Physicochemical and antimicrobial properties of cassava starch films with ferulic or cinnamic acid

Ramón Ordoñez[*], Lorena Atarés, Amparo Chiralt

Instituto Universitario de Ingeniería de Alimentos para el Desarrollo. Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

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ABSTRACT

Plasticised cassava starch films with 1–2% ferulic or cinnamic acid were obtained by melt blending and compression moulding. Partial losses (about 40%) of the more thermo-sensitive ferulic acid occurred during processing. Incorporation of phenolic acids did not provoke remarkable changes in the oxygen and water vapour permeability of starch films, but these became less water soluble (13–25% reduction), more extensible and less resistant to break, especially with the highest concentration of ferulic acid. Films with 2% of either acid effectively inhibited the growth of *E. coli* and *L. innocua* in tests carried out in culture medium; being cinnamic acid more effective than ferulic and *L. innocua* more sensitive than *E. coli*; a Log CFU reduction, with respect to the control, higher than 6 was obtained for *L. innocua* with 2% of cinnamic acid in the films. The growth of both strains was also inhibited in chicken breast (log CFU reduction>2) with 2% of either acid. In fresh-cut melon, the *L. innocua* growth was significantly inhibited by films with 2% cinnamic acid, but lower growth inhibition of *E. coli* in fresh-cut melon was observed.

1. Introduction

The food industry currently relies on mostly single-use, petroleum-derived non-biodegradable plastic materials to satisfy the food packaging requirements. Specifically in Europe, only 30% of the plastic produced is recycled (Gong, Putnam, You, & Zhao, 2020; Moreno, Cárdenas, Atarés, & Chiralt, 2017). The environmental impact of plastic use and mishandling is reaching a critical point (Lestari & Trihadiningrum, 2019; Qi, Jones, Li, Liu, & Yan, 2020; Rodrigues et al., 2019). Furthermore, consumers demand more sustainable and less processed products with fewer or no synthetic additives (Carocho, Barreiro, Morales, & Ferreira, 2014; Kushwah, Dhir, & Sagar, 2019). However, food safety needs to be ensured and both packaging and additives are needed for this. The development of active packaging materials using biodegradable polymers and naturally-occurring antimicrobial/antioxidant compounds is necessary to improve food preservation and extend shelf-life in a more sustainable way (Atarés & Chiralt, 2016).

Starch is a naturally occurring polymer, constituted by amylose and amylopectin, which can be obtained from a variety of renewable sources, such as cassava, potato or maize (Domene-López, Delgado-Marín, Martin-Gullon, García-Quesada, & Montalbán, 2019). Starch has proven to be a good alternative for traditional food packaging, since it can be

thermo-processed with plasticisers to obtain adequate materials (Menzel, González-Martínez, Chiralt, & Vilaplana, 2019; Ochoa-Yepes, Di Giogio, Goyanes, Mauri, & Famá, 2019). It is cheap and provides transparent films with excellent oxygen barrier capacity (Ortega-Toro, Muñoz, Talens, & Chiralt, 2016; Tampau, González-Martínez, & Chiralt, 2018). However, given its hydrophilic nature, starch films are very sensitive and permeable to water vapour, making applications on high moisture foods very difficult (Acosta, Jiménez, Cháfer, González-Martínez, & Chiralt, 2015; Ochoa-Yepes et al., 2019). Several strategies to overcome this drawback have been studied, such as promoting crosslinking with different chemicals or combining starch with other polymers with complementary properties, either by blending them or using multilayer film assemblies (Fang, Fu, Tao, Liu, & Cui, 2020; Muller, González-Martínez, & Chiralt, 2017; Ochoa-Yepes et al., 2019; Requena, Vargas, & Chiralt, 2018).

Phenolic acids are characterised by the presence of a benzene ring, a carboxylic group and at least one hydroxyl or methoxyl group (Lima et al., 2016). The antioxidant and antimicrobial activities of these naturally-occurring compounds have been studied in the last few years (Guil-Guerrero et al., 2016; Lima et al., 2019). Specifically, ferulic and cinnamic acids have shown effective growth inhibition of both gram positive and negative bacterial strains at concentrations below 1 mg/g

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(Meira, Holley, Bordin, Macedo, & Luciano, 2017; Miyague, Macedo, Meca, Holley, & Luciano, 2015; Olasupo, Fitzgerald, Gasson, & Narbad, 2003). The incorporation of highly active phenolic acids, such as ferulic and cinnamic, into a biodegradable polymeric matrix, such as starch, could result in a filmogenic material with antimicrobial properties for food packaging. Although previous studies (Benbettaïeb, Karbowiak, Brachais, & Debeaufort, 2015; Fabra, Hambleton, Talens, Debeaufort, & Chiralt, 2011) analysed the effect of ferulic acid on the properties of sodium caseinate and chitosan films, antibacterial capacity of these films was not reported.

The aim of this study was to analyse the effect of the incorporation of different ratios of cinnamic and ferulic acids into thermoplastic cassava starch films, by analysing the changes induced in the films' functional properties as packaging material. The antimicrobial action of the phenolic acid-loaded films in both the culture media and different food substrates was also analysed.

