Incorporation of natural antioxidants from rice straw into renewable starch films

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ABSTRACT

This study showed that rice straw waste is a valuable source for the extraction of water-soluble phenolic compounds that can be successfully incorporated into bioactive starch-based films. The major phenolic compounds in the extract were identified as ferulic, p-coumaric and protocatechuic acid using UHPLC-MS. Homogeneous films with antioxidant properties were produced by melt blending and compression molding and the changes in the physico-chemical properties were evaluated. The produced antioxidant starch films were slightly reddish-colored and exhibited good in-vitro antiradical scavenging activity against DPPH*. The addition of the antioxidant extract improved the oxygen barrier properties without negatively affecting the thermal and the water vapor barrier properties. However, antioxidant starch films turned more brittle with increasing amount of the antioxidant extract, which was probably due to interactions of phenolic compounds with the starch chains. The film forming process induced chain scission of starch molecules in all films, shown in a decrease in molecular weight of native starch from 9.1×10^6 Da to values as low as $1.0–3.5 \times 10^6$ Da. This study aids a circular economy by recycling rice straw for the production of bioactive food packaging.

1. Introduction

Rice is the most important crop throughout Asia. According to the Food and Agriculture Organization of the United Nations, the annual world rice production in 2017 was estimated 770 million tons, with China and India together accounting for almost 50% of the world market and a production of 2.98 million tons alone in Europe (FAOSTAT, 2017). After harvest, rice straw is typically threshed and burned, resulting in a loss of valuable soil and nutrients and, at the same time, contributing heavily to air pollution. The amount of burned rice crop residues are calculated to be 87 million tons globally and within Europe around 0.3 million tons (FAOSTAT, 2016). Hence, new viable alternatives are needed to utilize this crop residue [1]. For instance, rice straw has been shown to be a valuable source of bioactive compounds, such as

gallic acid, caffeic acid, pyrogallol and many flavonoids [1–3]. In addition, the phenolic extracts of rice straw have shown antioxidant activity based on several free radical scavenging activity assays [2,3], which to the best of our knowledge, has not been utilized yet. Hence, bioactive extracts from rice straw are of interest as additive for bio-based packaging materials to produce active food packaging, which has successfully been shown for other agro-industrial and food wastes to obtain active biodegradable films for packaging [4–7]. Active packaging technologies provide added properties to the packaging, such as antimicrobial and antioxidant activity and, therefore, positively affecting the shelf-life of a food product [8]. Such bioactive additives origin often from natural plant extracts rich in phenolic compounds that enhance antioxidant and antimicrobial properties in edible films. In particular, recent efforts have been made to utilize plant by-products such as fruit peels and seed hulls as source of active compounds for edible packaging [4,7,9,10]. For example, dried and grinded blueberry peel was incorporated into starch to manufacture starch films with enhanced properties, i.e. UV-light barrier and lower water solubility [11]. Furthermore, phenolic extracts

from sunflower hulls were successfully incorporated into fully bio-based and antioxidant starch films [9].

Starch has successfully been used in different active film preparations and has shown to work as carrier of antioxidants and antimicrobials, which could be released into food or the surrounding environment [9,11,12]. Starch is a biopolymer that is well-known for its ability to form transparent thermoplastic materials with excellent oxygen barrier properties using thermal-mechanical treatments [13]. However, in its native form, starch films are very brittle and hydrophilic, hence, plasticizers such as water or glycerol are used to produce flexible films and chemical modifications like cross-linking are needed to overcome its hydrophilic character [14,15]. For example, Mathew and Abraham [16] used oxidized ferulic acid to cross-link starch-chitosan blend films and improve its physico-chemical properties.

This study aims to valorize an antioxidant extract from rice straw using optimized extraction conditions, analyzing its active compounds and properties. Then, the extract will be used to produce potential biodegradable antioxidant packaging material by its incorporation into potato starch films using melt blending and compression-molding. The influence of the extract on molecular structure of starch films will be evaluated as well as the antioxidant, antimicrobial, barrier, mechanical and thermal properties.

2. Material and methods

2.1. Material and chemicals

Rice straw was collected from rice fields (Albufera, Valencia, Spain), dried, milled and sieved to < 0.5 mm particle size. Potato starch was supplied by Roquette (France) with an amylose content of 27%, which was calculated as the area under the curve of the branch chain-length distribution of debranched starch according to Vilaplana, Hasjim and Gilbert [18]. Glycerol, sodium carbonate, methanol and ethanol were purchased from PanReac Quimica S.L.U. (Castellar del Vallés, Barcelona, Spain). Gallic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteau reagent (2N), p-coumaric, ferulic and protocatechuic acids were purchased from Sigma-Aldrich (Saint Louis, USA). All other reagents and solvents were of analytical grade. Phosphate buffered saline, tryptone soy broth, tryptone soy agar and thiazolyl blue tetrazolium bromide (MTT) reagents were purchased by Scharlab (Barcelona, Spain). *Escherichia coli* (CECT 101) and *Listeria innocua* (CECT 910) were obtained from the Spanish Type Collection (CECT, University de Valencia, Spain).

2.2. Extraction and characterization of phenolic compounds from rice straw

Extraction of phenolic compounds was carried out at a rice straw: solvent ratio of 1:10 for 1 h at room temperature, the same material was subsequently extracted a second and a third time. Three different solvents were tested including water, 80% MeOH in water and 80% EtOH in water. Subsequently, the organic solvent was evaporated at 35 °C under vacuum, the extract was re-dissolved in water and then lyophilized to determine the exact dry weight. All extractions were done in triplicates.

2.2.1. Total phenolic content

The amount of total phenolic content was analyzed using Folin-Ciocalteau reagent and results are expressed as mg gallic acid equivalents (GAE) per 100 g dry weight of rice straw using a gallic acid standard calibration as described elsewhere [9].

2.2.2. Analysis of phenolic compounds using UHPLC-HRMS

Ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) analysis of rice straw extracts were performed using an Ultimate UHPLC system equipped with a photodiode array detector (Dionex), and a Q-exactive quadrupole Orbitrap mass spectrometry system (ThermoFisher Scientific) (LC-HRMS), as described previously by Grosso, Farina, Giorgi, Nardi, Diretto and Lucretti [19] and Cappelli, Giovannini, Basso, Demurtas, Diretto, Santi, Girelli, Bacchetta and Mariani [20] with slight modifications. LC separation of phenolic compounds was carried injecting 5 μl of samples on a Luna C18 reverse-phase column (150 × 2.1 mm, 3 μm; Phenomenex). Metabolites were ionized in a heated electrospray ionization (HESI) source, with nitrogen used as sheath and auxiliary gas, set to 40 and 20 units, respectively. The vaporizer and capillary temperature were set to 270 °C and 300 °C, respectively. The discharge current was set to 5 μA, and S-lens RF level was set at 50. The acquisition was performed in the mass range 110–1600 m/z, both in positive and in negative ion mode with the following parameters: resolution 70,000, microscan 1, AGC target $1xe^6$, and maximum injection time 50. Phenolics targeted analysis was carried out by compiling, first of all, all previously identified compounds in rice straw, by using literature search [3,21,22], as well as using a custom *in house* database. Metabolite identification was achieved by comparing chromatographic and MS properties of each compound with authentic standards, if available; on the basis of the m/z accurate masses, as found in the Pubchem database for monoisotopic masses, or in the Metabolomics Fiehn Lab Mass Spectrometry Adduct Calculator for adduct ions; and, on the basis of mass fragmentation, as found in Metlin, or through the comparison between experimental and theoretical mass fragmentation, performed using MassFrontier 7.0 software (Thermofisher Scientific). Quantification of each phenolic compound was performed by calculating the relative contents to the formononetin internal standard levels. For the three compounds showing the highest ion intensity (p-coumaric, ferulic and protocatechuic acid), absolute amounts were calculated performing external calibration curves with authentical standards.

2.2.3. Antiradical activity of rice straw extract using DPPH* assay

The assay was in accordance with Brand-Williams, Cuvelier and Berset [23] including small modifications (using 0.06 mM DPPH in methanol and 2 h of reaction time). All characterizations were done in triplicates and results were expressed as efficient concentration of antioxidant in the raw material to decrease the initial DPPH* concentration by 50% (EC_{50}, mg dried rice straw/ mg DPPH*).

