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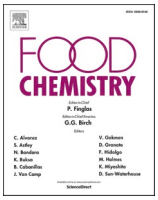

Starch-polyester bilayer films with phenolic acids for pork meat preservation

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ABSTRACT

Starch (S) films containing gellan gum (90:10) and polyester (P) blend films (PLA:PHBV, 75:25) with and without ferulic, *p*-coumaric or protocatechuic acid at 2% (w/w) were obtained by melt-blending and compression moulding for the purposes of obtaining S—P bilayers by thermo-compressing both monolayers together. These were characterised as to their mechanical and barrier properties and as to their performance as packaging materials for pork meat slices. The incorporation of phenolic acids promoted the water vapour and oxygen barrier capacity of bilayers while reducing their stiffness and resistance to break, mainly in the case of protocatechuic acid. Phenolic acids significantly improved the antioxidant capacity of the bilayer films, reducing the lipid oxidation of packaged meat during storage. Phenolic acid loaded bilayers also reduced the microbial counts of meat, mainly for lactic acid bacteria. These effects positively affected the development of the sample pH and colour parameters throughout storage. Active starch-polyester bilayer films exhibited great potential as a means of extending the shelf-life and improving the quality preservation of pork meat.

1. Introduction

Active food packaging is an emerging technology as compared to traditional "inert packaging", since it incorporates active compounds, such as oxygen scavengers and antioxidant or antimicrobial compounds, extending the shelf-life of food (Yildirim et al., 2018). Active food packaging is a field in dynamic development that presents broad market prospects and takes advantage of the possible interactions between the packaging and the food for the benefit of improving its quality and acceptability (Kuai et al., 2021).

The use of biodegradable materials for the purposes of producing active packaging is necessary in order to reduce the environmental impact of plastics, given that landfilling of postconsumer plastic waste continuously threatens the environment and the ecological system because of the presence of plastic waste and microplastics in rivers and marine and coastal environments (Muthuraj et al., 2018). Likewise, the development of biodegradable laminates to better adapt the materials to the food packaging requirements is a useful strategy as it occurs in synthetic plastics. The combination of biodegradable polymers with complementary barrier properties, such as hydrophilic (e.g. starch) and hydrophobic (e.g. polyesters) polymers, permits the production of materials with suitable barrier and mechanical properties and improved functionality for food packaging (Muller et al., 2017). Starch-based films are materials of poor resistance, materials that are highly water sensitive with a great oxygen barrier capacity (Olivato et al., 2013), whereas PLA and PHBV are hydrophobic polymers that yield stiff packaging materials with good mechanical performance and water vapour barrier capacity. However, the low chemical affinity of hydrophilic and hydrophobic polymers makes it necessary to add compatibilisers to promote their interfacial affinity (Encalada et al., 2018). In a previous study, Hernández-García et al. (2021a) obtained bilayer films that consisted of PEG-plasticised PLA-PHBV (75:25) blend film and glycerol-plasticised cassava starch films with 10% gellan gum with adequate functional properties to meet food packaging requirements. These bilayers presented improved oxygen and water vapour barrier properties, as compared to the starch-based or polyester-based monolayers, with a good mechanical performance.

Packaging represents a critical tool for providing meat with physical protection, while controlling microbiological growth and oxidative processes during distribution and retailing. During storage, meat deteriorates due to pigment oxidation, oxidative rancidity, microbial growth and surface dehydration (Bou et al., 2009; Mazzola & Sarantópoulos, 2019). Colour, which is one of the principal attributes evaluated by consumers during the purchase, can be affected by other

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inherent variables, such as pH, oxidation processes or microbial contamination (Mazzola & Sarantópoulos, 2019). Bacterial growth is the most important contributing factor for meat spoilage. The presence of microorganisms can also cause enzymatic deterioration and oxidation. After microbial deterioration, oxidative processes are the most significant, since they affect lipids, pigments, proteins, and vitamins (Domínguez et al., 2019). Packaging of meat reduces oxygen exposure and may inhibit lipid oxidation (Xiao et al., 2011). Therefore, the development of biodegradable, active materials for meat packaging is a challenge that requires the use of materials with low oxygen transmission rates and with antioxidant/antimicrobial compounds to extend meat shelf-life, ensuring quality and safety (Jalal et al., 2018; Cenci-Goga et al., 2020). Pork is one of the most commonly-consumed meat products in the world (McGlone, 2013). Due to its high lipid content, lipid oxidation can occur during storage, reducing the quality and shortening the shelf life (Hong et al., 2009). Therefore, one of the challenges as regards pork meat packaging is the incorporation of antioxidants into the materials in order to inhibit lipid oxidation and microbial growth.

Phenolic compounds are considered effective antioxidants due to their ability to deactivate and stabilise free radicals, acting as hydrogen donors from the phenolic hydroxyls (Christaki et al., 2012; Kalogianni et al., 2020). Ferulic, *p*-coumaric and protocatechuic acids exhibited quite similar antioxidant activity, quantified as 1.9, 2.2 and 1.2 mM relative to TROLOX, respectively (Rice-Evans, et al., 1996) while also exhibiting antimicrobial activity (Alves et al., 2013; Miyague et al., 2015). In this sense, these have been used to enhance meat product preservation. Ferulic (Ijabadeniyi et al., 2021), *p*-coumaric (Chen et al., 2020) and protocatechuic (Yin & Chao, 2008) acids have been incorporated into meat products in order to extend their shelf-life during cold storage. Ferulic acid was incorporated into chitosan-based coatings that were applied to extend the shelf-life of beef (El-Refai et al., 2017) and pork (Wang et al., 2021) meat. Ferulic, *p*-coumaric or protocatechuic acids were incorporated into PEG-plasticised PLA:PHBV films, obtaining antibacterial films with improved tensile and barrier properties of potential use as active food packaging materials (Hernández-García et al., 2021b). Nevertheless, to the best of our knowledge, the promotion of their oxygen barrier capacity by means of lamination with starch films was not previously studied so as to better meet the meat packaging requirements. Moreover, there are no reported studies on the incorporation of these active compounds on bilayer films with improved barrier capacity by combining hydrophilic (starch) and hydrophobic (polyester blends) polymer sheets with complementary barrier properties.

