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Active packaging based on PLA/Green Tea Extract

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Trabalho efetuado sob a orientação de
Doutor Arsénio Vasconcelos de Sá
Professora Doutora Ana Vera Alves Machado

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RESUMO

Ao longo dos anos, a indústria dos plásticos tem vindo a evoluir de forma gradual, sendo que a presença dos mesmos já faz parte do nosso dia-a-dia. Os polímeros sintéticos, devido às suas propriedades (baixo custo, versatilidade, peso, entre outras), têm sido cada vez mais aplicados numa vasta gama de sectores, principalmente na produção de embalagens. Apesar da utilização de polímeros sintéticos ser benéfica, a acumulação deste tipo de materiais no meio ambiente tem vindo a causar sérios problemas ambientais, visto serem extremamente resistentes a degradação. Uma das alternativas para a resolução deste problema passa pelo uso de polímeros biodegradáveis de origem natural, visando substituir os polímeros sintéticos, em particular na produção de embalagens com um ciclo de vida curto. Simultaneamente, o aumento da população e a constante exigência por produtos alimentares com qualidade, segurança e saudáveis tem contribuído para o desenvolvimento de novos sistemas por parte da indústria. O desperdício alimentar ao longo da cadeia de produção motivou, também, o desenvolvimento destes novos sistemas. Assim, foram desenvolvidas novas abordagens na fabricação de embalagens, entre as quais a embalagem ativa. Esta embalagem é capaz de interagir com o meio envolvente, contribuindo para a preservação das propriedades do alimento e aumentar o seu tempo de preservação.

Esta dissertação foca-se no desenvolvimento de um material para embalagem alimentar ativa, baseada em ácido poliláctico (PLA), um polímero biodegradável, ao qual foi adicionado extrato de chá verde (ECV), de modo a conferir propriedades antioxidantes. A compatibilidade entre PLA e o ECV foi avaliada através da produção de misturas de ambos no fundido. Após o estudo de compatibilidade, foram produzidos filmes por extrusão de filme tubular. Adicionaram-se diferentes quantidades de ECV ao PLA de modo a avaliar a sua influência na capacidade antioxidante dos filmes. Os materiais produzidos foram analisados por análise termo gravimétrica (TGA), calorimetria diferencial de varrimento (DSC) e espectroscopia de infravermelhos (FTIR). Foram também realizados ensaios mecânicos e ensaios para avaliar a capacidade antioxidante e determinar as propriedades de barreira ao vapor de água. Os resultados mostraram que a adição do extrato de chá verde confere capacidade antioxidante aos filmes, estando esta dependente da quantidade de ECV adicionado. Verificou-se que os filmes que contêm ECV apresentam estabilidade térmica e propriedades mecânicas inferiores às dos filmes sem ECV. No entanto, possuem melhores propriedades de barreira ao vapor de água. Estes filmes mostram-se eficientes na preservação de vários alimentos, incluindo maçã, batata e salmão fumado, retardando processos oxidativos que levam à sua degradação.

Palavras-chave: Embalagem ativa, PLA, extrato de chá verde, antioxidante, biodegradável.

ABSTRACT

Over the years, plastic industry has been growing, and plastics are present in our daily basis. Synthetic polymers, due to their excellent properties (low cost, versatility, weight, among others), have been extensively used in a wide range of applications, especially in packaging production. Although the use of synthetic polymers has proven to be benefic, their accumulation in the environment has been one of the main causes of environmental issues, since they are extremely resistant to degradation. One of the alternatives for this problem has been the incorporation of biodegradable polymers of natural origin to replace synthetic polymers, in particular in the production of packages with a short life-cycle. At the same time, population growth and constant demand for quality, safety and healthy food products contributed to the development of new food packaging systems. The amount of food waste generated along the supply chain also played an important role in the development of these new systems. Thus, new approaches were developed in the manufacture of packaging, being active packaging one of these newer systems. This type of package interacts with the surrounding environment contributing to the preservation of foods' properties and increasing their shelf-life.

This dissertation was focused on the development of a material with application as active food packaging, based on polylactic acid (PLA), a biodegradable polymer, to which was added green tea extract (GTE), in order to confer antioxidant properties. The compatibility between PLA and GTE was evaluated by producing mixtures of both materials in the melt. After this compatibility study, films of the mixture were produced by using blown film extrusion. Different amounts of GTE were added to PLA to evaluate its influence on the films' antioxidant capacity. The produced materials were analyzed by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR). The films' mechanical properties were evaluated, as well as their antioxidant capacity and water-vapor barrier properties. The results showed that the addition of green tea extract confers antioxidant capacity to the films, which is dependent on the quantity of GTE added. It was observed that films containing GTE have lower thermal stability and mechanical properties when compared with films without GTE. Nevertheless, they showed improved water-vapor barrier properties. These films proved to be efficient in the preservation of food, including apple, potatoes and smoked salmon, inhibiting oxidative phenomena that result in their spoilage.

Keywords: Active packaging, PLA, green tea extract, antioxidant, biodegradable.

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LIST OF ACRONYMS AND ABBREVIATIONS

O_2	Oxygen
H_2O	Water
CO_2	Carbon dioxide
PLA	Polylactic acid
GTE	Green tea extract
PE	Polyethylene
PET	Polyethylene terephthalate
PP	Polypropylene
PS	Polystyrene
EPS	Expanded polystyrene
LDPE	Low density polyethylene
HDPE	High density polyethylene
PVC	Polyvinyl chloride
PUR	Polyurethane
PHA	Polyhydroxyalkanoates
CH_4	Methane
EOs	Essential Oils
WVT	Water-vapor transmission
WVP	Water-vapor permeability
TGA	Thermogravimetric analysis
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared spectroscopy
g	Grams
%	Percentage
MPa	MegaPascal
°C	Degrees Celsius
C_2H_4	Ethylene
M_w	Molecular weight
rpm	Rotations per minute
KBr	Potassium Bromide

$CaCl_2.6H_2O$	Calcium chloride hexahydrate
RH	Relative humidity
ECV	Extrato de chá verde
Mm	Millimetres
T	Time
wt.%	Weight percentage
σ_{stress}	Yield stress
$\sigma_{strength}$	Tensile strength
ε_{break}	Strain at break
ε_{strain}	Yield strain
E_{Young}	Young's modulus
DPPH	2,2-diphenyl-1-picrylhydrazyl
TE	Trolox equivalents
GAE	Gallic acid equivalents
ECE	Epicatechin equivalents

1. INTRODUCTION

1.1 Motivation

Over the years, the consumption rate of synthetic polymers has grown in an astonishing fashion and it has become one of the most attractive materials in the industry, especially for packaging applications. These materials possess optimal characteristics, such as excellent mechanical and optical properties, ease to process, low cost of the raw materials and good aesthetic qualities. However, the massive increase of plastics production and consumption has been associated to environmental issues, especially due to their inappropriate disposal. For that reason, the packaging industry is investing in the development of new packaging systems, focused in the implementation and use of natural and biodegradable materials. The use of biodegradable materials, especially biopolymers, for packaging represents a viable solution to the growing environmental issues, since they possess similar properties to synthetic polymers and are biodegradable.

Active packaging, one of the newer packaging systems, has attracted high interest in recent years. This type of packaging consists in the incorporation of active agents, which can possess antioxidant or antimicrobial properties, for instance, into a polymer matrix to increase the products' shelf-life. Although this represents a viable approach to guarantee food preservation and a solution to environmental issues, the incorporation of this technology in the European packaging market is a slow process, due to the existence of a strict regulation.

The present work is focused on the development of a material with application as an active packaging system based on the incorporation of green tea extract (GTE), known for its antioxidant properties, into polylactic acid (PLA), a biodegradable polymer.

2. STATE OF THE ART

2.1 European consumption of plastics

Plastics' consumption has been growing at a continuous rate, and it quickly became one of the most used materials worldwide, especially by the packaging industry, that looked at plastics as a viable option to replace traditional packaging materials, including cellulose-based materials (paper), glass and metal [1–3]. This increase in consumption can be associated with populational growth, life-style changes (less time spent shopping for fresh products and cooking) and market globalization (products are distributed to longer distances, thus increasing the need for longer storage times, due to the nature of the existence of a wide variety of products), resulting in higher consumption of disposable products, including packed and ready-to-eat foods [4–7]. Figure 1 depicts the major applications of synthetic polymers in industry and its evolution over the years. It is possible to conclude that the packaging sector plays a fundamental role when it comes to polymer consumption. Also, it is an ever-growing sector due to the increase of the consumption of packed products, as already mentioned.

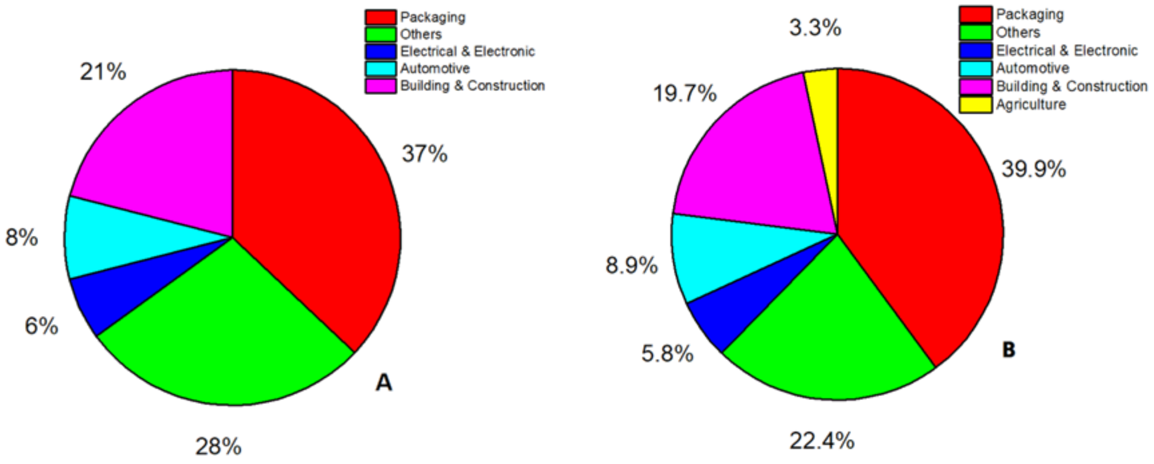

Figure 1: Major applications of synthetic polymers in the European industry in 2006 (A) and in 2015 (B). Adapted from [8,9].

Generally, packaging is an element used to, not only assure mechanical support, preservation, protection, and containment to all types of products, but also include related information, representing an essential market that can be found in every industry [4,10]. In terms of food packaging, its main purpose is to ensure the protection of foodstuffs against external deteriorative effects, such as oxygen (O_2), heat, microorganisms, light, presence or absence of moisture, among others, thus preserving food quality and extending products' shelf-life [4,11]. It is important to assure that there are minimal interactions between the packaging material and the food, in order to prevent the migration of substances that could compromise food quality and safety [11,12].

2.1.1 Packaging Materials

As it is demonstrated in Figure 2, there is a wide variety of petroleum based polymers that are used in a wide variety of applications. Polymers such as polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), low density polyethylene (LDPE), high density polyethylene (HDPE), polystyrene (PS), polyvinyl chloride (PVC) and polyurethane (PUR) are the most commonly used materials for packaging applications [2,13–15]. Their extensive use in the packaging industry is mainly due to their properties, such as low cost of raw materials, light weight, good gas barrier properties to O_2, water (H_2O), carbon dioxide (CO_2), transparency, heat seal ability, chemical resistance, good mechanical properties and easy to process [2,4,10,13–17].

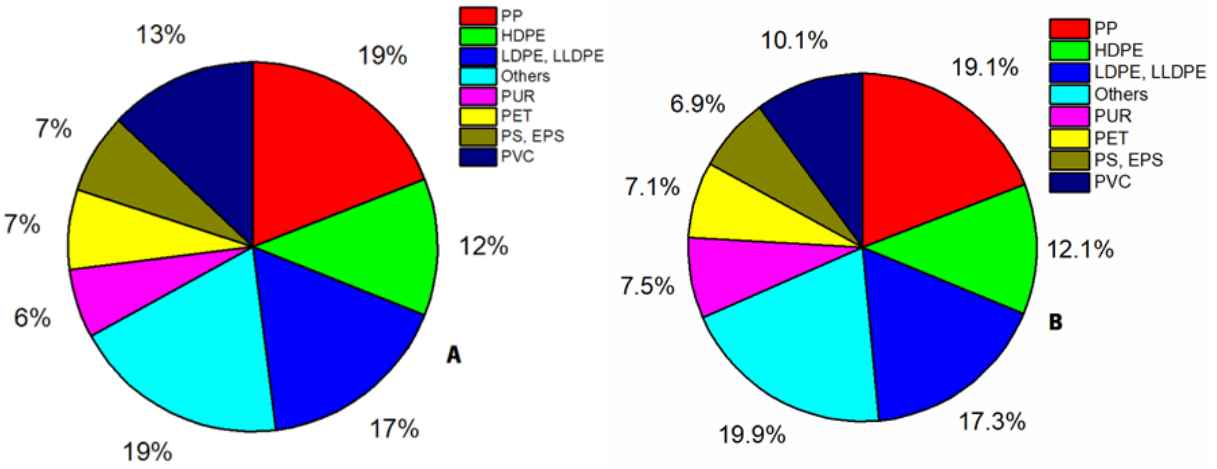

Figure 2: Plastics demand in Europe in 2006 (A) and 2015 (B). Adapted from [8,9].

However, these materials have disadvantages, including non-biodegradability, fossil fuels derived, use of high amounts of energy in their production, among others [2,18]. Due to these aspects and their longevity and widespread use in disposable packaging, they are associated to severe environmental issues [3,12–14,19–21]. Depending on the applications, plastic materials can be recycled [13]. However, there are situations that their recycling is not a viable option, due to the presence of other materials (mixtures), additives (plasticizers) and contamination of the package by foodstuffs, for instance [3,13,17]. Usually, contaminated end-of-life products are disposed in landfills, however, when done inappropriately, lead to several environmental problems [3,14].

Thus, attempting to counteract and reduce the environmental impact caused by synthetic packaging, biodegradable materials are being look at as an potential replacement of these materials in packaging applications [4,13]. The main objective of using biodegradable materials is associated to their capacity to mimic the biomass's lifecycle. Therefore, these materials should be renewable and able to biodegrade into natural non-harmful products, including H_2O, CO_2 and methane (CH_4), contributing to conservation of fossil resources [10,19,22,23].