2.2.4. Antimicrobial activity of rice straw extract

Antimicrobial activity against gram-positive and gram-negative bacteria of rice straw extracts were tested against *E. coli* (CECT 101) and *Listeria innocua* (CECT 910) using the colorimetric thiazolyl blue tetrazolium bromide (MTT) assay on 96-well microtiter plates according to Houdkova, Rondevaldova, Doskocil and Kokoska [24]. Cell metabolic activity was determined measuring the yellow to purple color change due to the NAD(P)H-dependent cellular oxidoreductase enzyme activity, which reflects the number of viable cells present. The two bacterial strains were grown on tryptone soy buffer (TSB) and diluted to a working solution of 10^5 colony forming units (CFU). The freeze-dried rice straw extracts were dissolved in TSB to a concentration of 100 mg/ ml. The minimum inhibitory concentration (MIC) determination of both bacteria strains were performed in 96-well plates with the following scheme: for each bacteria strain, 100 μl of the 10^5 CFU working solution of each bacteria strain were added to the wells and 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 μl sample solution were added and completed with the appropriate amount of TSB to give a final volume of 200 μl in each well. The plates were incubated at 37 °C for 24 h. Afterwards, 10 μl MTT solution were added to each well and incubated again for 4 h at 37 °C. Finally, the bacterial cell growth was checked visually by observing a change of color. The amount of sample that showed no purple colour formation indicates the approximate MIC.

Table 1
Total phenolic content in mg GAE/100 g dry milled rice straw using different extraction solvent, and repeated extractions (stirred with magnetic stirrer at 300 rpm at room temperature for 1 h), individual phenolic acid content and EC_{50} values in mg dry rice straw per mg DPPH* of 1st extract at same extraction conditions.

	Extraction solvent*		
	H_2O	80% MeOH	80% EtOH
Total phenolic content			
1st Extraction	225 ± 21	153 ± 22	138 ± 1
2nd Extraction	107 ± 31	56 ± 6	62 ± 13
3rd Extraction	33 ± 3	40 ± 8	34 ± 3
Individual phenolic acid content			
Ferulic acid [mg/g extract]	36 ± 5	4.3 ± 0.4	4.0 ± 0.3
p-coumaric acid [mg/g extract]	16 ± 3	14 ± 3	8.6 ± 0.1
Protocatechuic acid [mg/g extract]	25 ± 4	3.2 ± 0.4	4.0 ± 0.3
$^1EC_{50}$ *values*			
mg dry rice straw/ mg DPPH	153	334	400
mg freeze-dried extract/mg DPPH*	12.0	20.3	19.8

* Yield of freeze-dried extract using water 7.82 wt%, MeOH 6.09 wt% and EtOH 4.96 wt%.

2.3. Antioxidant starch film preparation and characterization

2.3.1. Melt blending and compression molding of starch film with aqueous rice straw extract

Native potato starch was blended with glycerol in a 1:0.25 (w, w) ratio, using 40 g starch and 10 g glycerol (control starch film referred as CS_10G). To produce the antioxidant starch films, glycerol was substituted by three different amounts of the antioxidant rice straw extract, 2 g, 3 g and 4 g (starch films with antioxidant rice straw extract referred as RS_2,3,4A; sample abbreviations see Table 2) and another starch formulation with 10 g glycerol and 3 g antioxidant extract (starch film with rice extract referred as CRS_3A). Furthermore, one starch formulation with 40 g starch and 7 g glycerol was prepared for comparison (referred as CS_7G). The blends were mixed using an internal mixer (Haake PolyLab QC, Thermo Fisher Scientific, Germany) at 160 °C for 7 min and 50 rpm. Afterwards, films of about 200 μm thickness were produced by compression-molding at 160 °C and 30 bar (2 min) followed by 130 bar (6 min), and were conditioned in a desiccator containing a saturated solution of magnesium nitrate at 53% relative humidity (RH) for 7 days before further analysis.

2.3.2. Changes in molecular structure of starch using size-exclusion chromatography

Changes in molecular structure of starch during thermal processing were determined by analyzing molecular weight distribution of starch molecules using size-exclusion chromatography (SEC) and changes in branch chain-length after debranching with isoamylase. The analysis was carried out according to Vilaplana and Gilbert [25]. In brief, laser light and refractive index detection (DRI) were used to determine size, molar mass and branch chain-length distribution of starch. Starch polymers were separated by their hydrodynamic volume (V_h), which for branched polymers are equivalent to the corresponding hydrodynamic radius R_h with $V_h = \frac{3}{4}\pi R_h^3$. The size distribution was measured with DRI to obtain the weight distributions $w(\log V_h)$.

2.3.3. Physical properties of the starch films

2.3.3.1. Appearance and microstructure of the film.
Digital pictures of all films were taken using a conventional camera and thickness of the conditioned films were measured using a digital electronic micrometer with an accuracy of 0.001 mm (Palmer model COMECTA, Barcelona). Cross-section of all starch films were analyzed using a field emission scanning electron microscope (FESEM, ZEISS ULTRA 55 model, Zeiss, Germany). For the FESEM analysis, all films were previously dehydrated
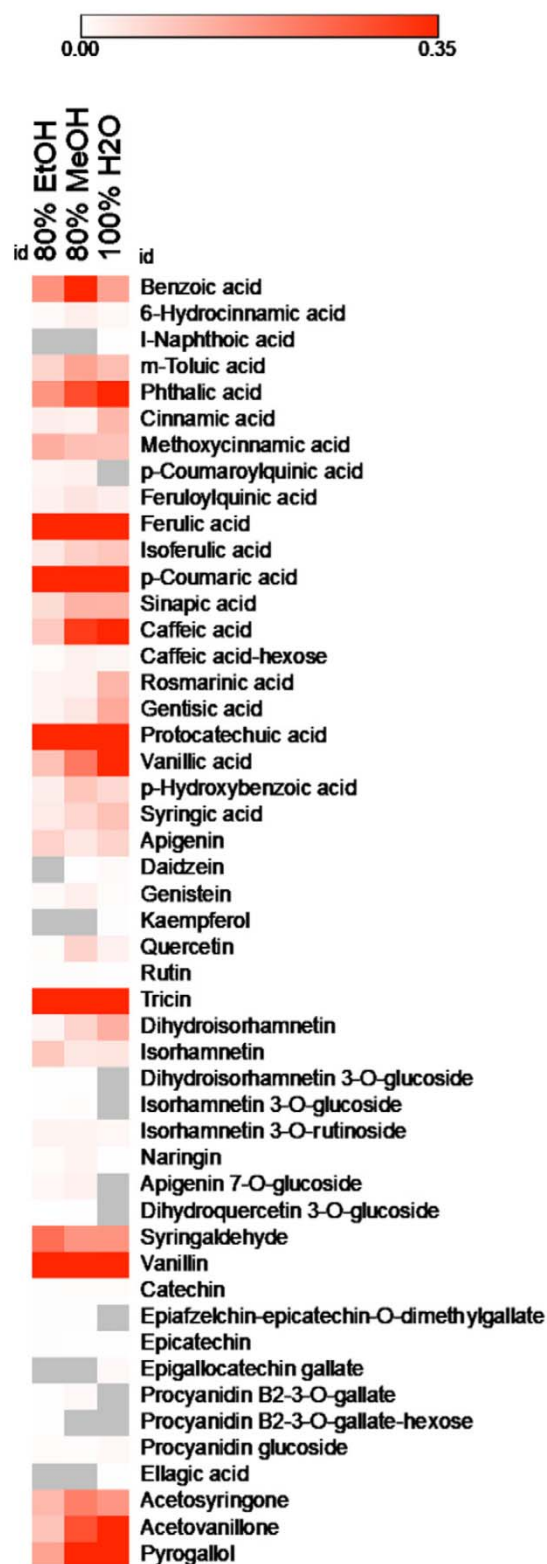

Fig. 1. Heatmap visualization of phenolic compounds detected in rice straw extracted with water, 80%MeOH or 80% EtOH. For each metabolite, data represent the fold level with respect to the signal intensity of the internal standard (formononetin).

at 0% RH over phosphorous pentoxide and cryo-fractured using liquid nitrogen, and subsequently placed on graphite stickers and gold coated. Images of cross-sections were taken using an acceleration voltage of 1.5 kV.