The aim of this study was to evaluate the physical properties of starch-polyester (PLA:PHBV blend) bilayer films incorporating phenolic acids (ferulic, *p*-coumaric and protocatechuic acid) into the polyester layer, and to evaluate the performance of such films at extending the shelf-life of pork meat during cold storage.

2. Materials and methods

2.1. Materials

Cassava starch (9% amylose) was purchased by Quimidroga S.A. (Barcelona, Spain). Negatively charged, low acyl gellan gum KELKOGEL F (MW 3-5x10^5) was supplied from Premium Ingredients (Murcia, Spain). Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) ENMAT Y1000P with 3% hydroxyvalerate was supplied by Helian Polymers B.V. (Belfeld, Holland) and amorphous PLA 4060D, density of 1.24 g/cm^3 and average molecular weight of 106,226 D with 40% of low molecular weight fraction (275 D) as reported by Muller et al. (2017), was supplied by Natureworks (U.S.A). The plasticiser, poly(ethylene glycol) with a molecular weight of 1000 Da (PEG1000), was purchased from Sigma-Aldrich (Steinheim, Germany). The glycerol was obtained from Panreac Química S.L.U. (Castellar del Vallés, Barcelona, Spain).

For sample conditioning purposes, magnesium nitrate-6-hydrate ($Mg(NO_3)_2$) was supplied by Panreac Química, S.A. (Castellar del Vallés, Barcelona, Spain). Glacial acetic acid and petroleum ether, used to carry out the different analyses, were provided by Panreac Química S.L.U. (Barcelona, Spain).

Pork meat was purchased from a local supermarket (Consum, Valencia, Spain) and processed in the laboratory. Microbiological media: buffered peptone water, Violet Bile Red agar (VRB) and Plate Count agar (PCA) were provided by Scharlab (Barcelona, Spain). Man, Rogosa and Sharpe agar (MRS) was provided by Lankem-Labbox (Barcelona, Spain).

2.2. Preparation of bilayer films

For the preparation of cassava starch films, starch and gellan gum were mixed in the correct proportion to obtain a starch:gum ratio of 9:1, using glycerol (0.30 g/g of starch) as a plasticiser. Films were obtained by melt blending and compression moulding. The melt blending process was carried out in an internal mixer (HAAKE™ PolyLab™ QC, Thermo Fisher Scientific, Germany) at 130 °C, rotor speed 50 rpm, for 10 min and 50 g of blend were processed in each batch. After processing, the obtained blends were cold ground in a refrigerated batch mill (Model M20, IKA, Germany). The obtained powder was conditioned at 25 °C and 53% relative humidity (RH) for one week. Four grams of the conditioned powder were required to obtain each film (160 mm in diameter); this powder was put onto Teflon sheets and preheated at 160 °C for 1 min in a hot-plate press (Model LP20, Labtech Engineering, Thailand). The films were obtained by compressing at 160 °C for 2 min at 50 bars, followed by 6 min at 100 bars and a final cooling cycle for 3 min until the temperature reached about 70 °C, according to that described by Hernández-García et al. (2021a). The obtained films were conditioned at 25 °C and 53% RH until used to obtain bilayer films.

PLA: PHBV films were prepared by melt-blending and compression moulding in a ratio of 3:1, using PEG1000 (15 g/100 g polymer) as a plasticiser, and with or without a constant amount of phenolic acids (ferulic, *p*-coumaric or protocatechuic acid) of 2% (w/w) with respect to the polymer matrix (polymer plus plasticiser). The concentration of the acids was determined on the basis of their minimum inhibitory concentration (MIC) of several bacteria (in the range of 1 mg/L); thus, assuming a total release of the active compound from the film into the test medium, the MIC values would be exceeded. The melt blending process (50 g per batch) was carried out in an internal mixer (HAAKE™ PolyLab™ QC, Thermo Fisher Scientific, Germany) at 170 °C, rotor speed 50 rpm, for 12 min. After processing, blends were cold ground in a refrigerated batch mill (Model M20, IKA, Germany) and conditioned at 25 °C. Three grams of the conditioned powder were required to obtain each film (160 mm in diameter); this powder was put onto Teflon sheets and preheated at 200 °C for 5 min in a hot-plate press (Model LP20, Labtech Engineering, Thailand). The films were obtained by compressing at 200 °C for 4 min at 100 bars and a final cooling cycle for 3 min until the temperature reached about 70 °C, according to that described by Hernández-García et al. (2021a).

Starch/polyester bilayer films, with or without phenolic acids, were obtained by thermocompressing the polyester film together with the cassava starch-gellan gum film in the hydraulic press (Model LP20, Labtech Engineering, Thailand) at 180 °C and 100 bars for 2 min and then cooling it down until 80 °C over 2 min. The bilayer films were stored at 25 °C and 53% RH until their analyses.

2.3. Characterisation of tensile and barrier properties of the bilayer films

The mechanical properties of the bilayer films were evaluated using a universal testing machine (Stable Micro System TA-XT plus, Surrey, United Kingdom), following standard method ASTM D882 (ASTM, 2001). The thickness of twelve preconditioned film samples at 25 °C and 53% RH of 25 mm × 100 mm was measured at six random points using an electronic digital micrometer (Comecta S.A., Barcelona, Spain). The samples were positioned in tension test clips (model A/TG, Stable Micro

Systems Haslemere, United Kingdom) and subjected to a tensile test at a speed of 50 mm min^{-1} until break. The force distance curves obtained in the test were transformed into Henky stress–strain curves that made it possible to obtain the mechanical parameters: elastic modulus (EM), tensile strength (TS) and elongation at break (E).