So, a wide variety of biopolymers, which can be obtained from renewable and biodegradable resources, such as PLA, chitosan, thermoplastic starch, polyhydroxyalkanoates (PHA), among others, have been employed in packaging [4,14,24]. Figure 3 summarizes the existing natural biopolymers according to their origin.

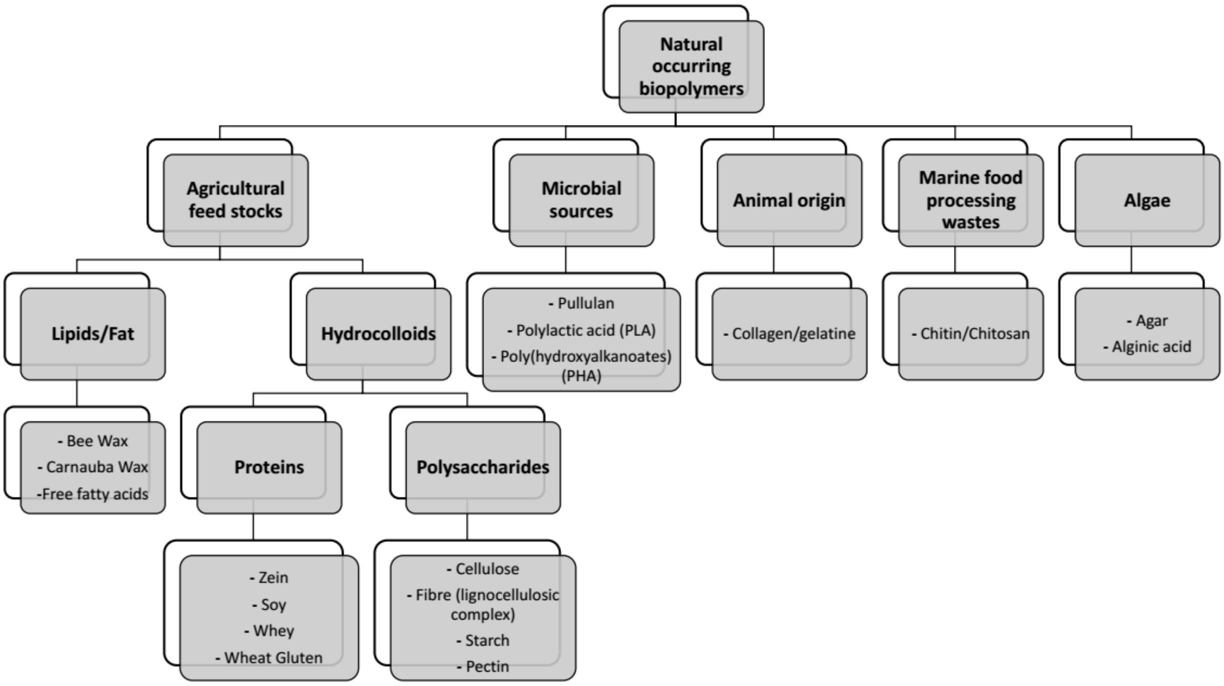

Figure 3: Natural biopolymers and their sources. Adapted from [10,25].

The use of these materials has several advantages, especially when it comes to be applied in packaging, since they are biodegradable, contribute to the conservation of fossil fuels, possess similar mechanical performance as synthetic polymers, among others [15,26].

However, the use of biopolymers in the production of packaging materials has several disadvantages, including their higher cost compared with the petroleum-based polymers, the use of land and resources to produce raw materials (agricultural crops) and lower performances in terms of thermal stability, melt strength and higher permeability to H_2O and O_2 [3,23,27].

2.1.2 Development of new packaging systems

In order to keep up with population growth and changes in society, the packaging industry need to develop new systems able to preserve and maintain food safety and quality during all stages of the supply chain, until it reaches its final destination [4,6,7,13]. This resulted in the development of the concepts of active and intelligent packaging [4]. While active packaging is focused on the interactions between package, food and environment, in order to extend the shelf-life of a product, intelligent packaging *"monitors the condition of the packaged food and the environment surrounding it"* [4,13,28].

Based on these concepts, it is possible to produce a wide diversity of active and intelligent mechanisms that provide the packaging industry with the opportunity to develop a specific package for a specific food product [4]. They can promote ideal packaging conditions to extend shelf-life, but for that it is essential to understand how food's unique spoilage mechanisms work and how they can be controlled [4].

However, there are some issues regarding the acceptance of these technologies, especially in Europe [13]. The existence of strict regulations (European Regulation 450/2009/EC [28]) on the materials that can be in contact with food, difficulty on the diffusion and implementation of active and intelligent packaging on the market, and lack of knowledge on their potential benefits in food preservation contribute to its slower penetration in the European market [4,12]. Also, the higher costs associated with them and the lack of acceptance also contribute to the slow implementation on the market [4].

Although, there are possible solutions for this type of restraints. For example, the cost of the technology can be greatly reduced by expanding the range of applications in packaging, if consumers are willing to pay for the added benefits, and guarantee that consumers get the necessary information regarding the benefits of active and intelligent materials in food preservation [4,7,12].

2.2 **Active packaging**

Active packaging can be defined as an innovative packaging strategy that was developed in order to fulfill the demands of an ever-growing society [6]. It is a system based in the interactions between package, product and environment, in order to extend the food products' shelf-life or to enhance its sensory properties, while assuring product quality and safety [6,29]. However, due to market globalization, the packaging industry has been forced to develop an considerable amount of packaging systems that are capable to preserve and protect food products from external influences, maintaining food quality until it reaches its final destination [6,29]. Also, the implementation of this technology can have a positive impact on the cost-effectiveness of a product, by reducing food waste caused by food spoilage [4,17].

As a result of this, a wide variety of different active packaging systems for the food industry, including O_2 scavengers, CO_2 absorbers, ethylene (C_2H_4) scavengers, antimicrobial, antioxidant, among others, were developed to enhance the shelf-life of a wide range of food products [6,29]. Extensive knowledge related to this system and mechanisms of actions can be found in the literature [11,30].

Regarding this technology, the current focus is to potentiate the incorporation of biopolymers in the production of active packaging systems [13,31]. This focus corresponds to the popular demand for a more extensive use of materials obtained from natural resources and the growing concerns about the environmental impact caused by the over usage of synthetic polymers. Furthermore, due to their properties, they can be used as a potential replacement for synthetic polymers for packaging applications [31]. Although there is a wide variety of biopolymers available in nature (Figure 3), only a few can be used in packaging applications [13], including PLA [5,16,32–34], protein films [31], chitosan [35,36], among others [37,38].

The demand for minimally processed food and the raising health concerns about the use of synthetic additives for food preservation [30,31] lead the packaging industry to replace such additives, by natural ones (obtained from plant extracts, herbs or spices) [39,40]. The incorporation of natural preservation agents in food packaging became a viable due to the fact that they can prevent microbial proliferation and oxidative activity, and by doing so, extend the shelf-life of food products [39]. Plant extracts or essential oils (EO's) are the most used natural products in the packaging industry, since they are rich in phenolic compounds, known for their high antioxidant and antimicrobial properties [31,39].

The selection of the natural additive should be based on several aspects, including activity, availability, cost effectiveness, consumer awareness and effect on the sensory properties that it may have on the final product [39,40]. Also, the compatibility between the natural extract and the polymer matrix is of extreme importance to assure a homogeneous distribution of the extract that can potentiate the efficiency of its antioxidant and/or antimicrobial effect [39,40].

Several additives rich in phenolic compounds have been incorporated into synthetic or biodegradable polymers to produce materials with antioxidant and antimicrobial capacity. Extracts of rosemary [1,41,42], grapefruit [43], green tea [31,35,37,44,45] and black tea [31,46] are some examples of natural additives tested in the production of active packaging systems.

2.2.1 Antioxidant activity

Antioxidant activity can be defined *"as the delay or inhibition of oxidative reactions with lipids or other molecules by inhibiting the initiation and propagation step of lipid oxidation by forming stable radicals A·, which are non-reactive or form non-radical products"* [39].

It is known that lipids play a critical role in food quality, especially when it comes to nutrition (essential fatty acids), health promotion (omega-3 fatty acids), among others [47]. Their oxidation is one of the main degradation phenomena, and represents one of the main concerns in the food industry [40,48]. It occurs when oxygen free radicals attack foodstuffs with high lipid content, especially unsaturated fatty acids, leading to loss of quality and safety during the stages of production, transport, processing or storage, reducing drastically the shelf-life and quality of packed products [37,40,43,47,48]. This results in the loss of nutritional value, due to degradation of essential nutrients, production of off-flavors and odors, and color changes, which turns food products inadequate for human consumption, causing economic impact to the food industry and leading to environmental issues [43,49].

Briefly, lipids' oxidation can be described as "*a free radical chain reaction between unsaturated fatty acids and reactive oxygen species"* [48]. This process is dependent on the food type and it can be accelerated by the presence of reactive species and physicochemical environment [48,50]. It can be divided into three stages [47,48]: initiation; propagation; and termination. Equations 1 to 6 represent the mechanism of lipid oxidation [39,48].

$$\mathrm{RH} \xrightarrow{\text{Initiator}} \mathrm{R}^{\bullet} + \mathrm{H}^{\bullet} \quad (1)$$

$$\mathrm{R}^{\bullet} + \mathrm{O}_2 \rightarrow \mathrm{ROO}^{\bullet} \quad (2)$$

$$\mathrm{ROO}^{\bullet} + \mathrm{RH} \rightarrow \mathrm{ROOH} + \mathrm{R}^{\bullet} \quad (3)$$

$$\mathrm{ROO}^{\bullet} + \mathrm{ROO}^{\bullet} \rightarrow \mathrm{ROOH} + \mathrm{O}_2 \quad (4)$$

$$\mathrm{ROO}^{\bullet} + \mathrm{R}^{\bullet} \rightarrow \mathrm{ROOR} \quad (5)$$

$$\mathrm{R}^{\bullet} + \mathrm{R}^{\bullet} \rightarrow \mathrm{RR} \quad (6)$$

The initiation step corresponds to the formation of a fatty acid radical, known as an alkyl radical (R·). This radical is formed by abstraction of a hydrogen in the presence of an initiator, such as heat, light, among others (Eq. 1) [48].

The propagation step corresponds to the reaction between O_2 and the alkyl radical, resulting in the production of a peroxyl radical (ROO·) (Eq. 2). This peroxyl radical can abstract a hydrogen from another unsaturated fatty acid (RH) and form a stable lipid hydroperoxide (ROOH) and an alkyl radical (Eq. 2) [48]. The decomposition of the hydroperoxides (homolytic cleavage) into alkoxyl radicals (RO·) and hydroxyl radicals (·OH) can lead to the formation of secondary lipid oxidation products, such as aldehydes, ketones, alcohols, among others, responsible for the appearance of off-flavors and off-odors in foodstuffs [48].

The termination step corresponds to the interaction of two free radicals, which can be two peroxyl radicals (Eq. 4), a peroxyl radical and an alkyl radical (Eq. 5) and two alkyl radicals (Eq. 6) [48]. Generally, depending on the food product, this step does not have a significant importance, since food already suffered from severe rancidity before terminal reactions can occur [48].

An antioxidant (A) can prevent the propagation stage by reacting with the ROO· radicals forming stable ROOH and A· radicals, which are non-reactive species.

Also, there are other oxidation mechanisms that can be inhibited by antioxidant compounds. In the case of fruits and vegetables, the oxidative degradation is related to the presence of polyphenol oxidases (PPOs), enzymes able to catalyze the oxidation of phenolic compounds into quinones in the presence of oxygen, producing the characteristic brown or black pigments in the wounded tissues [51,52]. Figure 4 represents the enzymatic oxidation of a phenolic compound.

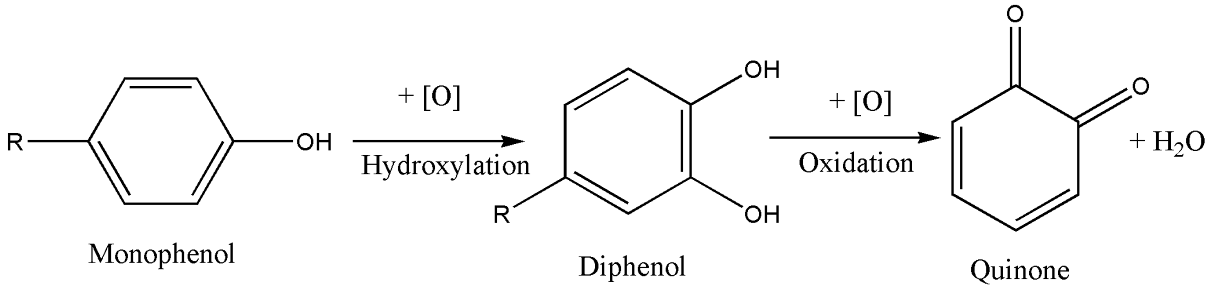

Figure 4: Scheme of the enzymatic oxidation of a phenolic compound. Adapted from [52].

2.3 Biodegradation

Biodegradation can be defined as the chemical process that converts organic materials into CO_2, H_2O and biomass, which can result from microorganisms intervention [15]. This process depends on the presence of three indispensable factors: conditions (temperature, humidity, presence of microorganisms, etc.); environment (industrial, compositing plant, garden, etc.) and the material [53]. The phenomenon of biodegradation of polymers occurs in two different stages. In a first stage occurs a primary degradation, where polymer chains are fragmented by hydrolysis, or other type of oxidative reactions, into smaller chains. This step can be initiated by different factors, including heat, microbial enzymes, etc. In a second stage, the smaller chains are assimilated by microorganisms and converted into H_2O, biomass, CO_2, and CH_4 (if biodegradation occurs in anaerobic conditions) [15,53]. Figure 5 represents the general degradation process of polymers.