Table 2

Sample abbreviation and composition of starch-glycerol films with and without antioxidant extract from rice straw (AOE). Number-average molecular weight M_n, weight-average molecular weight M_w and polydispersity D for branched starches using light scattering and peak maximum of degree of polymerization X_{DP} in the three regions of debranched samples and height ratio AP2/AP1 (AP – amylopectin, AM – amylose) using the DRI calibration.

Sample	Composition [g]			Branched Starch			Debranched Starch			
Abbreviation	starch	Gly	AOE	$M_w[10^6$ Da]	$M_n[10^6$ Da]	D	$X_{DP,AP1}$	$X_{DP,AP2}$	AP2/AP1	$X_{DP,AM}$
Native starch				9.10	8.07	1.13	26	48	1.07	7475
CS_7G*	40	7	–	1.03	0.61	1.70	24	46	0.94	464
CS_10G*	40	10	–	3.57	2.36	1.36	24	50	0.93	1240
CRS_3A	40	10	3	2.24	1.48	1.52	24	48	0.99	1162
RS_2A	40	8	2	1.75	1.11	1.57	25	48	0.99	965
RS_3A	40	7	3	1.58	0.84	1.89	26	48	1.02	764
RS_4A	40	6	4	1.11	0.28	3.99	26	48	1.02	527

* Data from reference samples from [9], CS – control starch films with 7 g and 10 g Glycerol (G), RS – starch films with 2, 3 or 4 g antioxidant rice straw extract, CRS – controls starch film with 10 g glycerol and 3 g antioxidant rice straw extract.

2.3.3.2. Color and transparency. Color measurement was carried out using a MINOLTA spectrocolorimeter (Model CM-3600d, Tokyo, Japan) measuring the reflection spectra from 400 to 700 nm (10 nm bandwidth, specular component included) of the films backed on black and white plates at three points of the same film sample. The internal transmittance was measured by applying the Kubelka-Munk theory of the multiple dispersion of reflection spectrum using the reflection spectra of the white and black backgrounds. The CIELab color coordinates
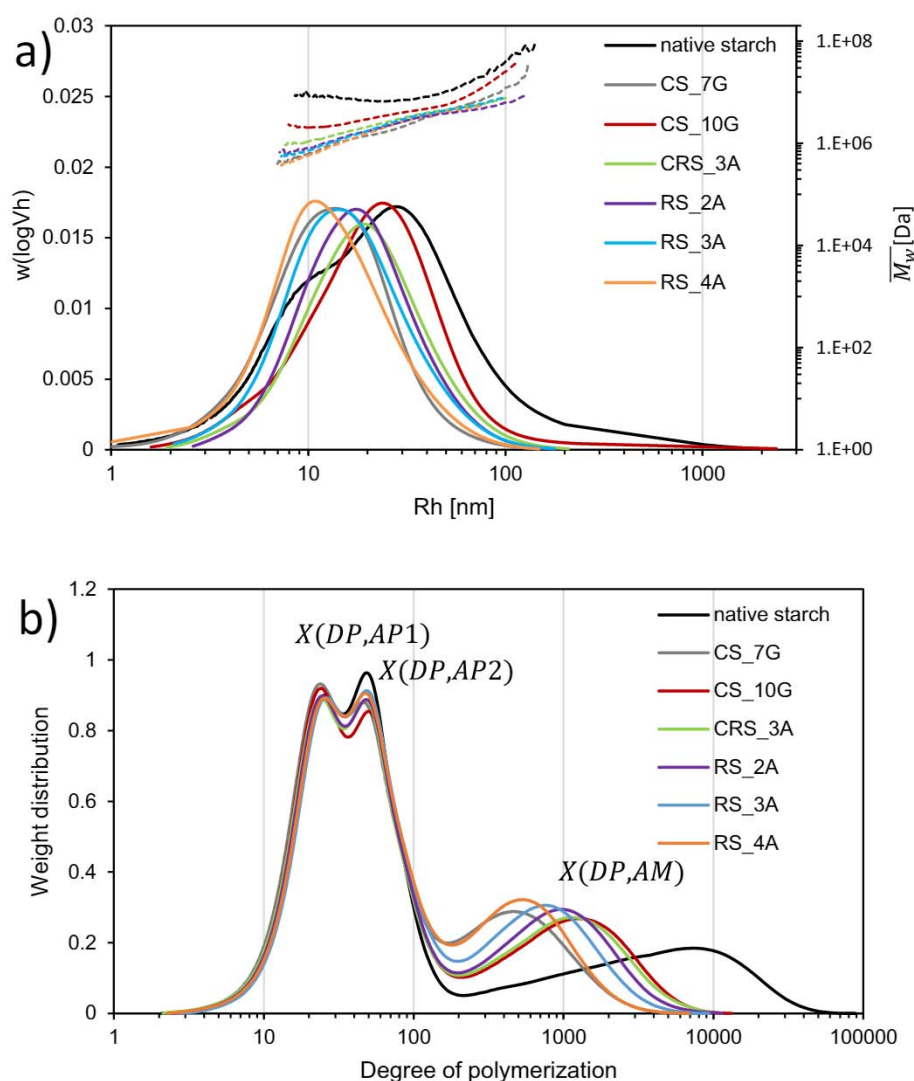

Fig. 2. (a) SEC weight distribution and weight-average molecular weight Mw as function of hydrodynamic radius (Rh) and (b) SEC weight distribution of debranched starches as function of their degree of polymerization for native potato starch and all starch films with rice straw antioxidant extract dissolved in DMSO/LiBr 0.5%.

(illuminant D65 and observer 10°) were obtained from the reflectance of an infinitely thick layer of the material [26].

2.3.3.3. Barrier properties. Oxygen permeability of starch films was determined at 53% RH and 25 °C using an Ox-Tran equipment (MOCON Model 1/50, Minneapolis, USA). Oxygen permeability was calculated by multiplying oxygen transmission rate with the average film thickness of each starch film. Water vapor permeability of starch films was determined at a 53–100% RH gradient using the ASTM E96-95 gravimetric method (1995) for hydrophilic films. Thereby, films were placed in cups with water (100% RH) and cups were put in desiccators equilibrated to 53% RH using a saturated magnesium nitrate solution and weight loss was measured periodically over 24 h. Water vapor permeability was calculated by taking film thickness into account. Both barrier measurements were carried out in duplicates.

2.3.3.4. Equilibrium moisture content. The moisture content of all produced starch films, which were previously conditioned at 53% RH for one week, were determined gravimetrically before and after drying. The films were dried at 60 °C for 48 h under vacuum and kept at 0% relative humidity for 2 days at room temperature in sealed chambers containing phosphorous pentoxide until constant weight.

2.3.3.5. Mechanical properties. At least six replicates of each film formulation were used to determine tensile properties using a Universal testing machine (Stable Micro System TA, XT plus, Haslemere, England) and following the ASTM standard method. Therefore, the films were cut into 25 mm × 80 mm pieces and were conditioned at 25 °C and 53% RH for one week. The film stripes were mounted into the equipment, and a stretching of 50 mm/ min was performed. Tensile strength, elongation and Younǵs modulus were calculated from the stress–strain curves, based on the average film thickness measured at six points for each film.

2.3.3.6. Thermogravimetric analysis. The thermal stability of dried starch films was determined using a thermogravimetric analyzer (TGA/SDTA 851e, Mettler Toledo, Schwarzenbach, Switzerland) by heating the samples from 25 °C to 600 °C at 10 K/ min. The glass transition temperature of dried films was obtained using differential scanning calorimetry (DSC 1 StareSystem, Mettler-Toledo, Inc., Switzerland) and two heating cycles. First, samples were heated from 25 °C to 160 °C at 5 K/ min, held for 5 min at 160 °C, cooled to 10 °C and rested for 5 min, and then a second heating cycle was performed to 160 °C at 10 K/ min. All characterizations were carried out in triplicates.

2.3.4. In-vitro antioxidant activity of active starch films

About 1 g of each film was suspended in 50.0 ml water using a roto-stator homogenizer for 1 min at 9,000 rpm and afterwards, the solution was stirred with a magnetic stirrer for 12 h at 200 rpm and room temperature. An aliquot of the sample dispersion was used for the DPPH* assay as described above and, if needed, filtered using a 0.45 μm nylon filter before reading the absorbance at 515 nm. All measurements were carried out in triplicates.

