PLA films loaded with green tea and rosemary polyphenolic extracts as an active packaging for almond and beef

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ABSTRACT

In the present study, RE presented higher antioxidant capacity and higher content of total phenolic compounds than GTE. While the main phenolic compounds identified in RE were carnosic acid, carnosol and rosmarinic acid, in GTE catechins, rutin and gallic acid were the main identifies compounds. Extracts were incorporated into PLA active films, followed by the evaluation of its properties. The potential of the active PLA films to extend foods shelf-life was tested in almonds and beef. PLA/4GTE presented the highest water vapor permeability and opacity, while PLA/4RE presented the highest O_2 permeability. PLA/2 GTE and PLA/4GTE presented the highest total content in phenolic compounds at the end of 10 days (at 40 °C). Regarding the almond packaged with the active films, PLA with RE films were the most effective in the reduction of oxidation, allowing to obtain the lowest lipid oxidation products, at the end of 60 days of storage at room temperature. In addition, active PLA films inhibited the formation of MDA content in beef stored for 11 days. This study shows that these PLA active packages can contribute for delaying lipid oxidation in foodstuffs with high fat content.

1. Introduction

Currently, food packaging is mainly based on highly durable non-biodegradable plastics, such as polyethylene and polyethylene terephthalate (Asgher et al., 2020; Paletta et al., 2019). Their high versatility and wide application promote an annual production of more than 300 million tons of plastic (Paletta et al., 2019). Being a non-biodegradable product, its accumulation in the environment causes serious concerns and imbalances (Paletta et al., 2019). Finding suitable substitutes for these materials has become a priority for the scientific and industry communities.

One of the most promising candidates to substitute conventional polymers in food packaging is polylactic acid (PLA) due to its high biocompatibility and biodegradability (Piergiovanni & Limbo, 2016; Fernanda Vilarinho et al., 2018). Indeed, this compound is already widely used in medical applications (Li et al., 2020; Riaz et al., 2018; Singhvi et al., 2019). Moreover, PLA is produced through the polymerization of lactic acid of natural sources such as corn and sugarcane, can be manufactured by most polymer processing equipment, and is easily molded. Several additives, such as nanoparticles, plasticizers, and

bioactive compounds, allow the improvement of PLA oxygen barrier and water vapor permeability (Martins et al., 2018; Piergiovanni & Limbo, 2016; Fernanda Vilarinho et al., 2018).

The main function of food packaging is to protect food against several external factors responsible for accelerating food spoilage, such as radiation, microorganisms contamination, oxygen and humidity (Brody et al., 2008; Dainelli et al., 2008). Conventional food packaging cannot interact with the packed food, meaning, they should be inert and migration from the package constituents to the packaged food should not occur (Brody et al., 2001; European Commission Regulation No. 1935/2004 (European Commission, European Parliament and the Council of the European Union, 711 European Commission, & European Parliament and the Council of the European Union., 2004) and its amendments). On the opposite, active food packaging is intentionally manufactured to positively interact with the packed foods, by absorbing or releasing compounds from/into the food matrix (Azevedo et al., 2022; European Commission Regulation No. 1935/2004 (European Commission, European Parliament and the Council of the European Union, 711 European Commission, & European Parliament and the Council of the European Union., 2004)).

The interest in PLA-based active food packaging has been growing, either incorporated with antioxidant and antimicrobial compounds or with nanoparticles to improve its mechanical and barrier properties (Martins et al., 2018; Rojas et al., 2021; Tawakkal et al., 2014; Vilarinho et al., 2021). Rojas et al. (2021) and Velásquez et al. (2021) recently review the incorporation of active agents, in the form of plant extracts and/or essential oils, in PLA for active food packaging materials. The authors clearly stated that the addition of active agents decreased PLA' elastic modulus and increased PLA' elongation at break. Also, the addition of active agents in low percentages translates into higher microbiological growth inhibition (Rojas et al., 2021). Another important parameter is the chosen technique for the active agents' incorporation in the PLA matrix. Melting-based technics are the most common processes but, when dealing with thermosensitive agents, such as essential oils or plant extracts, other techniques must be considered such as casting and electrospinning (Velásquez et al., 2021).

Lipid oxidation is one of the main causes of food spoilage, especially in meat and meat products (Andrade et al., 2022; Domínguez et al., 2019). Unsaturated fatty acids are very susceptible to oxidization, reacting with molecular oxygen, producing hydroperoxides, (primary oxidation) which are highly unstable and will decompose resulting in secondary compounds, such as hydrocarbons, aldehydes, ketones, alcohols, esters and acids (Amaral et al., 2018; Domínguez et al., 2019). One process of protecting food against lipid oxidation, is through the addition and presence of antioxidant compounds, such as phenolic compounds from natural sources.

Rosemary (*Rosmarinus oficinallis* L.) is an aromatic plant from the Lamiaceae family with proven antioxidant and antimicrobial activities, through their main bioactive compounds rosmarinic acid, carnosic acid and carnosol (Andrade et al., 2018; Ribeiro-Santos et al., 2015). Rosmarinic acid is commonly found in the Lamiaceae family and has proven to have antioxidant, antibacterial, anti-inflammatory, and antiviral activities (Ferraro et al., 2015; Marchev et al., 2021; Petersen, 2003; S.-J. Wang et al., 2019; Zhao et al., 2018). Carnosic acid and carnosol, an oxidative derivative of carnosic acid, have demonstrated antioxidant and antibacterial activities (Campo et al., 2000; Guitard et al., 2016; Moreno et al., 2006; Nakagawa et al., 2020; Pavić et al., 2019; H. Wang et al., 2011). The application of the rosemary extract as a food additive is approved by the Regulation (EU) nº 231/2012(European Commission, (2012)) from the European Commission and its subsequent amendments. Green tea, obtained from *Camellia sinensis* L. leaves, is a rich source of catechins, flavonoids and phenolic acids, powerful antioxidants (Martins et al., 2018). Catechins, the most abundant flavonoids in green tea, have shown antioxidant, anti-inflammatory and anti-viral activities (Babu & Liu, 2009; Higdon & Frei, 2003), even against SARS-CoV-2 (Kicker et al., 2022). Among catechins, epigallocatechin gallate (EGCG) is the most abundant in green tea leaves and is known for its health benefits, such as neuroprotective effects against neurodegenerative diseases like Alzheimer's and Parkinson's (Bae et al., 2020). According to the extensive review by Hu et al. (2018), the adults' safe intake level of EGCG is 338 mg/day.

In the literature, there are several examples of the use of green tea extracts to delay or inhibit lipid oxidation and to inhibit pathogens growth. Castro et al. (2019) incorporated a commercial green tea extract into a whey protein-based coating and successfully inhibited the lipid oxidation of salmon for 14 days of storage, when compared with the control. Also, Robalo et al. (2022) incorporated a green tea extract into a whey protein-based film, which successfully delayed the lipid oxidation of goat cheese and inhibited the microbiological load. Martins et al. (2018) delayed the lipid oxidation of smoked salmon for 60 days with a PLA-based active packaging loaded with a commercial green tea extract. Vilarinho et al. (2021) developed a PLA active packaging with green tea extract and cellulose nanocrystals to inhibit the lipid oxidation of salami slices. Additionally, Zeid et al. (2019) proved the efficiency of a PLA-based active food packaging with rosemary essential oil on preserving fresh rainbow trout for 6 days. Darie-Niţă et al. (2018) developed a PLA-based active packaging with an ethanolic rosemary extract. The incorporation of the extract improved the elongation at break, rheological properties and antimicrobial activity against *Bacillus cereus*, *Salmonella* Typhimurium and *Escherichia coli* (Darie-Niţă et al., 2018). The present study resorts to ethanolic extracts of rosemary and green tea extracts and studies a possible synergic effect between the two extracts. Therefore, the main objective of this study was to evaluate the capacities and properties of ethanolic extracts obtained from rosemary and green tea, incorporated into PLA films, and to study their capacity to extend the shelf-life of almonds and beef.