2. Materials and methods

2.1. Materials

Cassava starch produced by Asia Co., LDT (Kalasin, Thailand) and purchased from Quimidroga S.A. (Barcelona, Spain), as well as glycerol as plasticiser (Panreac Química, Barcelona, Spain) were used to obtain films. Cinnamic and ferulic acids (Sigma-Aldrich, Saint Louis, USA) were used as antimicrobial compounds. Magnesium chloride and phosphorus pentoxide, used to control the sample relative humidity (RH) were supplied by Panreac Química (Barcelona, Spain). Tryptone soy broth, tryptone soy agar, phosphate-buffered saline, and peptone water for microbial test were purchased from Scharlab (Barcelona, Spain). Strains of *Listeria innocua* (CECT 910) and *Escherichia coli* (CECT 101) were from the Spanish Type Collection (CECT, University of Valencia, Spain).

2.2. Film preparation

Glycerol (G) was added to cassava starch in a ratio of 0.30 g/g starch. Cinnamic (C) or ferulic (F) acids were added at 0, 1 or 2 g acid/100 g dry film. Melt blending of components was carried out in an internal mixer (Haake PolyLab QC, Thermo Fisher Scientific, Germany) at 130 °C and 50 rpm for 10 min. The obtained melts were cold ground and conditioned at 33% RH and 25 °C in desiccators containing an oversaturated solution of $MgCl_2$. Films of about 20 cm in diameter were obtained with 4 g of conditioned samples, using a hydraulic heating press (LP20, Labtech engineering, Thailand). Samples were preheated at 160 °C for 1 min and compressed at 5 MPa for 2 min, plus 10 MPa for 6 min. Finally, a 3 min cooling step to 70 °C was applied. The films obtained were conditioned at 33% RH and 25 °C for at least 7 days before characterization. The different samples were coded as S (starch films without actives), 1F, 2F (starch with ferulic acid at 1 or 2% w/w); and 1C, 2C (starch with cinnamic acid at 1 or 2% w/w).

2.3. Characterization of the films

2.3.1. Thermal analysis

The thermal properties of the films were measured using a differential scanning calorimeter (DSC, 1 StareSystem, Mettler-Toledo, Switzerland) and a thermogravimetrical analyser (TGA/SDTA 851e, Mettler-Toledo, Switzerland). Prior to the analyses, all the samples were conditioned at 0% RH for 1 week in a desiccator with P_2O_5. DSC samples (about 10 mg) were placed into aluminium pans and sealed. Samples were heated from room temperature to 160 °C at 10 K/min, maintaining 160 °C for 5 min, cooling at 50 K/min to −10 °C, maintaining at −10 °C for 5 min and applying a second heating to 160 °C at 10 K/min. An empty aluminium pan was used as reference. For TGA analyses, samples of about 10 mg were placed into alumina crucibles and heated from 25 to 600 °C at 10 K/min, under a nitrogen flow (50 mL/min). Both analyses were performed in duplicate for each sample.

2.3.2. Retention of active compounds, moisture content and water solubility of the films

The final content of ferulic and cinnamic acids in the films was determined through methanol extraction and spectrophotometric quantification. Strip samples (about 100 mg) preconditioned at 0% RH with P_2O_5, were immersed in 10 ml methanol while being gently stirred for 48 h. Methanol extracts were filtered and properly diluted for the absorbance measurements at the wavelength of maximum absorbance (320 nm for F and 270 nm for C), by using a UV–visible spectrophotometer (Thermoscientific Evolution 201, USA). The respective methanol extract of the film without active compounds was used as blank. The absorbance measurement was transformed into a concentration using the previously determined calibration curve for each active compound. All measurements were taken in triplicate.

The moisture content of films conditioned at a specific RH was gravimetrically determined by completely drying the conditioned films in a vacuum oven at 60 °C for 48 h and their subsequent conditioning at 0% RH (using P_2O_5) at room temperature until constant weight (Cano, Cháfer, Chiralt, & González-Martínez, 2015). The results were expressed as g water/100 g of film.

In order to quantify the film's water solubility, the samples conditioned at 0% RH were immersed in vials with 10 ml distilled water and gently stirred for 24 h. The content of the vials was filtered and the liquid phase was dried in a vacuum chamber at 60 °C until reaching constant weight. Solubility was expressed as the total solid content of the aqueous phase (solubilised film) with respect to the mass of the initial sample.

2.3.3. Mechanical, barrier and optical properties

A texture analyser TA-XT plus (Stable Micro Systems, Haslemere, England) was used to determine the tensile properties of each film formulation, following the ASTM D882 method (American Society for Testing Materials, 2002). Film strips (25 mm × 100 mm) were mounted on tensile grips (50 mm separation) and stretched at 50 mm/min. Tensile strength at break (TS), elastic modulus (EM) and elongation at break (%E) were obtained from the stress-strain curves. A hand-held electronic digital micrometer (Comecta S.A., Barcelona, Spain) was used to measure film thickness to the closest 0.001 mm, at six random points in each strip. Eight replicates per sample were performed.

Water vapour permeability (WVP) was measured gravimetrically following the ASTM E96-95 method (American Society for Testing Materials, 1995), using Payne permeability cups (Elcometer SPRL, Hermelle/s Argenteau, Belgium) of 3.5 cm in diameter. Cups were filled with 5 ml of distilled water and mounted with circular film samples. The cups were placed into desiccators containing $MgCl_2$ oversaturated solution and kept at 25 °C. Cups were weighed periodically (1.5 h–24 h) using an analytical balance (±0.00001 g). The rate of weight loss over time was used to calculate WVP (American Society for Testing Materials, 1995) when the stationary state was reached. The measurements were taken in triplicate for each sample.