The water vapour permeability (WVP) of the films was determined according to standard method ASTM E96-95 (ASTM, 1995), considering the modification proposed by McHugh et al. (1993). Prior to the test, the thickness of every sample was measured at six points, as described above. Three round film samples of each formulation were placed on Payne permeability cups of 3.5 cm diameter (Elcometer SPRL, Hermelle/s Argenteau, Belgium). The temperature was 25 °C and the relative humidity gradient was 53–100%. 5 mL distilled water was placed (100% RH) inside each cup, and then the cups were placed inside desiccators containing an oversaturated magnesium-nitrate solution in order to generate 53% RH. The cups were weighed periodically every 1.5 h for 24 h using an analytical balance (ME36S, Sartorius, Germany, ±0.00001 g), until the steady state was reached. The WVP was calculated from the steady-state permeation slopes obtained from the regression analysis of weight loss data as a function of time, as described by Cano et al., (2014).

The oxygen permeability (OP) of the different bilayers was determined according to standard method ASTM F1927 (ASTM, 2010) by using an OX-TRAN 1/50 system (Mocon, Minneapolis, USA). Prior to the test, three samples of each formulation were conditioned to 53% RH and 25 °C. The film thickness was measured with a digital electronic micrometer (Palmer, COMECTA, Barcelona, Spain) to the nearest 0.001 mm at six random positions. The area of exposure of each sample to pure nitrogen flow on one side and pure oxygen flow on the other side was 50 cm^2. The equipment took oxygen transmission rate measurements at intervals of 20 min until equilibrium was reached. The oxygen transmission rate was transformed into oxygen permeability using average film thickness and oxygen pressure in the equipment.

2.4. Application of the bilayer films for pork meat packaging purposes

Pork meat was purchased from a local supermarket (Consum, Valencia, Spain). To avoid cross contamination during sample preparation, devices and work surfaces were disinfected with 96% ethanol (Panreac S.A., Barcelona, Spain) and every film was sterilized by exposure to ultraviolet (UV) light for 1 h in a laminar flow cabinet (Bio II Advance, Telstar, Terrasa, Spain).

Packaging bags (8 cm × 11 cm) were prepared with the different bilayer films by thermo-sealing (SAECO Vacio Press Elite, Barcelona, Spain). The pork meat was cut into 10 g slices using a professional slicer (Smarty 250 IX, Manconi, Italy) and immediately placed inside the bags that were heat sealed and vacuum packed (SAECO Vacio Press Elite, Barcelona, Spain). All of these procedures were carried out in a laminar flow cabinet (Bio II Advance, Telstar, Terrasa, Spain). All of the samples were stored at 5 °C and 48% RH for 3, 7, 11 and 15 days, after which times the bags were weighed to determine the sample weight loss with respect to the initial weight, by using an analytical balance (Sartorius, Goettingen, Germany). Likewise, throughout cold storage, the pH, lipid oxidation, colour changes and microbial counts were monitored in the packaged pork meat slices.

The pH was determined using a digital pH meter by means of the direct insertion of the electrode probe (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) into the pork meat slice. Six measurements were taken, in duplicate for each packaging treatment and time.

The oxidative stability of pork meat was measured by means of the peroxide index (PI) and thiobarbituric acid reactive substances (TBARs) on day 0 and after 15 storage days. Prior to taking the PI measurements, fat was extracted from the pork meat samples. For this purpose, 5 g of minced pork sample were weighed and mixed with 10 g of sea sand and dried for 24 h at 100 °C. Then, Soxhlet equipment was used to extract the meat fat with petroleum ether using for 3 h and the solvent was evaporated to obtain solvent free fat. All of the extractions were performed in triplicate and the peroxide index was also evaluated in triplicate using the method described by Talón et al. (2019). The results were expressed as meq O_2/kg extracted fat. The TBARs in the meat samples were measured in triplicate as described by Siu and Draper (1978). The results were expressed as mg of malondialdehyde (MDA)/kg of meat sample.

The CIE-L* a* b* colour coordinates of the packaged pork meat were obtained using the illuminant D65/ 10° observer from the reflection spectra of the sample surface measured at six random points using the MINOLTA colorimeter spectrum (model CM-5, Minolta Co., Tokyo, Japan) and using a black and a white standard background to calculate the L*, a* and b* coordinates from the reflectance of an infinite thickness sample (Hutchings, 1999). The colour parameters, chroma (Cab*) and hue (hab*) were also determined by using Equations (1) and (2), respectively. The total colour difference after packaged meat samples were cold stored for different times with respect to day 0 was calculated using Equation (3). Three packaged pork meat samples with each bilayer film were analysed in duplicate after each storage time.

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

$$h^*_{ab} = arctg\frac{b^*}{a^*} \quad (2)$$

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

Microbiological analyses of the packaged pork meat slices were carried out after different storage times (0, 3, 7, 11 and 15 days). A total of 10 g of meat sample were aseptically taken from the film bags using sterile forceps in the laminar flow cabinet (Bio II Advance, Telstar, Terrassa, Spain) and subsequently placed in sterile bags (Stomacher 440 Classic Strainer Bags, Worthing, UK) with 90 mL of peptone water (Scharlab, Barcelona, Spain). The Stomacher bags were homogenised for 3 min using a homogeniser (IUL Instruments, Barcelona, Spain) and the homogenate was then 10-fold serially diluted using TSB and used to enumerate total viable counts, total coliforms and lactic acid bacteria. The total viable counts and total coliforms were enumerated in PCA and VRB plates, respectively, after incubation at 37 °C for 48 h. The lactic acid bacteria were enumerated using MRS plates after incubation at 30 °C for 72 h. The results of bacterial counts were converted to $\log_{10}$ colony-forming units per gram of sample (log CFU/g) prior to statistical analyses.

