential as a transformative additive in sustainable recycling practices.

The integration of advanced analytical methodologies and theoretical approaches enriched the understanding of decontamination and structural integrity challenges in PLA recycling. Techniques like UPLC-QTOF-MS^E enabled precise identification and quantification of oligomers, while theoretical calculations elucidated interactions between contaminants, solvents, and the polymer matrix. These insights are crucial for designing more effective recycling systems that balance efficiency, material integrity, and safety.

This research contributes to the broader goals of sustainability and circular economy principles by demonstrating the feasibility of returning bio-based polymers like PLA to their original applications. The results emphasize optimizing recycling protocols not only to meet stringent food safety but also to reduce the environmental impact of biodegradable plastics. By addressing challenges such as NIAS formation, contaminant

migration, and polymer degradation, the study provides a solid foundation for advancing sustainable packaging solutions.

In conclusion, this study highlights the importance of optimizing parameters, controlling contaminants, and leveraging innovative additives like CDI to enhance the recycling process for PLA. By aligning with international food safety regulations and environmentally responsible recycling practices. Ultimately, these insights support the development of a circular economy, where biodegradable polymers can be effectively recycled and reused, reducing reliance on virgin materials and mitigating the environmental impact of plastic waste.

Conclusión General

Los hallazgos de este estudio demuestran avances significativos en la optimización del proceso de reciclaje de polímeros biodegradables, como el poli (ácido láctico) (PLA), que mediante un Diseño de Experimentos (DoE), la investigación analizó de manera sistemática los parámetros clave de lavado que influyen en la degradación del PLA durante el reciclaje. Este método permitió una evaluación exhaustiva de variables como temperatura, tiempo, concentración de surfactantes y niveles de hidróxido de sodio, estableciendo condiciones óptimas para minimizar la degradación al tiempo que se logra una descontaminación efectiva.

Uno de los principales hallazgos fue el papel crítico de la concentración de surfactantes (tensoactivos) en la mitigación de la degradación, especialmente cuando se combina con temperaturas más altas y tiempos de lavado prolongados. Por otro lado, se observó que los tiempos de lavado prolongados agravaban significativamente la degradación, particularmente en combinación con temperaturas elevadas y concentraciones de hidróxido de sodio. Estos resultados proporcionan un marco claro para personalizar los protocolos de lavado según el nivel de contaminación del PLA posconsumo, mejorando la sostenibilidad y eficiencia del proceso de reciclaje.

El estudio también exploró las complejas interacciones entre el PLA y los contaminantes, particularmente la formación de sustancias no intencionadamente añadidas (NIAS) durante el reciclaje. La identificación de 33 compuestos volátiles y semivolátiles, principalmente derivados de la degradación de las cadenas del biopolímero, subrayó la dinámica intrincada del proceso de reciclaje. En particular, la detección de dimetil-1,4-dioxano-2,5-diona (lactida) en todas las muestras destacó la importancia de monitorizar estas sustancias, dado que no están incluidas en la lista positiva del Reglamento Europeo EU/10/2011. La categorización de los tipos de NIAS antes y después del reciclaje, incluyendo aquellas originadas por aditivos, degradación de cadenas y cambios inducidos por el reciclaje, aporta información valiosa para refinar las estrategias de descontaminación y garantizar la seguridad de los materiales reciclados.

La investigación sobre compuestos odorantes subrayó aún más su impacto en el perfil sensorial y de seguridad del PLA reciclado. Técnicas analíticas avanzadas como HS-SPME-GC-MS y HS-SPME-GC-O-MS identificaron 14 compuestos odorantes categorizados como tostados, florales, verdes y químicos. Compuestos como nonanal,

decanal, dodecanal y 2,4-di-terc-butilfenol se atribuyeron a procesos de contaminación y reacciones posteriores de hidrólisis. Abordar estos olores indeseados es fundamental, ya que pueden comprometer tanto la calidad sensorial como la seguridad de los productos alimenticios. Estos hallazgos enfatizan la necesidad de medidas específicas para mitigar o eliminar estos compuestos durante el reciclaje.