2.4. Statistical analysis

IBM SPSS Statistics 25.0.0 software was used for analysis of variance (ANOVA) and Tukeýs HSD post hoc test in case of equal replicates and Gabriel post hoc test for unequal amount of replicates. In case of
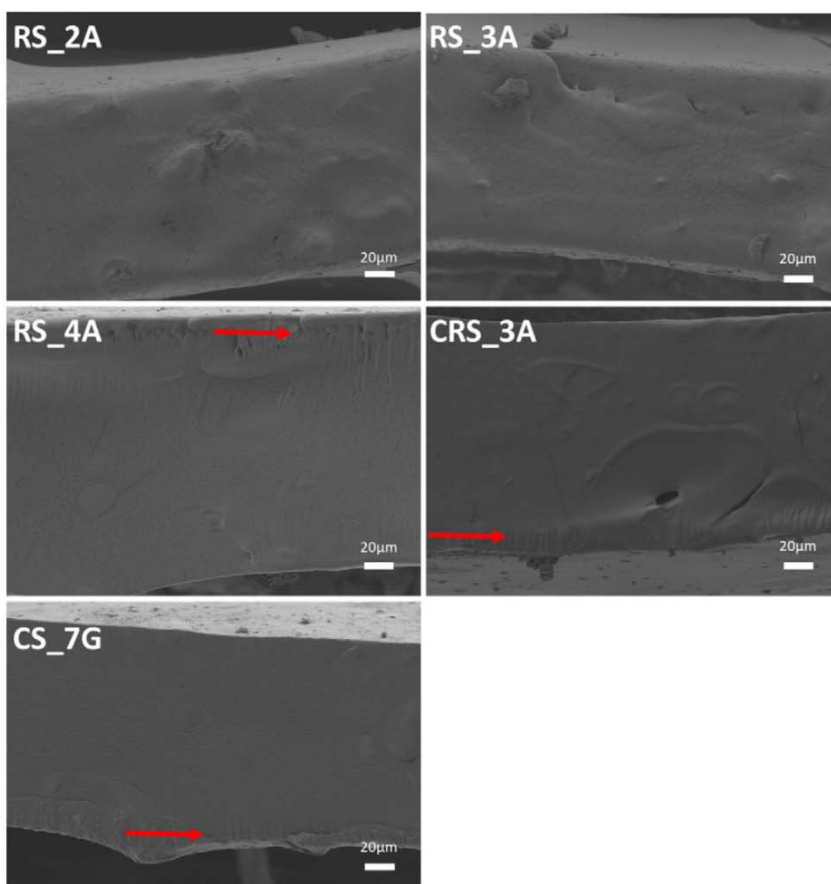

Fig. 3. FESEM images of cross sections of starch films with 2, 3 and 4 g antioxidant extract (RS_2A, RS_3A, RS_4A), with 3 g antioxidant extract and 10 g glycerol (CRS_3A) and without antioxidant extract and 7 g glycerol (CS_7G).

duplicate analysis, a simple *t*-test was used at p < 0.05. Heatmap of rice straw phenolic compounds was generated as previously reported [27].

3. Results and discussion

3.1. Extraction of phenolic compounds from rice straw

3.1.1. Total phenolic acid content using Folin-Ciocalteau reagent

The efficiency of the extraction of phenolic compounds using water, aqueous 80% MeOH or aqueous 80% EtOH was evaluated based on the total phenolic content (Table 1). Water was the most efficient solvent to extract phenolic compounds, resulting in 225 mg GAE/100 g dry rice straw. Subsequent extraction of the material (2nd and 3rd extraction) showed additional extraction of remaining phenolic compounds but at low concentrations. Similar amounts of 221 mg GAE were reported earlier in rice straw extracts by others [3].

3.1.2. Identification of phenolic compounds

A detailed phenolic compound characterization was carried out on all three extracts, the aqueous, methanolic and ethanolic extract,
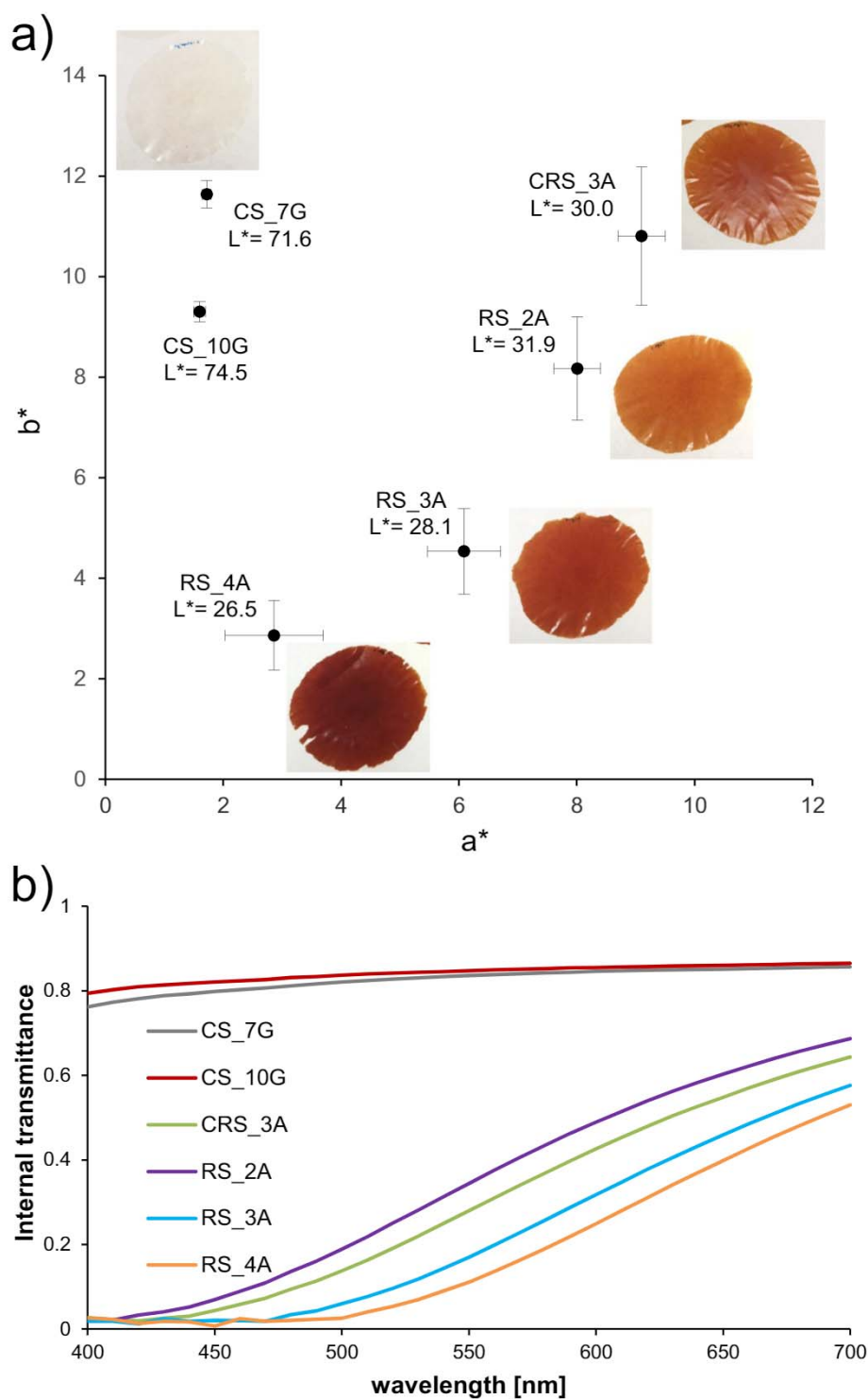

Fig. 4. (a) CIELab coordinates and digital images of starch films. Data labels contain L* value, (b) internal transmittance of starch films between 400 and 700 nm.

using UHPLC-MS. A graphical representation of the identified phenolic acids is shown in the heat map in Fig. 1 (and Supplementary Table 1 including all relative amounts), where intensive red color indicates high abundance of a compound. The three most abundant phenolic acids were ferulic, p-coumaric and protocatechuic acid (Table 1). The aqueous extract showed highest content of these three acids, which is in accordance with the trend shown in the total phenolic content measured by Folin-reagent above (Table 1). The discrepancy between the individual phenolic acid content and the higher phenolic contents determined by Folin-reagent are due to its lower specificity in the presence of reducing interferants like soluble proteins [28]. Nevertheless, the phenolic acid composition is in agreement with the seven major phenolic acids reported earlier by Elzaawely, Maswada, El-Sayed and Ahmed [3] in alkaline pretreated rice straw extracts namely ferulic acid, protocatechuic acid, p-coumaric acid, caffeic acid, syringic acid, rosmarinic acid and cinnamic acid.