The oxygen permeability (OP) was determined by following the ASTM D3985-05 method (American Society for Testing Materials, 2010, pp. 1–7) using Ox-Tran 1/50 equipment (Mocon, Minneapolis, USA). The film test area was 50 cm^2 and measurements were taken at 25 °C and 53% RH. OP was calculated by dividing the oxygen transmission rate by the difference between the oxygen partial pressure on both sides of the film and multiplying by the average film thickness. Two replicates per formulation were obtained.

The measurement of colour coordinates and internal transmittance was taken with a CM-5 spectrocolorimeter (Konica Minolta, Inc., Japan). Reflection spectra (400–700 nm) of the films were obtained for three film replicates and at three points per replicate, backed on both black and white plates. Internal transmittance (T_i) was calculated by applying the Kubelka-Munk theory of multiple dispersion (Hutchings, 1999). CIEL*a*b* colour coordinates were obtained from the determined

reflectance of an infinitely thick layer of material, considering the D65 illuminant and 10° observer (Cano, Jiménez, Cháfer, Gónzalez, & Chiralt, 2014). Finally, the lightness (L*), chroma (C_ab*) and hue (h_ab*) psychometric coordinates were calculated (Mahy, Van Eycken, & Oosterlinck, 1994).

2.4. Antibacterial effectiveness of the films

2.4.1. Minimal inhibitory concentration of phenolic acids

Minimal inhibitory concentrations (MIC) of both ferulic and cinnamic acids against *E. coli* and *L. innocua* were determined using the thiazyl blue tetrazolium bromide (MTT) colorimetric assay, using a 96-well disposable sterile microtiter plate(Requena, Vargas, & Chiralt, 2019b). Stock solutions of each compound were obtained using DMSO. Diluted solutions ranging between 0.5 and 2.0 mg/mL were prepared using TBS broth medium, and 100 μL aliquots of each dilution were placed in the corresponding wells. Plates were inoculated with 100 μL of bacterial suspension (10^5 CFU/mL) and covered with a sterile sealer mat to act as vapour barrier. Sterile and growth controls were prepared for each plate, and the outer wells were left empty in order to prevent border effect. After 24 h of incubation at 37 °C, 10 μL of MTT reconstituted in PBS at 5 mg/mL were added to all the wells, and the plate was left to incubate for 4 h before MIC was assessed by observation of the plate. MIC was considered as the lowest concentration at which no purple colour was observed in the well. Experiments were carried out in duplicate inside a laminar flow sterile cabinet.

2.4.2. Antimicrobial activity of films tested with culture medium

Tryptic Soy Agar (TSA, Scharlab, Barcelona, Spain) was used for culture medium tests, following the methodology described by previous studies (Requena, Vargas, & Chiralt, 2019a; Tampau et al., 2018). 10 mL samples of TSA were poured into 55 mm petri dishes and inoculated with 100 μL of *E. coli* or *L. innocua* bacterial suspension (10^6 CFU/mL) on the plate surface using a L-form rod to uniformly spread the inoculum. Then, samples were covered with 55 mm round film samples. A non-covered inoculated control was also included. Dishes were closed with their lids, sealed with Parafilm^tm and incubated for 6 days at 10 °C in a culture chamber. After incubation, TSA and films were homogenised for 3 min in buffer peptone water (Scharlab, Barcelona, Spain) by means of a Masticator paddle blender (IUL Instruments, Barcelona, Spain). Serial dilutions were plated and covered with selective media: violet red vile agar (VRBA) for *E. coli* and palcam agar base (PAB) enriched with palcam selective supplement for *Listeria* (Scharlab, Barcelona, Spain), as described by Requena et al. (2019a). After 48 h incubation at 37 °C, the colonies were counted. Each film formulation and control was tested in duplicate. Microbial counts were also performed in duplicate for each sample.

2.4.3. Antimicrobial activity of films tested with food matrices

The antibacterial activity of the films was also tested on two different food matrices, namely chicken breast and melon (var. *Cucumis melo*). Chicken breast and melon were selected as respective models of protein-rich and carbohydrate-rich matrices. These products were purchased from the local market and handled in sterile conditions inside a laminar flow cabinet. The chicken breast was cut into thin fillets, while the fresh melon was peeled and cut into thin slices. Samples (about 10 g) were shaped as cylinders (55 mm in diameter and about 6 mm thickness) and placed on petri dishes of 55 mm in diameter. The samples were inoculated with 100 μL of gram-negative (*E. coli*) or gram-positive (*L. innocua)* bacterial suspension (10^6 CFU/mL), by uniformly spreading the inoculum on the sample surface using a L-form rod. Then, samples were covered with the films, closed with their lids, sealed with Parafilm^tm and incubated for 6 days at 10 °C in a culture chamber. Non-covered growth controls were included. After incubation, samples were processed as described in section 2.4.2 to determine bacterial counts.

2.5. Statistical analysis

A statistical analysis of data was performed through analysis of variance (ANOVA) and regression analyses using Statgraphics Centurion XVII software. Fisher's least significant difference was used at 95% confidence level.

3. Results and discussion

3.1. Physical properties of films

A DSC analysis of the obtained films revealed the glass transition of the plasticised starch that was affected by the presence of phenolic acids. Table 1 shows the midpoint T_g values of the different samples. The T_g values found for glycerol plasticised starch films (S) were similar to those previously reported by other authors for the same type of starch (Menzel et al., 2019; Muller et al., 2017). Acid incorporation led to a decrease in T_g values, coherently with the proportion of acid. This could be due to a partial reduction of the starch chain molecular weight associated with a hydrolytic effect provoked by the acids during melt blending, when high temperatures and shear forces were applied to the material (Menzel et al., 2019). The T_g reduction was higher for the ferulic blends than for the cinnamic, thus indicating a greater hydrolytic potential of the former as its concentration increases.

