UNIVERSITAT POLITÈCNICA DE VALÈNCIA

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Extractos acuosos activos de la paja de arroz para mejorar la capacidad de conservación del PLA de carne de cerdo.

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EXTRACTOS ACUOSOS ACTIVOS DE LA PAJA DE ARROZ PARA MEJORAR LA CAPACIDAD DE CONSERVACIÓN DEL PLA DE CARNE DE CERDO.

ACTIVE AQUEOUS EXTRACTS FROM RICE STRAW TO IMPROVE THE PRESERVATION CAPACITY OF PLA IN PORK MEAT.

Nuria Bas Gil

RESUMEN

La paja de arroz es un residuo lignocelulósico agroindustrial producido en todo el mundo en grandes cantidades, altamente rico en compuestos fenólicos con propiedades bioactivas. En este estudio, se han utilizados extractos acuosos de este residuo para la obtención de films activos de PLA. Los extractos obtenidos con agua como disolvente limpio, procedían de la combinación de ultrasonidos y tratamiento térmico a reflujo (USHT) y la extracción con agua subcrítica a 160ºC (SWE160) y 180ºC (SWE180). Los films de PLA con y sin incorporación de cada uno de los extractos al 6% fueron caracterizados en su microestructura y propiedades funcionales como material de envase (mecánicas, de barrera al vapor de agua y al oxígeno y ópticas) y probados en su efectividad como envase activo para carne de cerdo. La microestructura de las películas se modificó con la incorporación de los extractos, así como sus propiedades ópticas. En cuanto a sus propiedades mecánicas, las películas activas fueron más frágiles y menos extensibles que el film control de PLA, debido a las interacciones entre los compuestos presentes y las cadenas poliméricas, pero mostraron buenas propiedades de barrera al oxígeno y al vapor de agua. Tras la aplicación en carne de cerdo, los filetes envasados con el film PLA-SWE180 mantuvieron mejor el color, con menos cambios de pH durante el almacenamiento, menores valores de TBARS y menores recuentos microbiológicos. Estos resultados sugieren que el material PLA-SWE180 puede alargar la vida útil de productos cárnicos, manteniendo su calidad y seguridad. El material podía ser un sustituto de materiales de envasado tradicionales, reduciendo el impacto medioambiental de plásticos de origen petroquímico y aportando propiedades bioactivas que mejoran la calidad y seguridad de los alimentos envasados, permitiendo, a su vez, la valorización de la paja de arroz.

RESUM

La palla d'arròs és un residu lignocel•lulósic agroindustrial produït a tot el món en grans quantitats, altament ric en compostos fenòlics amb propietats bioactives. En aquest estudi, s'han utilitzat extractes aquosos d'aquest residu per a l'obtenció de films actius de PLA. Els extractes obtinguts amb aigua com a dissolvent net, procedien de la combinació d'ultrasons i tractament tèrmic a reflux (USHT) i l'extracció amb aigua subcrítica a 160 °C (SWE160) i 180 °C

(SWE180). Els films de PLA amb i sense incorporació de cadascun dels extractes al 6% van ser caracteritzats en la seua microestructura i propietats funcionals com a material d'envàs (mecàniques, de barrera al vapor d'aigua i a l'oxigen i òptiques) i provats en la seua efectivitat com a envàs actiu per a carn de porc. La microestructura de les pel·lícules es va modificar amb la incorporació dels extractes, així com les seues propietats òptiques. Quant a les seues propietats mecàniques, les pel·lícules actives van ser més fràgils i menys extensibles que el film control de PLA, a causa de les interaccions entre els compostos presents i les cadenes polimèriques, però van mostrar bones propietats de barrera a l'oxigen i al vapor d'aigua. Després de l'aplicació en carn de porc, els filets envasats amb el film PLA-SWE180 van mantenir millor el color, amb menys canvis de pH durant l'emmagatzematge, menors valors de TBARS i menors recomptes microbiològics. Aquests resultats suggereixen que el material PLA-SWE180 pot allargar la vida útil de productes carnis, mantenint la seua qualitat i seguretat. El material podia ser un substitut de materials d'envasament tradicionals, reduint l'impacte mediambiental de plàstics d'origen petroquímic i aportant propietats bioactives que milloren la qualitat i seguretat dels aliments envasats, permetent, al seu torn, la valorització de la palla d'arròs.

ABSTRACT

Rice straw is an agro-industrial lignocellulosic residue produced worldwide in large quantities, highly rich in phenolic compounds with bioactive properties. In this study, aqueous extracts of this residue have been to obtain PLA active films. The extracts obtained with water as a clean solvent were prepared by a combination of ultrasound and reflux heat treatment (USHT) and extraction with subcritical water at 160ºC (SWE 160) and 180ºC (SWE 180). PLA films with and without incorporation of each extract at 6 % wt. were characterised in terms of their microstructure and functional properties (mechanical, water vapour and oxygen barrier and optical) and tested as to their effectiveness as active packaging material for pork meat preservation. The microstructure of the films and its optical properties were modified with the incorporation of the extracts. Regarding their mechanical properties, the active films were more brittle and less stretchable than neat PLA film due to interactions between the extract compounds and the polymer chains, although they showed good barrier properties to oxygen and water vapour. Upon application to the pork meat, the samples packaged with PLA-SWE180 film maintained better color, with less pH changes during the storage, lower TBARS values and lower microbiological counts. These results suggest that the PLA-SWE 180 material can extend the shelf life of meat products, while maintaining their quality and safety. The material could be a substitute for traditional packaging materials, reducing the environmental impact of petrochemical-based plastics and providing bioactive properties that improve the quality and safety of packaged foods, while allowing the valorisation of rice straw.

KEY WORDS: Polylactic acid, Rice straw, Bioactive extract, Subcritical water extraction, Ultrasound and thermal treatment, Pork meat preservation, Active Films.

1. INTRODUCTION

Rice straw (RS) is, globally, the most produced crop residue with a waste factor (residue to product ratio) of 1.757 and with an availability of residue of 999.587 $x10^3$ tons (Lahr, 2015). This phenomenon is due to the high rice production worldwide, as rice is considered highly nutritious, full filling and inexpensive in many countries. Spain positions itself in second place on rice production in the European Union, just behind Italy with 28% of the total production, and, according to the Food and Agriculture Organization, there was a total rice production of 739,000 in the 2020 campaign (FAO, 2021).

One kilogram of rice grain provides 1.5 kg of RS (Peanparkdee et al. 2019), a problematic waste to manage and without monetary value. The current management of this residue supposes a risk for the environment and for human health, as the primary management method is its burning in fields. This is an inappropriate practice of waste disposal since it releases harmful dioxins, such as polychlorinated dibenzo-p-dioxins or dibenzofurans, and can result in greenhouse gas (CH_4, CO_2, and N_2O) emissions. Additionally, polycyclic aromatic hydrocarbons produced from incomplete combustion of straw can enter the lungs, causing serious respiratory problems, asthma, allergies, and other serious health effects (Yang et al. 2006). In this regard, there is currently a growing requirement to find new ways to dispose of the organic waste in a more economical, eco-friendly, and healthy manner.