3.1.3. Antiradical activity of rice straw extract using DPPH* assay

The antioxidant capacity of the three different extracts was determined using DPPH* assay and EC_{50} values were calculated, which were referred to the dry rice straw raw material and the resulting freeze-dried extract basis (Table 1), were calculated. The aqueous extract showed the lowest EC_{50} values, corresponding to the high amount of phenolic compound with a high activity towards DPPH radicals. The extract from aqueous 80% MeOH showed a twofold increase of EC_{50} values, assuming that only 50% of phenolic compounds/ antioxidants of the rice straw material were extracted compared to the aqueous extract. However, the total phenolic content was about 75% compared to the water extract (Table 1), which implies that the nature of the extracted phenolic compounds differed strongly in activity depending on the solvent. It is well-known that the antioxidant activity depends on the type and polarity of the extracting solvent and the purity of active compound and that there is discussable correlation between antioxidant activity and phenolic content and composition [29]. In conclusion, water was the most efficient solvent to extract phenolic compounds with highest antioxidant activity, compared to methanol and ethanol extracts.

3.1.4. Antibacterial activity of rice straw extract against E. coli and L. innocua

The in-vitro activity against two bacteria, the gram-negative bacteria E. coli and the gram-positive bacteria L. innocua, was tested for the water extract. All tested concentrations (10–100 mg/ ml) showed bacteria growth indicated by a blue-purple staining after adding the indicator solution MTT (see Supplementary Fig. 1). Even a concentration as high as 100 mg freeze-dried extract/ml buffer solution did not show any inhibition effect on the bacteria growth.

3.2. Starch films with antioxidant extract

The aqueous extract was chosen for the production of antioxidant starch films, since it had highest total phenolic content and highest antioxidant activity.

3.2.1. Molecular changes in starch during film preparation

3.2.1.1. Molecular weight distribution and branch-chain length distribution. Changes in in the distribution of molar size, molar weight and the branch chain-length of starch are shown in Table 2 and Fig. 2. The SEC size distribution of the fully branched starch polymers are shown in Fig. 2a. The starting material, native potato starch, showed two peaks: a left-shoulder peak of amylose at $R_h < 20$ nm and the peak of the amylopectin fraction of starch at $R_h > 20$ nm. The SEC profile of all the produced starch films showed a narrowing of the two peaks towards smaller polymers and the disappearance of the left shoulder peak (Fig. 2a). Hence, there was an overlapping of the altered fractions of amylopectin and amylose in the elution profiles. The film forming process including melt blending and compression molding at 160 °C induced starch degradation, resulting in the peak shift of the SEC chromatogram (Fig. 2a) and the decrease of the weight-average molecular weight M_w (R_h) in Table 2. A similar shift in the size distribution and decrease in molar weight was reported previously during the extrusion process of starch by Liu, Halley and Gilbert [30] due to shear-induced chain scission of starch molecules. In addition, the starch degradation was more pronounced with decreasing glycerol content and increasing antioxidant extract content in the film. Glycerol protected starch macromolecules against the hydrolytic cleavage during the shear and thermal processing [31] as seen in lower M_w (Table 2).

Debranched starch samples are linear molecules and hence, their branch chain-length distributions can be directly obtained by universal calibration using pullulan standards. The SEC distribution gives the number of (debranched) chains with DP X (degree of polymerization of X glucose molecules), which is calculated by dividing the molecular weight (M_w) of the branches by the molecular weight of the anhydroglucose monomers (162 g mol^{-1}). All SEC weight distribution of the debranched starch films (Fig. 2b) exhibited two main peaks: debranched amylose chains at > DP100 and a distinct bimodal peak < DP100 for the debranched amylopectin chains. The two distinct maxima's in amylopectin fraction originate from the single-lamella and lamella-spanning branches in amylopectin. The first maximum at DP26

Table 3
Physical properties of starch films.

Sample	Thickness [mm]	Moisture %	TGA Onset [°C]	Peak [°C]	T_g Onset [°C]	Midpoint [°C]	EC_{50} Exp.[mg film/ mg DPPH*]	Theor.[mg film/ mg DPPH*]	Tensile properties Elongation [%]	Tensile strength [MPa]	Youngś modulus
CS_7G	0.216[b]	7.17 ± 0.03[a]	229 ± 0.03[d]	285 ± 0.5[d]	100 ± 2[b]	117 ± 10[c]	n.d.	n.d.	25.3 ± 3.94[a]	5.36 ± 0.93[a]	79 ± 20[c]
CS_10G	0.188[a]	12.5 ± 0.12[e]	228 ± 0.1[d]	284 ± 0.5[d]	54 ± 8[a]	70 ± 7[ab]	n.d.	n.d.	26.0 ± 5.95[a]	4.73 ± 1.11[a]	55 ± 21[c]
CRS_3A	0.186[a]	9.79 ± 0.05[d]	204 ± 0.2[bc]	269 ± 1.0[b]	46 ± 6[a]	57 ± 4[a]	103 ± 29[ab]	214	14.1 ± 2.90[b]	11.4 ± 0.91[b]	374 ± 26[b]
RS_2A	0.208[b]	8.72 ± 0.48[c]	205 ± 1.8[c]	274 ± 0.6[c]	62 ± 8[a]	75 ± 6[ab]	119 ± 14[b]	300	3.55 ± 0.78[c]	12.1 ± 2.07[bc]	503 ± 55[b]
RS_3A	0.223[c]	7.92 ± 0.18[b]	202 ± 0.8[ab]	269 ± 0.4[b]	68 ± 2[a]	84 ± 5[ab]	105 ± 5[ab]	200	2.90 ± 0.52[d]	14.7 ± 2.90[c]	671 ± 40[a]
RS_4A	0.235[c]	7.21 ± 0.16[a]	201 ± 1.2[a]	265 ± 0.5[a]	72 ± 14[ab]	89 ± 12[bc]	72 ± 4[ab]	150	-*	-*	-*

Thickness - average of 48 replicates; moisture content - average of triplicates, TGA peak - average of triplicates, EC_{50} values from DPPH* assay - average of triplicates, tensile testing - average of 6–8 replicates. Small letters correspond to significant difference using Tukeýs HSD post hoc test and Gabriel post hoc test for tensile properties. n.d. – not determined, *films broke during mounting. EC_{50} exp – experimental determined EC_{50} values, EC_{50} theor. – theoretical determined EC50 values.
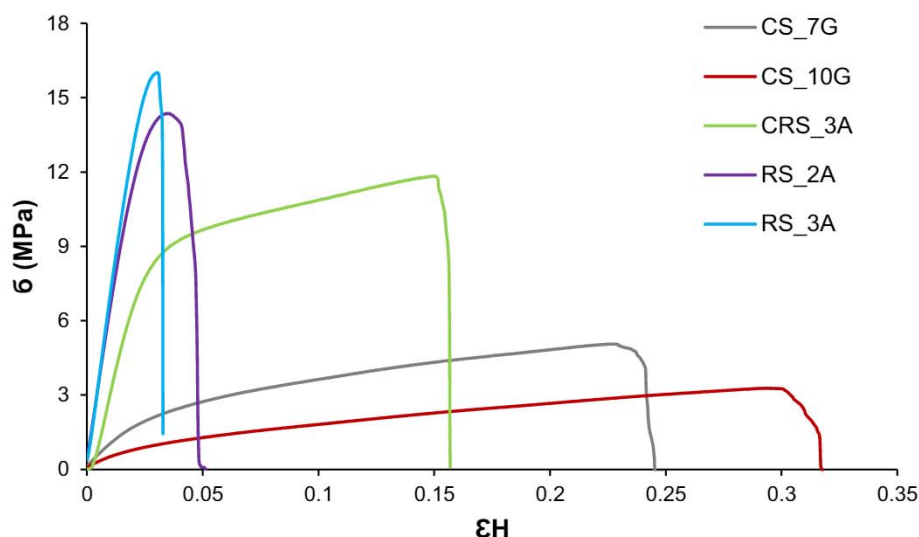
Fig. 5. Stress-strain curves of starch films. (Starch film RS_4A not included).

represents the chain length to fit the 9 nm span of a lamella repeated distance, whereas the maximum DP48 represents the minimum number of glucose units to space between 2 clusters [32]. Starch films with increasing antioxidant extract and lower glycerol content showed a gradually shift of the debranched amylose population to the left to lower chain-length (Fig. 2b). That implies that also amylose branches were degraded during processing. However, the bimodal shaped amylopectin population remained in all starch films (constant AP2/AP1 ratio in Table 2), which indicates that the degradation process has no selectivity towards structural features of the amylopectin molecule. The same random cleavage of amylopectin molecules has been reported before [30].