The TGA analyses of the films showed the mass loss curves of each film formulation as a function of the temperature, which agreed with those described by other authors (Mathew & Abraham, 2008) for starch films. Fig. 1 shows the mass loss curves absolute and derivative for each sample. A first, progressive mass loss took place below 240 °C, at which point bonded water was evaporated, glycerol was evaporated/degraded (Muller et al., 2017) and phenolic acids could also be partially degraded. The TGA curves of pure phenolic acids revealed that ferulic decomposition started at 167 °C, with the peak at 212 °C, while cinnamic started degradation at 180 °C reaching a maximum at 215 °C. In fact, samples with ferulic acid exhibited greater mass loss in this first step, while samples with cinnamic acid presented a shoulder in the main weight loss step starting at about 250 °C, which could be attributed to a slightly retarded cinnamic acid degradation in the presence of a polymer. The second and more intense mass loss step occurred between 250 °C and 300 °C, when the degradation of the main polymeric chains occurred. The onset and peak temperatures of the polymer degradation are shown in Table 1.

The incorporation of phenolic acids into the starch matrices slightly increased the onset and peak temperatures of starch degradation, the ferulic acid exerting the greatest effect. These changes could be attributed to differences in the mean molecular weight of the starch polymers associated with the hydrolytic effect of phenolic acids during melt blending and compression moulding (Menzel et al., 2019).

Table 1
Glass transition temperature (second heating) and onset and peak temperature of the polymer thermal degradation of the different starch films without and with 1 or 2% of ferulic (F) or cinnamic (C) acids.

	DSC	TGA	
	Tg (°C)	Onset (°C)	Peak (°C)
S	98 ± 11[b]	247 ± 1[a]	282± 1[ab]
1F	75 ± 2[ab]	258 ± 2[c]	286 ± 1[b]
2F	56 ± 13[a]	252 ± 1[ab]	285 ± 2[ab]
1C	97 ± 3[b]	253 ± 3[b]	281± 4[a]
2C	80 ± 2[ab]	255 ± 1[bc]	285 ±2[ab]

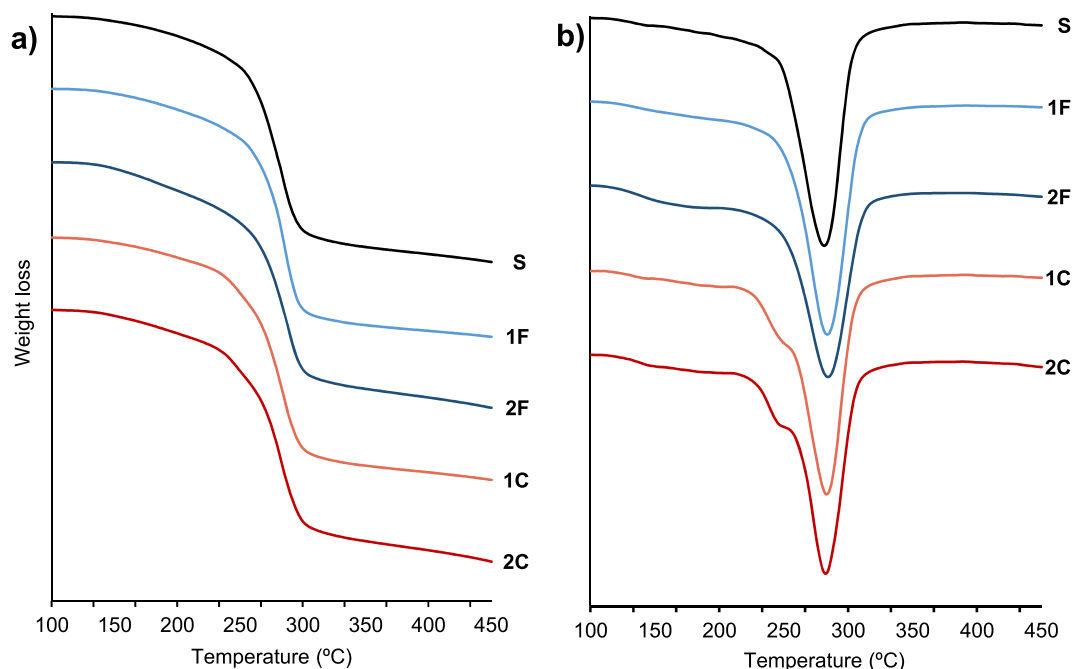Superscript letters in each column indicate significative differences between groups (p < 0.05).

Fig. 1. TGA curves of starch films with and without phenolic acids, a) weight *vs* temperature curve and b) first derivative of weight loss *vs* temperature. Starch without active compounds (S); starch with ferulic acid at 1 and 2% w/w (1F, 2F); starch with cinnamic acid at 1 and 2% w/w (1C, 2C).