RS is chemically composed of 31-47% cellulose, 15-35% hemicellulose and 5-24% lignin (Freitas et al. 2020). Its composition also contains a variable quantity of ash (about 15%) and different nutrients and other compounds. This composition changes according to the season and the plant variety, type of tissue, and growing stage. While biogenic silica represents 67-82% of the ashes (Abril et al. 2009), phenolic compounds represent a very promising fraction for the revalorization of this agro-food waste, due to their antimicrobial and antioxidant capacities. These compounds are mainly in the form of *p*-hydroxybenzoic acids and hydroxycinnamic acids and their derivatives, as well as their glycosidic esters. The antioxidant potential of phenolic compounds is associated with their capability to act as free-radical scavengers and inhibit lipoxygenase enzyme activity. Likewise, phenolic compounds can act as secondary antioxidants by decomposing hydroperoxyl radicals formed in the initial phase of oxidation. The antimicrobial character of these compounds is associated to their capacity to inhibit extracellular microbial enzymes, destabilize the cytoplasmic membrane, and decrease the amounts of substrates needed for microbial survival (Collazo-Bigliardi et al. 2019). For example, bioactive extract from RS has been incorporated into potato starch-based films to produce edible packaging for food preservation (Menzel et al. 2019). These films have been proven to have great antiradical scavenging activity while exhibited an improved barrier capacity to oxygen without affecting the water vapor barrier properties.

To properly extract the bioactive compounds from the RS, a standardized protocol must be performed to ensure purity, quality, and integrity of the extract. There are currently many different extraction protocols, and each is

more adequate depending on the target yield of extract and extracted compounds.

Combined ultrasound-reflux heating extraction is a method proposed by Freitas et al. (2020) that uses water as a solvent and ultrasound pre-treatment to enhance the extraction yield. Water has been shown to be the best solvent to extract the phenolic compounds compared to other solvents, such as methanol or ethanol, giving higher values of extract yield (Menzel et al. 2020). The application of ultrasound facilitates de release of phenolic compounds as it provokes the reduction of particle size, collisions between the particles and bumping with bubbles, thereby fractionating cells and causing the release of the target compounds. This phenomenon is called cavitation (Machado et al., 2019). Additionally, application of ultrasound to aqueous extraction processes represents a more sustainable alternative to other traditional methods, which use large amounts of solvents and produce toxic and corrosive waste. The application of heat in the extraction methods has also been studied previously. The heating time determines the process yield but can also affect the stability of target compounds. The amount of phenolic compounds in biological samples increases when heating is applied, but at a specific time. Therefore, process parameters must be optimized in order to obtain a good yield in bioactive compounds. A sequential combination of ultrasound step and reflux heating treatment has been optimized to obtain extracts with excellent phenolic yield and bioactivity (Freitas et al 2020), which could be used in the pharmaceutical, and/or food industry.

Subcritical water extraction (SWE) is an extraction procedure that is particularly interesting from an environmental point of view. When high temperature and pressure combinations are reached, above normal boiling point at atmospheric conditions, but below the water critical point (374 ºC, 22 MPa), the water reaches the so called “subcritical state”. In the subcritical state, liquid water presents some changes in its properties, such as a decrease in viscosity, dielectric constant, and surface tension, as well as an increase in its penetrating power and disruption of interactions between the analytes and the plant matrix. Moreover, high temperatures increase the solubility of the compounds present in the plant, which further boosts the selective extraction of bioactive compounds from the plant matrix. Furthermore, effluent treatment costs are reduced and, therefore there are no emissions of volatile organic solvents, and problems associated with the residual content of toxic solvents in the finished products disappear (Gbashi et al. 2016).

Most of today's plastics and synthetic polymers are obtained from petrochemical resources. It is estimated that by 2050, 12 thousand million tons of these plastics will be accumulated in our natural environment, with the environmental repercussions this involves. But few to no changes have been made due to the practical and economic benefits these convey, such as their optimal toughness, durability, barrier properties, and low production cost (Sheldon, 2020). Nonetheless, conventional plastics are persistent in the environment, so inadequate disposal treatment of plastic material waste is a significant source of environmental pollution and, even, significant disturbance

of nature. For all these reasons, it is essential for decision makers and for the plastics industry to achieve the necessary conditions to replace non-degradable polymers with degradable plastics, especially for applications in the packaging industry (Colvin, 1995).

Biodegradable and biobased plastics represent a greener option to resolve the environmental problems we face today. The main advantage these have is that they transform naturally to water, carbon dioxide, and microbial biomass, not being harmful. On the contrary, they have a key disadvantage: their production cost. Therefore the ideal material to substitute current plastics, would be a biobased and biodegradable polymer that maintains the desired characteristics (durability, strength, barrier capacity and food compatibility) without increasing too much its cost.

Poly (lactic acid), PLA, is a compostable thermoplastic polyester derived from the α-hydroxy-acids and its precursor is lactic acid, a chiral molecule (Serna et al. 2003). Lactic acid can be obtained chemically or biotechnologically. Biotechnological production is based on the fermentation of carbohydrate-rich substrates by microorganisms. *Lactobacillus delbrueckii* is the microorganism used in industrial production, since it has two main advantages: it efficiently consumes glucose and it is thermophilic with an optimal growth temperature in the range of 45 to 62ºC (which reduces cooling and sterilization costs) (García, 1993).

The physical and mechanical properties of the material depend on the composition of the polymer, its molecular weight, and its crystallinity. PLA has mechanical properties in the same range as some petrochemical polymers, except for low elongation. However, this property can be refined during polymerization or by post-polymerization modifications (e.g. by plasticizers). Additionally, barrier properties to water vapour, have been proven to be adequate for food packaging purposes, although the oxygen barrier capacity is limited (Rojas-Lema et al. 2020).

PLA is recognized as safe and can be used as a packaging material for food (Södergard, 2000). PLA has been already used as packaging material for yogurt, butter, margarine, and spreadable cheeses. These packages have fulfilled functions of mechanical protection, barrier to moisture, light, fats, and gases. PLA-coated paper containers for packaging beverages have also been developed since they also serve as a moisture barrier (Haugaard, 2000). In this sense, the development of biodegradable PLA-based films incorporating active substances, such as bioactive extracts obtained from agro-industrial waste, has great potential to extend the shelf-life of packaged foods, maintaining their quality parameters, while contributing to the correct waste management and boosting the circular economy.

The aim of this study was to incorporate aqueous RW extracts obtained by different procedures (US-heating method: USHT, and SWE at 160 and 180ºC) into PLA films in order to obtain active materials for meat packaging. The different extracts were incorporated into PLA films which were characterized as to their microstructure, functional properties as packaging material

(mechanical, barrier and optical properties) and as to their capacity to preserve pork meat during cold storage.

2. MATERIALS AND METHODS

2.1. Materials

Rice straw (RS) (*Oryza sativa* L var. J. Sendra) was collected from L'Albufera paddy field (Valencia, Spain). Amorphous PLA 4060D, with density 1.24 g/cm^3 and average molecular weight of 106,226 D was purchased from Natureworks (U.S.A). Sulfuric acid, trichloroacetic acid, sodium carbonate, magnesium nitrate, and di-Phosphorous pentoxide were acquired from PanReac Química S.L.U (Castellar del Vallés, Barcelona, Spain). 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (> 97 % purity), 2-thiobarbituric acid (> 98 % purity), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS) (> 98 % purity), glucose, arabinose, ethanol (98 % purity), and methanol (> 99.9 purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and D(+)-Xylose from Merck KGaA (Darmstadt, Germany).