2. Materials and methods

2.1. Reagents and materials

Absolute ethanol (ACS grade reagent, for analysis), methanol (ACS grade reagent ($\geq$99.8%) and for HPLC, $\geq$ 99.9%), chloroform (Supra-Solv®, for gas chromatography ECD and FID), sodium carbonate anhydrous (ACS grade reagent), sodium nitrite (ACS grade reagent), sodium hydroxide (ACS grade reagent), petroleum ether (ACS grade reagent, bp 40–60 °C), barium chloride dihydrate (ACS grade reagent), iron(II) sulfate (pro analysis), iron(III) chloride (anhydrous for synthesis), hydrochloric acid, glacial acetic acid (for HPLC, $\geq$99.9%), Folin-Cioucalteu's phenol reagent, were acquired from Merck (Darmstadt, Germany). *n*-Hexane (SupraSolv®, for gas chromatography ECD and FID) was acquired from Honeywell. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Tween®40, β-carotene ($\geq$93%), linoleic acid (analytical standard), aluminum chloride, trichloroacetic acid (ACS grade reagent, $\geq$99.0%), 2-thiobarbituric acid ($\geq$98%), 1,1,3,3-tetramethoxypropane (97%), 2,2-diphenyl-1-picrylhydrazyl, xylenol orange sodium (spectrophotometric grade), 2,4-diphenilhydrazin and hexanal (98%), were acquired from Sigma-Aldrich (Madrid, Spain). Ultra-pure water was obtained through a Milli-Q® purification system (Millipore Corp., Belford, USA).

Concerning the used equipment, a compact stirrer Edmund Bühler™ Shaker KS 15 A (Hechingen, Germany), an Eppendorf AG 5804 R centrifuge (Hamburg, Germany), a rotary evaporator Büchi model R-210 (Labortechnik, Switzerland), a Thermo Scientific Evolution 300 LC spectrophotometer, a RSLAB-6PRO Vortex, Ultra-Turrax IKA® DI 25basic, a Grindomix GM 300 (Retsch) and a Grant Instruments™ QB Series Dry Block Heating System (Cambridge, England) were used.

2.2. Rosemary and green tea extraction

Rosemary (*Rosmarinus officinalis* L.) dried leaves were obtained on a local store in Lisbon, Portugal. Dried leaves of green tea (*Camellia*

sinensis L.), variety "*Encosta de Bruma*", were purchased from Gorreana, Azores, Portugal. As soon as the plants arrived to the laboratory, the leaves were ground in the Grindomix and extraction was made according to the method described by Andrade et al. (2018). Briefly, the powder was mixed with absolute ethanol in a 1:10 ratio. Then, the mixtures were agitated in the compact stirrer for 30 min, centrifuged for 15 min at 4025 *g*, at 10 °C, and the supernatant was evaporated until dryness in a rotary evaporator at 35 °C. The extract was removed with the help of a spatula, vacuum packaged and stored at −20 °C, protected from the light, until further use.

2.3. Antioxidant assays

For the evaluation of the antioxidant activity of the extracts, both rosemary extract (RE) and green tea extract (GTE) were diluted in absolute ethanol at 1.0 mg/mL and 0.1 mg/mL. All the assays were performed in triplicate. Also, to evaluate possible synergic effects, 3 solutions containing both extracts at 0.1 mg/mL were made: S1 containing 50% of each extract (RE and GTE); S2 containing 75% of the rosemary extract (RE) and 25% of green tea extract (GTE); and S3 containing 75% of GTE extract and 25% of RE.

2.3.1. DPPH radical scavenging assay

The original method was described by Moure et al. (2001) and adapted by Andrade et al. (2018). In short, 50 μL of sample (for the blanks, 50 μL of absolute ethanol) were mixed with 2 mL of a methanolic DPPH• solution (14.2 μg/mL). The samples were left for 30 min, protected from the light and, at the end of this period, the absorbance was measured in the spectrophotometer at 515 nm. The DPPH• Inhibition Percentage (IP) was calculated according to the Eq. 1.

$$IP(\%) = \frac{Ab - As}{Ab} \times 100 \tag{1}$$

where, *Ab* stands for the absorbance of the blank and *As* stands for the absorbance of the sample. A calibration curve, using trolox as standard was drawn, and the results are also expressed in mg of trolox equivalents per g of sample (mg TE/g).

2.3.2. Beta-carotene bleaching assay

The performed method was originally described by Miller (1971) and adapted by Andrade et al. (2018). Briefly, a solution of β-carotene in chloroform was prepared (2 mg/mL). Then, for the β-carotene:linoleic acid emulsion, 20 mg of linoleic acid were mixed with 200 mg of Tween®40 and 1 mL of the β-carotene solution. The chloroform was evaporated under a constant flow of nitrogen and 50 mL of ultrapure water were added. The emulsion was vigorously agitated. To 200 μL of sample, 5 mL of the emulsion were added. For the blanks, 200 μL of ethanol were used and the absorbance was immediately read, in the spectrophotometer at 470 nm. The samples and the blanks were kept at 50 °C in the heating block for 2 h and the absorbances were measured, at 470 nm, while warm. The Antioxidant Activity Coefficient (AAC) was calculated according to the Eq. 2.

$$AAC = \frac{As - Ab2}{Ab0 - Ab2} \times 1000 \tag{2}$$

where, *As* stands for the absorbance of the samples, *Ab0* stands for the initial absorbance of the blanks and *Ab2* stands for the absorbance of the blanks at the end of 2 h.

2.4. Total Content in Phenolic Compounds (TPC) and Flavonoids (TFC)

The method used to quantify the total content of the extracts in phenolic compounds was described by Erkan et al. (2008). In sum, 7.5 mL of Folin-Ciocalteu (10%, v/v) were added to 1 mL of sample and the mixture was homogenized. After 5 min, 7.5 mL of sodium carbonate aqueous solution (60 mg/mL) were added, the mixtures were once again homogenized and kept in the dark for 2 h. At the end of this period, the absorbance was measured at 725 nm. A standard calibration curve using gallic acid in ethanol was drawn and the results are expressed in mg of gallic acid equivalents per mL (mg GAE/g).

For the total quantification of flavonoids, the method described by Yoo et al. (2008) was performed. Succinctly, 4 mL of ultrapure water and 300 μL of an aqueous solution of sodium nitrite (5%, w/v) were added to 1 mL of sample and the mixture was homogenized. At the end of 5 min, 600 μL of aluminum chloride (10%, w/v) were added and the mixture was homogenized. At the end of 6 min, 2 mL of an aqueous solution of sodium hydroxide (1 M, w/v), and 2.1 mL of ultrapure water were added. The mixture was homogenized, and the absorbance was measured in the spectrophotometer at 510 nm. Epicatechin in ethanol was used as a standard for the calibration curve. The results are expressed in mg of epicatechin equivalents per mL (mg ECE/g).

2.5. Determination and quantification of individual phenolic compounds and catechins

Identification and quantification of individual phenolic compounds and catechins were performed in a UPLC® ACQUITY™ (Waters, Milford, MA, EUA) equipped with a DAD detector, using an ACQUITY™ UPLC® BEH C18 (2.1 × 150 mm, 1.7 μm particle size) column. Column was kept at 35 °C and samples at 5 °C and the injection volume was 20 μL. Mobile phase A was ultra-pure water acidified with glacial acetic acid at 0.1% (v/v) and mobile phase B was acetonitrile with glacial acetic acid at 0.1% (v/v) with a flow of 0.5 mL/min. A standard curve using a mix of 14 phenolic compounds diluted in methanol acidified with glacial acetic acid at 0.1% (v/v) was drawn. The gradient was as follows: 0 min, 99% A; 2 min, 99% A; 2.5 min, 98% A; 3 min, 95% A, 15 min, 80% A; 18 min, 70% A, 20 min, 60% A; 23 min, 10% A; 25 min, 99% A. The 14 individual phenolic compounds were gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, rutin, rosmarinic acid, quercetin, apigenin, kaempferol, hesperetin, carnosol and carnosic acid.

For the identification of individual catechins, same column and mobile phases were used. The gradient was as follows: 0–2 min, 99% A; 2.5 min, 98% A; 3 min, 95% A; 15 min, 80% A; 17 min, 99% A. The flow was kept at 0.3 mL/min, the column at 35 °C and samples at 5 °C. The injection volume was 20 μL. A standard curve using a mix of 5 catechins and gallic acid diluted in methanol acidified with glacial acetic acid at 0.1% (v/v) was drawn. The identified catechins were gallocatechin (GC), catechin, epicatechin (EC), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG). The calibration curve equations, detection and quantification limits, the coefficient of determination (r^2) and range of concentrations are present in the supplementary material.