2.5. Statistical analysis

The statistical analyses of the data were performed through an analysis of variance (ANOVA) using Statgraphics Centurion XVII-X64 software (Manugistics Corp., Rockville, Md.). Both a one-way and multifactor ANOVA were used to analyse the influence of the kind of packaging and storage time on the properties of packaged meat. Fisher's least significant difference (LSD) procedure was used at the 95% confidence level.

3. Results and discussion

3.1. Tensile and barrier properties of the films

Films with good mechanical strength and extensibility are generally required for food packaging applications to withstand external stress during the processing, transportation, handling and storage of packaged food products (Aloui et al., 2021). Fig. 1 shows the typical stress–strain curves of the obtained bilayers and the mechanical parameters (elastic modulus, tensile strength, deformation at break) are shown in Table 1.

S—P films did not exhibit plastic deformation, since after elastic deformation these films broke without any signs of deformation

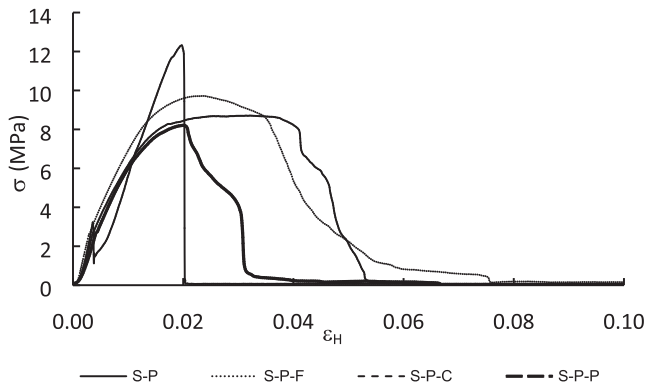

Fig. 1. Stress–strain curves of starch-gellan/PLA-PHBV bilayer films (S—P) and those containing ferulic acid (S—P—F), *p*-coumaric acid (S—P—C) and protocatechuic acid (S—P—P).

exceeding the elastic limit. These bilayers exhibited a small peak at low deformation that can be attributed to a partial layer detaching during the film's extension until the simultaneous fracture of both layers. In contrast, bilayer films containing phenolic acids presented plastic deformation when exceeding the elastic limit, especially with the incorporation of ferulic and *p*-coumaric acids. The fracture of the layers in the laminate occurred separately, the starch layer being more extensible after the first fracture of the P layer. Therefore, the incorporation of phenolic acids yielded more plasticised polyester-starch bilayer films, while mainly promoting the extensibility of the starch layer. Although the tensile behaviour of laminates is determined by the particular properties of each bilayer and the interlayer adhesion forces, it would also be affected by the partial compound migration between layers during thermocompresion that could modify the initial properties of each layer. Specifically, water and glycerol could migrate from the starch sheet to the polyester sheet and PEG and phenolic acids could migrate into the starch layer, all of which would affect the expected tensile behaviour. The less extensible, more resistant and stiffer polyester sheet will mainly determine the bilayer's tensile properties; however, these can also be affected by the starch layer's contribution to the tensile strength, interlayer adhesion forces and compound migration.

Even though the incorporation of phenolic acids slightly promoted the extensibility of bilayers (measured at the first fracture point), it led to a decrease in the elastic modulus and tensile stress at break. In contrast, these compounds promoted the stiffness and resistance to break (by about 17 and 30 %, respectively) in the polyester blend monolayers (Hernández-García et al., 2021b). This suggests that the acid migration into the starch sheets notably affected the mechanical contribution of the starch layers to the laminate. Previous studies showed a marked decrease in the elastic modulus and resistance to break, with highly enhanced elongation, when phenolic acids were incorporated into the film matrix (Ordoñez et al., 2021). Therefore, the partial migration of phenolic acids into the starch layer could explain the tensile behaviour of the active bilayers.

As shown in Table 1, the incorporation of the three acids caused a significant decrease ($p < 0.05$) in the thickness of the films, with the films containing *p*-coumaric acid being the thinnest. This could be associated with the changes promoted by phenolic acids in the interchain forces of the polymeric matrix, which affect the flowability and extension of the films during the first and the second compression moulding steps.

As concerns the barrier properties, the water vapour and oxygen permeability of packaging materials are critical parameters for the shelf-life of the packaged foods since they can affect the microbial growth, fat oxidation and texture and water loss (Kerry & Tyuftin, 2017). As shown in Table 1, the incorporation of the phenolic acids into the polyester layer resulted in a significant improvement ($p < 0.05$) in the barrier properties of the bilayers. The polyester layer provides the laminate with a greater water vapour barrier capacity, whereas the starch layer contributes to an enhancement of the oxygen barrier capacity. Despite the fact that the phenolic acids reduced the WVP and OP values of polyester monolayers by about 30 % (Hernández-García et al., 2021b), they promoted the OP in starch monolayers, without there being any notable effect on WVP; this was mainly due to matrix plasticisation (Ordoñez et al., 2021). The significant reduction in WVP caused by phenolic acids in the bilayers reflects the positive effect of the phenolic acids in the polyester sheet. This has been explained both by the possible reaction of the end chain OH groups with phenolic acid molecules during thermal processing, as well as by the hydrogen bonding between the phenol groups and the oxygen of the polyester group, which further limits the water solubility in the films and so the permeation capability of water molecules. The decreased oxygen permeability promoted by the addition of phenolic acids to the polyester monolayer could also explain the observed reduction in the laminates. The interactions between the phenols and the polyester chains, tightening the polymer matrix, limit mass transport phenomena through the films (Benbettaïeb et al., 2019; Contardi et al., 2019).