The different RS aqueous extracts were supplied, as a freeze-dried powder, by the research group of the Instituto de Ingeniería de Alimentos para el Desarrollo (IAD). These were obtained from dried, milled and sieved (particle size lower than 0.5 mm) RS (Oryza sativa L.), *J. Sendra var*., from L'Albufera rice fields, Valencia, Spain, supplied by the "Banco de Paja" (Valencia, Spain). The USHT extract was obtained by applying a 30 min ultrasound pre-treatment, followed by reflux in water for 1 h (Freitas et al. 2020), and the SWE160 and SWE180 extracts were obtained by 30 min SWE at 160 and 180ºC, respectively, as described by Freitas et al. (2022a). The antioxidant and antibacterial properties of the extracts, as characterized by Freitas et al. (2022a), are shown in Table 1.

Table 1. Total phenolic content (TPC), DPPH scavenging capacity (EC_{50}), and minimum inhibitory concentration (MIC) for *L. innocua* and *E. coli* of the bioactive extracts obtained with different extraction methods (From Freitas et al. 2022)

	USHT	**SWE-160**	**SWE-180**
TPC (mg GAE. g^{-1} dry extract)[(1)]	37.1 ± 0.4^c	51.1 ± 2.4^b	82.5 ± 3.2^a
EC_{50} (mg dry extract. mg^{-1} DPPH) [(2)]	6.3 ± 0.3^a	2.0 ± 0.1^b	1.2 ± 0.1^c
MIC *L. innocua* (mg.mL^{-1})	>200	50 ± 3^a	30 ± 3^b
MIC *E. coli* (mg.mL^{-1})	>200	>200	182 ± 3

(1) GAE: gallic acid equivalent.

(2) Amount of extract necessary to reduce by 50% the DPPH radical.

2.2. Preparation of films

Firstly, PLA was conditioned in a desiccator containing P_2O5 for 2 days to remove residual water. Films were obtained by melt blending of components in an internal mixer (HAAKETM PolyLabTM QC, Thermo Fisher Scientific, Germany) at 160 ºC and 50 rpm, for 6 min. Four formulations of PLA films were obtained as follows: control films of PLA without RS extract (PLA sample) with, films containing RS extract obtained using the Ultrasound-reflux heating method (PLA-USHT sample), and films containing RS extract obtained by SWE at 160 and 180 ºC (samples PLA-SWE160 and PLA-SWE180). The incorporation of the active extracts in the films was performed by adding to the control mixture (PLA) a percentage of 6% of RS extract (with respect to the total polymer mass) as described by Freitas et al. (2022b). After milling the solid blend, the films were obtained by compression-molding, using a hydraulic press (Model LP20, Labtech Engineering, Thailand) performing 3 cycles (preheating, heating and cooling), of 3 min each at 160 ºC (except cooling; until about 70 ºC) and 100 bars.

2.3. Film characterization

2.3.1. Microstructural properties

To study the internal physical structure of the films, High resolution Field Emission Scanning Electron Microscopy (FESEM) was used. Firstly, the films were cryo-fractured by immersion in liquid nitrogen, covered with platinum using an EM MED020 sputter coater (Leica BioSystems, Barcelona, Spain). Then the images were taken under vacuum and at a 2.0 kV acceleration voltage, using a Field Emission Scanning Electron microscope (Auriga Compact, Zeiis, Oxford Instruments).

2.3.2. Optical properties

These were measured by applying the Kubelka-Munk theory of multiple scattering (Hutchings, 1999), using a spectro-colorimeter CM-3600d (Minolta Co. Tokyo, Japan). The film color coordinates, L* (lightness), a* (a* > 0 is red, a* < 0 is green), and b* (b* > 0 is yellow, and b* < 0 is blue) were obtained from the infinite reflectance (Rojas-Lema et al., 2020), using D65 illuminant and 10º observer, as reference system. Chroma (saturation) (C_{ab}*) and hue angle (h_{ab}*) were obtained applying equations 1 and 2. Lastly, equation 3 was used to determine the total color difference (ΔE*), considering as standard the color parameters of the neat PLA film (L_0*, a_0*, and b_0*).

$$C_{ab}{}^{*} = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h_{ab}{}^{*} = arctg\left(\frac{b^{*}}{a^{*}}\right) \quad (2)$$

$$\Delta E^{*}{}_{a,b} = \sqrt{(\Delta L^{*})^{2} + (\Delta a^{*})^{2} + (\Delta b^{*})^{2}} \quad (3)$$

Where $\Delta L^{*} = (L^{*} - L_0^{*}),\ \Delta a^{*} = (a^{*} - a_0^{*}), \Delta b^{*} = (b^{*} - b_0^{*})$.

The UV-Vis transmission spectra of the films, between 200 and 900 nm, were also obtained, using a UV-visible spectrophotometer (Evolution 201, Thermo Scientific) in light transmission mode for the evaluation of the light barrier properties of the films.

2.3.3. Mechanical properties

Tensile strength (TS), elastic modulus (EM) and the elongation at break (EB) of the films were determined using the standardized ASTM D882 method (ASTM, 2001) and a universal test machine (TA.XTplus model, Stable Micro Systems, Haslemere, England). After cutting all the film formulations with dimensions 2.5 x 10 cm, these were mounted in the film-extension grips of the machine and lengthened at cross-head speed of 12.5 mm/min until breaking. Additionally, thickness of the films was measured using a Palmer digital micrometer (accuracy of 0.001 mm) at ten random film positions.

2.3.4. Barrier properties

Water vapor permeability (WVP) of the films was determined according to ASTM E96/E96M (ASTM, 2005) gravimetric methodology, following the modifications proposed by McHugh et al. (1993). The films were cut (Ø = 3.5 cm), placed and sealed on Payne permeability cups (Elcometer SPRL, Hermelle/s Argenteau, Belgium) containing distilled water (5 mL) to reach 100% relative humidity (RH) inside the cup. The airtight cups, to which a fan was adapted, were placed in a desiccator with oversaturated $Mg(NO_3)_2$ to obtain 53% RH, at 25ºC and weighed every 1.5 hours with an analytical balance (± 0.0001 g) throughout 25 h. Finally, WVP was calculated from the slope of the weight loss-time curves.

For the analysis of oxygen permeability, a modified version of the ASTM Standard Method D3985-05 (2010) was followed. The films were exposed to pure oxygen flow, evaluating the oxygen transfer rate using an Oxygen Permeation Analyzer (Model 8101e,Systech Illinois, USA) The tests were conducted in continuous mode at 25°C and 53% RH. The OP was calculated by dividing the oxygen transfer rate by the difference in oxygen pressure between the two sides of the film and multiplying by the average thickness of the film.

2.4. Capacity of the films to preserve meat

Fresh pork meat was purchased from the local market and fillets were cut into 22 ± 1 g pieces, in an aseptic environment, and packaged in thermo-sealed bags of the different films. The films were cut to 12 cm x 7 cm and sealed using a vacuum sealer (Vacio Press, Saeco) to obtain bags that were thermo-sealed after the meat sample introduction. Samples were packaged in duplicate for the analyses at each time. The packaged samples were stored at 4 °C for 16 days, and the meat quality parameters described below were evaluated after 0, 7 and 16 days of storage in the samples packaged, using two different sample bags for each time.