3.2.1.2. Microstructure of starch films. All starch film preparations with the antioxidant extract were able to produce continuous and handy films. The cross-sections of films are shown in Fig. 3, where no notables changes in the microstruture of the films due to the AOE incorporation were observed. All films displayed an homogeneous structure without any large cracks, wholes or phase separations. However, visible fine fissures were observed throughout all the films. In some films, such as RS_4A, CRS_3A and CS_7G, the presence of a heterogeneously fracture layer near the film surface indicates the progress of crystallization in these zones (indicated by red arrow), surely due to the greater molecular mobility associated with diffusion of water vapor to the films surface.

3.2.1.3. Optical properties - color and transparency of starch films. The antioxidant extract conferred a red-brownish colour to the starch films compared to starch-glycerol films (Fig. 4a). The color of the films became darker with higher AOE contents while maintaining similar hue (around 45°). Likewise, the lightness L parameter decreased with addition of antioxidant extract. In case of the films with same amount of antioxidant extract (CRS_3A and RS_3A) higher L values can be explained due to a dilution effect of glycerol in CRS_3A films with high glycerol content. The control glycerol-starch samples were slightly yellow (higher b value) but lighter (high L value).

The internal transmittance in the visible region from 400 to 800 nm is shown in Fig. 4b. At short wavelength 400–500 nm all films with added antioxidant extract absorbed light due to the selective absorption of the incorporated compounds and coherently with their concentration in the film. At longer wavelengths, the films turned more transparent with internal transmittance values between 40% and 60%.

3.2.2. Mechanical, thermal and barrier properties of the films

3.2.2.1. Moisture content. The moisture content of all starch films is given in Table 3. The increased glycerol content resulted in higher moisture content. Glycerol can interact with starch chains, increasing the molecular mobility and enhancing the free volume in the film network, which increases the accessibility of hydroxyl groups to interact with water by hydrogen bonding. Furthermore, glycerol has a hydrophilic character, which also increases the affinity to water [33]. The antioxidant extract reduced that effect, as deduced from the lower moisture content of films with the antioxidant extract. The added phenolic compounds replaced a part of the glycerol and could react with hydroxyl groups of starch chains, which, in turn, lowers their affinity to water.

3.2.2.2. Tensile properties. The mechanical behavior of starch films is shown in the stress-strain curves in Fig. 5. The maximum strength that a sample can withhold at rupture (tensile strength), the maximum elongation before rupture (elongation) and the initial linear relation between tension and deformation (Youngś modulus) are given in Table 3. Starch-glycerol films had a short elastic behavior in the initial part of the curve as seen by linear stress-strain relationship (slope corresponding to Youngś modulus), followed by a plastic behavior with an increase of deformation with low increase of stress until the rupture of the
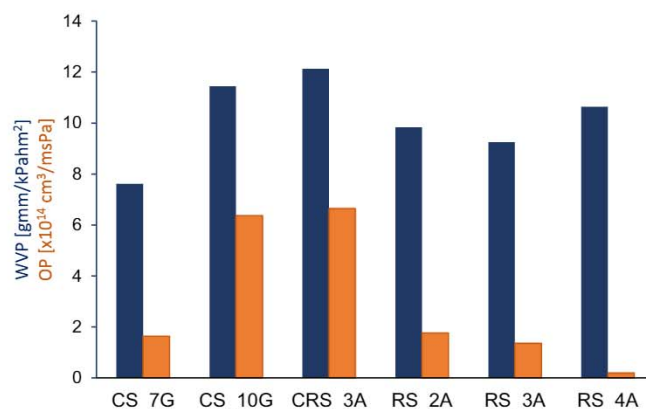

Fig. 6. Water vapor permeability (WVP, blue) and oxygen permeability (OP, orange) of starch films.

film. That behavior is related to the plasticizing effect of glycerol, which increased the space within and between starch polymers and reduced the inter-chain hydrogen bonding resulting in gliding of starch chains and material elongation [34]. The addition of antioxidant extract to the starch-glycerol film resulted in higher tensile strength but lower extensibility of the films. The incorporation of the antioxidant extract in the film CRS_3A provoked a reduction of elongation at break from 26% (in CS_10G films) to 14% while Youngs modulus and tensile strength increased. That effect was more pronounced in starch films where glycerol was partially replaced with the antioxidant extract. The film RS_4A broke almost immediately during the tensile test and hence, no

values of tensile parameters were presented. This evidences that the phenolic compounds were able to interact with the starch chains probably through induced cross-linking between starch chains hindering the intermolecular movement and making the films more rigid. An increase in tensile strength and decrease in elongation with increasing ferulic acid content has been reported in starch-chitosan films and was attributed to cross-linking between the phenolic acid and carbohydrate matrix [16]. Ferulic acid can induce cross-links between polysaccharides through radical dimerization, which could explain the mechanical properties of the films [35].
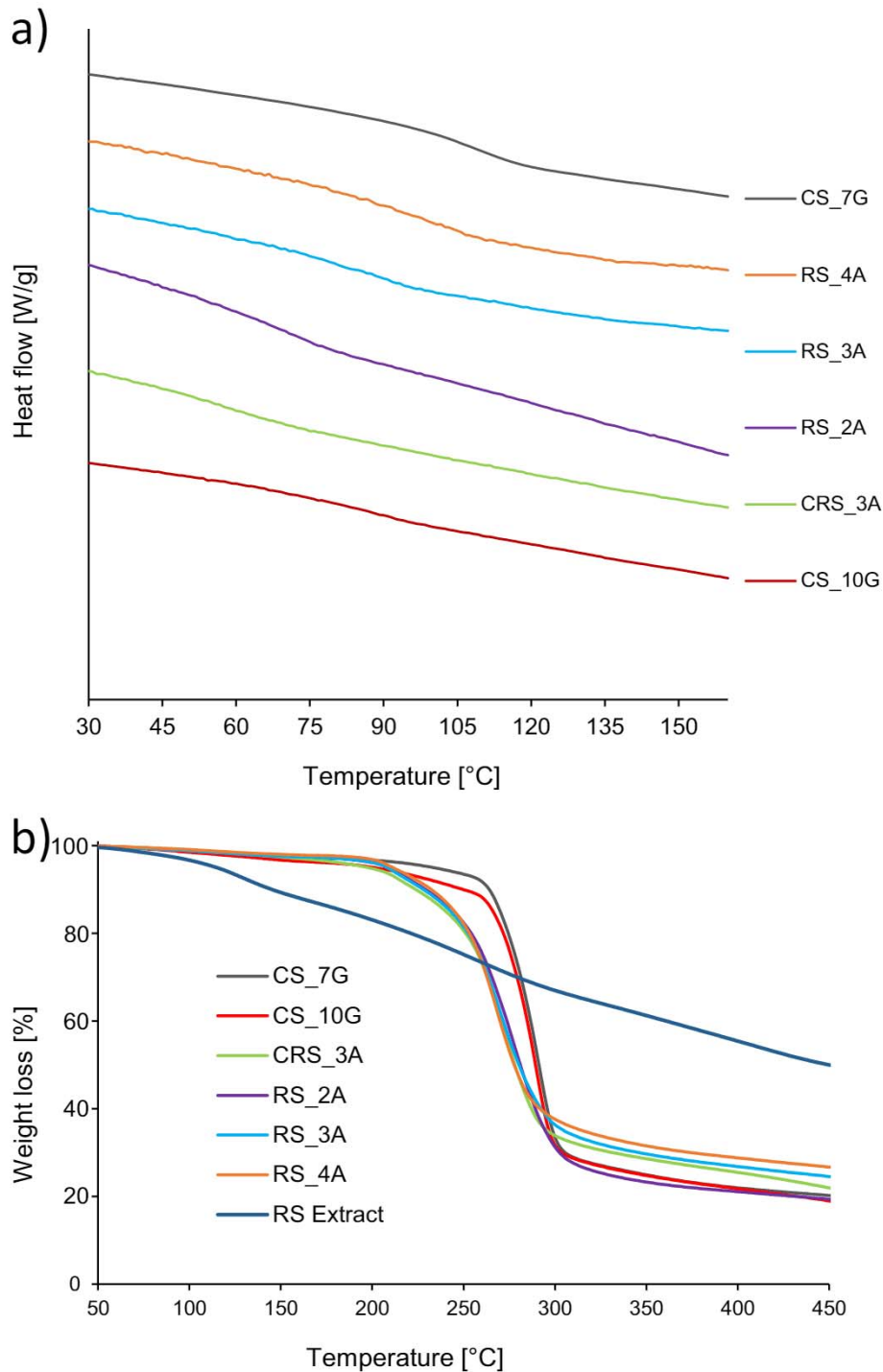

Fig. 7. (a) DSC curves of glycerol-starch films and (b) TGA curves of glycerol-starch films.