2.6. Preparation of rosemary and green tea PLA films

Five different active PLA films were prepared, first by melt mixing 2% and 4% of GTE (PLA/2GT and PLA/4GT), 2% and 4% of RE (PLA/2 R and PLA/4 R), and, to evaluate the potential synergistic effect of the two extracts, 2% of RE and 2% of GTE (PLA/2GT/2 R) in a PLA matrix, this was performed in a twin screw extruder, Leistritz AG LSM 34 6 L, using a constant barrel temperature of 170 ºC, a screw speed of 125 rpm and feed rate of 4 kg.h-1. All the compounds were dried in a vacuum oven at 60 ºC, overnight prior to blown film extrusion. Monolayer films were prepared using a Periplast single screw extruder. The thickness of the films was in 50–60 μm range. The processing conditions were constant, with a screw speed of 50 rpm, 170 ºC on the first heating zone, 175 ºC on the second zone and 180 ºC on the remaining zones. A digital micrometer (No. 293–340, Mitutoyo, Kanagawa, Japan) was used to measure the thickness measurement of the films.

2.6.1. Fourier Transform Infrared spectroscopy (FTIR)

FTIR spectroscopy of the films was performed using a Bruker FT-IR VERTEX 80/80 v (Boston, USA) in Attenuated Total Reflectance mode (ATR) with a platinum crystal was used to obtain the FTIR spectra. The measurements were recorded from 4000 to 400 cm^{-1} wavenumber, at a resolution of 4 cm^{-1} and 64 scans. All the analyses were performed in duplicate.

2.6.2. Scanning electron microscopy (SEM)

The cross section of the films was examined by SEM (Quanta FEG 650, FEI, USA). Films were broken with nitrogen liquid and cross section of films were affixed on carbon adhesive tape and sputter-coated with gold. An acceleration voltage of 5 kV at different magnifications was applied to obtain the images.

2.6.3. Water Vapor Permeability assays

Water vapor permeability (WVP) was determined gravimetrically according to the ASTM E96/E96M – 10 standard method using Elcometer 5100 Payne Permeability Cups. Films were sealed on cups with distilled water and put into a controlled environmental chamber at 21

$\pm$ 1 °C and 0 $\pm$ 2% relative humidity (RH) with silica gel. Cups were weighed one time for day and weight loss was measured over time until steady state was reached (for 10 days). Water vapor transmission rate (WVTR) was calculated by dividing the slope of a linear regression of weight loss *versus* time by film area. The WVP (g.m^{-1}.s^{-1}. Pa^{-1}) was determined by Eq. 3:

$$WVP = \frac{(WVTR x L)}{\Delta P} \tag{3}$$

where L is the film thickness (m) and ΔP is the water vapor partial pressure difference (Pa) across the two sides of the film. At least two replicates were made for each film sample.

2.6.4. Opacity

The opacity can be described as the ratio between the absorbance and the material thickness, meaning that the higher the opacity level, the less transparent the material is (Guzman-Puyol et al., 2022). A Minolta colorimeter (CR 400; Minolta, Japan) was used to determine the film opacity, through the relationship of the film opacity on a black standard and a white standard. Three replicates were carried out with ten measurements for each sample.

2.6.5. Oxygen permeability

The oxygen permeability was determined using a Gas Diffusion Permeameter DP-100A (Porous Materials, Inc. Ithaca, New York, USA) with the pressure increase method, at a temperature of 23 $\pm$ 2 °C with a pressure of 760 torr for 3 h. Samples with a diameter of 4 cm were placed in the holder of the permeameter chamber and oxygen permeability was measured. Before analyses, the samples were stored at 23 $\pm$ 2 °C. Three measurements were performed for each film.

2.6.6. Mechanical properties

The tensile properties were determined based on the ISO 527–1/− 3 standard, using an INSTRON 5969 mechanical testing machine, with a 50 kN load cell. The tests were carried out at a speed of 200 mm/min and a temperature of 23 °C. Five specimens of type 2 (160 mm × 25 mm) of the ISO 527–3 standard were tested in the longitudinal (LD) and transversal (TD) direction. Tensile strength (σ_{max}, MPa), elongation-at-break (ε_{max}, %), and Young's modulus ($E_{1\%}$, MPa) were determined from stress-strain curves. The Young Modulus was determined by secant modulus at 1% strain and an initial distance of 50 mm was used.

2.6.7. Antioxidant capacity and quantification of the total phenolics and flavonoids of loaded PLA films

In order to evaluate the potential antioxidant capacity and total content in phenolic compounds and flavonoids of the PLA incorporated with the extracts, an accelerated migration assay was performed according to the method described by López-de-Dicastillo et al. (2012) with a few changes. Circles with 6 cm^2 of the PLA films were cut and, the surface intended to be in contact with the food was placed in migration cells and in contact with 10 mL ethanol 95% (v/v), as a simulant of fatty food. The migration cells were stored in an oven at 40 $\pm$ 1 °C, protected from the light, for 10 days. At the end of this period, the antioxidant capacity assays (see Section 2.3) and the assays to determine the total content in phenolic compounds and flavonoids (see Section 2.4) were performed.

2.7. Packaging of the foodstuffs

Two model foodstuffs, almond and beef, were selected based on their high content in unsaturated fatty acids. Almond was acquired in a local store in Lisbon, Portugal, still in the hard shell. As soon as it arrived at the laboratory, the hard shell was manually separated and, for the peel removal, the almond was placed in hot water (80 $\pm$ 1 °C) for 5 min, and manually peeled. Then, the almonds were ground in a Grindomix, and 35 $\pm$ 1 g were immediately vacuum packaged and sealed, in 10 cm × 10 cm squares, in a vacuum chamber machine (MULTIVAC Model C70) with the active PLA films. The packaged almond was stored at room temperature (21 $\pm$ 1 °C) and in an oven at 40 $\pm$ 1 °C (accelerated assay), protected from the light, for a maximum time period of 60 and 30 days, respectively. The almond at room temperature was analyzed at the end of 7, 14, 21, 30, 45 and 60 days of storage and the almond stored at 40 °C was analyzed at the end of 2, 4, 7, 14, 21 and 30 days of storage.

The beef from the cow's neck was acquired from a local butcher shop in Lisbon, Portugal, in 35 $\pm$ 2 g pieces. As soon as it arrived at the laboratory, the meat was vacuum packaged and sealed in a vacuum chamber machine (MULTIVAC Model C70) with the active PLA films and stored at 4 $\pm$ 1 °C, for a maximum time period of 11 days, protected from the light. The lipid oxidation as well as the antimicrobial assays were performed at the end of 1, 4, 6, 8 and 11 days of storage.

2.8. Lipid oxidation evaluation

2.8.1. Thiobarbituric acid reactive substances (TBARS)

The TBARS assay, one of the most common assays performed to evaluate lipid oxidation status, measures the malonaldehyde (MDA) content, which is an aldehyde formed during the primary oxidation being a result of the peroxidation of polyunsaturated fatty acids (Castro et al., 2019; D. D. Miller, 1998; Osawa et al., 2005; Ross & Smith, 2006). The applied method was initial described by Rosmini et al. (1996) and altered/adapted by Andrade et al. (2021). Briefly, 5 g of sample were mixed with 20 mL of an aqueous solution of trichloroacetic acid (7.5%, w/v). The mixture was homogenized in the compact stirrer, for 1 h, at 400–450 rpm, and filtered using a paper filter Whatman No. 1. Then, 2.5 mL of the filtered liquid were mixed with 2.5 mL of an aqueous solution of thiobarbituric acid (2.88 mg/mL), homogenized and kept at 95 °C for 30 min, followed by a rapid cooling in ice for 15 min. The absorbance of the samples was measured in a spectrophotometer at 530 nm, against the blank (2.5 mL of water with 2.5 mL of thiobarbituric acid solution). The calibration curve was drawn using 1,1,3, 3-tetramethoxypropane as standard. The results are expressed in mg of MDA/kg of sample.

2.8.2. Peroxide value determination

The samples' fat was extracted using the method described by Vilarinho et al. (2018). Briefly, 10 $\pm$ 1 g of sample were mixed with 100 mL of petroleum ether and mixed for 1 h in the compact stirrer at

350–400 rpm. The solutions were filtered through a paper filter What-man No. 4 with 1 g of sodium carbonate anhydrous. The petroleum ether was evaporated in the rotary evaporator at 35 °C.

The determination of the peroxide value was performed according to the method described by Shantha and Decker (1994). In the first place, a solution of iron(II) chloride was prepared: 50 mL of an aqueous solution of barium chloride dihydrate (8 mg/mL) were slowly added to 50 mL of an aqueous solution of iron(II) sulfate solution, under constant stirring. Then, 2 mL of hydrochloric acid (10 N) were added, and the solution was left to rest until the barium precipitate was formed. Then, the clear supernatant was removed to an amber glass and stored, protected from the light, for a maximum time period of 1 week.