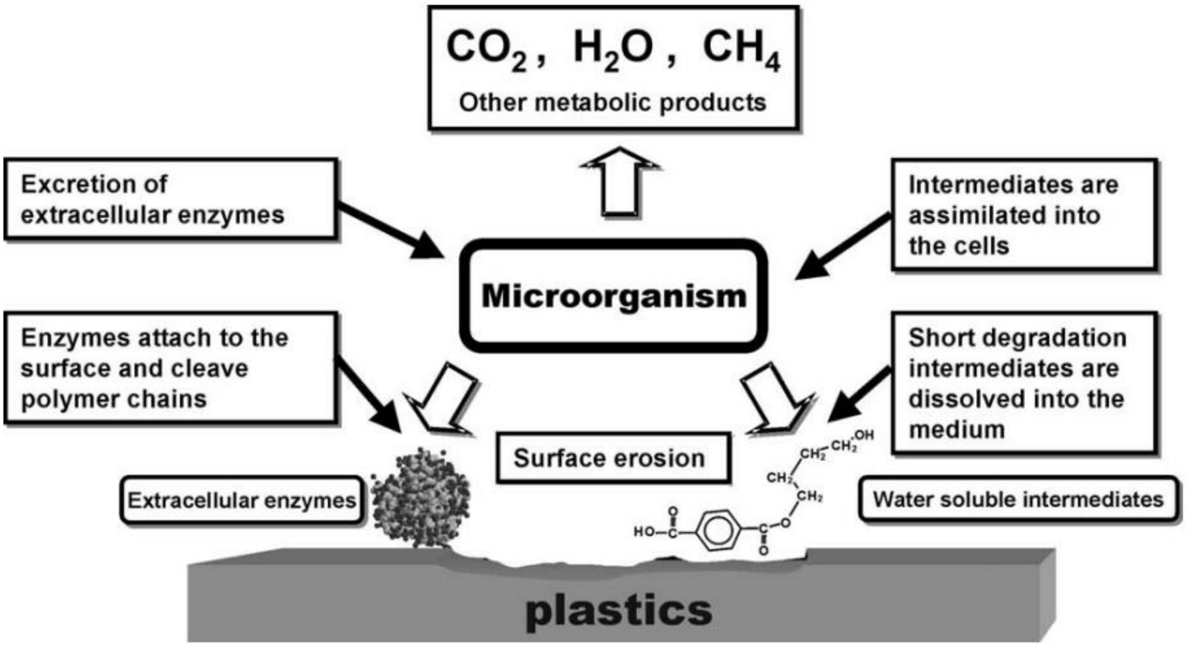

Figure 5: Scheme of the general degradation process of a polymer [21].

2.4 **Polylactic acid**

PLA is a highly versatile, biodegradable and bio-based aliphatic polyester that can be produced during the fermentation of agricultural products rich in starch, such as maize, wheat, corn, tapioca, among others [5,24,27,32,42,53,54]. At an industrial scale, PLA can be produced by direct polymerization of lactic acid or by ring-opening polymerization of lactide dimers, being the last one the most widely used production process in the industry [23,53,55]. The production of lactic acid is a process that involves the starch hydrolysis to dextrose, which, in its turn, is converted into lactic acid by fermentation [3,23,27,56].
In Figure 6 is depicted the chemical conversion of lactic acid into PLA through ring-opening polymerization.

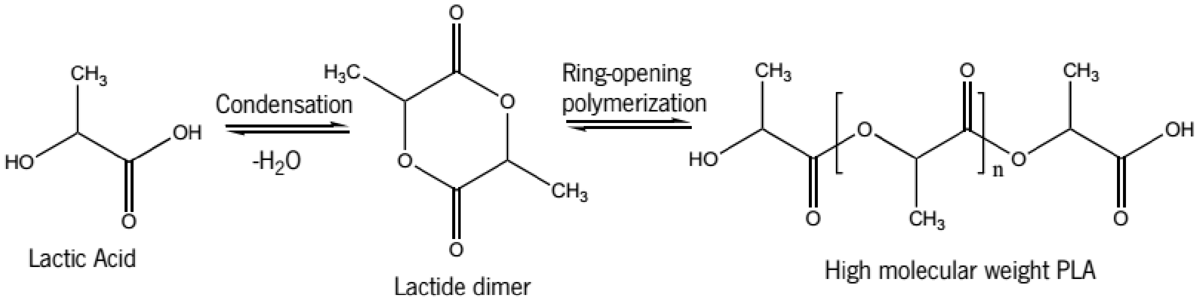

Figure 6: Conversion of lactic acid into PLA through ring-opening polymerization. Adapted from [56].

PLA can be used for packaging applications due to its good mechanical properties (high tensile strength, Young's modulus and good flexural strength), biocompatibility, good processability, biodegradability and monomer renewability [20,24,55,57].
However, it has several drawbacks, such as inherent brittleness, poor barrier properties to H_2O and O_2, low thermal resistance, low-flexibility, low resistance to hydrolysis and slow crystallization rate, which can affect the performance of this material in certain packaging applications (hot packaging) [15,20,24,34,58].
Currently, PLA is considered one of the most attractive biopolymers on the market, especially due to its relevance in the production of short-term or disposable goods, such as throwaway cutlery (plates, cups, drinking straws, etc.), bags, film packaging and biomedical applications [20,24]. Additionally, it can be used to produce rigid and flexible food packaging and "durable" products for the automotive and electronics industry [24,34].

2.4.1 Biodegradability of PLA

Biodegradability is a process where a material is chemically converted into CO_2, O_2, among others. PLA in particular, first suffers hydrolysis of ester [C(O)-O-C] bonds in the main polymer chain by reaction with H_2O (Figure 7) [15,59]. This results in the production of compounds with lower molecular weight (M_w), such as lactic acid oligomers [59], which are assimilated by microorganisms and further converted into H_2O, CO_2 and biomass [60].

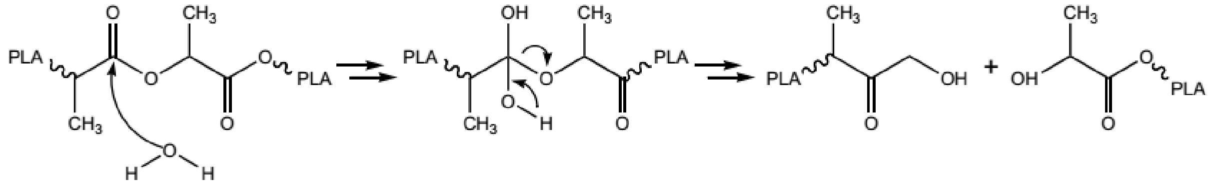

Figure 7: Hydrolysis of PLA. Adapted from [61].

2.4.2 Studies related with the use of PLA in active packaging

Among the biopolymers used in the packaging industry, PLA is considered one of the most important [24]. Several reports can be found in the literature investigating the influence of natural compounds, such as plant extracts and EO's on its properties.

Arrieta *et al.* [34] demonstrated that the addition of limonene influenced the mechanical properties of PLA, such as increasing the elongation at break and decreasing the elastic modulus. They also observed that the incorporation of limonene reduces the barrier properties of PLA, due to an increase of the molecular mobility. Ruiz-Cabello *et al.* [5] studied the incorporation of *Allium spp.* on PLA films to produce a material for packaging of ready-to-eat salads. The active substance did not affect the optical and mechanical properties of the material. However, it was observed that the active substance can reduce significantly the microbial activity, extending the salads' shelf-life. Qin *et al.* [42] studied the incorporation of bergamot, lemongrass, rosemary or clove essential oils in PLA. The mechanical and antimicrobial properties of PLA/EO's were superior to those of pristine PLA. They also reported that the water-vapor permeability (WVP) of PLA/EO films was higher when compared to the film of pure PLA. Samsudin *et al.* [33] studied the incorporation of marigold flower extract into PLA for the production of films to be used in the packaging of fatty-food. The marigold extract decreased the WVP by 21%, when compared to pristine PLA. They also report that the extract did not influence the PLA's thermal properties and O_2 permeability.

2.5 Green tea extract

Tea, derived from *Camellia sinesis* (L.), is considered to be one of the most consumed beverages in the world [39]. This is mainly due to the benefits that they have to human health, such as antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic activity, among others [39].

Different varieties of tea are obtained by fermentation and heating of tea leaves. This promotes the polymerization of catechins and conformational changes, which have a direct impact on the tea's properties [39,50]. Depending on the degree of fermentation, tea can be classified in three principal types, non-fermented-green tea, which possesses the higher concentration of phenolic compounds, semi-fermented-oolong tea and fermented-black tea, which possess the lowest concentration of phenolic compounds [39,50].

Tea's chemical composition is rather complex, consisting of polyphenols (flavonoids and catechins), alkaloids (caffeine, theobromine, theophylline, etc.), volatile oils, polysaccharides, amino acids, lipids, uncharacterized compounds, among others [31,39,50,62]. Of all components, catechins are the most important natural compounds present in tea, especially due to their antioxidant activity. In the recent years, there was an emerging interest in polyphenolic compounds, due to their numerous benefits for human health [63].

Figure 8 depicts the chemical structures of the different catechins that can be found in green tea.

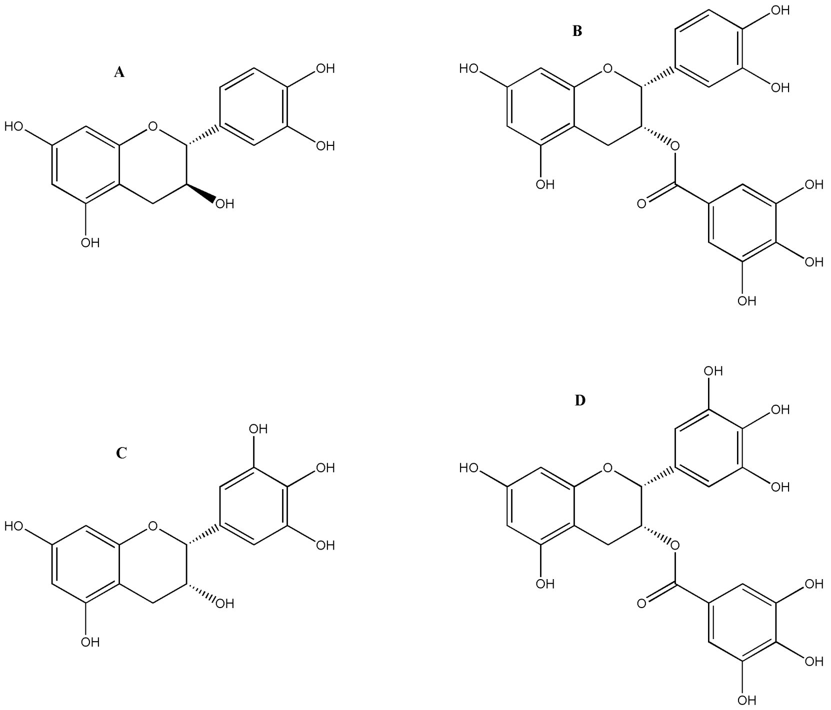

Figure 8: Chemical structures of catechins that can be found in green tea extract. Epicatechin (A), epicatechin-3-gallate (B), epigallocatechin (C) and epigallocatechin-3-gallate (D). Adapted from [50].

Polyphenols have the ability to scavenge free radicals, terminating the radical chain reactions that occur during lipids' oxidation [36,50,62,64]. This radical scavenging activity is directly dependent of the molecular structure and pattern of the hydroxyl groups of polyphenols [64]. These substances play an extremely important role in the control or delay of rancidity, discoloration and deterioration of foodstuffs [50].

2.5.1 Developed work using GTE in active packaging

Several studies were conducted evaluating the effect of GTE on packaging materials, including the products' properties, and antioxidant and/or antimicrobial capacity. Peng *et al.* [22] studied the influence of tea extracts (green tea extract and black tea extract) in chitosan-based films. The authors observed a decrease of WVP and an enhancement of the antioxidant capacity, especially when GTE was added. Colon *et al.* [43] studied the role of catechins in the antioxidant capacity of a film layer containing green tea, green coffee and grapefruit extract. The authors observed that GTE showed the strongest antioxidant activity, which was associated to the higher content of catechins in GTE. Yang *et al.* [31] studied the incorporation of tea extracts, including GTE into protein films to produce films with antioxidant capacity. The authors reported that the addition of GTE did not affect the mechanical properties of the film. In terms of radical scavenging activity, they reported that the protein film containing GTE showed a higher antioxidant activity. Medina-Jaramillo *et al.* [38] studied the influence of green tea extract and basil in cassava starch and glycerol films. The authors observed that the film containing GTE showed significant antioxidant activity, which was associated to a higher content of phenolic compounds. The addition of GTE and basil to the thermoplastic starch reduced the material's water vapor permeability. Siripatrawan *et al.* [35] studied the influence of GTE on the physical properties of a chitosan-based film and evaluated the antioxidant activity. The authors reported that the incorporation of GTE into chitosan resulted in an improvement of its mechanical and water vapor barrier properties and enhanced its antioxidant activity. Giménez *et al.* [65] reported the influence of GTE on the physical properties of agar and agar-fish gelatin films. The authors observed that the addition of GTE reduced the tensile strength and elongation at break of both films. The water vapor permeability and water resistance of both films were not affected, but the GTE promoted higher water solubility.

2.6 **Objectives**

This thesis is focused on the development of a material based on PLA, a biodegradable polymer, with antioxidant capacity, for potential use as active packaging. GTE was expected to have a good compatibility with the PLA matrix and was used to endow the polymer with antioxidant capacity.

One of the objectives of the work was to produce films of the prepared material by blown film extrusion. It was also expected to evaluate the films performance in the preservation of sliced apples and potatoes as a well as smoked salmon.

3. EXPERIMENTAL

3.1 Materials

PLA pellets (Ingeo 4032D, Natureworks™), green tea extract (MyProtein, Batch L704906709), potassium bromide (99%+, Arcos organics) and calcium chloride hexahydrate ($CaCl_2.6H_2O$, extra pure, Riel de Haën) were dried before use. Red apples (royal gala), red potatoes (désirée) and smoked salmon were bought at a local market.

3.2 Internal mixing

PLA and GTE mixtures were prepared in a Haake™ Reomix Lab Mixer and the experimental data was acquired with the Haake™ PolyLab™ OS System.