2.4.1. Color analysis

A Spectro-colorimeter (CM-3600d, Minolta Co., Japan), with an optical window of 9 mm, was used to determine from the reflectance of meat samples, backed with a white plate and covered with optical glass, and the color coordinates (L^*, a^*, b^*, C_{ab}^*, and h_{ab}^*), using with D65 illuminant and at 10º observer, as reference system. The total color difference of the samples (ΔE^*), with respect to the initial values of color coordinates, was evaluated throughout the storage by applying Equation 3,

2.4.2. Analyses of pH and mass changes

The pH analysis was performed immersing an electrode probe (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) into the pork meat fillets. In addition, the weight loss was determined by weighing the fillets at 0, 7 and 16 days of storage.

2.4.3. Analyses of oxidation using TBARS assay

The antioxidant capacity of the active films was evaluated by measuring the TBARS index of the meat (Siu and Draper, 1978). Samples of about 10 g were homogenized with 50 mL of distilled water and 50 mL of 10 % trichloroacetic acid (w/v) using an Ultra-turrax homogenizer (ULTRA-TURRAX®, Model T 25 D, IKA®, Germany) for 2 min. After, vacuum filtration of the suspension using a qualitative filter (Filterlab) was performed and 8 mL of the filtrate was mixed with 2 mL of 2-thiobarbituric acid (0.06 mol.L-1) applying heat (80 ºC) for 90 min. Lastly, the absorbance was measured at 532 nm and the TBARS index was expressed as mg malonaldehyde (MDA) per kg of meat using 1,1,3,3-tetramethoxypropane as standard (0.5 – 12 µM).

2.4.4. Microbiological controls

The initial culture (meat samples) was diluted in 0.1% Buffered Peptone Water (1:10 ratio) and homogenized in a stomacher for 3 min, obtaining a 10^{-1} dilution. Afterward, a 1 mL aliquot of the mother culture was transferred into 9 mL of tryptic soy broth (TSB), and subsequent serial dilutions were obtained. Then 1 mL of each dilution was added to the appropriate medium corresponding to the microorganisms tested (total viable (TV), psychotropic bacteria (PB), total coliforms (TC), and lactic acid bacteria (LA)). To determine the bacterial count, plates with 25-250 colonies were selected and the results were expressed as log colony-forming units per gram of meat (log $CFU.g^{-1}$). Table 2 summarizes the culture media and the incubation conditions used in each case.

TABLE 2. Growing medium and incubation conditions for the microbiological tests performed.

Microbiological test	Growing medium	Incubation conditions
Lactic acid bacteria (LA)	MRS agar	30 °C for 72 h
Total coliforms (TC)	Chromogenic medium Brilliance E. coli/Coliforms Selective Agar	37 °C for 24 h
Total viable (TV)	PCA plates	37 °C for 48 h

Psychotropic bacteria (PB)	PCA plates	4 ºC for 7 days

2.5. Statistical analysis

Both the analysis of variance ANOVA and Tukey's studentized range HSD (honestly significant difference) test were used to check for significant differences between the film treatments and the pork meat samples, using the least significant difference (α) of 5%. The 17th version of Minitab statistical program was used for this purpose.

3. RESULTS AND DISCUSSION

3.1. Film properties

3.1.1. Microstructural properties

Figure 2 shows the FESEM images of the cross-section of the control and active PLA films. PLA films without active extracts showed a homogeneous surface pattern, typical of amorphous PLA, where brittle and rubbery domains can be observed. The plastic cryofracture remains smooth due to the homogeneous matrix lacking any inclusions, as previously observed by other authors (Muller et al., 2017, Bhasney et al. 2018, Hernández-García et al., 2021, Freitas et al., 2022). In general, once bioactive extracts were incorporated into the films, a more granular surface pattern was observed, especially for the PLA-USHT sample. RS extracts have a large set of bioactive compounds, including p-coumaric, ferulic and caffeic acids, tricine and vanillin as identified by Menzel et al. (2018), which can establish hydrogen bonds with the carbonyl group of PLA chains. The extraction technique and operating parameters will determine the proportion and composition of the extracts, which may affect the intensity and type of interaction with the polymer matrix. Thus, the higher number of aggregates observed in the PLA-USHT film may be due to the more hydrophilic composition of the USHT extract, which makes it less miscible in the PLA matrix than those obtained from SWE. In fact, the high temperatures and pressures applied in SWE markedly decreases the dielectric constant of water, which has the potential to extract more hydrophobic components, similar to organic solvents, and thus more compatible with the PLA matrix.

When comparing SWE treatments, the PLA-SWE160 and PLA-SWE180 films showed a presence of small holes dispersed through the polymer matrix, which may be due to the presence of more volatile compounds that could be released during the thermocompression step. Therefore, the results revealed that the incorporation of RS extract produced by subcritical water extraction procedures, into PLA films not only modified the chain arrangement and the degree of compaction of the matrix, but also integrated different number of active compounds, which could positively increase the microbial and antioxidant properties of the films.

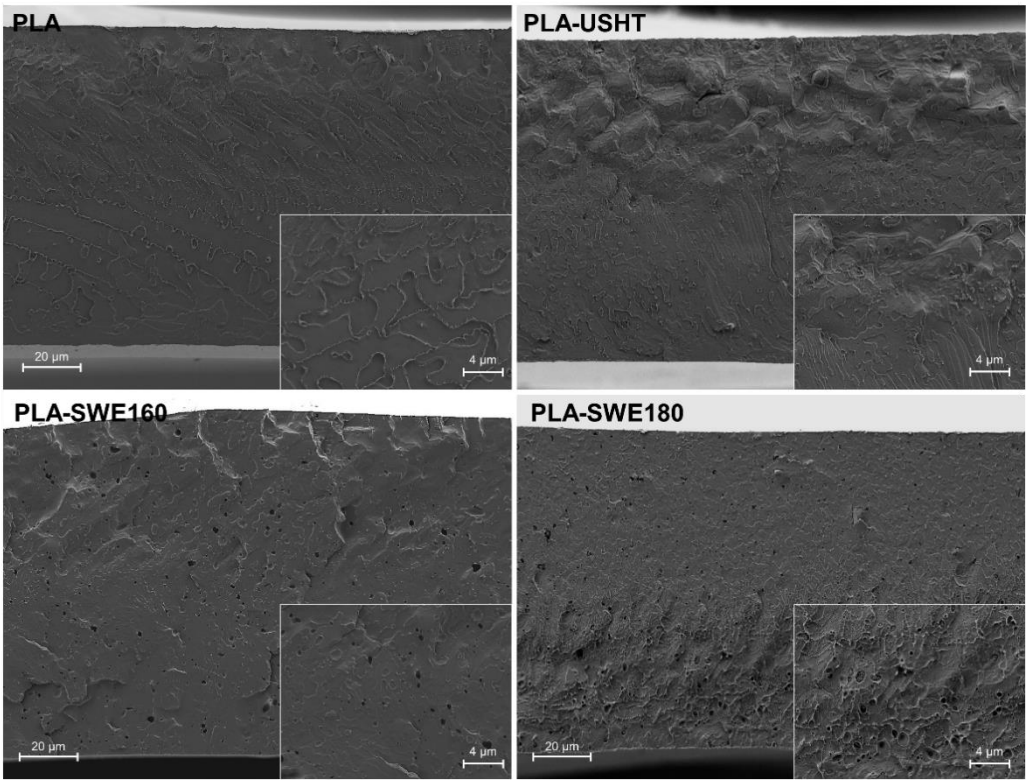

FIGURE 2. FESEM micrographs of the cryo-fractured PLA films with or without different active extracts. The images were taken at 600× magnification with insets at 2000×.