A mixture of 50 mg of fat and 9.8 mL of a chloroform-methanol solution (70:30, v/v) was prepared and mixed for 2–4 s in a vortex. Then, 50 μL of xylenol orange sodium (10 mM) were added and the solution was again mixed, for 2–4 s. Then, 50 μL of the iron(II) solution were added, the solution was mixed and, after 5 min, the absorbance was measured at 560 nm. The blank contained all the reagents with the exception of the sample. A calibration curve was drawn using iron(III) chloride as standard. The peroxide values were calculated through the Eq. 4 and are expressed in milliequivalents of oxygen per kilogram of sample (meq O_2/kg).

$$PV = \frac{(A_s - A_b) \times m}{55.84 \times m_0} \qquad (4)$$

where, As stands for the absorbance of the sample, Ab stands for the absorbance of the blank, m stands for the slope of the calibration curve, m_0 stands for the samples mass in grams and 55.84 is the atomic weight of iron.

2.9. Microbiological assays

Regarding the microbiologic analysis performed on samples of beef meat packaged with the control and active PLA films, the enumeration of microorganisms that are able to grow after aerobic incubation at 30 °C was performed, according to the automated test TEMPO® AC-Validated by AFNOR with certificate Nº BIO 12/35–05/13. The microbiological examinations were only performed in meat samples, since the normal degradation during the almonds shelf-life is not usually due to the action of microorganisms but to lipid oxidation (rancidity), which cause, mainly, organoleptic alterations.

2.10. Statistical analyses

All the assays were performed in triplicate and the results are expressed as means ± standard deviation. The statistical analysis was performed using a one-way analysis of variance (ANOVA) and ANOVA with Repeated Measures, performed in the Software IBM® SPSS® Statistics, version 27.0.1.0, and differences among mean values were processed by the Tukey test.

3. Results and discussion

3.1. Antioxidant capacity and total content in phenolic compounds and flavonoids

The results of the antioxidant capacity assays and of the total content in phenolic compounds and total flavonoids are presented in Table 1. GTE presented an EC_{50} of 0.14 mg/mL, lower than the EC_{50} presented by the RE (0.53 mg/mL). These results are close to the results found by Castro et al. (2019). However, RE presented significantly a higher antioxidant capacity coefficient and higher content in total phenolic compounds than GTE. Comparing with the results found by Castro et al. (2019), the values obtained for the TPC and TFC in GTE were similar, but the obtained RE presented higher content in phenolic compounds and flavonoids. Since the extraction method used by Castro et al. (2019) was the same, the differences detected could be due to edaphoclimatic conditions during plant development. Also, the isolated extracts presented higher values than the solutions combining both extracts, therefore no synergic effect was detected (Table 1).

3.2. Quantification of individual phenolic compounds and catechins

Table 2 presents the content of individual compounds in ethanolic extracts of RE and GTE. Carnosol (35.81 mg/g), carnosic acid (104.49 mg/g) and rosmarinic acid (25.60 mg/g) were identified in the rosemary extract. Catechin (C) was also identified in the rosemary extract, but below the quantification limit. All the five catechins were identified on the GTE, but only catechin (321.28 mg/g), epicatechin (EC) (31.30 mg/g), and EGCG (148.11 mg/g) were quantified. GTE also presented gallic acid (4.49 mg/g) and rutin (15.55 mg/g).

The analyzed GTE exhibited higher contents in C, EC, EGCG and rutin than the green tea extracts developed and analyzed by Bae et al. (2020), Das & Eun (2018) and Vilarinho et al. (2021). Bae et al. (2020) and Das and Eun, (2018) managed to quantify GCG (59.0 ± 08 mg/g; between 6.0 and 11.9 mg/g, respectively) that, although present in the GTE, was below the limit of quantification (12.9 μg/mL). Das and Eun (2018) also quantified GC (between 0.5 and 3.6 mg/g) that was not identified in the GTE. The commercial green tea extract analyzed by Vilarinho et al. (2021) presented higher content in gallic acid (6.64 mg/g) and the authors were able to identify chlorogenic acid (2.65 mg/g).

The differences between the extracts' phenolic content can be mainly assigned to the different extraction methods used. The green tea extract obtained by Bae et al. (2020) (HTP-GTE) and the different extracts from

Table 1

Antioxidant assays and total content of the extracts in phenolic compounds and flavonoids.

Samples	DPPH• assay (Inhibition %)	TE (mg TE/g)	EC_{50} (mg/mL)	β-Carotene assay (AAC)	TPC (GAE mg/g)	TFC (ECE mg/g)
RE (1 mg/mL)	92.34 ± 0.28[a]	161.64 ± 0.49	0.53	817.86 ± 18.74[p]	857.14 ± 18.16 [*,g]	611.93 ± 5.55 [*,m]
GTE (1 mg/mL)	90.07 ± 0.14[b]	157.75 ± 0.24	0.14	478.20 ± 42.85[q]	474.39 ± 11.05 [*,h]	174.25 ± 2.24 [n]
S1 (50% GTE + 50% RE)	31.60 ± 0.27[c]	57.19 ± 0.47	-	n.d.	339.55 ± 5.89[i]	59.22 ± 8.99 [l]
S2 (75% RE + 25% GTE)	22.06 ± 0.29[d]	40.77 ± 0.50	-	n.d.	267.07 ± 20.64[j]	73.47 ± 13.03 [l]
S3 (25% RE + 75% GTE)	38.21 ± 1.17 [f]	68.55 ± 2.00	-	n.d.	388.52 ± 8.37[k]	194.97 ± 6.82[°]

*Due to their high content in phenolic compounds and flavonoids, these values are for the extracts at 0.1 mg/mL.

Different letters in the same column mean significant differences (p < 0.05).

Legend: TE – Trolox equivalents; AAC – Antioxidant Activity Coefficient; TPC – Total Phenolic Content; TFC – Total Flavonoids Content; GAE – Gallic Acid Equivalents; ECE – Epicatechin Equivalents; GTE – Green Tea Extract; RE – Rosemary Extract; n.d. – not detected.

Table 2

Individual phenolic compounds identified in the rosemary (RE) and green tea (GTE) extracts.

Phenolic group	Compound	Wavelength (nm)	RE (mg/g)	GTE (mg/g)
Flavan-3-ols	(-)-Gallocatechin	280	n.d.	< LoQ
	(+)-Catechin	280	< LoQ	321.28 ± 5.15[a]
	(-)-Epicatechin	280	n.d.	31.30 ± 0.44[b]
	(-)-Epigallocatechin gallate	280	n.d.	148.11 ± 1.60[c]
	(-)-Gallocatechin gallate	280	n.d.	< LoQ
Benzoic acids derivatives	Gallic acid	270	n.d.	4.49 ± 0.07
	Protocatechuic acid	260	n.d.	n.d.
	Gentisic acid	325	n.d.	n.d.
Terpenes	Carnosol	280	35.81 ± 0.35	n.d.
	Carnosic acid	280	104.49 ± 5.23	n.d.
Flavanones	Hesperetin	280	n.d.	n.d.
Cinnamic acids derivatives	*p*-coumaric acid	320	n.d.	n.d.
	Ferulic acid	320	n.d.	n.d.
	Chlorogenic acid	325	n.d.	n.d.
	Caffeic acid	325	n.d.	n.d.
	Rosmarinic acid	330	25.60 ± 2.14	n.d.
Flavones	Apigenin	330	n.d.	n.d.
	Kaempferol	360	n.d.	n.d.
Flavonols	Rutin	360	n.d.	15.55 ± 0.60
	Quercetin	360	n.d.	n.d.
	Total content		**165.34**	**478.19**

Das and Eun (2018) were obtained with heat application, which can affect some compounds, especially EGCG, due to its thermosensitivity. Within extraction methods' selection, the extraction solvent is of major importance. The use of water or an ethanol-water solution can be beneficial for the extraction of some compounds but, in general, water is less efficient than organic solvents for compounds extraction. In addition, the use of water can delay the concentration of the extract, that is the evaporation of the extraction (Alara et al., 2021).

The analyzed RE showed higher content in carnosic acid than the extracts analyzed by Gonçalves et al. (2019) and Lefebvre et al. (2021). However, the different extracts developed by Lefebvre et al. (2021) presented higher concentrations of carnosol (between 9 and 43 mg/g) and rosmarinic acid (78 mg/g). The work developed by Gonçalves et al. (2019) employed a simple extraction method, using only water at room temperature. No carnosol or carnosic acid were identified, but the authors managed to quantify other phenolic compounds such as yunnaneic acid F (5.6 mg/g), luteolin-O-glucuronide (3.9 mg/g) and sagerinic acid isomer (2.44 mg/g). Also, the RE presented higher content in carnosol, carnosic acid and rosmarinic acid than the extract developed by Kanakidi et al. (2022). These authors produced rosemary extracts with and without ultrasonic assisted extraction, using acetone, water, and a mixture of both.