As opposed to the limited oxygen or water vapour barrier properties of biodegradable monolayer films, the obtained bilayer films exhibited mechanical and barrier properties that were adequate for the purposes of meeting the meat packaging requirements (Nguyen et al., 2021) while the barrier properties were notably improved by the incorporation of phenolic acids. The reduction in WVP and OP could impact positively on how well these materials preserve meat during storage. Additionally, the potential antimicrobial and antioxidant action of these compounds would also contribute to enhancing meat quality and safety during storage.

3.2. *Changes in the quality parameters of packaged pork meat throughout cold storage*

The weight losses of the pork meat samples packaged in the bilayer

Table 1
Tensile properties (Elastic modulus: EM, tensile strength: TS and deformation at break: %E), thickness, barrier properties (WTR: water transmission rate, WVP: water vapour permeability, OTR: oxygen transmission rate and OP: oxygen permeability) of starch-gellan/PLA-PHBV bilayer films (S—P) and bilayer films containing ferulic acid (S—P—F), *p*-coumaric acid (S—P—C) and protocatechuic acid (S—P—P). Mean values ± standard deviation.

Sample	EM (MPa)	TS (MPa)	E (%)	Thickness (μm)	WTR (g/d.m^2)	WVP (g·mm·kPa^{-1}·h^{-1}·m^{-2})	OTR (cm^3/d.m^2)	OPx10^{14} (cm^3·m^{-1}·s^{-1}·Pa^{-1})
S—P	834 ± 75^{a}	12.0 ± 2.0^{a}	2.0 ± 0.2^{a}	244 ± 3^{a}	190 ± 30^{a}	1.14 ± 0.20^{a}	0.354 ± 0.02^{ab}	0.93 ± 0.09^{a}
S—P—F	822 ± 157^{ab}	9.0 ± 1.0^{ab}	2.0 ± 1.0^{a}	217 ± 2^{b}	70 ± 14^{b}	0.40 ± 0.04^{b}	0.347 ± 0.01^{a}	0.76 ± 0.01^{b}
S—P—C	718 ± 90^{ab}	8.0 ± 2.0^{ab}	3.0 ± 1.0^{a}	204 ± 2^{c}	58 ± 7^{b}	0.41 ± 0.05^{b}	0.325 ± 0.01^{bc}	0.73 ± 0.04^{bc}
S—P—P	633 ± 60^{b}	8.0 ± 1.0^{b}	2.0 ± 1.0^{a}	215 ± 1^{b}	86 ± 30^{b}	0.54 ± 0.20^{b}	0.280 ± 0.05^{c}	0.62 ± 0.11^{c}

Different superscript letters (a-c) within the same column indicate significant differences between formulations ($p < 0.05$).

films are shown in Fig. 2. The greatest weight losses throughout the cold storage period were obtained in the samples packaged in S—P films, which exhibited significantly higher WTR, as compared to bilayer films with phenolic acids. Coherently, the samples packaged in films with *p*-coumaric or ferulic acid, which exhibited the lowest WTR, had the smallest weight loss.

Fig. 3 shows the changes in the pH of the different packaged pork meat samples during cold storage. The pH values were significantly ($p < 0.05$) affected by the bilayer composition and the storage time, as can be observed in Fig. 3. The pH of fresh pork meat typically ranges between 5.10 and 6.36 and consumers are more likely to prefer a pH of 5.7 to 6.1 (Xiong et al., 2020; Kim et al., 2016). As shown in Fig. 3, the initial pH value of pork meat was 5.6, which was consistent with the values reported by other authors (Zhang et al., 2018, Xiong et al., 2020; Wang et al., 2021). The pH value of all of the samples increased over storage time, which is coherent with the production of alkaline compounds due to proteolytic reactions during storage and microbial activity (Arancibia et al., 2015; Daniloski et al., 2019). As compared with samples packaged in phenolic-free S—P bilayer films, the samples packaged in bilayers with phenolic acids (S—P—F, S—P—C and S—P—P) showed lower pH values throughout storage, which suggests a better meat preservation and is coherent with the lower oxidation level and total microbial counts of these samples, commented on below.

When applied to pork meat samples, the antioxidant properties of the starch-polyester bilayer films, containing or not phenolic acids, were assessed by monitoring the PI and TBARs values at the beginning of cold storage and after 15 days. Unsaturated fatty acids react with molecular oxygen through a radical mechanism, giving rise to odourless, highly unstable, first oxidation products known as hydroperoxides; these, in turn, give rise to secondary compounds, such as hydrocarbons, aldehydes and esters, among others (Ross & Smith, 2006), which cause off-flavours and off-odours in meat. As shown in Table 2, the PI of pork meat samples increased after 15 days of storage, the PI of the control samples (S—P) being the highest at the end of storage. The PI values of the samples packaged in films with phenolic acids were lower, especially those packaged in the films containing protocatechuic acid, which is coherent with the fact that these films exhibited the lowest OTR values (Table 1). The three acids exhibited radical scavenging capacity and their release into the meat sample could limit its oxidation. Specifically, the activity relative to TROLOX was 1.9, 2.2 and 1.2 mM, respectively for ferulic, p-coumaric and protocatechuic acids (Rice-Evans, et al., 1996). Therefore, both, the radical scavenging capacity and the OP reduction should contribute to prevent meat oxidation. Nevertheless, the reduction of oxygen permeability could play a more important role as deduced from the relative antioxidant capacity of the acids and the higher efficacy of films with protocatechuic acid at reducing meat oxidation.