3.1.2. Optical properties

Figure 3 shows the UV-vis transmittance spectra (200-800 nm) of the PLA films with and without different actives extracts from RS. This measure is essential in food packaging applications as UV light has the potential to break protein and lipid bonds in food matrices, promoting photooxidation processes that result in food deterioration. Control PLA films show high transmittance values in the UV light wavelength range (230 – 400 nm) suggesting that these films have poor UV light barrier properties. Nevertheless, the active extracts conferred great light-barrier capacity to the PLA films, mainly the SWE extracts. Besides exhibiting higher blocking of visible light, the PLA-SWE160 and PLA-SWE180 films had a total light absorption in the UV region, which could prevent deterioration of food components by photoreactions. The fact that the PLA-USHT film showed a slightly higher light transmittance than the PLA-SWE160 and PLA-SWE180 films agrees with the lower concentration of phenolics in the active extract USHT (Freitas et al, 2022). Therefore, it is expected that the higher the amount of phenolics in the films, the more UV light is blocked.

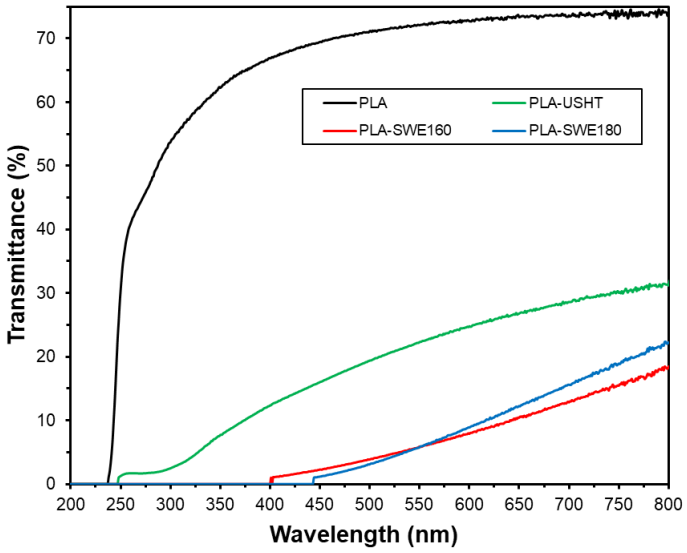

FIGURE 3. UV-Vis spectra of the PLA films with different RS extracts.

The color parameters, typically lightness, chroma, hue angle and total color difference are shown in Table 3. Lightness (*L**) of the films decreased when RS extract was incorporated although there were no significant differences between active films. This result suggests that less light can be reflected off the surface of the active films due to the extract solids present. Due to the presence of colored compounds, extracts have the capability of absorbing light, being a useful characteristic for food packaging applications. Color saturation (C_{ab}*) increased significantly when RS extracts were incorporated, as previously described by other authors (Menzel et al, 2019). Also, the differences between active films were statistically significant, corresponding to more vibrant-colored films when the SWE extracts were incorporated. This is due to the difference in compound composition amongst extracts: at different temperatures, different compounds are extracted and/or formed during the process, resulting in materials with different colorations. Besides, PLA-SWE180 reached higher C_{ab}*, probably because of the browning reactions, such as Maillard and caramelization reactions occurring in the process, giving rise to more colored extracts and films. The reduced hue angle (h_{ab}*) of the active films suggest that these turned more reddish once RS extract was incorporated. Using Equation 3, the total color difference (*ΔE**) of each active film with respect to the PLA control film, was calculated. As summarized in Table 3, the films were visually different (ΔE* ≥ 5). Menzel et al. (2019) also observed this tendency when incorporating aqueous extracts from sunflower hulls into starch-based films and Freitas et al. (2022b) when incorporating RS extract into PLA-based films.

TABLE 3. Optical properties in terms of lightness (L*), chroma (C_{ab}*), and hue angle (h_{ab}*) of the PLA films with different RS extracts. Mean values and standard deviations. Different subscript letters in the same column indicate significant differences by the Tukey test ($p < 0.05$).

Formulation	L^*	C_{ab}^*	h_{ab}^*	ΔE^*
PLA	90.7 ± 0.1^{a}	2.7 ± 0.1^{d}	99.0 ± 0.2^{a}	-
PLA-USHT	68.3 ± 2.2^{b}	31.9 ± 1.8^{c}	77.5 ± 1.0^{bc}	36.7 ± 2.8^{c}
PLA-SWE160	69.0 ± 2.2^{b}	42.0 ± 2.1^{b}	79.0 ± 1.3^{b}	45.1 ± 2.9^{b}
PLA-SWE180	61.0 ± 3.3^{b}	49.2 ± 1.3^{a}	75.2 ± 2.2^{c}	55.7 ± 3.0^{a}

3.1.3. Mechanical properties

Thicknesses and tensile properties of the PLA films are shown in Table 4. Thicknesses did not significantly change when RS extracts were incorporated, showing an average value of 0.140 mm. In comparison to other studies (Almeida et al., 2021, Bajíc et al., 2019, Baron et al., 2017, Quihui-Cota et al., 2017), the thickness values of the films are higher, which is a positive factor for food packaging applications, since it enhances barrier properties for diffusion of permeant molecules, resulting in an increased shelf-life f packaged food (Mihindukulasuriya & Lim, 2014). The active films showed a decrease in TS and E values, regardless of the extract incorporated. This suggests that there were interactions between the compounds present in the extract and the polymer matrix, which led to a weakening of the interchain forces of PLA, giving rise to a more brittle and less stretchable material, as reported in previous studies (Freitas et al., 2022b). Likewise, the presence of a wide range of phenolic acids in the extracts could partially hydrolyse the PLA chains, thus worsening their tensile properties. As regards the EM values, the films incorporated with USHT extract showed an increase in their stiffness, although it did not statistically differ from the PLA-SWE160 films. These differences could be attributed to the composition profile of the active extracts, either by the type of extraction technique or by the process parameters. Thus, different compositions of the extracts can greatly influence the type and intensity of interaction with the polymer matrix, as well as the degree of hydrolysis of the PLA chains, modifying the film tensile properties to a different extent.

TABLE 4. Thicknesses and tensile properties, typically TS, E, and EM of PLA films containing different RS extracts. Mean values and standard deviations. *TS: tensile strength, E: elongation at break, EM: elastic modulus. Different letters in the same column indicate significant differences between films by the Tukey test ($\alpha = 0.05$).

Formulation	Thickness (mm)	TS (MPa)	E (%)	EM (MPa)
PLA	0.140 ± 0.005^{a}	43.6 ± 1.8^{a}	4.3 ± 1.2^{a}	1842 ± 18^{b}
PLA-USHT	0.141 ± 0.006^{a}	35.9 ± 1.6^{b}	2.5 ± 0.2^{b}	1889 ± 16^{a}
PLA-SWE160	0.140 ± 0.007^{a}	37.2 ± 2.2^{b}	2.9 ± 0.4^{b}	1857 ± 35^{ab}
PLA-SWE180	0.141 ± 0.005^{a}	34.6 ± 1.8^{b}	2.8 ± 0.5^{b}	1830 ± 22^{b}

3.1.4. Barrier properties

Barrier properties to water vapour (WVP) and oxygen (OP) are summarized in Figure 4. The permeability of a given material depends mainly on the solubility and diffusion rate of the permeant in the matrix. The incorporation of hydrophilic components of the extracts into PLA promotes the solubility of the water molecules in the polymer matrix, which could explain the increase of the WVP in active films. Other authors (Roy and Rhim, 2020), Srisa and Harnkarnsujarit, 2020 and Freitas et al., 2022b) also observed an increase of WVP values of PLA when hydrophilic substances were incorporated into the polymer matrix.