El estudio demostró la eficacia del proceso de reciclaje para reducir los niveles de contaminantes, con reducciones de hasta el 80% en sustancias como tetracosano, heptano y tolueno. Un ciclo de reciclaje completo resultó ser más efectivo que pasos aislados de lavado o reciclaje mecánico. La investigación también reveló interacciones matizadas entre el PLA y los contaminantes, influenciadas por factores como el volumen molecular, el tipo de simulante alimentario, la temperatura de prueba y las dinámicas polímero-contaminante. El análisis teórico de interacciones moleculares, incluidos las cálculos de estructura electrónica y el análisis de esfera de Hansen, proporcionó marcos valiosos para comprender estas interacciones y optimizar los protocolos de reciclaje.

Un aspecto particularmente innovador fue el uso de carbodiimida (CDI) como agente antihidrólisis y extensor de cadenas en el reciclaje de PLA. La incorporación de CDI mediante extrusión de doble husillo mejoró las propriedades físicas del PLA, como el aumento de la masa molar y la viscosidad, al tiempo que redujo la formación de oligómeros. Las pruebas de migración con simulantes alimentarios, incluidos etanol y ácido acético, confirmaron que el CDI disminuye significativamente la migración de oligómeros, mejorando el perfil de seguridad del PLA reciclado. El doble papel del CDI en prevenir la hidrólisis y extender las cadenas del polímero destaca su potencial como un aditivo transformador en prácticas de reciclaje sostenibles.

La integración de metodologías analíticas avanzadas y enfoques teóricos enriqueció la comprensión de los desafíos relacionados con la descontaminación y la integridad estructural en el reciclaje del PLA. Técnicas como UPLC-QTOF-MS^E permitieron una identificación y cuantificación precisa de oligómeros, mientras que los cálculos teóricos elucidaron las interacciones entre contaminantes, solvente y la matriz polimérica. Estos conocimientos son cruciales para diseñar sistemas de reciclaje más efectivos que equilibren la eficiencia, la integridad del material y la seguridad.

Esta investigación contribuye a los objetivos más amplios de sostenibilidad y principios de economía circular al demostrar la viabilidad de devolver polímeros de base

biológica como el PLA a sus aplicaciones originales. Los resultados enfatizan la optimización de los protocolos de reciclaje no solo para cumplir con estrictas normativas de seguridad alimentario, sino también para reducir el impacto ambiental de los plásticos biodegradables. Al abordar desafíos como la formación de NIAS, la migración de contaminantes y la degradación del polímero, el estudio establece una base sólida para avanzar en soluciones de envasado sostenible.

En conclusión, este estudio destaca la importancia de optimizar los parámetros, controlar los contaminantes y aprovechar aditivos innovadores como el CDI para mejorar el proceso de reciclaje del PLA. Al alinearse con las normativas internacionales de seguridad alimentaria y prácticas de reciclaje ambientalmente responsables, estos conocimientos apoyan el desarrollo de una economía circular en la que los polímeros biodegradables puedan reciclarse y reutilizarse eficazmente, reduciendo la dependencia de materiales vírgenes y mitigando el impacto ambiental de los residuos plásticos.

Conclusões Gerais

Os pontos principais deste estudo demonstram avanços significativos na otimização do processo de reciclagem de polímeros biodegradáveis, como o poli(ácido lático) (PLA). Utilizando a metodologia de Desenho de Experimentos (DoE), a pesquisa analisou de forma sistemática os parâmetros-chave de lavagem que influenciam a degradação do PLA durante a reciclagem. Esse método permitiu uma avaliação abrangente de variáveis como temperatura, tempo, concentração de tensoativos e níveis de hidróxido de sódio, estabelecendo condições ótimas para minimizar a degradação sem comprometer a eficiência de descontaminação.

Um dos principais resultados foi o papel crítico da concentração de tensoativos na mitigação da degradação, especialmente em combinação com temperaturas mais elevadas e tempos prolongados de lavagem. Por outro lado, verificou-se que tempos excessivos de lavagem, intensificam a degradação, sobretudo quando associados a altas temperaturas e concentrações de hidróxido de sódio. Esses resultados fornecem, um referencial claro para a personalização dos protocolos de lavagem de acordo com o nível de contaminação do PLA pós-consumo, favorecendo um processo de reciclagem mais eficiente e sustentável.