Lefebvre et al. (2021) quantified different compounds in several fractions of rosemary extracts obtained with supercritical fluid extraction with CO_2 and different percentages of ethanol and water. Although these authors managed to obtain an extract with higher amounts of rosmarinic acid and carnosol, this methodology is more expensive and the apparatus and materials are difficult to find in a common laboratory.

3.3. Films characterization

3.3.1. FTIR and SEM

FTIR analysis (Fig. 1) did not show any differences between PLA film and the active PLA films (with extracts), meaning that the PLA structure was not altered with the addition of the GTE and the RE. The spectrum of the PLA matches the PLA spectrum described by Jiang et al. (2020). These authors developed a PLA with ginger and angelica essential oils which did not affect the structure of the PLA (Jiang et al., 2020). Fig. 2 presents the SEM images for the control and active PLA films. No visual significant differences were found between the active and control PLA.

3.3.2. Water vapor and oxygen permeability, and opacity

The WVP of films seems to be unaffected by the addition of the extracts with the exception of the PLA/4GT, which resulted in an increase ($p > 0.05$) of WVP values (Table 3). However, O_2 permeability was only increased with the PLA/4 R film. Comparing with the literature, the WVP values for neat PLA were lower than the ones reported by Radusin et al. (2019) (9.21×10^{-11} g m/Pa/s) and Vilarinho et al. (2021) (approximately 1.8×10^{-11} g/m s Pa). Radusin et al. (2019) revealed that the addition of the active substance, a garlic extract, decreased the
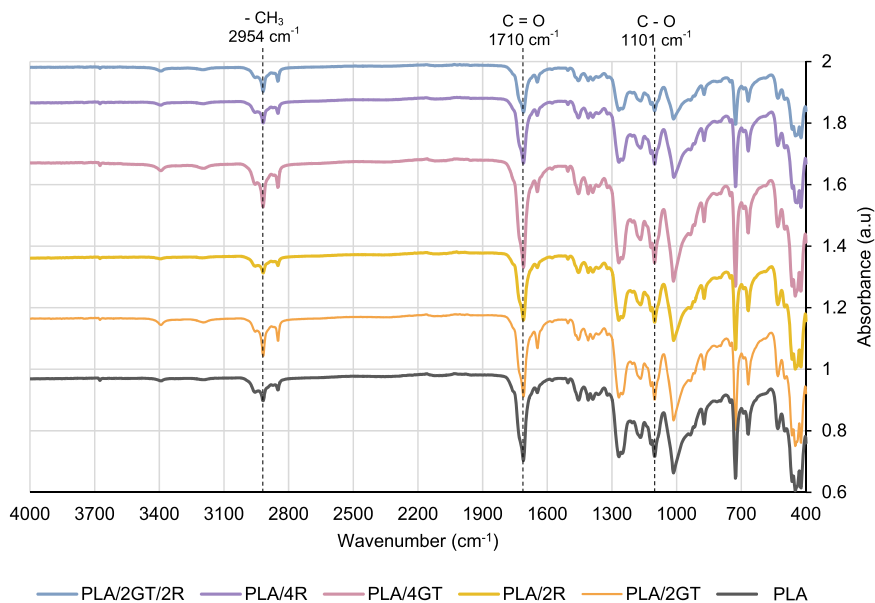
Fig. 1. FTIR spectrum of the control (PLA) and active (PLA/2GT, PLA/4GT, PLA/2 R, PLA/4 R, PLA/2GT/2 R) PLA films.
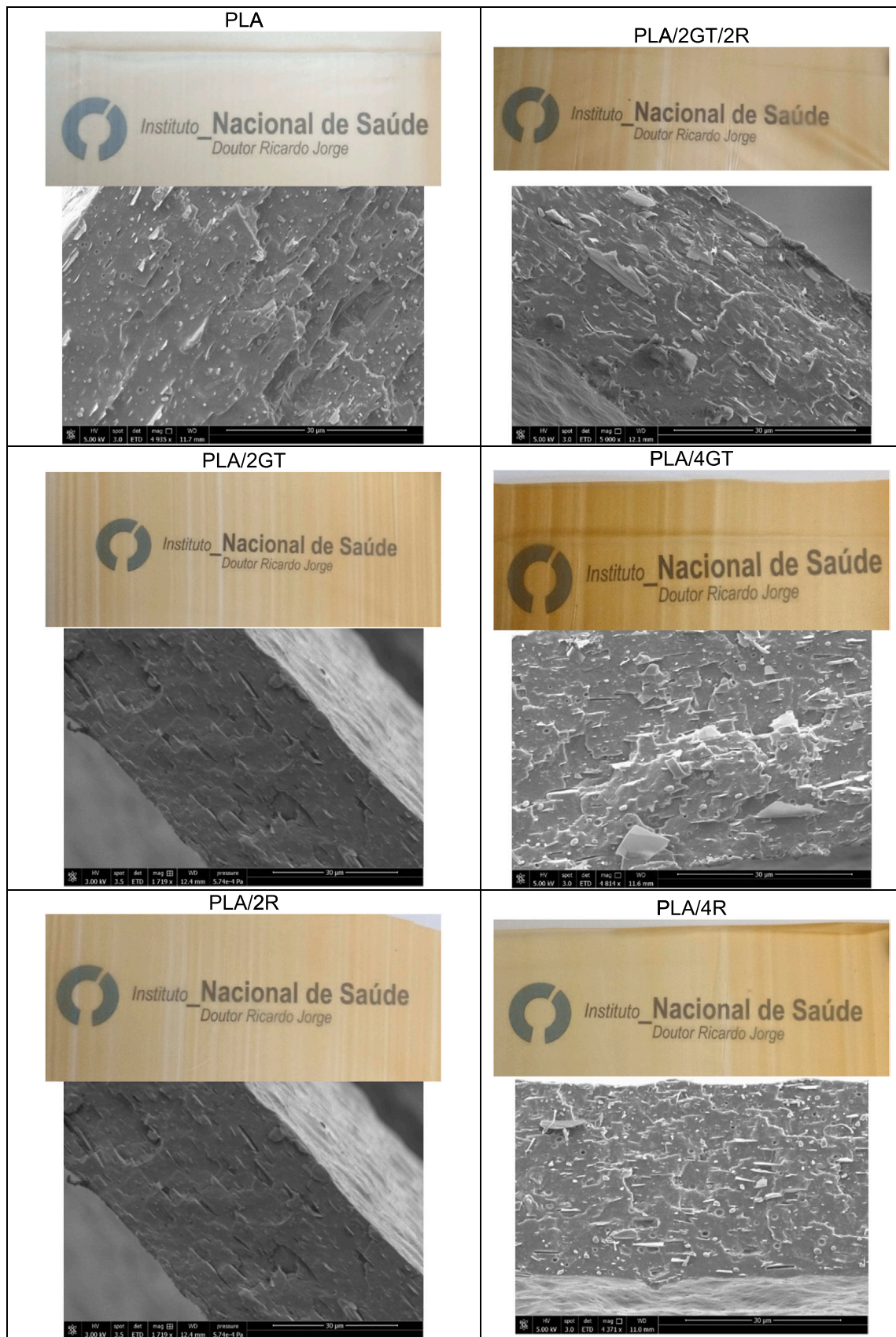
Fig. 2. Control (PLA) and active (PLA/2GT, PLA/4GT, PLA/2 R, PLA/4 R, PLA/2GT/2 R) PLA films) films over the Portuguese National Institute of Health logo and cross-section images of control (PLA) and active (PLA/2GT, PLA/4GT, PLA/2 R, PLA/4 R, PLA/2GT/2 R) PLA films obtained by SEM.

Table 3

Water vapor permeability (WVP), opacity and O_2 permeability (O_2P) of the control (PLA) and active (PLA/2GT, PLA/4GT, PLA/2 R, PLA/4 R, PLA/2GT/2 R) PLA films.