In contrast, the PLA-USHT, PLA-SWE160, PLA-SWE180 films exhibited a decrease of the OP values, with respect to the control film, of about 16, 20, and 24 %, respectively. This behaviour could be attributed to the antioxidant effect of phenolics in the extracts, which could have additional oxygen scavenging effect, thus decreasing the permeation of oxygen molecules through the films (Bonilla et al., 2013). The reduced OP supposes an advantage for food packaging purposes, as the chances of the foodstuff degrading due to oxidation reactions can be greatly reduced.

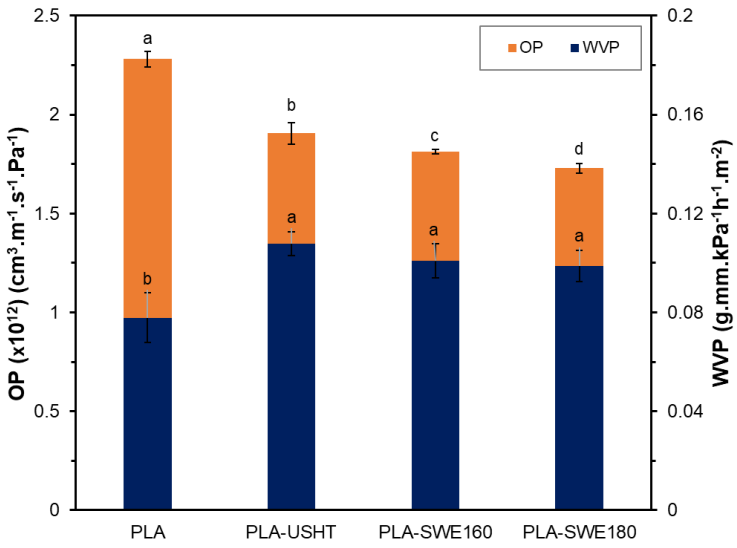

FIGURE 4. Oxygen permeability (OP) and water vapour permeability (WVP) of the PLA films.

3.2. Meat preservation by packaging in the thermo-sealed film bags

3.2.1. Color changes

Color parameters are a key factor when evaluating the quality of foodstuffs, particularly meats. Luminosity (L*), Chroma (C_{ab}*), hue angle (h_{ab}*) and total color difference (ΔE*) of the meat throughout the storage are plotted in Figures 5a, b, c and d, respectively. All samples showed a decrease in L* and C_{ab}* with time, as also reported by other authors analysing the effect of chitosan-based films in pork meat (Bonilla et al., 2014). L* values are related to the water retention capacity of the samples. Lower luminosity suggests a darker color of the meat due to the loss of water throughout storage time. Specifically,

reduction in water content leads to changes in the light reflection of the sample due to differences in the surface pigment content (Hutchings, 1999). The lowest *L** value was reached by the fillet packaged in control film, in coherence with the highest weight loss (Figure 6a). C_{ab}* or color saturation could indicate the maintenance of meat quality during the storage. As shown in Figure 5, the meat samples packed with the active films were those that exhibited the least changes in the values of the C_{ab}* parameter, regardless of the extraction treatment applied. This behaviour could be due to the antioxidant capacity of the phenolic compounds present in the RS extract, that delay autoxidation reactions by scavenging free radicals, thus maintaining the fillet's color stability. Decrease in *L** and C_{ab}* values can be related to the conversion of myoglobin to metmyoglobin, associated with the heme group oxidation. In the same sense, although h_{ab}* values increased in all samples throughout time, the ones that increased the least were those packaged in active films. This suggest that the reddish color of the meat was lost fastest when control films were used, in accordance with higher ratio of myoglobin oxidation. Regarding the ΔE^* values shown in figure 5d, obvious differences in color ($\Delta E^*>5$) were observed at day 10 for meats in contact with control films and around day 14 for meats in contact with active films, which evidence there were less physicochemical changes in samples in contact with active films.

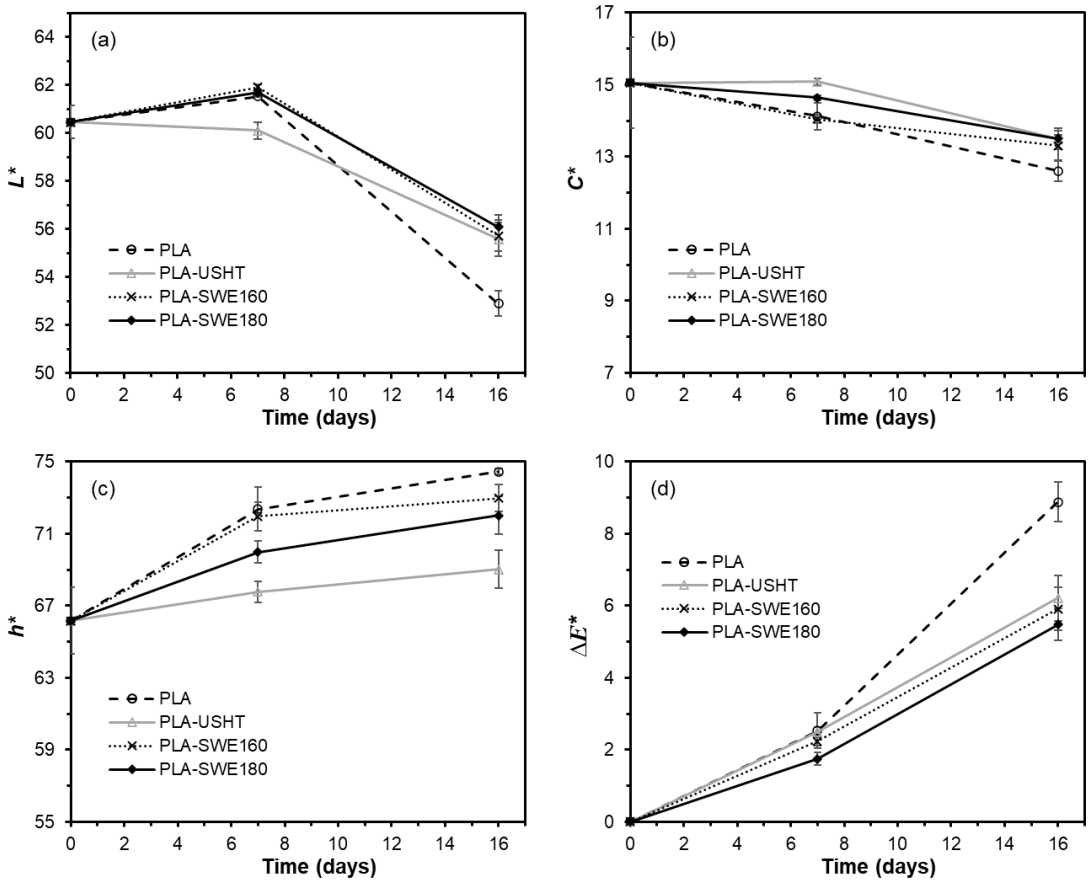

FIGURE 5. Color parameters L*, C*, h*, and ΔE* of the pork meat packed in the PLA films with different extracts stored at 4 °C for 16 days.