Before processing, PLA and GTE were dried at 60 °C in a vacuum oven overnight, to minimize the presence of humidity, due to the hygroscopic behavior of both materials. The mixtures were processed using the following conditions: 175 °C, counter rotating double screws at 100 rpm, total sample weight of 48 grams (g) (Table 1) and 5 minutes of residence time. In a first stage, PLA pellets were loaded on the batch mixer and melted for 4 minutes. Then, different amounts of GTE (1 wt.%, 2 wt.%, and 4 wt.%) were added to PLA and mixed for 1 minute, to minimize the GTE thermal degradation.

Table 1: Mixtures composition. The samples were identified according to the percentage of GTE added.

Sample	PLA (g)	GTE (g)
PLA 1% GTE	47.5	0.483
PLA 2% GTE	47.0	0.965
PLA 4% GTE	46.1	1.934

3.3 Preparation of a PLA and GTE masterbatch mixture

A masterbatch mixture with approximately 8 wt.% of GTE was produced in a Leistritz AG LSM 34 6L co-rotating twin screw extruder with 8 heating zones, at 190 °C, with a screw speed of 100 rpm, a throughput of 1.5 kg/h and 5 minutes of residence time. Before processing, PLA and GTE were dried at 80 °C for 4 hours in a vacuum oven. To minimize GTE degradation, the powder was added on a forward part of the extruder by a secondary feed system. The mixture was extruded as filaments, cooled in water, dried and granulated.

3.4 Blown film extrusion

Films of the PLA and GTE mixtures were produced with a Periplast blown extruder, with a single screw and the following conditions: 50 rpm, 170 °C on the first heating zone and 175 °C on the remaining zones. Both materials (PLA and masterbatch) were dried before the extrusion at 60 °C in a vacuum oven overnight. Different amounts of the masterbatch mixture with 8 wt.% GTE were added to PLA to produce films containing 1 wt.% and 2 wt.% of GTE (Table 2).

Table 2: Mass of PLA and PLA masterbatch added to produce films with 1 wt.% and 2 wt.% of GTE.

Sample	PLA (g)	Masterbatch (g)
PLA 1% GTE film	867	131
PLA 2% GTE film	737	263

3.5 Thermogravimetric analysis (TGA)

Thermogravimetric measurements were performed on a TA Q500 thermobalance. Pristine PLA was heated from 40 °C to 550 °C at 10 °C.min^{-1}, while the rest of the samples were heated from 40 °C to 900 °C at 10 °C.min^{-1} under a nitrogen atmosphere (60 mL.min^{-1}).

3.6 Differential scanning calorimetry (DSC)

The thermal behaviour of PLA and GTE was determined using a NETZSCH DSC 200 F3 under inert atmosphere (nitrogen). The samples were sealed in aluminium pans and placed in the equipment. GTE sample was heated from 30 °C to 150 °C at a heating rate of 10 °C.min^{-1} and then cooled to 30 °C with at 20 °C.min^{-1}. PLA sample was heated from 30 °C to 250 °C at 10 °C.min^{-1}, cooled to 30 °C with at 20 °C.min^{-1} and reheated to 250 °C. Samples of the films were analysed using the same parameters used to pristine PLA.

3.7 Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were acquired using a 4100 Jasco spectrometer in the range of 4500-400 cm^{-1}, by averaging 32 scans and using a resolution of 8 cm^{-1}. Translucent thin films, prepared by compression molding in a hot press at 170 °C under a pressure of 10 tons, of pristine PLA, Haake™ samples, masterbatch mixture and extruded films were used in the analysis. A translucent sample disc of GTE (10 wt.% in KBr) was used to acquire the FTIR spectrum.

3.8 Mechanical properties

The tensile properties of the films were determined at room temperature using a Zwick/Roell Z005 equipment, following the ASTM D 882 – 02 standard [66].

The mechanical properties were determined using longitudinal specimen. Five specimens of each sample were prepared with 310 x 20 mm and tested using an initial grip separation of 250 mm and an elongation rate of 25 $mm.min^{-1}$. Stress/strain curves were used to determine yield stress (σ_{stress}), yield strain (ε_{strain}), tensile strength ($\sigma_{strength}$), strain at break (ε_{break}) and Young's modulus (E_{Young}).

3.9 Water vapor transmission assays

The water vapor barrier properties of the extruded films were determined based on the ASTM E 96/E 96M – 05 standard [67]. The desiccant method was used to determine the value of water vapor transmission (WVT). The films were placed in test dishes, with a surface diameter of 69.5 mm, filled with approximately 25 g of $CaCl_2.6H_2O$, previously dried at 150 °C in a vacuum oven. The assay was conducted at room temperature in triplicate. The weight was measure along the assay and until a relative humidity (RH) of 10% has been achieved.

3.10 Evaluation of food's preservation

3.10.1 Sliced apples and sliced potatoes

To evaluate the films' capacity to preserve food's quality, samples (red apple, red potato) were packed in small bags (70 mm x 50 mm), sealed, refrigerated at 4 °C, and monitored for 3 days. Unpacked samples were also included. The assay was performed in triplicate. Samples were collected at controlled time periods and photographed to visually compare the spoilage evolution.

3.10.2 Smoked salmon

This assay was carried out by Cristiana Martins as part of her master's thesis.

Smoked salmon slices (acquired in a local market), with approximately 40 g were packed with PLA films without GTE (control) and with 1 wt.% and 2 wt.% of GTE. The packages were vacuum sealed to promote an optimal contact of the films with the salmon. The samples, refrigerated at 5 °C, were monitored for 60 days and evaluated in different time periods (0, 15, 30, 45 and 60 days).

The sample's preservation was evaluated by studying the lipid's oxidation, based on the determination of the *p*-anisidine value, according to the British Standard Method BS 684-2.24-1998 [68].

3.11 **Characterization of GTE**

This characterization was performed by Cristiana Martins as part of her master's thesis.

3.11.1 Antioxidant Capacity

The 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay, adapted from the method reported by Moure *et. al* [69], was used in the evaluation of the antioxidant capacity of GTE.

50 µl of a methanolic solution of GTE (0.1 mg/mL) were added to 2 mL of a methanolic solution of DPPH· (14.2 µg/mL), protected from the light. After 30 minutes, the absorbance of DPPH· was measured at 515 nm.

The inhibition percentage (IP%) of the DPPH radical was determined based on the reduction of the initial absorbance, according to equation 7, where IP (%) is the inhibition percentage of DPPH radical, Ac stands for the control absorbance, and AA30 corresponds to the sample's absorbance after 30 minutes.

$$\mathrm{IP}\ (\%) = \frac{\mathrm{Ac}-\mathrm{AA30}}{\mathrm{Ac}} \times 100 \quad (7)$$

The antioxidant activity was evaluated by comparison to 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) activity, known as Trolox Equivalent Antioxidant Activity (TEAC), and it was measured in µg ET/mL [70].

3.11.2 Quantification of phenolic compounds

The quantification of total phenolic compounds was performed according to the method reported by Erkan *et al.* [71].

7.5 mL of an aqueous solution of Folin-Cioucalteu reagent (1:10 v/v) was added to 1 mL of a GTE solution and the mixture was left to rest at room temperature for 5 minutes. Then, 7.5 mL of an aqueous solution of sodium carbonate (Na_2CO_3, 60 mg/ml) were added to the mixture. After 120 minutes in the dark, the mixture's absorbance was measured at 725 nm. The assay was performed in triplicate, using solutions with 0.1 mg/mL, and the results expressed in gallic acid equivalents (GAE).

3.11.3 Quantification of flavonoids

The quantification of the total flavonoid content was performed based on the method reported by Yoo *et al*. [72].

In a test tube, 1 mL of the GTE sample was diluted to 5 mL by addition of 4 mL of ultrapure water. 0.3 mL of sodium nitrite (5% w/v) were added to the solution. 0.6 mL of aluminium chloride (10% w/v) were added 5 minutes after. Upon 6 minutes, 2 mL of 1 M sodium hydroxide and 2.1 mL of distilled water were added to the mixture. The mixture's absorbance was measured at 510 nm. The assay was performed in triplicate, using solutions with 0.5 mg/mL, and the results expressed in epicatechin equivalents per g of sample (mg of ECE/g of sample).

4. RESULTS AND DISCUSSION

4.1 Characterization of the raw materials

The compatibility of PLA and GTE is a crucial aspect to produce functional materials. This condition was investigated by producing mixtures of both materials, containing different amounts of GTE. These mixtures were produced by internal mixing, using processing conditions that would minimize the GTE's degradation.

PLA is a biodegradable polymer, with a thermal degradation that can be classified as complex, dependent on several conditions, such as moisture, oxygen, among others [61,73]. As it was reported in several studies, PLA's thermal degradation can occur by several mechanisms, such as hydrolysis by trace amounts of water or by random oxidative chain scission, among others, which target the ester groups present in PLA, resulting in a molecular weight reduction and formation of lactide monomers and oligomers [61,73–75]. Observing the PLA's thermogram (Figure 9), it is possible to observe a profile with a single degradation step, occurring at 321.36 °C, resulting in a weight loss of 98.59%. This behaviour is similar to that reported by other authors [61,74,76].

GTE is a natural extract, very sensitive to high temperatures, as it can be seen on its thermogram (Figure 9), meaning that it can easily lose antioxidant activity due to the degradation of its active compounds. In its thermogram is possible to identify a more complex degradation profile. From 145 °C to 442 °C, GTE's degradation profile is related with to the glycosylation of catechins and of other components, resulting in a weight loss of 46.8%. Catechins, due to heating, start to suffer a "caramelization" process, causing the weight loss that is observed in Figure 9. This region, since corresponds to a temperature range in which PLA is thermally stable, can be used to evaluate the GTE presence in the mixtures. The steady decrease of weight that is verified between 442 °C and 900 °C can be related to the degradation of aromatic structures. It is also possible to observe that at 900 °C, the percentage of weight residue is 32.44%. These results are in accordance with the literature [77–79].

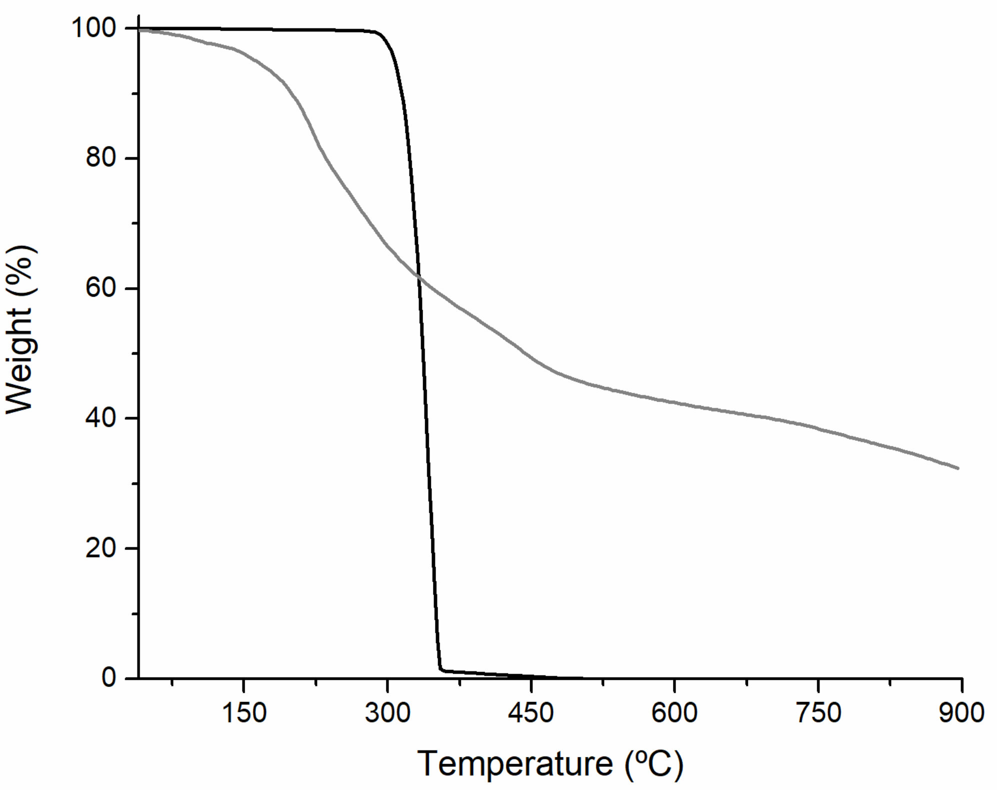

Figure 9: TGA thermograms of pristine PLA (black) and GTE (grey).

Another method that was used to characterize the raw materials was FTIR spectroscopy (Figure 10). In this test, the main objective was to identify each of the materials' characteristic chemical bonds that are present in their chemical structure of PLA or of the components in the extract mixture.

In the PLA's spectrum, three peaks at 2994 cm^{-1}, 2944 cm^{-1} and 2881 cm^{-1} were attributed to the C-H stretching. The peak located at 1760 cm^{-1} was associated to the C=O stretching. The peak at 1465 cm^{-1} was assigned to C-H bending. The stretching band located at 1176 cm^{-1} indicated the presence of the C-O-C bond, which can be associated with the ester groups. It is also possible to identify the presence of C-CH_3 stretching at the peak of 1045 cm^{-1}. The results are in accordance with those reported in the literature [80].

Regarding the GTE's spectrum, the broad band centered at 3347 cm^{-1} was attributed to the O-H stretching. The peak at 2927 cm^{-1} was assigned to the C-H stretching. The peak at 1697 cm^{-1} was associated with C=O stretching. It was also identified a C=C stretching band centered at 1629 cm^{-1}, which is characteristic of cyclic alkenes (benzene rings). The peak at 1367 cm^{-1} can be identified as the O-H bending. The peaks at 1238 cm^{-1} and 1039 cm^{-1} were associated to the presence of C-O stretching, which can be related to the presence of aromatic ether groups in. Due to the complexity of components that are present in GTE, it is difficult to associate a certain chemical bond to a certain component, since most of the components have similar chemical bonds on their structure. Similar analysis of the GTE's spectrum can be found in the literature [79,81].