O estudo também explorou as complexas interações entre o PLA e contaminantes, em particular a formação de substâncias não intencionalmente adicionadas (NAIS) durante o processo de reciclagem. Foram identificados 33 compostos voláteis e semivoláteis, principalmente derivados da degradação das cadeias do biopolímero, o que evidencia a complexidade da dinâmica de reciclagem. A detecção consistente de dimetil-1,4-dioxano-2,5-diona (lactida) em todas as amostras destacou a importância do monitoramento dessas substâncias, considerando que não constam na lista positiva do Regulamento Europeu EU/10/2011. A catalogação das NIAS antes e após o processo – incluindo aquelas originadas de aditivos, da degradação de cadeias e de modificações induzidas pela reciclagem – fornece informações valiosas para aprimorar estratégias de descontaminação e garantir a segurança dos materiais reciclados.

A investigação sobre compostos odorantes ressaltou ainda mais sua relevância no perfil sensorial e de segurança do PLA reciclado. Técnicas avançadas como HS-SPME-GC-MS e HS-SPME-GC-O-MS identificaram 14 compostos odorantes, classificados em

grupos de odores: torradas, florais, verdes e químicos. Substâncias como nonanal, decanal, dodecanal e 2,4-di-terc-butilfenol foram associadas a processos de contaminação e reações subsequentes de hidrolise. O controle desses compostos é essencial, já que odores indesejados podem comprometer tanto a qualidade sensorial quanto a segurança de embalagens alimentícias.

A eficiência do processo de reciclagem também foi confirmada pela redução significativa de contaminantes, com diminuições de até 80% em substâncias como tetracosano, heptano e tolueno. O ciclo completo de reciclagem mostrou-se mais eficaz do que etapas isoladas de lavagem ou reciclagem mecânica. Além disso, as interações entre o PLA e os contaminantes foram influenciadas por fatores como volume molecular, tipo de simulante alimentício, temperatura de ensaio e dinâmicas de interações polímero-contaminante. O suporte teórico, por meio de cálculos de interação moleculares, estrutura eletrônica e análise da esfera de solubilidade de Hansen, contribuiu para uma compreensão detalhada desses mecanismos e para o aperfeiçoamento dos protocolos de reciclagem.

Um aspecto inovador foi o uso da carbodiimida (CDI) como agente anti-hidrólise e extensor de cadeias no processo de reciclagem do PLA. A incorporação de CDI por extrusão em dupla rosca melhorou significativamente as propriedades físico-químicas do PLA, incluindo aumento da massa molar e da viscosidade, além da redução na formação de oligômeros. Ensaios de migração com simulantes alimentícios (etanol e ácido acético) confirmaram que a CDI reduz de forma expressiva a migração de oligômeros, elevando o perfil de segurança do PLA reciclado. Esse duplo papel da CDI – prevenir a hidrolise e promover a extensão da cadeia polimérica - evidencia seu potencial como aditivo estratégico para uma reciclagem sustentável.

A integração de metodologias analíticas avançadas e abordagens teóricas ampliou a compreensão dos desafios relacionados à descontaminação e à integridade estrutural no processo de reciclagem do PLA. Técnicas como UPLC-QTOF-MSE possibilitaram a identificação e quantificação precisa de oligômeros, enquanto análises teóricas esclareceram as interações entre contaminantes, solventes e a matriz polimérica. Esses conhecimentos são fundamentais para projetar sistemas de reciclagem mais eficientes, equilibrando desempenho, segurança e preservação da estrutura polimérica.

Este trabalho contribuiu para os objetivos globais de sustentabilidade e para os princípios da economia circular, ao demonstrar a viabilidade de reinserir biopolímeros como o PLA em suas aplicações originais. Os resultados reforçam a importância de otimizar protocolos de reciclagem não apenas para atender as rigorosas normas de segurança alimentar, mas também, para mitigar o impacto ambiental dos plásticos biodegradáveis. Ao abordar desafios como a formação de NIAS, a migração de contaminantes e a degradação do polímero, o estudo estabelece bases solidas para o avanço de soluções de embalagens sustentáveis.

Em conclusão, esta pesquisa evidencia a relevância da optimização de parâmetros de lavagem, do controle de contaminantes e do uso de aditivos inovadores como a CDI para aprimorar a reciclagem do PLA. Ao alinhar-se às normas internacionais de segurança alimentar e a práticas ambientalmente responsáveis, os resultados apoiam o desenvolvimento de uma economia circular em que polímeros biodegradáveis possam ser reciclados e reutilizados de forma eficaz, reduzindo a dependência de materiais virgens e os pactos ambientais associados aos resíduos plásticos.