Another factor affecting mechanical properties is the decreasing moisture content in films with added antioxidant extract. Water is a well-known plasticizer and lower amount of water molecules in the film reduced the molecular mobility in the starch network [36]. The effect of the lower molecular weight of the starch chains was not reflected in the tensile behavior of the films containing extract, which is more clearly affected by the interactions of starch chains with the phenolic compounds. The obtained results are consistent with the glass transition temperature (T_g) of the starch matrix reflected in Fig. 7a and Table 3. As expected, both glycerol and the antioxidant extract had a plasticizing effect in starch matrix depending on their concentration in the film. However, this effect was more marked for glycerol and its substitution by the extract in the formulations implied an increased tendency in the T_g value in line with the ratio of glycerol substitution. This hinders the plastic deformation of the films observed in the tensile curves when the substitution ratio increases. On the basis of tensile behavior, maintaining the glycerol content but incorporating additionally the extract (sample CRS_3A) is recommended, resulting in films with adequate plastic behavior and enhanced elastic modulus and tensile strength.

3.2.2.3. Barrier properties of starch films. The barrier properties were determined on all films in terms of water vapor (WVP) and oxygen permeability (OP) and are shown in Fig. 6. Both WVP and OP were affected by the plasticization effect of glycerol and water content, resulting in increased values with higher glycerol or water content. However, the antioxidant extract seemed to limit the mass transport of oxygen molecules through the films. For instance, no significant differences in the OP were observed for samples CS_10G and CRS_3A, with the same glycerol-starch ratio, but with lower T_g value of CRS_3A associated to the plasticizing effect of the extract. A similar comparison could be made for samples CS_7G and RS_3A. This suggests that the mentioned molecular interactions of phenolic compounds and starch hydroxyl groups, not only results in higher tensile strength of the matrices, but also contributes to decrease the permeability of the membranes, limiting the OP. Starch films with the highest amount of antioxidant extract (film RS_4A) showed the greatest improvement of oxygen barrier properties compared to the control starch-glycerol films, without compromising the WVP values. Indeed, the reduced glycerol content as well as the lower moisture content in the films with the antioxidant extract reduce the hydrophilicity of the films and restrict oxygen passage throughout the polymeric network [37].

3.2.2.4. Thermal degradation. The onset and peak temperature of thermal degradation of starch films is reported in Table 3. As shown in the TGA curves in Fig. 7b, the starch-glycerol films showed a small weight loss until the onset of the polymer thermal decomposition around 230 °C, which can be related to evaporation and decomposition of glycerol and bonded water in these films. At 230–300 °C, the thermal dehydration, depolymerization and thermal decomposition of starch occurred in all films [16,38]. The addition of the phenolic compounds shifted the onset temperature to around 200 °C, which can be attributed to the degradation of the extract compounds [39] as seen in Fig. 7b. On the contrary, the lower weight loss at 300 °C in starch films with added extract can be related to the higher ash content incorporated from the antioxidant extract. Fig. 7b also includes the thermo-degradation curve of the extract, showing a progressive weight loss from about 100 °C. This suggests that part of the extract compounds with higher thermo-sensitivity could be lost during the film processing at 160 °C. The presence of extract in the film reduced the thermal stability of the polymer, which provides clues that it promotes structural changes such as starch dehydration and depolymerization, as it has been previously discussed.

3.2.3. In vitro antioxidant activity of starch films

The aqueous rice straw extract was included in starch films to promote antioxidant activity in the films. The *in-vitro* activity was calculated using DPPH* assay after dispersing the films in water. The calculated EC_{50} values are shown in Table 3. The films containing the antioxidant extract exhibited increasing activity against DPPH radicals, which correlated with increasing antioxidant extract content in the films. Theoretical EC_{50} values (Table 3) of all starch films were calculated based on the amount of the antioxidant extract in the film and its EC_{50} value, e.g. CRS_3A contains 3 g rice straw extract per 53 g total starch formulation (see table 2).

The experimental EC_{50} values were lower than the theoretical value, which was unexpected. Normally, a decrease in the antioxidant capacity upon incorporation in the films can be attributed to the partial degradation and/or evaporation of the phenolic compounds during the film thermo-processing, which has been shown previously in cassava starch films with added phenolic compounds from green tea [40]. However, all starch films exhibited higher antioxidant activity, probably due to degradation by-products of the antioxidant extract during the thermal processing, which could interact with the DPPH radicals, and needs further investigations. Nonetheless, the produced antioxidant starch films are considered suitable as edible package for food products such as nuts. The antioxidant activity of the extracts lies in the chemical action of the antioxidant compounds which has been reported to be useful in the prevention of lipid oxidation in nuts [41,42].

4. Conclusions

This study demonstrates the successful valorization of rice straw to develop environmental friendly antioxidant food packaging materials based on potato starch. Rice straw is a source of phenolic compounds with latent antioxidant activity that were easy to extract with water and gentle stirring at room temperature. The incorporation of this aqueous extract from rice straw affected the physicochemical properties of the starch films enhancing the antioxidant properties of the produced starch films in terms of DPPH radical activity. The produced films were moderately transparent with a slight red-brownish color and very homogenous, as shown by the SEM results. All films showed very good oxygen barrier properties comparable to the commonly used synthetic plastic EVOH. The inclusion of phenolic acids promoted the degradation of starch during the melt-blending and compression molding process, resulting in lower molar mass and shorter amylose branches. Our developed antioxidant starch films can be easily produced at low cost, they are edible and renewable, and they are excellent candidates as oxygen barrier in multi-layer packaging, with the possibility to replace synthetic coatings. Furthermore, the films incorporating rice straw extracts could be used as packaging to prolong the shelf life of food products like nuts or ready-to-serve fruit salads due to their antioxidant activity.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2019.09.222.

References

[1] M. Peanparkdee, S. Iwamoto, Bioactive compounds from by-products of rice cultivation and rice processing: Extraction and application in the food and pharmaceutical industries, Trends Food Sci. Technol. 86 (2019) 109–117.

[2] E. Karimi, P. Mehrabanjoubani, M. Keshavarzian, E. Oskoueian, H.Z. Jaafar, A. Abdolzadeh, Identification and quantification of phenolic and flavonoid components in straw and seed husk of some rice varieties (Oryza sativa L.) and their antioxidant properties, J. Sci. Food Agric. 94 (11) (2014) 2324–2330.

[3] A.A. Elzaawely, H.F. Maswada, M. El-Sayed, M.E. Ahmed, Phenolic compounds and antioxidant activity of rice straw extract, Int. Lett. Nat. Sci. 64 (2017) 1–9.

[4] A. Riaz, S. Lei, H.M.S. Akhtar, P. Wan, D. Chen, S. Jabbar, M. Abid, M.M. Hashim, X. Zeng, Preparation and characterization of chitosan-based antimicrobial active food packaging film incorporated with apple peel polyphenols, Int. J. Biol. Macromol. 114 (2018) 547–555.

[5] M.S. Alsaggaf, S.H. Moussa, A.A. Tayel, Application of fungal chitosan incorporated with pomegranate peel extract as edible coating for microbiological, chemical and sensorial quality enhancement of Nile tilapia fillets, Int. J. Biol. Macromol. 99 (2017) 499–505.

[6] T. de Moraes Crizel, T.M. Haas Costa, A. de Oliveira Rios, S. Hickmann Flôres, Valorization of food-grade industrial waste in the obtaining active biodegradable films for packaging, Ind. Crops Prod. 87 (2016) 218–228.