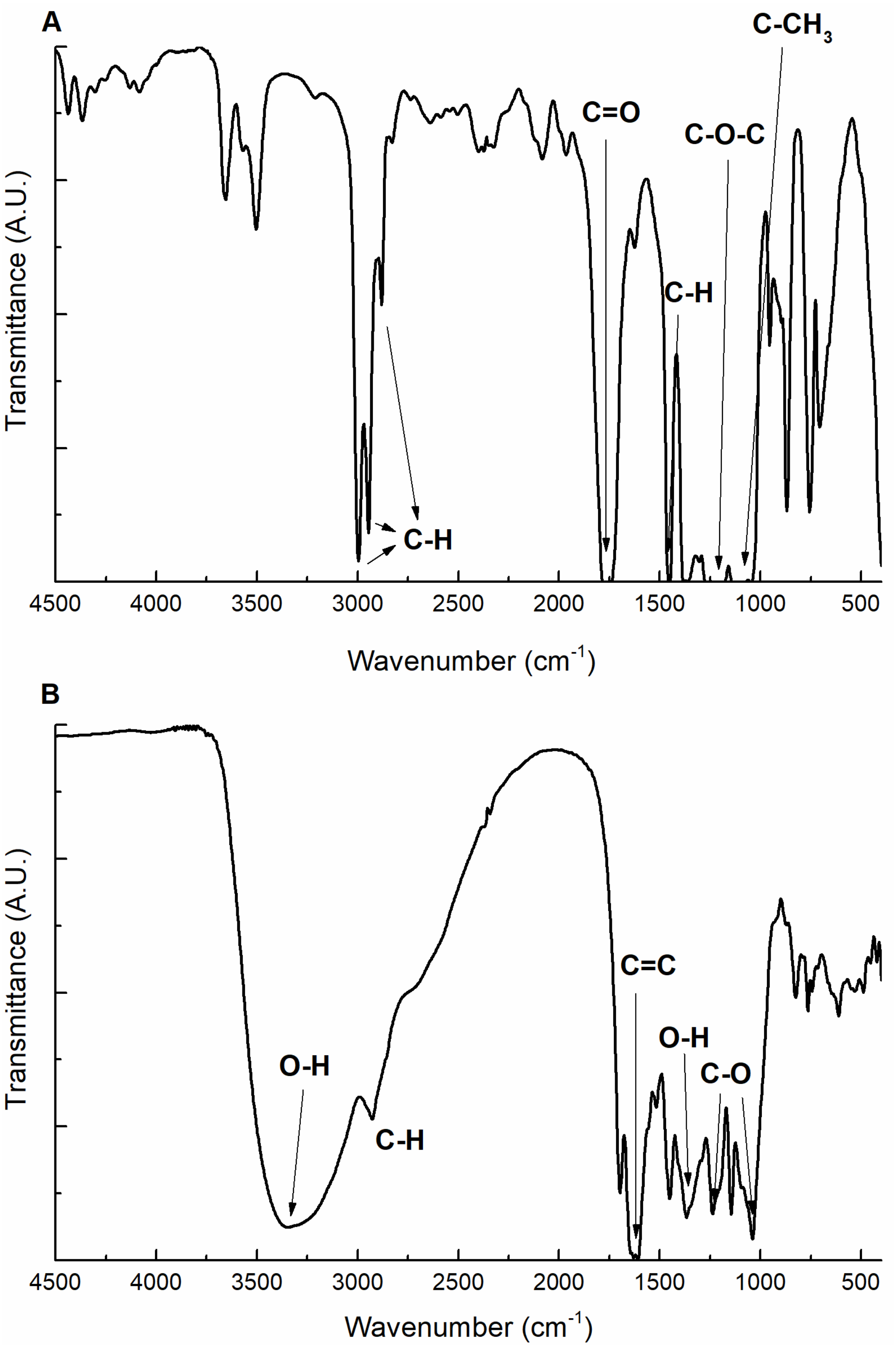

Figure 10: FTIR spectra of pristine PLA (A) and GTE (B).

The GTE antioxidant activity, based on the DPPH· method [69,70], the amount of phenolic compounds [71] and flavonoids [72] were determined by Cristiana Martins as part of her master's thesis. It was found that GTE has a DPPH radical inhibition of 917 ± 8.7 μg TE/g extract. The total phenolic compounds in GTE was quantified as 416 ± 9.95 mg GAE/g of extract. An amount of 148 ± 0.21 mg ECE/g of extract was determined as the total content of flavonoids in GTE.

4.2 **Characterization of the obtained mixtures**

The thermograms of the mixtures (Figure 11) show a single degradation step for PLA 1% GTE, occurring at 310.18 °C, resulting in a weight loss of 97.69%. A single degradation step is also observed for PLA 2% GTE and for PLA 4% GTE, occurring at 295.04 °C and 292.16 °C with a weight loss of 97.06% and 94.24%, respectively. Thus, increasing the amount of GTE reduces the thermal stability of the mixture, making the material more susceptible to thermal degradation.

Another aspect observed was related to the residual material at 900 °C, which increases with the amount of GTE, as expected. As it was observed for the GTE, it is not completely degraded at this temperature, while PLA does (Figure 9).

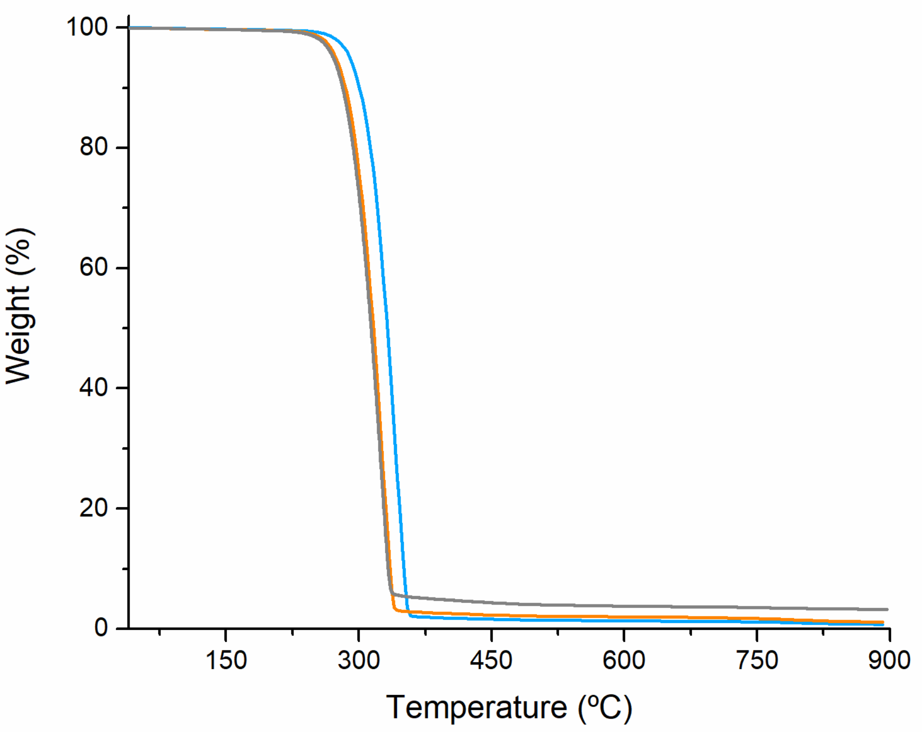

Figure 11: TGA thermograms of the mixtures produced by internal mixing. PLA 1% GTE (light blue), PLA 2% GTE (orange) and PLA 4% GTE (grey).

The mixtures were also characterized by FTIR (Figure 12). This analysis was performed to confirm the presence of GTE, which can provide some data on the miscibility between PLA and GTE. Based on the FTIR analysis of both raw materials (Figure 10), the presence of GTE can also be confirmed by the appearance of characteristic O-H and C=C stretching band in the mixtures' spectra. As it is expected, the addition of higher amounts of GTE resulted in the appearance of more pronounced vibrational bands related to the extract [22].

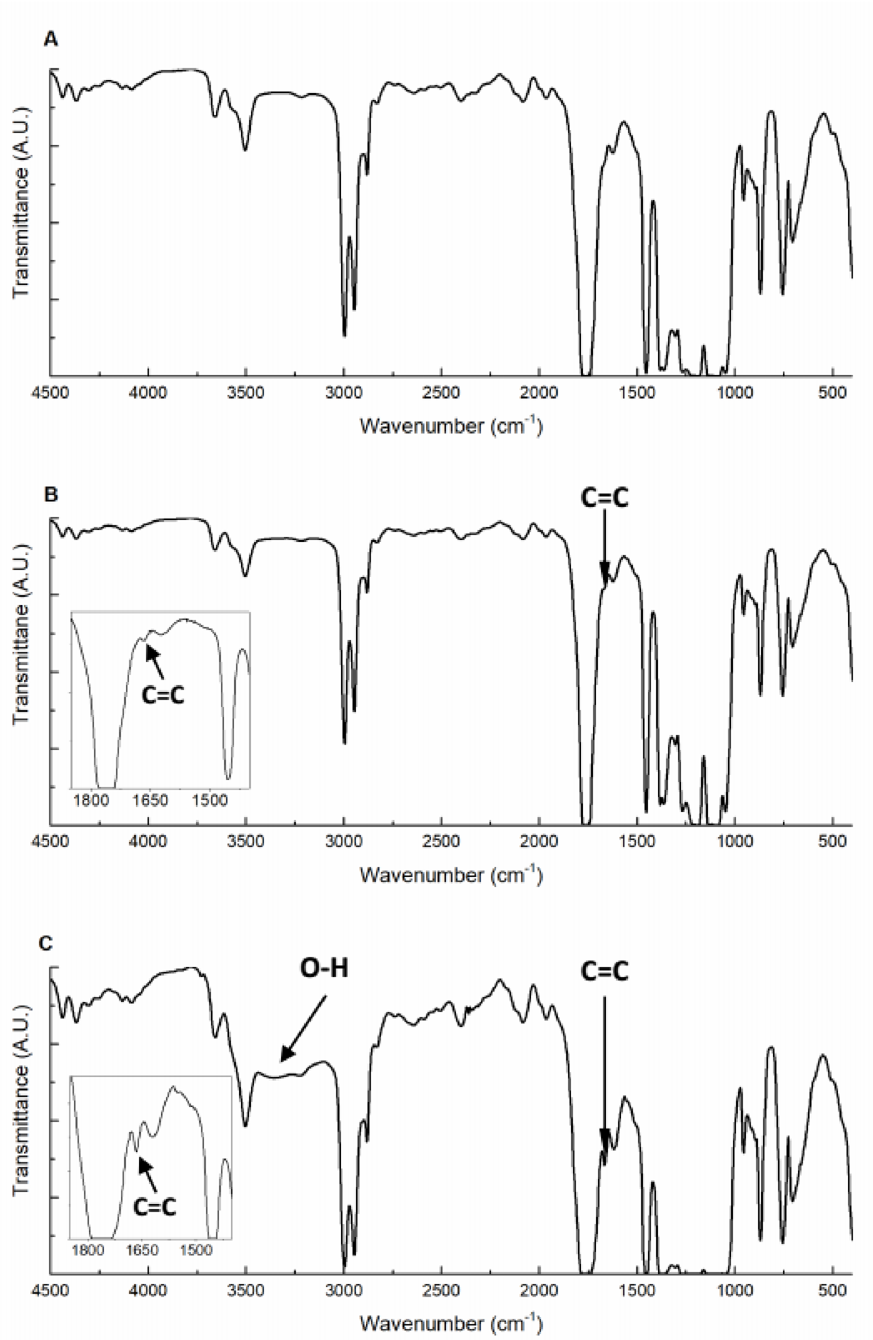

Figure 12: FTIR spectra of the PLA 1% GTE (A), PLA 2% GTE (B) and PLA 4% GTE (C) mixtures. The insets were acquired with a resolution of 2 cm^{-1} to allow a more detailed perspective of the GTE's characteristic vibrational bands.

Based on the mixtures characterization and on their visual aspect, it was decided that the sample containing 4 wt.% of GTE would not be used in the production films, since this amount of GTE darken the PLA in such a way that it became almost opaque. This is not an ideal property for the production of an active package for food products. Thus, it was decided to produce films containing only 1 wt.% and 2 wt.% of GTE.

4.3 **Production of PLA-based films**

4.3.1 Production of a PLA/GTE masterbatch

A PLA/GTE masterbatch mixture was produced to allow the preparation of more homogeneous mixtures that would promote a better dispersion of GTE within the PLA matrix during the extrusion. The thermal stability of this masterbatch was studied by TGA (Figure 13). Its thermogram exhibits a degradation profile similar to that observed for the mixtures prepared in the Haake (Figure 11). The most pronounced degradation occurs at 295.36, resulting in weight loss of 97.4%. Nonetheless, there is a small initial weight loss and, at 900 °C, it still remains 2.33% of the initial sample weight, both related to the GTE. The weight loss at 900 °C, since it is only related to GTE, allowed to estimate a GTE content in the mixture of 7.41%.

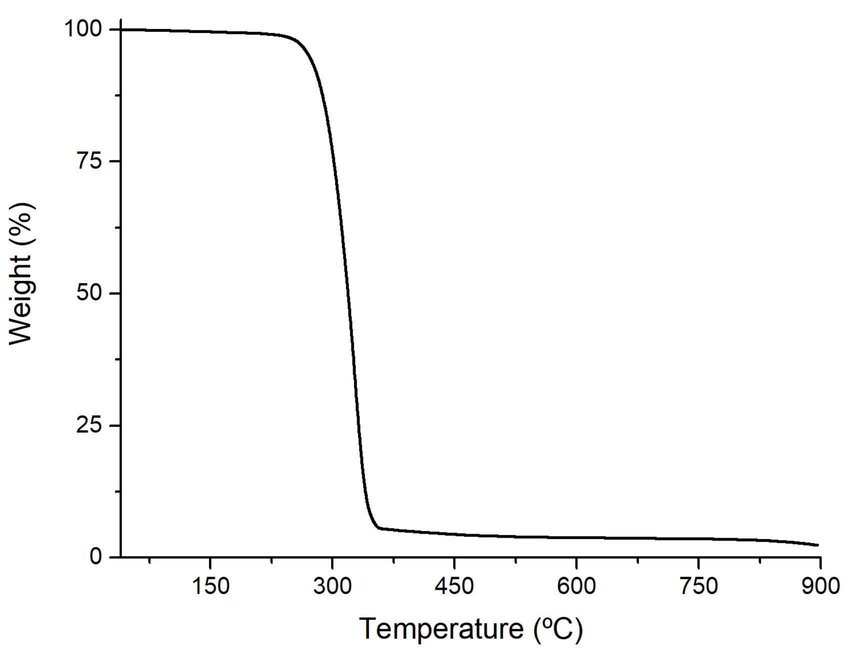

Figure 13: Thermogram of PLA Masterbatch.