3.2. Active compound retention, moisture content and water solubility

Table 2 shows the active compound retention (AC) expressed as g of ferulic or cinnamic acid per 100 g of the compound initially incorporated into the film formulation. Both films containing ferulic acid showed acid retention values of around 60%, which could be due to its partial degradation during the melt blending and compression moulding steps, as a result of the thermal and shear stress. Although TGA on pure ferulic showed an initial degradation at nearly 170 °C, previous studies

Table 2
Properties (Mean values ± standard deviations) of starch films with phenolic acids conditioned at 33% RH; active compound retention (AC, %), moisture (M), water solubility (WS), thickness (t), tensile strength (TS), elongation at break (E), elastic modulus (EM), water vapour permeability (WVP), oxygen permeability (OP), lightness (L^*), hue (h_{ab}^*) and chrome (C_{ab}^*).

	S	1F	2F	1C	2C
AC (%)	–	61 ± 3^a	59 ± 2^a	90 ± 3^b	97 ± 4^c
M (%)	7.6 ± 0.3^{ab}	7.9 ± 0.2^b	8.2 ± 0.3^b	7.2 ± 0.3^a	7.9 ± 0.2^b
WS (%)	86 ± 2^d	73 ± 3^c	71 ± 5^{bc}	61 ± 2^a	67 ± 2^b
t (µm)	142 ± 10^a	137 ± 14^a	143 ± 13^a	135 ± 12^a	143 ± 13^a
TS (MPa)	17 ± 4^c	17 ± 3^c	8.4 ± 0.9^a	11 ± 2^b	9.6 ± 1.2^{ab}
EM (MPa)	830 ± 110^e	720 ± 90^d	280 ± 40^a	550 ± 100^c	440 ± 80^b
E (%)	4 ± 2^a	6 ± 2^a	30 ± 5^c	12 ± 5^b	14 ± 3^b
WVP (g·mm/ kPa·h·m²)	10 ± 2^{ab}	9 ± 2^a	10 ± 1^{ab}	14 ± 2^c	13 ± 2^{bc}
OP (x10^{14}·cm³/ m·s·Pa)	4.2 ± 0.4^a	5.2 ± 0.4^{bc}	5.8 ± 0.5^c	4.7 ± 0.1^{ab}	4.6 ± 0.1^{ab}
L^*	76.4 ± 0.7^a	77.5 ± 0.7^b	76.9 ± 1.3^{ab}	77.0 ± 0.7^{ab}	79.9 ± 0.8^c
h_{ab}^*	76.8 ± 1.2^c	76.7 ± 0.5^c	79.4 ± 0.3^d	74.7 ± 0.2^b	72.0 ± 0.2^a
C_{ab}^*	11.5 ± 0.8^b	12.3 ± 0.5^c	16.4 ± 0.7^d	12.2 ± 0.6^{bc}	10.2 ± 0.3^a

S: starch without active compound, 1F: with 1% ferulic acid, 2F: with 2% ferulic acid, 1C: with 1% cinnamic acid, 2C: with 2% cinnamic acid. Superscript letters in each column indicate significant differences between groups (p < 0.05).

(Yilmaz, Özvural, & Vural, 2011) reported the degradation of phenolic compounds at temperatures as low as 50 °C when exposed to high pressures. Additionally, chemical interactions between ferulic acid, through hydrogen bonds between phenolic group and the starch hydroxyls, could be limiting the release of this acid from the film matrix into the extraction solvent. In contrast, cinnamic formulations presented higher compound retention values (around 90%), hence reduced acid loss, coherently with the greater thermal stability of cinnamic acid.

The incorporation of the phenolic acids did not remarkably affect the equilibrium moisture content (M) of the films (Table 2), and nor was any clear pattern observed as a result of the proportion of acid in the film. The obtained values were coherent with those previously reported for cassava starch/glycerol films (Cano et al., 2014; Menzel et al., 2019). In contrast, the film's water solubility (WS) was clearly reduced by the addition of phenolic acids. Given its hydrophilic nature, films of pure starch exhibit high WS (Cano et al., 2014). The concentration of ferulic acid did not affect the WS reduction and the films g with cinnamic acid (especially 1C) presented the most marked reduction in WS. This suggests that interactions between the carboxyl groups of acids and hydroxyls of starch chains reduced the water affinity of the matrix, inhibiting the formation of hydrogen bonds with water molecules, thus reducing the water solubility. The milder effect of ferulic acid could be attributed to the phenolic group in the aromatic ring that also favours the water interactions with the chain bonded phenolic compound.

3.3. Mechanical, barrier and optical properties

Table 2 also shows the mean values of film thickness (t) and the tensile parameters of conditioned films: tensile strength (TS), Young's modulus (EM) and percentage of elongation at break (%E). No significant differences in film thickness were observed (p > 0.05), while the values were similar to those previously reported for cassava starch/ glycerol monolayers processed under the same conditions (Muller et al., 2017).

As concerns tensile behaviour, Fig. 2 shows the stress-strain curves of every film formulation in which a significant effect of phenolic acid incorporation can be observed. Formulations with 1% ferulic acid did not notably differ from pure starch films and exhibited similar tensile
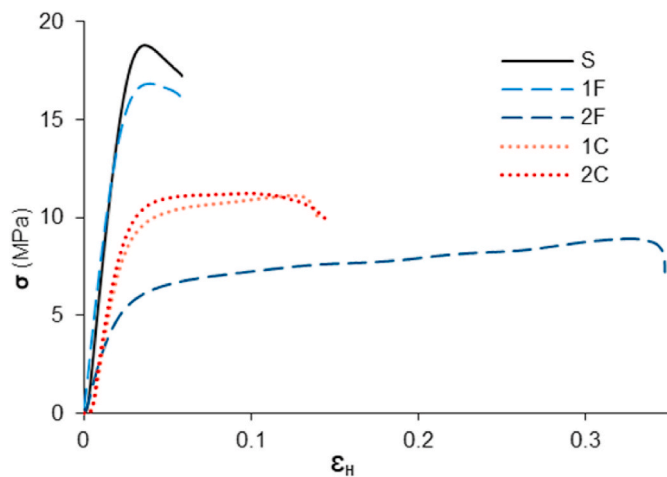
Fig. 2. Stress-strain curves of the films. Starch without active compound (S); starch with ferulic acid at 1 and 2% w/w (1F, 2F); starch with cinnamic acid at 1 and 2% w/w (1C, 2C).