Samples	WVP x 10^{-12} (g m^{-1} Pa^{-1} s^{-1})	Opacity (%)	O_2P x 10^{-14} (mL.cm/(Pa.s.cm^2))
PLA	5.54 ± 1.11^a	24.21 ± 1.70^a	4.16 ± 0.25^a
PLA/2GT	5.83 ± 2.14^a	22.66 ± 1.23^a	4.87 ± 0.07^{a,b}
PLA/4GT	9.78 ± 1.24^b	26.62 ± 1.06^a	5.27 ± 0.75^{a,b,c}
PLA/2 R	3.60 ± 0.16^a	21.82 ± 0.27^b	5.73 ± 0.18^{b,c}
PLA/4 R	5.03 ± 2.11^a	20.90 ± 1.12^b	6.54 ± 0.75^c
PLA/2GT/2 R	4.94 ± 1.93^a	20.96 ± 2.03^b	6.43 ± 0.42^c

Different letters stand for significant differences.

Legend: GT – Green Tea extract; R – Rosemary extract

WVP values (6.86 ×10^{-11} g m/Pa/s). Vilarinho et al. (2021) also incorporated a commercial GTE in PLA films, which decreases the PLA WVP values with the exception of the PLA with 1% of GTE.

Based on the results from Table 3, PLA with RE (2% and 4%) seems to be the best candidates for food packaging, since they have the lowest WVP. However, the PLA/4 R presented the highest O_2 permeability values.

Regarding the films' opacity, significant differences between the control and active PLA can be found with the addition of the extracts with exception of PLA/4GT. The addition of the extracts made the PLA less opaque (Table 3). Images of the control and the active PLA films can be observed in Fig. 2.

3.3.3. Mechanical properties

The results for the mechanical properties of the PLA films are indicated in Table 4. Observing the results from the transversal direction, the addition of the extracts decreased the tensile strength and elongation at break values when compared with PLA without extracts, especially in the PLA/4GT and PLA/2GT/2 R. Observing the results of the longitudinal direction, the addition of the extracts seems to have no effect on the tensile strength. By decreasing the tensile strength and elongation at break the addition of the extracts weakened the PLA. An increase on the Young's modulus can be observed with the addition of the RE and 2% of GTE.

Martins et al. (2018) evaluated the tensile strength through the longitudinal direction. The authors developed a control PLA film with a higher tensile strength value (40.2 MPa), which decreased with the addition of 2% of green tea extract (35.4 MPa).

3.3.4. Antioxidant evaluation of the active films

Table 5 shows the antioxidant capacity (DPPH and β-carotene assay,

Table 4

Mechanical properties of the control (PLA) and active (PLA/2GT, PLA/4GT, PLA/2 R, PLA/4 R, PLA/2GT/2 R) PLA films. Different letters stand for significant differences. Uppercase letters indicate the significant differences between the LD cuts, and the lowercase letters indicate differences for the TD cut.

Samples	Young's modulus (E$_{1\%}$, MPa)	Tensile strength (σ_{max}, MPa)	Elongation at break ε_{max} (%)
PLA TD	124.89 ± 25.49^a	12.52 ± 0.42^a	260.11 ± 37.91^a
PLA LD	184.17 ± 45.01^A	15.63 ± 1.23^A	432.89 ± 29.42^A
PLA/2 R TD	145.00 ± 30.03^b	10.88 ± 0.84^b	245.84 ± 84.35^b
PLA/2 R LD	224.62 ± 23.00^B	16.14 ± 2.30^B	442.21 ± 17.22^B
PLA/4 R TD	156.70 ± 36.21^c	10.07 ± 0.43^c	129.31 ± 28.25^c
PLA/4 R LD	173.82 ± 61.41^C	15.60 ± 1.02^C	435.50 ± 25.09^C
PLA2GT TD	133.93 ± 24.29^d	10.29 ± 0.16^d	121.95 ± 2.91^d
PLA/2GT LD	196.56 ± 25.27^D	15.57 ± 0.53^D	437.40 ± 24.25^D
PLA/4GT TD	114.90 ± 48.20^e	9.17 ± 0.23^e	76.38 ± 17.05^e
PLA/4GT LD	228.79 ± 22.72^E	15.76 ± 0.97^E	435.86 ± 26.01^E
PLA/2GT/2 R TD	109.13 ± 28.24^f	9.43 ± 0.43^f	126.78 ± 32.84^f
PLA/2GT/2 R LD	182.07 ± 47.90^F	13.50 ± 1.39^F	263.18 ± 53.95^F

Legend: GT – Green tea extract; R – Rosemary extract; Longitudinal (LD) and transversal (TD) direction

Table 5

Antioxidant capacity and total content in phenolic compounds and total content on flavonoids from of the fatty food simulant after contact with the control (PLA) and active (PLA/2GT, PLA/4GT, PLA/2 R, PLA/4 R, PLA/2GT/2 R) PLA films during 10 days at 40 ºC.

Samples	DPPH$^\bullet$ assay (Inhibition %)	β-Carotene assay (AAC)	TPC (GAE µg/mL)	TFC (ECE µg/mL)
PLA/2GT	18.48 ± 2.22^a	85.86 ± 8.46^f	19.39 ± 0.33^k	15.20 ± 0.78^{n,o}
PLA/4GT	42.69 ± 2.22^b	75.28 ± 2.80^f	42.39 ± 0.56^l	26.40 ± 0.16^q
PLA/2 R	5.80 ± 0.25^c	160.66 ± 5.03^h	11.72 ± 0.37^j	18.71 ± 0.89^p
PLA/4 R	1.65 ± 0.44^d	173.22 ± 13.69^h	12.63 ± 0.36^j	14.21 ± 0.55^n
PLA/2GT/2 R	10.15 ± 0.38^e	177.33 ± 37.76^g	18.24 ± 0.42^m	16.20 ± 0.99^{n,o}

Legend: n.d. – non detected; AAC – Antioxidant Activity Coefficient; TPC – Total Phenolic Content; TFC – Total Flavonoids Content; GAE – Gallic Acid Equivalents; ECE – Epicatechin Equivalents; GT – Green Tea Extract; R – Rosemary Extract

TPC and TFC) of the active PLA films after 10 days at 40 °C in contact with ethanol 95% (v/v). PLA/4GT presented the highest DPPH$^\bullet$ inhibition and the highest content in total phenolic compounds and flavonoids. In sum, the PLA with GTE presented the best antioxidant results in all assays with the exception of the β-carotene bleaching assay, in which PLA/4 R presented the highest value, followed by the PLA/2 R.

3.4. Lipid oxidation evaluation of almond

TBARS results of the almond, packaged at different temperatures, can be seen in Fig. 3 and Fig. 4. The MDA equivalent (MDA eq.) values of the almond packaged with PLA and stored at room temperature were significantly higher, over the 60 days of storage, than the almond packaged with PLA/2 R, PLA/4 R and PLA/2GT/2 R. The PLA/2GT and PLA/4GT only presented significantly lower MDA eq. values until the 14th day of storage, suggesting that the RE has a better performance in delaying the lipid oxidation of almond. Also, the PLA/4 R′ almond presented lower MDA eq. values than the PLA' almond by 30%, 40% and 50%, at 30, 45 and 60th day of storage, respectively. Similar results can be seen in the accelerated assay (at 40 ºC), in which the almond packaged with PLA/4 R presented significantly lower MDA eq. values than the PLA packaged almond, for 21 days of storage.

In the study led by Castro et al. (2019) a commercial GTE was used to delay salmon' lipid oxidation in a whey protein-based coating. The results showed that the GTE presented lower MDA eq. values for 5, 7, 14 and 17 days of storage than the control (Castro et al., 2019). However, in the study performed by Andrade et al. (2019), the salami slices packaged with a whey protein-based coating with RE exhibited lower MDA eq. values at the 7th, 15th, 60th and 90th day of storage than the salami packaged with the control whey-protein package. Martins et al. (2018) reported that a PLA package with 1% and 2% of a GTE delayed the smoked salmon' lipid oxidation for 45 days of storage, when compared with the control PLA. On the other hand, Vilarinho et al. (2021) presented higher MDA eq. values, for salami slices packaged with a PLA with only 1% of a GTE, than the control PLA. The results found in the literature suggest that, although the GT extracts have higher content in phenolic compounds and higher antioxidant activity, they seem to accelerate foods' lipid oxidation process. On the contrary, the rosemary extracts seem to effectively prolong foods' shelf-life, by reducing the MDA eq. values.