In the FTIR spectrum of the PLA/GTE masterbatch (Figure 14), it is possible to identify PLA's characteristic vibrational bands, such as the C-H stretching, located at 2994 cm^{-1}, 2944 cm^{-1} and 2881 cm^{-1}, C=O stretching at 1760 cm^{-1} and the [C(O)-O-C] stretching at 1176 cm^{-1}. We can also identify GTE's characteristic vibrational bands, such as O-H stretching centered at 3347 cm^{-1}, C=C stretching at 1666 cm^{-1}, O-H bending at 1367 cm^{-1} and the C-O stretching at 1238 cm^{-1} and 1039 cm^{1}.

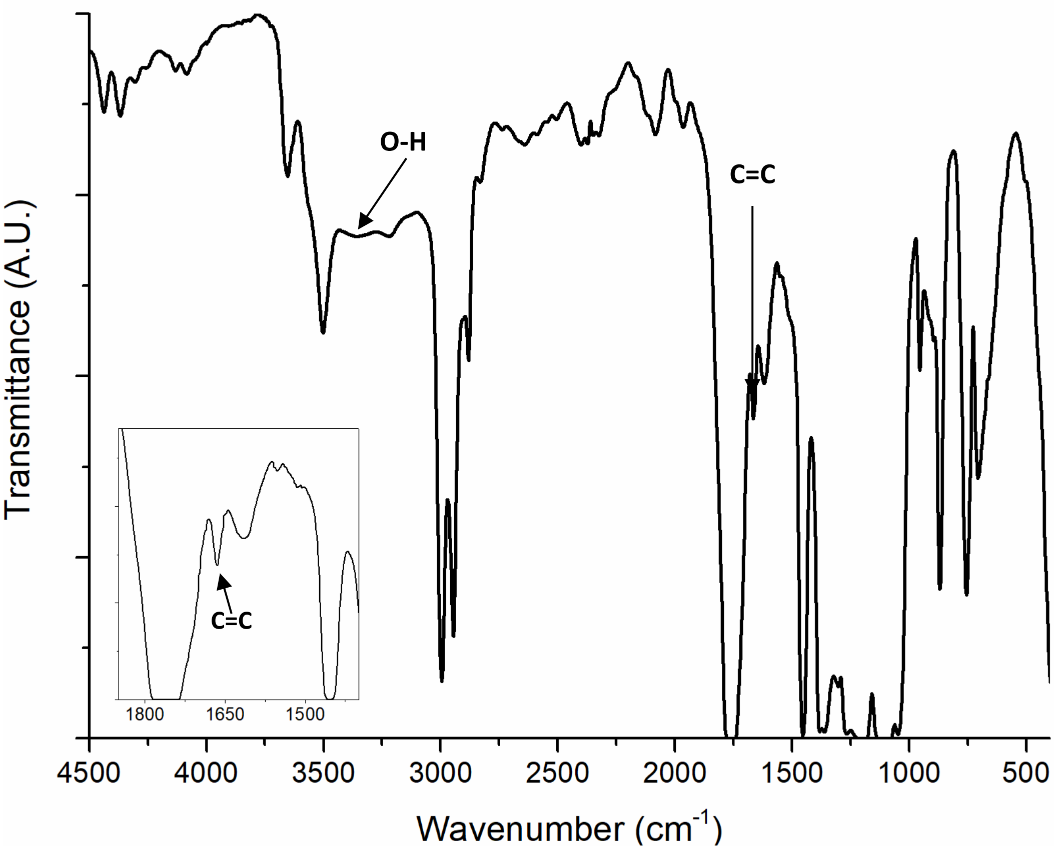

Figure 14: FTIR spectrum of PLA/GTE masterbatch. The inset was acquired with a resolution of 2 cm^{-1} to allow a more detailed observation of the GTE's characteristic vibrational bands.

4.4 **Characterization of the films**

Figure 15 depicts the PLA-based films produced. As expected, films containing GTE have a brownish color and are less transparent, which are aspects dependent on the amount of GTE added.

Figure 15: Visual appearance of the films of PLA (A), PLA 1% GTE film (B) and PLA 2% GTE film (C).

The films showed a slight different thermal stability (Figure 16) than the mixtures prepared in the Haake (Figure 11). In this case, the major degradation step occurs at higher temperatures, 304.4 °C, resulting in a weight loss of 98.76% and 97.57%, respectively for PLA 1% GTE film and PLA 2% GTE film. In what concerns the amount the residue at 900 °C, it is not proportional to the amount of GTE, in contrast to what was observed for the mixtures prepared in the Haake mixer. In this case, the GTE was exposed to high temperatures for a second time, which results in a different thermal behavior. Nevertheless, at lower temperatures, the film with 2 wt.% GTE shows a higher weight loss, which is in agreement with the higher content of extract.

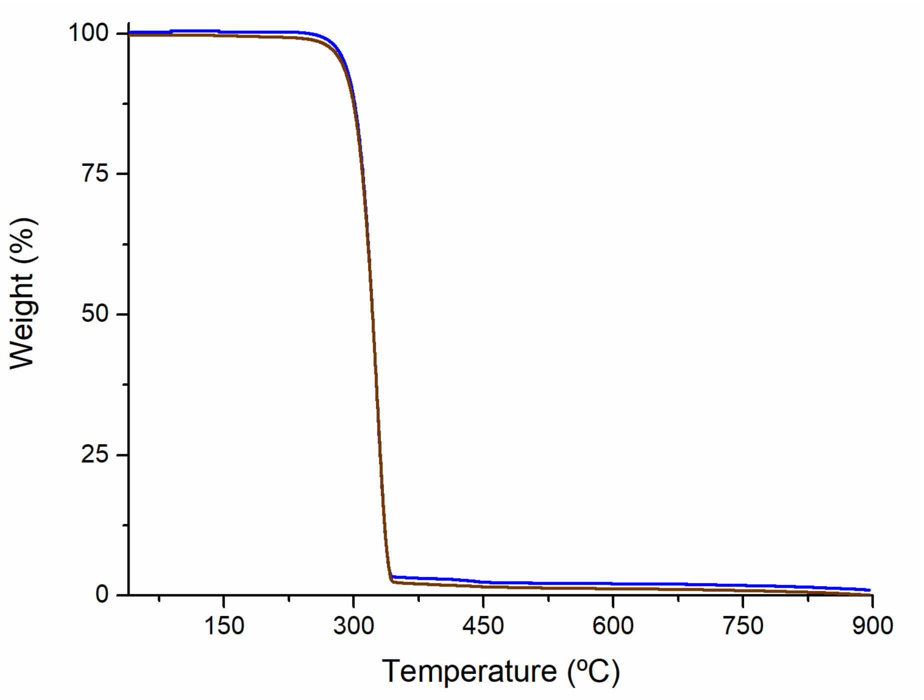

Figure 16: Thermogram of PLA 1% GTE film (blue) and PLA 2% GTE film (brown).

Figure 17 depicts the thermograms obtained for pristine PLA, GTE and the two extruded films, and in Table 3 are indicated the glass transition temperature (T_g), melting temperature (T_m) and cold crystallization temperature (T_{cc}) determined for each material.

In the GTE's thermogram, since it is a mixture of low M_w compounds, it only presents a T_m peak at 112.5 °C. In the cooling cycle, the appearance of a crystallization peak at 136.2 °C was attributed to the crystallization of GTE components.

In the first heating cycle, the PLA's thermogram shows a slight T_g peak at 76.5 °C and a T_m peak at 171.7 °C. This is consistent with the behavior of a semi-crystalline PLA. In the cooling cycle, PLA does not show any crystallization, since the rapid cooling rate (20 °C/min) inhibits the rearrangement of the PLA chains into crystalline regions, thus the material became amorphous. In the second heating cycle, PLA's thermogram exhibits a well-defined T_g peak at 67.5 °C. The non-existence of a T_m peak confirms that the sample is amorphous. This type of behavior on PLA is reported in literature by Signori *et al.* [76].

On the thermogram of the extruded films' first heating cycle, it was possible to observe T_g peaks around 68 °C for both samples, which corresponds to reduction of the T_g when compared to pristine PLA. This shows that GTE enhances the molecular mobility. This type of behavior is in accordance with other studies where natural compounds reduced the PLA's T_g [5,34]. It was also possible to observe the occurrence of cold crystallization with at 111 °C and 109.5 °C (T_{cc}), respectively for PLA 1% GTE film and PLA 2% GTE film. This phenomenon occurs at temperatures close to the GTE's T_m (112.5 °C). This could mean that upon its melt, the GTE promotes a higher molecular mobility that result in the rearrangement of amorphous regions into crystalline domains [76]. Regarding the T_m of the extruded films, for PLA 1% GTE film the melting occurred at 174.9 °C while for PLA 2% GTE film occurred at 172 °C, which is superior to the T_m of pristine PLA. This could be related to the effect of GTE on the PLA's crystalline structure. As observed for pristine PLA, the rapid cooling of the samples does not promote the formation of crystalline regions.

In the second heating cycle, it was observed lower T_g, 64.3 °C for PLA 1% GTE film and 62.3 °C for PLA 2% GTE film. Regarding the cold crystallization, it is noticeable that the T_{cc} peaks are slightly sharper and appear at higher temperatures. The PLA's melting is highly affected in the second heating. Not only it occurs at lower temperatures, but it is also related to the melting of two crystalline structures.

Badia *et al.* [82] observed a similar behavior when studying the effect of reprocessing PLA on its thermal properties.

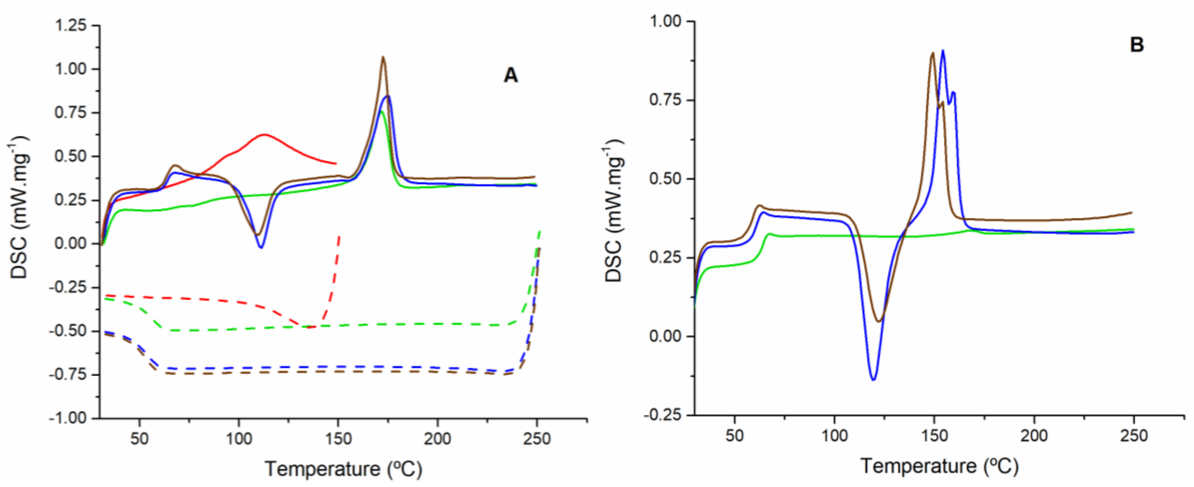

Figure 17: DSC thermograms of pristine PLA (green), GTE (red), PLA 1% GTE film (blue) and PLA 2% GTE film (brown). First heating cycle (solid lines) and cooling cycle (dash lines) (A) and second heating cycle (B).

Table 3: Temperatures of the events observed in the DSC thermograms.

Sample	**First heating cycle**			**Second heating cycle**		
	T_g (°C)	T_{cc} (°C)	T_m (°C)	T_g (°C)	T_{cc} (°C)	T_m (°C)
PLA	76.5	-	171.7	67.5	-	-
GTE	-	-	112.5	-	-	-
PLA 1% GTE film	68.0	111	174.9	64.3	119.4	154.2 and 158.8
PLA 2% GTE film	68.0	109.5	172.6	62.3	122.1	149.0 and 154.1

A FTIR analysis was performed to confirm the films' composition (Figure 18). The FTIR spectra of the extruded films are similar to those obtained with the mixtures with the same amount of GTE prepared in the Hakke (Figure 12). The characteristic peaks of GTE related to O-H stretching, centered at 3347 cm^{-1} are not noticeable on the FTIR spectra of the extruded films. However, it is noticeable the C=C stretching vibrational band at 1666 cm^{-1}. Once again, the low GTE content in the films does not have a high impact on the FTIR spectra.