The TBARs assay measures the amount of malondialdehyde (MDA)

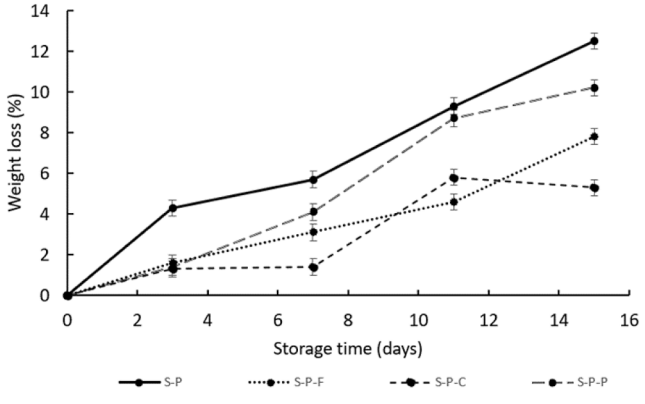

Fig. 2. Development of sample weight loss (%) during storage. Mean values and 95% LSD intervals.

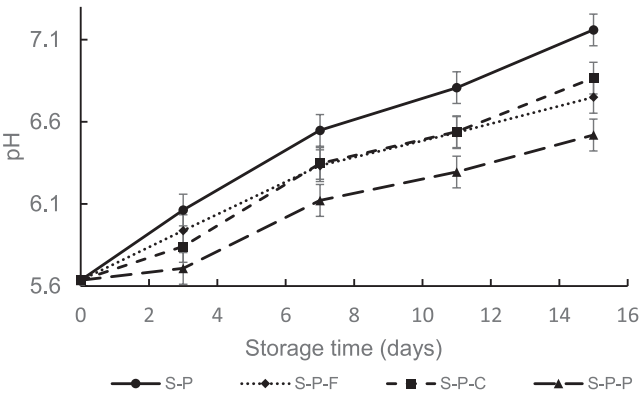

Fig. 3. Development of the pH of the pork meat samples packaged in starch-gellan/PLA-PHBV bilayer films without phenolic acids and with ferulic acid (S—P—F), *p*-coumaric acid (S—P—C) and protocatechuic acid (S—P—P), throughout 15 days at 5 °C. Average values and 95% LSD intervals.

Table 2
TBARs and Peroxide Index of fresh samples (t = 0) and of those stored for 15 days at 5 °C, packaged in bilayer films without phenolic acids (S—P), with ferulic acid (S—P—F), *p*-coumaric acid (S—P—C) and protocatechuic acid (S—P—P). Mean values ± standard deviation.

	t = 0 d	t = 15 d			
	Fresh sample	S—P	S—P—F	S—P—C	S—P—P
TBARs (mg MDA/ kg meat sample)	0.31 ± 0.05	1.01 ± 0.04^{a}	0.80 ± 0.07^{b}	0.75 ± 0.05^{b}	0.68 ± 0.06^{b}
Peroxide index (meq O2/ kg extracted fat)	0.6 ± 0.1	4.4 ± 0.5^{a}	4.1 ± 0.5^{ab}	4.0 ± 0.6^{ab}	3.1 ± 0.6^{b}

Different superscript letters (a-b) within the same row indicate significant differences between formulations ($p < 0.05$).

produced by the secondary products of polyunsaturated fatty acid peroxidation (Song et al., 2014). The off-flavours in pork meat can generally be detected by consumers when the TBARs value is above the threshold of 0.5 mg MDA/kg (Sheard et al., 2000). As shown in Table 2, the TBARs value of packaged pork meat increased after 15 days of storage and surpassed the abovementioned limit. Nevertheless, although the samples packaged with S—P films exhibited the highest TBARs value, the addition of phenolic acids to the bilayers led to a significant reduction ($p < 0.05$) in the TBARs value of pork meat samples, which must be attribute to both the radical scavenging capacity of the released phenolic acids to the meat sample and the OP reduction promoted by these compounds in the packaging film, as commented on above. The samples packaged in films with protocatechuic acid (S—P—P) exhibited the lowest TBARs value, which also agrees with the fact that this bilayer had the lowest OTR values (Table 2).

In terms of lipid oxidation, the results are coherent with the oxygen permeability values of the films, and also suggest that ferulic, *p*-coumaric and protocatechuic acids exerted their radical scavenging capacity when released from the laminate, thus effectively retarding lipid oxidation. A similar effect was reported in previous studies into the incorporation of ferulic acid into chitosan-based films (Wang et al., 2021) when applied to refrigerated pork meat.

Surface colour is an important visual quality indicator of fresh meat (Pathare et al., 2013). Fig. 4 shows the changes in the colour parameters, lightness (L*), chroma (C_{ab}*) and hue (h_{ab}*), as a function of storage time. All of the colour parameters were significantly ($p < 0.05$) affected by the type of film and storage time, as shown in Fig. 4. After three days of storage, L* significantly decreased in every sample, indicating that the packaged meat became darker. This could be, in part, explained by the changes in pH throughout the storage time (Fig. 2), which affect the