Peroxide results from the almond packaged with the PLA films and stored at room temperature can be observed in Fig. 5. At the 21st, 30th, 45th and 60th day of storage, the almond packaged with the PLA/4 R presented lower peroxides values than the almond packaged with the
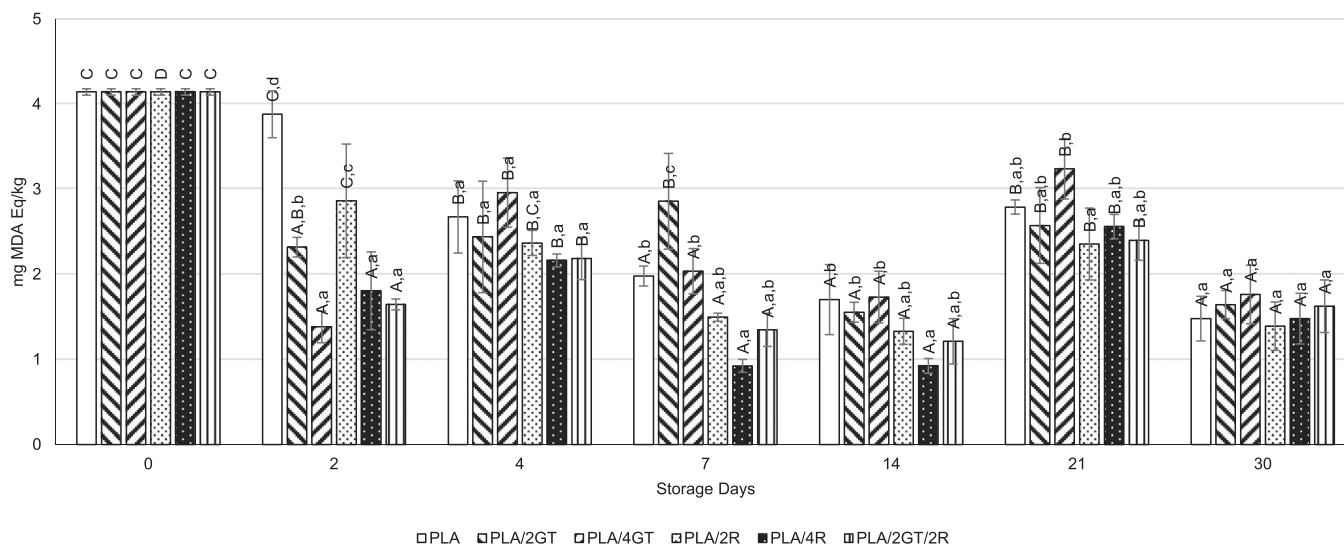
Fig. 3. TBARS assay results for almond packaged with the active and control (PLA) and active (PLA/2GT, PLA/4GT, PLA/2 R, PLA/4 R, PLA/2GT/2 R) PLA films stored at 40 ºC. The lowercase letters compare samples within the same storage day. The uppercase letters compare the same packaging for different storage days. Different letters, for either lowercase/uppercase comparison, represent results with significant differences.
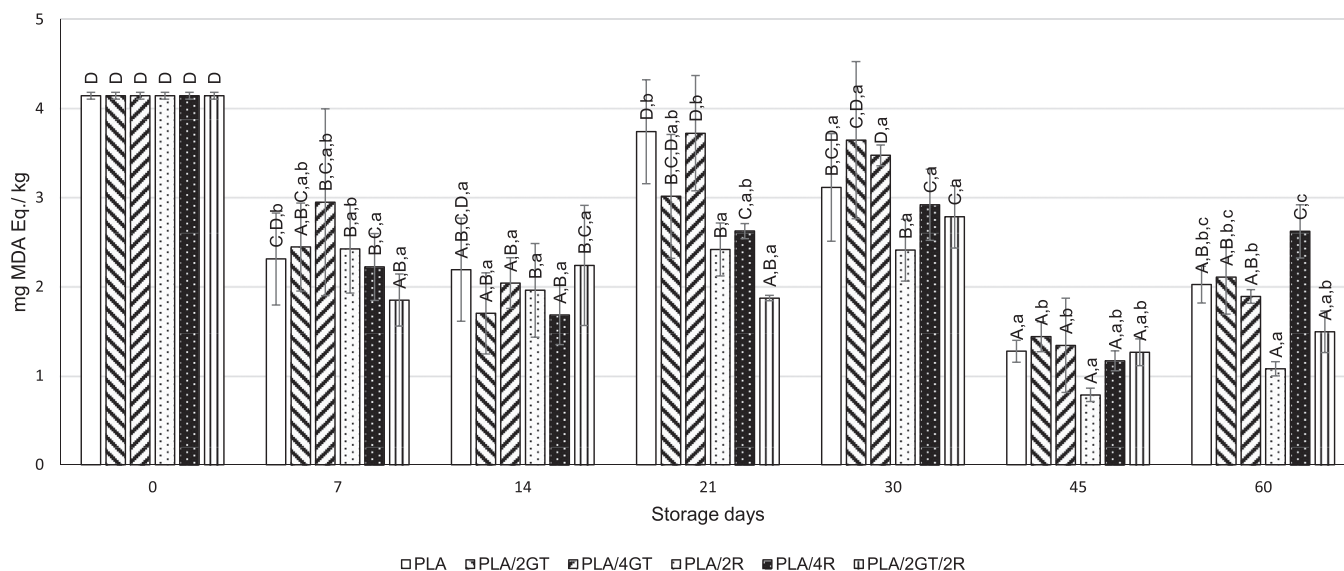
Fig. 4. TBARS assay results from the almond packaged with the control (PLA) and active (PLA/2GT, PLA/4GT, PLA/2 R, PLA/4 R, PLA/2GT/2 R) PLA films stored at 21 ºC. The lowercase letters compare samples within the same storage day. The uppercase letters compare the same packaging for different storage days. Different letters, for either lowercase/uppercase comparison, represent results with significant differences.

control PLA and the other active films, supporting the PLA/4 R TBARS results. Hexanal was not detected in any of the samples.

The *in vitro* antioxidant assays (see Sections 3.1 and 3.3.4) and the O_2 permeability (see Section 3.3.2), pointed that PLA incorporated with GTE was the better film for inhibiting a fatty foods' lipid oxidation. However, the *in vivo* results suggest that the GTE has a prooxidant effect in food, whereas the RE delays it.

### 3.5. Lipid oxidation and microbiological analysis for meat

The TBARS results of the meat are presented in Fig. 6. The MDA values are lower than the ones obtained in the almond assay. Also, for the total of the storage days, all the active films presented a lower MDA value than the meat packaged with the control PLA. At the end of the 8th and 11th day, PLA/4GT and PLA/2GT/2 R presented the lower MDA values, suggesting that the PLA with the GTE presents a higher lipid

oxidation inhibition for shorter times.