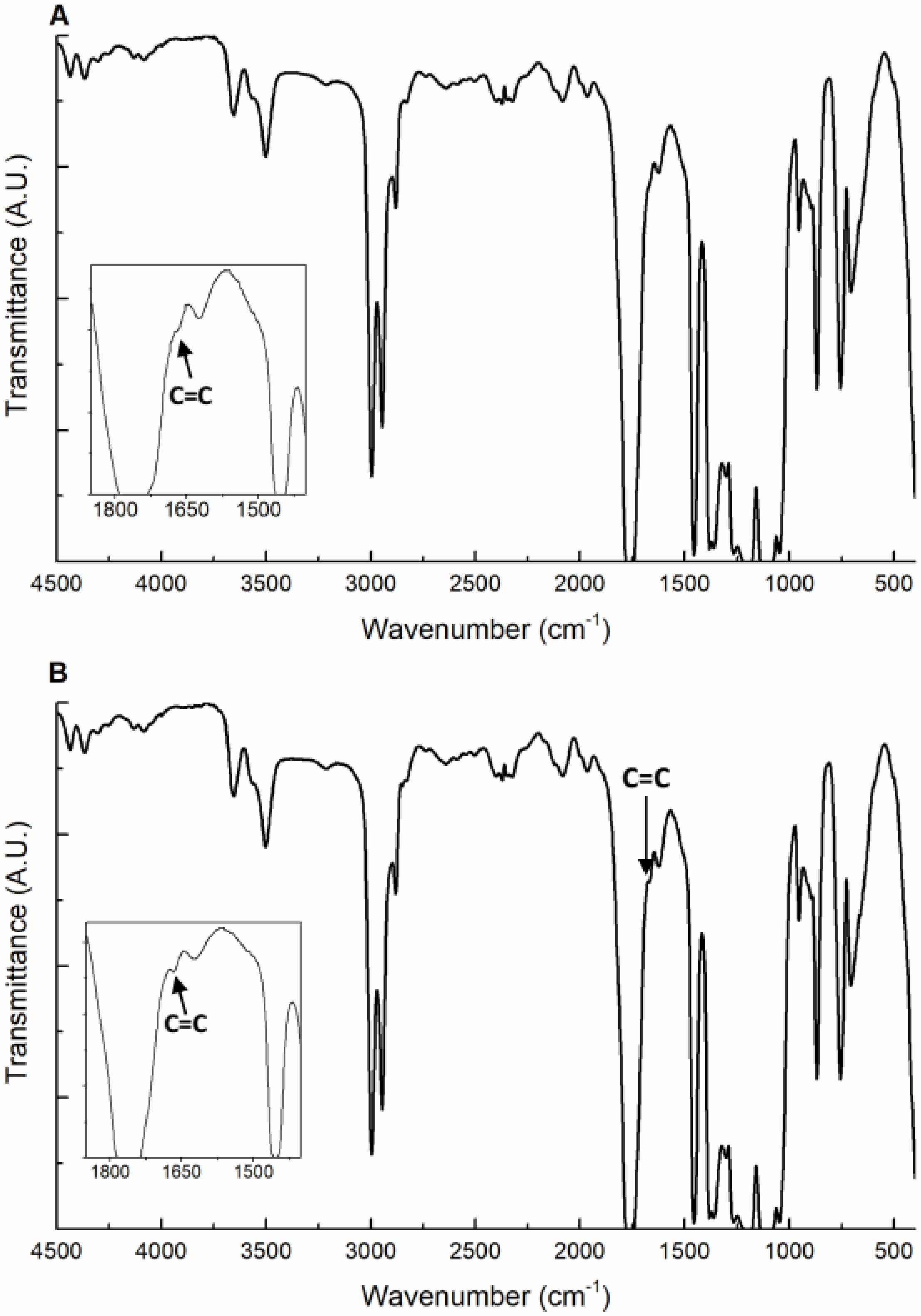

Figure 18: FTIR spectra of PLA 1% GTE film (A) and PLA 2% GTE film (B). The insets were acquired with a resolution of 2 cm^{-1} to allow a more detailed observation of the GTE's characteristic vibrational bands.

The mechanical performance, and the effect of GTE content of the extruded films were evaluated by tensile assays (Figure 19 and Table 4).

In terms of the GTE's influence on the mechanical properties of PLA, it was observed that its incorporation resulted in the reduction of the yield stress. The addition of 1 wt.% of GTE caused a reduction of 8.2%, while 2 wt.% GTE reduced the yield stress by 0.3%. The addition of GTE also had an impact on the PLA's tensile strength. It was observed that 1 wt.% of GTE caused a reduction of 11.9%, while 2 wt.% reduced 12.0%. Regarding the yield strain, it is possible to conclude that it is enhanced by the GTE. The addition of 1 wt.% GTE caused an increase of 0.3%, while 2 wt.% GTE increased the yield strain by 0.1%. Also, the addition of GTE increased the strain at break of PLA, 9.6% due to 1 wt.% GTE and 36% due to 2 wt.% GTE. The increase of both yield strain and strain at break is correlated with the increase of molecular mobility caused by the addition of GTE. Regarding the effect of GTE on the Young's modulus of PLA, it was observed that the addition of 1 wt.% of GTE caused a reduction of 5.3%, while 2 wt.% GTE caused an increase of 2.9%. Despite the differences observed, the addition of GTE only had a significant impact in the strain at break of the PLA 2% GTE film.

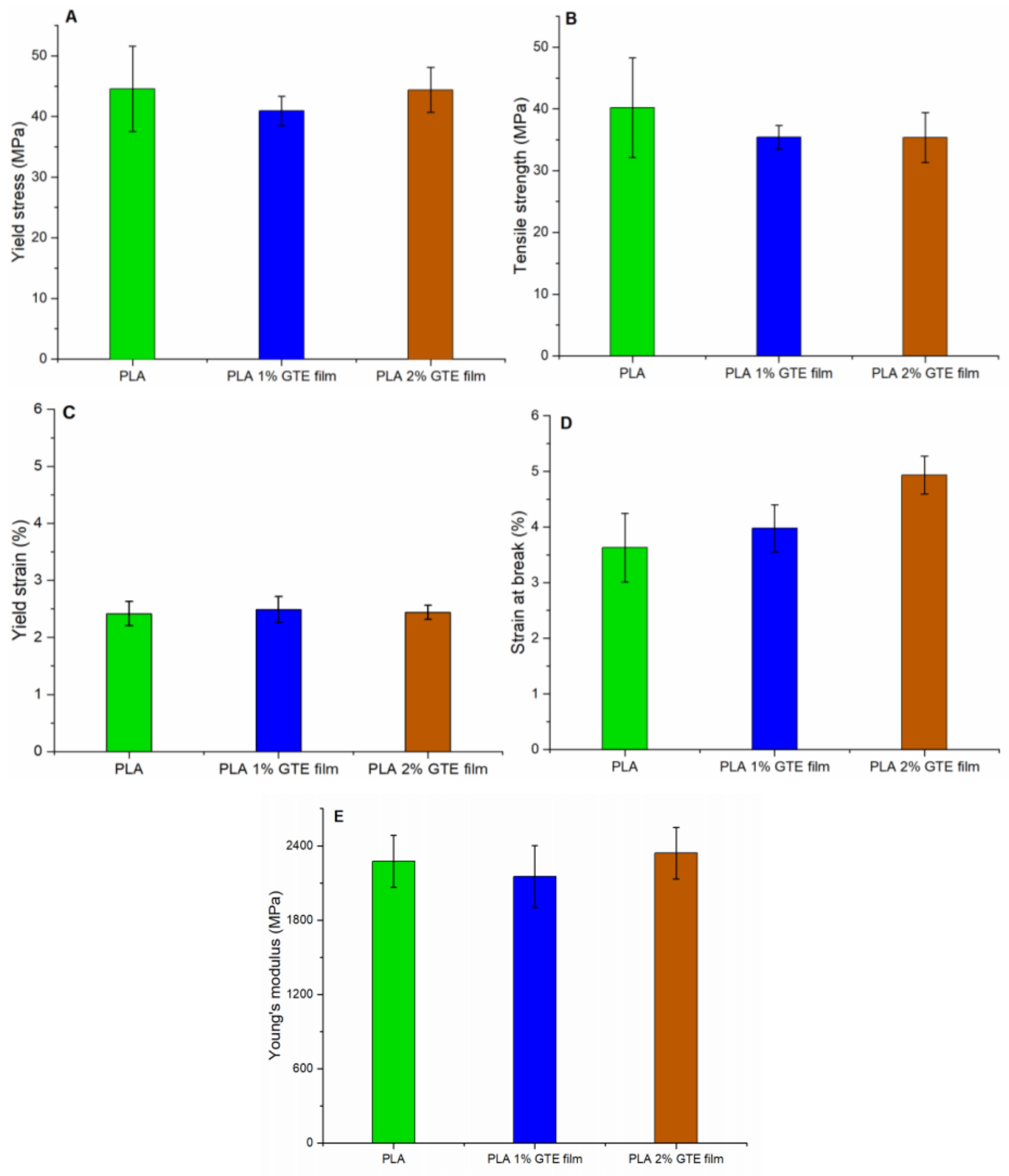

Figure 19: Mechanical properties of the extruded films: A) yield stress, B) tensile strength, C) yield strain, D) strain at break and E) Young's modulus.

Table 4: Mechanical properties of the extruded films and their standard deviation (SD).

Sample	σ_{stress} **± SD (MPa)**	$\sigma_{strength}$ **± SD (MPa)**	ε_{strain} **± SD (%)**	ε_{break} **± SD (%)**	E_{Young} **± SD (MPa)**
PLA	44.55 ± 7.042	40.21 ± 8.100	2.42 ± 0.214	3.63 ± 0.62	2274.6 ± 210.75
PLA 1% GTE film	40.91 ± 2.438	35.42 ± 1.945	2.49 ± 0.226	3.98 ± 0.43	2152.2 ± 249.84
PLA 2% GTE film	44.39 ± 3.686	35.37 ± 4.061	2.44 ± 0.123	4.94 ± 0.34	2340.1 ± 209.62

As it is well documented in the literature, biopolymers, such as PLA possess low barrier properties, which limits their applications for packaging, especially on food products that are highly susceptible to O_2 or H_2O [45].

The water-vapor transmission (WVT) of PLA films was evaluated to understand the influence of GTE on the PLA's barrier properties. The assays were performed following the desiccant method (ASTM E 96/E 96M – 05) [67], monitoring the desiccant weight until a relative humidity (RH) minimum of 10% was achieved. In Figure 20 is depicted the amount of water absorbed by the desiccant over time. The samples' conditions and the WVT rates determined are summarized in Table 5.

From the results, it is possible to conclude that the addition of GTE improves the PLA's water-vapor barrier properties, decreasing 21.5% and 34.9% the water vapor transmission when 1 wt.% of GTE and 2 wt.% of GTE, respectively, were added.

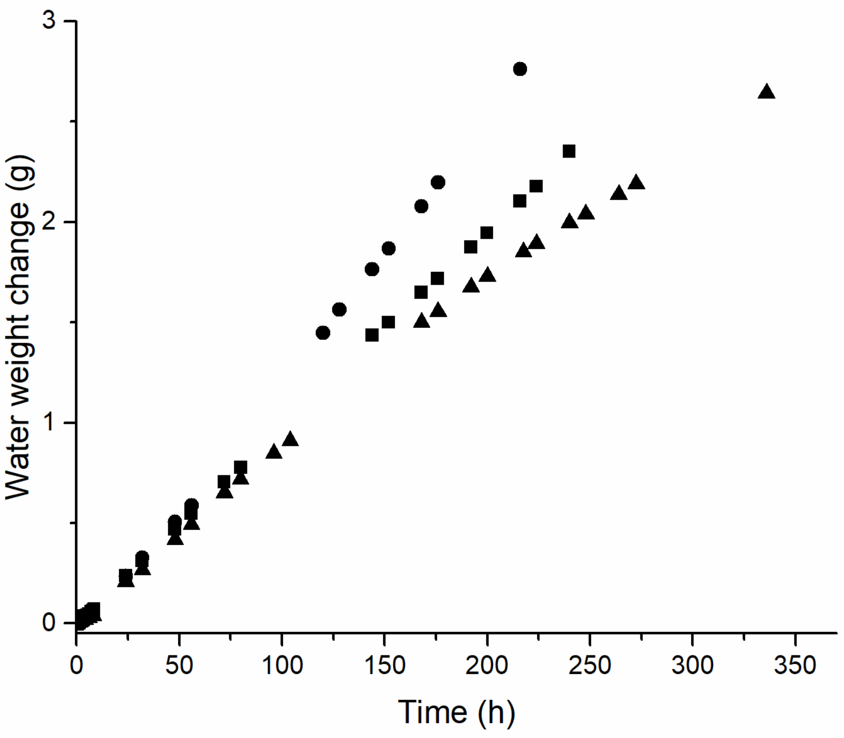

Figure 20: Evolution of the amount of water absorbed by the desiccant over time: PLA (circle), PLA 1% GTE film (square) and PLA 2% GTE film (triangle).

Table 5: Contact area, films' thickness, WVT rates calculated and their standard deviation (SD). Their results are the mean of three independent replicas.

Sample	Contact area (m^2)	Film thickness ± SD (mm)	WVT ± SD ($g.h^{-1}.m^{-2}$)
PLA	0.039	0.05 ± 0.01	3.35 ± 0.68
PLA 1% GTE film		0.05 ± 0.01	2.63 ± 0.48
PLA 2% GTE film		0.08 ± 0.02	2.18 ± 0.40

4.5 **Food preservation assays**

The PLA/GTE films were produced aiming the development of a material with antioxidant properties that could be used as an active packaging system, inhibiting food's oxidative degradation. To evaluate its potential to serve this purpose, several assays of food preservation were conducted, evaluating the spoilage of sliced apples, sliced potatoes and smoked salmon. These products were selected since they are very common in people's diet, whose lifestyle demands for ready to eat or ready to cook food.

4.5.1 Preservation of sliced apple

Apples, upon being sliced are immediately subjected to oxidative degradation due to polyphenol oxidases (PPOs), which are enzymes that catalyze the oxidation of phenolic compounds (i.e. chlorogenic acid, catechin and epicatechin) into quinones, in the presence of oxygen, producing the characteristic brown pigments in the wounded tissues [51,83]. This phenomenon results in the loss of nutritional quality and appearance during the food supply chain, reducing consumer's acceptability and causing implications on the economy of food producers and food industry [51].

It was expected that the presence of GTE in the PLA films would prevent this oxidative degradation. To evaluate its food preservation capacity, the efficiency of two films containing GTE was tested against PLA films without GTE. The spoilage evolution of all samples was compared to that of an unpacked specimen preserved under the same conditions. The assay was conducted during 48h (Figure 21). After 1h, it appears that there are no significant changes to be reported on the sliced apple's surface. After 5h, the sample exposed to atmospheric conditions shows some oxidative spoilage while the packed samples show less spoilage.

After 24h, the packed sample, with higher impact for that packed with PLA 2% GTE film, revealed a lower degree of oxidation than the unpacked sample. After 48h, the unpacked sample is extensively oxidized. The sample packed with pristine PLA shows an oxidation degree lower than the control sample, nevertheless higher than the remaining samples. The results showed that higher amounts of GTE have the ability to delay the oxidative spoilage of sliced apples.