3.2.2. pH changes

The development of pH along the storage time of all packaged samples is shown in Figure 6b. All meat samples presented mean pH values within the limits required by current legislation (Mettler-Toledo, 2022) for fresh pork meat (5.5≤ pH ≤6.2). It is to be expected that pH values increase during time, due to the degrading of proteins to secondary metabolites with basic amines by the action of endogenous or microbial lytic enzymes (Karabagias et al. 2011). The meat samples with less pH change were those packaged in PLA-SWE (both 160 and 180) films, followed by PLA-USHT and PLA, respectively, suggesting that the first packaging option is better to maintain the pH of the meat. Similar pH changes were obtained when double-layered active films containing pomegranate peel extract were used to package pork meats (Shuaifeng et al., 2016).

Figure 6 shows the change in weight loss (by liquid phase separation) of the samples during the cold storage. The weight loss of the meat is related to the water retention capacity of meat proteins. This can be influenced by both meat processing factors (salt addition, temperature, grinding, and cutting) and intrinsic factors, such as pH changes. The meat samples packed with the active films exhibited the lowest weight losses with respect to those in contact with the control PLA film, coherently with the lowest pH change, while weight losses could also be affected by oxidation reactions and protein changes produced by spoilage microorganisms or enzymes. Greater stability of the meat matrix could be inferred for the samples packaged in the active films.

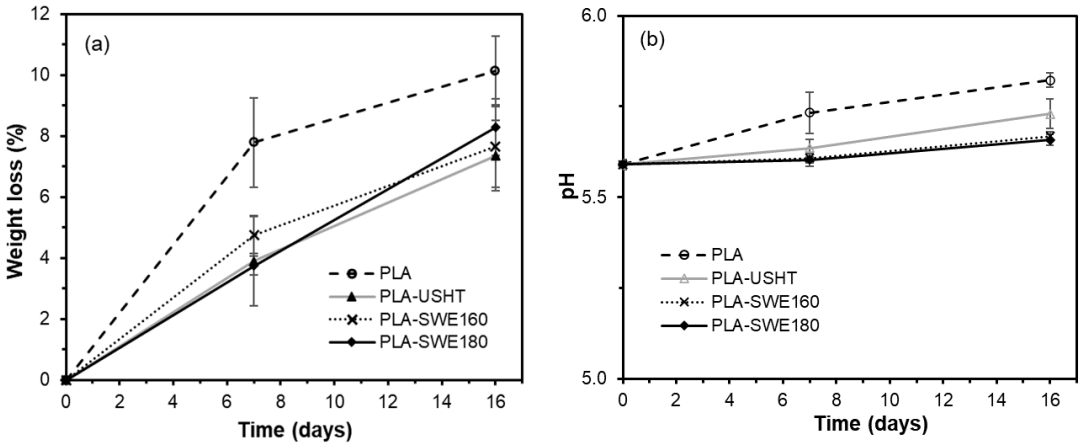

FIGURE 6. Weight loss and pH values of the pork meat packed in PLA films with different extracts stored at 4 °C for 16 days.

3.2.3. Meat oxidation

Lipid oxidation of the pork meat throughout the storage time is followed through TBARS index (Ojagh et al. 2010), which is shown in Figure 7 for the different packaging methods. The results are presented in mg of malondialdehyde (MDA) per kg of meat, which is one of the several end-products formed during the decomposition of hydroperoxides (Hernández et al. 2009). Samples in contact with control PLA films exhibited a significant increase in TBARS

values, suggesting that these had the highest rates of lipid oxidation during storage. This may be due to the presence of oxidant microorganisms and/or enzymes in the meat, which could promote the oxidation of fats and myoglobin (Moreno et al., 2018), enhanced by the oxygen action. In contrast, active films showed slow MDA levels at each storage time, although an overall slight increase is observed with time. The samples with the lowest TBARS values are those packaged in PLA-SWE180 films, which also showed the highest antioxidant activity, as deduced from the lowest EC_{50} value of the SWE180 extract (Table 1), which represent the extract concentration necessary to reduce by 50 % the concentration of the DPPH radial (Brandi-Williams et al., 1995). This extract also showed the highest concentration of polyphenols (PPC value in Table 1), which could delay the oxidation reactions in the meat. The differences between samples packaged in the different active films may be attributed to factors such as the compound profile in the RS extracts incorporated, and the respective antioxidant action. In fact, the higher the antioxidant activity of the extract (lower EC_{50} values) the lower the TBARS values in the meat. The reduction of the oxygen permeability of the films when active extracts were incorporated will also contribute to prevent the oxygen permeation through the package material, therefore reducing the rate of the oxidation reactions. Similar results have been reported by Shuaifeng et al. (2016) in pork meat samples packaged in PPE-PE/PET bilayers incorporated with pomegranate peel active extracts.

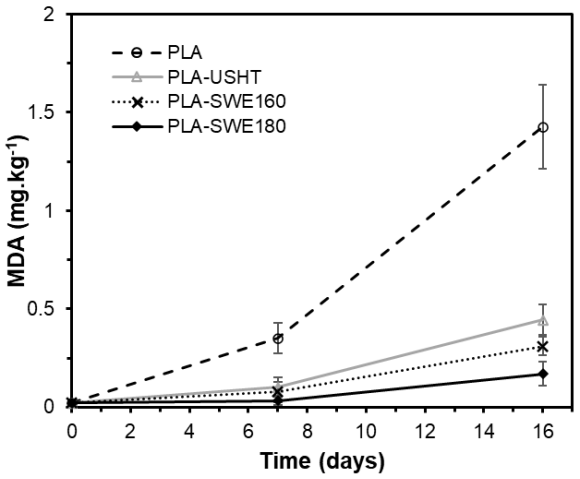

FIGURE 7. TBARS values of the packaged meat in contact with different PLA films stored at 4 °C for 16 days.

3.2.4. Microbiological stability

The microbial counts in the pork meat at different storage times, typically total viable counts (TV), lactic acid bacteria (LA), total coliforms (TC) and psychotrophic bacteria (PB) are shown in Figure 8. The pork spoilage is closely related to bacterial or microbial contamination, therefore total viable counts must be analysed during storage to determine whether the foodstuff satisfies or exceeds the acceptable levels of contamination (Shuaifeng et al., 2016). Considering that the maximum TV value permitted in pork meats by law is 6 log CFU.g-1 (Commission Regulation No 2073/2005), all the fillets

demonstrated poor quality values after the 16 days, except those packaged in PLA-SWE160 and PLA-SWE180 active films, which agrees with the higher antibacterial activity of the corresponding extracts shown in table 1. These extracts exhibited lower MIC values for gram positive (*L. innocua*) and gram negative (*E. coli*) bacteria, which could also imply higher capacity to control the microbial growth in the packaged meat, as deduced from the obtained lower bacterial counts at each storage time. The antimicrobial compounds could also be released from the films to the meat surface with different rate, resulting in different microbiological quality of the packaged samples. Additionally, film's oxygen barrier capacity could also influence microbial growth in the meat. As previously discussed, the oxygen-scavenging capacity of the compounds present in the RS extracts obtained by SWE, additionally prevent the diffusion of the oxygen molecules through the polymer matrix, thereby affecting the aerobic bacteria growth. When comparing the active films, it was observed that the meat samples in contact with the PLA-USHT film exhibited very similar results to those packaged in control films, suggesting that the extraction process there was not effective to extract antimicrobial compounds, whereas SWE gave rise to antibacterial extracts, as found by Freitas et al. (2022a) and reflected in the MIC value of Table 1. Likewise, a significant difference between the meat counts for samples packaged in PLA-SWE160 and PLA-SWE180 films was observed, in agreement with higher antibacterial action of the SWE180 extract. Thus, 180 ºC represent an optimal condition to obtain RS extract with better antimicrobial properties.