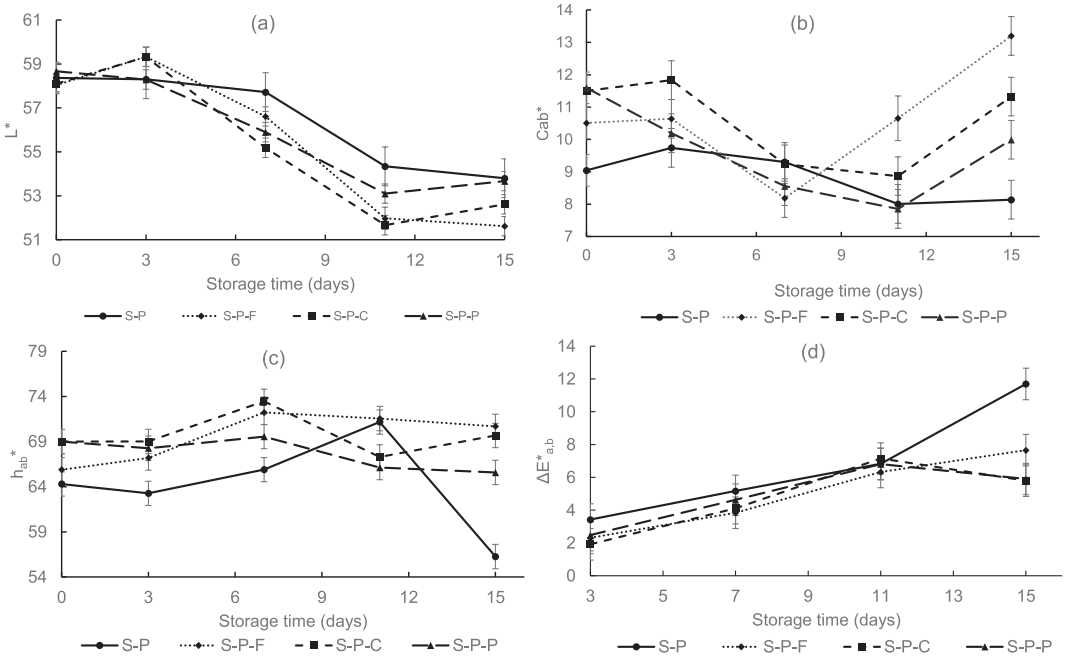

Fig. 4. Development of the colour parameters of pork meat samples packaged in starch-gellan/PLA-PHBV bilayer films without (S—P) and with ferulic acid (S—P—F), *p*-coumaric acid (S—P—C) and protocatechuic acid (S—P—P), throughout 15 days at 5 °C. a) L*, lightness; b) C_{ab}*, chroma c) h_{ab}*, hue d) total colour difference, ΔE*. Average values and 95% LSD intervals.

meat water holding capacity (Barbut et al., 2005) and the sample weight loss, which mainly occur at surface level (Fig. 4). This led to changes in the selective light absorption on the sample surface, due to the changes in the refractive index of the material and the surface concentration of the pigments (Hutchings, 1999). These changes also affect the values of the chromatic parameters and so, the chroma and hue values. The oxidation of lipids and myoglobin in meat also leads to discoloration, and these processes often appear to be linked (the oxidation of one of these compounds produces chemical species that promote the oxidation of the other) (Faustman et al., 2010). In fact, several studies have reported that antioxidants act to preserve the fresh meat colour.

The pH influence on meat colour has also been described. A lower pH in pork meat has been associated with greater reflectance, which leads to an increase in lightness (L*) and a decrease in the relative amount of the reduced form of myoglobin (Mb). At the same time, a lower pH is accompanied by a greater susceptibility of muscle pigments to oxygenation and oxidation (Karamucki et al., 2013). Meat yellowness increases due to an increase in the relative amounts of the oxygenated and oxidised forms of myoglobin (MbO_2 and MetMb) at the expense of the reduced form.

The type of film packaging slightly affected the colour development of meat during storage. Significant differences could be observed in the case of the samples packaged in films incorporating phenolic acids, mainly at the end of the storage period. The pork meat samples packaged in the active bilayers exhibited hue and chrome values that were significantly higher than the samples packaged in the S—P film, with lower hue and a less saturated colour.

The total colour difference after each storage time with respect to the initial values is shown in Fig. 4d. The total colour differences in the pork meat samples packaged in films containing phenolic acids did not exceed the usual tolerance limit for food products ($\Delta E < 5$) (Hutchings, 1999), thus indicating a better colour preservation in the samples packaged in antioxidant films with lower OTR values.

Fig. 5 shows the progress throughout the storage (up to 15 days) of the total aerobic counts, coliforms, and lactic acid bacteria of pork meat packaged in starch-polyester bilayer films containing or not phenolic acids. The total viable counts are an important microbiological indicator for the purposes of evaluating the sanitary quality and safety of meat. It is the quantitative sanitary standard used to identify the process conditions and contamination degree of meat (Commission Regulation, 2005). Total viable counts (Fig. 5a) were significantly ($p < 0.05$) affected by the type of food packaging and the storage time. In all of the meat samples, the total viable counts increased during the storage time, which is mainly related to the proliferation of psychotrophic bacteria (Ercolini et al., 2009; Xiong et al., 2020). The initial total viable count of the fresh samples was 2.8 log CFU/g. Similarly, Xiong et al. (2020) and Wang and Lihua (2018) reported counts of fresh pork of around 2.51 and 2.2–2.7 log CFU/g respectively. On days 3, 7 and 15, the incorporation of phenolic acids had a significant effect ($p > 0.05$) on the total viable counts. S—P—P was the formulation that showed the greatest reduction in total viable counts. The incorporation of protocatechuic acid led to a log reduction of 0.7, 0.5, 0.8 and 0.6 log on days 3, 7, 11 and 15, respectively. These results suggested that active bilayers incorporated with ferulic acid, *p*-coumaric acid and protocatechuic acids were capable of reducing the total viable counts of pork during cold storage, with the protocatechuic acid being slightly more effective. However, the decrease in microbial growth as compared with that of the bilayer films without phenolic acids (control) was smaller than 2 log, which is what is usually considered as being significant in microbial growth studies. This coincides with the results obtained in a previous study in which the antimicrobial performance of polyester films with the same phenolic acids was evaluated *in vitro* (Hernández-García et al., 2021b). The limited antimicrobial action of phenolic acids was attributed to the slow and scarce release of the active compounds into the culture medium, as deduced from the release kinetics of the compounds in aqueous food simulants, previously studied (Hernández-García et al., 2021b). According to Huang et al. (2013), a total viable count of 7 log can be used as the threshold for the quality of pork meat. All of the samples, except S—P, were below this threshold after the 15-day storage period, indicating that the incorporation of phenolic acids was effective at preventing the pork from microbial spoilage, despite their slow release. Nevertheless, the low oxygen permeability of the bilayer films also contributed to extend the meat shelf-life, limiting the oxygen availability for microbial growth.