Session V: Publications

Publications

During my doctoral studies, I published several papers in international journals, both as first author and in collaboration with researchers from various research groups and external partners. The works listed below span during the academic cotutor-ship, highlighting the contributions of both institutions involved in the collaboration.

- **Paiva, R.**; Pereira-Filho, E. R.; Wrona, M.; Cruz, S. A. Optimization of Washing Parameters to Minimize the Degradation of Poly(Lactic Acid) Using Design of Experiments: A Contribution to the Recycling Chain. *J Polym Environ* 2024, *32* (4), 1650–1657. https://doi.org/10.1007/s10924-023-03075-7.
- **Paiva, R.**; Wrona, M.; Nerín, C.; Gavril, G.-L.; Cruz, S. A. Volatile Compounds and Off-Odors Analysis of Recycled PLA for Packaging Applications: An Essential Factor for Ensuring Food Safety and Quality. *J Polym Environ* 2024, *32* (12), 6687–6697. https://doi.org/10.1007/s10924-024-03409-z.
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ANNEX I

Chapter 3

Biobased Polymer Recycling: A Comprehensive Dive into the Recycling Process of PLA and Its Decontamination Efficacy

Table 3.1 Limits of detection (LOD) and quantification (LOQ) for the surrogates present in DCM/ethanol after total dilution analysis.

Surrogates	Solvent		
	DCM/EtOH		
	LOD (ng/g)	LOQ (ng/g)	r
Benzophenone	6.53	21.77	0.996
Tetracosane	5.63	18.76	0.998
Toluene	12.95	43.17	0.990
Heptane	1.23	4.09	0.997
Chloroform	28.96	96.56	0.999

Table 3.2 Limits of detection (LOD) and quantification (LOQ) for the surrogates present in 10% ethanol and 3% acetic acid after migration study.

Surrogates	Food Simulants				
	EtOH 10%		HAc 3%		
	LOD (ng/g)	LOQ (ng/g)	LOD (ng/g)	LOQ (ng/g)	r (range)
Benzophenone	0.10	0.34	0.11	0.37	0.997 - 0.998
Tetracosane	0.10	0.32	0.10	0.34	0.993 - 0.999
Toluene	0.17	0.58	0.16	0.54	0.991 - 0.994
Heptane	0.09	0.28	0.09	0.30	0.995 - 0.997
Chloroform	0.11	0.38	0.12	0.39	0.996 - 0.997

Table 3.3 Concentration of surrogates for each sample in the different simulants and under different migration test conditions.

Food simulants		**Concentration migrants (mg kg^{-1}) EtOH 10%**				**Concentration migrants (mg kg^{-1}) HAc 3%**			
Samples		**PLAc**	**PLAcw**	**PLAcr**	**PLAcwr**	**PLAc**	**PLAcw**	**PLAcr**	**PLAcwr**
Surrogates	**Migration conditions**								
Benzophenone	10 days 40 °C	9.918 (±1.824)	4.298 (±1.386)	5.965 (0.049)	3.711 (0.188)	0.016 (0.002)	0.017 (0.003)	0.022 (0.01)	0.030 (0.003)
	10 days 60 °C	17.096 (1.292)	3.730 (0.053)	28.958 (0.785)	20.637 (1.985)	12.404 (0.50)	10.632 (1.09)	10.312 (1.08)	9.044 (3.78)
Tetracosane	10 days 40 °C	16.382 (8.998)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	10 days 60 °C	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Toluene	10 days 40 °C	18.819 (2.565)	13.819 (3.93)	0.300 (0.084)	0.149 (0.060)	3.486 (0.051)	2.201 (0.50)	0.092 (0.03)	0.1 (0.06)
	10 days 60 °C	34.309 (7.760)	17.40 (2.418)	0.365 (0.034)	0.148 (0.082)	28.040 (3.50)	29.506 (8.60)	0.267 (0.11)	0.391 (0.22)
Heptane	10 days 40 °C	0.987 (0.143)	0.287 (0.009)	0.038 (0.023)	0.038 (0.013)	21.721 (3.55)	1.288 (0.10)	0.156(0.002)	0.143 (0.03)
	10 days 60 °C	2.846 (0.682)	0.656 (0.167)	0.047 (0.012)	0.028 (0.006)	5.025 (0.701)	12.285 (3.07)	0.790 (0.13)	0.220 (0.03)
Chloroform	10 days 40 °C	13.265 (2.097)	8.134 (1.251)	0.521 (0.005)	0.657 (0.031)	19.027 (0.360)	18.208 (1.63)	0.377 (0.02)	0.366 (0.01)
	10 days 60 °C	85.979 (4.217)	31.857 (10.563)	0.643 (0.041)	0.611 (0.020)	47.635 (8.55)	36.723 (6.44)	0.351 (0.04)	0.335 (0.01)