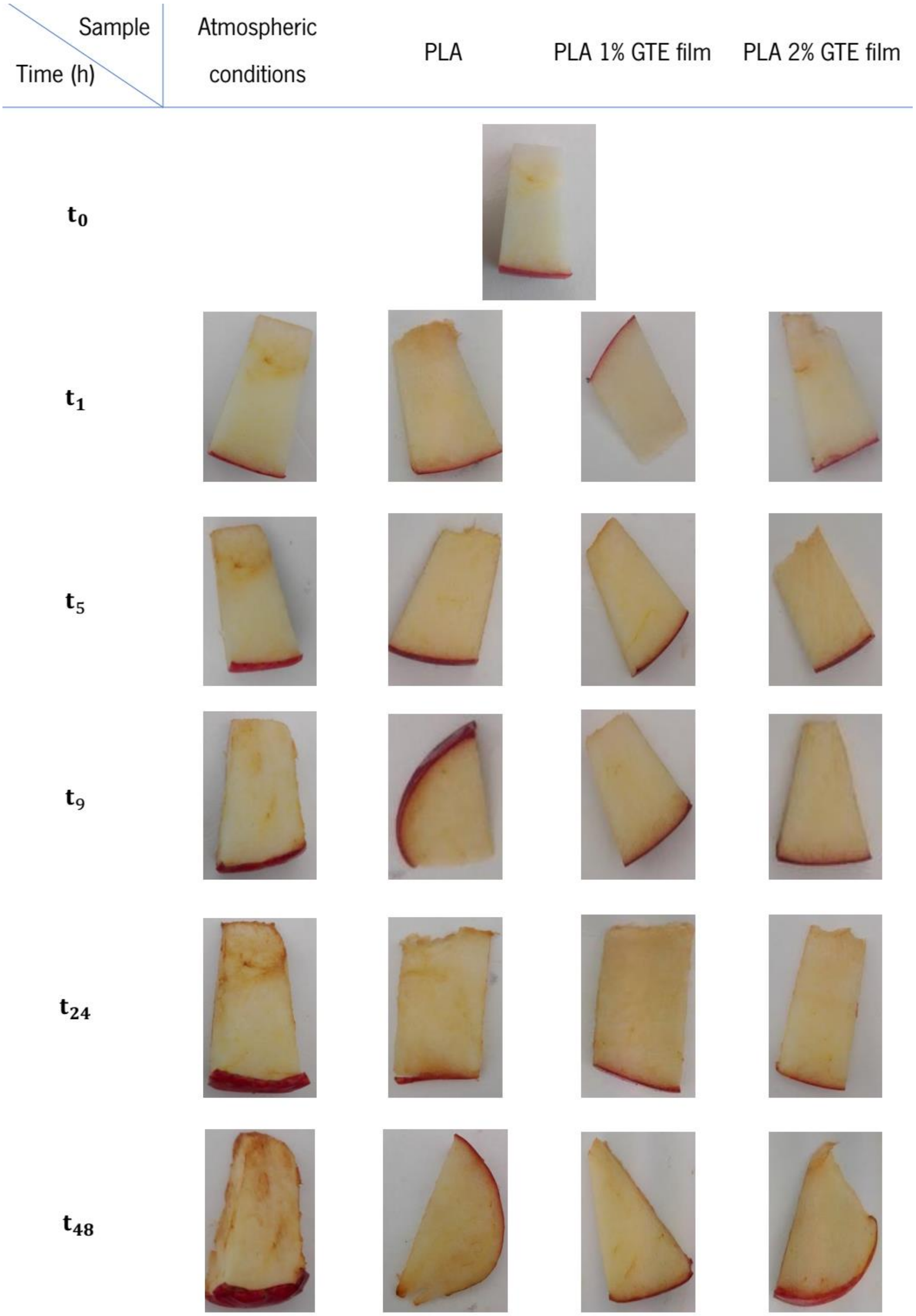

Figure 21: Evolution of the oxidative spoilage of sliced apples, stored in different conditions, over 48h.

4.5.2 Sliced red potatoes

Potatoes, like apples, have PPOs that promote their oxidative degradation. The general mechanism of enzymatic browning is represented in Figure 4, where plants with high quantities of oxidable *o*-diphenols are transformed into reddish/brown *o*-quinones, which are the main responsible for the browning [84].

As it was performed for sliced apples, the PLA/GTE films were tested for the preservation of sliced potatoes. Once more, the assay was conducted with samples packed with pristine PLA, PLA/GTE mixtures and unpacked (Figure 22).

After 1h, the sample placed on atmospheric conditions reveals signs of oxidation near the peel. For the packed samples, the oxidation has not started. The unpacked samples degraded rapidly and after 24 h showed an advanced degradation. The packed samples, at the same time, showed evidences of oxidative degradation, however in a lower extent. After 48 h, the unpacked sample is completely degraded.

Once more, the packed sample in PLA 2% GTE film, despite of being oxidized, was the one with the lower degree of degradation.

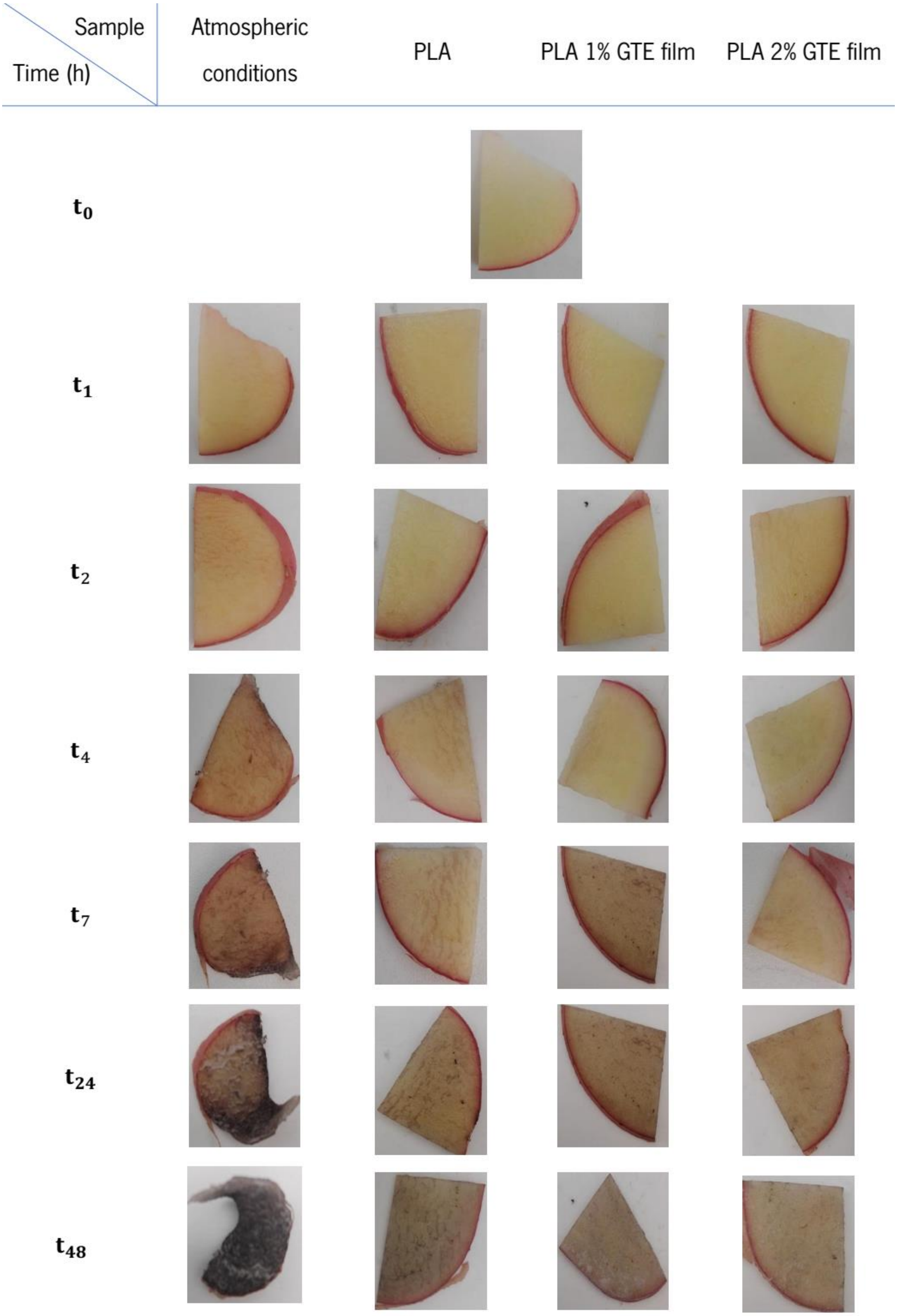

Figure 22: Evolution of the oxidative spoilage of sliced potatoes, stored in different conditions, over 48h.

4.5.3 Salmon

The evaluation of smoked salmon preservation was carried out by Cristiana Martins as part of her master's thesis.

Smoked salmon slices were packed with PLA films without GTE (control) and with 1 wt.% and 2 wt.% of GTE.

It was found that the presence of GTE in the PLA films inhibits the lipids' oxidation when compared to pristine PLA, with expectation of the sample packed with PLA 2% GTE film for which was observed a higher *p*-anisidine value after 60 days (Figure 23).

These results showed that the films with GTE can inhibit lipids' oxidation, thus evidence the potential of the developed films to be used as an active packaging material.

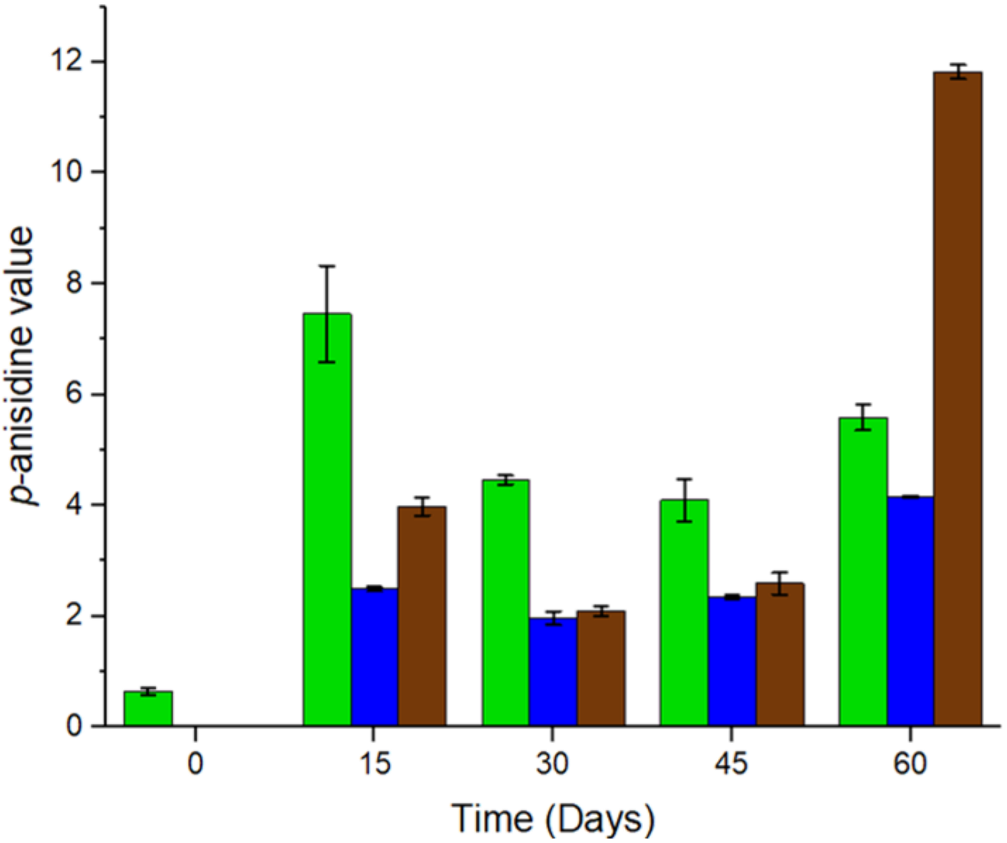

Figure 23: *p*-anisidine value, determined at different time points, in smoked salmon samples packed with PLA film (green), PLA 1% GTE film (blue) and PLA 2% GTE film (brown).

5. CONCLUSIONS

This thesis aimed to develop films of PLA containing different amounts of GTE, which was expected to endow the films with antioxidant capacity that could be used to produce active packaging. Mixtures of PLA with different amounts of GTE (1 wt.%, 2 wt.% and 4 wt.%) were produced using a Haake™ batch mixer. These mixtures were important to prove the compatibility of both materials. The prepared materials revealed that the GTE reduces the thermal stability of PLA. Additionally, they allowed us to understand how the color of the materials was affected by the GTE. 4 wt.% of GTE darken the PLA in such a way that it became almost opaque. Thus, it was decided to produce films containing only 1 wt.% and 2 wt.% of GTE.

Films of the PLA and GTE were produced by blown film extrusion. To facilitate the mixture and promote a better dispersion of GTE within the PLA matrix, GTE was added as a masterbatch with approximately 8 wt.% of extract.

The addition of GTE influences the mechanical properties of PLA. 1 wt.% of GTE decreased 8.2% of the PLA's yield stress, while 2% of GTE only induced a slight reduction (0.3%). In what concerns the tensile strength, the impact was similar, 1 wt.% of GTE reduced 11.9% and 2 wt.% GTE reduced this property by 12.0%. Young's modulus suffered opposite impacts. 1 wt.% of GTE reduced its value by 5.3% while 2 wt.% GTE enhanced it by 2.9%. The two films containing GTE had their yield strain and strain at break increased, with special emphasis to a 36% enhancement of the latter due to the 2 wt.% of extract.

Regarding the films' barrier properties, in particular the water-vapor transmission, it was observed that the addition of higher amounts of GTE decreased the WVT rate, thus improving the PLA's barrier efficiency to H_2O.

The films' capacity to act as an active packaging was evaluated studying the spoilage of sliced apples, red potatoes, and smoked salmon, packed with the extruded films. The results showed that it was possible to slow down the food oxidation when GTE was added to PLA, specially using 2 wt.% of GTE.

The results obtained during this work showed that PLA and GTE can be used to produce a material with antioxidant capacity with potential application to produce active food packaging.

6. FUTURE PERSPECTIVES

The work presented during this thesis shows that is possible to incorporate GTE into PLA and produce an active package.

However, it is important to develop additional efforts to optimize the production of the films. The assays of food preservation with the sliced apples and sliced potatoes gave only preliminary insights about the material efficiency. It is important to perform assays that allow to better understand the PLA/GTE films potential.

Additional assays to characterize the barrier properties should be performed.

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