Fiore et al. (2021) studied the incorporation of chitosan and rosemary essential oil in PLA packages in minced chicken breasts. The authors found that the incorporation of only 1% and 2% of rosemary essential oil protects the chicken meat against lipid oxidation until the 14th day of storage, with MDA values below 0.5 mg/kg, similar to the results in this study for the RE and GTE. On the other hand, Yoon et al. (2021) demonstrated that the direct addition of rosemary and green tea extracts seems to increase the MDA value of pork sausages when compared to the control. However, the MDA values presented by these authors were lower (between 0.094 and 0.142 mg/kg) than the MDA values present in this study (Yoon et al., 2021). Zhang et al. (2019) developed a gelatin film incorporated with different percentages of one of the most abundant bioactive compound of rosemary extract, rosmarinic acid. The authors found that active films inhibit the MDA formation in Chinese bacon for 60 days, when compared to the control
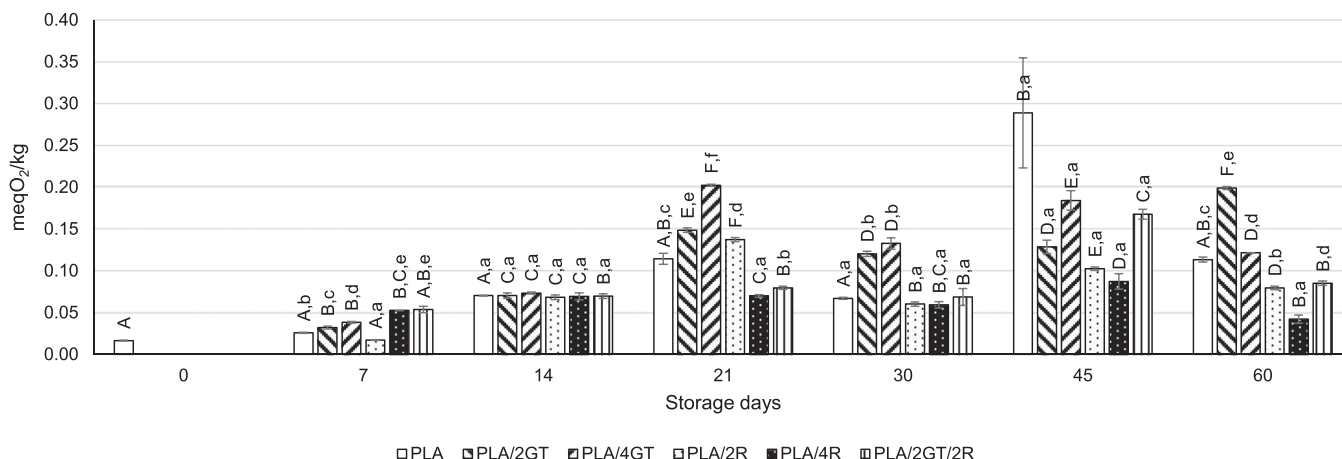
Fig. 5. Peroxide value results for almond packed with the control (PLA) and active (PLA/2GT, PLA/4GT, PLA/2 R, PLA/4 R, PLA/2GT/2 R) PLA films stored at 21 °C. The lowercase letters compare samples within the same storage day. The uppercase letters compare the same packaging for different storage days. Different letters, for either lowercase/uppercase comparison, represent results with significant differences.
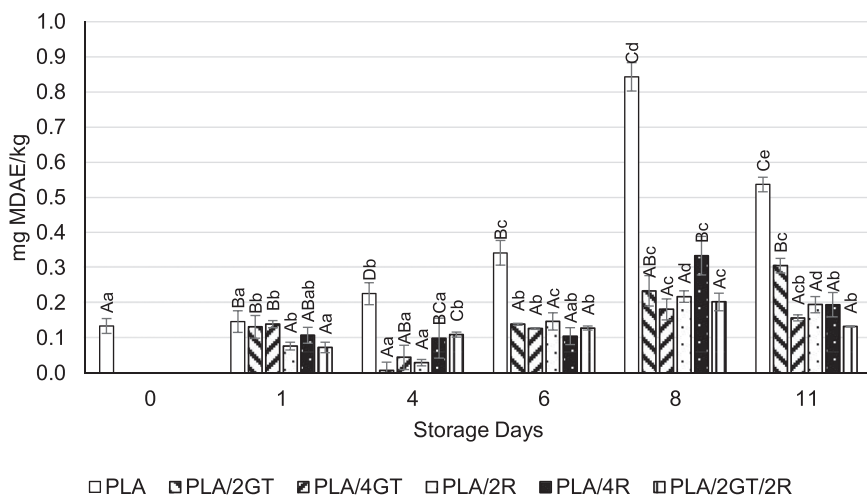
Fig. 6. Results of the Thiobarbituric Reactive Substances from the meat packed with the control (PLA) and active (PLA/2GT, PLA/4GT, PLA/2 R, PLA/4 R, PLA/2GT/2 R) PLA films. The lowercase letters compare samples within the same storage day. The lowercase letters compare the same packaging for different storage days (for instance, PLA at 0 and 1st storage day present no significant differences, has identified by the lowercase "a". On the other hand, at 1st storage day, PLA/2 R and PLA/4 R present no significant differences, has identified by the uppercase "A"). Different letters, for either lowercase/uppercase comparison, represent results with significant differences.

sample.

Regarding the microbiological assay (Table 6), a significant inhibition of the microbial growth was observed between the 6th and 8th storage day on the meat packaged with PLA/2 R and PLA/4 R films, and between the 8th and 11th day of storage on the meat packaged with PLA/2GT. Andrade et al. (2018) showed that a similar rosemary extract presented antimicrobial activity against *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium perfringens*, but not against *Escherichia coli*. The antimicrobial activity of rosemary extract against *L. monocytogenes* and *S. aureus* was also described by Gonçalves et al.

(2019) and Gazwi et al. (2020), respectively.

Macroscopically, it was not possible to identify differences (e.g. microbial growth, green color) between the samples over the storage time or between the samples wrapped with the different PLA packages. It should also be highlighted that, the meat did not present any odor change.

4. Conclusions

Food grade extracts from dried green tea and rosemary leaves were successfully obtained through an easy and simple method. Both extracts presented high antioxidant capacity and a high content in total phenolic compounds and total flavonoids. GTE stands out for its high content in EGCG and RE for its high content in carnosic acid. The extracts were incorporated into PLA at different percentages, resulting in resistant, visually attractive and homogenous packages. PLA/2 R and PLA/4 R presented interesting results on inhibiting almond's lipid oxidation for longer periods (45–60 days) at room temperature and 21–30 days at 40 °C. On the other hand, PLA/2GT and PLA/4GT seemed to act as a pro-oxidation agent in the almond test. However, PLA/4GT showed promising results on inhibiting beef's lipid oxidation for short times and lower storage temperature (until 11 days at 4 °C).

It is of utmost importance to standardize the plant based active extracts used in the development active packaging in order to better

Table 6
Aerobic colony average count at 30 °C, for the beef meat packaged with the control (PLA) and active (PLA/2GT, PLA/4GT, PLA/2 R, PLA/4 R, PLA/2GT/2 R) PLA films.

Storage time (days)	PLA	PLA/2GT	PLA/4GT	PLA/2 R	PLA/4 R	PLA/2GT/2 R
	(Log CFU/mL)					
0	4.32	4.32	4.32	4.32	4.32	4.32
1	4.57	4.57	3.86	4.32	4.64	4.38
4	6.28	6.04	5.00	5.23	5.52	5.76
6	6.63	7.41	6.72	6.92	7.08	6.48
8	6.98	7.78	7.46	6.76	6.41	7.15
11	7.49	7.65	7.49	6.98	7.34	7.36

CFU – Colony Forming Units

control their quality and composition and consequently the effectiveness of the new packaging materials. Further studies are needed in these PLA active packages, in particular, sensorial analysis to evaluate the possible organoleptic changes that can occur with the migration of the volatile active compounds present in the extracts; selective antimicrobial assays to determine the fully antimicrobial activity spectrum of these extracts and the application of these active packages to other food matrices either with high fat content or prone to microbiological spoilage.

CRediT authorship contribution statement

Ana Sanches Silva, Mariana Andrade, Miguel Cerqueira: **Conceptualization,** Ideas, formulation, or evolution of overarching research goals and aims.; Ana Gabriela Azevedo, Carolina Barros, Cássia Barbosa, Mariana Andrade, Miguel Cerqueira: **Data curation,** Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later re-use.; Ana Gabriela Azevedo, Mariana Andrade, Miguel Cerqueira: **Formal analysis,** Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data.; Ana Sanches Silva, Ana Vera Machado, Fernando Ramos, Miguel Cerqueira: **Funding acquisition,** Acquisition of the financial support for the project leading to this publication.; Ana Gabriela Azevedo, Carolina Barros, Cássia Barbosa; Mariana Andrade: **Investigation,** Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection.; Anabela Coelho, Cássia Barbosa, Mariana Andrade, Rosália Furtado: **Methodology,** Development or design of methodology; creation of models.; Ana Sanches Silva, Ana Vera Machado, Fernando Ramos, Miguel Cerqueira: **Project administration,** Management and coordination responsibility for the research activity planning and execution.; Ana Sanches Silva, Ana Vera Machado, Cristina Belo Correia, Fernando Ramos, Margarida Saraiva, Miguel Cerqueira: **Resources,** Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools; Ana Gabriela Azevedo, Carolina Barros, Mariana Andrade, Miguel Cerqueira: **Software,** Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components.; Ana Sanches Silva, Ana Vera Machado, Fernanda Vilarinho, Fernando Ramos, Miguel Cerqueira: **Supervision,** Supervision Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team.; Ana Sanches Silva, Ana Vera Machado, Cristina Belo Correia, Fernanda Vilarinho, Fernando Ramos, Margarida Saraiva, Miguel Cerqueira: **Validation,** Validation Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs.; Ana Gabriela Azevedo, Carolina Barros, Mariana Andrade, Miguel Cerqueira: **Visualization,** Visualization Preparation, creation and/or presentation of the published work, specifically visualization/ data presentation.; Ana Gabriela Azevedo, Mariana Andrade, Miguel Cerqueira: **Writing - original draft,** Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation).; Ana Sanches Silva, Ana Vera Machado, Cristina Belo Correia, Fernanda Vilarinho, Fernando Ramos, Margarida Saraiva: **Writing - review & editing,** Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision–including pre- or post-publication stages.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2023.101041.

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