Fig. 3. Internal transmittance (T_i) spectra (400–700 nm) of the films. Starch without active compound (S); starch with ferulic acid at 1 and 2% w/w (1F, 2F); starch with cinnamic acid at 1 and 2% w/w (1C, 2C).

parameters. In contrast, 2% of ferulic acid provoked a strong plasticising effect, as revealed by the sharp increase in the film's extensibility (elongation at break, 6 times higher) and the reduction in the elastic modulus (EM), by more than 500 MPa, and resistance to break (TS). This mechanical plasticisation was consistent with the notable Tg decrease observed in DSC analyses for the highest concentration of ferulic acid. Coherently, it is remarkable that these films with 2% F became very sticky when conditioned at a RH higher than 33%.

Films containing cinnamic acid (1 and 2%) exhibited very similar tensile behaviour, while being more extensible (3 times more) than starch films and having a reduced elastic modulus and lower resistance to break. This was coherent with the similar, small change in the Tg values of the starch matrix observed in DSC analyses for films with 1 and 2% of cinnamic acid. These results indicated that the starch matrix was highly sensitive to the hydrolytic action of ferulic acid as a function of its concentration, whereas the increase in the cinnamic acid content in the matrix did not provoke such important changes in the properties of the starch matrix.

The barrier properties (OP and WVP values) of the film formulations are also shown in Table 2. Both OP and WVP values were in the range reported in previous studies (Menzel et al., 2019; Muller et al., 2017) for cassava starch films and were hardly affected by the presence of the phenolic acids. Ferulic acid did not significantly affect the water vapour barrier capacity of the films at 1 or 2%, despite the observed differences in the matrix plasticisation, although it did slightly promote OP. However, Mathew and Abraham (2008) reported an improvement in both the water vapour and oxygen barrier capacity after the addition of a low ratio of ferulic acid in starch/chitosan films.

Although all the films were transparent and homogenous, with 2% C they exhibited a certain whiteness. This is reflected in Fig. 3, where the internal transmittance (Ti) spectra are shown with lower values for the 2C sample, while similar spectra were observed for the rest of the films. Colour parameters, lightness, hue and chroma (Table 2) revealed the greater lightness of the less transparent 2C films and the slightly yellowish and more saturated colour of films with 2% ferulic acid.

3.4. Antibacterial effectiveness of the films

The MIC values of phenolic acids for *E coli* and *L. Innocua* were determined to evaluate their antibacterial effectiveness against these specific strains. For *E. coli*, this value was 0.80 mg/mL for ferulic acid, slightly lower than that reported by Meira et al. (2017). However, the MIC value found for *L. innocua* with ferulic acid was 0.70 mg/mL, in the range of that reported by Miyague et al. (2015) who observed a marked
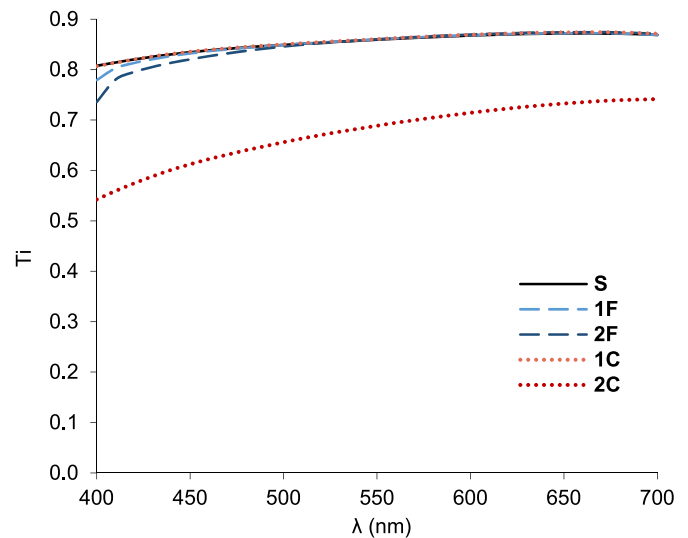
effect of the pH on the MIC values of *Listeria*. In the case of cinnamic acid, the MIC values were 0.70 and 0.65 mg/mL, respectively, for *E. coli* and *L. innocua*. These were also in the range of those previously reported by other authors (Miyague et al., 2015; Olasupo et al., 2003). These values indicate that cinnamic acid was more effective than ferulic acid against both bacteria and that both compounds exhibited a greater antibacterial effect against *L. innocua* than against *E. coli*. These MIC values were used to determine the dose of active compounds required in the films in order to obtain active films against the tested bacteria, assuming a complete active release in the culture medium or food substrate.