[7] M. Mushtaq, A. Gani, A. Gani, H.A. Punoo, F. Masoodi, Use of pomegranate peel extract incorporated zein film with improved properties for prolonged shelf life of fresh Himalayan cheese (Kalari/kradi), Innov. Food Sci. Emerg. Technol. 48 (2018) 25–32.

[8] S. Yildirim, B. Röcker, M.K. Pettersen, J. Nilsen-Nygaard, Z. Ayhan, R. Rutkaite, T. Radusin, P. Suminska, B. Marcos, V. Coma, Active packaging applications for food, Compr. Rev. Food Sci. Food Saf. 17 (1) (2017) 165–199.

[9] C. Menzel, C. González-Martínez, A. Chiralt, F. Vilaplana, Antioxidant starch films containing sunflower hull extracts, Carbohydr. Polym. 214 (2019) 142–151.

[10] X. Wang, H. Yong, L. Gao, L. Li, M. Jin, J. Liu, Preparation and characterization of antioxidant and pH-sensitive films based on chitosan and black soybean seed coat extract, Food Hydrocolloids (2018).

[11] C.L. Luchese, J. Uranga, J.C. Spada, I.C. Tessaro, K. de la Caba, Valorisation of blueberry waste and use of compression to manufacture sustainable starch films with enhanced properties, Int. J. Biol. Macromol. 115 (2018) 955–960.

[12] A. Ali, Y. Chen, H. Liu, L. Yu, Z. Baloch, S. Khalid, J. Zhu, L. Chen, Starch-based antimicrobial films functionalized by pomegranate peel, Int. J. Biol. Macromol. (2018).

[13] P. Forssell, R. Lahtinen, M. Lahelin, P. Myllärinen, Oxygen permeability of amylose and amylopectin films, Carbohydr. Polym. 47 (2) (2002) 125–129.

[14] E. Olsson, C. Menzel, C. Johansson, R. Andersson, K. Koch, L. Järnström, The effect of pH on hydrolysis, cross-linking and barrier properties of starch barriers containing citric acid, Carbohydr. Polym. 98 (2) (2013) 1505–1513.

[15] C. Menzel, E. Olsson, T.S. Plivelic, R. Andersson, C. Johansson, R. Kuktaite, L. Järnström, K. Koch, Molecular structure of citric acid cross-linked starch films, Carbohydr. Polym. 96 (1) (2013) 270–276.

[16] S. Mathew, T.E. Abraham, Characterisation of ferulic acid incorporated starch–chitosan blend films, Food Hydrocolloids 22 (5) (2008) 826–835.

[18] F. Vilaplana, J. Hasjim, R.G. Gilbert, Amylose content in starches: Toward optimal definition and validating experimental methods, Carbohydr. Polym. 88 (1) (2012) 103–111.

[19] V. Grosso, A. Farina, D. Giorgi, L. Nardi, G. Diretto, S. Lucretti, A high-throughput flow cytometry system for early screening of in vitro made polyploids in Dendrobium hybrids, Plant Cell, Tissue Organ Culture (PCTOC) 132 (1) (2018) 57–70.

[20] G. Cappelli, D. Giovannini, A.L. Basso, O.C. Demurtas, G. Diretto, C. Santi, G. Girelli, L. Bacchetta, F. Mariani, A Corylus avellana L. extract enhances human macrophage bactericidal response against Staphylococcus aureus by increasing the expression of anti-inflammatory and iron metabolism genes, J. Funct. Foods 45 (2018) 499–511.

[21] M. Ciulu, M. Cádiz-Gurrea, A. Segura-Carretero, Extraction and analysis of phenolic compounds in rice: a review, Molecules 23 (11) (2018) 2890.

[22] D. She, X. Nie, F. Xu, Z. Geng, H. Jia, G. Jones, M. Baird, Physico-chemical characterization of different alcohol-soluble lignins from rice straw, Cellul. Chem. Technol. 46 (3) (2012) 207.

[23] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT - Food Sci. Technol. 28 (1) (1995) 25–30.

[24] M. Houdkova, J. Rondevaldova, I. Doskocil, L. Kokoska, Evaluation of antibacterial potential and toxicity of plant volatile compounds using new broth microdilution volatilization method and modified MTT assay, Fitoterapia 118 (2017) 56–62.

[25] F. Vilaplana, R.G. Gilbert, Two-dimensional size/branch length distributions of a branched polymer, Macromolecules 43 (17) (2010) 7321–7329.

[26] J.B. Hutchings, Instrumental Specification, Food colour and appearance, Springer, 1999 199–237.

[27] G. Diretto, X. Jin, T. Capell, C. Zhu, L. Gomez-Gomez, Differential accumulation of pelargonidin glycosides in petals at three different developmental stages of the orange-flowered gentian (Gentiana lutea L. var. aurantiaca), PLoS ONE 14(2) (2019), e0212062.

[28] J.C. Sánchez-Rangel, J. Benavides, J.B. Heredia, L. Cisneros-Zevallos, D.A. Jacobo-Velázquez, The Folin-Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination, Anal. Methods 5 (21) (2013) 5990–5999.

[29] A. Moure, J.M. Cruz, D. Franco, J.M. Domínguez, J. Sineiro, H. Domínguez, M.A.J. Núñez, J.C. Parajó, Natural antioxidants from residual sources, Food Chem. 72 (2) (2001) 145–171.

[30] W.-C. Liu, P.J. Halley, R.G. Gilbert, Mechanism of degradation of starch, a highly branched polymer, during extrusion, Macromolecules 43 (6) (2010) 2855–2864.

[31] A.J.F. Carvalho, M.D. Zambon, A.A.S. Curvelo, A. Gandini, Size exclusion chromatography characterization of thermoplastic starch composites 1. Influence of plasticizer and fibre content, Polym. Degrad. Stab. 79 (1) (2003) 133–138.

[32] J.V. Castro, C. Dumas, H. Chiou, M.A. Fitzgerald, R.G. Gilbert, Mechanistic information from analysis of molecular weight distributions of starch, Biomacromolecules 6 (4) (2005) 2248–2259.

[33] V.D. Alves, S. Mali, A. Beléia, M.V.E. Grossmann, Effect of glycerol and amylose enrichment on cassava starch film properties, J. Food Eng. 78 (3) (2007) 941–946.

[34] P. Myllärinen, R. Partanen, J. Seppälä, P. Forssell, Effect of glycerol on behaviour of amylose and amylopectin films, Carbohydr. Polym. 50 (4) (2002) 355–361.

[35] R.D. Hatfield, J. Ralph, J.H. Grabber, Cell wall cross-linking by ferulates and diferulates in grasses, J. Sci. Food Agric. 79 (3) (1999) 403–407.

[36] S.H. Hulleman, F.H. Janssen, H. Feil, The role of water during plasticization of native starches, Polymer 39 (10) (1998) 2043–2048.

[37] I. Arvanitoyannis, E. Psomiadou, A. Nakayama, Edible films made from sodium casemate, starches, sugars or glycerol. Part 1, Carbohydr. Polym. 31 (4) (1996) 179–192.

[38] C.T. Greenwood, The Thermal Degradation of Starch, in: M.L. Wolfrom, R.S. Tipson (Eds.), Advances in Carbohydrate Chemistry, Academic Press, 1967, pp. 483–515.

[39] Y. Chai, M. Wang, G. Zhang, Interaction between Amylose and Tea Polyphenols Modulates the Postprandial Glycemic Response to High-Amylose Maize Starch, J. Agric. Food. Chem. 61 (36) (2013) 8608–8615.

[40] K.K.N.C.L Perazzo, A.C. de Vasconcelos Conceição, J.C.P. dos Santos, D. de Jesus Assis, C.O. Souza, J.I. Druzian, Properties and antioxidant action of actives cassava starch films incorporated with green tea and palm oil extracts, PloS one 9 (9) (2014), e105199.

[41] L. Atarés, R. Pérez-Masiá, A. Chiralt, The role of some antioxidants in the HPMC film properties and lipid protection in coated toasted almonds, J. Food Eng. 104 (4) (2011) 649–656.

[42] J. Bonilla, L. Atarés, M. Vargas, A. Chiralt, Edible films and coatings to prevent the detrimental effect of oxygen on food quality: Possibilities and limitations, J. Food Eng. 110 (2) (2012) 208–213.

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