Table 3.4 Results of the F calculated, F critical and P-values for the migration tests conducted in the two food simulants under the established test conditions.

Food Simulants		EtOH 10%			HAc 3%		
Surrogates	Migration Conditions	F calculated	F critical	P-Value	F calculated	F critical	P-value
Benzophenone	10 days 40 °C	9,332	6,591	0,028	2,626	4,066	0,122
	10 days 60 °C	125,678	6,591	0,0002	0,907	6,591	0,512
Tetracosane	10 days 40 °C	6,629	6,591	0,049	-	-	-
	10 days 60 °C	-	-	-	-	-	-
Toluene	10 days 40 °C	32,792	6,591	0,002	15,186	4,066	0,001
	10 days 60 °C	32,150	6,591	0,002	25,020	6,591	0,004
Heptane	10 days 40 °C	77,011	6,591	0,0005	76,491	6,591	0,0005
	10 days 60 °C	28,770	6,591	0,003	24,968	6,591	0,004
Chloroform	10 days 40 °C	50,70	6,591	0,001	803,52	4,066	2,9E-10
	10 days 60 °C	53,324	6,591	0,001	42,121	6,591	0,002

ANNEX II

Chapter 4

How carbodiimide modulates oligomer migration and molar mass in recycled poly (lactic acid): A study using UPLC-QTOF-MS^{E}

The time sweep measurements were performed in an Anton Paar MCR 302 at 200°C, using 25 mm diameter plates with a gap distance of 1 mm, under an inert nitrogen atmosphere. The samples were submitted at a frequency of 1 Hz with an oscillatory shear deformation defined by a strain of 3% deformation during 3600 s.

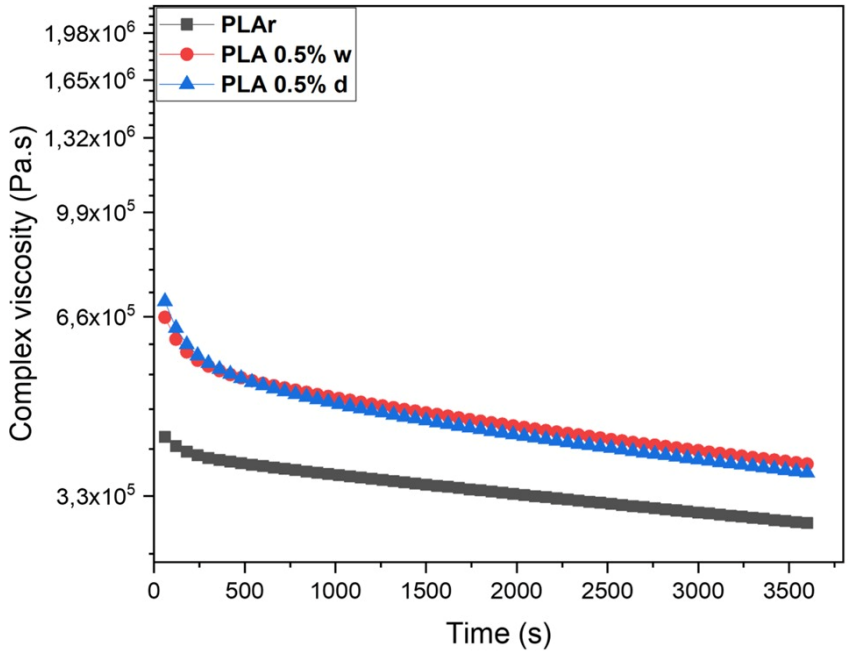

Figure 4.1. Time sweep measurement for PLA samples without and with 0.5% CDI for wet and dry samples.