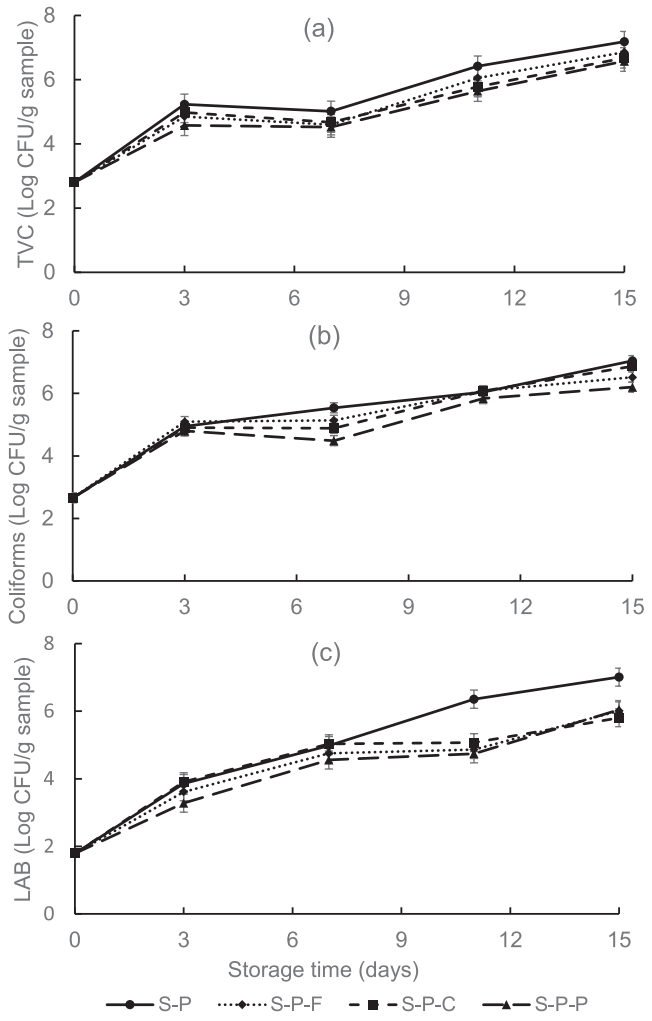

Fig. 5. Microbial counts obtained for the pork meat samples packaged in starch-gellan-/PLA-PHBV bilayer films (S—P) and those containing ferulic acid (S—P—F), *p*-coumaric acid (S—P—C) and protocatechuic acid (S—P—P) throughout 15 days at 5 °C. a) total aerobic counts, b) total coliforms, c) lactic acid bacteria. Average values and 95% LSD intervals.

The microbial counts of total coliforms throughout the storage time are shown in Fig. 5b. Total coliform counts were significantly ($p < 0.05$) affected by the type of packaging and the storage time. While the incorporation of phenolic acids did not have a significant effect ($p > 0.05$) on total coliform counts on day 3, it did have a significant effect on days 7 and 15, which is coherent with the slow compound release. Protocatechuic acid was the most effective, showing log reductions of 1.1 and 0.8 log on days 7 and 15 of storage, respectively.

Lactic acid bacteria are the dominant group of microorganisms isolated from meat and vacuum-packed meat products (Xu et al., 2018). The initial count of lactic acid bacteria in the fresh pork samples was 1.79 log CFU/g (Fig. 5c), in the range of that reported in other studies (Xu et al., 2018). Lactic acid bacteria counts were also significantly ($p < 0.05$) affected by the type of food packaging and the storage time. At the end of the storage, the greatest log reduction (1.2 log) also occurred in samples packaged in the bilayers with *p*-coumaric acid.

Protocatechuic acid was the phenolic acid that led to the greatest microbial inhibition, especially against total coliforms and lactic acid bacteria. The inhibition did not occur at the beginning, coherently with the slow release of these kinds of compounds from the polyester matrix, as previously reported (Hernández-García et al., 2021b). In fact, the antibacterial action was mainly detected from the 7th storage day onwards.

4. Conclusion

The incorporation of 2% (w/w) ferulic, *p*-coumaric or protocatechuic acid into starch-polyester bilayer films, obtained by melt blending and compression moulding, reduced their stiffness and resistance to break but significantly improved their barrier capacity to water vapour and oxygen. The films containing protocatechuic acid experienced the greatest improvement. This implied that the functional properties of bilayers with phenolic acids were better for meat packaging purposes. Additionally, the antioxidant and antimicrobial properties of phenolic acids may also contribute to an improvement in meat preservation. In fact, ferulic, *p*-coumaric and protocatechuic acids in the films led to a reduction in the meat weight loss and less lipid oxidation during storage, while the increase in sample pH was smaller than that in the samples packaged without phenolic acids. Microbial growth was also inhibited in meat samples packaged in films with phenolic acids, with the active films with protocatechuic acid being the most effective, especially against total coliforms and lactic acid bacteria. It would be necessary to carry out further studies in order to achieve a faster release of phenolic acids from the films for the purposes of reaching greater microbial growth inhibition, thus better ensuring the shelf-life extension of pork meat.

CRediT authorship contribution statement

Eva Hernández-García: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **María Vargas:** Supervision, Writing – review & editing. **Amparo Chiralt:** Supervision, Writing – review & editing.

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.

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