Fig. 4a shows the microbial counts obtained in the antibacterial test in the culture medium and Table 3 shows the reduction obtained in the log CFU, with respect to the uncovered control plates. The counts in TSA samples covered with phenolic-free starch films were similar to those obtained in the uncovered control plates; hence, the antimicrobial effectiveness (count reduction) of the rest of the films could be attributed to the action of the incorporated phenolic acids. In fact, the antimicrobial effectiveness of the films was highly dependent on the phenolic acid concentration and those containing the lowest proportion (1F and 1C) did not exhibit a significant inhibitory effect against the tested bacteria, according to the small amount potentially released of the active, compared to the bacteria MIC. In contrast, a reduction of more than 2 log in the bacterial counts of *L. Innocua* was obtained for films with 2 wt% of both acids; cinnamic acid being much more effective. However, these active compounds at 2 wt% were less effective against *E coli*, where lower growth inhibition was observed. In general, a more remarkable growth inhibition was observed for films containing cinnamic acid than for those with ferulic acid, while the films were more effective against *L. innocua* than against *E. coli*, coherently with the respective MIC values. It is remarkable that films with 2 wt% cinnamic acid provoked a reduction of more than 6 log CFU in the listeria counts in the culture medium after 6 incubation days.

Fig. 4b and c shows the results of the antibacterial tests performed on chicken breast and fresh-cut melon with the films containing 1 and 2 wt % of phenolic acids. As shown in Fig. 4b, the starch films with 2 wt % phenolic acids effectively protected chicken breast from bacterial growth (more than 2 Log reduction), both for *E. coli* and *L. innocua*. In this food matrix, cinnamic acid was also more active than ferulic acid against both bacteria and films with 2 wt % of cinnamic acid exhibited an almost bacteriostatic effect in both cases.

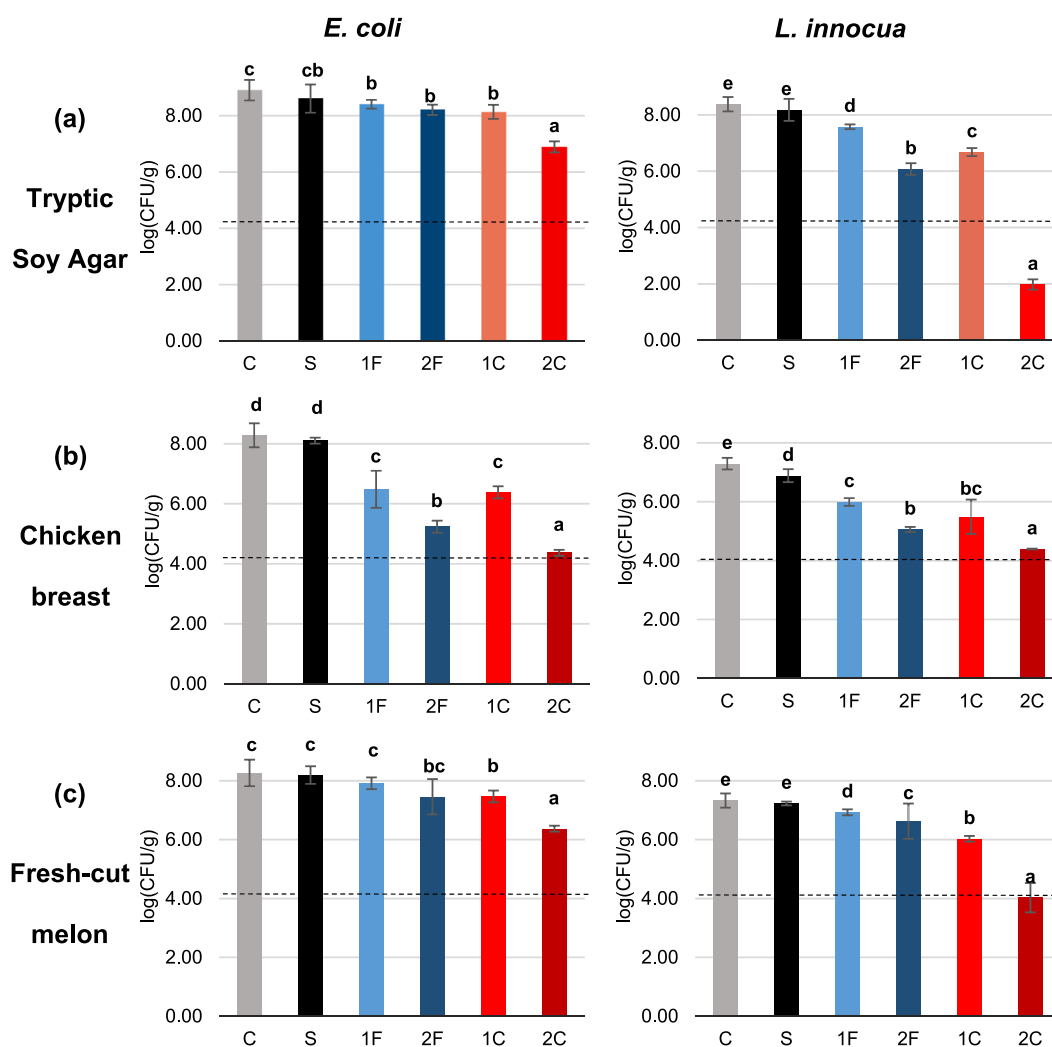As compared to TSA and chicken breast, the effectiveness of the films

Fig. 4. Microbial counts for *E. coli* and *L. innocua,* obtained after 6 days of incubation at 10 °C in (a) TSA culture media, chicken breast (b) and fresh-cut melon (c). Uncovered control sample (C) and samples covered with films of active-free starch (S), with 1 or 2 wt% ferulic acid (1F, 2F) and with 1 or 2 wt % cinnamic acid (1C, 2C). Dotted lines show the initial microbial counts. Different letters above the bars show homogeneous sample groups (p < 0.05).