Lactic acid bacteria are prominent in pork meats and are a menace to the integrity of the food product during storage time due to their fermentation reactions which result in spoilage and possible hazardous products for human consumption. As previously studied by Han et al. (2014), *Lactobacillus sakei* is the most dominant LA associated with pork meat samples, regardless of the origin or packaging, followed by *Lactococcus lactis* and *Pediococcus pentosaceus*. Fillets packaged in PLA-SWE180 showed the lowest LA counts during the storage period, with values reaching a maximum of 4.5 log CFU/g of sample. Following with the TC count, *Escherichia coli* is the most problematic in pork meat samples, mainly obtained by bad manipulation and contamination with visceral content. The ingestion of these coliforms by consumers supposes a clear risk of getting a foodborne disease when eaten raw (Bantawa et al., 2018). Samples in contact with PLA-SWE180 had significantly lower TC values than those in contact with the control and other active films (PLA-USHT and PLA-SWE160) during storage, which indicates the greater capacity of tis extract to control the growth of coliform bacteria. Finally, psychrotrophic bacteria (PB) growth in the meat samples have been studied. These bacteria are of major interest as they have complex skills to adapt to extreme conditions of life. Additionally, foods of animal origin contaminated with psychrotrophic bacteria could present organoleptic changes, even if they are kept at temperatures considered optimal for conservation (Vasut, 2009). The PB counts of the pork meat samples increased in all cases until the end of storage (16 days), but this increase was slow down for samples packaged in films with SWE extracts, especially with

SWE180 extract, which confirms the higher capacity of this extract to preserve meat products from microbial spoilage.

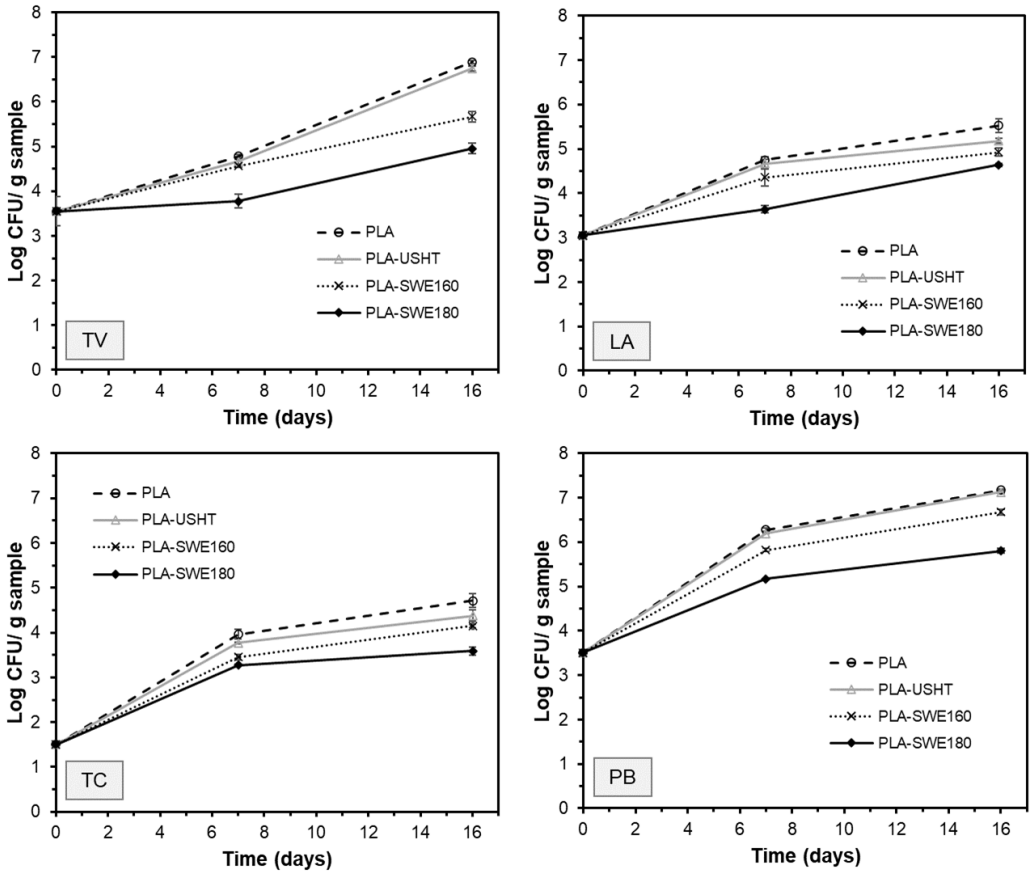

FIGURE 8. Development of the microbial counts, typically total viable counts (TV), lactic acid bacteria (LA), total coliforms (TC), and psychrotrophic bacteria (PB) of pork meat packed with PLA films stored at 4 °C for 16 days.

4. CONCLUSIONS

Incorporating RS active extracts from different aqueous extraction methods into PLA-based films was an interesting strategy to obtain active, sustainable food packaging materials, while supposed a valorisation method of rice straw. The incorporation of RS extracts provoked more heterogenous PLA matrices, due to the partial miscibility of the extract components in the polymer matrix. Structural differences were affected by the extract composition, which, in turn was determined by the extraction method. The structural differences affected the tensile and barrier properties of the films, which become less extensible and stiff, with lower water vapor barrier capacity, than the PLA control film. Nevertheless, all active films exhibited lower oxygen permeability than the control films and a great capacity to absorb UV, which supposed great advantages to prevent oxidation reactions of packaged food. Active films also exhibit color due to the colored nature of the incorporated extracts.

The food preservation capacity of the PLA active films was validated in pork meat samples, which were packaged in the different film bags and cold stored. The active films were effective in maintaining the quality parameters of the packaged pork meat, which showed lower changes in weight loss, pH values, and color parameters than that observed in control samples, packed in PLA films without extract. Likewise, the active extracts were able to prevent the oxidation of the meat as well as to mitigate the inhibit the microbial growth during storage. The bioactive effectiveness of the films was more remarkable for those with SWE extracts, especially for PLA-SWE180 film, which exhibited the highest antioxidant and antibacterial activity. This can be attributed to the different composition of extracts, depending on the extraction process.

Therefore, PLA active films produced with RS extracts showed adequate properties for food packaging applications. In particular, PLA-SWE180 films was the most adequate for this purpose, and it could considered as a potential substitute for single-use plastics in food packaging, reducing the plastic contamination, contributing to the rice straw valorisation and circular economy and extending the food shelf life, decreasing the food losses.

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