Table 3
Log CFU reduction for *E. coli* and *L. innocua* in TSA and food matrices obtained with the different films: active-free starch (S), with 1 or 2 wt% ferulic acid (1F, 2F) and with 1 or 2 wt% cinnamic acid (1C, 2C). Mean values and standard deviations.

		TSA	Chicken	Melon
S	*E. coli*	0.3 ± 0.5^{a1}	0.2 ± 0.1^{a1}	0.1 ± 0.1^{a1}
	L. innocua	0.2 ± 0.4^{a1}	0.4 ± 0.2^{a1}	0.1 ± 0.1^{a1}
1F	*E. coli*	0.5 ± 0.2^{a1}	1.8 ± 0.2^{a2}	0.4 ± 0.2^{a1}
	L. innocua	0.8 ± 0.1^{a2}	1.3 ± 0.1^{a2}	0.4 ± 0.1^{a1}
2F	*E. coli*	0.7 ± 0.2^{a1}	3.1 ± 0.2^{b2}	0.8 ± 0.1^{a1}
	L. innocua	2.3 ± 0.2^{b2}	2.2 ± 0.2^{b1}	0.7 ± 0.6^{a1}
1C	*E. coli*	0.8 ± 0.2^{1}	1.9 ± 0.2^{a2}	0.8 ± 0.2^{a1}
	L. innocua	1.7 ± 0.1^{2}	1.8 ± 0.1^{a2}	1.3 ± 0.1^{b1}
2C	*E. coli*	2.0 ± 0.2^{a1}	4.0 ± 0.1^{b2}	1.9 ± 0.1^{b1}
	L. innocua	6.4 ± 0.2^{b2}	2.9 ± 0.0^{a1}	3.3 ± 0.5^{c2}

Superscript letters (a-c) in each column and numbers (1–2) in lines indicate significant differences between values (p < 0.05).

against *E.coli* in fresh-cut melon was less remarkable. The growth of both bacteria was only significantly with inhibited (more than 2 log reduction) by cinnamic acid at 2%, while ferulic acid slightly reduced the CFU counts of both bacteria at the same concentration. Nevertheless, a notable antilisterial effect was observed in this food matrix for films

containing cinnamic acid, with more than 3 log CFU reduction. Requena et al. (2019a) also found a very slight growth inhibition of these bacteria in fresh-cut melon coated with active films containing carvacrol and eugenol. This was explained both by the fact that melon tissue provides a source of good nutrients for the bacteria and by the potential interactions of the active compounds with the melon components that could limit their antibacterial action. However, the antilisterial effect observed for cinnamic acid from starch films in this food matrix opens the possibility of their use for controlling the growth of this foodborne pathogen, resistant at low temperatures, such as those used for the preservation of fresh-cut fruits.

A comparison of bacterial growth inhibition in TSA and food matrices reveals the significant role that the interactions between the food components, bacterial cells and released active compounds play in the antimicrobial effectiveness of the films. Food nutrients promote bacterial growth to a different extent, and these can also interact with active compounds limiting or enhancing their ability to act against the cells. The amount of active released from the films, as well as the release rate, also plays an important role in the active film's effectiveness. An amount that is sufficient to reach the MIC value of bacteria is required, and its release should occur when the bacterial growth is still in the exponential phase when bacteria are more sensitive to the antimicrobial action. In highly water sensitive starch films, a fast and almost complete release of acids could be expected due to the relaxation of the polymer

matrix in contact with the moist food. Polymer relaxation associated with the film hydration favours the diffusion of active compound into the food in contact with the film. In this sense, phenolic acid-starch coatings could be used as active layers for food contact in multilayer assemblies with other hydrophobic polymer sheets to obtain active laminate materials with adequate functional properties for food packaging.

4. Conclusions

Active starch films with ferulic or cinnamic acid could be obtained by thermo-processing with small modifications of the films' functional properties. Incorporation did not notably affect the barrier properties of the starch films, although these became less water soluble, more extensible, and less resistant to break. Both cinnamic and ferulic acids conferred antibacterial activity to the starch films against *E. coli* and *L. innocua,* which was probed in culture medium and food systems (chicken breast and fresh cut melon). Films with cinnamic acid were more effective than with ferulic acid and both were more active against *L. Innocua* than against *E. coli.* The antibacterial activity of the films was affected by the kind of acid and strain and the food substrate. The highest effectiveness was obtained for films with cinnamic acid, against *L. innocua,* in chicken. breast and fresh-cut melon.

Therefore, although highly hydrophilic starch films had limitations for wet food packaging, starch layers with 2% ferulic or cinnamic acid could be used as active layers for food contact in multilayer assemblies, combined with other hydrophobic polymer sheets of complementary properties, to obtain active laminates that could provide remarkable antimicrobial capacity and, in particular, antilisterial activity.

CRediT authorship contribution statement

Ramón Ordoñez: Investigation, Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Lorena Atarés:** Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Amparo Chiralt:** Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration.

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 (Antimicrobial cassava starch films with ferulic or cinnamic acid) submitted to the journal LWT – Food Science and Technology.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111242.

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